

**Systematics of *Bonatea*  
(Orchidaceae): Species Boundaries  
and Phylogeny**

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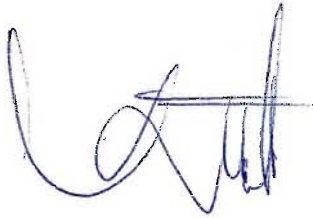
## ABSTRACT

*Bonatea* Willd. (Orchidaceae: Habernariinae) is a small genus confined to the African continent and Arabia. Phylogenetic and morphometric analyses were undertaken in order to evaluate phylogenetic relationships and species delimitations within *Bonatea*. In the phylogenetic analyses, little congruence was found between ITS and matK molecular data, while morphological results were largely congruent with those of the ITS region. There is little sequence variation within and between *Bonatea* species, which could indicate a recent and rapid radiation. The generic characters for *Bonatea* were re-evaluated. *Bonatea* is closely related to *Habenaria* but differs in having a galeate middle rostellum lobe that is clearly separated from the vertical anther thecae. By contrast, species of *Habenaria* have short anthers that are slightly arcuate and flank the rostellum. Morphometric analyses were used to determine taxon boundaries within the *Bonatea speciosa* and *Bonatea cassidea* complexes, respectively. Principle component and cluster analyses of morphological variation support the recognition of *Bonatea antennifera* Rolfe, *Bonatea boltonii* (Harv.) Bolus and *Bonatea speciosa* (L.f.) Willd. as distinct species. Morphological evidence supports the inclusion of *Bonatea porrecta* (Bolos) Summerh. and *Bonatea volkensiana* (Kraenzl.) Rolfe in the *B. speciosa* clade and this is corroborated by molecular data for the former. Clinal variation in petal lobe dimensions and colour across the distribution range of *Bonatea cassidea* Sond. encompasses the taxon *Bonatea saundersiae* (Harv.) T.Durand & Schinz, which is reduced to synonymy. *Bonatea saundersioides* (Kraenzl. & Schltr.) Cortesi, the sister species to *B. cassidea*, also exhibits colour variation in its petals. A revision of *Bonatea* is presented recognizing 14 species. *Bonatea eminii* (Kraenzl.) Rolfe was excluded due to insufficient information. Full descriptions are provided with diagnostic characters and distributional maps. *Bonatea bracteata* G.McDonald & McMurtry and *Bonatea tentaculifera* Summerh.

are removed from *Bonatea* based on their rostellum structure which is inconsistent with the revised generic concept. *Bonatea bracteata* was transferred as *Habenaria transvaalensis* Schltr. and *B. tentaculifera* was renamed *Habenaria bonateoides* M.Ponsie, as the specific epithet is currently occupied within *Habenaria*.

## **PREFACE**

I hereby declare that this project, submitted in the fulfillment of the requirement for the degree of Master of Science in the School of Biological and Conservation Sciences, University of KwaZulu-Natal, Pietermaritzburg, is the result of my own investigation, except where acknowledgement of other work is specifically indicated in the text.

A handwritten signature in blue ink, appearing to read 'M. Ponsie', with a horizontal line crossing through the middle of the letters.

Mariaan E. Ponsie

2006

A handwritten signature in black ink, appearing to read 'S. D. Johnson', with a horizontal line crossing through the middle of the letters.

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## LIST OF ABBREVIATIONS

°C	degrees Celsius
%	percentage
±	plus minus
ABI	Applied Biosystems INC.
BLFU	Bloemfontein: Geo. Potts Herbarium, South Africa
BOL	Cape Town: Bolus Herbarium, South Africa
BM	London: Herbarium, United Kingdom
bp	base pair
BR	Meise: Herbarium, Belgium
ca.	approximately
cf.	confer
CI	consistency index
cpDNA	chloroplast DNA
DNA	deoxy-ribonucleic acid
EA	Nairobi: Herbarium, Kenya
ed.	editor
eds.	editors
ETS	external transcribed spacer
FT	Florence: Herbarium, Italy
G	gamma distribution
GLOW	Nelspruit: Herbarium, South Africa
GRA	Grahamstown: Herbarium, South Africa
GTR	general time reversible
HKY	Hasegawa-Kishino-Yano
holo.	holotype
I	invariable sites
IGS	intergenic spacer
ill.	illustration
in obs.	in observation
iso.	isotype
ITS	internal transcribed spacer
J	Gauteng: Charles E. Moss Herbarium, South Africa

JC	Jukes and Cantor
K	Kew: Herbarium, United Kingdom
KEI	Umtata: Herbarium, South Africa
km	kilometer
KMG	Kimberley: Herbarium, South Africa
K2P	Kimura 2 parameter
lecto.	lectotype
lectosyn.	lectosyntype
-ln L	negative natural log likelihood
LYD	Lydenburg: Herbarium, South Africa
m	meter
matK	maturase K
MgCl <sub>2</sub>	magnesium chloride
min.	minute
ML	Maximum Likelihood
μl	microlitre
mm	millimeter
mM	millimolar
MP	Maximum Parsimony
NBG	Cape Town: Compton Herbarium, South Africa
ng	nanogram
NH	Durban: Natal Herbarium, South Africa
NJ	Neighbor Joining
NNI	Nearest Neighbour Interchange
nom. illegit.	<i>nomen illegitimum</i> (illegitimate name)
nom. nov. pro	<i>nomen novum pro</i> (new name)
nom. nud.	<i>nomen nudum</i> (naked name)
nom. superfl.	<i>nomen superfluum</i> (superfluous name)
non	not
nrDNA	nuclear ribosomal DNA
NU	Pietermaritzburg: Bews Herbarium, South Africa
p	probability
P	Paris: Herbarium, France
PAUP	Phylogenetic Analysis Using Parsimony
PCA	principle components analysis

PCR	polymerase chain reaction
pl.	plate
pmol	picamol
PRE	Tshwane: National Herbarium, South Africa
PRU	Tshwane: H. G. W. J. Schweickerdt Herbarium, South Africa
PUC	Potchefstroom: Herbarium, South Africa
$r^2$	coefficient of determination
RI	retention index
rRNA	ribosomal ribonucleic acid
S	Stockholm: Herbarium, Sweden
SACW	successive approximations character weighting
SAM	Cape Town: South African Museum Herbarium, South Africa
sec.	second
<i>sensu</i>	in the sense of
<i>s.n.</i>	<i>sine numero</i> (without number)
spp.	Species (singular or plural)
SPR	Subtree Pruning and Regrafting
STE	Stellenbosch: Stellenbosch Herbarium, South Africa
syn.	syntype
syn. nov.	new syntype
t.	tablate
<i>Taq</i>	<i>Thermus aquaticus</i>
TBR	Tree Bisection Reconnection
TCD	Dublin: Herbarium, Ireland
UNIN	Polokwane: Herbarium, South Africa
UPGMA	Unweighted Pair-Group Method with Arithmetic Mean
UPS	Uppsala: Botanical Museum (Fytoteket), Sweden
W	Wien: Herbarium, Austria

## INTRODUCTION

### ***Bonatea***

The Orchidaceae is one of the largest, most highly modified monocotyledonous families (Porter, 1959; Dressler, 1993), remarkable for its great variety of floral morphology and pollinator relationships (Dressler, 1981). It is distributed worldwide and includes an estimated 19,500 species, three-quarters of which are epiphytic (Dressler, 1993). Within the Orchideae, a tribe of terrestrial orchids, the subtribe Habernariinae includes 23 genera, including the two that were studied here, namely *Bonatea* Willdenow and *Habenaria* Willdenow.

*Bonatea* was established by Carl Ludwig von Willdenow in 1805 and named in honour of Guiseppe Antonio Bonato (1753-1836), professor of botany at the University of Padua in Italy (la Croix and la Croix, 1997). The genus contains 15 species (Summerhayes, 1968; Schelpe and Linder, 1999), some being endemic to South Africa, whilst others are found along the east African seaboard with the exception of one species that extends into Yemen. This terrestrial genus is closely related to *Habenaria*, also established by Willdenow in 1805, and both genera display distinctive green, or green and white flowers (Linder and Kurzweil, 1999). '*Habenaria*' is derived from the Latin *habena* or reins, presumably referring to the strap-like petal and labellum lobes, and long, slender spur found in many species. It is one of the largest terrestrial genera, with over 600 species found in tropical, subtropical, and warm temperate areas around the world. The greatest number of species is present in Central and south Central Africa, with only 35 species occurring in South Africa (la Croix and la Croix, 1997; Linder and Kurzweil, 1999).



*Bonatea* acquired generic recognition due to its distinct cucullate rostellum, tooth in the spur mouth, and the basal fusion of the lip with the lateral sepals, the anterior lobe of the petals and the stigmatic processes (Willdenow, 1805). However, some taxonomists did not believe this status was warranted, and as a consequence 11 of the 16 species have at one time or another been included within *Habenaria*. Kraenzlin (1893) even went as far as sinking *Bonatea* to a sectional status within *Habenaria*. Kraenzlin's perception of the *Bonatea* sect was however polyphyletic, with placement of current *Bonatea* taxa within *Habenaria* sections *Ceratopetalae*, *Replicatae* and *Tridactylae*. This arrangement was rejected by Rolfe (1898) and subsequent taxonomists (Schelpe, 1966; Summerhayes, 1968; Williamson, 1977; Stewart *et al.*, 1982; la Croix & Cribb, 1995; Linder and Kurzweil, 1999) who continued to recognize the genus, even though it was thought likely that retaining *Bonatea* as a distinct genus would render *Habenaria* paraphyletic (Linder and Kurzweil, 1999).

Many of the species within *Bonatea* are quite rare, with four species – *Bonatea eminii* (Kraenzl.) Rolfe, *Bonatea saundersiae* (Harv.) T.Durand & Schinz, *Bonatea stereophylla* (Kraenzl.) Summerh. and *Bonatea tentaculifera* Summerh. – known from only 1-3 sightings. No full revision of eastern and southern African species has ever been published and no molecular analyses have been undertaken on the group. In addition, species boundaries within the *Bonatea speciosa* complex are uncertain, requiring an examination of the characters delimiting *Bonatea antennifera* Rolfe, *Bonatea boltonii* (Harv.) Bolus and *Bonatea speciosa* (L. f.) Willd.

## **Taxonomy**

During the 18<sup>th</sup> century, Linnaeus laid the framework for a classification system by dividing organisms into a hierarchical series of taxonomic categories, starting with kingdom, and progressing down through phylum, class, order, family and genus to species. Delimitation and identification of taxa proceeds through taxonomic studies (Burns-Balogh and Funk, 1986), where variation amongst taxa is described and this data manipulated to construct estimates of relationships (Woodland, 1997). This can be achieved either intuitively or methodologically, using phenetics, cladistics (Burns-Balogh and Funk, 1986) and other methods.

Intuitive or artificial classification is largely subjective and does not necessarily reflect any particular lineage or relationship, as it involves the categorizing or sorting of systems based on obvious or convenient informative characters (Woodland, 1997). In contrast, the various phenetic approaches concentrate on reflecting the total similarity of the organisms in question. Phenetic studies eliminate some of the problems of intuitive classification, such as circular reasoning, appeal to authority and intuition. Yet in spite of this, how variable characters and character states are subjectively grouped, can affect the character weighting, which in turn affects the inter-relationships between the taxa (Burns-Balogh and Funk, 1986). Thus, the most serious objection to phenetics based on morphological data is that overall similarity may provide little indication of actual phylogenetic relationships (Woodland, 1997).

Cladistics, or phylogenetic systematics, is a discipline first formalized by Hennig in 1966. Cladistic techniques seek to reconstruct the evolutionary relationship among groups of taxa that can then be represented as a branching hierarchical tree called a cladogram. The primary principle of phylogenetic classification is the recognition of monophyletic groups, or clades, which include all the descendents from

a single ancestral species or common ancestor (Dressler, 1993; Judd *et al.*, 1999). Monophyletic groups are delimited by shared derived character states called synapomorphies, whilst derived character states unique to one taxon are called autapomorphies. Ancestral character states, termed plesiomorphies, cannot be used to infer relationships.

A phylogenetic classification is however, not easily achieved under the constraints of current rules of botanical and zoological nomenclature, as not all existing groups are monophyletic (Backland and Bremer, 1998). This is due to the problems of polyphyly and paraphyly. Polyphyletic groups reflect descent from unrelated ancestral species. Consequently, the group does not include the most recent common ancestor of all the taxa, nor does the ancestor necessarily possess the characters shared by the members of the group. Paraphyletic groups include the common ancestor and some but not all of its descendents owing to some of the lines falling into another group (Dressler 1993; Judd *et al.*, 1999).

Inherent to phylogenetic systematics are the concepts of homology, homoplasy and parsimony. Homology applies to characters inherited through a common ancestor, either with or without modification. Characters can be homologous either due to similarity of anatomical structure, position or development, similarity in behavioral traits, or, on a finer scale, due to special similarity of nucleotides and amino acids. Congruence with other characters provides the most compelling evidence for homology.

Homoplasy is when a character has arisen in totally unrelated species either through reversals, or parallel or convergent evolution (Burns-Balogh and Funk, 1986; Dressler, 1993). Reversals occur when a derived character state changes back to the ancestral state. Parallelism on the other hand, refers to those cases in which similar character states evolve independently in two or more different groups, including

closely allied groups. Homoplasy is most commonly the result of convergent evolution, where similar or related organisms occur under similar ecological conditions (Dressler, 1993; Judd *et al.*, 1999).

Parsimony accepts the simplest explanation for the data (Burns-Balogh and Funk, 1986). A cladogram is constructed such that the fewest *ad hoc* assumptions are required to explain the available data, minimising the number of changes from one character state to the next. This minimisation of the total tree length is equivalent to seeking trees that imply the least homoplasy. One must however always remember, that evolution need not proceed in the most parsimonious, most likely, or most efficient fashion.

### **Morphology**

This study uses morphological and DNA sequence data as a source of phylogenetic information for resolving the status of, and relationships within, a single plant genus. The use of data from many sources is fundamental to testing phylogenetic hypotheses, as it is possible to obtain comprehensive descriptions and interpretations of evolutionary events that are not attainable by focusing solely on one approach (Moritz and Hillis, 1996).

Morphological characters are useful at different hierarchical levels and therefore must be appropriate to the taxonomic problem being investigated. The characters can vary in phylogenetic informativeness, be discrete or continuously variable, binary or multistate.

Comparative studies have shown that morphological change and molecular divergence are quite independent, responding to different evolutionary pressures and following different rules (Moritz and Hillis, 1996; Barrier, Robichaux and Purugganan, 2001). Also, morphological traits may be subject to considerable variation and plasticity solely as a

function of environment (Donoghue and Sanderson, 1992). The use of nucleotide sequence comparisons for estimating phylogenetic relationships has attracted widespread attention among botanists studying a variety of plant groups, and is especially attractive for the orchidologist, given the complexity and overwhelming diversity of orchid flowers and vegetative structures within Orchidaceae (Cameron *et al.*, 1999). As each nucleotide position represents a single character it is possible to accumulate large data sets consisting of many characters in a relatively short space of time.

### **The chloroplast genome**

The chloroplast genome is well suited for evolutionary and phylogenetic studies as chloroplast DNA (cpDNA) is a relatively abundant component of plant total DNA, which facilitates extraction and analysis (Clegg and Zurawski, 1992). The majority of the approximately 120 genes in the chloroplast genome are essentially single copy (Palmer *et al.*, 1988; Sugura, 1992), in contrast to nuclear genes which are mostly members of multigene families (Soltis and Soltis, 1998). A critical feature of cpDNA, from the standpoint of phylogenetic studies at lower taxonomic levels, is its mode of inheritance (Palmer *et al.*, 1988). In most angiosperms examined to date it is inherited clonally, through the maternal parent (Palmer *et al.*, 1988; Soltis and Soltis, 1998).

The influence of hybridisation can largely be ignored since the chloroplast genome of only one parent is being followed (Harris and Ingram, 1991). However, the implicit assumption of only one mode of plastid transmission within a family, genus, or even species could have significant effects on a reconstructed phylogeny because of the sensitivity of cladistic methods to character state change (Harris and Ingram, 1991). Lineage sorting, chloroplast capture through hybridization, and chloroplast transfer or interspecific gene exchange by introgression, are all factors that may lead to incongruence between

phylogenetic hypotheses based on organelle DNA and the actual organismal phylogenies (Soltis and Kuzoff, 1995).

The chloroplast genome is relatively conserved and contains an inverted repeat region that is flanked by a small and a large single copy region. The presence of this inverted repeat is strongly correlated with the stability of the chloroplast genome, in which rearrangements are rare (Palmer *et al.*, 1988; Clegg and Zurawski, 1992). Also, chloroplast coding genes are not subject to the complex processes of gene conversion and intragenic recombination that often affect nuclear sequence evolution. Neither do chloroplasts and nuclei use the same enzymes for replication and repair, resulting in their vulnerability to different kinds and levels of natural mutagens, and thus different constraints on sequence evolution (Palmer *et al.*, 1988; Clegg and Zurawski, 1992).

About 20 chloroplast genes have been determined to be sufficiently large (>1kb) and widespread to be generally useful in comparative sequencing studies (Olmstead and Palmer, 1994). Among these protein coding regions, the maturase K gene (*matK*, previously designated ORFK of ORF509) is one of the most rapidly evolving (Steele and Vilgalys, 1994; Soltis and Soltis, 1998). In all photosynthetic land plants so far examined, *matK* is positioned between the 5' and 3' exons of the *trnK* gene, which is located in the large single copy region of the chloroplast genome, upstream of the *psbA* gene near the inverted repeat (Liang and Hilu, 1996; Soltis and Soltis, 1998). *matK* constitutes approximately 1550 bp of this 2600 bp *trnK* region and is easily amplified owing to several conserved regions over its length (Steele and Vilgalys, 1994), and is itself located between two highly conserved, flanking coding regions that include the *trnK* exons and the genes *rps16* and *psbA* (Steele and Vilgalys, 1994; Liang and Hilu, 1996; Soltis and Soltis, 1998). The substitution rate is relatively even across the *matK*

gene, yet slightly higher between base pair positions 600 and 900 (Whitten, Williams and Chase, 2000). Sequence comparisons reveal that insertions and deletions (indels), although relatively rare events in *matK* (Plunkett, Soltis and Soltis, 1997), are often phylogenetically informative (Downie and Palmer, 1992; Soltis and Soltis, 1998; Mort *et al.*, 2001).

### **The nuclear genome**

Despite the enormity of the nuclear genome and the large number and diversity of genes that it includes, identification of nuclear DNA regions that are useful for phylogenetic comparisons with chloroplast DNA data has been problematic (Baldwin, 1992). Eukaryotic nuclear ribosomal DNA has several features that promote its use for phylogenetic analyses at the rank of family and below (Baldwin *et al.*, 1995; Meerow, 2000). Firstly, the nrDNA gene units belong to a multigene family and are thus highly repeated in the plant nuclear genome (Baldwin *et al.*, 1995; Fritz, Cockburn and Seawright, 1995). Secondly, differential rates of evolution exist between the spacers and coding regions, with the Internal Transcribed Spacer (ITS) regions being the most appropriate for within and between species comparisons (Baldwin *et al.*, 1995; Fritz *et al.*, 1995). And lastly, from a practical standpoint, the small size of the ITS region and its location between highly conserved flanking sequences, makes this region easy to amplify by polymerase chain reaction (PCR) using universal eukaryotic primers designed by White *et al.* (1990).

The typical eukaryotic nuclear ribosomal DNA (nrDNA) gene array consists of tandemly repeating coding units that are preceded by an external transcribed spacer (ETS) and separated from the next repeat unit by a long nontranscribed intergenic spacer (IGS) (Hamby and Zimmer, 1992; Baldwin *et al.*, 1995; Bateman, Pridgeon and Chase, 1997). Each coding unit is further subdivided into three genes (18S,

5.8S and 26S genes), which are separated by two internal transcribed spacers (ITS1 and ITS2) (Suh *et al.*, 1993; Elder and Turner, 1995; Soltis and Soltis, 1998). The internal transcribed spacers (ITS1 and ITS2) evolve at or near the neutral rate (Bakker, Olsen and Stam, 1995), and hence, due to their relatively high rate of nucleotide substitution, are capable of resolving taxa that have recently diverged (Savard, Michaud and Bousquet, 1993; Kim and Jansen, 1996; Erdogan and Mehlenbacher, 2000).

The majority of variation between angiosperm ITS sequences is attributable to point mutations, transitions and transversions, rather than length mutations, where G+C or A+T rich sequences are inserted or possibly deleted (Baldwin *et al.*, 1995; Torres, Ganai and Hemleben, 1990). Consequently, both ITS1 and ITS2 sequences exhibit high levels of variation in both length and sequence (Kim and Jansen, 1996; Douzery *et al.*, 1999), and, when the boundary of the 5.8S gene is taken from Hershkovitz and Lewis (1996), ITS1 is found to range from 187 to 298 bp and ITS2 from 187 to 252 bp in flowering plants (Campbell *et al.*, 1995).

An analysis of plant ITS sequences shows that the G+C content of ITS1 nearly equals that of ITS2, although it can range from 20% to 90% in different taxa (Torres *et al.*, 1990; Soltis and Soltis, 1998). Furthermore, this balanced G+C content in a given species seems to be similar to that of the expansion segments in the 28S rRNA coding region. Thus the ITS spacers show co-evolution, with respect to each other and with respect to the expansion segments (Torres *et al.*, 1990).

Sliding windows of nucleotide divergence also show that ITS1 and ITS2 have distinct regional heterogeneity, owing to small regions within the ITS spacers that are under greater selection (Gaut and Weir, 1994; Buckler and Holtsford, 1996; Soltis and Soltis, 1998). On the converse,



there is a widespread existence of nrDNA pseudogenes (Buckler, Ippolito and Holtsford, 1997), which are apparently subject to no functional constraints irrespective of expression patterns and accumulate nucleotide substitutions at a rate equal to the mutation rate (Li, Luo and Wu, 1985). Identification of potential nrDNA pseudogene sequences is essential for inferring species relationships (Bailey *et al.*, 2003).

Most phylogenetic analyses of DNA sequences assume, explicitly or implicitly, that neighbouring nucleotides have evolved independently of one another (Muse and Weir, 1992), as well as in the absence of positive selection, as suggested by the neutral hypothesis (Kimura, 1969). Ribosomal ribonucleic acid (rRNA) however, forms a distinct secondary structure that is dependent on Watson-Crick (G-C, A-U) and wobble base (G-U) pairing interactions in the helical duplex regions (Wheeler and Honeycutt, 1988; Dixon and Hillis, 1993; Varani and McClain, 2000). This implies that some selection pressure is responsible for maintaining the stem loop structures (Torres *et al.*, 1990), which are dependent on the fidelity of pairing between distantly related bases (Gerbi, 1985; Wheeler and Honeycutt, 1988). Conversely, the helical regions of the molecule are often quite variable in sequence, which shows that precise sequence of bases within the helical regions is of relatively little importance, as long as the positioning of these regions within the secondary structure is correct (Savill, Hoyle and Higgs, 2001). As only independently varying qualities are viable characters in phylogenetic reconstruction (Wheeler and Honeycutt, 1988), base pairs that co-evolve in the stem loop regions violate the assumption of base pair independence (Dixon and Hillis, 1993). The mal-effects are further amplified by an additional factor: there are two to three times as many potentially varying positions in the double-stranded areas as in the single-stranded regions, which leaves the data set biased in favour of

the positions that pair (Wheeler and Honeycutt, 1988; Dixon and Hillis, 1993).

Molecular analysis of nrDNA genes has revealed that individual repeats within a gene family evolve non-independently in a concerted pattern within genomes (Elder and Turner, 1995), resulting in a sequence similarity of repeating units that is greater within than among species (Li *et al.*, 1985). However, even though gene homogenisation appears to be the rule (Hillis and Dixon, 1991), the presence of intragenomic ITS variation has been detected from comparisons of direct and cloned sequences in a range of vascular plant groups (Jeandroz, Roy and Bousquet, 1997; Whittall *et al.*, 2000; Mayol and Rosselló, 2001).

Polymorphism of nrDNA unit types can be attributed to one or a combination of factors that affect the rate of concerted evolution, unequal crossover and gene conversion, and can also result from sexual recombination, segregation, polyploidisation events and interspecific hybridisation in the nuclear DNA of closely related taxa (Baldwin *et al.*, Liston *et al.*, 1996; 1995; Jeandroz *et al.*, 1997; Whittall *et al.*, 2000; Mayol and Rosselló, 2001). Collectively, these features yield complex hierarchical relationships among genes within and between species (Sanderson and Doyle, 1992), which can result in the possible sampling of sequences with different evolutionary histories (Baldwin *et al.*, 1995; Buckler *et al.*, 1996). This presents problems of homology assessment (Baldwin, 1992), and it thus becomes necessary to make distinctions between orthologous sequences (those that have originated due to speciation) and paralogous sequences (those that have originated via intragenomic duplication), in order to assess the evolutionary homology of multilocus sequences being used to construct phylogenies (Sanderson and Doyle, 1992; Elder and Turner, 1995).

In general, interspersions of conserved and variable domains through both ITS spacers, promotes sequence alignment in the ITS region (Baldwin *et al.*, 1995). However, if rate heterogeneity is a general property of ITS sequences, both in terms of regional as well as character covariance, it necessitates caution in phylogenetic inference, as most phylogeny algorithms perform poorly when substitution rates vary among nucleotide sites. Similarly, polymorphisms complicate inference of species trees from gene trees, and can result in misleading phylogenetic conclusions (Soltis and Soltis, 1998; Bailey *et al.*, 2003). A degree of caution is thus probably warranted in using nrDNA for phylogenetic analysis (Hillis and Dixon, 1991). However, the inclusion of multiple sources of data, from other molecular sources as well as non-molecular evidence from morphology and biogeography, will aid in clarifying the phylogenetic signal (Sanderson and Doyle, 1992; Whittall *et al.*, 2000; Mayol and Rosselló, 2001).

### **Phylogenetic analysis**

The inference of phylogeny for a particular data set can be achieved via several computational methods. There are several program packages available, such as PHYLIP and Mega, but the most widely used in the field of plant systematics is the program PAUP\* (Phylogenetic Analysis Using Parsimony; Swofford, 1998).

There are two approaches to tree construction: algorithmic and tree-searching. Algorithmic methods, such as Neighbor Joining (NJ) and Unweighted Pair-Group Method with Arithmetic Mean (UPGMA), have two advantages in that they are fast and yield only a single tree from any given data set. These distance methods convert the aligned sequences into a distance matrix of pairwise differences between the sequences, from which branching order and branch lengths are computed. Of interest to this study are the discrete data or tree searching methods, which all use the multiple sequence alignment

directly by comparing characters within each column in the alignment (Hall, 2001; Baldauf, 2003).

When the number of taxa is small, it is possible to evaluate each of the possible trees by using an exhaustive search method with a branch-addition algorithm. This however is often impractical, as for 10 taxa there are already 34459425 trees. Thus, for up to 20 taxa, PAUP offers an alternative 'branch and bound' algorithm, which also guarantees finding the best tree but does not require searching every tree. When a data set includes too many taxa, it is beyond an exact computational solution and heuristic algorithms must be employed.

The heuristic search procedure can be interpreted as a two step process. Firstly a starting tree is generated, then 'branch swapping' is carried out to search the 'tree space' for more parsimonious solutions than the original starting tree. There are three widely used methods of branch-swapping, namely Nearest Neighbour Interchange (NNI), Subtree Pruning and Regrafting (SPR), and Tree Bisection Reconnection (TBR). These methods do not necessarily guarantee finding the best tree, but involve a trade off between the certainty of finding the best tree and the need to find a tree within a realistic time scale. Tree-searching methods are used with different optimality criteria, including Maximum Parsimony (MP), Maximum Likelihood (ML) and Bayesian analysis; each with their own analysis efficiency, robustness, computational speed and discriminating ability (Hall, 2001).

Under parsimony the following statistics are used to evaluate the amount of homoplasy in the data. The ensemble consistency index (CI) is a measure of the average fit of characters to a tree. Although the CI does reveal the amount of homoplasy, it fails to distinguish synapomorphies (phylogenetically informative) from autapomorphies (phylogenetically uninformative), since both have individual CI scores

of 1.0. The retention index (RI) provides a more specific measure to determine the extent of synapomorphy among characters and shows the proportion of character similarities that can be attributed to synapomorphy. A high value should give us some confidence that the branching pattern shown is largely determined by non-homoplastic characters. A factor used for character weighting is the rescaled consistency index (RC), which is equivalent to  $CI \times RI$ , and does not rely on *a priori* assumptions concerning the characters.

Phylogenetic data sets often do not yield a single tree. Thus following an initial search it is possible to reweight the characters through Successive Approximations Character Weighting (SACW) (Farris, 1969), yielding cladograms in which the homoplasious 'noise' is suppressed. Such *a posteriori* weighting of characters is objective in the sense that the reweighting values are automatically derived from the fit of each character on the initial cladograms. The weighting of each character thus relies entirely on its performance relative to all the other characters considered, and not on the subjective judgement of the researcher.

Morphological characters of unordered and ordered type can be traced onto the cladogram. Ambiguities in character tracings can be resolved so as to choose the assignments that delay (DELTRAN) or accelerate (ACCTRAN) character transformations within the internal nodes of a tree. The DELTRAN option shows those most-parsimonious assignments that delay changes away from the root - thus leading to a preference for two origins of a character and maximizing parallel changes, whilst, the ACCTRAN option shows those assignments that accelerate changes towards the root - thus leading to a preference for a single origin followed by a subsequent reversal (Maddison and Maddison, 1992).

Maximum parsimony (MP) is a simple but popular technique for reconstructing relationships. MP is based on the assumption that the best tree is the one that requires the fewest number of changes to explain the data in the alignment. All trees are given a length, equal to the minimum number of character state transformations needed to explain the tree, and the tree with the lowest length is the most parsimonious (MP) tree.

MP is not always accurate, nor is the most parsimonious tree necessarily unique. Parsimony methods are particularly sensitive to lineages having experienced rapid rates of change, and have a high probability of producing an erroneous tree in the presence of long branch effects (Felsenstein, 1978). To overcome these problems, a different approach is required.

Maximum likelihood (ML) is a popular statistical method used to make inferences about parameters of the underlying probability distribution of a given data set (Huelsenbeck and Crandall, 1997). The advantages of the ML method are that it allows the user to specify the evolutionary model they want to use and provides a simple way to compare trees according to their likelihoods i.e. using KH- and SH-tests. The program MODELTEST can be used to establish the model of nucleotide substitution that best fits the data (Posada and Crandall, 1998), allowing for the subsequent search of the tree with the largest log likelihood (lnL).

ML estimations often have lower variance than other methods (i.e., it is frequently the estimation method least affected by sampling error), and tend to be robust to many violations of the assumptions in the evolutionary model. Even with very short sequences ML tends to outperform alternative methods such as parsimony or distance methods (Hall, 2001). A disadvantage of ML is that even with simple models of

evolutionary change the computational task is enormous. Thus ML is considerably slower than either parsimony or NJ, and it is not difficult to exceed the capacity of even the most up to date desktop computer.

Bayesian analysis is a variant of ML, and has the ability to include prior information regarding relationships into the process of phylogeny estimation by stating a prior probability distribution of trees (Archibald, Mort and Crawford, 2003). Bayesian analysis is similar to ML in that the user postulates a model of nucleotide substitution and the program searches for the best trees that are consistent with both the model and with the data. However, instead of seeking the tree that maximizes the probability of observing the data given the tree, Bayesian analysis seeks the tree that maximizes the probability of the tree given the data and the model for evolution. And, instead of producing a single tree, Bayesian analysis produces a set of trees of roughly equal likelihoods (Hall, 2001). The frequency of a given clade in that set of trees is virtually identical to the probability of that clade, so no bootstrapping is necessary to assess the confidence in the structure of the tree. These posterior probabilities however, are not directly comparable with bootstrap values (Douady *et al.*, 2003). A sampling process, the Markov Chain Monte Carlo simulation method, is used to sample trees from the distribution of posterior probabilities and to approximate the posterior probability of a tree (Green, 1995). This increases the speed of analysis permitting more extensive searches to be performed than previously possible in a model-based system (Huelsenbeck *et al.*, 2001).

Once a phylogenetic tree has been generated, it is necessary to employ some method for determining the level of internal support attributable to the nodes of the various reconstructed branches. Methods that can be used to evaluate tree robustness are Bremer support, also known as

the “decay index” (Bremer, 1994), bootstrapping (Felsenstein, 1985) and jackknifing (Farris *et al.*, 1996).

Bremer support is calculated through the number of additional tree steps needed until a given branch arrangement collapses in the strict consensus tree. The decay index for the node is thus the number of steps that the tree is longer than the shortest tree (Zander, 2004). In phylogenetic analyses nonparametric bootstrapping is the most commonly used method. This is done by taking random subsamples of the data with replacement (pseudoreplicates) and building trees from each of these. The frequency with which a given branch is found upon analysis of the pseudoreplicate data sets is recorded as the bootstrap proportion. These proportions can then be used to assess the reliability of individual branches in the optimal tree (Baldauf, 2003). The jackknife is a different type of statistical test where subsets of the data matrix are created by subsampling the characters or taxa without replacement. The frequency with which a given branch is recovered after multiple analyses of the data set is recorded as the jackknife proportion.

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## **Aims**

Part of the challenge of systematics is to determine what went on in the past. This project aims to ascertain the relationships among the species of *Bonatea*, as well as the relationship of this genus to *Habenaria*.

These concepts will be explored and further background and details provided in the main body of the thesis. In summary, the aims of this study are:

1. Establish a phylogeny for *Bonatea* based on molecular and morphological data
2. Determine the relationship of the genus to *Habenaria*
3. Determine patterns of morphological evolution in the genus
4. Determine species delimitations by examination of the distribution, habitat and morphological differences of sister taxa
5. Provide a full revision of the genus, including both southern and east African species

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## CHAPTER 1

### **A molecular and morphological phylogeny of *Bonatea* (Orchidaceae)**

#### **ABSTRACT**

The genus *Bonatea* is widely distributed throughout southern and eastern Africa. Considerable debate surrounds the generic status of *Bonatea*, but there have been neither previous studies of evolutionary relationships among *Bonatea* species, nor any tests of the monophyly of the genus in relation to its close relative *Habenaria*. We investigated phylogenetic relationships among *Bonatea* and selected *Habenaria* species using morphology, as well as sequences of the chloroplast gene *matK* and of the internal transcribed spacer (ITS) regions of nuclear ribosomal DNA. Neither the ITS, *matK*, or both data sets combined yielded well-resolved and reasonably supported phylogenetic structure for *Bonatea*. There is poor congruence between ITS and *matK* data for interspecific relationships in *Bonatea*, whilst the morphological results are largely congruent with the ITS analysis. Relative to *Habenaria*, there is little sequence variation among *Bonatea* species, which could indicate a recent and rapid radiation. Although the sampled *Bonatea* species form a clade, more extensive sampling of *Habenaria* would be required to establish whether or not the genus is monophyletic.

#### **INTRODUCTION**

The genus *Bonatea* Willd. is a member of the subtribe Habernariinae (Orchidaceae) (Dressler, 1993). *Bonatea* consists of 15 species (Summerhayes, 1968; Schelpe and Linder, 1999) that are found mainly in forest and savannah biomes; with its centre of diversity on the

eastern seaboard of southern Africa. The genus extends through east Africa into Sudan, with one species in Yemen.

Considerable debate exists surrounding the generic status of *Bonatea* (Kurzweil and Weber 1992). Characters such as rostellum morphology, perianth adnation to the gynostemium, stigma shape and the occurrence of a tooth in the mouth of the spur have been cited as defining *Bonatea* (Rolfe, 1898; Rolfe, 1913; Summerhayes, 1949; Williamson, 1977; Stewart, 1996; la Croix and la Croix, 1997; Schelpe and Linder, 1999). However, with the exception of the galeate rostellum, the character states supposedly synapomorphic for *Bonatea* occur sporadically in *Habenaria*, and it has been suggested that the genus is merely a specialized section of *Habenaria* (Schelpe and Linder, 1999). Thus only a broad consideration of *Habenaria* will resolve this question of *Bonatea* monophyly.

An analysis of one morphological and two molecular data sets was performed to estimate phylogenetic relationships within the genus. The DNA regions sequenced were the *matK* region of the chloroplast genome and the internal transcribed spacers (ITS1 and ITS2) of the nuclear ribosomal DNA (nrDNA) repeat, and the intervening 5.8S rRNA gene. These rapidly evolving regions are appropriate for both within species and between species comparisons (Downie and Palmer, 1992; Baldwin *et al.*, 1995; Johnson and Soltis, 1995; Soltis and Soltis, 1998; Padgett, Les and Crow, 1999; Stanford, Harden and Parks, 2000; Whitten, Williams and Chase, 2000). The *matK* gene, approximately 1550 bp in length, codes for a maturase enzyme and is one of the most rapidly evolving protein coding regions in the chloroplast genome (Soltis and Soltis, 1998). Nucleotide substitution rates can vary greatly among different plants (Wolfe, Li and Sharp, 1987) and as the rate of evolution of *matK* is slower than ITS nrDNA, limited specimens and only a select region of the *matK* gene was sequenced to evaluate its

usefulness at the inter- and intra-generic level. Many recent studies have indicated that directly combining independent molecular data sets using regions with different levels of variation provides resolution at different areas of the cladogram, as well as increasing phylogenetic resolution and improving support levels (Chase and Cox, 1998; Whitten *et al.*, 2000).

With certain caveats (Jenner, 2004; Weins, 2004), phylogenetic information can also be obtained from morphological data. Comparative studies have shown that morphological change and molecular divergence are quite independent, responding to different evolutionary pressures and following different rules (Moritz and Hillis, 1996; Barrier, Robichaux and Purugganan, 2001.). Hence, the use of data from many sources is fundamental to obtain comprehensive descriptions and interpretations of evolutionary events that are not attainable by focusing solely on one approach (Moritz and Hillis, 1996).

Some controversy surrounds the delimitation of taxa within the *Bonatea speciosa* complex. Sommerville (1982) and Schelpe and Linder (1999) recognize a single species, *Bonatea speciosa* with two varieties (var. *speciosa* (L.f.) Willd. and var. *antennifera* (Rolfe) Sommerville), and conflate *Bonatea boltonii* (Harv.) Bolus within the typical variety. Following morphological investigation, we distinguish the constituent taxa *Bonatea antennifera* Rolfe (Rolfe, 1905), *Bonatea boltonii* (Harv.) Bolus (Harvey, 1859) and *Bonatea speciosa* (L.f.) Willd. (Linnaeus, 1781) in order to evaluate their taxonomic status.

This study aims to (i) establish a phylogeny for *Bonatea* based on morphological and molecular data, (ii) determine, as much as possible, the relationship of this genus to *Habenaria*.

## MATERIALS AND METHODS

### Morphology

Morphological, distributional and ecological data were derived from herbarium material, field observations, fresh plants in cultivation and literature. Herbarium specimens of *Bonatea* and *Habenaria* species were loaned and digital images of type specimens were obtained from the following herbaria: BLFU, BOL, BM, BR, EA, FT, GLOW, J, K, KEI, KMG, LYD, NBG, NH, NU, P, PRE, PRU, PUC, S, SAM, STE, TCD, UNIN and UPS. All measurements were made using a digital calliper.

Nineteen taxa (Appendix 1) were analysed for 45 character states (Appendix 2). *Bonatea eminii* (Kraenzl.) Rolfe was not included in the morphological analysis due to insufficient information. The species is known from a single collection (Type: Dodoma, Uyansi, Ipunguli (Pungusi), in the Mgunda-Mkali, 24km SW of Itigi on the central Tanzanian railway, *Stuhlmann 420* (B, holo. †)) which was destroyed during the bombing of Berlin in World War II. Morphological characters were coded as unordered and equally weighted (Hawkins, Hughes and Scotland, 1997), thus making no assumptions about the primitive or derived state of each character (Nixon and Carpenter, 1993). Quantitative characters were subdivided at boundaries coinciding with complete breaks in the variation (Stevens, 1991). The tree was rooted with *Habenaria lithophila* Schltr., one of the *Habenaria* species in the molecular study most distantly related to *Bonatea*.

No objective coding was possible for spur and ovary measurements due to a lack of discontinuity. Leaf margin characters 9 and 10 (Appendix 2) were not merged because half the *Bonatea* species show both smooth and undulate margins perianth within their range of variation. Flower colour is the product of a variety of evolutionary processes and these necessitate careful coding. Green parts are poorly reflective and the



product of the pigment chlorophyll, held in plastids within the perianth mesophyll (characters 23 and 30). By contrast, white parts of the flower are strongly reflective due to structural modification of the perianth cells (characters 16, 22 and 29). Lateral sepal and lower petal lobe posture (characters 17, 18, 26 and 27) were described from horizontal and vertical planes. Characters of the gynostemium were generated from rehydrated flowers. The caudicle canal extension (character 38) refers to the free part of the structure.

### **DNA extraction, PCR Amplification and Sequencing**

Total genomic DNA from fresh or silica gel-dried leaf tissue of individual plants was extracted using the QIAGEN DNeasy™ Plant Mini Kit. The manufacturer's protocol was followed, with the exception of steps 12 and 13, where 50µl elutions with Buffer AE were performed. The 36 specimens extracted and sources of the material are listed in Appendix 4. Fresh material of *B. eminii*, *Bonatea stereophylla* (Kraenzl.) Summerh. and *Bonatea tentaculifera* Summerh. was not available for the study owing to the rarity of these species. *Bonatea rabaiensis* (Rendle) Rolfe and *Bonatea volkensiana* (Kraenzl.) Rolfe similarly were excluded, owing to difficulty in obtaining material from east Africa.

The polymerase chain reaction (PCR) was used to amplify the ITS1, 5.8S and ITS2 region with primers ITS5HP (5' ggAAggAgAAgTCgTAACAagg 3'; Suh *et al.*, 1993) and ITS4 (5' TCCTCCgCTTATTgATATgC 3'; White *et al.*, 1990). Where multiple PCR products resulted, alternative primers were subsequently utilised, i.e., AB101 (5' ACgAATTCATggTCCggTgAAgTgTTCg 3'; the same as 17SE Sun *et al.*, 1994) and AB102 (5' TAGAATTCcccggTTCgCTCgCCgTTAC 3'; the same as 26SE Sun *et al.*, 1994). Primers matK-F1 (5' gTTCAAATCCTTCAATgCTgg 3') and matK-R1 (5' C(CT)AATACAgTACAAAATT(gT)AgC 3') were developed using the freeware software Amplify v1.2â (Bill Engels © 1992), to amplify the

most variable 630bp region within the matK gene (Whitten, Williams and Chase, 2000). PCR amplifications were performed in a Perkin Elmer GeneAmp PCR System 2400. ITS PCRs used the following cycling conditions: 95°C for 10 minutes, 30 cycles of (94°C for 45 sec., 55°C for 45 sec., 72°C for 60 sec.), 72°C for 6 min., 4°C hold. The reaction volume was 50µl, and the reaction mixture contained: 1X Supertherm PCR buffer, 1.5mM MgCl<sub>2</sub>, 200µM of each dNTP, 2.5pmol or 6pmol of each PCR primer for primer pairs AB101/AB102 and ITS5HP/ITS4 respectively, 1 unit of Supertherm GOLD Taq DNA polymerase, 50-250ng genomic DNA and sterile water. MatK PCRs used the following cycling conditions: 95°C for 10 minutes, 30 cycles of (94°C for 30 sec., 54°C for 30 sec., 72°C for 60 sec.), 72°C for 6 min., 4°C hold. PCR reaction conditions were as for ITS, except that 2.5mM MgCl<sub>2</sub> and 10pmol of each PCR primer was used. The PCR products were then purified using the Qiagen QIAquick® PCR Purification Kit and eluted in 30 to 50µl of sterile water.

Thirty one ITS and 22 matK PCR fragments were sequenced in both directions, with an additional 17 ITS and 3 matK sequences being obtained from published works (Table 1) (Aceto *et al.*, 1999; Douzery *et al.*, 1999; Goldman *et al.*, 2001; Kores *et al.*, 2001; Szalanski *et al.*, 2001; Clements *et al.*, 2002; Bateman *et al.*, 2003). Sequences from Bateman *et al.* (2003) are not in the public domain and thus were obtained from the senior author. We sampled 11 species of *Bonatea* (69%), 16 species of *Habenaria* (ca. 2.5%) and 7 species from 4 additional genera of the tribe Orchideae to serve as outgroups. Only one representative of each *Bonatea* species was sequenced for the matK gene, except for *B. antennifera* for which two representatives were sequenced because of an original misidentification. We were unable to obtain a matK sequence for *B. steudneri* MP40 as the DNA had degraded. The matK outgroup '*Brachycorythis* spp.' is a composite of 2 congeneric species (see Table 1).

Cycle sequencing was performed using the Applied Biosystems BigDye® Terminator v3.1 Cycle Sequencing Kit under the following conditions: 35 cycles of (96°C for 10 sec., 54°C or 55°C for 30 sec., 60°C for 4 min.), 4°C hold. The reaction volume was 20ul and the reaction mixture contained: 6µl 2.5X Cycle Sequencing Reaction Buffer (200mM Tris buffer, 5mM MgCl<sub>2</sub>.6H<sub>2</sub>O, pH9), 2µl BigDye® Terminator v3.1 Cycle Sequencing Ready Reaction Mix, 0.1-1 ul of the purified PCR product, 3.2pmol of primer and sterile water. The cycle sequencing reactions were cleaned using ethanol precipitation following the manufacturer's standard protocol and fractionated on an Applied Biosystems ABI PRISM® 3100 Genetic Analyzer.

### **Phylogenetic Analysis**

DNA sequence chromatograms were assembled and edited using the Staden package (Staden, 1996). The consensus sequences were automatically aligned using ClustalX (Thompson *et al.*, 1997), and then manually corrected using Se-Al v2.0a11 (Rambaut, 1996).

Identification of the boundaries of the ITS regions and coding sequences followed Campbell *et al.* (1995). Sections of the ITS gene with ambiguous alignments were excluded from the final data set.

Phylogenetic trees were inferred using maximum likelihood (ML) and maximum parsimony (MP) criteria as implemented in PAUP\*4.0b10 for Macintosh (Swofford, 1998). Mean and absolute pairwise distances for the ITS and matK data sets were calculated in PAUP. Partition Homogeneity tests were performed on the ITS, matK and morphology data partitions to ascertain whether ITS+matK and ITS+matK+morphology should be combined.

### **Maximum likelihood analyses**

The most appropriate ML nucleotide substitution model and parameter estimates were determined by running likelihood ratio tests for models JC, K2P, HKY and GTR, with and without gamma-distribution and

invariable sites parameters (Fratini *et al.*, 1997). Based on the results of the Chi-square analysis, the GTR+G+I and GTR+G models were the best fits for the ITS and matK data sets, respectively. Branch support was assessed using 1000 bootstrap resamplings under the ML criterion (Felsenstein, 1985). Select ML and MP constraint tests, detailed in the results, were applied to the ITS data set.

### **Maximum parsimony analyses**

The morphology matrix (Appendix 3) was analysed with the Branch and Bound search option. Successive approximations character weighting (SACW; Farris, 1969) was applied in an attempt to increase resolution of the trees. Decay Indices for all nodes were calculated using Branch and Bound searches in which less parsimonious trees were retained and consensus trees used to ascertain the number of additional tree steps needed until a given node collapsed (Bremer, 1994). All morphological matrix characters were mapped onto the morphological cladogram using Winclada (Nixon, 2002) using both ACCTRAN (Fast) and DELTRAN (Slow) optimizations. Spur length, which was not part of the matrix, was plotted onto the cladogram to assess the evolution of this character. Assuming that all *Bonatea* species are moth-pollinated (cf. Johnson and Liltved, 1997), spur length should reflect whether a species is pollinated by short- or long-tongued moths. The character was divided into two states 0 (< 50 mm) and 1 (> 50 mm).

The combined ITS and matK data set was reduced to include only one of each taxon with data for both genes, with the exception of *B. steudneri* MP40 which, although lacking the matK sequence, was included to provide the maximum complement of *Bonatea* species. A heuristic search was conducted using default conditions with 1000 random addition replicates, and the characters unordered and equally weighted. Gaps were treated as missing data. Clade support was estimated using 1000 bootstrap replicates, each with 1000 random addition replicates.

**Table 1.** Vouchers and GenBank/EMBL accession numbers (bold for new sequences) of the taxa investigated. Vouchers of previous publications have been abbreviated to senior author initials under the Specimen code.

Taxon	Specimen code	Voucher	Accession number ITS	Accession number matK
<i>Bonatea antennifera</i> (Rolfe) Sommerville	MP01	Truter JT251 (NU)	<b>DQ522049</b>	
<i>Bonatea antennifera</i> (Rolfe) Sommerville	MP02	Truter JT419 (NU)	<b>DQ522050</b>	<b>DQ522081</b>
<i>Bonatea antennifera</i> (Rolfe) Sommerville	MP17	Truter JT137 (NU)	<b>DQ522051</b>	<b>DQ522082</b>
<i>Bonatea antennifera</i> (Rolfe) Sommerville	MP18	Truter s.n. (NU)	<b>DQ522052</b>	
<i>Bonatea boltonii</i> (Harv.) Bolus	MP08	Truter JT25(NU)	<b>DQ522053</b>	
<i>Bonatea boltonii</i> (Harv.) Bolus	MP21	Truter JT164 (NU)	<b>DQ522054</b>	<b>DQ522083</b>
<i>Bonatea boltonii</i> (Harv.) Bolus	MP27	Peter CP475 (NU)	<b>DQ522055</b>	
<i>Bonatea boltonii</i> (Harv.) Bolus	MP28	Van der Hooven s.n. (NU)	<b>DQ522056</b>	
<i>Bonatea bracteata</i> G.McDonald & McMurtry	MP09	Truter JT687 (NU)	<b>DQ522057</b>	
<i>Bonatea bracteata</i> G.McDonald & McMurtry	MP10	Truter JT262 (NU)	<b>DQ522058</b>	<b>DQ522084</b>
<i>Bonatea cassidea</i> Sond.	MP20	Truter JT965 (NU)	<b>DQ522059</b>	<b>DQ522085</b>
<i>Bonatea lamprophylla</i> J.L.Stewart	MP03	Truter JT991 (NU)	<b>DQ522060</b>	
<i>Bonatea lamprophylla</i> J.L.Stewart	MP04	Truter JT996 (NU)	<b>DQ522061</b>	<b>DQ522086</b>
<i>Bonatea polypodantha</i> (Rchb.f.) L.Bolus	MP11	Truter s.n. (NU)	<b>DQ522062</b>	
<i>Bonatea polypodantha</i> (Rchb.f.) L.Bolus	MP12	Truter s.n. (NU)	<b>DQ522063</b>	<b>DQ522087</b>
<i>Bonatea porrecta</i> (Bolus) Summerh.	MP19	Truter s.n. (NU)	<b>DQ522064</b>	<b>DQ522088</b>
<i>Bonatea pulchella</i> Summerh.	MP13	Truter s.n. (NU)	<b>DQ522065</b>	<b>DQ522089</b>
<i>Bonatea pulchella</i> Summerh.	MP14	Truter s.n. (NU)	<b>DQ522066</b>	
<i>Bonatea saundersioides</i> Kraenzl. & Schltr.	MP15	Truter JT85 (NU)	<b>DQ522067</b>	<b>DQ522090</b>
<i>Bonatea saundersioides</i> Kraenzl. & Schltr.	MP16	Truter JT667 (NU)	<b>DQ522068</b>	
<i>Bonatea speciosa</i> (L.f.) Willd.	MP05	Truter JT1376 (NU)	<b>DQ522069</b>	
<i>Bonatea speciosa</i> (L.f.) Willd.	MP06	Truter s.n. (NU)	<b>DQ522070</b>	<b>DQ522091</b>
<i>Bonatea speciosa</i> (L.f.) Willd.	MP07	Truter s.n. (NU)	<b>DQ522071</b>	

<i>Bonatea speciosa</i> (L.f.) Willd.	ED	Roux s.n. (NBG)	AJ000121	
<i>Bonatea steudneri</i> (Rchb.f.) T.Durand & Schinz	MP40	Bytebier B2336 (STE)	<b>DQ522072</b>	
<i>Brachycorythis pubescens</i> Harv.	MP31	Peter CP3925 (GRA)		<b>DQ522102</b>
<i>Brachycorythis macrantha</i> (Lindl.) Summerh.	ED	Chase O-593 (K)	AJ000146	
<i>Habenaria arenaria</i> Lindl.	MP23	Truter s.n. (NU)	<b>DQ522073</b>	<b>DQ522092</b>
<i>Habenaria arenaria</i> Lindl.	ED	Chase O-1135 (K)	AJ000139	
<i>Habenaria clavata</i> (Lindl.) Rchb.f.	MP33	Peter CP4103 (GRA)		<b>DQ522093</b>
<i>Habenaria clavata</i> (Lindl.) Rchb.f.	MP34	Peter CP4185 (GRA)	<b>DQ522074</b>	<b>DQ522094</b>
<i>Habenaria delavayi</i> Finet	RB	Luo 161 (K)	Unpublished	
<i>Habenaria dives</i> Rchb.f.	MP35	Peter CP4104 (GRA)	<b>DQ522075</b>	<b>DQ522095</b>
<i>Habenaria laevigata</i> Lindl.	MP26	Johnson s.n. (NU)	<b>DQ522076</b>	<b>DQ522096</b>
<i>Habenaria laevigata</i> Lindl.	MP36	Peter CP4082 (GRA)		<b>DQ522097</b>
<i>Habenaria lithophila</i> Schltr.	MP37	Peter CP4085 (GRA)	<b>DQ522077</b>	<b>DQ522098</b>
<i>Habenaria lithophila</i> Schltr.	MP38	Peter CP4087 (GRA)	<b>DQ522078</b>	<b>DQ522099</b>
<i>Habenaria odontopetala</i> Rchb.f.	RB	Inoue s.n. (EBH/K)	Unpublished	
<i>Habenaria praestans</i> Rendle	MP39	Peter CP4186 (GRA)	<b>DQ522079</b>	<b>DQ522100</b>
<i>Habenaria procera</i> Lindl.	RB	LaCroix 1152 (K)	Unpublished	
<i>Habenaria propinquier</i> Rchb.f.	MC	Roberts ORG 2076 (CANB)	AF348035	
<i>Habenaria repens</i> Nutt.	PK	Chase O-381 (K)		AJ310036
<i>Habenaria sagittifera</i> Rchb.f.	RB	Inoue s.n. (EBH/K)	Unpublished	
<i>Habenaria socotrana</i> Balf.f.	RB	Bateman 199 (EBH)	Unpublished	
<i>Habenaria tibestica</i> Schltr.	RB	Bateman 186 (EBH)	Unpublished	
<i>Habenaria tridactylites</i> Lindl.	RB	Bateman 194 (EBH)	Unpublished	
<i>Habenaria tridens</i> Lindl.	MP25	Truter JT182 (NU)	<b>DQ522080</b>	<b>DQ522101</b>
<i>Herminium 'Platanthera' latilabris</i> Lindl.	RB	Bateman 392 (EBH)	Unpublished	
<i>Hemipilia 'Habenaria' purpureopunctata</i> K.Y.Lang	RB	Luo 014 (K)	Unpublished	

<i>Ophrys apifera</i> Huds.	PK	Chase O-536 (K)		AJ310049
<i>Platanthera chlorantha</i> (Custer) Rchb.	MP32	Peter s.n. (NU)		
<i>Platanthera chlorantha</i> (Custer) Rchb.	SA	Collector unknown (NAP)	PCZ94117/PCZ94118	
<i>Platanthera ciliaris</i> Lindl.	DG	Chase O-318 (K)		AF263678
<i>Platanthera praeclara</i> Sheviak & M.L.Bowles	AS	Collector unknown (NEB)	AF301445	
<i>Stenoglottis longifolia</i> Hook.f.	ED	Chase O-1136 (K)	AJ000124	

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*Note.* AS = Szalanski *et al.* 2001; DG = Goldman *et al.* 2001; ED = Douzery *et al.* 1999; MC = Clements *et al.* 2002; PK = Kores *et al.* 2001; RB = Bateman *et al.* 2003; SA = Aceto *et al.* 1999.

RESULTS

Summary statistics for the analyses performed using equally-weighted parsimony are shown in Table 2 for each data set partition. In addition, the SACW parsimony analysis of the morphological data recovered a single most parsimonious MP tree, shown in Figure 1.

Table 2. Summary statistics for parsimony analysis.

	Characters	No. (%) variable	No. (%) inform <sup>a</sup>	No. trees	No. steps	CI	RI
Morphology	45	45 (100)	39 (87)	9	96	0.511	0.711
ITS <sup>b</sup>	732	360 (49)	246 (34)	1872	902	0.548	0.727
matK	630	97 (15)	53 (8)	34	130	0.762	0.881
ITS+matK <sup>b, c</sup>	1362	343 (25)	168 (12)	53	530	0.675	0.768

CI = consistency index, excluding uninformative characters; RI = retention index.

<sup>a</sup>Parsimony informative characters.

<sup>b</sup>Minus align ambiguous characters.

<sup>c</sup>Only taxa with both data partitions, except for *B. steudneri* MP40 with ITS only.

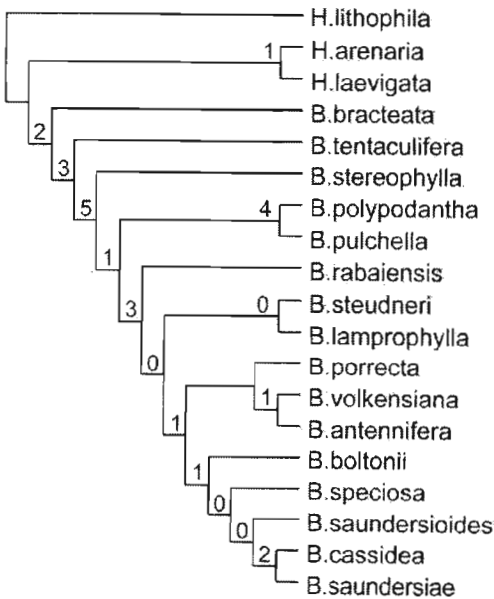


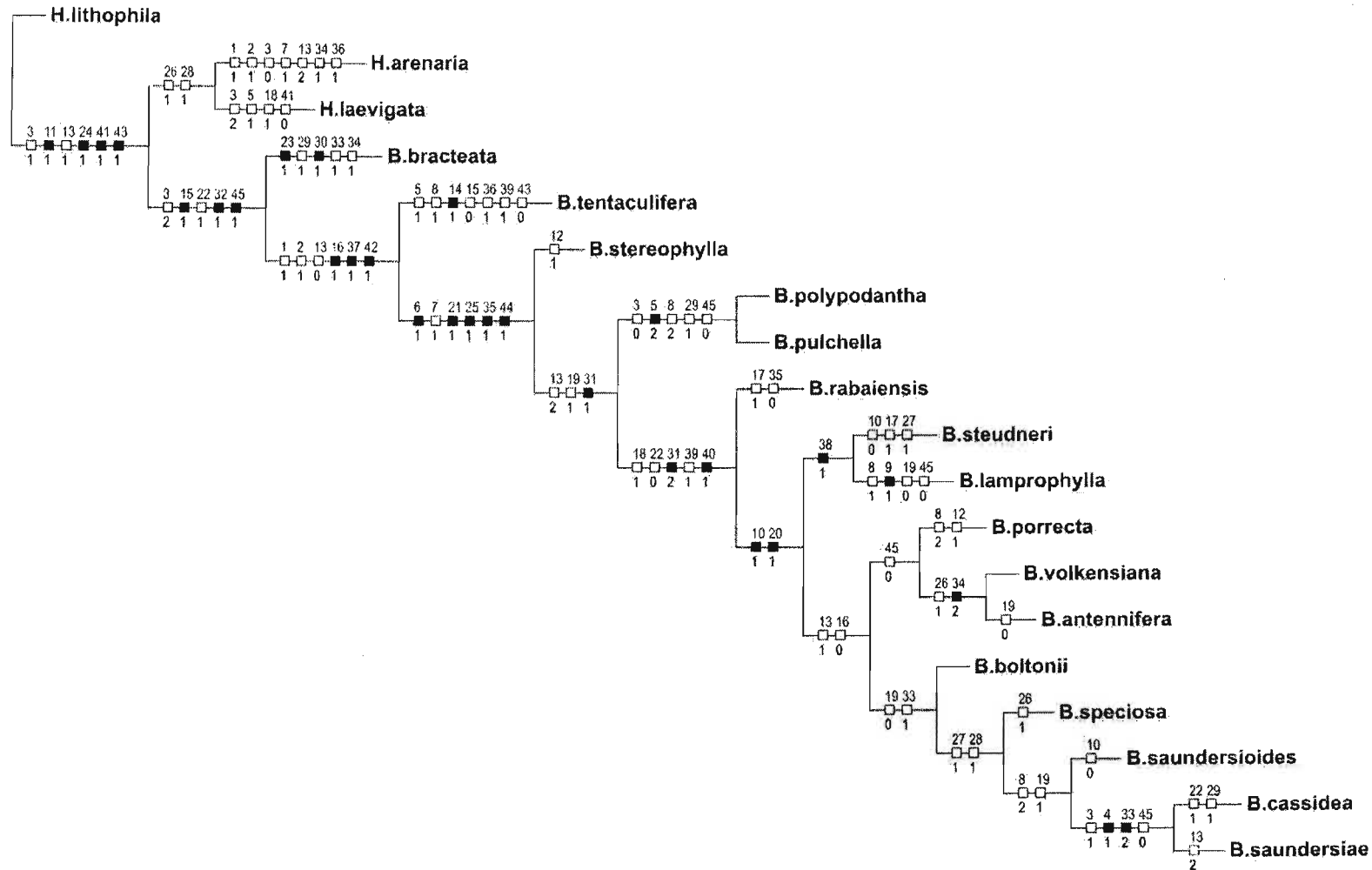
Figure 1. Single Maximum Parsimony tree obtained after Successive Approximations Character Weighting of morphological data (Length = 35.276; CI = 0.703, excluding uninformative characters; RI = 0.881). Numbers are decay values.



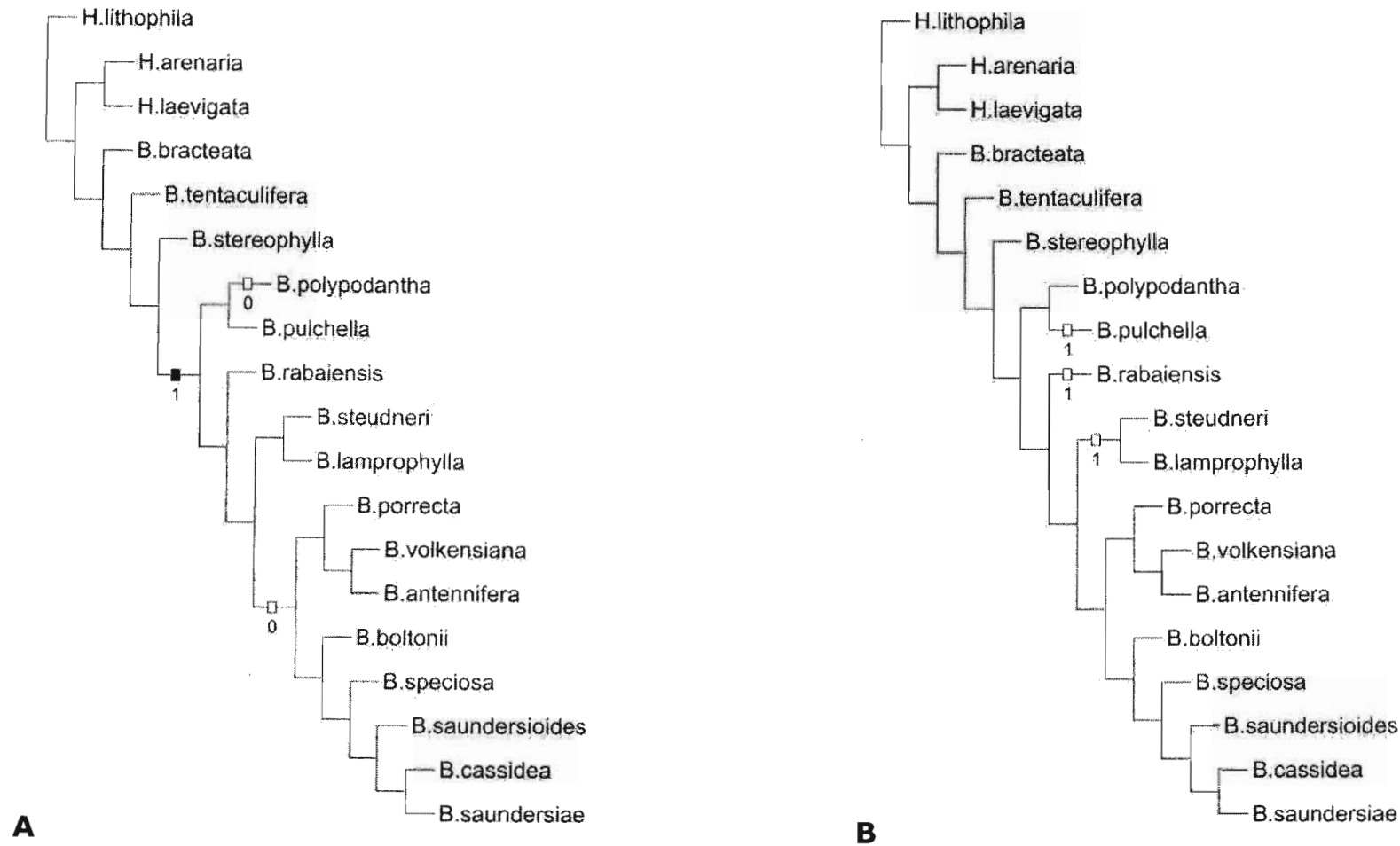
### **Morphological data**

Forty five morphological characters were used in the cladistic analysis. Of these, 6 characters were uninformative and 39 characters were phylogenetically informative. Branch and bound analysis of the equally-weighted data set produced 9 most parsimonious trees (not shown) of 96 steps with largely congruent basal nodes but conflict amongst the subterminal nodes. Two rounds of SACW resulted in the single tree represented in Figure 1, with length of 35.276.

ACCTRAN and DELTRAN optimisation produced similar character distributions on the reweighted morphological cladogram (Figure 2). Five synapomorphies support the monophyly of a group including all *Bonatea* species except for *B. bracteata* and *B. tentaculifera*. The Bremer support value for this clade (5) also strongly supports its monophyly, though Bremer support for monophyly of the entire genus is weaker (2). Contrasting results were obtained under ACCTRAN and DELTRAN optimisations for mapping of 'Spur length' (Figure 3).



**Figure 2.** Character matrix (Appendix 3) mapped onto the reweighted morphological cladogram under ACCTRAN optimisation. Synapomorphies are indicated by solid bars and homoplasies are indicated by hollow bars.



**Figure 3.** Spur length, (0) short < 50 mm and (1) long > 50 mm, mapped onto the reweighted morphological cladogram under ACCTRAN (A) and DELTRAN (B) optimisation. Synapomorphies are indicated by solid bars and homoplasies are indicated by hollow bars.

### DNA sequence data

Aligned sequences of ITS and matK were 770 and 630 bases long, respectively. Overall, ITS had the greater maximum sequence divergence in outgroup-ingroup comparisons (0–20.6% ITS vs. 0–5.7% matK), and similarly, had the greater ingroup sequence divergence (0–4.2% ITS vs. 0–1.4% matK) (see Table 3). ITS also contained more parsimony-informative characters than matK (246 vs. 53 informative characters).

**Table 3.** Uncorrected % sequence divergence for select *Bonatea* and *Habenaria* species. The ITS and matK data sets are below and above the bisecting diagonal line, respectively. *B. steudneri* MP40 is without a matK sequence and represented by '?'.

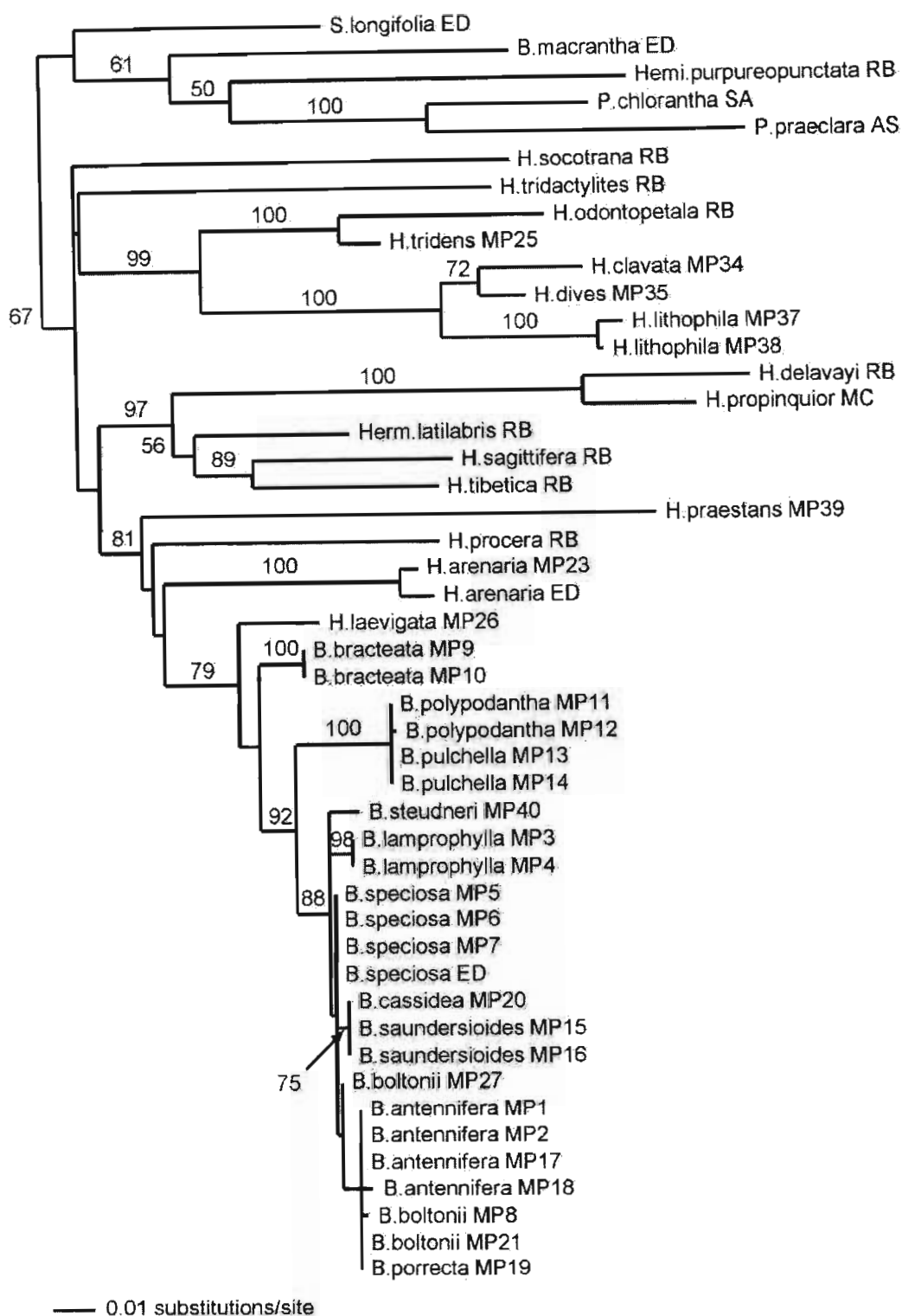
	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1 <i>B. antennifera</i> MP17	-	0	6	2	3	5	2	5	2	1	?	6	6	15
2 <i>B. boltonii</i> MP21	0	-	6	2	3	5	2	5	2	1	?	6	6	15
3 <i>B. bracteata</i> MP10	21	21	-	8	7	9	8	9	8	7	?	8	6	17
4 <i>B. cassidea</i> MP20	5	5	20	-	5	7	0	7	4	3	?	8	8	17
5 <i>B. lamprophylla</i> MP04	7	7	22	5	-	4	5	6	5	4	?	8	7	18
6 <i>B. polypodantha</i> MP12	24	24	29	23	23	-	7	2	7	6	?	11	9	20
7 <i>B. porrecta</i> MP19	0	0	18	2	4	21	-	7	4	3	?	8	8	17
8 <i>B. pulchella</i> MP13	23	23	28	22	22	1	20	-	7	6	?	11	9	20
9 <i>B. saundersioides</i> MP15	6	6	21	0	7	24	3	23	-	3	?	8	8	17
10 <i>B. speciosa</i> MP6	4	4	19	1	5	22	1	21	2	-	?	7	7	16
11 <i>B. steudneri</i> MP40	7	7	22	6	8	23	4	22	7	5	-	?	?	?
12 <i>H. arenaria</i> MP23	59	59	54	58	58	67	54	66	59	57	59	-	8	17
13 <i>H. laevigata</i> MP26	27	27	20	26	28	33	24	32	27	25	28	54	-	17
14 <i>H. lithophila</i> MP38	94	94	95	95	95	102	92	101	97	95	94	103	95	-

### ITS data only

The ITS sequences varied in length from 618–654 nucleotides. The relatively large difference is owing to a 25bp deletion within ITS1 for *Habenaria tridens* Lindl. For the ingroup, the ITS sequences varied from 644–654 nucleotides. Several short indels were inferred in aligning sequences. The final data set, excluding ambiguously-aligned sites, comprised 732 characters. A heuristic search of the ITS data recovered 1872 equally most parsimonious trees (not shown) of 902 steps, the strict consensus offering little resolution. The maximum likelihood

analysis produced three trees under the GTR + G + I model, with a likelihood of  $-\ln L = 5380.50$ . Figure 4 represents one of the three trees that most closely resembles their strict consensus. The remaining two trees showed minor differences within the *B. antennifera* | *B. boltonii* | *B. porrecta* group. *Bonatea* was recovered as monophyletic although without bootstrap support (<50%).

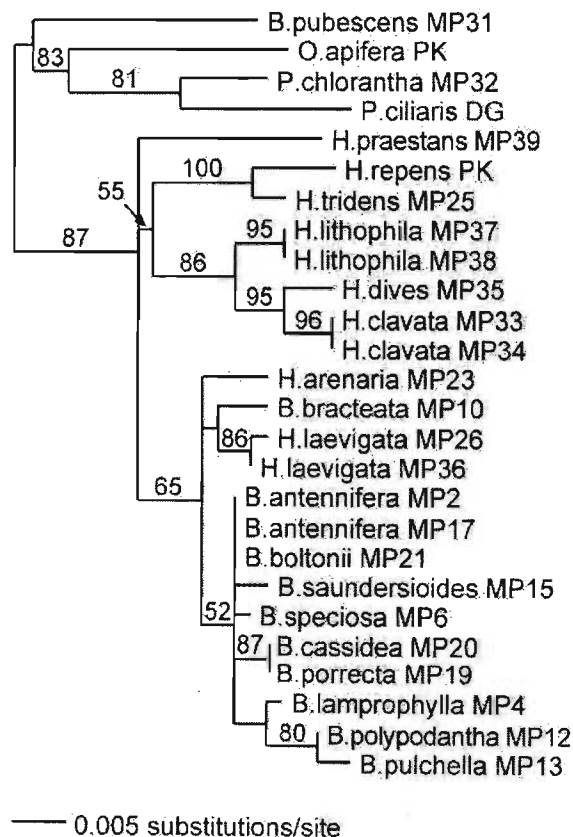
ML and MP searches were performed whilst enforcing topological constraints or reverse constraints, the resulting trees compared with Kishino-Hasegawa tests (K-H tests; Kishino and Hasegawa, 1989) and Shimodaira-Hasegawa tests (S-H tests; Shimodaira and Hasegawa, 1999). ML and MP constraint and reverse constraint topologies for the inclusion of *H. laevigata* MP26 within the *Bonatea* clade were non-significant. *Bonatea* monophyly also was explored by excluding *B. bracteata* from the genus. ML reverse constraint trees derived in this manner were statistically significant ( $p = 0.046$ ; S-H test). Thus, the tree excluding *B. bracteata* from *Bonatea* is significantly different to the tree including it, i.e., ML analyses provide statistically significant support for *Bonatea* monophyly even though bootstrap support is < 50%.



**Figure 4.** One of three Maximum Likelihood trees most closely resembling the strict consensus tree for the 732 base pairs of the ITS data set obtained under GTR + G + I model,  $-\ln L = 5380.50$ . Numbers are bootstrap percentages. Specimen codes in Table 1.

### matK data only

The aligned matK sequences comprised 630 characters with no sequence length variation. A heuristic search of the matK data recovered 34 most parsimonious trees (not shown) of 130 steps with the strict consensus tree offering little resolution within *Bonatea*. ML analysis under the GTR + G model produced a single tree with a likelihood of  $-\ln L = 1629.33$  (Figure 5). All but one species of *Bonatea* are recovered as monophyletic. *Bonatea bracteata* is recovered instead as sister-group to *H. laevigata*. Relationships among *H. arenaria*, (*H. laevigata* + *B. bracteata*), and the remaining *Bonatea* are unresolved.

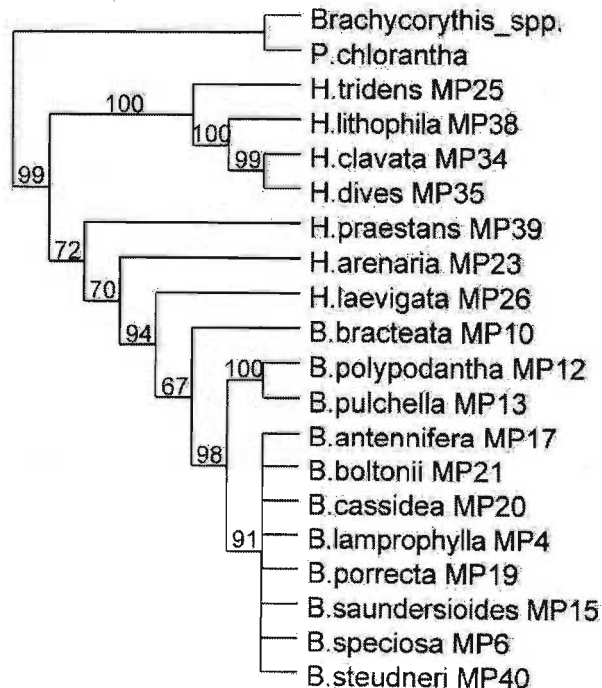


**Figure 5.** The single Maximum Likelihood topology for 630 base pairs of the matK region obtained under GTR + G model,  $-\ln L = 1629.33$ . Numbers are bootstrap percentages. Specimen codes in Table 1.

## Combined analysis

As the overall topologies of the three independent phylogenies were different, a Partition Homogeneity test was performed to test for possible incongruence. There was no significant difference between the two molecular data sets, but a highly significant difference between the matK, ITS and morphology data sets ( $p = 0.003$ ).

Maximum sequence divergence for all taxa and for ingroup taxa only, excluding *B. steudneri* MP40 owing to the missing matK data set, was 0–11.6% and 0–2.9%, respectively. A heuristic search of the combined molecular data recovered 53 equally most parsimonious trees of 530 steps, the strict consensus of which is shown in Figure 6. *Bonatea* is recovered as monophyletic but within *Bonatea* only basal relationships are well resolved.



**Figure 6.** The strict consensus of 53 most-parsimonious trees for combined ITS and matK data sets (Length = 530; CI = 0.675, excluding uninformative characters; RI = 0.768). Numbers are bootstrap percentages. Specimen codes in Table 1.



## DISCUSSION

Neither the ITS, matK, or both data sets combined, yielded well-resolved, well-supported cladograms for *Bonatea*. *Habenaria* is paraphyletic for analyses based on ITS and matK data sets. There is poor congruence between ITS and matK data for interspecific relationships in *Bonatea*, with good resolution obtained for the basal portions of the trees.

The combined analysis recovered *Bonatea* as monophyletic with 67% bootstrap support. The node for all *Bonatea* species excluding *B. bracteata* is robustly supported with 98% bootstrap support. Evidence from cladograms based on the matK region and morphology also suggest that *B. bracteata* is sister to the remaining species of *Bonatea*. The lack of a galeate middle rostellum lobe in *B. bracteata*, as well as in *B. tentaculifera*, suggests closer affinities to *Habenaria* than to the *Bonatea* in-group. Inclusion of a broader representation of African *Habenaria* species will give a clearer indication of the relationship between the two genera.

The morphological results largely are congruent with the ITS analysis. *Bonatea polypodantha* and *B. pulchella* are closely allied with 100% bootstrap support (Figure 4). The remaining species are grouped with 88% bootstrap support however relationships among them are not resolved conclusively. *Bonatea cassidea*, *B. saundersiae* and *B. saundersioides* are closely related, as are *B. antennifera*, *B. boltonii* and *B. porrecta*. Interestingly the supposed strong alliance between members of the *B. speciosa* complex *sensu* Schelpe and Linder (1999) is not supported by our results (Figure 1 and Figure 4).

Patterns of spur length evolution across the genus are inconclusive as alternative hypotheses are equally parsimonious. Long spurs appear to

have originated either three times independently (in *B. pulchella*, *B. rabaiensis*, and the common ancestor of *B. steudneri* and *B. lamprophylla*), or, once near the base of *Bonatea* with two later reversals to short spurs (Figure 3). Marked differences in spur length between the close relatives *B. polypodantha* and *B. pulchella* (Figure 3) indicate a possible role for pollinator-driven ecological speciation in the genus.

An increasing number of studies find that relationships among species in rapidly radiating lineages cannot be resolved with conventional sequence variation, even using hypervariable regions such as ITS (Givnish, 2000). This necessitates the need for alternative analyses, and techniques for consideration include: ISSRs (Levi *et al.*, 2003; Archibald, Wolfe and Johnson, 2004), microsatellites (Hughes *et al.*, 2004; Pellegrino *et al.*, 2005) and AFLPs (McLenachan *et al.*, 2000). RAPDs (Bailey, Hughes and Harris, 2004) can be used to obtain candidate “genes” (i.e. bands), which are then excised from agarose gels, cloned, and sequenced. Alternative low copy nuclear genes (examples listed in Mort and Crawford (2004)) are being employed on a more frequent basis for resolving lower-level phylogenetic relationships. Also, as the rates of morphological evolution generally are not correlated with the rates of molecular evolution, regulatory genes responsible for bringing about morphological change are showing promise at resolving relationships in recently diverged genera (Barrier *et al.*, 2001; Purugganan and Robichaux, 2005).

Although a molecular clock approach was not considered feasible in this study, owing to the lack of calibration points, the genus *Bonatea* is undoubtedly the result of a recent radiation. The species show marked incongruities in rates of morphological and molecular evolution, as documented in other organisms such as African rift lake cichlids (Meyer *et al.*, 1990; Johnson *et al.*, 1996), columbines (Hodges and Arnold,

1994) and the Hawaiian silversword alliance (Baldwin and Sanderson, 1998).

Future work should focus on the inclusion of morphology, ITS and matK data for all *Bonatea* species not sampled here, the inclusion of a comprehensive range of *Habenaria* species, and, the use of genetic markers (as mentioned above) that are capable of fine-scale resolution for resolving relationships within rapidly radiating lineages. *Bonatea* species appear to differ in their ecological niches and further investigation may aid in clarifying the relationships within this genus.

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## APPENDICES

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### Appendix 1. Taxa included in morphological analysis.

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*Bonatea antennifera* (Rolfe) Sommerville  
*Bonatea boltonii* (Harv.) Bolus  
*Bonatea bracteata* G.McDonald & McMurtry  
*Bonatea cassidea* Sond.  
*Bonatea lamprophylla* J.L.Stewart  
*Bonatea polypodantha* (Rchb.f.) L.Bolus  
*Bonatea porrecta* (Bolus) Summerh.  
*Bonatea pulchella* Summerh.  
*Bonatea rabaiensis* (Rendle) Rolfe  
*Bonatea saundersiae* (Harv.) T.Durand & Schinz  
*Bonatea saundersioides* Kraenzl. and Schltr.  
*Bonatea speciosa* (L.f.) Willd.  
*Bonatea stereophylla* (Kraenzl.) Summerh.  
*Bonatea steudneri* (Rchb.f.) T.Durand & Schinz  
*Bonatea tentaculifera* Summerh.  
*Bonatea volkensiana* (Kraenzl.) Rolfe  
  
*Habenaria arenaria* Lindl.  
*Habenaria laevigata* Lindl.  
*Habenaria lithophila* Schltr.

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**Appendix 2.** Morphological characters and character states. All multistate characters are unordered.

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1. Tuber number: (0) single, (1) multiple
  2. Tuber shape: (0) ovoid, (1) oblong-obovoid
  3. Basal internodes: (0) short, (1) intermediate, (2) long
  4. Gradation of basal leaves into cauline leaves: (0) no, (1) yes
  5. Bract shape: (0) ovate, (1) oblong, (2) lanceolate
  6. Bracts: (0) flat, (1) vaginate
  7. Bracts: (0) longer than ovary, (1) shorter than ovary
  8. Leaf shape: (0) oblong, (1) ovate, (2) lanceolate
  9. Leaf margin smooth: (0) yes, (1) no
  10. Leaf margin undulate: (0) no, (1) yes
  11. Leaf base: (0) cuneate, (1) sheathed
  12. Leaves at anthesis: (0) yes, (1) no
  13. Inflorescence spacing: (0) dense, (1) intermediate, (2) lax
  14. Sepal-petal complex: (0) elongate, (1) orbicular
  15. Sepal costae: (0) absent, (1) present.
  16. Lateral sepals white: (0) no, (1) yes
  17. Lateral sepals: (0) patent, (1) porrect
  18. Lateral sepals deflexed: (0) no, (1) yes
  19. Lateral sepals: (0) oblong-lanceolate, (1) ovate
  20. Lateral sepals with posticus tooth: (0) no, (1) yes
  21. Lateral sepals adnate to stigmatic processes: (0) no, (1) yes
  22. Upper petal lobe white: (0) no, (1) yes
  23. Upper petal lobe green: (0) yes, (1) no
  24. Upper petals adnate to upper sepals: (0) no, (1) yes
  25. Lower petal lobes adnate to stigmatic processes: (0) no, (1) yes
  26. Lower petal lobes: (0) patent, (1) porrect
  27. Lower petal lobes deflexed: (0) no, (1) yes
  28. Lower petal lobes: (0) linear, (1) lanceolate to oblanceolate
  29. Lip mid-lobe white: (0) no, (1) yes
  30. Lip mid-lobe green: (0) yes, (1) no
  31. Lip mid-lobe: (0) straight, (1) weakly geniculate, (2) medial fold
  32. Lip mid-lobe: (0) longer than side-lobes, (1) shorter than side-lobes
  33. Lip side-lobes: (0) linear, (1) linear-lanceolate (2) broad
  34. Lip side-lobes: (0) laterally descending, (1) laterally perpendicular, (2) porrect
  35. Tooth in spur: (0) absent, (1) present
  36. Tooth between lateral labellum lobes: (0) no, (1) yes
  37. Pollinia: (0) spherical, (1) obovoid
  38. Caudicle canal extension: (0) 1.0–6.8 mm (1) 7.5–17.4mm
  39. Rostellum papillae: (0) absent, (1) present
  40. Rostellum margin: (0) smooth, (1) ciliate
  41. Rostellum mid-lobe prominent: (0) no, (1) yes
  42. Rostellum mid-lobe fused to lateral lobes: (0) no, (1) yes
  43. Rostellum lateral lobes prominent: (0) yes, (1) no
  44. Rostellum lateral lobes fused with caudicle canals: (0) no, (1) yes
  45. Stigmatic processes: (0) porrect, (1) deflexed
-

**Appendix 3.** Morphological matrix. ? = unknown state. Character descriptions are in Appendix 2.

Species	Character number									
	5	10	15	20	25	30	35	40	45	
<i>H. lithophila</i>	0	0	0	0	0	0	0	0	0	
<i>H. arenaria</i>	1	1	0	0	1	0	1	0	1	
<i>H. laevigata</i>	0	0	2	0	1	0	0	0	1	
<i>B. antennifera</i>	1	1	2	0	1	1	0	0	1	
<i>B. boltonii</i>	1	1	2	0	1	1	0	0	1	
<i>B. bracteata</i>	0	0	2	0	0	0	1	1	0	
<i>B. cassidea</i>	1	1	1	0	1	1	0	1	1	
<i>B. lamprophylla</i>	1	1	2	0	1	1	0	0	1	
<i>B. polypodantha</i>	1	1	0	2	1	1	0	1	1	
<i>B. porrecta</i>	1	1	2	0	1	1	0	0	1	
<i>B. pulchella</i>	1	1	0	2	1	1	0	1	1	
<i>B. rabaiensis</i>	1	1	2	0	1	1	0	0	1	
<i>B. saundersiae</i>	1	1	1	0	1	1	0	1	1	
<i>B. saundersioides</i>	1	1	2	0	1	1	0	1	1	
<i>B. speciosa</i>	1	1	2	0	1	1	0	1	1	
<i>B. stereophylla</i>	1	1	2	0	1	1	0	0	1	
<i>B. steudneri</i>	1	1	2	0	1	1	0	0	1	
<i>B. tentaculifera</i>	1	1	2	0	1	1	0	0	1	
<i>B. volkensiana</i>	1	1	2	0	1	1	0	0	1	

**Appendix 4.** Origin of materials used in the molecular-based phylogenetic analysis of *Bonatea*.

Species	Herbarium	Specimen code	Longitude & latitude	Locality and collection number
<i>Bonatea antennifera</i> (Rolfe) Sommerville				
	NU	MP01	24S 30E	Pilgrim's Rest: Burgerfort, Botha's Hoek, Lebowa, <i>Truter JT251</i>
	NU	MP02	27S 32E	Maputuland, Tshongwe, <i>Truter JT3419</i>
	NU	MP17	25S 28E	Pretoria: Roodeplaat Dam, <i>Truter JT137</i>
	NU	MP18	26S 28E	Johannesburg: Albertan, <i>Truter s.n.</i>
<i>Bonatea boltonii</i> (Harv.) Bolus				
	NU	MP08	23S 29E	Polokwane: Houtbosdorp, Mphome Mountain, Zaagkuil Farm, <i>Truter JT25</i>
	NU	MP21	29S 31E	Stanger: Durban, Bluff grasslands, <i>Truter JT164</i>
	NU	MP27	33S 26E	Grahamstown: Mountain Drive, ridge crest at the western end of Dassie Kranz, 25-30 meters east of the Vodacom Tower, <i>Peter CP475</i>
	NU	MP28	32S 27E	Stutterheim: Kubusi Forest, <i>Van der Hooven s.n.</i>
<i>Bonatea bracteata</i> G.McDonald & McMurtry				
	NU	MP09	23S 31E	Phalaborwa: George's Valley Farm, George's Valley, <i>Truter JT687</i>
	NU	MP10	25S 30E	Lydenburg: Dullstroom, <i>Truter JT262</i>
<i>Bonatea cassidea</i> Sond.				
	NU	MP20	30S 30E	Port Shepstone: Ixopo, Valkrans Valley, Watervale Farm, <i>Truter JT965</i>



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<i>Bonatea lamprophylla</i> J.L.Stewart				
	NU	MP03	27S 32E	Ubombo: Maputuland, Tshongwe, <i>Truter JT991</i>
	NU	MP04	27S 32E	Ubombo: Maputuland, Mabibi Beach, <i>Truter JT996</i>
<i>Bonatea polypodantha</i> (Rchb.f.) L.Bolus				
	NU	MP11	23S 29E	Polokwane: Houtbosdorp, <i>Truter s.n.</i>
	NU	MP12	25S 27E	Rustenburg: Hennops River Estate, <i>Truter s.n.</i>
<i>Bonatea porrecta</i> (Bolus) Summerh.				
	NU	MP19	30S 30E	Port Shepstone: Oribi Gorge, Fair Acres Estate, <i>Truter s.n.</i>
<i>Bonatea pulchella</i> Summerh.				
	NU	MP13	24S 30E	Pilgrim's Rest: Ohrigstad, Strydom Tunnel, <i>Truter s.n.</i>
	NU	MP14	27S 32E	Ubombo: Maputuland, Tshongwe, <i>Truter s.n.</i>
<i>Bonatea saundersioides</i> Kraenzl. & Schltr.				
	NU	MP15	25S 27E	Rustenburg: Rustenburg, <i>Truter JT85</i>
	NU	MP16	23S 31E	Phalaborwa: George's Valley Farm, George's Valley, <i>Truter JT667</i>
<i>Bonatea speciosa</i> (L.f.) Willd.				
	NU	MP05	31S 28E	Umtata: Mnyolo-Engcobo, <i>Truter JT1376</i>
	NU	MP06	34S 18E	Simonstown: Betty's Bay, <i>Truter s.n.</i>
	NU	MP07	32S 28E	Butterworth: Morgan's Bay, <i>Truter s.n.</i>
<i>Bonatea steudneri</i> (Rchb.f.) T.Durand & Schinz				
	STE	MP40	01S 36E	Nairobi: Nairobi, <i>Bytebier B2336</i>
<i>Brachycorythis pubescens</i> Harv.				
	GRA	MP31	29S 30E	Pietermaritzburg: Winston Park, Stockville Valley, <i>Peter CP3925</i>

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<i>Habenaria arenaria</i> Lindl.	NU	MP23	32S 28E	Butterworth: Kei Mouth, <i>Truter s.n.</i>
<i>Habenaria clavate</i> (Lindl.) Rchb.f.	GRA	MP33	29S 30E	Pietermaritzburg: Umlaas Road, <i>Peter CP4103</i>
	GRA	MP34	10S 33E	Nykia: Nykia Plateau, Dembo Bridge, <i>Peter CP4185</i>
<i>Habenaria dives</i> Rchb.f.	GRA	MP35	29S 30E	Pietermaritzburg): Umlaas Road, <i>Peter CP4104</i>
<i>Habenaria laevigata</i> Lindl.	NU	MP26	29S 29E	Underberg: Garden Castle, <i>Johnson s.n.</i>
	GRA	MP36	29S 29E	Underberg: Cobham, Pholela Valley, <i>Peter CP4082</i>
<i>Habenaria lithophila</i> Schltr.	GRA	MP37	29S 29E	Underberg: Garden Castle, <i>Peter CP4085</i>
	GRA	MP38	29S 29E	Underberg: Sani Pass, <i>Peter CP4087</i>
<i>Habenaria praestans</i> Rendle	GRA	MP39	10S 33E	Nykia: Nykia Plateau, Dembo Bridge, <i>Peter CP4186</i>
<i>Habenaria tridens</i> Lindl.	NU	MP25	27S 31E	Louwsburg: Ngome, <i>Truter JT182</i>
<i>Platanthera chlorantha</i> (Custer) Rchb.	NU	MP32	56N 16E	Öland: Skogsby, <i>Peter s.n.</i>

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## CHAPTER 2

### **A re-examination of the taxonomic boundary between *Habenaria* and *Bonatea* (Orchidaceae)**

#### **ABSTRACT**

Diagnostic characters for *Bonatea* (Orchidaceae) are re-evaluated. Morphological evidence suggests that *Bonatea bracteata* G.McDonald & McMurtry and *Bonatea tentaculifera* Summerh. are better placed in *Habenaria*. Indeed, *Bonatea bracteata* is found to be a synonym of *Habenaria transvaalensis* Schltr. *Bonatea tentaculifera* is renamed *Habenaria bonateoides* M.Ponsie. The remaining *Bonatea* species form a monophyletic group, distinct from *Habenaria* with respect to the clear separation of the galeate rostellum and anthers, and the extensive basal fusion of the perianth with the stigmatic processes, the lateral sepals and the anterior lobe of the petals.

#### **INTRODUCTION**

The genus *Bonatea* is mainly distributed along the eastern seaboard of southern and eastern Africa, with one species extending into Yemen. *Bonatea* is closely related to *Habenaria* but has been traditionally distinguished by a suite of characters, including its galeate middle rostellum lobe, the presence of a tooth in the spur mouth, its clavate stigmas, and the basal fusion of the lip with the lateral sepals, the anterior lobe of the petals and the stigmatic processes (Rolfe, 1898; Rolfe, 1913; Summerhayes, 1949; Williamson, 1977; Stewart, 1996; la Croix and la Croix, 1997; Schelpe and Linder, 1999). However, with the exception of the galeate rostellum these characters occur sporadically in *Habenaria*, and Kurzweil and Weber (1992) suggested that the genus is merely a specialized section of *Habenaria*.

Phylogenetic analyses, though preliminary, suggest that *Bonatea* is monophyletic (Chapter 1). Irrespective of the final taxonomic status of *Bonatea*, as a genus or as a section of *Habenaria*, it needs to be adequately circumscribed. A thorough study of the morphology of *Bonatea* and selected *Habenaria* species was thus undertaken in order to identify characters that could uniquely diagnose *Bonatea*.

## MATERIALS AND METHODS

Herbarium specimens of *Bonatea* were loaned and digital images of type specimens were obtained from the following herbaria: BLFU, BOL, BR, BM, EA, FT, GLOW, J, K, KEI, KMG, LYD, NBG, NH, NU, P, PRE, PRU, PUC, S, SAM, STE, TCD, UNIN and UPS. These formed the basis of a morphological and distributional study aimed at reassessing generic and species limits.

### Morphology

Characters associated with vegetative and reproductive morphology of all currently recognized *Bonatea* species were recorded (data not shown). Flowers from selected species of *Bonatea* and *Habenaria* were rehydrated and dissected to elucidate gynostemium structure (Figure 1). The literature was consulted to determine the structure of the rostellum in other African *Habenaria* species (Summerhayes, 1968; Williamson, 1977; Williamson 1980; la Croix and Cribb, 1995). Rostellum morphology of *B. tentaculifera* was reconstructed from herbarium sheet drawings (*Summerhayes K79099*), digital images of the type material housed at K and pressed specimens (*Botha s.n.*; *Amiyo s.n.*). Flower size was measured from the middle of the dorsal sepal to the apex of the lip sidelobes. All measurements were made using a digital caliper.

## RESULTS AND DISCUSSION

### Vegetative characters

With respect to vegetative morphology *B. bracteata* is anomalous, being the only *Bonatea* in which the fresh tubers are small (ca. 15–35 x 10–25 mm) ovoid and solitary. All other species have large (40–260 x 20–50 mm), elongated obovoid tubers in clusters of (2–) 3–11.

*Bonatea bracteata* and *B. tentaculifera* are the only species that produce stolons, reminiscent of those of the forest-dwelling *Habenaria malacophylla* Rchb.f. The subterranean organs of *B. tentaculifera* are multiple, and elongate obovoid in shape, conforming to the typical pattern in *Bonatea*. The bracts of *Bonatea* species are sheathing, except for *B. bracteata* and *B. tentaculifera* which have flat bracts.

### Floral characters

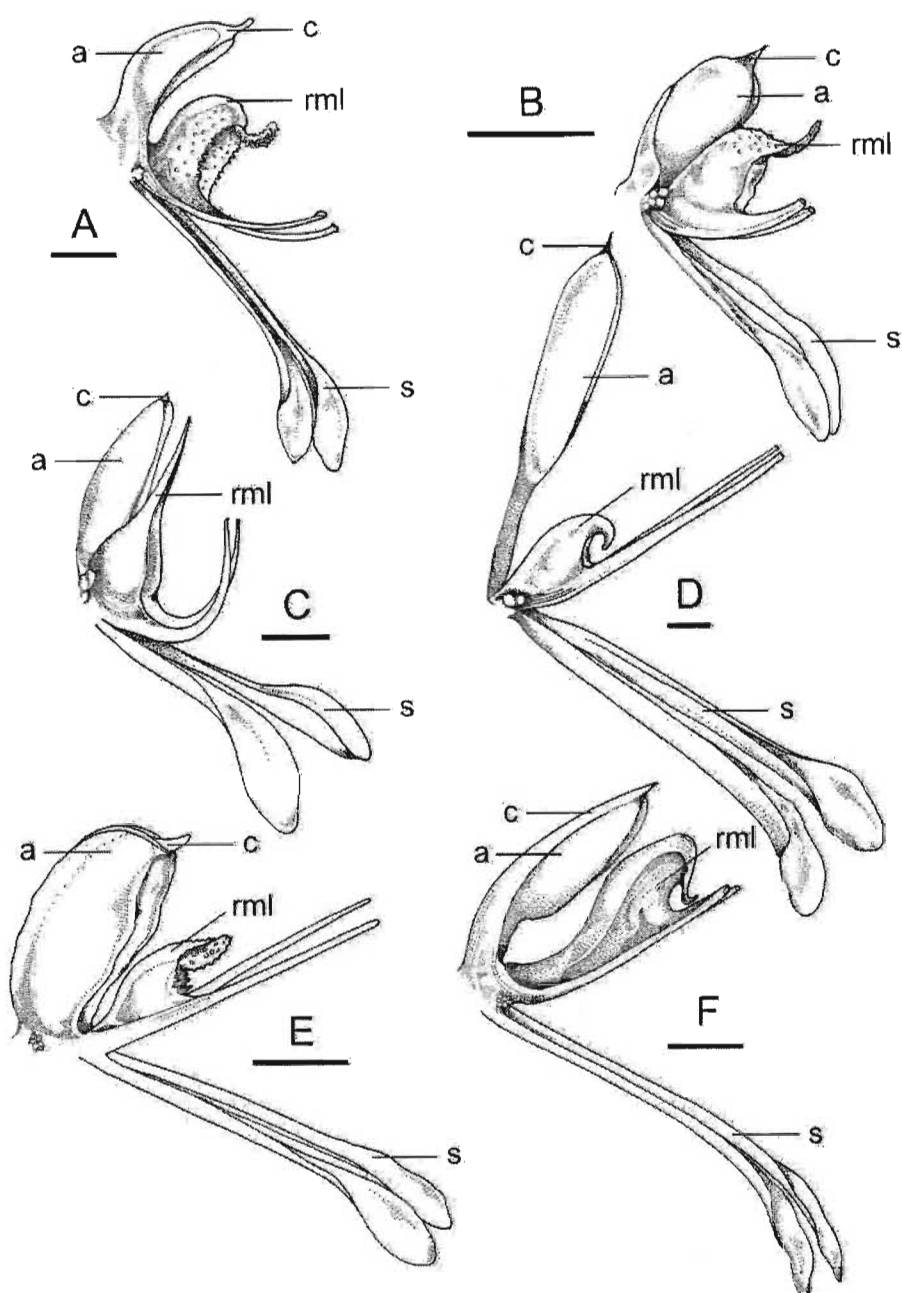
Many of the floral characters used to delimit *Bonatea* are not unique to the genus. Thus the tooth that occurs in the spur throat of most *Bonatea* species is absent from *Bonatea rabaiensis* Rendle but present in *Habenaria arenaria* Lindl. This structure is also absent from *B. bracteata* where the rostellum was misinterpreted as a spur tooth (McDonald, 1991). The tooth of *B. tentaculifera*, *H. arenaria* and *H. malacophylla* is distal to the spur throat, projecting at the split of the lip. This tooth appears to be different from that of *Bonatea*, which is always confined to the spur throat. In this respect, the type tooth found in *Bonatea* (yet secondarily lost in *B. rabaiensis*) could indeed qualify as a synapomorphy for the genus. Further sampling of *Habenaria* is required to confirm this.

Critical differences in the rostellum (Figure 1; see also Williamson, 1977; Williamson, 1980; Kurzweil, 1989; Kurzweil and Weber, 1992) separate *B. bracteata* and *B. tentaculifera* from the genus *Bonatea*. Kurzweil & Weber (1992) note the presence of the galeate rostellum as

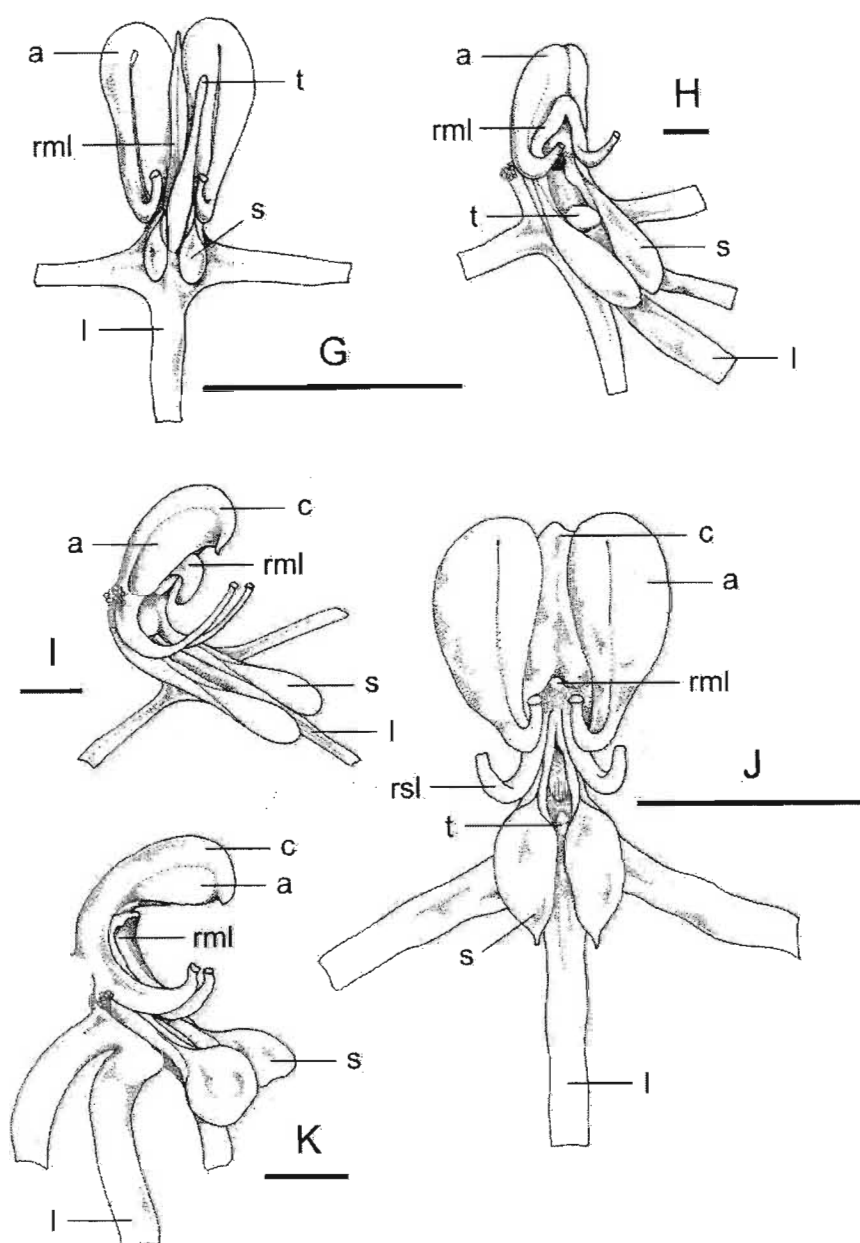
a consistent character delimiting *Bonatea* although they suggest that the character is inadequate support for generic circumscription. Diversification in shape has occurred within *Bonatea*, producing elongate-rostrate rostellum mid-lobes in *Bonatea stereophylla* (Kraenzl.) Summerh., *Bonatea pulchella* Summerh. and *Bonatea polypodantha* (Rchb.f.) L.Bolus. The remaining species of *Bonatea* all have distinctly galeate rostella. In addition, the anther thecae of *Bonatea* are vertical and diverge from the rostellum. By contrast, all southern African species of *Habenaria* have short anthers that are slightly arcuate and flank the rostellum. The latter pattern is also found in *B. bracteata* and *B. tentaculifera*, both of which lack the galeate middle rostellum lobe (Figure 2). In addition, little fusion exists between the stigmatic processes and either the lateral sepals or the anterior petal lobes in *B. bracteata* and *B. tentaculifera*.

### **Habitat**

African *Habenaria* are largely restricted to grassland habitats, whilst *Bonatea* species, including *B. bracteata* and *B. tentaculifera*, occur mostly in scrub-forest or woodland (Linder, Kurzweil and Johnson, 2005). All species are strongly fragrant at night and have cryptic green sepals and white petals that favour crepuscular and nocturnal pollination. The overlap of these species and their exploitation of the same nocturnal pollinators may have resulted in floral convergence and contributed to the taxonomic confusion surrounding the generic placement of *B. bracteata* and *B. tentaculifera*.

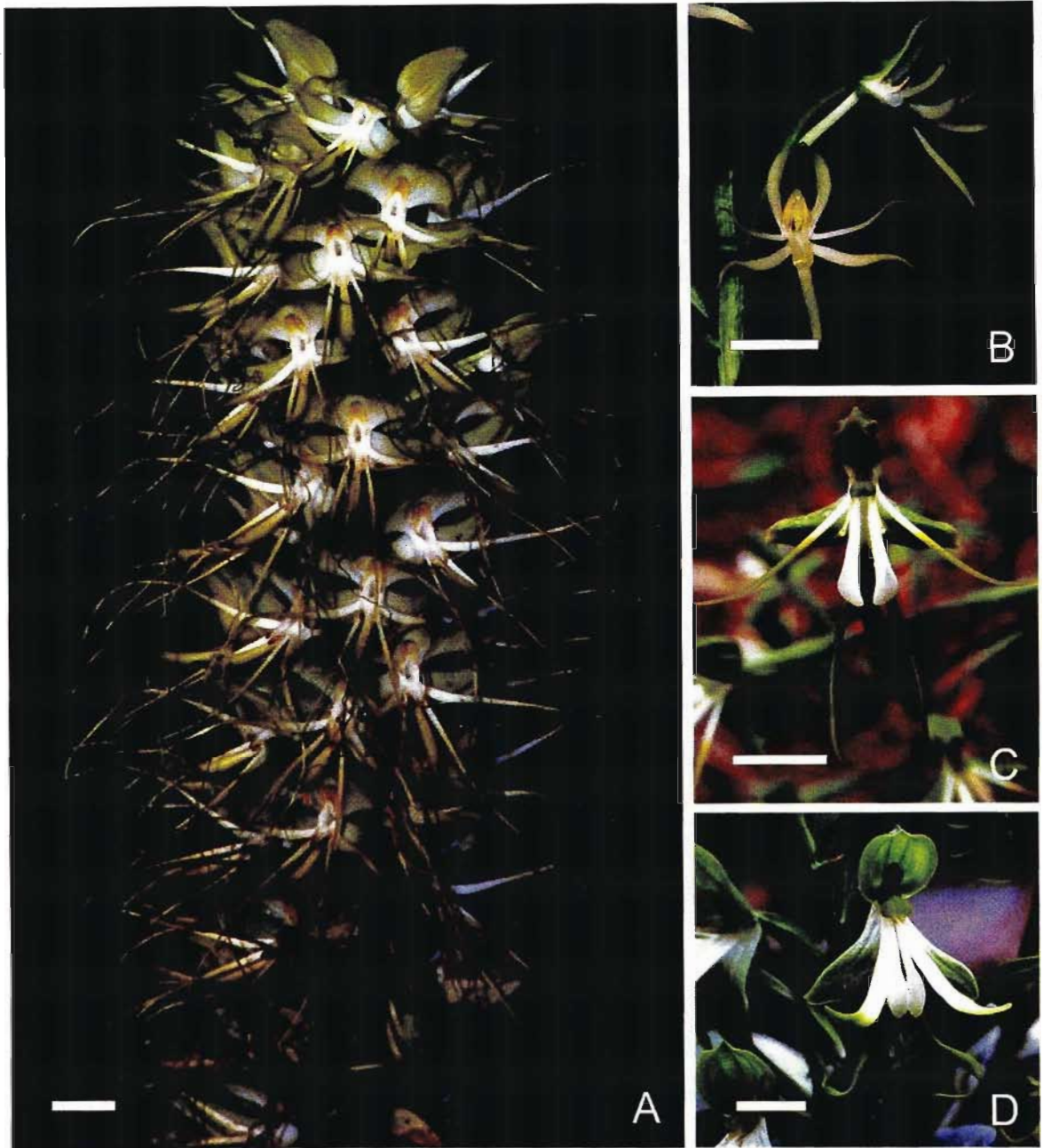


**Figure 1.** Rostellum diagrams of selected *Habenaria* and *Bonatea* species: *Bonatea speciosa* (A) [voucher: *Carnegie s.n.* (NU)], *Bonatea cassidea* (B) [voucher: *O'Connor 32* (NU)], *Bonatea pulchella* (C) [voucher: *Mogg 28138* (J)], *Bonatea lamprophylla* (D) [voucher: *O'Connor 604* (NU)], *Bonatea volkensiana* (E) [voucher: *Bally 9222* (EA)], *Bonatea rabaiensis* (F) [voucher: *Jeffery K238* (EA; PRE)]. Scale bar = 3 mm. Annotations: a = anther sac, c = connective, rml = rostellum middle lobe, s = stigmatic arm. Illustration T. J. Edwards.



**Figure 1 continued.** Rostellum diagrams of selected *Habenaria* and *Bonatea* species: *Habenaria arenaria* (G) [voucher: Ward 5713 (NU)], *Bonatea tentaculifera* (H) [voucher: Amiyo s.n. (NU)], *Bonatea bracteata* (I) [voucher: O'Connor 453 (NU)], *Habenaria malacophylla* (J) [voucher: Stewart 1736 (NU)] and *Habenaria barbertoni* (K) [voucher: O'Connor 615 (NU)]. Scale bar = 2 mm. Annotations: a = anther sac, c = connective, l = labellum, rml = rostellum middle lobe, rsl = rostellum side lobe, s = stigmatic arm, t = tooth. Illustration T. J. Edwards.





**Figure 2.** Similarity in floral design and divergence in rostellum structure of *Bonatea* species: *Bonatea tentaculifera* (A), photo M. Botha; *Bonatea bracteata* (B), photo M. O'Connor; *Bonatea porrecta* (C), photo M. Ponsie; *Bonatea speciosa* (D), photo M. Ponsie. A+B = galeate rostellum mid-lobe absent. C+D = galeate rostellum mid-lobe present. Scale bar = 10 mm.

## CONCLUSIONS

Based on morphological evidence it is necessary to remove *B. bracteata* and *B. tentaculifera* from *Bonatea* and place them within *Habenaria*.

This generic reconfiguration is important, whether the genera remain separate or are conflated, as it redefines *Bonatea* as a taxonomic unit. *Bonatea* can thus be diagnosed by the anther thecae which are vertical and diverge from the rostellum; the elongate-rostrate and galeate rostellum middle lobes; extensive fusion between the stigmatic processes, lateral sepals and the anterior petal lobes; a tooth in the spur mouth (lost in *B. rabaiensis*); and sheathing bracts. A full revision of *Habenaria* will provide a better insight into the evolution of these characters.

## TAXONOMIC TREATMENT

***Habenaria transvaalensis*** Schltr. in Bot. Jahrb. Syst. 20, Beibl. 50: 6, 32 (1895); Rolfe. in Fl. Cap. 5, 3: 126 (1913); Stewart *et al.* in Wild Orch. South. Africa: 88 (1982); Manning in Orchids of southern Africa: 120 (1999). Type: South Africa, [Mpumalanga], in shrubby vegetation around Barberton, 5000 ft, 17 January 1892, *Thorncroft* 466 (B<sup>+</sup>, holo.; NH!, iso.).

*Bonatea bracteata* G.McDonald & McMurtry in South African Orchid Journal 22: 29 (1991); la Croix & la Croix in African orchids in the wild and in cultivation: 154 (1997); Schelpe & Linder in Orchids of southern Africa: 139 (1999). Type: South Africa, [KwaZulu-Natal], Nkandla, Qudeni, Gonzaga Forest in bush, 6000 ft, 2 March 1935, *Gerstner* 588 (PRE!, holo.; NH!, iso.).

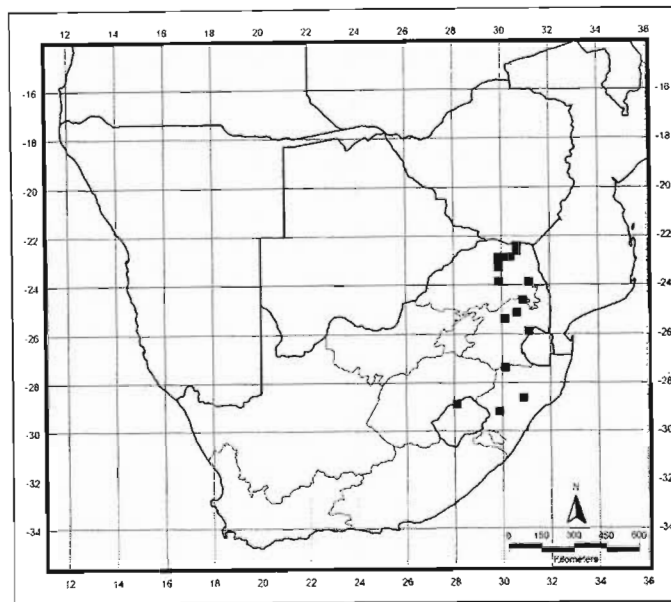
*Bonatea liparophylla* Schelpe nom. nud. in Stewart *et al.* in Wild Orch. South. Africa: 100, pl. 10.4 (1982).

Erect, decumbent or scandent terrestrial herb, 310–582 mm tall; tuber 1, ovoid, tomentose, stolons capable of tuber production at apex. *Stem* slender, 300–577 x 2.5–7.0 mm. *Leaves* 5–12, lax, cauline, lower sheathing, laminae reduced, ovate-elliptical to lanceolate, acuminate, margins entire, smooth, 38–102 x 18–44 mm. *Inflorescence* cylindrical, 49–226 x 41–75 mm, lax to dense, 6–21 flowered; *bracts* flat, ovate-lanceolate, acuminate, 16–38 x 5.5–12.5 mm. *Flowers* spreading, green and white, pedicle and ovary slender, sub-erect, 18–28 mm long. *Dorsal sepal* green, sub-erect, galeate, acute, 10–12 x 2.5–5.0 mm; *lateral sepals* green, patent, lanceolate-deltoid, acutimate to apiculate, base oblique, margins weakly revolute, 9–14 x 2.5–5.0 mm, adnate to lip and stigmatic processes for 3.0–4.5 mm. *Petals* white, deeply bifurcate, upper lobe erect, linear, acute to acuminate, 8.5–12.0 x 0.5–2.0 mm, adnate to dorsal sepal, lower lobe patent, horizontal, curved, linear, apiculate, 9–18 x 0.5–1.5 mm, adnate to claw of lip for 2.0–4.5 mm. *Lip* white, tripartite, claw 5.0–7.5 mm, median lobe descending, linear, acute, 9.0–14.5 x 0.5–2.0 mm, lateral lobes patent, horizontal, sometimes weakly deflexed, slightly curved, linear, apiculate, 10–18 x 0.5–1.5 mm; spur toothless, curved, 17–28 mm long, clavate tip 9.0–15.5 mm long. *Anther* thecae flanking the rostellum, narrowly cylindrical, 2.5–4.5 mm long, canals slender, 3.5–5.5 mm long. *Stigmatic processes* clavate, projecting forwards and deflexed, sub-parallel, 5–10 mm long, base adnate to lip. *Rostellum* 3-lobed, base auriculate, median lobe ligulate, apiculate, apex incurved, margin smooth, 2–3 mm long, lateral lobes linear, fused to anther canals, sub-erect to 45°, sub-parallel, 2.5–4.0 mm long. Flowering period: January to March.

## Habitat

Grassland and savannah, 500–2000 m altitude. *Habenaria transvaalensis* is found in forest or scrub margins, under shrubs or between big boulders; rarely in open grassland.

## Distribution



**Figure 3.** Known distribution of *Habenaria transvaalensis*.

*Habenaria transvaalensis* is restricted to the north-east portions of South Africa, occurring in north-west Lesotho, KwaZulu-Natal, Mpumalanga and Limpopo (Figure 3).

## Observations

*Habenaria transvaalensis* was described by Schlechter in 1895. Schelpe was unsure of its generic placement and in 1985 annotated specimens at PRE as "*Bonatea transvaalensis* (Schltr.) Schelpe" – but without any valid publication. Nonetheless, the lack of galeate rostellum middle lobe and the anomalous growth pattern support Schlechter's placement of the species within *Habenaria*. Subsequent authors considered the species to be a new *Bonatea* and, apparently unaware of the existing name, redescribed it with various other specific epithets (Stewart *et al.*,

1982; McDonald, 1991). Schlechter's specific epithet antedates all of these and thus holds precedence.

*Habenaria transvaalensis* has been previously confused with *Habenaria barbertoni* Kraenzl. & Schltr. The confusion is largely due to both taxa having few, similar sized flowers with white petal lobes and white labellums, as well as an overlap in distribution and flowering period. However, the species can be clearly separated by differences in the structure of the middle rostellum lobe (Figure 1I and Figure 1K), upper petal lobe shape and length compared to the lower petal lobe, as well as the length of the spur in relation to the ovary and pedicel.

McDonald in South African Orchid Journal **22**: 29 (1991), incorrectly record *Gerstner 588* (PRE!, iso.). The isotype is in fact housed at NH.

### **Specimens examined**

—**2229** (Waterpoort): Louis Trichardt, Minastone Farm, (–DD), *Breyer 19488* (PRE).

—**2230** (Messina): Zoutpansberg, main forest, (–BC), *Rodin 4028* (PRE); Khaku, Lake Fundudzi, (–CD), *McMurtry 2981* (Hortus Siccus McMurtrianus; PRE); road to Rambuda near Tshosa Mountain, (–CD), *O'Connor 453* (NU).

—**2329** (Polokwane): Louis Trichardt, (–BB), without collector SAM 68063 (SAM); Soekmekaar, (–BD), *Botha s.n.* (PRE); Haenertsburg, (–DD), *Grewcock 30* (PRE); in shade near Houtboschberg, 2000 m, (–DD), *Schlechter 4383* (BOL; K); Woodbush Range, Pickfontein 1078LS, E of Withoogte Nek, (–DD), *Winter 4717* (UNIN).

—**2530** (Lydenburg): E of Dullstroom, SE down Kruisfontein Road, Morgenzon Farm, (–AC), *McMurtry 4379* (Hortus Siccus McMurtrianus); between Nelspruit and Lydenburg, (–BA), *Harwood s.n.* (PRE).

- 2531** (Komatipoort): among shrubs near Barberton, (–CC), *Thorncraft* 466 (NH).
- 2828** (Bethlehem): Lesotho, Leribe, plateau in veld, (–CC), *Dieterlen* 480 (NH; PRE).
- 2830** (Dundee): Nkandla, Gonzaga Forest, (–DB), *Gerstner* 588 (NH; PRE).

***Habenaria bonateoides*** M.Ponsie, nom. nov. pro *Bonatea tentaculifera* Summerh. in Kew Bull. 4: 431 (1949); Summerhayes in Fl. Trop. E. Afr.: 141 (1968); Stewart in Orchids of Kenya: 93 (1996). Type: Kenya, Nairobi, [Nairobi] City Park in forest, 1650 m, 14 April 1945, *Mountford in Bally B4403* (K!, holo.).

### Description

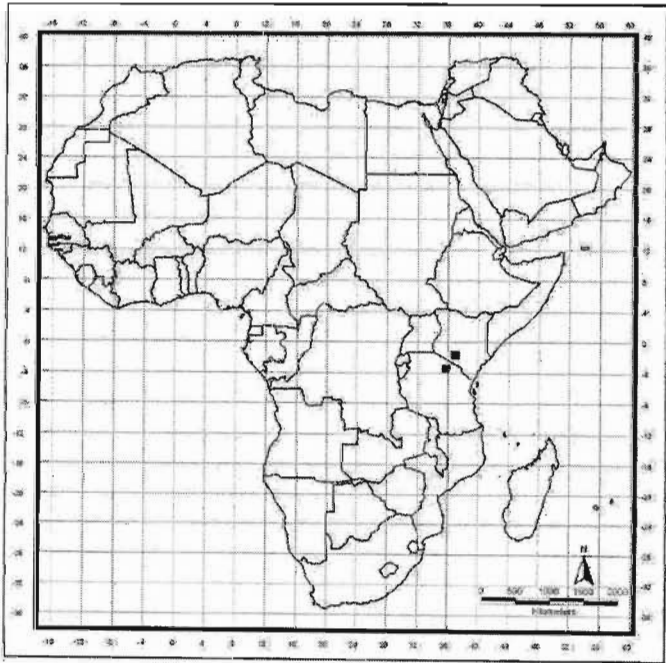
Erect, decumbent or scandent terrestrial herbs, 600–1500 mm tall; tubers 1–7, spreading, elongate-oblong, tomentose, young plants stoloniferous. *Stem* stout, 590–1495 x 8–15 mm. *Leaves* 7–12, lax, cauline, lower sheathing, laminae reduced, elliptical to elliptical-lanceolate, acute, margins entire, crisped, 47–144 x 23–76 mm. *Inflorescence* cylindrical, 148–500 x 100–132 mm, dense, 30–70 flowered; *bracts* similar to upper leaves, flat, lanceolate to ovate-lanceolate, acuminate, 27–41 x 6–12 mm. *Flowers* spreading, green, pedicel and ovary slender, erect, 21–29 mm long. *Dorsal sepal* erect, narrowly galeate, acute, 15–19 x 5–7 mm; *lateral sepals* patent, triangular, acuminate, base truncate, 14–20 x 4–7 mm, adnate to lip and stigmatic processes for 3.5–5.0 mm. *Petals* deeply bifurcate, upper lobe erect, falcate, acute to acuminate, 15–18 x 5.0–7.5 mm, adnate to dorsal sepal, lower lobe patent, horizontal, slightly connate with the lip, linear, tip curved, apiculate, 50–55 x 0.5–1.5 mm. *Lip* tripartite, claw 4–5 mm long, median lobe descending, curved forward, oblanceolate, acute, 13–21 x 1.5–3.5 mm, lateral lobes descending, diverging, linear,

apiculate, 65–72 x 0.5–1.0 mm; spur toothless, 22–30 mm long, clavate tip 10–13 mm long, residual tooth distal to mouth of spur. *Anther* thecae flanking the rostellum, narrowly cylindrical, 3.5–5.0 mm long, canals slender, 2–3 mm long. *Stigmatic processes* clavate, projecting forwards and deflexed, 4–7 mm long, base adnate to lip. *Rostellum* 3-lobed, base auriculate, median lobe erect, minutely papillate, rounded-deltoid, acute, margin smooth, 2–4 mm long, lateral lobes incurved, linear, fused to anther canals, deflexed, sub-parallel, 2 mm long. Flowering period: April to May.

**Habitat**

Forest and forest margins, at 1650–2000 m altitude.

**Distribution**



**Figure 4.** Known distribution of *Habenaria bonateoides*.

*Habenaria bonateoides* is restricted to east Africa occurring in Kenya and Tanzania (Figure 4).

## Observations

A new species epithet was chosen for *B. tentaculifera* to avoid confusion with species epithets derived from the basionym 'tentacula' (*Habenaria tentaculigera* Rchb.f., *Habenaria tentaculata* (Lindl.) Rchb.f. and *Habenaria tentaculata* Pfitzer). The specific epithet 'bonateoides' accentuates the resemblance to, and the initial placement of, the species within *Bonatea*.

*Habenaria bonateoides* was first discovered in 1945 (Mountford in Bally B4403), in a forest within the Nairobi City Park, Kenya. The species is clearly rare but widespread for it has now been collected in the Ngorongoro Crater, Tanzania (*Botha s.n.*; *Amiyo s.n.*). Summerhayes (1949) viewed the species as a link between the genus *Bonatea* and section *Pentaceras* of *Habenaria*, where this group has no tooth in the spur mouth and the rostellum middle lobe is comparatively small, triangular and flanked by the anther thecae. The fusion between the stigmatic lobes and the perianth is also very poorly developed.

Herbarium sheet drawings (Summerhayes K79099) incorrectly show the anthers as erect rather than arcuate and flanking the rostellum; probably a misinterpretation of the distorted pressed material.

## Specimens examined

—**0136** (Nairobi): Kenya, Nairobi City Park in forest, (–BD), Mountford in Bally B4403 (K).

—**0335** (Masai): Tanzania, Ngorongoro Crater, (–BA), *Botha s.n.* NU9949 (NU); Tanzania, Ngorongoro Crater, (–BA), *Amiyo s.n.* (NU).



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## CHAPTER 3

### Systematics of the *Bonatea speciosa* complex (Orchidaceae)

#### ABSTRACT

Species boundaries in rapidly evolving lineages are often blurred, necessitating analysis of the full range of variation within a complex in order to identify traits that can be used to diagnose taxa. *Bonatea speciosa*, as currently recognized, consists of two varieties: *Bonatea speciosa* var. *speciosa* (L.f.) Willd. and *Bonatea speciosa* var. *antennifera* (Rolfe) Sommerville. Morphological variation across the *Bonatea speciosa* complex (Orchidaceae) was explored using principal component and cluster analysis. Scatter plots were used to identify characters that can be used to diagnose taxa. This data, in conjunction with molecular, geographic and ecological data, support the recognition of three distinct species: *Bonatea antennifera* Rolfe, *Bonatea boltonii* (Harv.) Bolus and *Bonatea speciosa* (L.f.) Willd. Phylogenetic analyses (Chapter 1) suggest that *Bonatea porrecta* (Bolus) Summerh. and *Bonatea volkensiana* (Kraenzl.) Rolfe may also be embedded within the *B. speciosa* complex. A taxonomic revision of southern Africa taxa within the *B. speciosa* complex is presented.

#### INTRODUCTION

The argument about appropriate ways to define a species is still unresolved (Donoghue, 1985; de Queiroz and Donoghue, 1988; Mayr, 1992; Gavrillets, 2003). In terms of an operational species concept, biologists need to decide, either implicitly or explicitly, what level of difference between two groups makes each worthy of its own species designation (Bremer and Eriksson, 1992; Hendry *et al.*, 2000). Operational species concepts make use of diagnostic phenotypic

characters to resolve species boundaries (Agapow *et al.*, 2004; Sites and Marshall, 2004), but the selection of diagnostic characters and identification of their thresholds is a process that is seldom carried out in a rigorous quantitative manner.

Species complexes are usually symptomatic of recent adaptive radiation (Schluter, 1996; Givnish, 2001), where geographically defined groups of individuals diverge from one another and accumulate, through selective and stochastic processes, the numerous genetic and phenotypic attributes that distinguish them (Levin, 1993; Orr and Smith, 1998). Phylogenetic analyses often reveal limited molecular divergence during recent radiation events (Givnish, 2000; Chapter 1). Reliance on morphology, ecology and biogeographic data thus remains informative, with morphological characters providing the only practical means available to diagnose terminal units within these species complexes (Balfour and Linder, 1990; Johnson and Linder, 1995; Wendt *et al.*, 2000).

The *Bonatea speciosa* complex is poorly understood with taxonomic controversy surrounding the circumscription of three taxa, *Bonatea antennifera* Rolfe (Rolfe, 1905), *Bonatea boltonii* (Harv.) Bolus (Harvey, 1859) and *Bonatea speciosa* (L.f.) Willd. (Linnaeus, 1781). On the basis of variation in the lower petal lobe, Sommerville (1982) and Schelpe and Linder (1999) recognized a single species, *Bonatea speciosa* with two varieties (var. *speciosa* (L.f.) Willd. and var. *antennifera* (Rolfe) Sommerville), and conflated *B. boltonii* within the typical variety. By contrast, la Croix and Cribb (1995) recognize *B. antennifera* as a distinct species.

Phylogenetic analyses (Chapter 1) indicate that the *B. speciosa* complex may not be monophyletic. The complex may include *Bonatea porrecta* (Bolus) Summerh., *Bonatea volkensiana* (Kraenzl.) Rolfe,

*Bonatea cassidea* Sond., *Bonatea saundersiae* (Harv.) T.Durand & Schinz and *Bonatea saundersioides* (Kraenzl. & Schltr.) Cortesi. The taxonomy of the *Bonatea cassidea* complex is dealt with in Chapter 4.

The aim of this chapter was to determine whether *B. antennifera*, *B. boltonii* and *B. speciosa* have sufficient diagnostic molecular and morphological characters to be treated as distinct species, or whether they should be considered variants of *B. speciosa*, as was suggested by Sommerville (1982) and Schelpe and Linder (1999).

## **MATERIALS AND METHODS**

Morphological variation within the taxa was assessed by examination of fresh plants both *in situ* and in cultivation and preserved material deposited in the following herbaria: BLFU, BOL, GLOW, J, KEI, KMG, NBG, NH, NU, PRE, PRU, PUC, SAM, STE, UNIN and the private herbarium of D. McMurtry. Type digital images from BOL, K, S, TCD and UPS have also been examined. Specimens were assigned into tentative groupings based on the characters used in their original descriptions.

### **Molecular**

Sequence alignment for the internal transcribed spacer regions and the intervening 5.8S rRNA gene (ITS) was obtained from Chapter 1.

### **Morphology**

Both ordination and cluster analyses were used to evaluate the importance of quantitative characters in the recognition of species boundaries. The full data set (364 specimens) was analysed by principal components analysis (PCA) using the MVSP multivariate statistical package (Kovach, 1999). A small number of specimens were

not possible to group (*Strey 5880* (NH; PRE); *Galpin 8429* (PRE); *Sim 809* (NU)) but were included as 'undecided'. The resulting morphometric patterns are illustrated in ordination plots. Character loading from the ordination axes identified potential diagnostic characters that were investigated by scatter plots. 'Spur basal length' was measured from the tip of the spur to the top of the clavate basal portion. Leaf surface area was investigated as the different elements occupy different vegetation types with different water availability which affect transpiration rates and light exposure. The orientations of floral organs were evaluated for qualitative differences.

RESULTS

Molecular data

Recent molecular work on *Bonatea* (Chapter 1) hinted at a closer alliance between *B. boltonii* and *B. antennifera* than either of these entities have with *B. speciosa*. Examination of all ITS nucleotide differences for the *B. speciosa* complex reveal conservatism within *B. speciosa*. All specimens of *B. antennifera* and *B. boltonii* (excluding *B. boltonii* MP27) lack the -TTAA- stretch, with additional minor differences within the species and inconclusive differences between the species.

**Table 1.** Comparative summary of the 16 ITS nucleotide differences between *B. porrecta* and the *Bonatea speciosa* complex. All nucleotides in common with *B. speciosa* have been highlighted. All nucleotides are from single sites throughout the ITS region besides the -**TTAA**- stretch in bold. R = A/G and Y = C/T.

Taxa	Specimen Code	Nucleotides
<i>B. porrecta</i>	MP19	TYYC <b>Y</b> T----RYYTYR
<i>B. antennifera</i>	MP1	TTCCCT----GCTTTG
<i>B. antennifera</i>	MP2	TTCCCT----GCTTTG
<i>B. antennifera</i>	MP17	TT <b>C</b> YYT----GCTTTG
<i>B. antennifera</i>	MP18	TTY <b>Y</b> TT----GCTTTG
<i>B. boltonii</i>	MP8	TTCC <b>C</b> Y----GCTTTG
<i>B. boltonii</i>	MP21	TTCCCT----GCT <b>Y</b> TG
<i>B. boltonii</i>	MP28	TTCCCT----GCTCTG
<i>B. boltonii</i>	MP27	CCCCCT <b>TTA</b> AGCTTCG
<i>B. speciosa</i>	MP5	CCCCCT <b>TTA</b> AGCTTCA
<i>B. speciosa</i>	MP6	CCCCCT <b>Y</b> TAAGCTTCA
<i>B. speciosa</i>	MP7	CCCCCT <b>TTA</b> AGCTTCA
<i>B. speciosa</i>	ED*	CCCCCT <b>TTA</b> AGCTTCA

ED\* = Douzery *et al.* 1999

**Principal components analysis**

The main five variables contributing to the loading (Table 3), include three useful diagnostic characters ‘Lower petal lobe length’, ‘Stigmatic process length’ and the ‘Spur basal length’, whilst ‘Side lobe length’ and ‘Mid-lobe length’ overlap extensively within the complex. Three groups were evident in scatter plots (Figure 1). *Bonatea antennifera* individuals from northern KwaZulu-Natal (*O’Connor 510* (NU); *O’Connor 512* (NU); *Pooley 1398* (NU)) are long-spurred causing overlap with *B. speciosa* in the scatterplots. Individuals of *B. antennifera* embedded within *B. boltonii*, are anomalous specimens with unusually small flowers (*de Feijter 94* (PUC); *Kuun 23* (PUC; PRU)). Long-spurred specimens of *B. speciosa* from Inhaca Island, Mozambique, are found clustered in the top right corner (*Mogg 29486* (J); *Moss s.n.* (J); *Weintraub s.n.* (J); *Weintraub s.n.* (J); *Wolfowitz 5* (J)). The specimens that were undiagnosed group within *B. boltonii*.

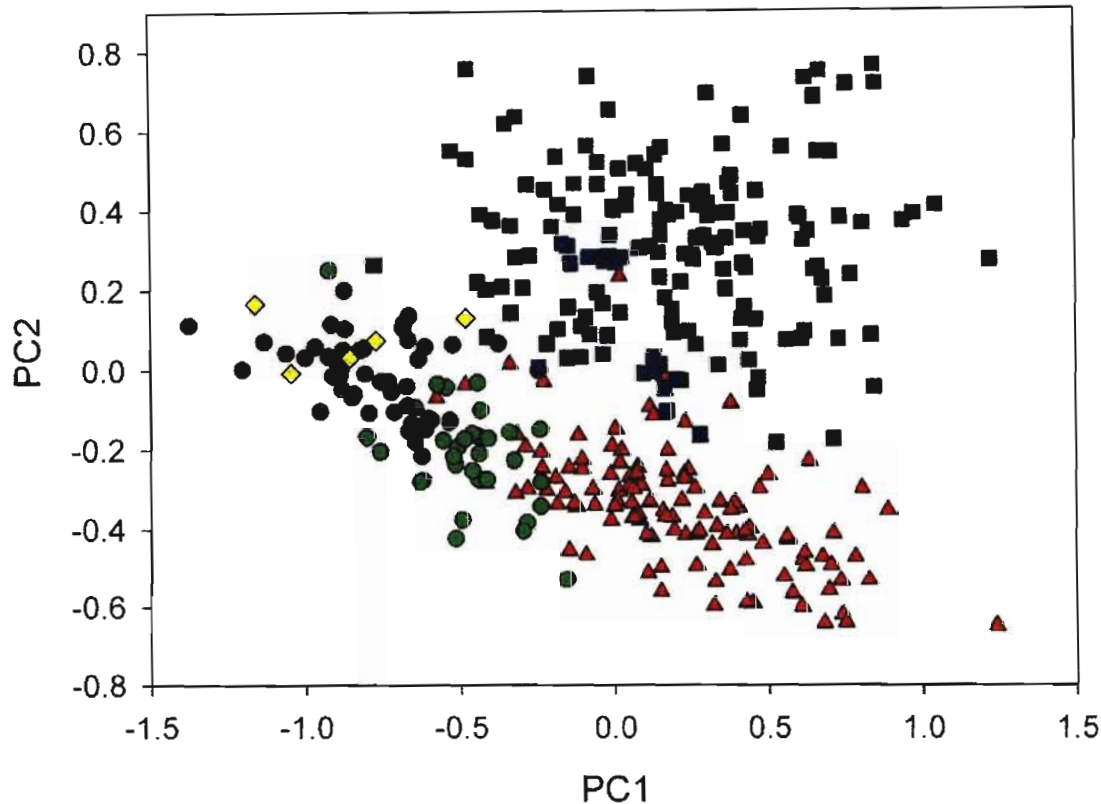
**Table 2.** Results of PCA on 14 variables of 364 specimens of the *Bonatea speciosa* complex. Eigenvalues, percentage of total variance explained by each axis and cumulative percentage along the first three axes.

Axis	Eigenvalues	Percentage of variance explained	Cumulative percentage of variance explained
1	88.56	49.67	49.67
2	40.42	22.67	72.33
3	26.48	14.85	87.17



**Table 3.** PCA variable loadings. The 5 highest loadings per axis are in bold.

Variable	Axis 1	Axis 2	Axis 3
Lateral sepal length	0.233	<b>0.202</b>	-0.048
Lateral sepal width	0.113	0.144	<b>-0.092</b>
Upper petal lobe length	0.263	0.179	-0.089
Upper petal lobe width	-0.008	-0.013	0.012
Lower petal lobe length	<b>0.569</b>	-0.152	<b>-0.112</b>
Lower petal lobe width	0.036	0.106	-0.046
Lip side lobe length	<b>0.460</b>	<b>-0.397</b>	0.011
Lip side lobe width	0.020	0.062	-0.029
Lip mid-lobe length	<b>0.331</b>	<b>-0.425</b>	<b>0.090</b>
Lip mid-lobe width	-0.006	-0.016	0.005
Stigmatic process length	<b>0.326</b>	<b>0.207</b>	<b>-0.093</b>
Stigmatic receptive surface length	0.048	0.067	-0.006
Spur length	0.129	0.137	<b>0.972</b>
Spur clavate apex	<b>0.303</b>	<b>0.681</b>	-0.062



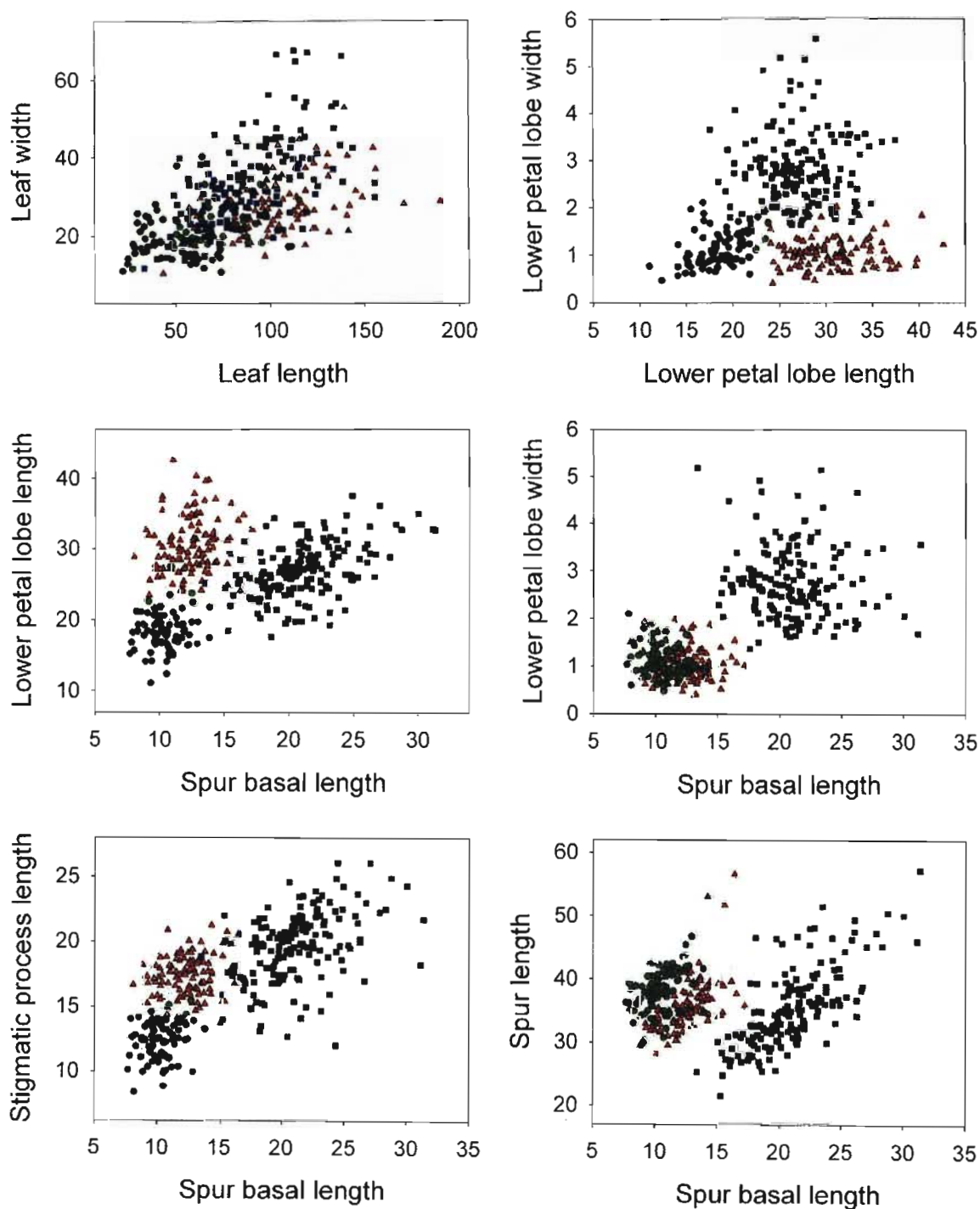
**Figure 1.** Scatter plot of first two components from a PCA performed on 14 variables of 119 specimens diagnosed *a priori* as *Bonatea antennifera* (▲), 81 specimens of *Bonatea boltonii* (●), 159 specimens of *Bonatea speciosa* (■) and 5 undecided specimens (◆).

## Scatter plots

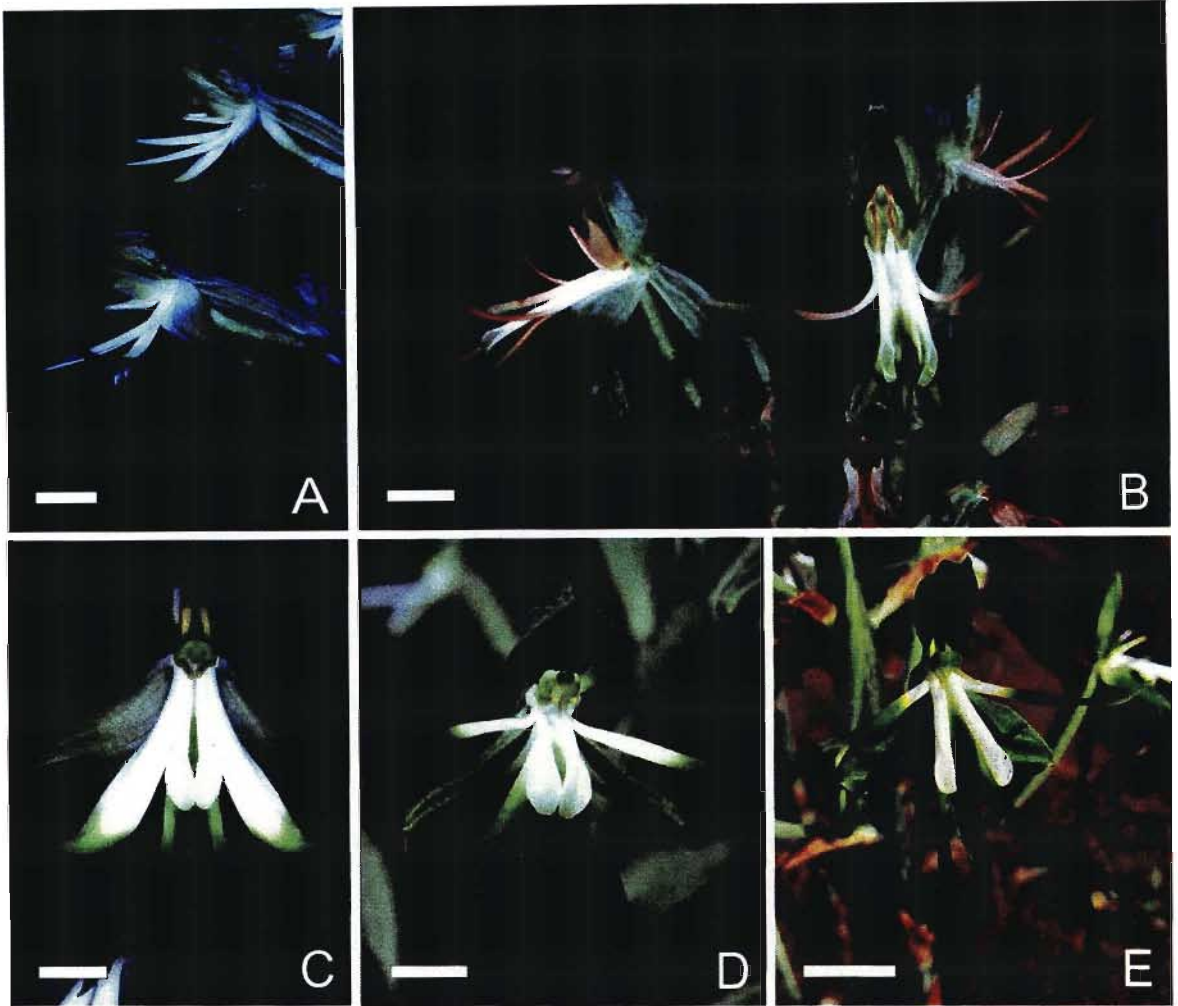
Leaf dimensions (Figure 2) show little diagnostic pattern.

Scatter plots combining 'Lower petal lobe length x Lower petal lobe width', 'Spur basal length x Lower petal lobe length' and 'Spur basal length x Stigmatic process length' reveal trait discontinuities. These correspond to previously described taxa that were included in more recent interpretations of *B. speciosa*. Slight overlap between the species occurs in a number of traits but combined suites of characters provide repeated spatial separation. Overall, the 'Lower petal lobe length', 'Stigmatic process length' and the 'Spur basal length' are the most reliable diagnostic characters.

'Spur basal length x Lower petal lobe width' and 'Spur basal length x Spur length' clearly separate *B. speciosa* from *B. antennifera* and *B. boltonii*. A wide range in spur length exists for *B. speciosa* (20–57 mm) compared to the narrow ranges of *B. boltonii* (27–51 mm) and *B. antennifera* (27–44(–58) mm). Specimens of *B. antennifera* from northern KwaZulu-Natal have spur lengths outside the range of the remaining population (51–58 mm). Similarly, specimens of *B. speciosa* from Inhaca Island, Mozambique, have long spurs (44–57 mm). Both species flower during June and July, which overlaps with the flowering period of other long-spurred *Bonatea* species such as *Bonatea pulchella* Summerh. (spur length 46–70 mm) and *Bonatea steudneri* (Rchb.f.) T.Durand & Schinz (spur length 75–184 mm).



**Figure 2.** Cluster plots comparing vegetative and floral traits for *Bonatea antennifera* (▲), *Bonatea boltonii* (●) and *Bonatea speciosa* (■).



**Figure 3.** Species comprising the *Bonatea speciosa* complex: *Bonatea antennifera* (A), photo D. McMurtry; *Bonatea antennifera* (B) from northern KwaZulu-Natal, photo M. O'Connor; *Bonatea speciosa* (C), photo R. Parsons; *Bonatea boltonii* (D), photo O. Kurze; *Bonatea porrecta* (E), photo G. Chittenden. Scale bar = 10 mm.

## DISCUSSION

The results of this study point to the existence of three morphologically distinct and diagnosable species within the entity currently recognized as *B. speciosa* complex. Rapid evolution within this complex has presumably restricted the accumulation of major molecular differences. Nevertheless, molecules show a stronger alliance between *B. boltonii* and *B. antennifera*, and this contradicts the taxonomic inclusion of *B. boltonii* within *B. speciosa* var. *speciosa* (Bolus, 1911; Stewart *et al.*, 1982; Schelpe and Linder, 1999).

Although *B. boltonii* and *B. antennifera* appear to be sister taxa, they are clearly distinct. Critical quantitative divides occur at 15 mm for 'Stigmatic process length' and 24 mm for 'Lower petal lobe length', with *B. boltonii* falling below the divide (Figure 2). Posture of the lower petal lobe and labellum side lobe are also significantly different in the species – both are porrect in *B. antennifera*, whilst spreading lower petal lobes and descending labellum side lobes occur in *B. boltonii* (Figure 3). *Bonatea speciosa* is distinctive with respect to its elongate basal spur length (> 15 mm) in combination with its wider lower petal lobes (> 2 mm) (Figure 2). The lower petal lobe posture is porrect and weakly to strongly deflexed, with descending labellum side lobes. All species have lanceolate labellum mid-lobes which are geniculate.

*Bonatea speciosa* and *B. antennifera* are largely allopatric, being constituents of coastal dune forest and savannah vegetation types, respectively, but both are sympatric with *B. boltonii* (Figure 4). Despite the overlapping distribution and flowering times, no intermediate forms have been observed, even in mixed populations (*Flanagan 647* (GRA)). *Bonatea speciosa* is distributed coastally from the Western Cape to Mozambique, and its wide range in spur length may be the result of local adaptation to different pollinators across this geographic range. Populations of *B. antennifera*, *B. boltonii* and *B. speciosa* found in northern KwaZulu-Natal flower at the same time, with selection

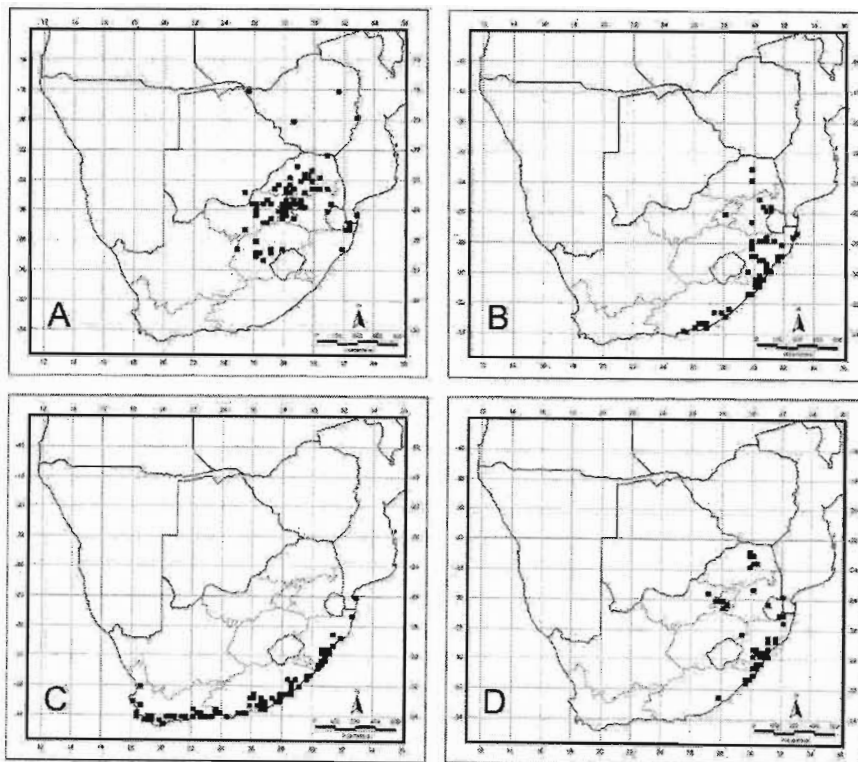
favouring larger flowers and longer spur length. This shift could be the result of local adaption to long tongued pollinators which visit *Bonatea pulchella* Summerh. and *Bonatea lamprophylla* J.L.Stewart; both long spurred *Bonatea*.

Distributionally, *B. porrecta* is sympatric with *B. antennifera*, *B. boltonii* and *B. speciosa*. No overlap of these taxa is known to occur with the east African species *B. volkensiana*. Morphologically, there is a close resemblance between the *B. speciosa* complex taxa, reflecting the recent migration and radiation of this genus within Africa (Chapter 1). This group of taxa warrant a detailed study of pollination syndromes and population genetics, which will elucidate evolutionary and speciation patterns.

## TAXONOMIC TREATMENT

### KEY TO TAXA IN THE *BONATEA SPECIOSA* COMPLEX

- 1a. Leaves withered at flowering ..... 1. *B. porrecta*
- 1b. Leaves present at flowering..... 2
- 2a. Clavate apex of spur > 15 mm long; lower petal lobe > 2mm wide; lower petal lobes projecting forwards and deflexed ..... 4. *B. speciosa*
- 2b. Clavate apex of spur < 15 mm long; lower petal lobe < 2mm wide ..... (3)
- 3a. Lower petal lobe (18–)24–43 × 0.5–2.0 mm, projecting forwards and ascending; stigmatic processes (11–)15–22 mm long..... 2. *B. antennifera*
- 3b. Lower petal lobes 11–24(–28) × 0.5–2.0(–2.5) mm, spreading horizontally; stigmatic processes 8–15 mm long ..... 3. *B. boltonii*



**Figure 4.** Known distribution of *Bonatea antennifera* (A), *Bonatea boltonii* (B), *Bonatea speciosa* (C) and *Bonatea porrecta* (D).

Refer to Chapter 5 for taxonomic descriptions.

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## CHAPTER 4

### **A morphometric analysis of species boundaries in the *Bonatea cassidea* complex**

#### **ABSTRACT**

Taxa with large geographical ranges often exhibit clinal variation. Detailed morphometric analysis may be required to establish the species boundaries in these taxa. Box and whisker plots in combination with multivariate PCA-based cluster analyses of herbarium material revealed extensive morphological variation within *Bonatea cassidea* Sond. Intraspecific variation was examined across seven populations of *B. cassidea* spanning its distribution range. The taxonomic status of *Bonatea saundersiae* (Harv.) T.Durand & Schinz is reconsidered in light of evidence that this taxon lacks clear diagnostic characters that would separate it from *B. cassidea*. The taxon is thus reduced to synonymy with *B. cassidea*. A synopsis of *B. cassidea* and its sister taxon *Bonatea saundersioides* (Kraenzl. & Schltr.) Cortesi is presented.

#### **INTRODUCTION**

Biological diversification and speciation have long been of interest in studies of adaptive evolution. The process of speciation is gradual and there will always be stages of incomplete speciation where populations are still acquiring the attributes of species (Grant, 1981; Ferguson, 2002). Variation is often random in disjunct populations founded by few individuals (Mayr, 1982; Mayr, 1992; García-Ramos and Kirkpatrick, 1997). However, in widespread species variation may reflect local adaptation to gradually shifting physical parameters to produce patterned changes or clines (Jonas and Geber, 1999). Natural selection, genetic variability, adaptation and gene flow all influence the formation

of species (Schluter, 2001; Via, 2002). For speciation to occur in 'incipient' populations (*sensu* Mayr, 1992), local entities must persist long enough for the accumulation of multiple changes (Harder, 2001). However, environmental and demographic fluctuations, as well as inbreeding, may limit the persistence of small isolated populations, especially if they occupy marginal niches (Levin, 1993).

A recent molecular evaluation of the southern African species of *Bonatea* Willd. (Chapter 1) provided information on species relationships within the genus and highlighted taxonomic problems. One of these problems concerns the circumscription of *Bonatea cassidea* Sond. and the taxonomic status of its close allies *Bonatea saundersiae* (Harv.) T.Durand & Schinz and *Bonatea saundersioides* (Kraenzl. & Schltr.) Cortesi. Morphologically *B. saundersiae* is closely allied to *B. cassidea*, but differs chiefly in its reduced rostellum middle lobe (Weale, 1869), markedly longer rostellum side lobes (Rolfe, 1913), and fewer flowers which are larger in size, with more arched side lobes and falcate tips (Schelpe and Linder, 1999). In the initial description of *B. saundersiae*, Harvey (1863) misdiagnosed the upper and lower petal lobes as undivided. During preliminary analysis of herbarium material, large morphological variability within *B. cassidea* was recorded which appears to grade into the northern species *B. saundersioides*. This northern species is distinguished from *B. cassidea* by its longer and narrower lower petal lobes and labellum side lobes (Rolfe, 1913; Schelpe and Linder, 1999). Principal component and cluster analysis, as well as scatter plots, were used to investigate the intraspecific variation of *B. cassidea* across its geographic range, and to assess its relationship with *B. saundersiae* and *B. saundersioides*.

## MATERIALS AND METHODS

### Materials

Morphological variation within the complex was assessed using fresh specimens (Table 1) and preserved material deposited in the following herbaria: GRA, J, NBG, NH, NU, PRE, SAM, the Umtamvuna Herbarium and the private herbarium of D. McMurtry. Type material or digital images from BOL, GRA, K, PRE, S, SAM and TCD have also been examined.

**Table 1.** Population material for *Bonatea cassidea* was collected from the following localities.

Province	Locality	Grid Reference	n
KwaZulu-Natal	Eshowe, Goedetrouw Dam	2831CB	13
KwaZulu-Natal	Ixopo, Watervale Farm	3030AA	10
KwaZulu-Natal	Port Edward, Horseshoe Dam	3030CA	8
Eastern Cape	East London, Kei Road	3227DA	13
Eastern Cape	East London, Casino Valley	3227DD	8
Eastern Cape	East London, Geneva Farm	3228CC	7
Eastern Cape	East London, Umtiza Forest	3327BB	2

We failed to rediscover populations at Kosi Bay, Amsterdam, Harold Johnson Nature Reserve, Cowies Hill, Port Alfred and the Alexandria State Forest.

### Morphology

#### Herbarium material

Box and whisker plots were used to explore the species boundaries of quantitative characters in *B. cassidea*, *B. saundersiae* and *B. saundersioides*. PCA-based ordination analysis was used to explore species boundaries of *B. cassidea*, *B. saundersioides* and *B. saundersiae* for 16 quantitative characters. The full data set (69



specimens) was analysed by ordination of the principal components using the MVSP multivariate statistical package (Kovach, 1999). The resulting morphometric patterns are illustrated in ordination plots. Scatter plots were used to investigate geographically variable characters of *B. cassidea* plotted against latitude and longitude.

### **Population material**

Similarly, PCA analysis and subsequent ordination plots were used to explore population boundaries of *B. cassidea* (61 specimens) for 6 quantitative characters. Scatter plots were used to investigate geographic variation for selected characters of *B. cassidea*.

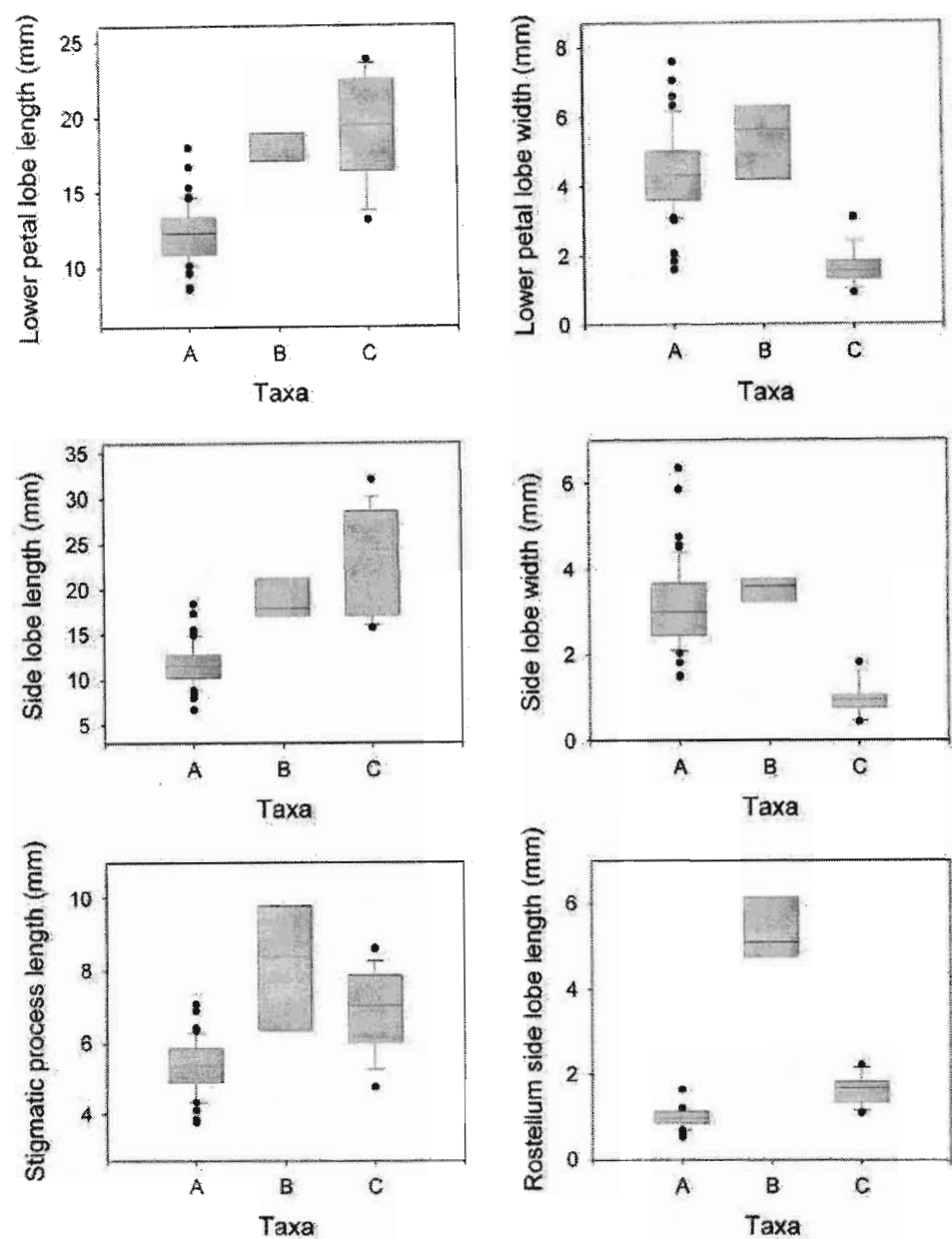
## **RESULTS**

### **Herbarium material analyses**

*Bonatea saundersioides* is morphologically distinct, with longer and narrower lower petal lobes and side lobes than its sister taxa (Figure 1). The length of the lower petal lobes and side lobes is variable, as small and large flower forms (*McMurtry 1209* (Hortus Siccus *McMurtrianus*); *Pooley 633* (NU); *O'Connor 511* (NU); *Botha s.n.* (PRE)) are present on an intra- and inter-population level. Although overlap is evident for all characters, *B. saundersioides* and *B. cassidea* can be differentiated from ordination plots (Figure 2).

The comparison of characters related to flower size of *B. cassidea* and *B. saundersiae* (Figure 1) show larger measurements on average for *B. saundersiae*, yet overlap with *B. cassidea* occurs for all measurements except the rostellum side lobe length. Consequently, specimens of *B. saundersiae* are found to cluster alongside *B. cassidea* in the ordination plot (Figure 2) along the same horizontal plane. *Bonatea cassidea* shows a wide variation in both 'lower petal lobe' and 'side lobe' length

and width. Investigation of this variation in a geographic context (Figure 3) resulted in a significant regression for 'lower petal lobe width' and 'side lobe width' against latitude.



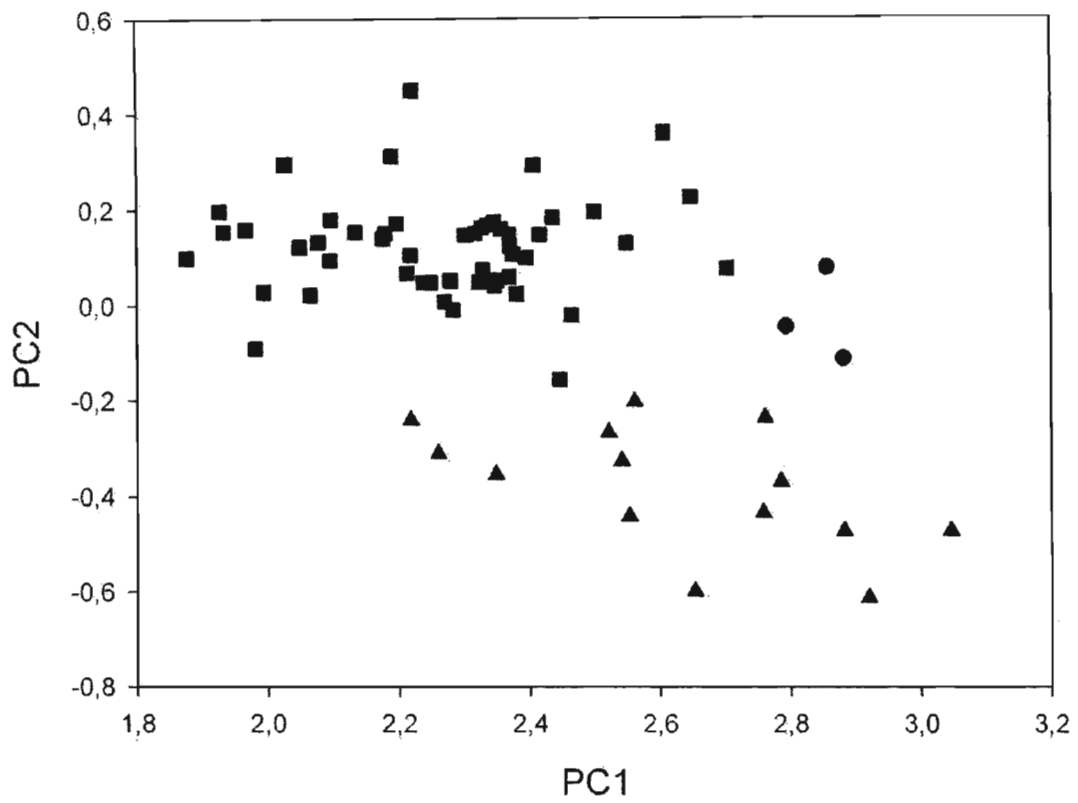
**Figure 1.** Box and whisker plots showing the range of variation in diagnostic characters (vertical line within the box is the mean; box limits, 25-75 % interquartile ranges; whiskers, 5 %-95 % ranges; dots, outliers). *Bonatea cassidea* (A) (n=52), *Bonatea saundersiae* (B) (n=3) and *Bonatea saundersioides* (C) (n=14).

**Table 2.** Results of PCA on 14 variables of 52, 14 and 3 specimens of *B. cassidea*, *B. saundersioides* and *B. saundersiae*, respectively. Eigenvalues, percentage of total variance explained by each axis and cumulative percentage along the first two axes.

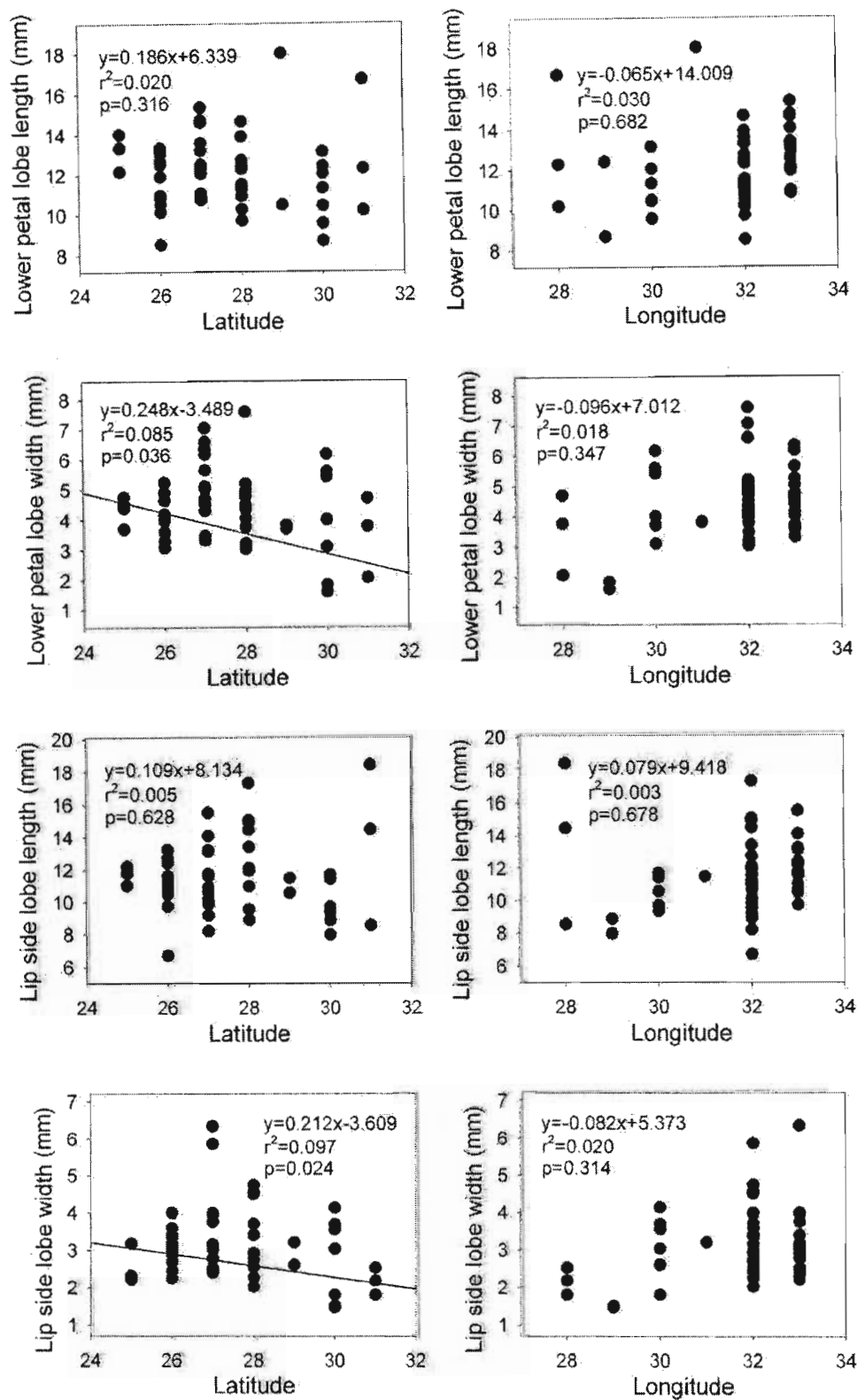
Axis	Eigenvalues	Percentage of variance explained	Cumulative percentage of variance explained
1	391.387	97.191	97.19
2	3.625	0.900	98.09

**Table 3.** PCA variable loadings. The 5 highest loadings for each axis are in bold.

Variable	Axis 1	Axis 2
Bract length	0.208	-0.239
Bract width	0.257	-0.041
Lateral sepal length	<b>0.371</b>	-0.003
Lateral sepal width	<b>0.293</b>	-0.079
Upper petal lobe length	<b>0.315</b>	-0.057
Upper petal lobe width	0.221	<b>0.315</b>
Lower petal lobe length	0.199	<b>-0.269</b>
Lower petal lobe width	0.123	<b>0.505</b>
Lip side lobe length	0.132	<b>-0.340</b>
Lip side lobe width	0.109	<b>0.507</b>
Lip mid-lobe length	0.212	-0.210
Lip mid-lobe width	0.171	0.212
Stigmatic process length	0.270	-0.109
Rostellum side lobe length	0.082	-0.089
Ovary length	<b>0.343</b>	-0.011
Spur length	<b>0.404</b>	0.154



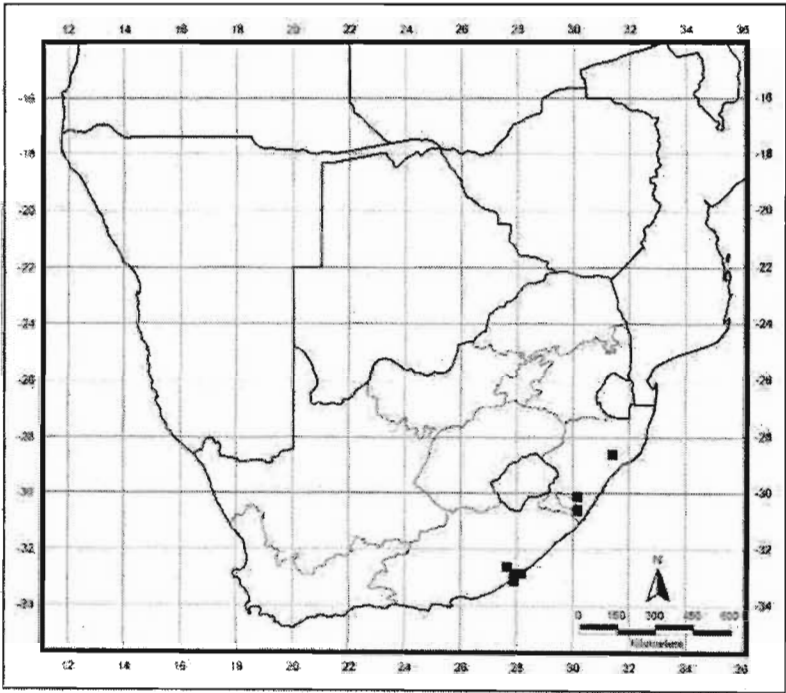
**Figure 2.** Scatter plot of first two components from a PCA performed on 14 variables of 52 specimens of *Bonatea cassidea* (■), 14 specimens of *Bonatea saundersioides* (▲) and 3 specimens of *Bonatea saundersiae* (●).



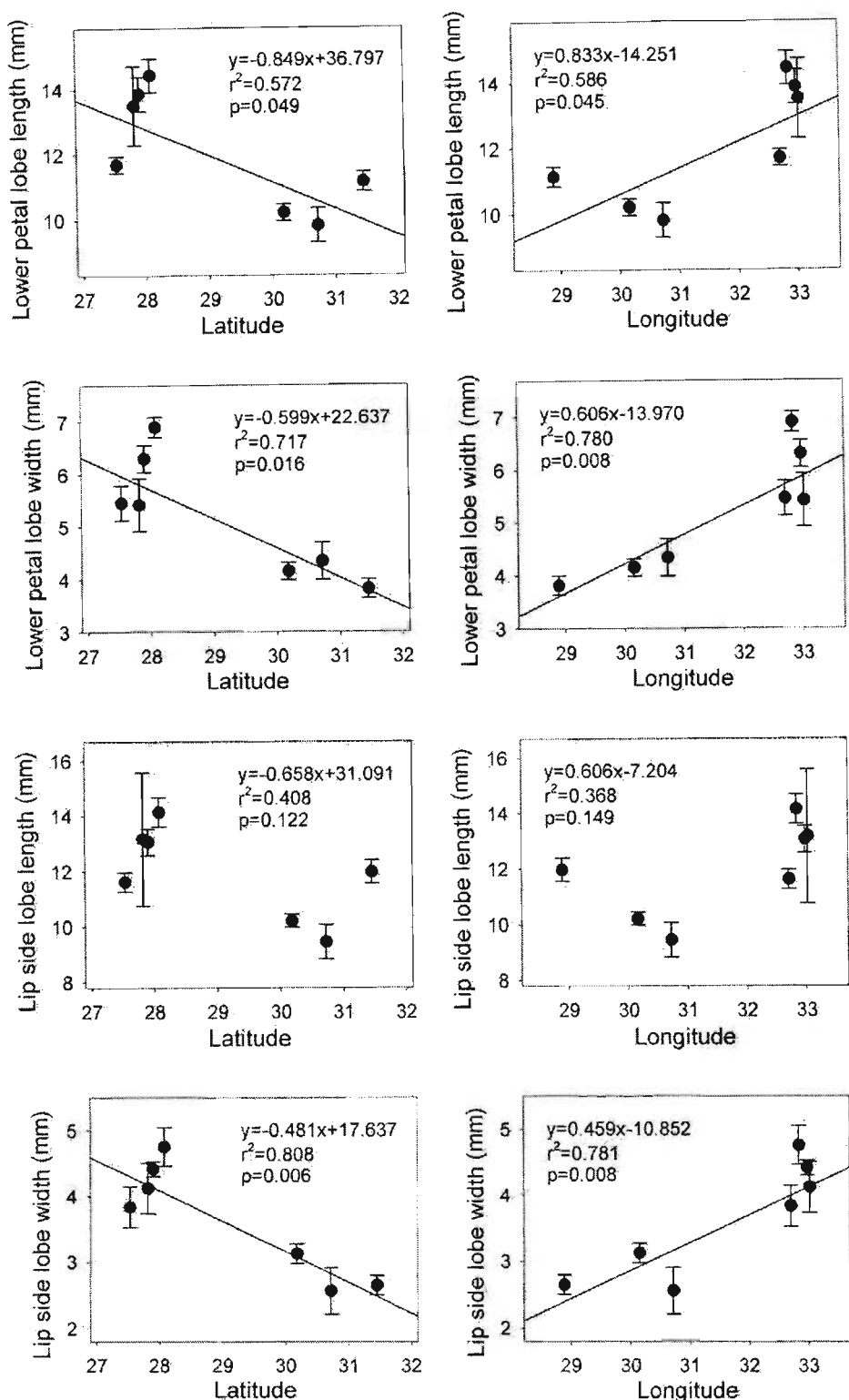
**Figure 3.** Relationships between floral traits and latitude and longitude for herbarium collections of populations belonging to the *Bonatea cassidea* complex.

**Population differentiation**

Data obtained by measuring plants from populations in the field (Figure 4) showed similar trends to those obtained from the herbarium material. There were significant changes against latitude and longitude for 'lower petal lobe width' and 'side lobe width', whilst 'lower petal lobe length' is just significant at the 5 % level (Figure 5).



**Figure 4.** Geographic distribution of the seven *Bonatea cassidea* populations studied.



**Figure 5.** Relationships between the dimensions of flowers and latitude and longitude in populations of the *Bonatea cassidea* complex (symbols represent the mean  $\pm$  standard error). Regressions are based on the mean values for each population.

A spatial separation of KwaZulu-Natal populations (solid symbols) and Eastern Cape populations (open symbols) is evident from the PCA scatter plot (Figure 6), with the individual populations showing weak clustering. Strong diagnostic characters contributing to the axis loading include 'lower petal lobe' and 'side lobe' length, as well as 'lower petal lobe' and 'side lobe' width (Table 5 and Figure 7). 'Mid-lobe length', although contributing strongly on both axes, is a poor diagnostic variable. Superimposed upon this morphological differentiation are subtle shifts in the distribution of floral pigments (Figure 7).

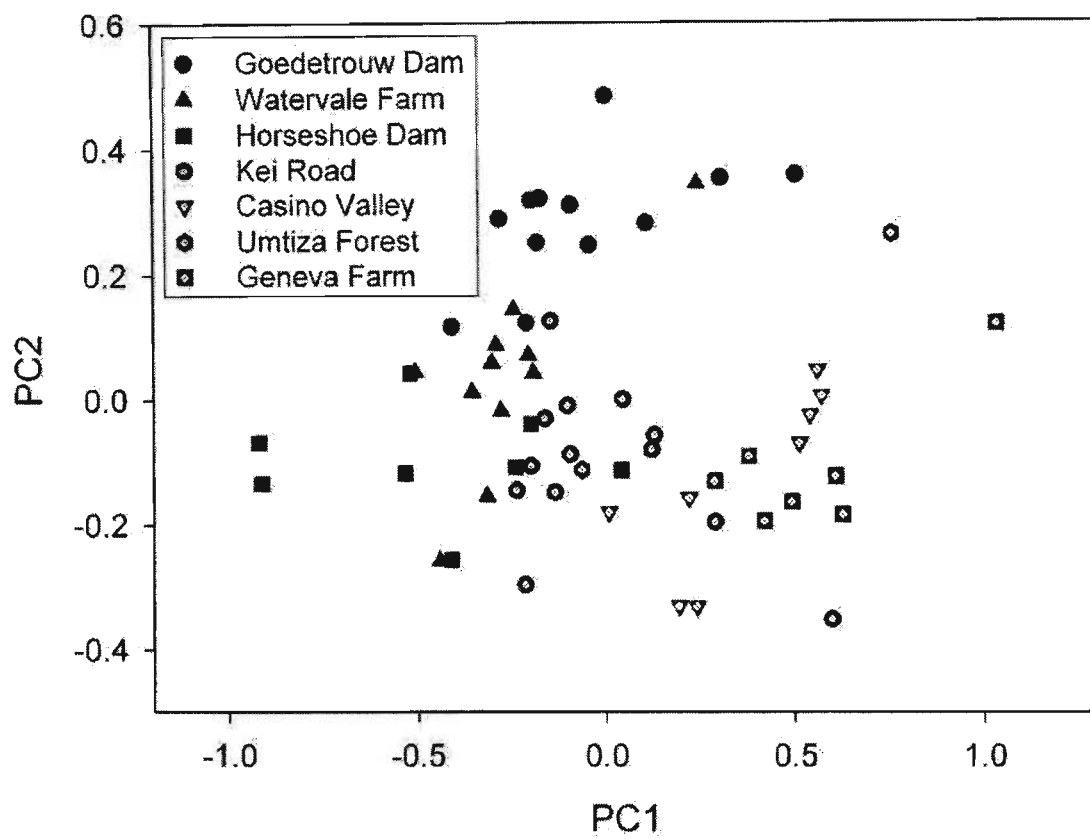
**Table 4.** Results of PCA on 6 variables of 61 specimens from the 7 *B. cassidea* populations. Eigenvalues, percentage of total variance explained by each axis and cumulative percentage along the first two axes.

Axis	Eigenvalues	Percentage of variance explained	Cumulative percentage of variance explained
1	9.492	72.69	72.69
2	2.339	17.91	90.60

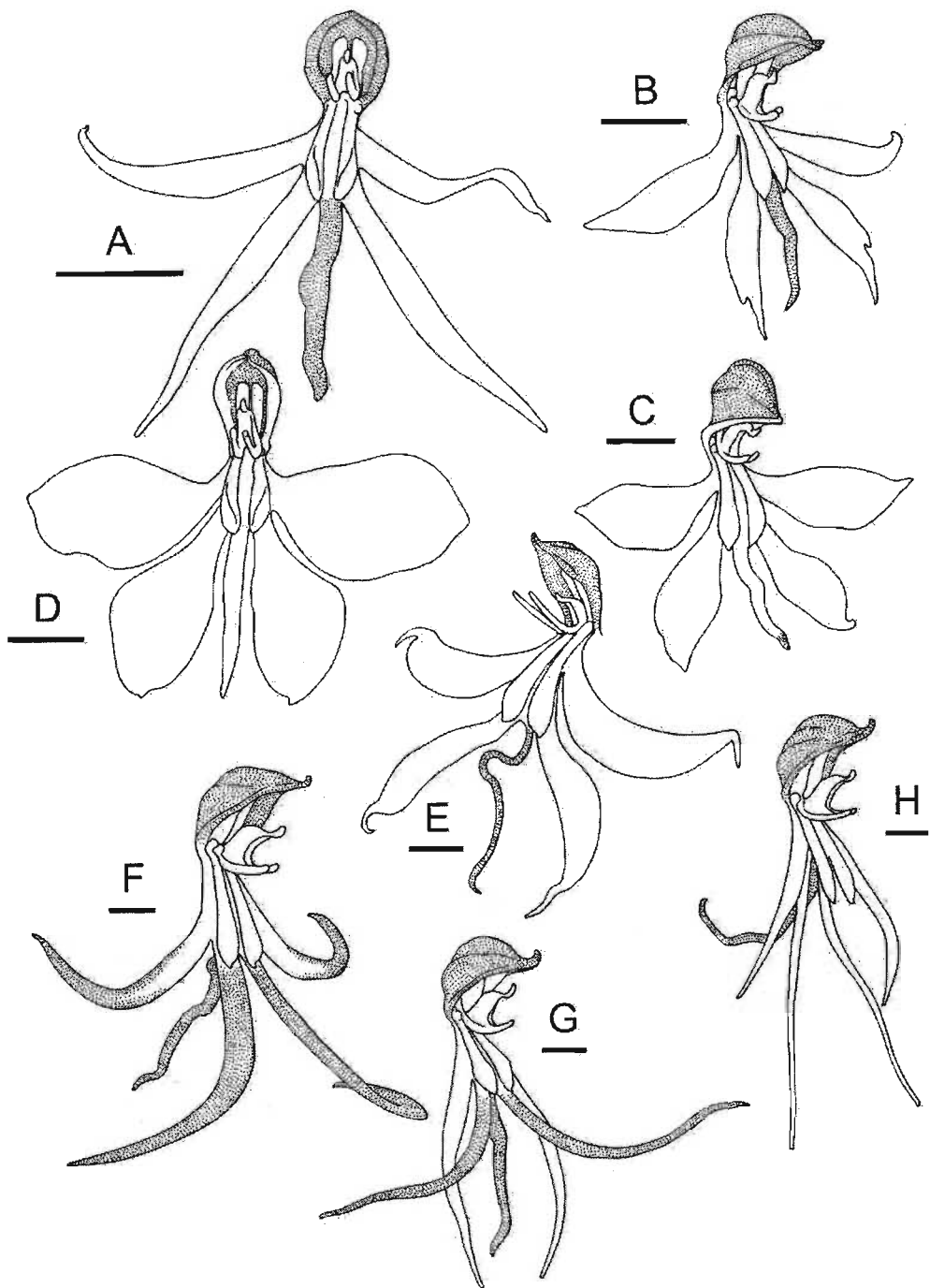
**Table 5.** PCA variable loadings. The 3 highest loadings for each axis are in bold.

Variable	Axis 1	Axis 2
Lower petal lobe length	<b>0.597</b>	-0.159
Lower petal lobe width	0.306	<b>-0.531</b>
Side lobe length	<b>0.621</b>	0.192
Side lobe width	0.254	<b>-0.362</b>
Mid-lobe length	<b>0.318</b>	<b>0.724</b>
Mid-lobe width	0.004	-0.003





**Figure 6.** Scatter plot of first two components from a PCA performed on 6 variables of 61 specimens from various *Bonatea cassidea* populations.



**Figure 7.** Morphological and colour variation for the *Bonatea cassidea* complex: Tugela River and Kosi Bay (A); Ixopo and Eshowe (B); Port Edward (C); East London (D); Umbilo River (E) (*Bonatea saundersiae*). Morphological and colour variation for *Bonatea saundersioides* (F-H). Small and large flower size variation is found for both *B. cassidea* and *B. saundersioides*. Flowers white, stipled areas green. Scale bar = 5 mm. Illustration A. J. Beaumont.

## DISCUSSION

*Bonatea cassidea* shows clinal variation in morphology across its distribution (Figure 3 and Figure 5). Woody vegetation is often disrupted in Africa due to discontinuity in suitable groundwater supply and to the prevalence of seasonal fire (White, 1983). *Bonatea cassidea* is a component of such communities and the patchy distribution of populations has contributed to the disruption of gene flow within the species. In the long term this may lead to significant morphological and genetic divergence which allows the recognition of distinct species. Our study reveals that ecotypic differentiation is occurring in northern and southern populations, but that these are linked through intermediate morphs that do not allow the recognition of infraspecific taxa. This range of morphological diversity requires consideration in conservation of the taxon, and necessitates protection of divergent populations to conserve the maximum range of genetic variation (Balfour and Linder, 1990; Goldstein *et al.*, 2005).

The clinal variation evident in the narrowing of petal segments in the flowers of *B. cassidea* reaches its climax in the north. This pattern is further developed in *B. saundersioides* (Figure 7); although, the latter taxon is unequivocally a species with longer and narrower lower petal lobes and side lobes (Figure 1) and a distribution largely allopatric to *B. cassidea* (Figure 8 and Figure 9). *Bonatea saundersiae*, on the other hand, is found to fall within the morphological range of *B. cassidea* (Figure 1 and Figure 7) and is reduced to synonymy. This 'incipient' taxon was restricted to the Umbilo River in KwaZulu-Natal (Figure 8) and appears extinct, with only three collections made during the mid nineteenth and early twentieth century. We revisited the area but were unable to trace populations.

## TAXONOMIC TREATMENT

***Bonatea cassidea*** Sond. in Linnaea 19: 81. (1847); Stewart *et al.* in Wild Orch. South. Africa: 101, pl. 10.6 (1982); la Croix & Cribb in Flora Zambesiaca, Orchidaceae 11: 46 (1995); la Croix & la Croix in African orchids in the wild and in cultivation: 155 (1997); Schelpe & Linder in Orchids of southern Africa: 141 (1999). Ill.: L.Bolus in Fl. Pl. South Africa, t. 302 (1928). *Habenaria cassidea* (Sond.) Rchb.f. in Ann. Bot. Syst. 1: 797 (1849); Bolus in J. Linn. Soc., Bot. 19: 340 (1882); Brown in Gard. Chron. 24: 307 (1885); Kraenzl. in Bot. Jahrb. Syst. 16: 80 (1893). Type: South Africa, [Eastern Cape], Uitenhage, Olifantshoek near Bushmans River, September, *Ecklon and Zeyher s.n.* (S!, holo.).

*Bonatea darwinii* Weale in J. Linn. Soc., Bot. 10: 470 (1869). Type: South Africa, [Eastern Cape], Bedford, Kagaberg, *Weale s.n.* (PRE!, lecto., selected here; SAM!, isolecto.); South Africa, [Eastern Cape], Bedford, shady places, *Weale s.n.* (BOL!, GRA!, syn.).

*Habenaria saundersiae* Harv. in Thes. Cap. 2: 29, fig. 147 (1863); Bolus in J. Linn. Soc., Bot. 19: 340 (1882); Kraenzl. in Bot. Jahrb. Syst. 16: 57-58 (1893), syn. nov. *Bonatea saundersiae* (Harv.) T.Durand & Schinz in Consp. Fl. Afr. 5: 89 (1895); Stewart *et al.* in Wild Orch. South. Africa: 101 (1982); Schelpe & Linder in Orchids of southern Africa: 141 (1999). Type: South Africa, [KwaZulu-Natal], [Durban], without precise locality or date, *Saunders sub Wheelwright s.n.* (TCD!, holo.).

### Description

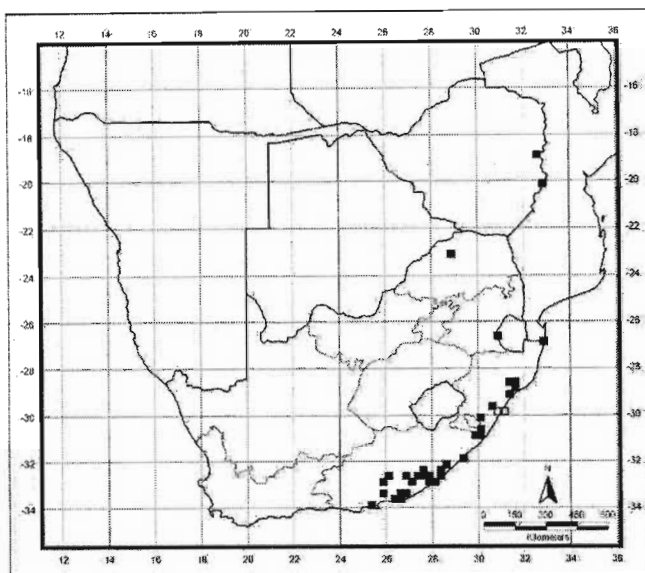
Erect, decumbent or scandent terrestrial herbs, 100–640 mm tall; *tubers* 1–8, spreading, elongate-oblong, tomentose. *Stem* slender, 95–630 × 1.5–5.0 mm. *Leaves* sometimes withered at flowering, 5–13, lax, cauline, lower leaves intermediately basal, sheathing, narrowly-

obovate to oblanceolate, acute to acuminate, margins entire, rarely undulate, 20–250 × 4–19 mm. *Inflorescence* cylindrical, 40–212 × 34–64(–80) mm, lax to dense, 3–35-flowered; *bracts* sometimes withered at flowering, similar to upper leaves, sheathing, linear-oblong to ovate-lanceolate, apiculate, 10–25 × 2.5–7.5 mm. *Flowers* spreading, green and white, pedicel and ovary sub-erect to erect, 14–32 mm long. *Dorsal sepal* green, sub-erect to erect, elliptical-oblong, galeate, acute to acuminate, 7.0–13.5 × 3–5 mm; *lateral sepals* green, patent, strongly deflexed, oblong-lanceolate to ovate, acute to apiculate, base oblique, margins weakly to strongly revolute with small acute tooth on lower margin, 7.0–15.5 × 2.0–6.5 mm, adnate to lip and stigmatic processes for 3–6 mm. *Petals* upper lobe green or white, erect, linear, acute to acuminate, 6–14 × 0.5–2.0 mm, adnate to dorsal sepal, lower lobe white, spreading horizontally, weakly to strongly deflexed, falcate, lanceolate to widely-obovate, tip curved, acute to apiculate, 8.5–15.0(–19.0) × (1.5–)3.0–8.0 mm, adnate to claw of lip for 1–5 mm. *Lip* claw 3–9 mm, median lobe green or white, descending, weakly to strongly geniculate, filiform to linear, acute, 5.5–17.0 × 0.5–2.0 mm, lateral lobes white, descending, diverging, falcate, narrowly to broadly-oblanceolate, tips curved, apiculate, 6.5–21.0 × 1.5–6.5 mm; spur toothed, curved, 12–26 mm long, clavate apex 8–17 mm long. *Anther* erect, acute, 2.5–5.5 mm long, canals slender, 3.5–5.5(–9.5) mm long. *Stigmatic processes* clavate, projecting forwards and deflexed, weakly divergent, 3.5–7.0(–10.5) mm long, base adnate to lip. *Rostellum* 3-lobed, base auriculate, median lobe narrowly-cucullate, apiculate, margin ciliate, 3.0–5.5 mm long, lateral lobes linear, fused to anther canals, sub-erect to 45°, sub-parallel, 0.5–2.0(–6.5) mm long. Flowering period: July to September.

## Habitat

*Acacia* savannah, forest and thicket, from 60 m to 1500 m altitude. *Bonatea cassidea* occurs consistently in open shady niches often in close proximity to water.

## Distribution



**Figure 8.** Known distribution of *Bonatea cassidea*. Hollow squares represent populations previously segregated as *Bonatea saundersiae*.

*Bonatea cassidea* ranges from Port Elizabeth in the Eastern Cape along the coast and near interior of South Africa as far north as Kosi Bay, with scattered records in Swaziland, Limpopo and the eastern highlands of Zimbabwe (Figure 8).

## Observations

Populations of *B. cassidea* show a gradient in morphology from the Eastern Cape through to northern KwaZulu-Natal - with the mid-lobe and upper petal lobes changing from white to green, and the lower petal lobes and side lobes decreasing in width. Little is known about pollinators of this species, except for remarks by Weale (1869) regarding visits by the Skipper Butterfly *Gomalia elma* Trimen.

### **Specimens examined**

*Bonatea cassidea*: McMurtry 2519 (Hortus Siccus McMurtrianus); Wells 1598 (NU; PRE); Abbott 7001 (NH); O'Connor 32 (NU); Nicholson 1762 (PRE; Umtamvuna Herbarium); McMurtry 2489 (Hortus Siccus McMurtrianus); Germishuizen 1212 (PRE); Weale s.n. (BOL; GRA); Weale s.n. (PRE; SAM); Giffen 86 (PRE); Holmes s.n. (GRA); Watson 3 (PRE); Johnston s.n. (NBG); Ranger 53 (PRE); Ranger s.n. (GRA); Pegler 780 (GRA; SAM); Flanagan 857 (NU; PRE; SAM); Howe s.n. (GRA); Crundall s.n. (PRE); Acocks 23917 (PRE); Wells 3071 (PRE); Acocks 16128 (PRE); MacOwan 1529 (PRE; SAM); Dodd s.n. (GRA); Dodd 7935 (GRA; PRE); Galpin 6534 (PRE); Dodd 25800 (PRE); McMurtry 929 (Hortus Siccus McMurtrianus); Venter 1084 (PRE); Lawn 1570 (NH); Lawn 1619 (NH). *Bonatea saundersiae*: without collector NH 12392 (NH); Sanderson 822 (NH); Wheelwright s.n. (TCD).

***Bonatea saundersioides*** (Kraenzl. & Schltr.) Cortesi in Ann. Bot. (Rome) 2: 363 (1905); Stewart *et al.* in Wild Orch. South. Africa: 100, pl. 10.5 (1982); la Croix & la Croix in African orchids in the wild and in cultivation: 156 (1997); Schelpe & Linder in Orchids of southern Africa: 140 (1999). *Habenaria saundersioides* Kraenzl. & Schltr. in Orchid. Gen. Sp. 1, 3: 181 (1897); Schlechter in Ann. Transvaal Mus. 10: 245 (1924). *Bonatea saundersioides* (Kraenzl. & Schltr.) Rolfe in Fl. Cap. 5, 3: 140 (1913), nom. illegit. superfl. Type: South Africa, [Mpumalanga], Barberton, Umvoti Creek, 3000 ft, April–May, *Culver 30* (K!, holo.; BOL!, iso.).

*Habenaria umvotensis* Rolfe in Fl. Cap. 5, 3: 133 (1913). Type: South Africa, [Mpumalanga], Barberton, Umvoti Creek, 3000 ft, April–May, *Culver 30* (BOL!, K!, syn.); South Africa, [Mpumalanga], Barberton, Umvoti Creek, shady places amongst rocks in wooded ravines, 3000 ft, May–June, *Galpin 954* (BOL!, K!, PRE!, syn.).

## Description

Erect, decumbent or scandent terrestrial herbs, 200–600 mm tall; *tubers* 1–8, spreading, elongate-oblong, tomentose. *Stem* slender to sturdy, 195–595 × 2.5–8.5 mm. *Leaves* sometimes withered at flowering, 5–12, lax, cauline, lower leaves intermediately basal, sheathing, lanceolate to narrowly-elliptical, acute to apiculate, margins entire, smooth, 54–193 × 7–28 mm. *Inflorescence* cylindrical, 65–262 × 52–95 mm, lax to dense, 5–41-flowered; *bracts* sometimes withered at flowering, similar to upper leaves, sheathing, ovate-lanceolate, apiculate, 17.5–30.5 × 4.0–8.5 mm. *Flowers* spreading, green and white, pedicel with ovary projecting forwards to sub-erect, 16–29 mm long. *Dorsal sepal* green, sub-erect, elliptical-oblong, galeate, acute to acuminate, 8.5–15.0 × 3.0–5.5 mm; *lateral sepals* green, patent, strongly deflexed, ovate-lanceolate, acuminate to apiculate, base oblique, margins weakly to strongly revolute with small acute tooth on lower margin, 10–15 × 3.5–6.5 mm, adnate to lip and stigmatic processes for 2.5–5.5 mm. *Petals* upper lobe green, erect, linear, acute to acuminate, 8–14 × 0.5–1.5 mm, adnate to dorsal sepal, lower lobe white, spreading horizontally, strongly deflexed, weakly to strongly falcate, linear to linear-lanceolate, tip curved, acuminate to apiculate, (13–)16–24 × 0.5–2.0(–3.0) mm, adnate to claw of lip for 2.5–4.5 mm. *Lip* claw 4.5–9.0 mm, median lobe green, descending, geniculate, sometimes apically recurved, linear, acute, 8.5–20.0 × 0.5–1.5 mm, lateral lobes green to white, descending, diverging, weakly to strongly falcate, linear, tips curved, acuminate to apiculate, 14–34 × 0.5–2.0 mm; spur toothed, curved, 16–30 mm long, clavate apex 9.5–24.0 mm long. *Anther* erect, acute, 3.0–5.5 mm long, canals slender, 5.0–8.0 mm long. *Stigmatic processes* clavate, projecting forwards and deflexed, weakly divergent, 4.5–9.0 mm long, base adnate to lip. *Rostellum* 3-lobed, base auriculate, median lobe narrowly-cucullate, apiculate, margin ciliate, 4.0–6.5 mm long, lateral lobes linear, fused to

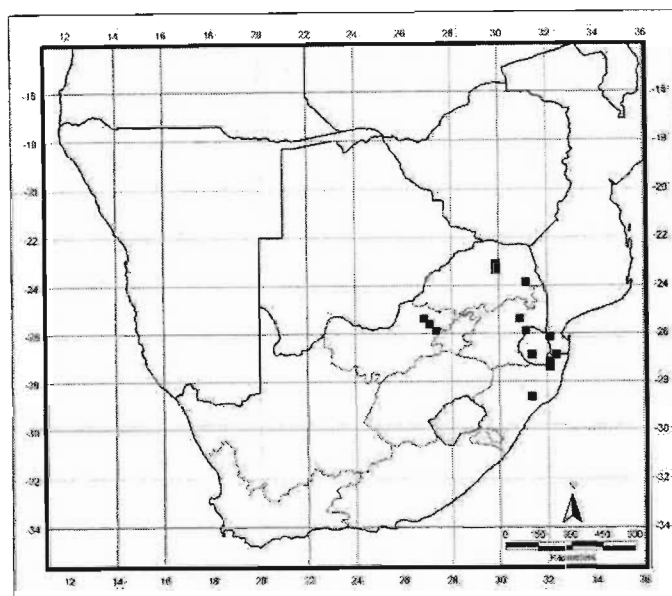


anther canals, projecting forwards to sub-erect 45°, sub-parallel, 1.0–2.5 mm long. Flowering period: rarely March to May, June to August.

### Habitat

Savannah and forest, at near sea level to 1500 m altitude.

### Distribution



**Figure 9.** Known distribution of *Bonatea saundersioides*.

*Bonatea saundersioides* is restricted to the north-eastern portion of southern Africa, occurring in KwaZulu-Natal, Mpumalanga, North-West, Limpopo Provinces and Swaziland (Figure 9).

### Observations

Colour variation occurs in the perianth of *B. saundersioides* with either green or white labellum side lobes. *Bonatea saundersioides* and sister taxa *Bonatea cassidea* Sond. are closely allied. The taxa have comparable rostellum, stigmatic process and spur structure, but *B. saundersioides* differs in its longer and narrower lower petal lobes and labellum side lobes. The species may extend into Mozambique, but the area is poorly botanized.

**Specimens examined**

Bartlett s.n. (J); Botha s.n. (PRE); Bartlett s.n. (J; PRE); McMurtry 1209 (Hortus Siccus McMurtrianus); Galpin 954 (PRE); Compton 28930 (PRE); Bayliss 3485 (PRE); Bayliss B/S6 (PRE); Pooley 633 (NU); O'Connor 511 (NU).

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## CHAPTER 5

### A revision of *Bonatea* (Orchidaceae)

#### ABSTRACT

A taxonomic revision and key to species of *Bonatea* Willd. is presented. Fourteen species are recognized, ten of which are found in South Africa. The genus has a distribution range that extends from southern Africa up the east African seaboard into Yemen. Full descriptions are provided with diagnostic characters and distribution maps. *Bonatea eminii* (Kraenzl.) Rolfe is known from a single collection destroyed in Berlin. It appears closely allied to *Bonatea steudneri* (Rchb.f.) T.Durand & Schinz but is excluded from the revision due to insufficient information.

#### INTRODUCTION

The genus *Bonatea* Willd. was named in honour of Guiseppe Antonio Bonato (Willdenow, 1805) and is a member of the subtribe Habernariinae (Orchidaceae). *Bonatea* consists of 14 species that are found mainly in forest and savannah biomes. The genus is most diverse on the eastern seaboard of southern Africa, extending through east Africa into Sudan, with one species in Yemen.

*Bonatea* is closely related to *Habenaria* but differs in the elongate-rostrate or galeate middle rostellum lobe that is clearly separated from the vertical anther thecae (Chapter 2). Many of the floral characters previously used to delimit *Bonatea*, such as clavate stigmas and the basal fusion of the lip with the lateral sepals, the anterior lobe of the petals and the stigmatic processes (Rolfe, 1898; Rolfe, 1913; Summerhayes, 1949; Williamson, 1977; Stewart, 1996; la Croix and la Croix, 1997; Schelpe and Linder, 1999) are not unique

synapomorphies. These characters occur sporadically in *Habenaria* but our sample of *Habenaria* was too limited to fully understand their origin. The presence of a tooth in the spur mouth appears distinct to *Bonatea* (albeit lost in *Bonatea rabaiensis* Rendle), being distal to the spur throat and projecting at the split of the lip in closely related *Habenaria* (ie. *H. bonateoides* M.Ponsie, *H. arenaria* Lindl. and *H. malacophylla* Rchb.f.). It has been suggested that the genus is merely a specialized section of *Habenaria* (Kurzweil and Weber, 1992), and that retaining *Bonatea* as a distinct genus may render *Habenaria* paraphyletic (Schelpe and Linder, 1999). Higher level phylogenetics of the Habenariinae are being explored (Dressler, 1993; Bateman *et al.*, 2003); however, only a broad consideration of *Habenaria* will resolve the circumscription and taxonomic status of *Bonatea*.

Recent descriptions of southern African (Schelpe and Linder, 1999) and east African (Summerhayes, 1968) members of *Bonatea*, account for 15 species, one of which includes two varieties. Taxonomic changes for *Bonatea* (Chapter 2, Chapter 3 and Chapter 4) necessitate a revision of the genus that provides detailed descriptions of all southern African and east African species. *Bonatea eminii* (Kraenzl.) Rolfe is not included due to insufficient information. The species is known from a single collection (Type: Dodoma, Uyansi, Ipunguli (Pungusi), in the Mgunda-Mkali, 24 km SW of Itigi on the central Tanzanian railway, *Stuhlmann 420* (B†, holo.)) which was destroyed during the bombing of Berlin in World War II. It is clear from the original description that the entity is closely allied to, if not conspecific with, *Bonatea steudneri* (Rchb.f.) T.Durand & Schinz. However, insufficient data is available to allow certainty of its identity.



*Bonatea* Willd. in Sp. Pl. 4: 43 (1805); Rolfe in Fl. Trop. Afr. 7: 252 (1898); Rolfe in Fl. Cap. 5: 138 (1913); Schelpe in An introduction to the South African orchids: 74 (1966); Summerhayes in Fl. Trop. E. Afr., Orchid.: 136 (1968); Williamson in The Orchids of S. Centr. Afr.: 68 (1977); Stewart *et al.* in Wild Orch. South. Africa: 97 (1982); la Croix & Cribb in Flora Zambesiaca, Orchidaceae 11: 43 (1995); Stewart in Orchids of Kenya: 92 (1996); Cribb & Thomas in Flora of Ethiopia and Eritrea: 231 (1997); la Croix & la Croix in African orchids in the wild and in cultivation: 153 (1997); Schelpe & Linder in Orchids of southern Africa: 136 (1999). Type species: *Bonatea speciosa* (L.f.) Willd.

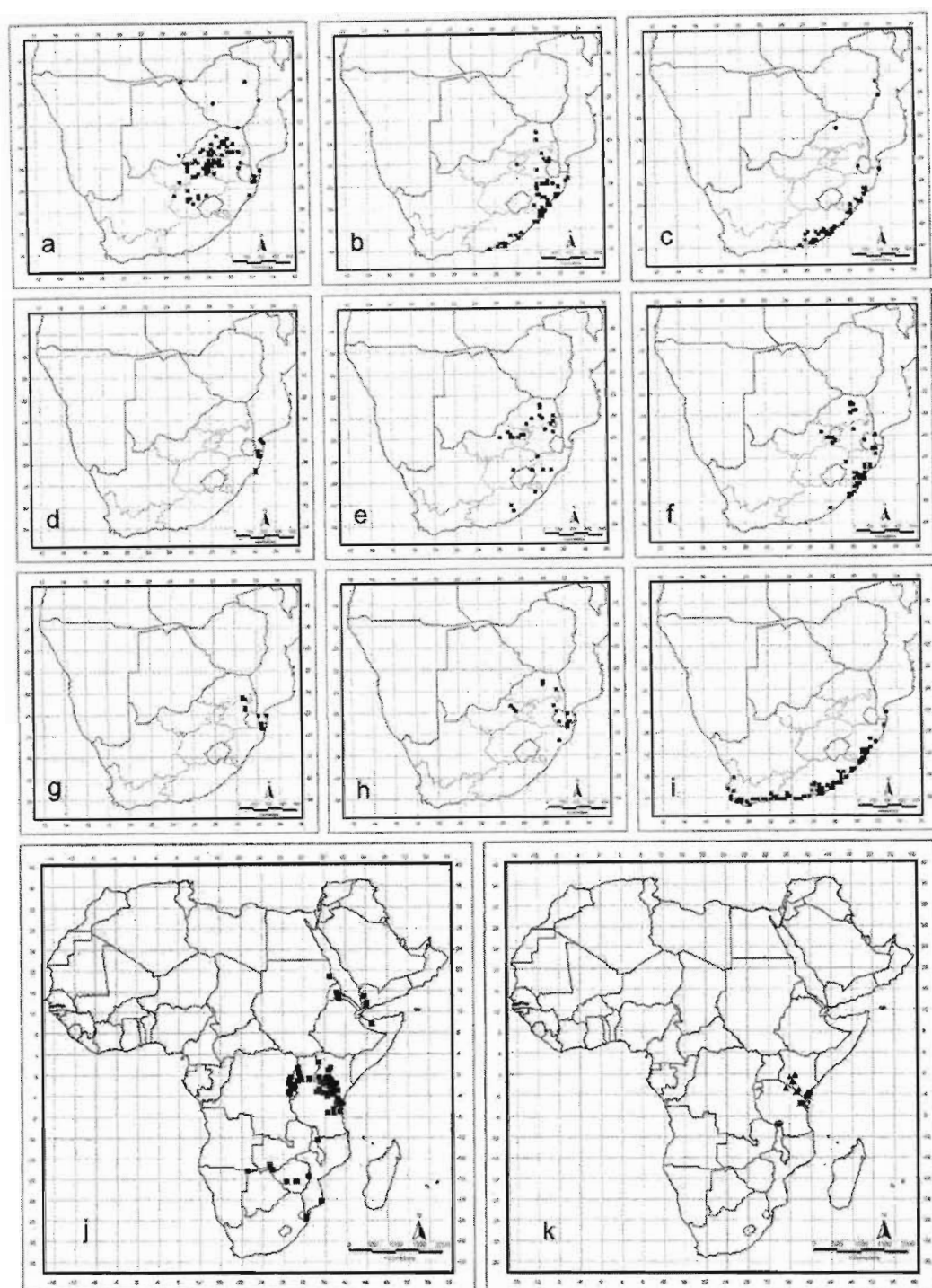
*Habenaria* Willd. sect. *Bonatea* (Willd.) Kraenzl. in Bot. Jahrb. Syst. 16: 56 (1893). Type species: *Habenaria bonatea* Rchb.f., nom. illegit. superfl. (= *Bonatea speciosa* (L.f.) Willd.).

Erect, decumbent or scandent terrestrial herbs; tubers multiple, clustered, elongate-oblong, tomentose; stem unbranched, slender to robust. Leaves sheathing, cauline or basal, oblanceolate or obovate to broadly-elliptical, sometimes withered at flowering. Inflorescence terminal, elongate, racemose, 2- to many-flowered, lax to dense; bracts ovate to lanceolate, sheathing. Flowers green and white (rarely greenish yellow), resupinate; sepals unequal, free, dorsal sepal galeate, lateral sepals oblique, reflexed, united basally with lower petal lobes, lip and stigmatic processes; petals deeply bipartite, upper petal lobe erect, linear, generally adpressed to margin of dorsal sepal to form a hood, lower petal lobe linear to broadly oblanceolate, adnate to base of lip and stigmatic arms; lip adnate to base of column, produced at base into descending spur, usually with tooth at mouth (absent in *B. rabaiensis*), tripartite from short, narrow base, lateral lobes linear to broadly oblanceolate, median lobe shorter than lateral lobes, filiform to narrowly-oblong, spur short or long, cylindrical, apex clavate. Column

short. *Anther* erect, loculi slightly divergent, canals usually elongate, adnate to lateral lobes of rostellum; staminodes lateral, small, auriculate, rugulose; pollinaria 2, pollinia sectile, caudicles long, slender, viscidia small, discoid, terminal. *Stigmatic processes* 2, elongate, adnate to base of lip, clavate to spatulate; *rostellum* 3-lobed, median lobe narrowly-cucullate to rostrate, lying well forward of anthers, lateral lobes well-developed. *Capsules* oblong to obovate.

## KEY TO SPECIES OF *BONATEA*

- 1a. Leaves in a basal rosette..... (2)
- 1b. Leaves cauline, not in a basal rosette..... (3)
- 2a. Spur 20–47 mm long ..... 2. *B. polypodantha*
- 2b. Spur 53–70 mm long ..... 3. *B. pulchella*
- 3a. Spur > 74 mm ..... (4)
- 3b. Spur < 69 mm ..... (5)
- 4a. Lip lateral lobes 35–60 mm long..... 5. *B. steudneri*
- 4b. Lip lateral lobes 106–135 mm long..... 6. *B. lamprophylla*
- 5a. Leaves withered at flowering time ..... (6)
- 5b. Leaves not withered at flowering time ..... (7)
- 6a. Lip median lobe straight; rostellum elongate-rostrate ..... 1. *B. stereophylla*
- 6b. Lip median lobe geniculate; rostellum cucullate..... 7. *B. porrecta*
- 7a. Tooth lacking in spur mouth..... 4. *B. rabaiensis*
- 7b. Tooth present in spur mouth..... (8)
- 8a. Rostellum lateral lobes longer than median lobe ..... 8. *B. volkensiana*
- 8b. Rostellum lateral lobes shorter than median lobe..... (9)
- 9a. Leaves linear-lanceolate; rostellum median lobe narrowly cucullate;  
bracts dry at anthesis ..... (10)
- 9b. Leaves ovate, oblong or broadly lanceolate; rostellum median  
lobe cucullate; bracts green at anthesis ..... (11)
- 10a. Lower petal lobes (13–)16–24 × 0.5–2.0(–3.0) mm..... 12. *B. saundersioides*
- 10b. Lower petal lobes 8–15(–19) × (1.5–)3.0–8.0 mm ..... 13. *B. cassidea*
- 11a. Clavate apex of spur > 15 mm long; lower petal lobe > 2mm wide;  
lower petal lobes projecting forwards and deflexed ..... 11. *B. speciosa*
- 11b. Clavate apex of spur < 15 mm long; lower petal lobe < 2mm wide..... (12)
- 12a. Lower petal lobe (18–)24–43 × 0.5–2.0 mm,  
projecting forwards and ascending;  
stigmatic processes (11–)15–22 mm long ..... 9. *B. antennifera*
- 12b. Lower petal lobes 11–24(–28) × 0.5–2.0(–2.5) mm,  
spreading horizontally;  
stigmatic processes 8–15 mm long ..... 10. *B. boltonii*



**Figure 1.** Known distribution of *Bonatea antennifera* (A), *Bonatea boltonii* (B), *Bonatea cassidea* (C), *Bonatea lamprophylla* (D), *Bonatea polypodantha* (E), *Bonatea porrecta* (F), *Bonatea pulchella* (G), *Bonatea saundersioides* (H), *Bonatea speciosa* (I), *Bonatea steudneri* (J), *Bonatea volkensiana* (K: ▲), *Bonatea rabaiensis* (K: ■), *Bonatea stereophylla* (K: ●).

## TAXONOMIC TREATMENT

**1. *Bonatea stereophylla*** (Kraenzl.) Summerh. in Kew Bull. 4: 430 (1949); Summerhayes in Fl. Trop. E. Afr.: 140 (1968). *Habenaria stereophylla* Kraenzl. in Bot. Jahrb. Syst. 30: 280 (1901b); Kraenzlin in Orchid. Gen. Sp. 1, 15: 950 (1901a). Type: Tanzania, Mt. Rungwe, Umuamba, flat slopes of the Kiwira Valley, 1500 m, Goetze 1339 (B†, holo.).

*Habenaria polychlamys* Schltr. in Bot. Jahrb. Syst. 53: 508 (1915). Type: Tanzania, Njombe District, Tandala, Ukinga mountains, on mountain meadows, 2100 m, April 1913, Stolz 2242 (B†, holo.; K!, iso.).

### Description

Erect, decumbent or scandent terrestrial herbs, 500–700 mm tall; *tubers* 2, spreading, elongate-oblong, tomentose. *Stem* robust, 490–695 × 8–10 mm. *Leaves* withered at flowering, 15–20, dense, lower sheathing, oblong, acute, 50–60 mm broad. *Inflorescence* cylindrical, 150 × 80 mm, dense, 10–20-flowered; *bracts* sometimes withered at flowering, lanceolate to ovate-lanceolate, acuminate, 30–40 mm long. *Flowers* spreading, greenish yellow, pedicel and ovary sub-erect, 30–35 mm long. *Dorsal sepal* green, erect, elliptical-lanceolate, galeate, acuminate, 20–25 × 8–10 mm; *lateral sepals* green, patent, decurved, narrowly-triangular, acuminate to apiculate, base oblique, 20 × 4 mm, adnate to lip and stigmatic processes for 3–4 mm. *Petal* upper lobe erect, linear, acute to acuminate, 18–20 × 1.5–2.0 mm, adnate to dorsal sepal, lower lobe weakly falcate, linear, acuminate, 17–27 × 1 mm, adnate to claw of lip for 2–3 mm. *Lip* claw 4.5–6.0 mm, median lobe descending, straight, linear, acute to acuminate, 15–21 × 1 mm, lateral lobes descending, linear, tips curved, apiculate, 21–25 × 1 mm;

spur toothed, curved, 28–35 mm long, clavate apex 18–25 mm long. *Anther* erect, acute, 12 mm long, canals slender, 8–10 mm long. *Stigmatic processes* clavate, projecting forwards and sometimes deflexed, 15–16 mm long, base adnate to lip. *Rostellum* 3-lobed, base auriculate, median lobe elongate-rostrate, acute, margin smooth, 8–11 mm long, lateral lobes linear, fused to anther canals, sub-erect to 45°, sub-parallel or slightly divergent, 4–6 mm long. Flowering period: April.

### **Habitat**

Grassland, to approximately 2100 m altitude.

### **Distribution**

*Bonatea stereophylla* is restricted to Tanzania (Figure 3K).

### **Observations**

*Bonatea stereophylla* is a rare species, known from only two collections. This deciduous species has a large distinctive tooth (> 5 mm long) in the spur mouth, an elongate-rostrate rostellum mid-lobe, straight labellum mid-lobe, narrowly triangular lateral sepals, and flowers described as yellowy green (Schlechter, 1915). Morphology suggests the closest allies are *B. polypodantha* (Rchb.f.) L.Bolus and *B. pulchella* Summerh.

### **Specimens examined**

Stolz 2242 (K).

**2. *Bonatea polypodantha*** (Rchb.f.) L.Bolus in Fl. Pl. South Africa 8: sub t. 302 (1928); Stewart *et al.* in Wild Orch. South. Africa: 102, pl. 10.8 (1982); la Croix & la Croix in African orchids in the wild and in cultivation: 155 (1997); Schelpe & Linder in Orchids of southern Africa: 142 (1999). Ill.: McDonald in Fl. Pl. Africa, t. 2072 (1993). *Habenaria*

*polypodantha* Rchb.f. in Otia Bot. Hamburg. 2: 97 (1881); Bolus in J. Linn. Soc., Bot. 19: 340 (1882); Kraenzl. in Bot. Jahrb. Syst. 16: 70 (1893); Rolfe in Fl. Cap. 5, 3: 127 (1913). Ill.: Bolus in Ic. Orch. Austr. Afr. 3: t. 23 (1913). Type: South Africa, [KwaZulu-Natal], without precise locality or date, *Gerrard 1554* (K!, holo.; BOL!, iso.).

*Bonatea insignis* (Schltr.) Summerh. in Kew Bull. 7: 463 (1951). *Habenaria insignis* Schltr. in Bot. Jahrb. Syst. 20, Beibl. 50: 1, 32 (1895); Rolfe in Fl. Cap. 5, 3: 127 (1913). Type: South Africa, [Limpopo], Maila's Kop, 2400–2500 ft, 16 February 1894, *Schlechter 4517* (B†, holo.; BOL!, PRE!, iso.).

### **Description**

Erect, decumbent or scandent terrestrial herbs, 44–330 mm tall; *tubers* 1–3, spreading, elongate-oblong, tomentose. *Stem* slender, 34–325 × 1.5–3.5 mm. *Leaves* 4–8, margins entire, smooth; basal leaves 1–3, dense, sheathing, lanceolate to broadly-elliptical, acute, 21–157 × 8–36 mm, middle leaves 2–5, lax, cauline, narrowly-lanceolate to lanceolate, acuminate, 11–29 × 3–12 mm. *Inflorescence* cylindrical, 42–172 × 39–95 mm, lax, 2–12-flowered; *bracts* similar to upper leaves, sheathing, lanceolate, acuminate to apiculate, 9.0–30.5 × 2–6 mm. *Flowers* spreading, pale green and white, pedicel and ovary sub-erect to erect, 19–36 mm long. *Dorsal sepal* pale green, erect, narrowly-elliptical, galeate, acute, 7–11 × 2.5–5.0 mm; *lateral sepals* pale green to white, patent, ovate, acuminate to apiculate, base oblique, 6–10 × 3.5–7.0 mm, adnate to lip and stigmatic processes for 2–5 mm. *Petals* pale green to white, upper lobe erect, linear, acute to acuminate, 7.0–12.0 × 0.5–1.5 mm, adnate to dorsal sepal, lower lobe spreading horizontally, weakly deflexed, linear-falcate, tip curved, acuminate to apiculate, 22.5–35.5 × 0.5–1.0 mm, adnate to claw of lip for 1.5–5.0 mm. *Lip* pale green to white, claw 3.5–8.5 mm, median lobe descending, curved forward, linear, acute to acuminate, 11–22 ×

0.5–1.5 mm, lateral lobes descending, strongly divergent, linear-falcate, tips curved, acuminate to apiculate, 25–48 × 0.5–1.5 mm; spur toothed, (20–)31–47 mm long, clavate apex 10.5–23.0 mm long, sometimes bent forward. *Anther* erect, acute, 3.0–4.5 mm long, canals slender, 4.0–7.5 mm long. *Stigmatic processes* clavate, projecting forwards and deflexed, sub-parallel, 6–11 mm long, base adnate to lip. *Rostellum* 3-lobed, base auriculate, median lobe narrowly triangular-rostrate, acuminate, margin smooth, 2.5–6.5 mm long, lateral lobes linear, fused to anther canals, sub-erect to 45°, sub-parallel, 2–5 mm long. Flowering period: January to April.

### **Habitat**

Grassland, savannah, forest and thicket, to 2000 m altitude.

### **Distribution**

*Bonatea polypodantha* is restricted to South Africa where it is widespread in the north-eastern part of the country in the Eastern Cape, KwaZulu-Natal, Free State, North-West, Gauteng, Mpumalanga and Limpopo (Figure 3E).

### **Observations**

*Bonatea polypodantha* and *B. pulchella* are distinct from other *Bonatea* species in their basal leaves and middle rostellum lobe that is narrowly-triangulate rostrate. *Bonatea polypodantha* differs from *B. pulchella* in its smaller stature and shorter spurs (< 50 mm).

### **Specimens examined**

Raal 259 (LYD; PRE); Venter s.n. (PRE); Venter 12400 (UNIN); Balkwill 4422 (J); Burrows 3497 (J); Ubbink 158 (PUC); Buys 436 (PRE; PUC); Repton 3760 (PRE); McMurtry 1211 (Hortus Siccus McMurtrianus); Kroker s.n. (J); Venter 615 (PRE); Mogg 34056 (PRE); McMurtry 4378 (Hortus Siccus McMurtrianus); McMurtry 5435 (Hortus Siccus



McMurtrianus); Rodin 3897 (PRE); Mogg s.n. (PRE); Schweickhardt 1061 (PRE); Vogts s.n. (PRE); van Rooyen 3444 (PRU); Meeuse 10032 (PRE); Breyer 13843 (PRE); du Preez s.n. (NBG); Edwards 1626 (NU); Incken 27 (NH); Acocks 22114 (PRE); Galpin 8180 (PRE); Gerrard 1554 (BOL; K); Schlechter 4517 (PRE).

**3. *Bonatea pulchella*** Summerh. in Kew Bull. 17: 529, fig. 9 (1964); Stewart *et al.* in Wild Orch. South. Africa: 102, pl. 10.9 (1982); la Croix & Cribb in Flora Zambesiaca, Orchidaceae 11: 46, fig. 16 (1995); la Croix & la Croix in African orchids in the wild and in cultivation: 156 (1997); Schelpe & Linder in Orchids of southern Africa: 142 (1999). Ill.: Schelpe in Fl. Pl. Africa, t. 1823 (1981). Type: Mozambique, [Maputo], Inhaca Island, Ponta Rasa, on calcareous rocks covered by dunes, in dense coastal forest, 10 July 1957, *Barbosa 7646* (K!, holo.).

### Description

Erect, decumbent or scandent terrestrial herbs, 98–322 mm tall; *tubers* 1–3, spreading, elongate-oblong, tomentose. *Stem* slender, 88–317 × 1.5–3.5 mm. *Leaves* 5–9, margins entire, smooth; basal leaves 2–5, dense, sheathing, lanceolate to broadly-elliptical, acute, 27–113 × 12–44 mm, middle leaves 2–4, lax, cauline, narrowly-lanceolate to lanceolate, acuminate, 13–39 × 2–9 mm. *Inflorescence* cylindrical, 62–155 × 48–116 mm, lax, 2–11-flowered; *bracts* similar to upper leaves, sheathing, lanceolate, acuminate to apiculate, 11.5–28.0 × 2.0–5.5 mm. *Flowers* spreading, pale green and white, pedicel and ovary sub-erect to erect, 27.0–44.5 mm long. *Dorsal sepal* pale green, erect, narrowly-elliptical, galeate, acute, 10.0–15.5 × 3.0–5.5 mm; *lateral sepals* pale green to white, patent, ovate, acuminate to apiculate, base oblique, 9.5–14.5 × 6–8 mm, adnate to lip and stigmatic processes for 4.0–6.5 mm. *Petals* pale green to white, upper lobe erect, linear, acute to acuminate, 9.0–15.0 × 0.5–1.5 mm, adnate to dorsal sepal, lower

lobe spreading horizontally, weakly deflexed, weakly to strongly falcate, linear, tip sometimes curved, acuminate to apiculate,  $36\text{--}50 \times 0.5\text{--}1.0$  mm, adnate to claw of lip for 2.5–5.5 mm. *Lip* pale green to white, claw 5.5–15.0 mm, median lobe descending, curved forward, linear, acute to acuminate,  $21.5\text{--}38.0 \times 0.5\text{--}1.5$  mm, lateral lobes descending, strongly divergent, linear, weakly to strongly falcate, tips curved, acuminate,  $36.0\text{--}57.5 \times 0.5\text{--}1.0$  mm; spur toothed, 53–70 mm long, clavate apex 15.5–24.0 mm long. *Anther* erect, acute, 3–5 mm long, canals slender, 7.0–11.5 mm long. *Stigmatic processes* clavate, projecting forwards and deflexed, sub-parallel, 8.5–14.0 mm long, base adnate to lip. *Rostellum* 3-lobed, base auriculate, median lobe narrowly-triangular rostrate, acuminate, margin smooth, 5–9 mm long, lateral lobes linear, fused to anther canals, sub-erect to 45°, sub-parallel, 4–6 mm long. Flowering period: April to July.

### **Habitat**

Grassland and savannah, to 1700 m altitude.

### **Distribution**

*Bonatea pulchella* is narrowly distributed in southern Africa, ranging from the coastal plain of northern KwaZulu-Natal and southern Mozambique, inland to Mpumalanga and the Limpopo provinces (Figure 3G).

### **Observations**

*Bonatea pulchella* is larger than its sister taxa *B. polypodantha*, with basal leaves and spurs 53–70 mm long. The hawkmoth *Hippotion osiris* Dalman (Sphingidae), with a proboscis length of 70 mm, is a possible pollinator as a specimen was caught in the vicinity of *B. pulchella* plants in Nelspruit (S. D. Johnson, unpublished data).

### Specimens examined

Strey 3237 (PRE); Strey 3789 (PRE); McMurtry 2449 (Hortus Siccus McMurtrianus); Buitendag 1044 (GLOW; PRE); Van Jaarsveld 468 (GLOW); Van Jaarsveld 2154 (NBG); Mogg 29486 (J); Mogg 28138 (J; PRE); Moss 20791 (PRE); Moss s.n. (J; PRE); van der Horst s.n. (J); Weintraub s.n. (J); Barbosa 7646 (K).

**4. *Bonatea rabaiensis*** (Rendle) Rolfe in Fl. Trop. Afr. 7: 253 (1898); Summerhayes in Fl. Trop. E. Afr.: 139 (1968); Stewart in Orchids of Kenya: 93 (1996); Cribb & Thomas in Flora of Ethiopia and Eritrea: 232 (1997). *Habenaria rabaiensis* Rendle in J. Linn. Soc., Bot. 30: 390 (1895). Type: Kenya, Rabai Hills near Mombasa, 1886, *Taylor s.n.* (BM!, hol.).

### Description

Erect, decumbent or scandent terrestrial herbs, 200–593 mm tall; *tubers* 2–4, spreading, elongate-oblong, tomentose. *Stem* slender, 195–583 × 1.5–6.0 mm. *Leaves* 3–10, lax, cauline, lower sheathing, blades reduced, oblanceolate to elliptical-lanceolate, acute, mucronate, margins entire, smooth, 40–138 × 14–46 mm. *Inflorescence* cylindrical, 65–143 × 58–131 mm, lax, 1–7-flowered; *bracts* sometimes withered at flowering, similar to upper leaves, sheathing, lanceolate to ovate-lanceolate, apiculate, 15.5–38.5 × 4.0–11.5 mm. *Flowers* spreading, green and white, pedicel and ovary sub-erect, 31–47 mm long. *Dorsal sepal* green, erect, elliptical to oblong-ovate, galeate, acute to acuminate, 13.5–22.0 × 5.5–7.5 mm; *lateral sepals* green, projecting forwards, strongly deflexed, ovate-lanceolate, apiculate, base oblique, margins weakly to strongly revolute, 10.5–19.0 × 6–9 mm, adnate to lip and stigmatic processes for 6.0–10.5 mm. *Petals* upper lobe erect, linear, acute to acuminate, 13.5–19.5 × 0.5–2.0 mm, adnate to dorsal sepal, lower lobe spreading horizontally,

weakly to strongly deflexed, linear-falcate, tip curved, acuminate to apiculate, 15.0–31.5 × 0.5–1.5 mm, adnate to claw of lip for 4–10 mm. *Lip* green, claw 10.0–18.5 mm, median lobe descending, geniculate, apically recurved, linear-lanceolate to narrowly-oblong, acute to acuminate, 9.0–16.5 × 0.5–1.5 mm, lateral lobes descending, diverging, linear-falcate, tips curved, acuminate, 17.0–33.5 × 0.5–1.5 mm; spur toothless, 49–68 mm long, clavate apex 20–31 mm long. *Anther* erect, apiculate, 4.5–10.0 mm long, canals slender, 10–17 mm long. *Stigmatic processes* clavate, projecting forwards and deflexed, sub-parallel, 14.5–18.5 mm long, base adnate to lip. *Rostellum* 3-lobed, base auriculate, median lobe cucullate, apex incurved, apiculate, margin ciliate, 6.5–9.5 mm long, lateral lobes linear, fused to anther canals, projecting forwards to sub-erect to 45°, sub-parallel, 2.0–3.5 mm long. Flowering period: May to June, rarely to August.

### **Habitat**

Forest and thicket, 50–1220 m altitude.

### **Distribution**

*Bonatea rabaiensis* is restricted to coastal east Africa in Tanzania and Kenya (Figure 3K).

### **Observations**

*Bonatea rabaiensis* is the only species of *Bonatea* to have no tooth in the mouth of the spur. This species has lateral sepals similar to *B. steudneri* and a spur length equivalent to *B. pulchella*. A hawkmoth of the genus *Nephele*, proboscis length ca. 70 mm, occurs within the coastal range of *B. rabaiensis* and may be a pollinator.

### **Specimens examined**

Jeffery K238 (EA; PRE); Luke and Robertson 2285 (EA); Robertson and Luke 6210 (EA); Taylor s.n. (BM); Magogo and Glover 1045 (BR; EA;

PRE); Robertson and Luke 5890 (EA); Greenways and Hoyle 8327 (EA; PRE).

**5. *Bonatea steudneri*** (Rchb.f.) T.Durand & Schinz in Consp. Fl. Afr. 5: 90 (1895); Rolfe in Fl. Trop. Afr. 7: 253 (1898); Summerhayes in Kew Bull. 17: 531 (1964); Summerhayes in Fl. Trop. E. Afr., Orchid.: 137, fig. 24 (1968); Piers in Orch. E. Afr.: 37, fig. 6-7 (1968); Stewart & Campbell in Orchids Trop. Afr.: 56, tab. 16 [photo] (1970); Lewalle in Bull. Jard. Bot. Nat. Belg. 42: 244 (1972); Williamson in Orchids S. Centr. Afr.: 68, fig. 31 [1] (1977); la Croix & Cribb in Flora Zambesiaca, Orchidaceae 11: 43, fig. 15, pl. 2 (1995); Stewart in Orchids of Kenya: 93 (1996); Cribb & Thomas in Flora of Ethiopia and Eritrea: 232 (1997); la Croix & la Croix in African orchids in the wild and in cultivation: 157 (1997); Schelpe & Linder in Orchids of southern Africa: 143 (1999). *Habenaria steudneri* Rchb.f. in Otia Bot. Hamburg.: 101 (1881); Kraenzl. in Bot. Jahrb. Syst. 16: 57 (1893); Kraenzlin in Orchid. Gen. Sp. 1, 3: 179 (1897). Type: Eritrea, Bogos, Keren, *Steudner* 700 (B<sup>+</sup>, holo.; W!, part of holo.).

*Bicornella arabica* Deflers in Voy. Yemen: 208, t. 6 (1889). *Habenaria arabica* (Deflers) Kraenzl. in Orchid. Gen. Sp. 1, 3: 184 (1897). *Bonatea arabica* (Deflers) Cortesi in Ann. Bot. (Rome) 2: 363 (1905). Type: Yemen, near Ibb, 1650 m, 1887, *Deflers* 659 (P!, lecto., selected here); Yemen, near Ibb, 1650–2000 m, 1887, *Deflers* 665 (P!, syn.).

*Habenaria ecaudata* Kraenzl. in Pflanzenw. Ost-Afrikas, C: 152 (1895); Kraenzlin in Orchid. Gen. Sp. 1, 14: 887 (1900); Rolfe in Fl. Trop. Afr. 7: 249 (1898). Type: Tanzania, Kilosa District, Usagara, near Mtondwe, *Stuhlmann* 8282 (B<sup>+</sup>, holo.).

*Habenaria kayseri* Kraenzl. in Bot. Jahrb. Syst. 19: 246 (1894); Kraenzlin in Orchid. Gen. Sp. 1, 3: 183 (1897). *Bonatea kayseri* (Kraenzl.) Rolfe in Fl. Trop. Afr. 7: 255 (1898); Robyns & Tournay in Fl. Spermat. Parc Nat. Albert 3: 428, t. 59 (1955). Type: Tanzania, Usambara Mountains, Mlalo, frequently in area on stones, March 1893, *Holst* 2443 (B<sup>+</sup>; K!, lecto., selected here); Tanzania, Usambara Mountains, Mlalo, in stony dry areas, *Holst* 635 (B<sup>+</sup>, syn.); Tanzania, Kilimanjaro, Marangu, spread everywhere on hills, *Volken* 630 (B<sup>+</sup>, syn.).

*Habenaria phillipsii* Rolfe in Bull. Misc. Inf. Kew 1895: 227 (1895); Kraenzlin in Orchid. Gen. Sp. 1, 3: 183 (1897). *Bonatea phillipsii* (Rolfe) Rolfe in Fl. Trop. Afr. 7: 254 (1898). Type: Somalia, Golis Range at Dara-as, in deep gorge near water, June 1895, *Lort Phillips* s.n. (K!, holo.).

*Bonatea pirottae* Cortesi in Ann. Bot. (Rome) 2: 362-365 (1905). *Habenaria pirottae* (Cortesi) Schltr. in Bot. Jahrb. Syst. 53: 508 (1915). Type: Eritrea, Amasen, Monti Arbaroba e Mahdet, 2200 m, 6 January 1901, *Pappi* 3392 (FT!, lecto., selected here); Eritrea, Amasen, Asmara, *Baldrati* D66 (FT!, syn.); Eritrea, Cohain, Addi Catina, 1800 m, 24 October 1902, *Pappi* 3002 (FT!, syn.); Eritrea, Amasen, Dongollo presso Ghinda, 2 April 1902, *Pappi* 4514 (FT!, syn.); Eritrea, Amasen, Asmara, 1-10 October 1902, *Tellini* 962 (FT!, syn.).

*Bonatea sudanensis* Rolfe in Bull. Misc. Inform. Kew 1910: 162 (1910). *Habenaria sudanensis* (Rolfe) Schltr. in Bot. Jahrb. Syst. 53: 508 (1915). Type: Sudan, Erkowit, February 1908, *Sillitoe* 1464 (K!, holo.).

*Bonatea ugandae* Rolfe ex Summerh. in Bull. Misc. Inform. Kew 1931: 383 (1931). Type: Uganda, Busoga District, Jinja, 3900 ft, May 1904, E. Brown 50 (K!, holo.).

*Bonatea ugandae* Rolfe nom. nud. in Orchid Rev. 14: 365, 368 (1906).

### **Description**

Erect, decumbent or scandent terrestrial herbs, 415–981 mm tall; *tubers* 1–6, spreading, elongate-oblong, tomentose. *Stem* robust, 405–976 × 4.0–15.5 mm. *Leaves* 7–20, lax to dense, cauline, lower sheathing, blades reduced, oblong-lanceolate to ovate-elliptical, acuminate, margins entire, smooth to undulate, 56–154 × 16–56 mm. *Inflorescence* cylindrical, 92–304 × 120–289 mm, lax, 5–35-flowered; *bracts* sometimes withered at flowering, similar to upper leaves, sheathing, lanceolate to ovate-lanceolate, apiculate, 24.5–50.5 × 5.5–15.0 mm. *Flowers* spreading, green and white, pedicel with ovary sub-erect, 44–80 mm long. *Dorsal sepal* green, erect, narrowly-elliptical, galeate, acute to acuminate, 19–25 × 5.5–9.0 mm; *lateral sepals* green, projecting forwards, weakly to strongly deflexed, ovate-lanceolate, acuminate to apiculate, base oblique, margins weakly to strongly revolute with acute tooth on lower margin, 17.5–27.5 × 8.5–12.5 mm, adnate to lip and stigmatic processes for 7.0–17.5 mm. *Petals* upper lobe green, erect, linear, acute to acuminate, 18–26 × 0.5–2.0 mm, adnate to dorsal sepal, lower lobe white with green tip, spreading horizontally, strongly deflexed, linear-falcate, tip curved, acuminate to apiculate, 39–75 × 0.5–2.0 mm, adnate to claw of lip for 5.0–15.5 mm. *Lip* green, claw 14.0–36.5 mm, median lobe descending, geniculate, apically recurved, linear-lanceolate to narrowly-oblong, acute to acuminate, 22–43 × 1.0–2.5 mm, lateral lobes descending, diverging, linear-falcate, tips curved, acuminate, 35.5–60.0 × 0.5–2.0 mm; spur toothed, 75–230 mm long, clavate apex 22–49 mm long. *Anther* erect, acute, 7.5–11.5 mm long, canals slender, 18.5–26.0 mm

long. *Stigmatic processes* clavate, projecting forwards and deflexed, sub-parallel, 24.5–33.5 mm long, base adnate to lip. *Rostellum* 3-lobed, base auriculate, median lobe cucullate, apex incurved, apiculate, margin ciliate, 6–10 mm long, lateral lobes linear, fused to anther canals, projecting forwards to sub-erect 45°, sub-parallel, 6.0–15.5 mm long. Flowering period: March to August, rarely to February.

### **Habitat**

Grassland, savannah, thicket and forest margins, to 2800 m altitude.

### **Distribution**

*Bonatea steudneri* is the most widespread species in the genus ranging from the Pongola floodplain in South Africa, through Mozambique, Zimbabwe, Namibia, Zambia, Malawi, Tanzania, Kenya, Rwanda, Congo, Uganda, Somalia, Ethiopia, Eritrea, Sudan to Yemen (Figure 3J).

### **Observations**

*Bonatea steudneri* has variable spur length probably owing to local adaptation to pollinators across its wide distribution range. This species has slightly smaller flowers with similar rostellum structure to *Bonatea lamprophylla* J.L.Stewart. It differs in its porrect, strongly deflexed lateral sepals and shorter labellum side lobes. Schelpe and Linder (1999) Figure 24 C is incorrectly labelled as *B. steudneri*. The image more closely approximates *Bonatea boltonii* (Harv.) Bolus, but there is no voucher reference to confirm this.

### **Specimens examined**

Greenway and Turner 10700 (PRE); Evans and Pole-Evans 1109 (PRE); Williams 57 (PRE); Mohamedi 9107 (PRE); Greenway 3386 (PRE); Verdcourt 278 (PRE); Verdcourt and Greenway 263 (PRE); Mainwaring 3014 (NBG); Eyles and Johnson 1064 (GRA); McMurtry 9929 (Hortus



Siccus McMurtrianus); Greatrex s.n. (PRE); Thompson s.n. (J); Ward 2735 (NH); Venter 12969 (PRE; UNIN); Steudner 700 (W); Holst 2443 (K); Lort Phillips s.n. (K); Pappi 3392 (FT); Baldrati D66 (FT); Pappi 3002 (FT); Pappi 4514 (FT); Tellini 962 (FT); Sillitoe 1464 (K); E. Brown 50 (K); Deflers 659 (P); Deflers 665 (P).

**6. *Bonatea lamprophylla*** J.L.Stewart in Amer. Orchid Soc. Bull. 47: 995 (1978); Stewart *et al.* in Wild Orch. South. Africa: 97, pl. 10.1 (1982); Schelpe & Linder in Orchids of southern Africa: 136 (1999). Type: South Africa, [KwaZulu-Natal], [Mabibi] Beach, growing in dune forest in deep shade on top of road, September–October 1971, *Jeppe s.n.* (NU!, holo.).

### Description

Erect, decumbent or scandent terrestrial herbs, 518–1054 mm tall; *tubers* 1–6, spreading, elongate-oblong, tomentose. *Stem* robust, 508–1049 × 5.5–12.5 mm. *Leaves* glossy, 5–15, lax, cauline, lower sheathing, blades reduced, cordate, acuminate, margins entire, undulate, 56–121 × 28–63 mm. *Inflorescence* cylindrical, 111–215 × 103–236 mm, lax, 5–16-flowered; *bracts* similar to upper leaves, sheathing, ovate-lanceolate, acuminate, 33–71 × 9.5–29.0 mm. *Flowers* spreading, green and white, pedicel and ovary sub-erect, 51.5–87.0 mm long. *Dorsal sepal* green, erect, narrowly-elliptical, galeate, acute to acuminate, 28.5–34.0 × 5.5–8.0 mm; *lateral sepals* green, patent, strongly deflexed, oblong-lanceolate, acuminate, base oblique, margins strongly revolute with acute tooth on lower margin, 28.5–33.5 × 9–13 mm, adnate to lip and stigmatic processes for 8–11 mm. *Petals* upper lobe green, erect, linear, acute to acuminate, 27.5–32.5 × 0.5–1.5 mm, adnate to dorsal sepal, lower lobe white with green tip, spreading horizontally, weakly deflexed, linear-falcate, tip curved, acuminate to apiculate, 34.5–54.0 × 0.5–1.5 mm, adnate to claw of lip

for 3.5–6.5 mm. *Lip* green, claw 17.5–22.5 mm, median lobe descending, geniculate, linear, acute to acuminate, 32–47 × 0.5–1.5 mm, lateral lobes descending, diverging, linear, tips curved, acuminate, 106–135 × 0.5–1.0 mm; spur toothed, 92–131 mm long, clavate apex 29–59 mm long. *Anther* erect, acute, 9.5–12.5 mm long, canals slender, 25.0–32.5 mm long. *Stigmatic processes* clavate, projecting forwards and deflexed, weakly divergent, 20.5–27.0 mm long, base adnate to lip. *Rostellum* 3-lobed, base auriculate, median lobe cucullate, apex incurved, apiculate, margin ciliate, 6.5–10.0 mm long, lateral lobes linear, fused to anther canals, sub-erect to 45°, sub-parallel, 14.0–17.5 mm long. Flowering period: September to November.

### **Habitat**

Savannah and coastal dune forest, at near sea level to 200 m altitude.

### **Distribution**

*Bonatea lamprophylla* is a coastal species, occurring from northern KwaZulu-Natal to southern Mozambique (Figure 3D).

### **Observations**

*Bonatea lamprophylla* is a striking species with the largest flowers in the genus. It is closely allied to *B. steudneri* but is easily distinguished by its exceptionally long labellum side lobes (106–135 mm compared to 35.5–60.0 mm for *B. steudneri*), and its glossy cordate dark green leaves with undulate margins.

An un-numbered specimen collected by Barbara Jeppe is listed as an isotype in the original description (*Jeppe s.n.* (BOL!, K!) South Africa, [KwaZulu-Natal], Lake Sibaya, [Mabibi] Beach, in heavy shade in dune forest and at roadside, 24 October 1976). This collection is relegated to

paratype status because it was not part of the original collection, differing in date.

### **Specimens examined**

Daintree s.n. (PRE); Mogg 30071 (J); Jeppe s.n. (PRE); Mogg 27678 (J; PRE); Mogg 27633 (J); Lubbe 376 (NH; PRU); O'Connor 604 (NU); Jeppe s.n. (BOL; K); Jeppe s.n. (NU); Jeppe s.n. (PRE).

**7. *Bonatea porrecta*** (Bolus) Summerh. in Kew Bull. 4: 430 (1949); Stewart *et al.* in Wild Orch. South. Africa: 100, pl. 10.3 (1982); la Croix & Cribb in Flora Zambesiaca, Orchidaceae 11: 46 (1995); la Croix & la Croix in African orchids in the wild and in cultivation: 156 (1997); Schelpe & Linder in Orchids of southern Africa: 139 (1999). *Habenaria porrecta* Bolus in J. Linn. Soc., Bot. 25: 167, fig. 5 (1889); Kraenzl. in Bot. Jahrb. Syst. 16: 71 (1893); Rolfe in Fl. Cap. 5, 3: 134 (1913). Type: South Africa, [KwaZulu-Natal], [Durban], without precise locality or date, *Gueinzus s.n.* (K!, lecto., selected here); South Africa, without precise locality or date, *McKen 11* (BOL!, syn.); South Africa, [KwaZulu-Natal], *Pappe 77* (K!, syn.); South Africa, [KwaZulu-Natal], [Durban], Green Vale, 1852, *Plant 52* (K!, syn.); South Africa, [KwaZulu-Natal], 1854, *Sanderson s.n.* (K!, syn.).

### **Description**

Erect, decumbent or scandent terrestrial herbs, 85–560 mm tall; *tubers* 1–10, spreading, elongate-oblong, tomentose. *Stem* slender to robust, 80–550 × 1.5–6.5 mm. *Leaves* sometimes withered at flowering, 5–16, lax to dense, cauline, lower sheathing, blades reduced, lanceolate to narrowly-oblong, acute to acuminate, margins entire, smooth to undulate, 39–131 × 7–25 mm. *Inflorescence* cylindrical, 41–237 × 50–131 mm, lax to dense, 3–36-flowered; *bracts* sometimes withered at flowering, similar to upper leaves, sheathing, ovate-lanceolate to

ovate-oblong, apiculate, 12–33 × 4.5–10.0 mm. *Flowers* spreading, green and white, pedicel and ovary sub-erect, 21.5–39.5 mm long. *Dorsal sepal* green, erect, elliptical-ovate, galeate, acute to acuminate, 8.5–15.0 × 3–5 mm; *lateral sepals* green, patent, slightly deflexed, widely oblong-ovate, acute to apiculate, base oblique, margins weakly to strongly revolute with acute tooth on lower margin, 8.0–14.5 × 4.5–8.5 mm, adnate to lip and stigmatic processes for 3.5–7.5 mm. *Petals* upper lobe green, erect, narrowly-falcate, acute to acuminate, 6.5–16.0 × 0.5–2.0 mm, adnate to dorsal sepal, lower lobe white with green tip, spreading horizontally, weakly deflexed, weakly to strongly falcate, linear, tip curved, sometimes falcate, acuminate, 12.5–24.5 × 0.5–1.5 mm, adnate to claw of lip for 2.0–5.5 mm. *Lip* green, claw 6–13 mm, median lobe descending, geniculate, sometimes apically recurved, linear-lanceolate to narrowly-oblong, acute to acuminate, 10.5–20.0 × 1.0–2.5 mm, lateral lobes descending, diverging, linear-falcate, tips curved, acuminate, 14–29 × 0.5–2.0 mm; spur toothed, 24–43 mm long, clavate apex 6.5–21.5 mm long. *Anther* erect, acute, 3.5–6.0 mm long, canals slender, 6–12 mm long. *Stigmatic processes* clavate to spatulate, projecting forwards and deflexed, sub-parallel, 8.5–15.5 mm long, base adnate to lip. *Rostellum* 3-lobed, base auriculate, median lobe cucullate, acute to acuminate, margin ciliate, 2–5 mm long, lateral lobes linear, fused to anther canals, projecting forwards to sub-erect 20°, weakly divergent, 2.0–5.5 mm long. Flowering period: June to September, rarely October.

### **Habitat**

Savannah, grassland, forest and thicket, at near sea level to 1800 m altitude.

### **Distribution**

*Bonatea porrecta* is scattered through the eastern half of southern Africa from the Eastern Cape through KwaZulu-Natal, Swaziland,

Mpumalanga, Gauteng, North-West to Limpopo Province. It is recorded just entering Mozambique along the Lebombo Mountains (Figure 3F).

### Observations

*Bonatea porrecta* is distinctive owing to its withered leaves at flowering. This species is similar to *B. boltonii* in flower size and spur morphology (both species averaging 35 mm for total spur length and 11 mm for the clavate apex). The rostellum in *B. porrecta* is however diminutive, with the free portion of the rostellum side lobes generally the length of the rostellum middle lobe. Schelpe and Linder (1999) record collections from south-east Botswana but we have not verified these. Kurzweil and Weber (1992) misidentified *Bayliss s.n.* (BOLp736 (spirit material)) as *B. porrecta*. The specimen drawn in Figure 1 (Kurzweil and Weber, 1992) is *Bonatea saundersioides* (Kraenzl. & Schltr.) Cortesi, which is easily distinguished by its very different lower petal lobes, rostellum and spur. Schelpe and Linder (1999) similarly misidentify *B. porrecta* as *B. saundersioides* in Figure 24 D.

### Specimens examined

Breyer 24387 (PRE); Van Wyk s.n. (PRE); Venter s.n. (UNIN); Venter 13542 (PRE; UNIN); Obermeyer 540 (PRE); Thode 5000 (NH); Compton 18083 (GRA); Gilliland s.n. (J); Von Maltitz s.n. (J); Gilliland s.n. (J); Reddy, Reddy and Reddy 2133 (PRE); McMurtry 3373 (Hortus Siccus McMurtrianus); Strang s.n. (J); McMurtry 911 (Hortus Siccus McMurtrianus); Schijf 60 (J); Moss 3617 (J); Barlett s.n. (J, PRE); Davidson 3163 (J); Karsten s.n. (PRE); Roberts s.n. (PRE); Compton 19713 (GRA); Ward 2381 (NH); Strey 5308 (NH); O'Connor 513 (NU); Stewart 1731 (NU); Garbutt 17 (NU); Butcher s.n. (NH); Lawn 1620 (NH); Daffey s.n. (NU); Forbes 1254 (NH); Commis 478 (NU); Lawson 1110 (NH); O'Connor 435 (NU); Wells 1641 (NU); Alexander 30 (NU); Alexander 31 (NU); Thomas 17 (NU); Chamier 8 (NU); Schelpe 3 (NU); Rennie s.n. (NU); Manning 263 (NU); Forbes 670 (NH); Thode 4159

(NH); Moonsamy s.n. (NH); Wood 5992 (NH); Wood 7168 (NH); Rudatis 1087 (NH); Anderson s.n. (NH); Rosenbrock s.n. (GRA); Behr s.n. (NBG); Van Wyk 8463 (NH; PRU); O'Connor 406 (KEI; NU); Balkwill, Norris, Manning and Hutchings 1892 (NU); O'Connor 405 (NU); Flanagan s.n. (PRE); Pappe 77 (K); McKen 11 (BOL; K); Plant 52 (K); Sanderson s.n. (K); Gueinzus s.n. (K).

**8. *Bonatea volkensiana*** (Kraenzl.) Rolfe in Fl. Trop. Afr. 7: 253 (1898); Robyns & Tournay in Fl. Spermat. Parc Nat. Albert 3: 426 (1955); Summerhayes in Fl. Trop. E. Afr.: 139 (1968); Stewart in Orchids of Kenya: 94 (1996). *Habenaria volkensiana* Kraenzl. in Bot. Jahrb. Syst. 19: 244 (1894); von Götzen in Durch Afr. Von Ost nach West: 376 (1895). Type: Tanzania, Kilimanjaro, Kwa Ngowe, many in sandy, grassy hills of landscape, east of Marangu up to Rombo, an area of Kenya at Kwa Iletto, 1450 m, June 1893, *Volkens* 342 (B†, holo.; BM!, iso.).

### Description

Erect, decumbent or scandent terrestrial herbs, 210–660 mm tall; *tubers* 2–10, spreading, elongate-oblong, tomentose. *Stem* slender to robust, 205–650 × 2.5–10.5 mm. *Leaves* 7–15, lax to dense, cauline, lower sheathing, blades reduced, lanceolate to oblanceolate, acute to acuminate, margins entire, smooth to undulate, 27–123 × 10–41 mm. *Inflorescence* cylindrical, 89–221 × 55–123 mm, lax to dense, 6–27-flowered; *bracts* similar to upper leaves, sheathing, lanceolate to ovate-lanceolate, apiculate, 16–35 × 5–12 mm. *Flowers* spreading, green and white, pedicel and ovary sub-erect to erect, 25–43 mm long. *Dorsal sepal* green, erect, elliptical-lanceolate, galeate, acute to acuminate, 13.0–18.5 × 4–8 mm; *lateral sepals* green, patent, strongly deflexed, widely oblong-ovate, acute to apiculate, base oblique, margins weakly revolute with acute tooth on lower margin, 11.5–17.5 × 5.5–9.5 mm,

adnate to lip and stigmatic processes for 4.5–8.0 mm. *Petals* upper lobe green, erect, linear, acute to acuminate, 12.5–19.5 × 0.5–2.0 mm, adnate to dorsal sepal, lower lobe white with green tip, projecting forwards, ascending, weakly divergent, linear-falcate, tip curved, acuminate, (21–)25–38.5 × 0.5–1.5 mm, adnate to claw of lip for 3–6 mm. *Lip* claw 10.0–14.5 mm, median lobe green, descending, geniculate, sometimes apically recurved, linear-lanceolate to narrowly-oblong, acute to acuminate, 11.5–25.0 × 1.0–2.5 mm, lateral lobes white with green tips, projecting forward, ascending, diverging, linear-falcate, tips curved, acuminate, (16.5–)20–32.0 × 0.5–1.5 mm; spur toothed, 23–36 mm long, clavate apex 8.0–16.5 mm long. *Anther* erect, acute, 5–8 mm long, canals slender, 9.0–14.5 mm long. *Stigmatic processes* clavate to spatulate, projecting forwards and deflexed, sub-parallel, 11.5–18.0 mm long, base adnate to lip. *Rostellum* 3-lobed, base auriculate, median lobe cucullate, acute to acuminate, margin ciliate, 3.0–5.5 mm long, lateral lobes linear, fused to anther canals, sub-erect to 45°, sub-parallel, 4.5–7.0 mm long. Flowering period: rarely January to February, March to June.

### **Habitat**

Grassland and savannah, 1000–2200 m altitude.

### **Distribution**

*Bonatea volkensiana* has been recorded from north-eastern Tanzania and south-western Kenya (Figure 3K).

### **Observations**

*Bonatea volkensiana* is distributed inland and is allopatric with coastal populations of *B. rabaiensis*. It is occasionally sympatric with *B. steudneri* (Donnelly 13799 (EA)) but hybrids have not been recorded. This species has a similar posture of the elongate rostellum side lobes (ascending >45°) to *B. steudneri* and *B. lamprophylla*, but the

morphology and posture of its lower petal lobes and labellum closely resemble *Bonatea antennifera* Rolfe.

### **Specimens examined**

Bullid 1584 (EA); Hingley 230 (EA); Gilbert 4999 (EA); Greenway 18568 (EA); Archer s.n. (EA); Bally 9222 (EA); Donelly 13799 (EA); Turner 13990 (EA); Greenway and Turner 13357 (EA; PRE); Bally 855 (EA); Bally 1096 (EA); Haarer 550 (EA); Volkens 342 (BM).

**9. *Bonatea antennifera*** Rolfe in Gard. Chron. 3: 450 (1905); Rolfe in Fl. Cap. 5, 3: 142 (1913); la Croix & Cribb in Flora Zambesiaca, Orchidaceae 11: 45 (1995); la Croix & la Croix in African orchids in the wild and in cultivation: 154 (1997); Verdoorn in Fl. Pl. Africa, t. 1405 (1964). *Habenaria antennifera* (Rolfe) Schltr. in Bot. Jahrb. Syst. 53: 508 (1915), nom. illegit., non *Habenaria antennifera* A.Rich. in Ann. Sc. Nat. Ser. 2, 14: 268 (1840). *Bonatea speciosa* var. *antennifera* (Rolfe) Sommerville in Contr. Bolus Herb. 10: 157 (1982); Stewart *et al.* in Wild Orch. South. Africa: 99, pl. 10.2b (1982); Schelpe & Linder in Orchids of southern Africa: 139 (1999). Type: [Zimbabwe], without precise locality or date, *Munro s.n.* (K!, holo.).

*Habenaria macrorchis* Schltr. [as *H. macrocorchis* in Schelpe & Linder in Orchids of southern Africa: 139 (1999)] in Ann. Transvaal Mus. 10: 245 (1924), nom. illegit. superfl.

### **Description**

Erect, decumbent or scandent terrestrial herbs, 260–1258 mm tall; *tubers* 1–12, spreading, elongate-oblong, tomentose. *Stem* robust, 255–1250 × 3.5–11.0 mm. *Leaves* 6–21, lax to dense, cauline, lower sheathing, blades reduced, oblong-lanceolate to oblong-ovate, acute to acuminate, margins entire, smooth to undulate, 42–190 × 10–53 mm.



*Inflorescence* cylindrical, 38–339 × 79–181 mm, lax to dense, 4–44-flowered; *bracts* similar to upper leaves, sheathing, ovate-lanceolate to ovate-oblong, acuminate to apiculate, 19.5–50.5 × 6.0–14.5 mm. *Flowers* spreading, green and white, pedicel and ovary sub-erect, 26.5–60.5 mm long. *Dorsal sepal* green, sub-erect to erect, elliptical to oblong-ovate, galeate, acute to acuminate, 14–23 × 5.0–8.5 mm; *lateral sepals* green, patent, strongly deflexed, oblong-lanceolate to ovate, acute to apiculate, base oblique, margins weakly to strongly revolute with acute tooth on lower margin, 13–26 × 5–10 mm, adnate to lip and stigmatic processes for 4.5–9.5 mm. *Petals* upper lobe green, erect, narrowly-falcate, acute to acuminate, 13–24 × 1.0–3.5 mm, adnate to dorsal sepal, lower lobe white with green tip, projecting forwards, ascending, weakly divergent, linear-falcate, tip curved, acuminate to apiculate, (18–)24–43 × 0.5–2.0 mm, adnate to claw of lip for 2.5–6.5 mm. *Lip* claw 6.0–14.0 mm, median lobe green, descending, geniculate, sometimes apically recurved, linear-lanceolate to narrowly-oblong, acute-acuminate, 15.0–33.5 × 1.0–3.5 mm, lateral lobes white with green tips, projecting forward, ascending, diverging, linear-falcate, tips curved, acuminate, 17.5–44.5 × 0.5–2.0 mm; spur toothed, 27–44(–58) mm long, clavate apex 8.0–15.0(–18.5) mm long. *Anther* erect, acute, 4.5–7.5 mm long, canals slender, 10–14 mm long. *Stigmatic processes* clavate to spatulate, projecting forwards and deflexed, sub-parallel, (11.0–)15.0–21.5 mm long, base adnate to lip. *Rostellum* 3-lobed, base auriculate, median lobe cucullate, acute to acuminate, margin ciliate, 7.5–11.5 mm long, lateral lobes linear, fused to anther canals, slightly deflexed to sub-erect 45°, sub-parallel, 2.0–5.5 mm long. Flowering period: March to May, rarely to July.

### **Habitat**

Grassland, savannah and forest fringes, at near sea level to 1700 m altitude.

### **Distribution**

*Bonatea antennifera* is widespread throughout north-western South Africa in KwaZulu-Natal, Free State, North-West, Gauteng, Mpumalanga and Limpopo provinces. It has also been recorded in south-eastern Botswana, southern Mozambique and from scattered populations in Zimbabwe (Figure 3A).

### **Observations**

Plants have characteristically glaucous leaves. Populations of *B. antennifera* from northern KwaZulu-Natal are atypical, flowering from June to July with markedly elongate spurs of 51–58 mm (typical flowering is from March to May and spur length 27–44 mm). Labellum fusion and anterior lobe adnation to the lip, as well as stigmatic process, rostellum side lobe and canal length, fall within the maximum size range for the species. The rostellum side lobes are distinctive in posture being semi-erect (45° angle) rather than porrect.

### **Specimens examined**

Netshiungani 1416 (PRE); Meeuse 10342 (PRE); Steyn 67 (PRE); Riley s.n. (NBG); Makgakga 101 (PRE); Fourie 1130 (PRE); Van Vuuren s.n. (UNIN); Van Vuuren 1461 (PRE); Bredenkamp 1085 (PRE; UNIN); Jacobsen 5312 (PRE); Hansen 3406 (PRE); Venter 1906 (PRE); Leistner 192 (PRE); Venter 11624 (UNIN); Leipoldt s.n. (PRE); Galpin M328 (PRE); Balkwill 1595 (NU); Barnard 385 (PRE); Pole-Evans 4698 (PRE); van der Meulen 440FM103 (PRE); Botha 2658 (PUC); Sutton 819 (PRE); Schlotfeldt s.n. (NBG); Rogers 18723 (PRE); Bischoff s.n. (PRU); Bremekamp s.n. (PRE); Erens s.n. (PRE); Van Rooyen and Schultze 4274 (PRU); without collector PRU s.n. (PRU); Smith 6024 (PRE); Oliver 7674 (PRE; STE); Moss 11265 (J); van Rooyen 3443 (PRU); Brusse 1933 (PRE); Bremekamp s.n. (PRU); de Wit s.n. (PRU); Leendertz 123 (PRE); Wasserfall and Collett s.n. (PRE); Howlett s.n. (PRE); Codd 3772 (PRE); Strater 14 (J); Salm 24 (J); McMurtry 4358

(Hortus Siccus McMurtrianus); Hayes-Palmer s.n. (NBG); Pole-Evans 112 (PRE); McMurtry 4983 (Hortus Siccus McMurtrianus); Frames s.n. (PRE); McMurtry 4520 (Hortus Siccus McMurtrianus); Wentzel 4 (PRU); McMurtry 4525 (Hortus Siccus McMurtrianus); King s.n. (PRE); Onderstall 1061 (GLOW); Kinges 1509 (PRE; PRU); Convent 19 (GRA); Schweickerdt 1069a (PRE); Van Jaarsveld 1848 (NBG); Mogg 23304 (J); Franklin 28 (J); Bartlett s.n. (J); McMurtry 6100 (Hortus Siccus McMurtrianus); Behr 447 (NBG); Bartlett and Mogg s.n. (J); Hartzer 188 (PRE); Bartlett s.n. (J); Westhuizen 647 (PUC); Theron 2 (PRE); Louw 1392 (PUC); McLea 3038 (SAM); Theron 298 (PUC); Theron 1439 (PRU); Theron 1547 (PRU; PUC); Ubbink 48 (PUC); Venter 191 (PRE); Mogg 21061 (J); Gilliland s.n. (J; PRE); Moss 11266 (J); Lucas 204 (J); McMurtry 2450 (Hortus Siccus McMurtrianus); Cron and Balkwill 261 (J); Bartlett s.n. (J); Moss s.n. (J); Moss s.n. (J); Ludick 7 (PRU); Mogg 37600 (PRE); Pooley 1398 (NU); O'Connor 512 (NH); O'Connor 510 (NU); Tafscolt s.n. (KMG); Atkinson 10 (J); Muller 1761 (PRE); Zietsman and Zietsman 359 (PRE); Kok 22 (BLFU); du Preez 699 (BLFU); Rossouw 26 (BLFU); Hanekom 884 (PRE); Müller 533 (NBG; PRE); Macnae 1347 (NBG); Feijter 94 (PUC); Kuun 23 (PRU; PUC); Moss and du Toit 20718 (J); Lawn 1557 (NH); Munro s.n. (K).

**10. *Bonatea boltonii*** (Harv.) Bolus in J. Linn. Soc., Bot. 19: 340 (1882); Rolfe in Fl. Cap. 5, 3: 140 (1913). *Habenaria boltonii* Harv. in Thes. Cap. 1: 55, fig. 1 (1859); Kraenzl. in Bot. Jahrb. Syst. 16: 142 (1893). *Habenaria bonatea* Rchb.f. var. *boltonii* (Harv.) Bolus in Ic. Orch. Austr. Afr. 2: t. 45 (1911), nom. illegit. superfl. Type: South Africa, [Eastern Cape], near Grahamstown, without precise locality or date, *Bolton s.n.* (TCD!, holo.).

## Description

Erect, decumbent or scandent terrestrial herbs, 100–750 mm tall; *tubers* 1–8, spreading, elongate-oblong, tomentose. *Stem* robust, 95–740 × 3.5–11.0 mm. *Leaves* 5–13, lax to dense, cauline, lower sheathing, blades reduced, oblong-lanceolate to widely-ovate, acute to acuminate, margins entire, smooth to undulate, 19–178 × 9–40 mm. *Inflorescence* cylindrical, 74–191 × 67–132 mm, lax to dense, 3–20-flowered; *bracts* similar to upper leaves, sheathing, ovate-lanceolate to ovate-oblong, acuminate to apiculate, 16.5–43.5 × 6.0–17.0 mm. *Flowers* spreading, green and white, pedicel and ovary sub-erect, 30–59 mm long. *Dorsal sepal* green, sub-erect to erect, elliptical-ovate, galeate, acute to acuminate, 12.5–18.5 × 4–7 mm; *lateral sepals* green, patent, slightly deflexed, oblong-lanceolate to oblong-ovate, acute to apiculate, base oblique, margins weakly to strongly revolute with acute tooth on lower margin, 10.0–20.5 × 4–8 mm, adnate to lip and stigmatic processes for 4.0–6.5 mm. *Petals* upper lobe green, erect, narrowly-falcate, acute to acuminate, 9.5–18.0 × 1–4 mm, adnate to dorsal sepal, lower lobe white with green tip, spreading horizontally, weakly deflexed, weakly to strongly falcate, linear to linear-lanceolate, tip curved, acuminate, 11.0–24(–27.5) × 0.5–2.0(–2.5) mm, adnate to claw of lip for 1.5–4.0 mm. *Lip* green, claw 6.5–12.0 mm, median lobe descending, geniculate, sometimes apically recurved, linear-lanceolate to narrowly-oblong, acute to acuminate, 7–27 × 1–3 mm, lateral lobes descending, diverging, falcate, linear-lanceolate, tips curved, acuminate, 10.5–32.0 × 0.5–3.0 mm; spur toothed, 27–51 mm long, clavate apex 7.0–14.5 mm long. *Anther* erect, acute, 4.5–7.0 mm long, canals slender, 6.5–11.0 mm long. *Stigmatic processes* very spatulate, projecting forwards and deflexed, sub-parallel, 8.5–15.0 mm long, base adnate to lip. *Rostellum* 3-lobed, base auriculate, median lobe cucullate, acute to acuminate, margin ciliate, 5–9 mm long, lateral lobes linear, fused to anther canals,

projecting forwards to sub-erect 20°, sub-parallel, 2.0–3.5 mm long.  
Flowering period: rarely August to November, December to March.

### **Habitat**

Grassland, savannah and dunes in full sun, rarely in forest and thicket, at near sea level to 1900 m altitude.

### **Distribution**

*Bonatea boltonii* is restricted to the eastern parts of South Africa, ranging from the Eastern Cape, through KwaZulu-Natal, into Mpumalanga and Limpopo (Figure 3B).

### **Observations**

Plants are generally short in stature, often with glaucous leaves. This taxon however, can reach heights comparable with *B. antennifera* and *Bonatea speciosa* (L.f.) Willd. when growing in tall vegetation such as forest margins and mixed grassland. *Bonatea boltonii* and *B. antennifera* are distinguished by a critical quantitative divide occurring at 15 mm for stigmatic process length and 24 mm for lower petal lobe length, with *B. boltonii* falling below the divide. Posture of the lower petal lobe and labellum side lobe are also significantly different in the species - both projecting forward, ascending in *B. antennifera*, whilst lateral, horizontal petal lobes and descending labellum side lobes occur in *B. boltonii*.

Harvey (1859) describes the petals as bipartite, although the figure shows simple petals. This error was perpetuated by Kraenzlin in his Monograph (1893), pp 142.

### **Specimens examined**

Schlechter 4381 (PRE); Venter 10483 (PRE; UNIN); McMurtry 4994 (Hortus Siccus McMurtrianus); Obermeyer 349 (PRE); Rogers s.n.

(PRE); Onderstall 1307 (PRE); Culver 18 (J); Schlieben 9592 (PRE); Galpin 769 (GRA; PRE); Holden s.n. (PRE); Hardeman s.n. (NBG); Smit 1373 (PRU); Mattison 20 (NU); Breyer 16956 (PRE); Jansen s.n. (NH); Lubbe 327 (NH; PRU); Sikhakhane 580 (NH; PRE); Robbeson 240 (PRU); Green 192 (NH); Acocks 10142 (NH); Thode 3889 (NH); Harrison 214 (NH; PRE); Williams 1069 (NH); Wood 12227 (J; NH); Rennie 1301 (NU); Greene 1020 (NH); Wylie s.n. (NH); Wood s.n. (SAM); Nair 9 (UDW); Hennessy 143 (UDW); Gathiram 10 (UDW); Gathiram 15 (UDW); Hall s.n. (NU); O'Connor 198 (NU); O'Connor 565 (NU); O'Connor s.n. (NU); Walsh 24 (NU); Germishuizen 1795 (PRE); Rudatis 1203 (NH); Pellew 31 (NU); Glen 273 (J; Umtamvuna Herbarium); Moss 10631 (J); Prosser 1426 (J); Mgemell s.n. (BLFU); Thomas 1 (NU); Germishuizen 1668 (PRE); Melvin s.n. (NH); Van Wyk and Matthews 7818 (KEI; NH; PRU); Jordaan 975 (NH); Flanagan 647 (SAM; PRE); Pegler 299 (PRE); Potts 1822 (BLFU); Glass s.n. (SAM); Black 30 (PRE); Noel 1131 (GRA); Peter 475 (NU); Norton s.n. (NBG); Bolton s.n. (TCD).

**11. *Bonatea speciosa*** (L.f.) Willd. in Sp. Pl. 4: 43 (1805); Bolus in J. Linn. Soc., Bot. 19: 340 (1882); Stewart *et al.* in Wild Orch. South Africa: 99 (1982); la Croix & Cribb in Flora Zambesiaca, Orchidaceae 11: 45 (1995); la Croix & la Croix in African orchids in the wild and in cultivation: 157 (1997); Schelpe & Linder in Orchids of southern Africa: 137 (1999). Ill.: Schelpe in An introduction to the South African orchids: 74, pl. 35 (1966). *Orchis speciosa* L.f. in Suppl. Pl.: 401 (1781). Type: South Africa, [Western Cape], Cape of Good Hope, without precise locality or date, *Herbarium Thunberg* 21246 (UPS!, lecto.); South Africa, [Western Cape], Cape of Good Hope, *Herbarium Thunberg* 21245 (UPS!, syn.).

*Habenaria bonatea* Rchb.f. in Otia Bot. Hamburg.: 101 (1881), nom. illegit. superfl.; Kraenzl. in Bot. Jahrb. Syst. 16: 57 (1893); Schltr. Bot. Jahrb. Syst. 20, Beibl. 50: 32 (1895); Bolus in Ic. Orch. Austr. Afr. 2: t. 44 (1911); Bolus in The orchids of the Cape Peninsula: t. 15 (1918). Type: as above.

*Habenaria bonatea* Pfitzer in Engl. & Prantl, Naturl. Pflanzenfam. 2, 6: 95 (1888), nom. illegit. superfl. Type: as above.

*Habenaria robusta* N.E.Br. in Gard. Chron., n.s., 24: 307 (1885), nom. illegit., non *Habenaria robusta* Welw. ex Rchb.f. in Flora 50: 102 (1867). Type: as above.

*Bonatea densiflora* Sond. in Linnaea 19: 80 (1847); Kraenzl. in Bot. Jahrb. Syst. 16: 68 (1893). *Habenaria densiflora* (Sond.) Rchb.f. in Ann. Bot. Syst. 1: 797 (1849); Bolus in J. Linn. Soc., Bot. 19: 340 (1882). Type: South Africa, [Eastern Cape], Balfour, Kat River near to the Missionary Institution, 2000–3000 ft, June, *Ecklon and Zeyher s.n.* (S!, holo.).

## Description

Erect, decumbent or scandent terrestrial herbs, 95–957 mm tall; *tubers* 1–8, spreading, elongate-oblong, tomentose. *Stem* robust, 90–950 × 2.5–14.0 mm. *Leaves* 4–19, lax to dense, cauline, lower sheathing, blades reduced, oblong-lanceolate to obovate, acute to acuminate, margins entire, smooth to undulate, 26–155 × 110–67 mm. *Inflorescence* cylindrical, 63–286 × 66.5–165.0 mm, lax to dense, 1–41-flowered; *bracts* similar to upper leaves, sheathing, ovate-lanceolate to ovate, acuminate to apiculate, 15–53 × 6–23 mm. *Flowers* spreading, green and white, pedicel and ovary sub-erect, 24–64 mm long. *Dorsal sepal* green, sub-erect to erect, elliptical to oblong-ovate, galeate, acute to acuminate, 15–25 × 4.5–9.5 mm; *lateral sepals* green, patent, slightly deflexed, oblong-lanceolate to ovate,

acute to apiculate, base oblique, margins weakly revolute with acute tooth on lower margin, 14.0–29.5 × 5.5–12.5 mm, adnate to lip and stigmatic processes for 5.0–13.5 mm. *Petals* upper lobe green, erect, narrowly-falcate, acute to acuminate, 13–26 × 1.0–3.5 mm, adnate to dorsal sepal, lower lobe white with green tip, projecting forwards, weakly to strongly deflexed, weakly to strongly divergent, falcate, linear to lanceolate, tip curved, acute to acuminate, (16–)19–39 × (1.5–)2.0–5.5 mm, adnate to claw of lip for 3.5–9.8 mm. *Lip* green, claw 9.0–25.5 mm, median lobe descending, geniculate, apically recurved, linear-lanceolate to narrowly-oblong, acute to acuminate, 9.0–27.5 × 0.5–3.0 mm, lateral lobes descending, diverging, falcate, linear-lanceolate, tips curved, acuminate, 11.5–39.0 × 1–4 mm; spur toothed, 20–57 mm long, clavate apex (13.5–)15.0–32.0 mm long. *Anther* erect, acute, 5.0–8.5 mm long, canals slender, 9.5–16.5 mm long. *Stigmatic processes* clavate to slightly spathulate, projecting forwards and deflexed, sub-parallel, (11.5–)14.0–27.5 mm long, base adnate to lip. *Rostellum* 3-lobed, base auriculate, median lobe cucullate, acute to acuminate, margin ciliate, 5.0–11.5 mm long, lateral lobes linear, fused to anther canals, slightly deflexed to sub-erect 45°, sub-parallel, 2.5–7.0 mm long. Flowering period: June to December, rarely to March.

### **Habitat**

Grassland, savannah, forest, thicket, fynbos and nama-karoo, but most common in dune forest, from sea level to 1300 m altitude.

### **Distribution**

*Bonatea speciosa* occurs along the seaboard of southern Africa from Lambert's Bay in the Western Province, through Eastern Cape and KwaZulu-Natal to southern Mozambique, extending shortly inland, especially in the Eastern Cape (Figure 3I).



## Observations

*Bonatea speciosa* is a morphologically variable species with a wide distribution on the eastern seaboard of South Africa and an extended flowering period. Populations from northern KwaZulu-Natal and southern Mozambique show a remarkable conformity; these populations flower between June and July, have large flowers, lax inflorescence and low flower numbers (5–7). The rostellum side lobes are distinctive in being semi-erect ( $45^\circ$ ).

*Bonatea speciosa* is distinct from *B. antennifera* and *B. boltonii* with respect to its longer dilated portion of the spur ( $> 15$  mm) in combination with its wider lower petal lobes ( $> 1.5$  mm). The lower petal lobe posture is porrect and weakly to strongly deflexed, with descending labellum side lobes. *B. speciosa* and *B. antennifera* are largely allopatric, being constituents of coastal dune forest and savannah vegetation types, respectively, but both overlap with *B. boltonii*. These three species also differ in their peak flowering period, with the majority of populations flowering from March to May (*B. antennifera*), June to December (*B. speciosa*) and December to March (*B. boltonii*).

In Schelpe and Linder in *Orchids of southern Africa*: 137 (1999), the collector of the syntype *Bonatea densiflora* Sond. is incorrectly noted as *Balfour s.n.*

## Specimens examined

Mogg 29486 (J); Moss s.n. (J); Weintraub s.n. (J); Wolfowitz 5 (J); Lawn 953 (NH); Carnegie s.n. (NU); O'Connor 515 (NU); Strey 6487 (NH; PRE); Wood s.n. (SAM); Forbes and Obermeyer 51 (NH; PRE); Moss 3615 (J); Ward 913 (PRE); Gibson s.n. (NU); Pentz 371 (PRE); McMurtry s.n. (Hortus Siccus McMurtrianus); Franks s.n. (NH); Wood 12226 (NH); Sanderson 1037 (NH); Ward 12594 (NH); Adams 211

(NU); without collector NH 17185 (NH); Ward 913 (NU); Thode 5770 (NH); Nixon 19 (NU); Newmark 11 (NU); King 115 (NU); Aubrey s.n. (NU); Ball 743 (PRE); Melvin s.n. (Umtamvuna Herbarium); Thode 4995 (NH); Fisher 313 (NU); Cohen s.n. (NBG); Dunstan s.n. (GRA); McNeil 112 (NU); Von Fintel 560 (NH); Coleman 882 (NH); Gordon-Gray 571 (NU); Kelly 17 (NBG); Venter 939 (PRE); Bayliss 7077 (NBG); Schelpe 2 (NU); Grant 2577 (GRA); Hutton 496 (GRA); Sim 808 (NU); Flanagan 647 (SAM; PRE); Peter 240 (NU); Flanagan 239 (GRA); Flanagan 810 (GRA); Flanagan 239 (PRE); Pegler 1435 (PRE); Lubke s.n. (GRA); Hutchings 1062 (KEI); Chater s.n. (STE); without collector SAM 52227 (SAM); Taylor s.n. (PRE); Stephany s.n. (PRE); Lange 15 (PRE); Morze 2035 (PRE); Van Niekerk 194 (PRE); Bower 605 (PRE); Taylor 3749 (NBG); Flanagan 239 (SAM); Pappe s.n. (SAM); Drège 96 (GRA); Paterson 12287 (PRE); MacOwan s.n. (SAM); White s.n. (GRA); Davies s.n. (GRA); Galpin 295 (PRE); Roux s.n. (NBG); Dieterlen 29 (NBG); Noel 7311 (GRA); Bolus s.n. (NBG); Glass 470 (NBG); Burrows 3315 (GRA); Muirhead s.n. (GRA); Fatherstone s.n. (GRA); Tyson s.n. (PRE); Dold 1229 (GRA); Sole s.n. (GRA); Tyson H12978 (PRE); Edwards 205 (NU); Werner s.n. (NBG); Norris s.n. (NBG); Batten s.n. (NBG; STE); Carter s.n. (NBG); Galpin 1877 (PRE); Gane 301 (GRA); Rattray 150 (GRA); Thode 7707 (STE); Stokoe 13731 (PRE); Kurzweil HK859 (NBG); Tapscott s.n. (SAM); Boucher 2421 (STE); Martin 229 (NBG); Ebersohn 287 (NBG); Compton 14103 (NBG); Radloff s.n. (STE); Marloth 7681 (PRE); Rogers 22604 (PRE); Purchell s.n. (SAM); Becquet 17870 (PRE); Cloete s.n. (SAM); Swart s.n. (STE); Galpin 4590 (PRE); Kramer s.n. (PRE); without collector STE 17225 (STE); Boucher 1678 (STE); Barker s.n. (NBG); Muir 1231 (PRE); Van der Byl 13376 (PRE); Hugo 1952 (PRE; STE); Hugo 1977 (STE); Morze 2030 (PRE); McWade s.n. (NBG); Chater s.n. (STE); Thomas s.n. (NBG); Rogers 17243 (GRA; J; STE); Jeppe s.n. (PRE); Thunberg 21246 (UPS); Thunberg 21245 (UPS); Ecklon and Zeyher s.n. (S).

**12. *Bonatea saundersioides*** (Kraenzl. & Schltr.) Cortesi in Ann. Bot. (Rome) 2: 363 (1905); Stewart *et al.* in Wild Orch. South. Africa: 100, pl. 10.5 (1982); la Croix & la Croix in African orchids in the wild and in cultivation: 156 (1997); Schelpe & Linder in Orchids of southern Africa: 140 (1999). *Habenaria saundersioides* Kraenzl. & Schltr. in Orchid. Gen. Sp. 1, 3: 181 (1897); Schlechter in Ann. Transvaal Mus. 10: 245 (1924). *Bonatea saundersioides* (Kraenzl. & Schltr.) Rolfe in Fl. Cap. 5, 3: 140 (1913), nom. illegit. superfl. Type: South Africa, [Mpumalanga], Barberton, Umvoti Creek, 3000 ft, April–May, *Culver 30* (K!, holo.; BOL!, iso.).

*Habenaria umvotensis* Rolfe in Fl. Cap. 5, 3: 133 (1913). Type: South Africa, [Mpumalanga], Barberton, Umvoti Creek, 3000 ft, April–May, *Culver 30* (BOL!, K!, syn.); South Africa, [Mpumalanga], Barberton, Umvoti Creek, shady places amongst rocks in wooded ravines, 3000 ft, May–June, *Galpin 954* (BOL!, K!, PRE!, syn.).

### Description

Erect, decumbent or scandent terrestrial herbs, 200–600 mm tall; *tubers* 1–8, spreading, elongate-oblong, tomentose. *Stem* slender to sturdy, 195–595 × 2.5–8.5 mm. *Leaves* sometimes withered at flowering, 5–12, lax, cauline, lower leaves intermediately basal, sheathing, lanceolate to narrowly-elliptical, acute to apiculate, margins entire, smooth, 54–193 × 7–28 mm. *Inflorescence* cylindrical, 65–262 × 52–95 mm, lax to dense, 5–41-flowered; *bracts* sometimes withered at flowering, similar to upper leaves, sheathing, ovate-lanceolate, apiculate, 17.5–30.5 × 4.0–8.5 mm. *Flowers* spreading, green and white, pedicel with ovary projecting forwards to sub-erect, 16–29 mm long. *Dorsal sepal* green, sub-erect, elliptical-oblong, galeate, acute to acuminate, 8.5–15.0 × 3.0–5.5 mm; *lateral sepals* green, patent, strongly deflexed, ovate-lanceolate, acuminate to apiculate, base oblique, margins weakly to strongly revolute with small acute tooth on

lower margin, 10–15 × 3.5–6.5 mm, adnate to lip and stigmatic processes for 2.5–5.5 mm. *Petals* upper lobe green, erect, linear, acute to acuminate, 8–14 × 0.5–1.5 mm, adnate to dorsal sepal, lower lobe white, spreading horizontally, strongly deflexed, weakly to strongly falcate, linear to linear-lanceolate, tip curved, acuminate to apiculate, (13–)16–24 × 0.5–2.0(–3.0) mm, adnate to claw of lip for 2.5–4.5 mm. *Lip* claw 4.5–9.0 mm, median lobe green, descending, geniculate, sometimes apically recurved, linear, acute, 8.5–20.0 × 0.5–1.5 mm, lateral lobes green to white, descending, diverging, weakly to strongly falcate, linear, tips curved, acuminate to apiculate, 14–34 × 0.5–2.0 mm; spur toothed, curved, 16–30 mm long, clavate apex 9.5–24.0 mm long. *Anther* erect, acute, 3.0–5.5 mm long, canals slender, 5.0–8.0 mm long. *Stigmatic processes* clavate, projecting forwards and deflexed, weakly divergent, 4.5–9.0 mm long, base adnate to lip. *Rostellum* 3-lobed, base auriculate, median lobe narrowly-cucullate, apiculate, margin ciliate, 4.0–6.5 mm long, lateral lobes linear, fused to anther canals, projecting forwards to sub-erect 45°, sub-parallel, 1.0–2.5 mm long. Flowering period: rarely March to May, June to August.

### **Habitat**

Savannah and forest, at near sea level to 1500 m altitude.

### **Distribution**

*Bonatea saundersioides* is restricted to the north-eastern portion of southern Africa, occurring in KwaZulu-Natal, Mpumalanga, North-West, Limpopo Provinces and Swaziland (Figure 3H).

### **Observations**

Colour variation occurs in the perianth of *B. saundersioides* with either green or white labellum side lobes. *Bonatea saundersioides* and sister taxa *Bonatea cassidea* Sond. are closely allied. The taxa have similar rostellum structure, stigmatic process and spur structure, but *B.*

*saundersioides* differs in its longer and narrower lower petal lobes and labellum side lobes. The species may extend into Mozambique, but the area is poorly botanized.

### **Specimens examined**

Bartlett s.n. (J); Botha s.n. (PRE); Bartlett s.n. (J; PRE); McMurtry 1209 (Hortus Siccus McMurtrianus); Galpin 954 (PRE); Compton 28930 (PRE); Bayliss 3485 (PRE); Bayliss B/S6 (PRE); Pooley 633 (NU); O'Connor 511 (NU); Culver 30 (BOL; K); Galpin 954 (BOL; K; PRE).

**13. *Bonatea cassidea*** Sond. in Linnaea 19: 81. (1847); Stewart *et al.* in Wild Orch. South. Africa: 101, pl. 10.6 (1982); la Croix & Cribb in Flora Zambesiaca, Orchidaceae 11: 46 (1995); la Croix & la Croix in African orchids in the wild and in cultivation: 155 (1997); Schelpe & Linder in Orchids of southern Africa: 141 (1999). Ill.: L.Bolus in Fl. Pl. South Africa, t. 302 (1928). *Habenaria cassidea* (Sond.) Rchb.f. in Ann. Bot. Syst. 1: 797 (1849); Bolus in J. Linn. Soc., Bot. 19: 340 (1882); Brown in Gard. Chron. 24: 307 (1885); Kraenzl. in Bot. Jahrb. Syst. 16: 80 (1893). Type: South Africa, [Eastern Cape], Uitenhage, Olifantshoek near Bushmans River, September, *Ecklon and Zeyher s.n.* (S!, holo.).

*Bonatea darwinii* Weale in J. Linn. Soc., Bot. 10: 470 (1869). Type: South Africa, [Eastern Cape], Bedford, Kagaberg, *Weale s.n.* (PRE!, lecto.; SAM!, islecto.); South Africa, [Eastern Cape], Bedford, shady places, *Weale s.n.* (BOL!, GRA!, syn.).

*Habenaria saundersiae* Harv. in Thes. Cap. 2: 29, fig. 147 (1863); Bolus in J. Linn. Soc., Bot. 19: 340 (1882); Kraenzl. in Bot. Jahrb. Syst. 16: 57-58 (1893), syn. nov. *Bonatea saundersiae* (Harv.) T.Durand & Schinz in Consp. Fl. Afr. 5: 89 (1895); Stewart *et al.* in Wild Orch.

South. Africa: 101 (1982); Schelpe & Linder in Orchids of southern Africa: 141 (1999). Type: South Africa, [KwaZulu-Natal], [Durban], without precise locality or date, *Saunders sub Wheelwright s.n.* (TCD!, holo.).

### Description

Erect, decumbent or scandent terrestrial herbs, 100–640 mm tall; *tubers* 1–8, spreading, elongate-oblong, tomentose. *Stem* slender, 95–630 × 1.5–5.0 mm. *Leaves* sometimes withered at flowering, 5–13, lax, cauline, lower leaves intermediately basal, sheathing, narrowly-obovate to oblanceolate, acute to acuminate, margins entire, rarely undulate, 20–250 × 4–19 mm. *Inflorescence* cylindrical, 40–212 × 34–64(–80) mm, lax to dense, 3–35-flowered; *bracts* sometimes withered at flowering, similar to upper leaves, sheathing, linear-oblong to ovate-lanceolate, apiculate, 10–25 × 2.5–7.5 mm. *Flowers* spreading, green and white, pedicel and ovary sub-erect to erect, 14–32 mm long. *Dorsal sepal* green, sub-erect to erect, elliptical-oblong, galeate, acute to acuminate, 7.0–13.5 × 3–5 mm; *lateral sepals* green, patent, strongly deflexed, oblong-lanceolate to ovate, acute to apiculate, base oblique, margins weakly to strongly revolute with small acute tooth on lower margin, 7.0–15.5 × 2.0–6.5 mm, adnate to lip and stigmatic processes for 3–6 mm. *Petals* upper lobe green or white, erect, linear, acute to acuminate, 6–14 × 0.5–2.0 mm, adnate to dorsal sepal, lower lobe white, spreading horizontally, weakly to strongly deflexed, falcate, lanceolate to widely-obovate, tip curved, acute to apiculate, 8.5–15.0(–19.0) × (1.5–)3.0–8.0 mm, adnate to claw of lip for 1–5 mm. *Lip* claw 3–9 mm, median lobe green or white, descending, weakly to strongly geniculate, filiform to linear, acute, 5.5–17.0 × 0.5–2.0 mm, lateral lobes white, descending, diverging, falcate, narrowly to broadly-ob lanceolate, tips curved, apiculate, 6.5–21.0 × 1.5–6.5 mm; spur toothed, curved, 12–26 mm long, clavate apex 8–17 mm long. *Anther* erect, acute, 2.5–5.5 mm long, canals slender, 3.5–5.5(–9.5) mm long.

*Stigmatic processes* clavate, projecting forwards and deflexed, weakly divergent, 3.5–7.0(–10.5) mm long, base adnate to lip. *Rostellum* 3-lobed, base auriculate, median lobe narrowly-cucullate, apiculate, margin ciliate, 3.0–5.5 mm long, lateral lobes linear, fused to anther canals, sub-erect to 45°, sub-parallel, 0.5–2.0(–6.5) mm long. Flowering period: July to September.

### **Habitat**

*Acacia* savannah, forest and thicket, from 60 m to 1500 m altitude. *B. cassidea* occurs consistently in open shady niches often in close proximity to water.

### **Distribution**

*Bonatea cassidea* ranges from Port Elizabeth in the Eastern Cape along the coast and near interior of South Africa as far north as Kosi Bay, with scattered records in Swaziland, Limpopo Province and the eastern highlands of Zimbabwe (Figure 3C).

### **Observations**

Populations of *B. cassidea* show a gradient in morphology from the Eastern Cape through to northern KwaZulu-Natal - with the mid-lobe and upper petal lobes changing from white to green, and the lower petal lobes and side lobes decreasing in width. Little is known about pollinators of this species, except for remarks by Weale (1869) regarding visits by the Skipper Butterfly *Gomalia elma* Trimen.

### **Specimens examined**

McMurtry 2519 (Hortus Siccus McMurtrianus); Wells 1598 (NU; PRE); Abbott 7001 (NH); O'Connor 32 (NU); Nicholson 1762 (PRE; Umtamvuna Herbarium); McMurtry 2489 (Hortus Siccus McMurtrianus); Germishuizen 1212 (PRE); Weale s.n. (BOL; GRA); Weale s.n. (PRE; SAM); Giffen 86 (PRE); Holmes s.n. (GRA); Watson 3 (PRE); Johnston

s.n. (NBG); Ranger 53 (PRE); Ranger s.n. (GRA); Pegler 780 (GRA; SAM); Flanagan 857 (NU; PRE; SAM); Howe s.n. (GRA); Crundall s.n. (PRE); Acocks 23917 (PRE); Wells 3071 (PRE); Acocks 16128 (PRE); MacOwan 1529 (PRE; SAM); Dodd s.n. (GRA); Dodd 7935 (GRA; PRE); Galpin 6534 (PRE); Dodd 25800 (PRE); McMurtry 929 (Hortus Siccus McMurtrianus); Venter 1084 (PRE); Lawn 1570 (NH); Ecklon and Zeyher s.n. (S); Lawn 1619 (NH): without collector NH 12392 (NH); Sanderson 822 (NH); Saunders sub Wheelwright s.n. (TCD).

### **Excluded names**

*Bonatea bracteata* G.McDonald & McMurtry = *Habenaria transvaalensis* Schltr.

*Bonatea tentaculifera* Summerh. = *Habenaria bonateoides* M.Ponsie



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## CONCLUSIONS

The initial aims of this thesis were to investigate species delimitations within *Bonatea* through examination of molecular, morphological and ecological differences; to determine the generic characters that define *Bonatea* as a monophyletic unit; to investigate the relationship of the genus to *Habenaria*; and, to provide a full revision of the genus including both southern and east African species.

Results suggest a recent and rapid radiation for the genus *Bonatea* with the consequence that only basal relationships can be resolved.

Members of the *Bonatea speciosa* complex (*Bonatea antennifera* Rolfe, *Bonatea boltonii* (Harv.) Bolus and *Bonatea speciosa* (L.f.) Willd.) were found to be distinct species; with *Bonatea porrecta* (Bolus) Summerh. and *Bonatea volkensiana* (Kraenzl.) Rolfe also embedded within the complex. *Bonatea saundersiae* (Harv.) T.Durand & Schinz was found to fall along the clinal range of variation within *Bonatea cassidea* Sond. and was thus reduced to synonymy of the latter. As a result of re-evaluation of the generic characters for *Bonatea*, *Bonatea bracteata* G.McDonald & McMurtry and *Bonatea tentaculifera* Summerh. are better placed in *Habenaria* as *Habenaria transvaalensis* Schltr. and *Habenaria bonateoides* M.Ponsie, respectively.

This thesis has contributed to a greater understanding of *Bonatea*, solving previous taxonomic problems and establishing *Bonatea* as a monophyletic clade. All hypothetical objectives were met, although the relationship of *Bonatea* to *Habenaria* is still uncertain. Future work should focus on the poorly known east African *Bonatea* species, greater sampling of *Habenaria* species, and use of techniques that are capable of resolving inter- and intra-generic relationships within rapidly radiating lineages.