

**The Phylogeny of *Brachycorythis*  
Lindl. and *Neobolusia* Schltr.**

David Chuba

SUPERVISOR: Prof. H. P. Linder.

Botany Department  
University of Cape Town  
Rondebosch, 7701, South Africa.  
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## ABSTRACT

The monophyly of the genus *Brachycorythis* has been tested. The phylogeny of the African genera of Orchidinae – Orchideae – Orchidaceae (*Schwartzkopffia*, *Brachycorythis*, *Neobolusia*, *Schizochilus*, *Dracomonticola*, *Holothrix* and *Bartholina*) is estimated with special attention to phylogenetic positions of *Brachycorythis* and *Neobolusia*. Pollen surfaces of twelve of the species are examined. Thirty-eight morphological and anatomical characters for 25 species of the species mentioned above are cladistically analysed and the stability of the different clades of the most parsimonious trees determined using various clade stability indices. The current delimitation of the Southern African Orchidinae genera, with *Neobolusia* and all the other genera being independent from *Brachycorythis* is supported. Pollen surface ornamentation also shows the only species of *Neobolusia* examined for this (*N. tysonii*) as being quite different from the rest of the genera studied in having intectate pollen whereas the rest have semi-tectate pollen. This has apparently further indicated support for the independence of *Neobolusia* from the genus *Brachycorythis*.

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## INTRODUCTION

*Brachycorythis* Lindl. and *Neobolusia* Schltr. belong to the subtribe Orchidinae (Orchideae, Orchidoideae, Orchidaceae), which presently contains 34 genera and approximately 361 species (Pridgeon et al., 1997). According to Dressler (1981) and Pridgeon et al. (1997), the subtribe is distributed throughout the Northern hemisphere, Asia and Africa. In tropical east Africa the tribe is represented by about 45 species in 9 genera (Summerhayes, 1968), whereas in South Central Africa it is represented by about 75 species in 10 genera (la Croix and Cribb, 1995). In Southern Africa it is represented by 42 species in 6 genera (Linder and Kurzweil, 1999). The Southern African Orchidinae genera include *Brachycorythis*, *Schwartzkopffia*, *Neobolusia*, *Schizochilus*, *Dracomonticola*, *Holothrix* and *Bartholina* (Kurzweil and Weber, 1991, Linder and Kurzweil 1995, Linder and Kurzweil, 1999).

The currently knowledge of the phylogenetic relationships at the generic level in the subtribe Orchidinae is quite poor, even though the knowledge of generic relationships in some other groups of the Orchidoideae such as Deseae has increased considerably in the past years (Dressler, 1981; Linder, 1986; Kurzweil, et al., 1991; Linder and Kurzweil, 1994; Linder & Kurzweil, 1995; Kores et al., 1997; Pridgeon et al., 1997).

Since the description of the genus *Brachycorythis* by Lindley in 1838, its taxonomy has under gone a lot of changes. Lindley (1838) described it as having a labellum (lip) with an inflated base or hypochile and the bluntly galeate perianth that is inserted very obliquely. However at the time Lindley only saw one species (*B. ovata*) and his opinion of the generic characters was based only on this species.

In 1844, Sonder described the new genus *Schizochilus* as having sepals that are free and equal, petals that are smaller than sepals and a trilobed lip. Later on Harvey and Reichenbach (1850) took a broad view of *Brachycorythis* and transferred to it two species of the genus *Schizochilus* Sond., a genus that was considered distinct by almost all the other authors.

On account of their different floral structure (sepals free, petals smaller than sepals and oblique, labellum with a fleshy callus and presence of a lip spur) and habits, Bentham and Hooker (1883), later on separated *Schizochilus* from *Brachycorythis* and transferred *B. tenuior* Rchb. f. and *B. macowaniana* Rchb. f. from *Brachycorythis* and placed them in *Schizochilus*, according to them, due to the presence of a spur on the lip. This view was also adopted by Pfitzer (1889). Later Schlechter (1895) however pointed out that the genus had been wrongly placed in *Diseae* by Bentham and Hooker (1883), and Pfitzer (1889). He viewed it as being closely allied to the genus *Platanthera*. He took this broader view of the genus by taking it as including the spurred species so as to incorporate the two genera *B. tenuior* and *B. macowaniana*, and also include *Schizochilus*. Though *Brachycorythis* is similar to *Platanthera* in many features, it can be differentiated by its peculiar habit, the tall slender column, the tendency for the petals to be fused to the lower part of the column (Summerhayes, 1955) and the coherence of the median sepal to the lateral sepals forming a hood. Schlechter (1895) also removed the species *B. tysonii* Bolus from *Brachycorythis* and placed it in his new genus *Neobolusia* Schltr.

In 1898, Rolfe dealt with the genus *Brachycorythis* and included in the genus only species with a boat shaped or hollowed-out but not spur like lip hypochile. He placed the spurred species in *Platanthera*.

At the same time Kraenzlin (1898), as opposed to Rolfe's view of the genus, included both spurred and non-spurred species in *Brachycorythis* but excluded *Schizochilus* and *Neobolusia*. He also pointed out that no line of demarcation could be drawn between the spurred and the boat shaped or hollowed out species. Rolfe also included at least one new clearly intermediate species, as regards spur development, *B. congoensis* Kraenzl.

In 1900 Kraenzlin described the genus *Schwartzkopffia*, based on a plant from Togo. He however did not mention anything about its affinity with *Brachycorythis*. The floral structure of *Schwartzkopffia* is very similar to *Brachycorythis* (Kurzweil and Weber, 1991), and Geerinck (1984) actually included it in *Brachycorythis*. Unlike

*Brachycorythis* however, *Schwartzkopffia* is a dwarf plant, generally not more than up to about 12 cm tall. It also differs from *Brachycorythis* by its leaves reduced to imbricate bracts covering the whole stem.

Rolfe (1912), in his treatment of the Southern African species of the genus *Brachycorythis*, in *Flora Capensis*, moved back species of *Neobolusia* to *Brachycorythis* based on its spurless hypochile. He also included in *Brachycorythis* a species originally described as *Platanthera virginea* by Bolus (1896). Rolfe initially defined this species as having petals about as long as the sepals but described the same species later on as having petals that are one third the length of sepals, the latter of which is true. He also transferred the spurred species, *B. tenuior* and *B. macowaniana* to *Platanthera*. Rolfe also placed *Schizochilus* in a separate genus in which it has been up to now. Schlechter (1915) later transferred *Brachycorythis virginea* to *Neobolusia* under the name *Neobolusia virginea* (Bolus) Schlecht.

In 1921, Schlechter after a comprehensive investigation, maintained *Schizochilus* and *Neobolusia* and divided *Brachycorythis* (in its wider sense) into four genera, *Brachycorythis* proper (with spurless flowers, petals united to the column, and naked viscidia), *Schwartzkopffia* Kraenzl. (species characterised by the leafless habit) *Gyladenia* Schltr (species with short spurs, a short column with free petals and naked viscidia), and *Diplacorchis* Schltr (species with long-spurred flowers and bursiculate viscidia). According to Schlechter (1921), the later three genera differed in the structure of the column as well as the labellum.

In 1925, Braid reviewed Schlechter's (1895) account and proposed some new species and varieties and expressed doubts about some of those recognised by Schlechter, but maintained the main lines of Schlechter's classification.

Summerhayes (1936) maintained the genera recognised by Schlechter, but later mentioned in his revision of the genus *Brachycorythis* (Summerhayes, 1955) that he had become increasingly doubtful of the distinctions between the genera separated from *Brachycorythis* by Schlechter. He re-examined the genera that Schlechter

worked on and stated that he was unable to find the distinctions described by Schlechter. In all the species examined, he found that the viscidia are quite naked and found no sign of any bursicle or similar structure (Summerhayes, 1955). With regard to the presence or absence of a spur, almost all intermediates are found between the long spurs such as found in *B. tenuior* and the apparently spurless hypochile found in *B. ovata* (the type species) (Summerhayes, 1955). The decurrence of the sides of the hypochile on to the epichile is also a very variable feature and again practically all intermediates can be found between the high abruptly terminating plates of species like *B. tenuior* and species like *B. buchananii* where there is no decurrence at all but the sides of the hypochile are continued as the margins of the hypochile. In view of the results of Summerhayes examination, he finally amalgamated all these genera under the original name *Brachycorythis*.

Dressler (1981, 1993), proposed a treatment of generic groups within Orchideae that separated *Neobolusia* from *Brachycorythis* based largely on the underground structures. He recognised four tentative alliances; (1) tuberoids lacking, (2) tuberoids palmate and attenuate (in which he included *Brachycorythis* Lindl. and *Schwartzkopffia*), (3) tuberoids spheroid (in which he included *Schizochilus* Sond.), (4) hairy African plants with basal leaves and with the petals and leaves frequently fimbriate (in which he included *Holothrix* Rich. ex. Lindl. and *Bartholina* R. Br.).

In 1995 Linder and Kurzweil found that the species known as *Neobolusia virginea* had earlier on been evidently misplaced in its genus. They described a new genus *Dracomonticola* and transferred to it this species, based on its spreading sepals, a lip with rudimentary side lobes, a rudimentary callus and petals that are about one third as long as the sepals (which links it to *Schizochilus*), the slight swelling at the lip base, the absence of a spur and the non drooping inflorescence (which differentiates it from *Schizochilus*). It also resembles *Neobolusia* and *Brachycorythis* by having a slightly saccate lip base. *Dracomonticola* however also differs from these two genera by its possession of a non-receptive stigmatic process and only two leaves, one basal and the other about midway up the stem. The genus *Dracomonticola* consists of only one species (Linder and Kurzweil 1995).



*Neobolusia* has frequently been considered as closely related to *Brachycorythis* (Bolus, 1893-1896; Schlechter, 1926; Senghas 1973-1974) and in modern literature it is maintained as a separate genus (Linder, 1980, Kurzweil and Weber, 1991). The genus *Brachycorythis* was referred to by Summerhayes (1955) as suggestive of a generalised sort of Orchidinae and based on its rather simple floral structure Kurzweil and Weber (1991), also suggested that it might be closer to the ancestral Orchidinae.

In this study *Holothrix* and *Bartholina* are used as outgroup taxa. The *Holothrix-  
Bartholina* alliance, according to Kurzweil and Weber (1991), is the most distinctive group in the entire sub tribe Orchidinae and according to Dressler (1981), “could well merit sub tribal status”. Szlachetko, (1991), actually placed *Bartholina* and *Holothrix* in two new subfamilies, Bartholininae and Herminiinae respectively. The *Holothrix-  
Bartholina* alliance is characterised by the flat basal leaves apparently appressed to the ground and the hairiness of the vegetative organs in some species and the frequent presence of a fimbriate lip and the petals (Dressler, 1981). The lateral gynostemium appendages correspond entirely to staminoides while filament excrescences are missing (Dressler, 1981). Both these characters are apparently shared derived characters (Kurzweil and Weber, 1991). Because of their distinctiveness in the entire tribe *Holothrix* and *Bartholina* are expected to show only slight relationship with the rest of the Orchidinae, hence the use of *Holothrix* and *Bartholina* as an outgroup taxon in this study.

It is evident that the existing data on the subtribe Orchidinae are quite inadequate as they are only mostly confined to details and a synthesis is badly needed (Dressler, 1981). Only Senghas’s (1973 - 1974) approach provides the most detailed treatment of generic groups in Orchideae so far, though it is criticised by Dressler (1981) because of much emphasis placed on the gynostemial characters. Of particular interest to this study is the generic position of genera *Brachycorythis*, *Neobolusia* and *Schwartzkopffia* whose separation into distinct genera have been said to appear unjustified (Kurzweil and Weber, 1991). However no phylogenetic analysis has yet been done to determine their possible phylogenetic positions.

In a more recent study, Pridgeon et al. (1997) using molecular sequence data found strong bootstrap support for the monophyly of subtribe Orchidinae (excluding *Holothrix*) as well as strong support for several genera within the subtribe. However none of the genera currently under study (except *Holothrix scropularia* Rchb. f., a member of our outgroup genus) were included in Pridgeon et al.'s (1997) study and molecular studies involving these taxa would further elucidate the phylogenetic relationships among the Orchidinae genera.

The aim of the study is to investigate the phylogeny of the African Orchideae genera *Brachycorythis* and *Neobolusia* and thus test the monophyly of *Brachycorythis*. Though a proper phylogenetic study would require a comprehensive study on a worldwide scale including information on such anatomical features as the ultra structure of pollen and seed cytology (Kurzweil and Weber, 1991, Pridgeon et al.'s (1997), including molecular work, this study included only vegetative floral morphology and pollen surface ornamentation structure and only the Southern African species. It is therefore only a contribution to the work required to fully understand the phylogenetic relationships at generic level in Orchidinae.

#### **MATERIALS AND METHODS:**

##### *Specimens*

The study was largely based on herbarium specimens and pickled material. The pickled specimens had been preserved in a mixture of water, 96% alcohol and glycerine (15:16:1), at the Bolus Herbarium (Botany Department, University of Cape Town). Twenty five wet and dry specimens (Table 1) consisting of 4 species of *Schizochilus*, 11 species of *Brachycorythis*, 2 species of *Neobolusia*, the single species of *Dracomonticola* and 5 species of *Holothrix* were examined. The dry specimens were also used to obtain vegetative characters. The species were selected on the basis of availability of pickled material and dried specimens. It would have been better to sample on phylogenetic divergence but the available material was not adequate for this.

**TABLE 1: Specimens used in the present study (pickled material and dried specimens) for vegetative and floral, morphological and anatomical characters, as well as pollen analysis. All specimens are deposited in the Bolus Herbarium.**

Specimens	Type of specimen	Collector (name & number)	Locality
<i>Brachycorythis congoensis</i>	Pickled	A. V. Hall, 667	Salisbury, Zimbabwe.
<i>B. inhambanensis</i> (Schltr.) Schltr.	Pickled	A. V. Hall, 222	Chimanimani Mts, Zimbabwe.
<i>B. macowaniana</i> Reichb. F	Pickled	Rosenbruck s.n.	Swellendam, R.S.A.
<i>B. ovata</i> Lindl.	Pickled	C. Latimer s.n.	East London, R.S.A.
<i>B. pubescens</i> Harv.	Pickled	E. Schelpe, s.n.	Drakensberg, R.S.A.
<i>Brachycorythis tenuior</i> (Rchb.f.) Summerhayes	Pickled	A. V. Hall, 666	Salisbury, Zimbabwe.
<i>Neobolusia tysonii</i> (H. Bol.) Schltr.	Pickled	H. P. Linder, 1986	Barberton, R.S.A.
<i>Schizochilus angustifolius</i> Rolfe	Pickled	E. Schelpe, 1799	Drakensberg, Cathedral park area, R.S.A.
<i>S. crenulatus</i> Linder	Pickled	A. V. Hall, 865	Graskop, R.S.A.
<i>S. flexuosus</i> Harv. Ex. Rolfe	Pickled	H. P. Linder, 2091	Bushmansnek, R.S.A.
<i>S. zeyheri</i> Sond.	Pickled	H. P. Linder, 2004	Belfast, R.S.A.
<i>Dracomonticola virginea</i>	Pickled	H.K. Kurzweil, 1320	Nanoles Nele, Lesotho.
<i>B. friesii</i> (Schltr.) Summerh.	Dry	E. A. Robinson, 5899	Mwinilunga, Zambia.

Table 1 continued

Specimens	Type of specimen	Collector (name & number)	Locality
<i>B. angolensis</i> (Schltr.) Schltr.	Dry	G Williamson, 1221	Kawambwa, Zambia.
<i>B. buchananii</i> (Schltr) Rolfe	Dry	R. K. Grosvenor, 500	Marandellas, Zimbabwe.
<i>B. congoensis</i>	Dry	O.B. Miller, 45759	Matobo district Zimbabwe.
<i>B. inhambanensis</i> (Schltr.) Schltr.	Dry	A & G, Hutchings and E. Plumstead, 1547	D.A. Port St. Johns, Mt. Thesiger, R.S.A.
<i>B. macowaniana</i> Reichb. f.	Dry	A. V. Hall, 1108	Humansdorp District, R.S.A.
<i>B. ovata</i> Lindl.	Dry	M. Rugters, s.n.	Port St. Johns, R.S.A.
<i>B. pleistophylla</i> Reichb. f.	Dry	O. B. Miller, 7551	Matobo district, Zimbabwe.
<i>B. pubescens</i> Harv.	Dry	A.V. Hall, 868	Pilgrim's Rest, District, R.S.A.
<i>B. tenuior</i> (Rchb.f.) Summerhayes	Dry	C. L. Leipoldt, 17075	Roos Senekal, 2529BB, R.S.A.
<i>B. velutina</i> Schltr.	Dry	Philomena, 5196	Inyanga, Zimbabwe.
<i>Bartholina ethelae</i> H. Bolus	Pickled	E. G. H. Oliver, 4776	Blackheath flats, R.S.A.
<i>D. virginea</i>	Pickled	F. K Hoener, 1631 and Boardman, 2	Sehlabathebe National Park, Matsa a Mafikeng, R.S.A.
<i>H. aspera</i> (Lindl.) Rchb. f.	Pickled	E. G. Oliver, s.n.	Clanwilliam, R.S.A.
<i>H. burchellii</i> (Lindl.) Rchb. f.	Pickled	H.P. Linder, 1584	Unknown.

Table 1 continued

Specimens	Type of specimen	Collector (name & number)	Locality
<i>H. hispidula</i>	Pickled	141/17 s.n.	Unknown.
<i>H. secunda</i> (Thunb.) Rechb. f.	Pickled	H. P. Linder, 1126	Clanwilliam, R.S.A.
<i>H. squamulosa</i> Lindl.	Pickled	s.n.	Near Kasteels Prt, R.S.A.
<i>N. stolzii</i> Schltr.	Dry	R. Grosvenor & J. Renz, 1110	Nyika Plateaux, Malawi.
<i>N. tysonii</i> (H. Bol.) Schltr.	Dry	H.P. Linder, 995 and Boardman, 40	Cape: Natatiele, Ramtseliso Gate, R.S.A.
<i>S. angustifolius</i> Rolfe	Dry	H.P. Linder, 4695	Natal, Bergville Cathedral Park Reserve, R.S.A.
<i>S. crenulatus</i> H.P. Linder	Dry	H.P. Linder, 850	Pilgrim's Rest, near God's Window, R.S.A.
<i>S. flexuosus</i> Harv. Ex. Rolfe	Dry	Boardman, 28	Transkei, Matatiele, Ramatseliso gate, R.S.A.
<i>S. zeyheri</i> Sond.	Dry	H.P. Linder, 2004	Transvaal, Belfast 2530 CA, R.S.A.

### *Morphological and anatomical characters*

Gross morphological characters such as the position, density and number of leaves, and the position and shape of the inflorescence were examined from herbarium specimens and verified from literature. For *Schwartzkopffia lastii* only the literature was used. Morphological and anatomical characters (Table 2) were then documented and scored for each of the selected species of the genera under study.

### *Dissection and drawing of flowers*

For each species, a single flower from the pickled material specimens was placed in a petri-dish containing distilled water, just enough to immerse it. The flower was then dissected using two pairs of forceps and examined under the Zeiss Stemi SV6 or Leica MS5 stereomicroscopes. Morphological and anatomical characters were noted and drawn. It was necessary sometimes to draw off some water, using a dropper, to reduce the movement of the specimens, or to add a bit of water for easy visibility of the specimen and to avoid desiccation. Both the lateral and ventral views of the whole flower were drawn using camera lucida, at appropriate magnifications (usually X8, X10, or X12), depending on the size of the flower. The lateral sepals were removed, and one drawn while spread out on a slide containing a drop of distilled water to prevent desiccation of the specimen. The dorsal sepal, the lateral petal and the lip were successively removed and drawn. At each stage of the dissection the remaining parts on the main floral structure were also drawn. The lengths of the spur, sepals and petals measured using the eye piece graticule to an accuracy of 0.1mm. The breadth of the lip blade was measured at the widest continuous point, and the length was measured from the tip of the epichile to the lowest point of the epichile. For the lips with a three lobed epichile, the lengths of the middle and lateral lobes were measured using the eye piece graticule to determine whether the lateral lobes were larger or smaller than the middle lobe (measurements were taken as shown in figure 1C).

For some specimens (Table 1), dissections and drawings were done using reconstructed herbarium specimens. For each of the species a flower was placed in a

50 ml beaker filled with about 40 ml water and with 2 drops of detergent added. This was then brought to boil and kept boiling gently for about 3 minutes. The specimen was then removed and dissected as above.

The lip was inspected for the presence of the callus and a lip spur, and if the latter was present, its size and shape were recorded. The shape of the ovary was also noted. For one of the species (*Schwartzkopffia lastii*), the data were collected only from herbarium specimens and from literature (la Croix and Cribb, 1995), as no pickled material specimens were available.

### *Pollen*

Pollen grains were obtained by scrapping them off from the anther cells of each pickled material specimen, onto a labelled SEM specimen stub containing a small drop of water and allowing them to air dry at room temperature for about 40 minutes. The stubs were numbered on white masking tape, attached to the side of the stub. The specimens were then sputter coated with gold/palladium (Au/Pd) and stored in a dessicator to keep them dry before viewing. Coating the specimens with gold/palladium (heavy metals), makes them electrically and thermally conductive, thus reducing charging of the specimen which reduces image quality (Watson et al., 1980). Such heavy metal coating also results in high yield of electrons, thus enhancing image resolution (Watson et al., 1980). The specimens were then viewed at x2000, x5000 and x10000 magnifications for each species, using the Leica stereoscan 440-(S440) scanning electron microscope. In SEM, electrons are directed at the surface of the sample, where they cause other electrons to be emitted. The scanning electron microscope then focuses these electrons onto a viewing screen. SEM thus reveals the surface structures of three-dimensional objects. The pollen was described and characters noted, particularly the surface ornamentation. Pictures of the whole grain and surface ornamentation details were stored electronically. These, or laser prints, were used to describe the surface micromorphology of the pollen.

### *Cladistic analysis*

The characters that were found to be cladistically informative are listed below (pages 14-21). Their distribution among the taxa in table 2.

#### *Character used for cladistic analysis*

The characters used in the cladistic analysis are described below. All multi-state characters were coded as non-additive.

**[1] Plant size;** 0 = dwarf up to 12cm tall 1; = more than 12cm tall.

There is a range of plant sizes amongst the species studied e.g. up to 80cm tall in *Schizochilus*, up to about 90cm in *Brachycorythis*, up to about 65cm tall in *Neobolusia*, but always less than 12 cm tall in *Schwartzkopffia*.

**[2] Stem;** 0 = completely covered with imbricate bracts; 1 = not covered with imbricate sheaths.

In *Schwartzkopffia* the short stem is completely covered by imbricate bracts (la Croix and Cribb, 1995) whereas in the other genera the leaves are usually reduced to bracts towards the tip of the stem and these do not completely cover the stem.

**[3] Leaves or bracts;** 0 = fully expanded present; 1 = reduced to bracts.

Fully expanded leaves are present in all species except *Schwartzkopffia lastii* where they are reduced to bracts. However there is a tendency for the leaves in all the species (excluding *Holothrix* and *Bartholina* species) to be broader at the base, getting smaller towards the tip.

**[4] Leaf position;** 0 = all basal; 1 = dense through out the stem; 2 = laxly arranged through out the stem; 3 = one at base and one midway; 4 = 2-5 clustered at base, the rest laxly arranged through out the stem.

Both the leaf number and position show a range of differences. In some species of *Brachycorythis* the leaves are quite dense, overlapping and found throughout the stem (e.g. *B. congoensis*) whereas in others (e.g. *N. tysonii*) the leaves are found through



out the stem, widely separated and not very numerous (i.e. up to 10). *Holothrix* and *Bartholina* species have one or two orbicular leaves appressed to the ground whereas *Dracomonticola virginea* has two leaves with one at the base and one midway up the culm. *Schizochilus* species have a number of basal leaves (i.e. up to 5) that are not appressed to the ground.

**[5] Basal leaves;** 0 = appressed to ground, 1 = not appressed to ground.

*Schizochilus* species usually have both basal and cauline leaves.

The basal leaves are usually not appressed to the ground. *Brachycorythis* mostly have cauline leaves. *Holothrix* and *Bartholina* species have basal leaves appressed to the ground whereas *Dracomonticola virginea* has one basal leaf which is not appressed to the ground and another cauline leaf about half way up the stem.

**[6] Shape of basal leaves;** 0 = linear, 1 = oblanceolate, 2 = orbicular.

In both *Holothrix* and *Bartholina* species the basal species are distinctly orbicular, whereas for *Schizochilus* the basal leaves are linear to oblanceolate.

**[7] Cauline leaves;** 0 = dense; 1 = laxly arranged.

There is a varying degree of density of leaves in all the species with cauline leaves. Leaves that overlap on the stem were treated as dense whereas those that are not overlapping were treated as laxly arranged. Most of the species of *Brachycorythis* have fairly densely arranged leaves and in *Neobolusia* leaves are usually laxly arranged.

**[8] Leaf number;** 0 = more than two; 1 = one or two.

The leaves are more than two in all genera *Holothrix* and *Dracomonticola*. However the difference between these two is as explained under character 15 below.

**[9] Inflorescence orientation;** 0 = slightly drooping; 1 = erect.

All species of *Brachycorythis*, *Schwartzkopffia*, *Neobolusia* and *Dracomonticola* have erect inflorescences whereas the inflorescences of *Schizochilus* are slightly drooping (nodding inflorescences) (la Croix and Cribb, 1995).

**[10] Inflorescence position;** 0 = about upper third of culm; 1 = about upper quarter or higher.

The inflorescence is positioned at about the upper third in the species of *Brachycorythis*, *Neobolusia* and *Holothrix* whereas it usually occupies a much higher position, about the upper quarter of the stem in or less in *Schizochilus* and *Dracomonticola*. *Bartholina* possesses a solitary flower found at the end of the stem.

**[11] Inflorescence density;** 0 = dense; 1 = laxly arranged

The inflorescence was considered dense if the flowers more or less completely obscured the stem and laxly arranged if not. Most of the species of *Brachycorythis* have dense inflorescences. However *B. friesii* and *B. pubescens* have fairly laxly arranged inflorescences. *Neobolusia* species and all the *Holothrix* and *Bartholina* also have laxly arranged inflorescences. Within *Schizochilus* of the four species studied two (*S. zeyheri* and *S. crenulatus*) have laxly arranged inflorescences

**[12] Flower number;** 0 = one; 1 = 2 to many.

In all *Brachycorythis* species the flower number is variable but generally up to 10 or more, in *Schizochilus* the flowers are about 5 to 50 (Linder, 1980) whereas in the *Schwartzkopffia* they are only up to 5 and in *Bartholina*, only one.

**[13] Floral bract;** 0 = longer than flower; 1 = not longer than flower.

All the species studied have floral bracts that are not longer than the flower except *B. macowaniana*, which has a very distinctly longer bract than the flower.

**[14] Ovary;** 0 = twisted; 1 = not twisted.

In most of the *Brachycorythis* species and *Holothrix burchellii*, the ovary shows three ribs corresponding to the carpels that make up the ovary. These are twisted to different degrees. Some species such as *Neobolusia stolzii* shows three ribs but the ovary is not twisted.

**[15] Sepals;** 0 = hairy; 1 = glabrous.

Hairy sepals are found within *Brachycorythis* in *B. pubescens* and *B. velutina*, and within *Holothrix* in *H. squamulosa* and *H. hispidula*. Even though the plant of *Bartholina ethelae* is generally hairy, the sepals are glabrous.

**[16] Lateral petals and dorsal sepal;** 0 = partially fused forming hood; 1 = free.

In *Brachycorythis* the lateral petals are appressed to the dorsal sepal such that they are partially fused together and form a hood around the gynostemium. In the rest of the species studied no such association is found.

**[17] Lateral sepal length;** 0 = less than or equal to 4.4 mm, 1 = above 4.4 mm.

All the *Holothrix* have a sepal of less than or equal to 4.4 mm and this separates them from *Bartholina*, with which they share a number of characters. Most of the species of *Brachycorythis* have sepal length of more than 4.4 mm, except *B. congoensis* and *B. buchananii*. In *Schizochilus* all the species studied except *S. angustifolius* have sepals more than 4.4 mm long.

**[18] Petals;** 0 = basally fused to gynostemium; 1 = free.

Fusion to the gynostemium is found only at the base of each gynostemium, as reported by Kurzweil and Weber (1991). In *Brachycorythis*, *Neobolusia* and *Schwartzkopffia*, they are fused by a small portion to the gynostemium at their basal part whereas in the other species, they are simply attached by their basal margins.

**[19] Petal and sepals;** 0 = equal; 1 = sepals larger than petals; 2 = petals larger than sepals.

In all the *Brachycorythis* species, the petals are equal or subequal to the sepals. In *Holothrix* and *Bartholina* the sepals are about 1/2 to 1/3 the length of the petals, whereas in *Schizochilus* species, *Dracomonticola*, *virginea*, *Schwartzkopffia lastii* and *Neobolusia stolzii* the petals are about 1/2 to 1/3 the length of the sepals.

**[20] Lip attachment;** 0 = broadly attached to gynostemium; 1 = narrowly attached to gynostemium.

In most of the species in the present study, the lip is broadly attached to the gynostemium (e.g. *B. ovata* Fig. 1b) except in *Neobolusia* (Fig.3) where the attachment is quite narrow.

**[21] Lip margin;** 0 = entire; 1 = serrated.

Lip margins in the species studied are mostly entire with *B. tenuior* showing slightly more or less serrated margins on the lateral lobes of the epichile and *B. congoensis* being distinctly serrated along the entire lip margin. Serration differs from lobing in the serrations are very small divisions that don't divide the lip deeply or into large parts (e.g. Fig 2a and 2b), whereas lobing involves division of the lip into large parts or deeply dividing it (e.g. Fig 1c and 2c). *B. tenuior* (Fig 2a and b) and *B. congoensis* have both features on the lip epichile.

**[22] hypochile;** 0 = sac; 1 = concave; 2 = flat.

The orchid lip is normally divided into two part, the epichile, which is the upper part, and the hypochile, which is the lower and usually smaller part of the lip. The hypochile shape ranges from flat in species of *Holothrix*, concave in species of *Schizochilus* and *Neobolusia* to sac like in some *Brachycorythis*, *Schwartzkopffia* and *Dracomonticola* species. The spurred species of *Brachycorythis* appear to have a saccate hypochile that continues into a spur.

**[23] hypochile margins;** 0 = decurrent onto epichile as two plates; 1 = continuous with sides of epichile.

In most of the species studied, the hypochile margins are continuous with the epichile margins, whereas in *B. tenuior* the hypochile margins are decurrent onto the epichile as two plates (Fig. 2b).

**[24] Lip spur;** 0 = present; 1 = absent.

The lip spur is a quite variable character in the species studied. Where it is absent the lower part of the lip (the hypochile) could be flat or concave or sometimes deeply depressed to form a sac. In cases where it is present, it is a more or less tube-like structure that protrudes downwards from the hypochile. It could be clavate or

subclavate, cylindrical or bifid, straight or curved. It is present in all *Holothrix* and *Schizochilus* species but absent in *Neobolusia*, *Dracomonticola*, *Schwartzkopffia* and some species of *Brachycorythis*. In *Brachycorythis* it ranges from absent in species such as *B. pubescens*, to well developed in species such as *B. tenuior*, *B. macowaniana* and *B. congoensis* in which the sac like hypochile continues into a conical or clavate spur.

**[25] Spur length;** 0 = more than 2 mm long; 1 = 2 mm long or less; 2 = Variable on same plant.

The measurements were taken from the lower part of the lateral sepal (Fig. 3b) to the tip of the spur. In the genus *Brachycorythis* all the spurred species have spurs more than 2 mm long. All *Holothrix* species have spurs less than 2 mm. In *Schizochilus* this character separates *S. angustifolius* from the rest of the species. In *Holothrix* the spur length is more than 2 mm in *H. secunda* and variable on the same plant in *H. burchelii*. The rest of the species of *Holothrix* have spurs 2 mm long or less. *Bartholina ethelae* has a spur longer than 2 mm.

**[26] Lip callus;** 0 = present; 1 = absent.

The lip callus is found in all species of *Schizochilus*, *Neobolusia* and some species of *Brachycorythis* e.g. *B. buchananii*, *B. pleistophylla* and *B. friesii*. It was not found in *Dracomonticola*, *Holothrix* or *Schwartzkopffia*.

**[27] Callus lobing;** 0 = one; 1 = three; 2 = filamentous.

The callus may be simple as in *Neobolusia tysonii*, three lobed as in *S. zeyheri*, *S. angustifolius* and *S. flexuosus*, or filamentous as in *S. crenulatus* (Fig. 2c).

**[28] Lip lobing;** 0 = 3-lobed; 1 = entire; 2 = more than three lobed.

In most of the species the lip epichile is divided into three lobes. For *Schizochilus* and *Brachycorythis* species, the lip is usually trilobed lobed to varying degrees. In *Holothrix* and *Bartholina ethelae* however, the lip epichile is mostly deeply or shallowly divided into five or more lobes, whereas it is entire in *Neobolusia tysonii*.

**[29] Lateral lobes of three lobed epichile;** 0 = well-developed 1 = not well developed.

The lobes however differ in their sizes and position on the epichile. The shape of the lip epichile is either simple or three lobed. There is a range of differences in three lobed epichiles from having lateral lobes near the base as in *B. friesii*, *D. virginea*, and *B. tenuior* to having them almost at the tip of the epichile as in *B. inhambanensis*, *B. pubescens*, *B. ovata*, *B. angolensis* and *B. velutina*. The sizes of the lobes also differ. To determine whether the lateral lobes of the epichile were well developed or not the size of the angle between the middle and the lateral lobes was used, if more than 90°, then the lateral lobes were taken not to be well developed (e.g. *B. ovata*, Fig. 1a and b), if vice versa, then the lateral lobes were taken to be well developed (e.g. *S. zeyheri* Fig. 1c).

**[30] Middle lobe of epichile;** 0 = larger than side lobes 1 = not larger than side lobes, lower than side, 2 = not larger than side lobes and lower than side lobes.

The lateral and middle lobes were measured as shown in fig. 1 to determine whether the middle lobe of the epichile was larger than the lateral lobes. The middle lobe is larger than the side lobes in *B. tenuior*, *B. congoensis*, *B. macowaniana* and *Dracomonticola virginea*. Whereas the middle lobe is of different sizes in *Brachycorythis* species, it is invariably longer than the lateral lobes in all the *Schizochilus* species (Linder, 1980). In the rest of the species the middle lobe of the epichile is not larger than side lobes but there are two forms of smaller lobes, one in which the middle lobe is lower than the side lobes as in *B. buchananii* and *B. pleistophylla* and the other in which the middle lobe is higher than the side lobes as in *B. ovata*, *B. pubescens*, *B. angolensis*, *B. velutina*, *B. friesii* and *Schwartzkopffia lastii*.

**[31] Larger middle lobe of epichile;** 0 = about twice larger, 1 = about three times larger, 2 = more than 3 times larger.

In all the *Schizochilus* species the middle lobe of the epichile is either about twice larger than the lateral lobe as in *S. zeyheri* and *S. crenulatus* or about three times larger as in *S. flexuosus* and *S. angustifolius*. In *B. congoensis*, *B. macowaniana* and *B. friesii*, the middle lobe is about twice larger than the lateral lobes. *B. tenuior* and

*Dracomonticola virginea* have middle lobes that are more than three times longer than the side lobes.

**[32] Lip epichile;** 0 = bent down more or less at its junction with the hypochile, 1 = not bent down.

Some of the *Brachycorythis* species (*B. macowaniana*, *B. pubescens* and *B. angolensis*) have a lip epichile that is bent down more or less at the junction of the epichile and the hypochile. This character state was not observed in the rest of the species studied.

**[33] Stigma sterile process;** 0 = present 1 = absent.

The stigma sterile process is found only in *Dracomonticola virginea*. This is a short, occasionally bilobed and non-receptive process that projects forward from the stigma (Linder and Kurzweil, 1995).

**[34] Anther;** 0 = elongate, 1 = not elongate.

In most of the species studied the anthers are sessile and more or less similar, whereas in *Bartholina ethelae*, it is most distinct in being elongate and possessing anther canals.

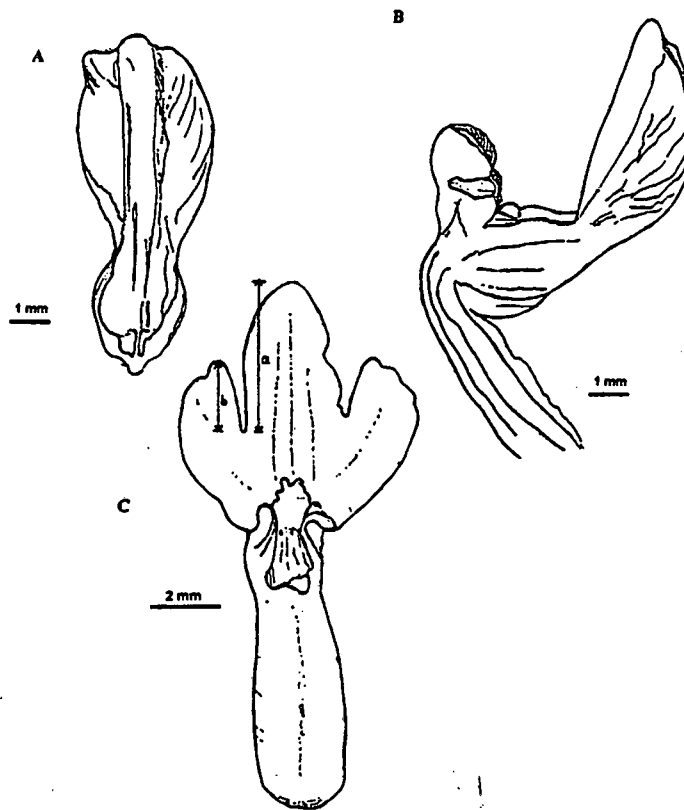
**[35] Pollen,** 0 = tectate, 1 = intectate.

**[36] Pollen surface,** 0 = reticulate, 1 = rugose

**[37] pollen lumina,** 0 = very small, 1 = small, 2 = large

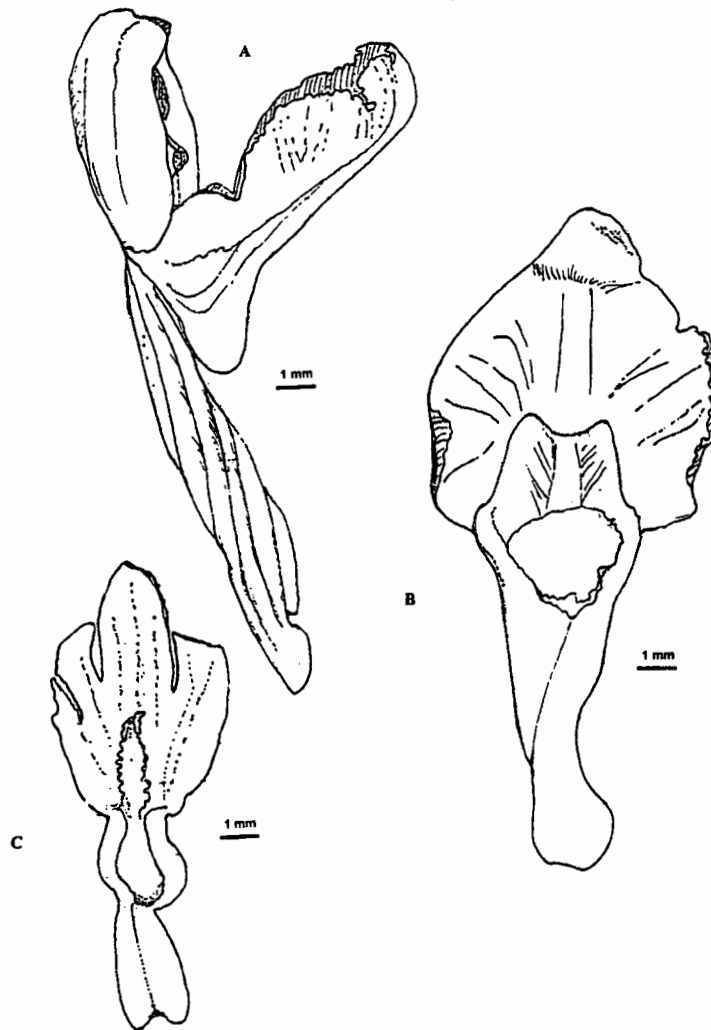
**[38] muri,** 0 = thin, 1 = thick, 2 = very thick

The pollen characters states are described in the results section.

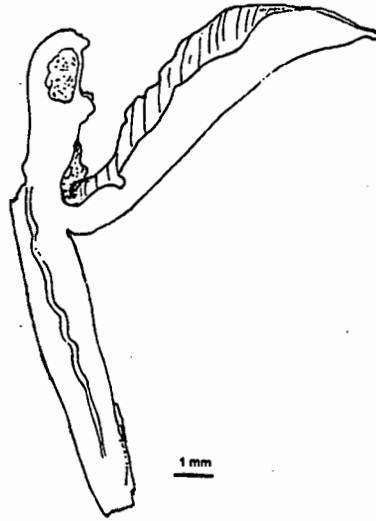


**Fig. 1: (a) Lip of *B. ovata* showing lateral lobes that are not well developed (i.e. size of the angle between the middle and the lateral lobes, less than  $90^{\circ}$ ) (C. Latimer s.n.). (b) Lateral view of *B. ovata* with sepals and petals removed showing the broad attachment of the lip to the gynostemium (C. Latimer s.n.); (c) Lip of *S. zeyheri* showing well developed (size of the angle between the middle and the lateral lobes, less than  $90^{\circ}$ ) (H. P. Linder, 2004), it also shows comparative measurements of the lateral and the middle lobes. 'a' = length of middle lobe, 'b' = length of lateral lobe. Drawn by D. Chuba.**





**Fig 2: (a) Lateral view of flower of *B. tenuior*; diagram showing three lobed with serrations along the lateral margins and hypochile margins of the lateral lobes (A. V. Hall, 666). (b) Ventral view of *B. tenuior* showing three lobed lip with serrations along the margins and hypochile margins decurrent onto the epichile as two plates (A. V. Hall, 666); (c) Three lobed lip of *Schizochilus crenulatus* with filamentous callus and a bifid spur (A. V. Hall, 865). Drawn by D. Chuba.**



**Fig 3: Lateral view of *Neobolusia tysonii* flower with sepals and petals removed, showing the narrow attachment of the lip to the gynostemium (H. P. Linder, 1986). Drawn by D. Chuba.**

**TABLE 2:** Distribution of characters states among the taxa in the study. Missing characters and inapplicable characters are both coded as “?” since they are treated in the same way by both Macclade version 3.07 and PAUP version 4.0 (the programmes used in the analysis). All other character states are as indicated in above. Four of the characters (4, 28, 37 and 38) were polymorphic. The generic abbreviations are as follows; *H.* = *Holothrix*, *Bar.* = *Bartholina*, *B.* = *Brachycorythis*, *N.* = *Neobolusia*, *S.* = *Schizochilus*, *D.* = *Dracomonticola* and *Schw.* = *Schwartzkopffia*.

<i>H. aspera</i>	10002?1111111110120021011?2????111?????
<i>H. burchellii</i>	110002?1111110110120021021?3????111????
<i>H. secunda</i>	110002?1111111110120021001?2????111????
<i>H. hispidula</i>	110002?1111111010120021011?2????111????
<i>H. squamulosa</i>	110002?1111111010120021011?2????111????
<i>Bar. ethelae</i>	110002?1101011111100021001?3????110????
<i>B. tenuior</i>	1101??00110110101000100001?01021110011
<i>B. inhambanensis</i>	1101??001101101010000011?1?002?0110011
<i>B. congoensis</i>	1101??00110110100000101001?00001110111
<i>B. ovata</i>	1101??001101101010000011?1?012?1110021
<i>B. pubescens</i>	1101??001111100010000011?1?002?1110111
<i>B. macowaniana</i>	1101??00110100101000001001?000001100?0
<i>B. buchananii</i>	1101??001101101000000011?00001?111????
<i>B. pleistophylla</i>	1101??001101101010000011?1?001?111????
<i>B. angolensis</i>	1101??10110110101000001001?012?011????
<i>B. velutina</i>	1101??001101100010000011?1?002?111????
<i>B. friesii</i>	1101??10111110101000001001?0020111????
<i>N. tysonii</i>	1101??101111111110010111?001???1111???
<i>N. stolzii</i>	1101??101111111110110111?001???111????
<i>S. zeyheri</i>	110210?000111111110011000100001110021
<i>S. flexuosus</i>	110211?000011111110011000100011110120
<i>S. crenulatus</i>	110210?000111111110011000200001110002
<i>S. angustifolius</i>	110210?0000111110110011011?00011110011
<i>D. virginea</i>	11031???1001111111100011?0001021010010
<i>Schw. lastii</i>	001?????10?1111110000011???002?111????

The data matrix (table 2) was analysed using parsimony analysis as implemented in PAUP version 4.0 (Swofford, 1998). Owing to the large size of the data set it was not possible to perform an exact method (Kitching, et al., 1998). The most parsimonious cladograms were therefore generated using the heuristic search option of PAUP version 4.0, using the stepwise addition of sequences, and with branches having maximum length zero collapsed to yield polychotomies. The Branch swapping option was done using the tree bisection–reconnection (TBR) algorithm with MULPARS option in effect, and initial ‘maximum trees saved’ setting of 100. The TBR branch-swapping algorithm divides the initial tree into two subsets by bisecting a branch between nodes and then pruning both resulting free branches leaving two disjoint subtrees. The two subtrees are then reconnected by creating linking branch between them. All possible bisections and reconnections are then evaluated. The trees in this analysis were outgrouped to *Holothrix burchellii*, *H. aspera*, *H. secunda*, *H. squamulosa*, *H. hispidula* and *Bartholina ethelae* (which differ from the rest of the taxa in having a multi-lobed (i.e. more than three lobed) lip and 1 or two basal leaves that are appressed to the ground).

The assumptions involved in designating these taxa as outgroup are; first, if a feature is present among many relatives of a monophyletic group, or best of all in the sister group, and if its homologue occurs, sometimes or universally, in the members of the monophyletic group, it will have been primitive for the group (Nixon and Carpenter, 1993). Second, the outgroup taxa are assumed to be sufficiently close to the in group taxa.

All transformations were weighted equally (Fitch parsimony). Fitch parsimony (Fitch, 1971, Hartigan, 1973) allows free transformation of a state into any other state with the cost of only one additional step in tree length, thus permitting free reversibility of transformations.

### Successive weighting

To obtain a single more resolved tree, successive weighting was applied (Farris, 1969, Carpenter, 1988). This assigns weights to characters. The characters that have low consistency index (incongruent with the other characters) receive low weight and those with high consistency index (i.e. congruent with the other characters) are given

higher weighting. However use of the consistency index does not result in completely homoplasious characters being assigned a value of 0, and therefore in this analysis the approach using the rescaled consistency index ( $rc = ri \times ci$ ) as the value of weight (Farris 1988), was followed. This is a preferred approach as it achieves a value of 0 for such characters. Successive weighting is also a check on whether homoplasy (i.e. low consistency index) could obscure character information (Farris 1969). The supporting characters for this cladogram and the node numbers were mapped onto it.

#### Strict Consensus Tree

In cladistic analysis, multiple parsimonious trees are often obtained due to different character state optimisations of homoplastic characters or from the choice of which characters should be homoplastic (Anderberg and Tehler, 1990). To synthesise one taxonomy from these many hypotheses the strict consensus was calculated using PAUP version 4.0 (Swofford, 1998). It illustrates components common to all the equally parsimonious cladograms of the analysis (Anderberg and Tehler, 1990), and thus provides a conservative estimate of the best single tree.

#### Forcing the topology

An analysis of the effects of placing *Neobolusia* in various places in the *Brachycorythis* clade was carried out in Macclade version 3.06 (Maddison and Maddison, 1992) using the tree manipulation facility. This was done to determine the cost of placing *Neobolusia* in different positions and thus possibly determine the strength or weakness of its current position.

#### Bremer support (Decay index)

This is the number of extra steps required before a clade is lost from the strict consensus tree of the near minimum length cladograms (Bremer, 1988). This is done to explore the stability of the data behind the cladogram (Bremer, 1988). To calculate the Bremer support, for the different clades in the strict consensus cladogram, all cladograms one step longer than the minimum were found. The strict consensus of these plus the most parsimonious cladograms was constructed. This process was repeated increasing the size of the sub-optimal cladograms by one step each time until all the clades were lost. Each time a clade was lost the number of extra steps was

noted and the number of extra steps required for this to occur is the Bremer support for that particular clade.

#### Bootstrap analysis

To further assess the support for the nodes in the estimated phylogeny Bootstrap analysis (Felsenstein, 1985) was conducted. The procedure goes on by random sampling either the character rows or columns in a data set to build up a bootstrap data set of the same size as the original data set, which is analysed to give a tree or a number of trees. This procedure is repeated at least 100 times and the percentage of occurrence of a particular node among the trees of the sample data sets is considered as an index of support. However this does not give true confidence limits in a statistical sense. Bootstrapping was conducted using the Heuristic (TBR algorithm with MULPARS effected) search with a random number seed of 1 and set to 100 replicates. All groups (nodes) with a frequency of greater than 50% were retained.

#### Character exclusion (Jackknife)

To further assess the stability of each clade obtained from analysis of the data set, the minimum number of characters that when removed cause resolution of the clade to be lost (i.e. to be absent from the strict consensus tree) (Davis, 1993) was determined. This was done by successive exclusion of characters using the character exclusion/inclusion facility of PAUP version 4.0. After each character exclusion, the general heuristic search was run followed by the consensus tree calculation. Changes in tree topology, if any, were then compared with the strict consensus tree calculated with all characters included. Character removal may also facilitate more detailed analysis of the character interactions that cause clades to be resolved under global parsimony (Davis, 1993).

#### Taxa exclusion (Jackknife approach)

Taxa exclusion or Jackknife approach (Lanyon, 1985) is another way of determining the stable portions or subsets of the estimated phylogeny. 'N' computer runs of the heuristic search were made (where N being the number of in-group taxa). In each run

a single taxon was omitted, thus producing 19 modifications of the original data set. This simulates extinctions and possible outcomes had it not been possible to analyse any one of the taxa (Lanyon, 1985). At each stage calculation of the strict consensus tree was performed. The resulting consensus trees, after every taxon exclusion step, were compared with the strict consensus tree calculated with all the taxa included.

## RESULTS

### Pollen surface ornamentation

The following is the description of the different pollen types observed for the species for which the pollen was available.

#### *1 S. zeyheri*

Semi-tectate, with irregular perforations or lumina, having diameters greater than the width of the pollen wall between them, thus forming an irregular reticulum held by free columellae that is quite distinct (Figure 4: 1b).

#### *2 S. crenulatus*

Semi-tectate, with irregular perforations or lumina, having diameters less than the width of the pollen wall between them, thus forming a more or less reticulate ornamentation. The columellae are not easily distinct (Figure 4: 2b).

#### *3 S. flexuosus*

Semi-tectate, with irregular perforations or lumina having diameters greater than the width of the pollen wall between them. They form a clearly distinct but rugulate reticulum held by free columellae (Figure 4: 3b).

#### *4 Schizochilus angustifolius*

Semi-tectate, with irregular perforations or lumina having diameters greater than the width of the pollen wall between them. They form a more or less irregular reticulum held by free columellae (Figure 5: 4b).

5 *Neobolusia tysonii*

Intectate, with the numerous columellae standing out to form flat pillars (pilate) (Figure 5: 5b).

6 *B. inhambanensis*

Semi-tectate, with irregular perforations or lumina having diameters smaller than the width of the pollen wall between them. They more or less form a rugulate reticulum. The walls or muri appear generally thicker than the lumina and slightly collapsed close together. The columellae are not easily distinct (Figure 5: 6b).

7 *B. macowaniana*

Semi-tectate, with irregular perforations or lumina with diameters larger than the width of the pollen wall between them. They more or less form a reticulate ornamentation. The walls or muri appear slightly collapsed but not very close together unlike in *B. inhambanensis*. A number of muri are also interrupted. The columellae are quite distinct in some places (Figure 6:7b).

8 *B. ovata*

Semi-tectate, with irregular perforations or lumina having diameters less than the width of the pollen wall between them, thus forming a more or less irregular reticulum. The columellae are not easily distinct (Figure 6: 8b).

9 *Brachycorythis congoensis*

Semi-tectate, with very irregular lumina. The muri or pollen wall between the perforations are also very irregularly arranged and not completely anastomosing. They form a rugose ornamentation. The walls or muri appear quite collapsed close together and columellae are not easily distinct (Figure 6: 9b).

10 *B. pubescens*

Semi-tectate, with very irregular or perforations or lumina. The muri or pollen wall between the perforations are also very irregularly arranged, collapsed close together



and not completely anastomosing, forming a more or less rugulose ornamentation. The columellae are not easily distinct (Figure 7 10b).

11 *B. tenuior*

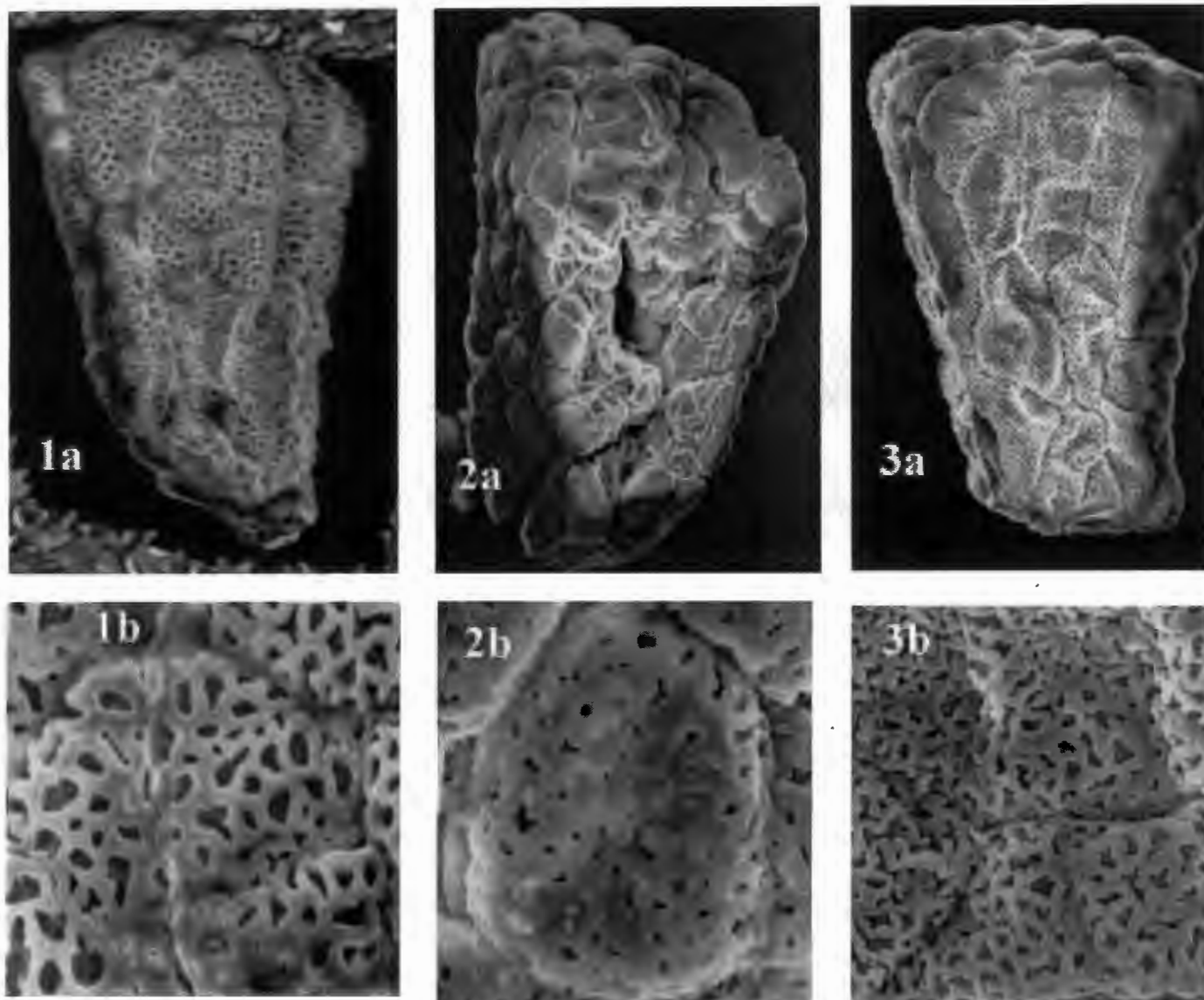
Semi-tectate, with irregular perforations or lumina having diameters less than the width of the pollen wall between them, thus forming a more or less reticulate ornamentation, with the muri are slightly collapsed close together. The columellae are not easily distinct (Figure 7: 11b).

12 *Dracomonticola virginea*

Semi-tectate, with irregular perforations or lumina having diameters greater than the width of the pollen wall between them. They form a clearly distinct but irregular reticulum held by free columellae (Figure 7: 12b). The ornamentation is quite similar to that of *B. macowaniana* except the muri are generally not interrupted.

The pollen surfaces are quite similar in the different genera: for example, the pollen surface ornamentation observed in *S. crenulatus* is similar to that observed in *B. ovata*. *Neobolusia tysonii* shows the most distinct type of ornamentation in lacking the tectum whereas all other species are semi-tectate. *B. macowaniana* (Figure 6: 7b) has very similar ornamentation to *Dracomonticola virginea* (Figure 7: 12b) and *S. flexuosus* (Figure 5: 3a & b). All the semi-tectate pollen in this study have either reticulate or rugose ornamentation.

The other morphological variation has been summarised above (pages 14 - 21)



**Figure 4: Scanning electron micrographs of *S. zeyheri* (1a, mg. x2000 and 1b, mg. x10000), *S. flexuosus* (2a, mg. x2000 and 2b, mg. x10000) and *S. crenulatus* (3a, mg. x2000 and 3b, mg. x10000). See text for description of the pollen surface ornamentation.**

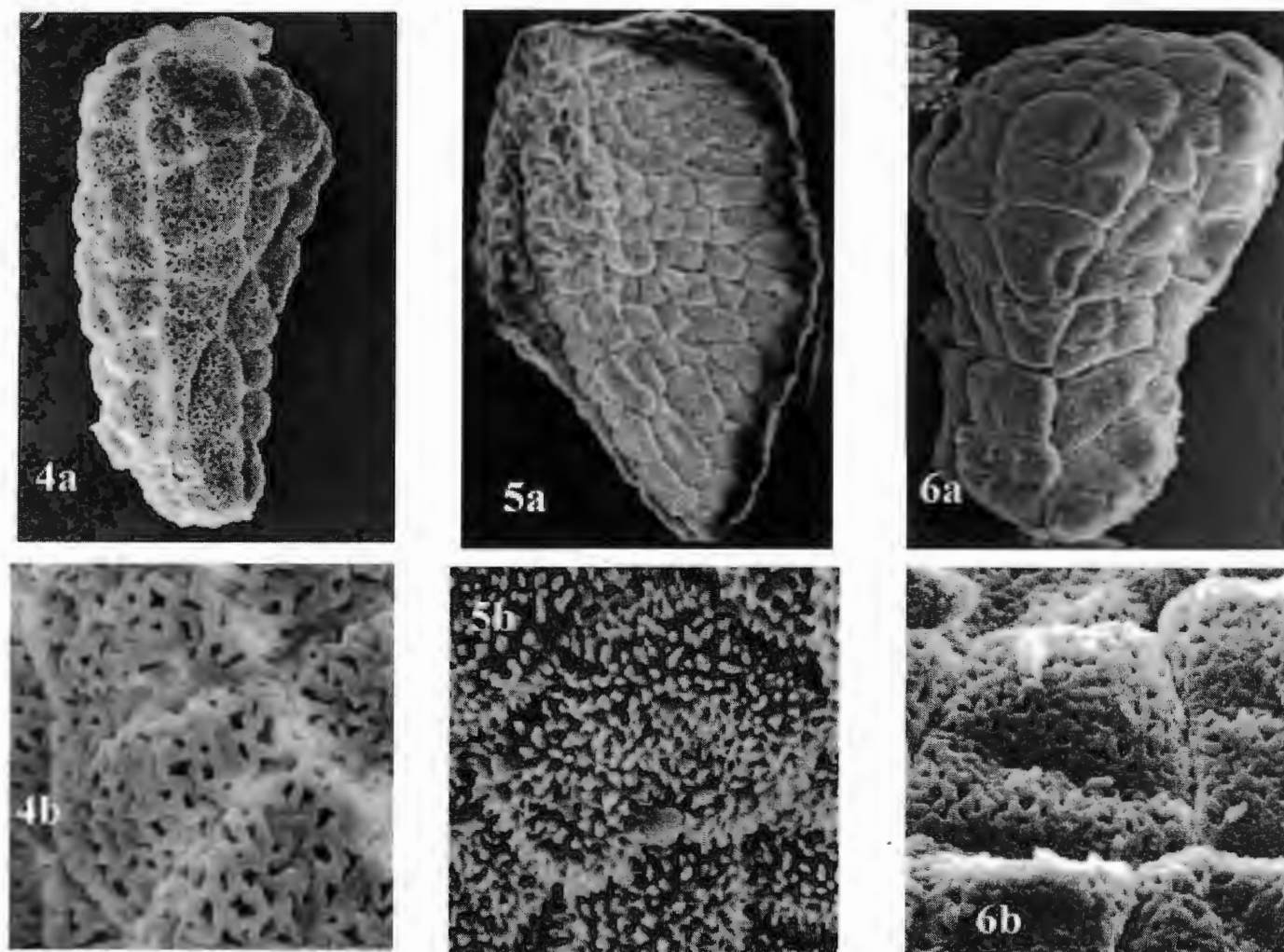
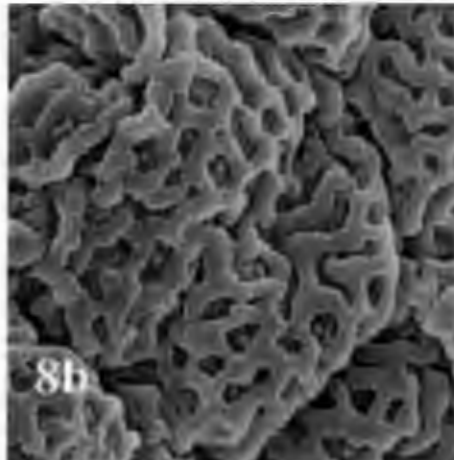
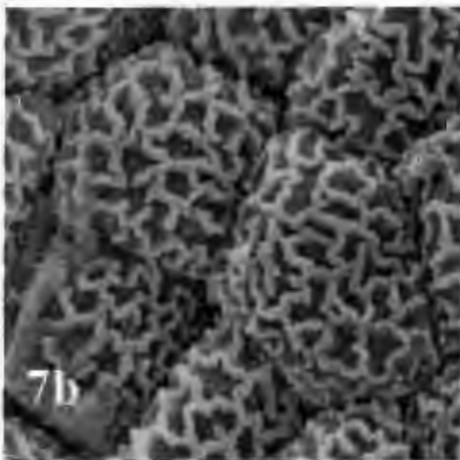
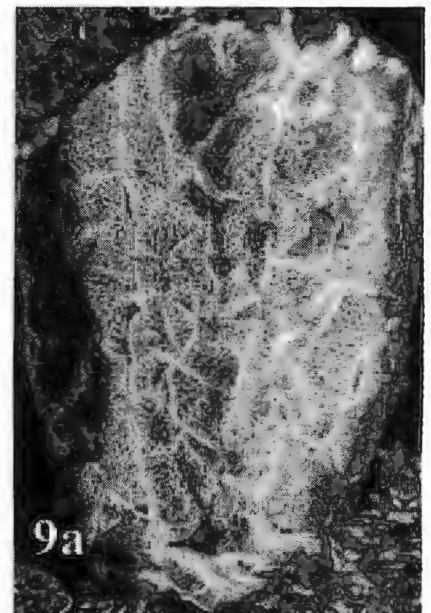


Figure 5: Scanning electron micrographs of *Schizochilus angustifolius* (4a, mg. x2000 and 4b, mg. x10000), *Neobolusia tysonii* (5a, mg. x2000 and 5b, mg. x10000) and *B. inhambanensis*, (6a, mg. x2000 and 6b, mg. x10000). See text for description of the pollen surface ornamentation.



**Figure 6: Scanning electron micrographs of 7 *B. macowaniana*, (7a, mg. x2000 and 7b, mg. x10000), *B. ovata*, (8a, mg. x2000 and 8b, mg. x10000) and *B. congoensis*, (9a, mg. x2000 and 9b, mg. x10000). See text for description of the pollen surface ornamentation.**

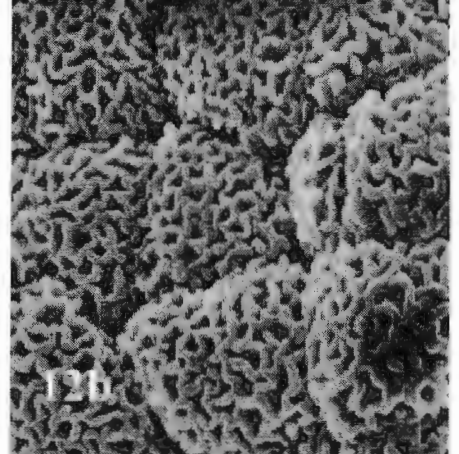
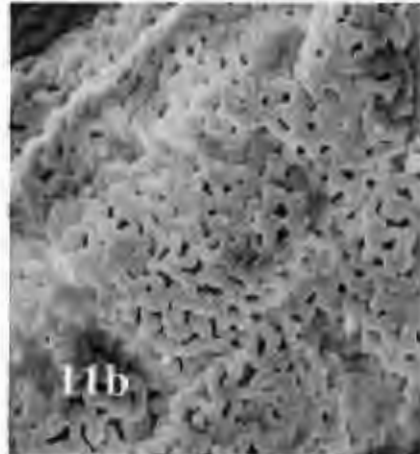
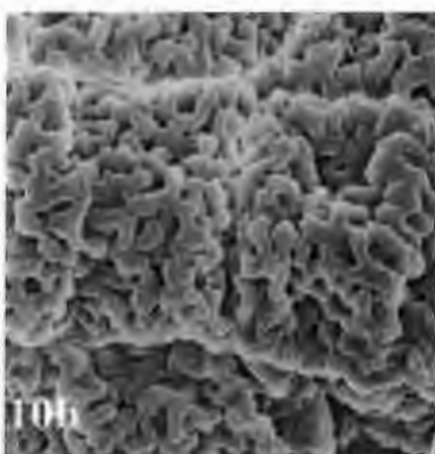
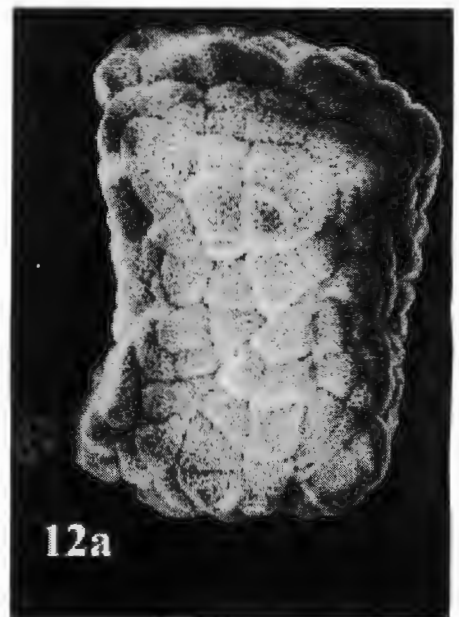


Figure 7: Scanning electron micrographs of *B. pubescens* (10a, mg. x2000 and 10b, mg. x10000), *B. tenuior* (11a, mg. x2000 and 11b, mg. x10000) and *Dracomonticola virginea* (12a, mg. x2000 and 12b, mg. x10000). See text for description of the pollen surface ornamentation.

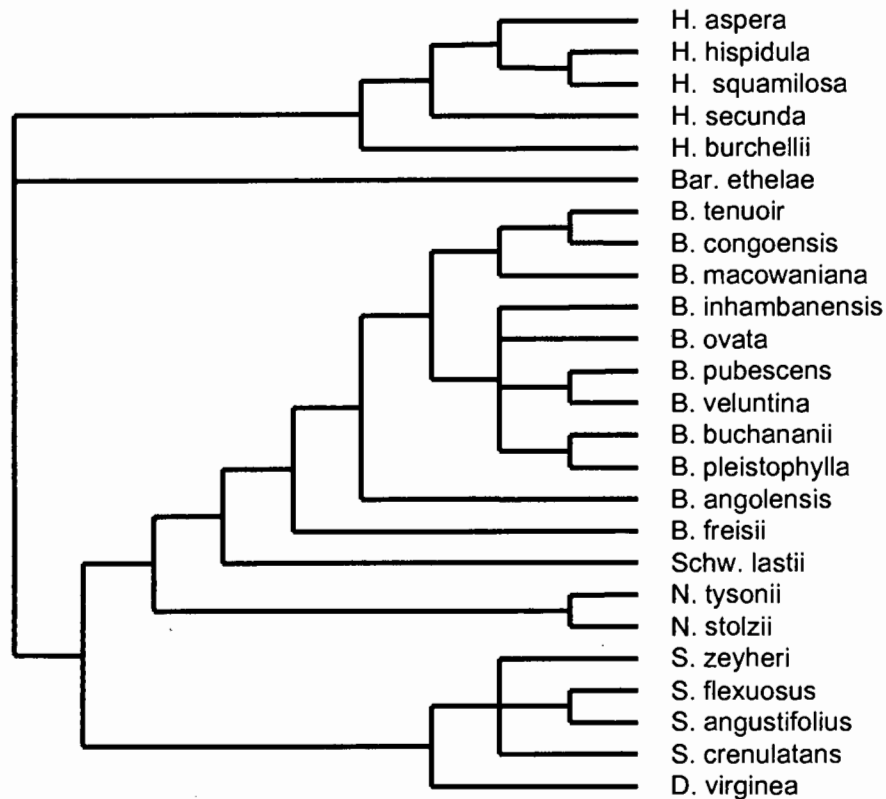
## *Cladistic analysis*

### *Parsimonious trees*

Cladistic analysis yielded 18 equally parsimonious cladograms, 82 steps long, with a consistency index (C.I.) of 0.622 and retention (R.I.) index of 0.801. All the eighteen most parsimonious cladograms showed two basal clades for the ingroup taxa, the *Brachycorythis* clade and the *Neobolusia*, *Schizochilus*, *Dracomonticola*, *Schwartzkopffia* clade. Within the latter clade are four smaller clades, the first one consisting of *Neobolusia* alone, the second consisting of consisting of *Schizochilus* alone, a third clade consisting of *Dracomonticola* and *Schizochilus* the fourth consisting of *Dracomonticola*, *Schizochilus* and *Schwartzkopffia* (Fig. 9). The 18 cladograms differed in the topology within the *Brachycorythis* clade and within the *Schizochilus* clade. In both cladograms *Neobolusia* is placed as sister to the *Schizochilus*, *Dracomonticola*, *Schwartzkopffia* clade.

### *Successive weighting*

The successive weighting was done only twice before the cladogram stabilised. One cladogram was found with a tree length of 39315 steps, consistency index (C.I.) of 0.846 and a retention index (R.I.) of 0.930. This cladogram is the topology presented in figure 8. However this tree differed from the consensus tree and from all the parsimonious trees in the position of *Schwartzkopffia*, which in the consensus tree appears at the base of the *Schizochilus*, *Dracomonticola* clade but occurs at the base of *Brachycorythis* in the successively weighted tree. One of the consensus trees however was the same as the consensus tree and this is the cladogram used to map the characters and for subsequent discussion.



**Fig 8:** The cladogram obtained after successively weighting the data; tree length = 39315 steps, consistency index (C.I.) = 0.846 and a retention index (R.I.) = 0.930. The Cladogram shows two basal clades, one consisting of *Brachycorythis*, *Schwartzkopffia* and *Neobolusia*, and the other one consisting of the *Schizochilus*, *Dracomonticola* *virginea* clade. *Schwartzkopffia* is placed at the base of the *Brachycorythis* clade and the *Neobolusia* clade at a more basal position next to *Schwartzkopffia lastii*.

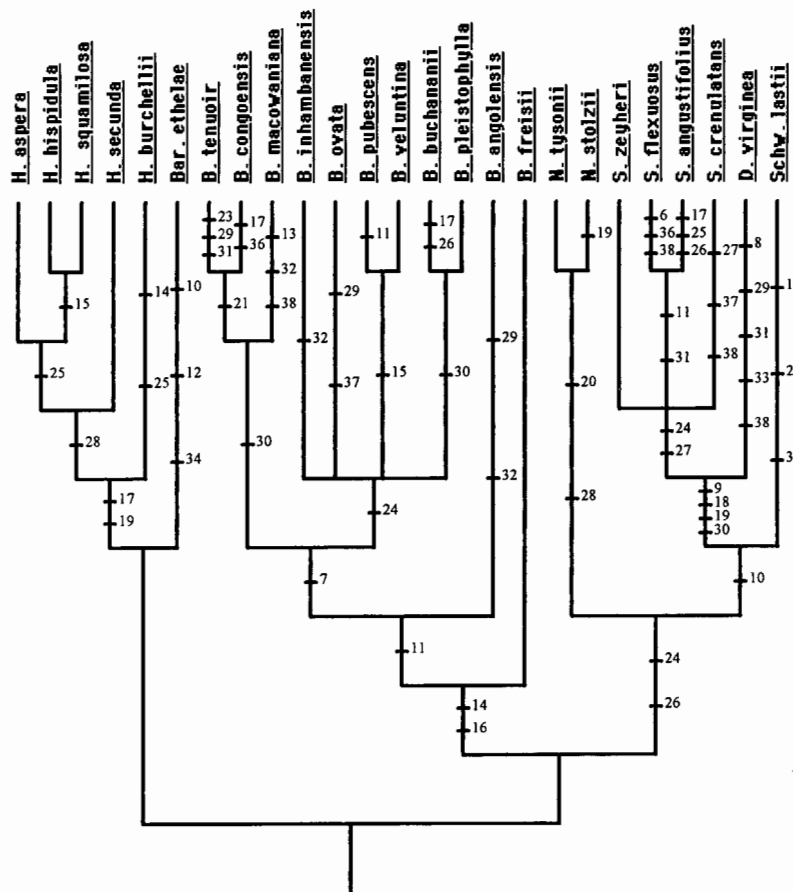
Strict Consensus Tree

The strict consensus tree calculated (Figure 9: L =82 C.I. = 0.622, and R.I. = 0.801) was exactly the same as one of the most parsimonious trees. Two basal clades were distinct amongst the ingroup taxa, the *Brachycorythis* clade and the *Neobolusia*, *Schizochilus*, *Dracomonticola*, *Schwartzkopffia* clade. Within the latter clade are four smaller clades, the first one consisting of *Neobolusia* alone, the second consisting of consisting of *Schizochilus* alone, a third clade consisting of *Dracomonticola* and *Schizochilus* the fourth consisting of *Dracomonticola*, *Schizochilus* and *Schwartzkopffia*. Figure 9 shows the strict consensus tree with all unambiguous character changes mapped onto it.

Eighteen homoplasies and 3 reversals were observed. Twelve autapomorphies were observed, 1 for *B. tenuior*, 1 for *Brachycorythis macowaniana*, 1 for *S. flexuosus*, 3 for *S. crenulatus*, 1 for *Dracomonticola virginea* and 3 for *Schwartzkopffia lastii*. The other 4 autapomorphies occur in the outgroup taxa, 1 for the *H. burchellii* and 3 for *Bartholina ethelae*.

Seven synapomorphies (table 3) were observed, 1 for the *Brachycorythis* clade, one for the clade including *B. tenuior*, *B. congoensis*, *Brachycorythis macowaniana*, *B. inhambanensis* and *B. ovata*, *B. pubescens*, *B. velutina* *B. buchananii* and *B. pleistophylla*, 1 for the *B. tenuior*, *B. congoensis* clade, 1 for the *B. buchananii*, *B. pleistophylla* clade, 1 for the *Neobolusia* clade and 2 for the *Schizochilus* clade. The last one occurs in the outgroup for the *Holothrix* clade.





**Fig. 9** Strict consensus of the eighteen most parsimonious trees, with all unambiguous character changes mapped onto it. Eighteen homoplasies and 3 reversals were observed. Twelve autapomorphies were observed, eight for the ingroup taxa and 4 for the outgroup taxa. Seven synapomorphies (see table 3) were observed, four in the ingroup and one in the outgroup. For more details see text.

**Table 3: Eight synapomorphies for the estimated phylogeny. Five were found in the in group taxa whereas three occur in the outgroup.**

Clade supported by node of interest	Synapormorphies and Character numbers
<i>Brachycorythis</i> clade	Lateral petals and dorsal sepal lightly fused and forming hood over the gynostemium (16)
<i>B. tenuior</i> , <i>B. congoensis</i> , <i>Brachycorythis macowaniana</i> , <i>B. inhambanensis</i> and <i>B. ovata</i> , <i>B. pubescens</i> , <i>B. veluntina</i> <i>B. buchananii</i> and <i>B. pleistophylla</i> clade	Dense cauline leaves (7)
<i>B. tenuior</i> , <i>B. congoensis</i> clade	Serrated lip margin (21)
<i>B. buchananii</i> , <i>B. pleistophylla</i> clade	Middle lobe of epichile smaller and lower than lateral lobes (30)
<i>Neobolusia</i> clade	Narrow lip attachment (20)
<i>Neobolusia</i> clade	Unlobbed or entire lip (28)
<i>Schizochilus</i> clade	Nodding inflorescence (9)
<i>Holothrix</i> clade.	Petals longer than sepals (19)

*Bremer support (Decay index)*

An analysis of the Bremer support for the nodes resulted in the loss of the node supporting the *Schizochilus* clade (node 10: Fig. 10). Two other nodes, one supporting *Schizochilus* and *Dracomonticola* clade (node 13) and the other one supporting the *Schizochilus*, *Dracomonticola* and *Schwartzkopffia* clade (node 16) also collapsed after addition of only one extra step. The basal node supporting the *Brachycorythis*, *Neobolusia*, *Schizochilus*, *Dracomonticola* and *Schwartzkopffia* clade (node 19) was lost after addition of two extra steps. Other nodes that required two extra steps to be lost were the one supporting the *Brachycorythis* clade (node 17) and the *Neobolusia* clade (node 5) required two extra steps to be lost (Fig. 10).

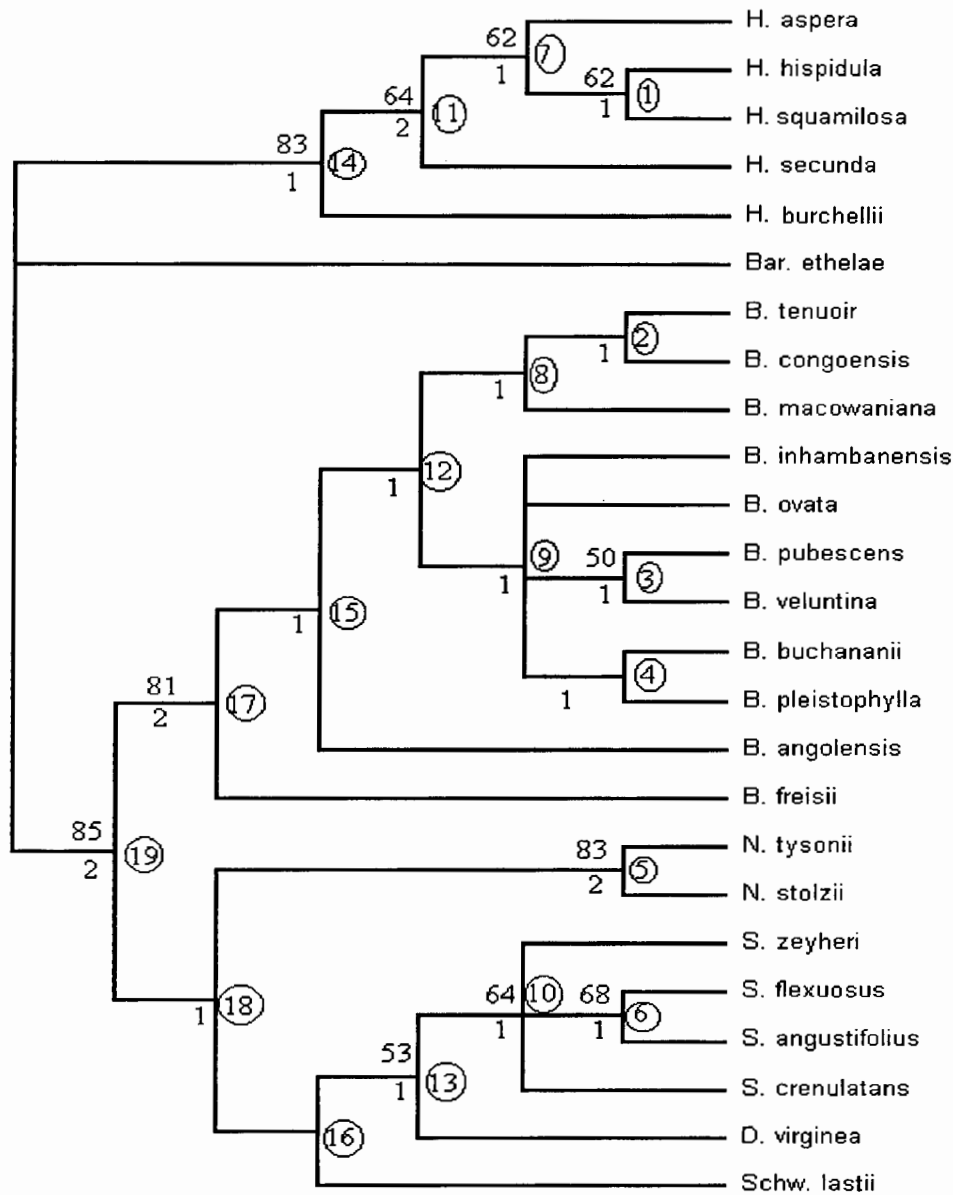


Fig. 10: One of the most parsimonious cladograms showing the bootstrap percentiles (obtained by bootstrapping the original matrix), and the Bremer support values for the nodes. The bootstrap values are indicated above the branches whereas the bremer support values are indicated below the branches. The circled values are the node numbers. The *Brachycorythis* clade occurred 81% of time in 100 replicates. The clade containing *Neobolusia* species have the highest support (83%). The clade consisting of *Schizochilus*, *Dracomonticola* and *Schwartzkopffia* clade occurred 58% of time. The node that supports the clade consisting of *Schizochilus* and *Dracomonticola virginea* and the node supporting the *Schizochilus* clade occurred in just over half of the replicates (53% and 64% respectively). Bremer support analysis shows addition of fundamental trees with one extra step to the consensus tree calculation resulting in the loss of nodes 1, 2, 3, 4, 6, 7, 8, 9, 10, 12, 13, 14, 15, 16 and 18. Nodes 5, 11, 17 and 19 collapse only after addition of two extra step.

### Bootstrap analysis

Bootstrap analysis (Fig. 10) yielded fairly high support, for a number of clades. There is fairly strong support (more than 80%) for the clade consisting of all species of *Brachycorythis* (81%) and the clade containing *Neobolusia* species (83%). The node that supports the clade consisting of *Schizochilus* and *Dracomonticola virginea* and the node supporting the *Schizochilus* clade had weak support (50 – 80%). All the clades within the *Brachycorythis* clade were poorly supported (less than 50%). Bootstrap analysis therefore suggests high support for the *Brachycorythis* and *Neobolusia* clades.

### Forcing the topology

An analysis of the cost of placing the *Neobolusia* clade in various places on the tree, showed that for a tree length of 82 (the shortest length found) *Neobolusia* clade can not be placed anywhere else on the tree. Placing the clade as sister to *Schwartzkopffia lastii* resulted in an increase of one step (tree length of 83). Placing it at the base of either the *Schizochilus* or *Brachycorythis* clades resulted in an increase of two steps (tree length of 84). For three extra steps (tree length of 85), it could be placed as sister to *Dracomonticola virginea* and for four extra steps (tree length of 86) as sister to *B. friesii*. Placing the clade anywhere else required more extra steps, five (tree length of 87) as sister to *S. flexuosus*, *S. angustifolius* clade or as sister to either *S. zeyheri*, *S. crenulatus* or *B. angolensis* and six (tree length of 88) as sister to either *S. flexuosus* or *S. angustifolius*. The highest number of extra steps (tree length of 89) was required for a position of the *Neobolusia* clade as sister to either *B. tenuior* or *B. congoensis*.

### Character exclusion (Jackknife)

Only exclusion of twelve out of 33 characters from the analysis had an effect on the tree topology. All node numbers in the following description refers to figure 10.

Exclusion of character seven (density of the cauline leaves) resulted in the collapse of nodes 6, 8, 9, 10, 12, 13, 15, 16 and 18.

Exclusion of character nine (inflorescence orientation) caused the loss of three nodes within *Brachycorythis* (nodes 12, 9 and 3). Within the *Schizochilus*, *Schwartzkopffia*, *Dracomonticola* clade nodes 6, 10 13 and 16 were lost. Node 18 also collapsed.

Exclusion of character 10 (inflorescence position) resulted in nodes 3, 6, 9, 10, 12, 13, 16 and 18 collapsing.

Exclusion of character eleven (inflorescence density) resulted in loss of nodes 4, 6, 9, 10, 13, 15, 16, 17, 18 and 19.

Nodes 4, 6, 9, 12, 16, 10, 13 and 18 collapsed as a result of removal of character nineteen (relative sizes of petals and sepals).

Exclusion of character twenty one (Lip margin, entire or serrated) resulted in loss of nodes only within the *Brachycorythis* clade (i.e. nodes 2, 4, 8 and 9). The rest of the topology remained the same.

The exclusion of character twenty six (presence or absence of a lip callus) results in the loss 3, 9, 10, 12, 13, 16 and 18.

When character twenty-eight (lip lobing) was removed the tree topology remains the same but nodes 7, 11 and 14, within the outgroup collapsed.

Exclusion of character thirty (relative size and position of middle lobe of the epichile) resulted in the loss of most of the nodes within *Brachycorythis* (nodes 2, 4, 8, 9 and 12). The rest of the tree topology remained the same.

The exclusion of character thirty one (relative size of larger middle lobe of lip epichile and side lobes) results in the loss nodes 3, 6, 9, 10, 12, 13, 16 and 18.

Taxa exclusion

Successive exclusion of eleven of the taxa in the analysis caused changes in the topology of the strict consensus tree. The node numbers refer to the cladogram in figure 10.

Nodes 6, 9, 10, 12, 13, 16 and 18 collapsed when *B. velutina* was excluded from the cladistic analysis.

Exclusion of *Bartholina ethelae* from the analysis resulted in nodes 9, 6, 7, 10, 11, 12, 13, 14, 16 and 18 being lost.

Only nodes 8 and 9 were lost as a result of the removal of *B. congoensis* from the analysis. The rest of the tree topology remained the same.

Exclusion of *B. friesii* resulted in the loss of nodes 3, 4, 6, 8, 10, 12, 13, 16 and 18

Exclusion of *Schwartzkopffia lastii* from the analysis resulted in nodes 6, 8, 10, 12 and 18 collapsing.

Only nodes 8 and 9 were lost as a result of the removal of *B. tenuior*. A new clade consisting of *B. congoensis* and *B. buchananii* was formed.

Exclusion of *Neobolusia tysonii* resulted in nodes 3, 9, 12, 13, 16 and 18 collapsing.

When *Dracomonticola virginea* was excluded from the cladistic analysis, nodes 9, 12, 13, 16 and 18 were lost.

Exclusion of *Brachycorythis macowaniana* resulted in the loss of nodes 2, 4 and 9. The rest of the topology remained the same.

Exclusion of *Schizochilus zeyheri* resulted in the loss of five nodes (2, 4, 8, 9 and 12) within *Brachycorythis*. The rest of the topology remained the same.

Exclusion of *Schizochilus crenulatus* resulted in the loss of nodes 3, 6, 9, 10, 12, 13, 16 and 18.

Exclusion of *Schizochilus angustifolius* resulted in the loss of five nodes (2, 4, 8, 9 and 12).

## DISCUSSION

The delimitation of generic groups in the subtribe Orchidinae has been made difficult by the striking intergeneric similarity of individual species in the currently designated groups (i.e. the highly homoplasious nature of many characters). It has been very difficult to find characters that exclusively distinguish members of each genus and hence the large number of shifts and counter shifts of species from one genus to another over the past 160 years. Based on morphological evidence, this study shows the genus *Brachycorythis* as being diagnosed by lateral petals and dorsal sepals lightly fused and forming a hood over the gynostemium. *Neobolusia* is diagnosed by narrow lip attachment to the gynostemium and by an unlobed lip whereas *Schizochilus* is diagnosed by the nodding inflorescence. The outgroup *Holothrix* is diagnosed by petals that are longer than sepals.

In this study the number of apomorphies defining the different clades is quite low (one to two for any particular clade) whereas the number of homoplasies are quite high (up to four for some clades). This high amount of homoplasy in the data set overwhelmed the synapomorphies since the tree yielded by successive weighting was different from the all the most parsimonious trees and the consensus tree in the position of *Schwartzkopffia* (Fig. 8), which suggests weak support for this position of *Schwartzkopffia* in the estimated phylogeny. The position of *Schwartzkopffia* in the most parsimonious trees may therefore only be due to inadequate information. The position of *Schwartzkopffia* could be confirmed by more information as mentioned below. The position of *Brachycorythis* and *Neobolusia* clades however, remains the same as in the strict consensus tree possibly suggesting fairly strong support for the two clades.

The strict consensus tree shows that the *Brachycorythis*, *Neobolusia* and *Schizochilus* clades were supported by all the most parsimonious cladograms. The analysis of the Bremer support for the nodes supporting the different clades showed fair support for both the *Brachycorythis* clade (2 extra steps) and the *Neobolusia* clades (2 extra steps) but not for the *Schizochilus* clade (1 step). This supports the monophyly of *Brachycorythis* as presently circumscribed and the maintenance of *Neobolusia* and *Schwartzkopffia* as independent taxa outside the genus *Brachycorythis*.

Of the twelve characters whose exclusion from the analysis result in topological changes, none causes any change in the inferred monophyly of *Brachycorythis* or *Neobolusia*. The position of *Schwartzkopffia lastii*, though little affected by the exclusion of characters from the analysis, remains separate from *Neobolusia* and *Brachycorythis* clades. Most of the nodes that collapse with the exclusion of characters are internal nodes within *Brachycorythis*, and the also the *Schizochilus* clade and its internal nodes. These results therefore seem to suggest uncertainty for the monophyly of *Schizochilus*. This may nevertheless only be due to inadequate information. For the exclusion of taxa from the cladistic analysis, the same situation is observed as in the case of character exclusion. The inferred monophyly of *Brachycorythis* and of *Neobolusia* is not affected by the exclusion of any taxon from the analysis.

The position of *Neobolusia* is not significantly supported (< 50%) by the bootstrap analysis. However the position of all the *Brachycorythis* species within one clade is well supported by the bootstrap analysis and this suggests that *Neobolusia* might most likely not possibly be placed within *Brachycorythis* even with additional information. The position of *Schizochilus*, *Dracomonticola* and *Schwartzkopffia* has poor bootstrap support, which makes it difficult to definitely confirm the delimitations of these taxa, and further studies are needed to clearly define the phylogenetic relationships of *Schizochilus*, *Dracomonticola* and *Schwartzkopffia*.



Since *Neobolusia* could only be placed at the base of the *Brachycorythis* clade for a single additional step, and within the clade for more steps, the placement of *Neobolusia* outside the clade seems justified. The low Bootstrap support therefore could only be due to inadequate information.

A relevant result of the analysis is indication of the monophyletic nature of the genus *Brachycorythis* as presently circumscribed. In fact the *Brachycorythis* clade includes both spurred and unspurred species as suggested by Summerhayes (1955).

Unlike Kurzweil and Weber's (1991) view that the generic separation of *Neobolusia* and *Schwartzkopffia* from *Brachycorythis* does not seem justified, all available data concur in indicating the monophyly of the genus *Brachycorythis* and existence of *Neobolusia* and *Schwartzkopffia* species as independent from the genus *Brachycorythis*. Therefore Schlechter's (1895) placement of *Neobolusia* into a separate genus seems most parsimonious. Even though some conspicuous morphological similarities had suggested in the past the inclusion of *Neobolusia* in *Brachycorythis*, further evidence from pollen analysis seems to indicate that at least one species, *Neobolusia tysonii* (the only species of *Neobolusia* analysed for pollen surface ornamentation) is actually different from *Brachycorythis* and any other genus studied in possessing intectate pollen (Fig. 5.5b) whereas all other species examined for pollen surface ornamentation show semi-tectate pollen. This apparent difference of *Neobolusia* from the other genera, would most likely be further confirmed by molecular studies of the genera. If we accept the inclusion of the genus *Neobolusia* into *Brachycorythis*, the resulting cladograms depart by at least 2 steps from maximum parsimony, further supporting the independence of this genus from *Brachycorythis*.

Another issue worth mentioning is that phylogenetic position of *Schwartzkopffia* at the base of the *Schizochilus* clade, which changes with successive weighting needs to be further confirmed by additional analyses.

### *Evolution of characters*

With regards to the evolution of characters, a number of trends have been observed. In Summerhayes's (1955) Revision of *Brachycorythis* he proposed an adaptation of species from mesophytic conditions to adaptation to savannah and grassland conditions with considerable dry periods. In terms of habitat there seems to be a change from plants growing to more than 12 cm high in all the plants of the ingroup taxa to a dwarf type of growth in *Schwartzkopffia*. This is probably an adaptation to growth in frequently burnt habitats. Other such adaptations in *Schwartzkopffia* are the complete covering of the stem by reduced, imbricate bracts, which as Summerhayes (1955) suggested may reasonably be looked upon as a special development adapted to growth in periodically burnt savannahs or short grass areas.

Evolution of leaf arrangement is shown to have taken place from being cauline in *Brachycorythis* to leaves concentrated at the base or reduced to only two, with one at the base and the other midway up the stem in *Dracomonticola virginea*. This line of evolutionary trend supports Summerhayes's (1955) earlier suggestions. There is also a clear trend of evolution of laxly arranged leaves to densely arranged ones. This probably is an adaptation from growth in mesophytic conditions to growth in growth in montane grasslands in the same general region as is the case for *B. henryi* (Schltr.) Summerh. and *B. obcordata* (Lindl.) Summerh. (Summerhayes, 1955). Sepals show a trend of development from being glabrous for example in most of the *Brachycorythis* species to being hairy in *B. velutina* and *B. pubescens*. This is probably an adaptation savannah type environments. This kind of evolutionary development is seen in *B. pubescens* which has a velvety covering of hairs to the leaves and has a wide distribution all over the savannah regions of Africa, and its allies *B. velutina* Schltr. and *B. pilosa* Summerh.

The presence of a lip spur is shown to be a primitive condition in Orchidinae. This also supports Summerhayes' earlier suggestion that in Orchidinae, the well developed spur, which in many orchids is considered as an advanced character is actually more primitive and that development has taken place in the direction of reduction of the

spur and its gradual replacement by a sac like hypochile (Summerhayes, 1955). The hypochile is shown to have progressed in *Brachycorythis* from being a flat to sac like. Spur length also shows a trend of reduction.

Generally the line of evolution of the characters are in line with Summerhayes' s (1955) suggestions. Summerhayes proposed that starting from the above basic group the other African species can be interpreted as derivatives increasingly adapted to savannah conditions and showing in other respects certain evolutionary trends with species such as *B. sceptrum* Schltr. and *B. congoensis* Kraenzl., being evidently intermediate in characters. Further pollen surface ornamentation data seems to suggest that *Neobolusia tysonii* pollen surface ornamentation is the most distinct from any of the rest of the species in the study group. The trend of evolution is from tectate to intectate ornamentation. Burn-Balogh (1983) suggested that Orchidoideae are primitively intectate whereas Chesselet and Linder (1993) proposed that development has taken place from semi-tectate to intectate pollen. The later is therefore supported by the current analyses.

A few lateral branches or even reversal are also present for individual cases, as is expected in such lines of evolutionary development (Summerhayes's 1955). An example given by Summerhayes is the occurrence in *B. tenuior* of a large spur which is, unlike that of other long spurred species, enlargement of the decurrent hypochile sides to produce what appear to be insect guides (Fig. 2b) and the typical *Brachycorythis* habit, which are evidently off the general line of evolution.

## CONCLUSION

The suggestion that *Neobolusia* could only be a highly derived member of the *Brachycorythis* (Kurzweil and Weber, 1991) seems likely to be adequately disapproved with addition of more information to the phylogenetic analysis. A complete examination of the genera of Orchidinae is beyond the scope of this study. However some inferences towards a phylogenetic hypothesis can be drawn on the results presented here. Though these results indicate fairly strong support for both the

*Brachycorythis* and *Neobolusia* clades, we can not at the moment rule out the possibility of including *Neobolusia* in the *Brachycorythis* clade with the addition of more information. It would also be interesting to see if *N. stolzii* also possesses intectate pollen. It is known that few point mutations in floral homeotic genes may dramatically change floral morphology (Coen & Meyerowitz 1991; Weigel & Meyerowitz, 1994). As a consequence remarkably different morphology may develop in closely allied taxa. It is also possible that adaptation to different environmental conditions and different selective pressures in orchid species would complicate morphology based phylogenetic inference. There is need for further work towards a detailed phylogenetic study, which would include information on anatomical features such as pollen ultra structure and seed cytology, as well as molecular sequence data. This would also have to include diverse species.

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## APPENDEX

Table of measurements taken in the study. LSL = Lateral sepal length; LPL = Lateral petal length; DSL = Dorsal sepal length; LBL = Lip blade length; LBW = Lip blade length; LLL = Lateral lip lobe length; MLL = middle lip lobe length. ? = inapplicable or absent

Specimens	LSL	LPL	DSL	LBW	LBL	MLL	LLL	Spur length
<i>B. angolensis</i>	9.8	8	7.9	8.5	6.5	1.5	2	2.5-4.5
<i>B. buchananii</i>	4.4	5.2	3.9	4.4	2.5	0.9	1.5	5.2-4.5
<i>B. congoensis</i> Kraenzl.	5.9	5.8	5.5	4.1	4.8	1.9	0.9	2-2.3
<i>B. friesii</i>	5.5	4.1	4.5	6	4.3	2.3	1	2.5-4.5
<i>B. inhambanensis</i> (Schltr.) Schltr.	5.2	4.5	5.2	3.2	3.35	0.6	1	?
<i>B. macowaniana</i> Reichb. F	2.7	2.6	2.6	2.8	3.8	0.7	0.3	2-2.5
<i>B. ovata</i> Lindl.	5.2	4.6	4.3	4	5	0.5	0.6	?
<i>B. pleistohylla</i>	6.9	6	7	9.8	11.2	0.3	4.2	?
<i>B. pubescens</i> Harv.	10	5	4.4	6.1	4.4	1.9	1.9	?
<i>B. tenuior</i> (Rchb.f.) Summerhayes	10	8.1	9.5	5.5	7	3	1.9	5-9
<i>B. velutina</i>	7	4.9	6.3	5.1	5.4	1.2	1.2	?
<i>Bartholina ethelae</i>	12	12	12.2	4.2	16.3	?	?	7-8
<i>D. virginea</i> (Bolus), H.P. Linder & Kurzweil	10	1.4	11.7	3.5	4.1	2.1	0.6	?
<i>H. aspera</i>	2.6	5.2	2.7	6.5	6.5	?	?	1-2.2
<i>H. burchellii</i> Rb.f.	3	5.5	2.6	1.4	3.4	?	?	1-3
<i>H. hispidula</i>	2.1	3.3	2.5	2.0	3.5	?	?	0.9-1.6
<i>H. secunda</i>	3	6.2	2.5	2.4	5.2	?	?	3.2-5.7
<i>H. squamulosa</i>	4.4	7.4	4.3	2.5	5.2	?	?	1.5-2.2
<i>N. stolzii</i>	11.5	6.1	9.5	6.5	4	?	?	?
<i>N. tysonii</i> (H. Bol.) Schltr.	8.6	6.3	7.7	3.4	8.4	?	?	?
<i>S. crenulatus</i> Linder	7.5	2.9	5.8	4.3	6	2.9	1.5	2.7-4
<i>S. flexuosus</i> Harv. Ex. Rolfe	6.5	3	6	3.75	5	2	0.6	3-4
<i>S. zeyheri</i> Sond.	6.9	3.8	7.1	5.8	5	3	1.4	2-6.3
<i>Schizochilus angustifolius</i> Rolfe	3.1	1.4	2.6	2.7	2.6	1.5	0.52	0.5-0.9