

ALLIACEAE

MICROMORPHOLOGY AND CYTOLOGY OF *PROTOTULBAGHIA SIEBERTII*, WITH NOTES ON ITS TAXONOMIC SIGNIFICANCE

Four genera of the Alliaceae are known to occur in sub-Saharan Africa, in total comprising 35 indigenous and two naturalized alien species (Table 2) (Archer 2003; Klopper *et al.* 2006). *Tulbaghia* L. is the most diverse and widespread genus of the family in southern Africa, but the other two indigenous sub-Saharan genera, *Allium* L. and *Prototulbaghia* Vosa, are each represented by a single restricted-range species, of which the latter has only been described recently (Vosa 2007). This note supplements the existing description of *P. siebertii* Vosa, which is endemic to the Sekhukhuneland Centre of Endemism (Van Wyk & Smith 2001), South Africa (Vosa *et al.* 2011), where it is restricted to the summit of the Leolo Mountain Range, Limpopo Province. Although macro-morphological characters of *Prototulbaghia* have been documented by Siebert *et al.* (2008), little is known about the micromorphology and cytology of *P. siebertii*.

Live material of *Prototulbaghia siebertii* was collected from the wild in November 2005 and cultivated in clay pots in a mixture of compost, peat, sand and garden soil. Plants were housed in a greenhouse at

TABLE 2.—Sub-Saharan genera of Alliaceae and no. species per genus recorded for *Flora of southern Africa (FSA)* (Archer 2003) and tropical African flowering plants (EPFAT*) respectively (Klopper *et al.* 2006)

Genera	Species per genus		Total
	FSA	EPFAT*	sub-Saharan Africa
<i>Allium</i>	1 + †1	2	3 + †1
† <i>Nothoscordum</i>	†1	†1	†1
<i>Prototulbaghia</i>	1	0	1
<i>Tulbaghia</i>	25	9	31
Total: 4	27 + †2	11 + †1	35 + †2

†alien taxa; * Enumération des plantes à fleurs d'Afrique tropicale.

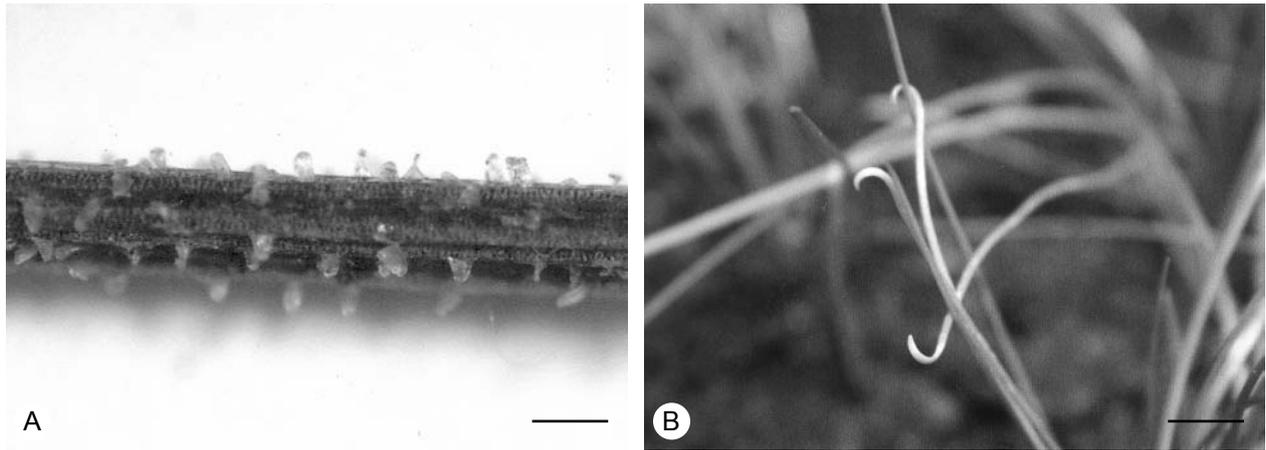


FIGURE 17.—*Prototulbaghia siebertii*. A, micrograph of floral scape showing very small glandular hairs; B, photograph of peculiar hooked tips of leaves. Plant grown from seed originally collected near Schoonoord, Leolo Mountains, Sekhukhuneland. Scale bars: A, 1 mm; B, 0.75 mm.

18 to 22°C. For mitotic preparations, actively growing root tips were collected at about 11:00, pretreated in an aqueous solution of 0.05 % colchicine for four hours at greenhouse temperature and fixed overnight in 1:3 glacial acetic acid/absolute alcohol (Vosa 2000). After a short hydrolysis in 1N hydrochloric acid at 60°C, the meristematic part of the tip was squashed in a drop of 1.5 % orcein in 45 % acetic acid and stained in Feulgen for one hour (Vosa 2000). For an assessment of meiosis in pollen mother cells, anthers were removed from young flower buds at an appropriate developmental stage, before colouring of the perianth, and squashed in 1.5 % aceto-orcein (Vosa 1961) without pretreatment or fixation on a microscope slide. The slide was left for six hours and pressed between filter paper. The slide was heated slightly over a flame, covered with a cover-slip and sealed with paraffin wax (Vosa 1961). Micrographs of the living material and of the cytological preparations were taken with a digital camera.

Observations of the finer morphological features of *Prototulbaghia*, both in living plants and herbarium specimens have revealed four further diagnostic characters of the inflorescence, leaves and anatomy. Most conspicuous is the scape (or peduncle) which is reddish brown in the lower portion during its early stages

of development and is covered by short, whitish, glandular hairs with somewhat recurved tips (Figure 17A). The glandular hairs on the scape are easily rubbed off, revealing underneath a single ridge of small translucent teeth with their tips directed proximally (Figure 18A).

Under higher magnification ($\times 10$), the surface of the leaves is somewhat pruinose, especially distally, and near the apex is a single row of tiny translucent, almost glass-like teeth directed toward the apex. The row of teeth forms a type of low central keel between two indistinct keels on the abaxial surface as described by Vosa (2007) (Figure 18B). The teeth on the distal part of the leaves are easily rubbed off.

The minute teeth on the leaves and scape of *Prototulbaghia siebertii* have not been recorded in any of the species of *Tulbaghia* so far studied. In the allied genus *Allium*, at least in section *Molium*, glandular hairs are present both on the floral scape and on the leaves (Mann 1959). However, many of the over 250 species of *Allium* have not been examined for this character.

The spreading, shiny, dark green leaves of *Prototulbaghia siebertii* have peculiar hooked apices (Figure 17B), which turn black in dried material. The apices of emerging young leaves are slightly coiled outwards,

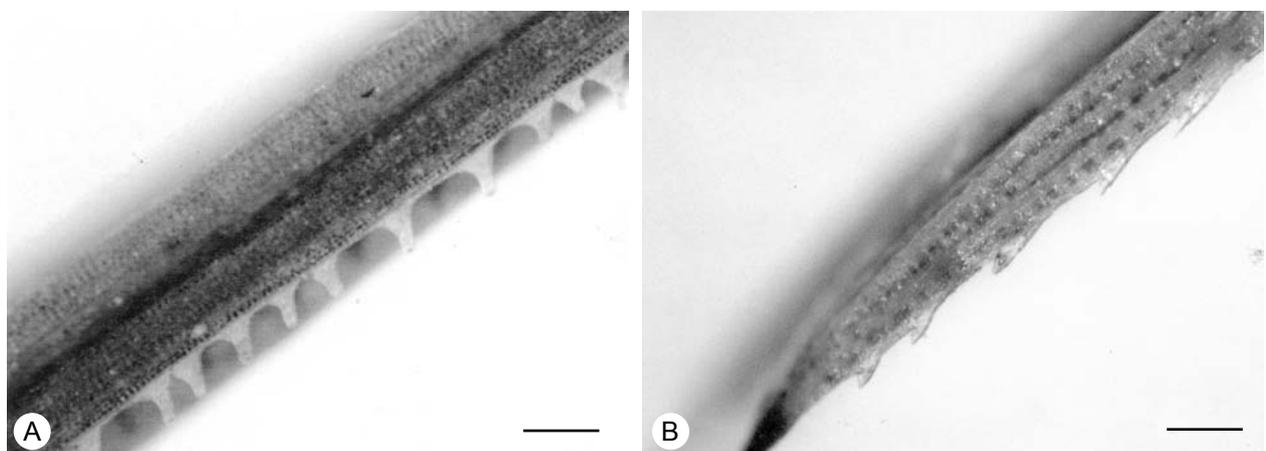


FIGURE 18.—*Prototulbaghia siebertii*. A, micrograph of floral scape showing ridge of tiny translucent teeth; B, micrograph of distal part of leaf showing tiny teeth directed towards apex of leaf. Scale bars: A, B, 1 mm.

the coils relaxing as the leaf develops, but remaining thickened and hook-like in mature leaves. Such hook-like leaf apices have not previously been recorded for the Alliaceae. Plants are easily uprooted when passing a hand or fingers through a clump, because of the entangled hooked apices. We speculate that the hooked leaves function as an additional mechanism of short range vegetative dispersal (Vosa 2007). So far the only other recorded instance of hooked leaf apices assisting in dispersal of a plant is known for *Junaea spectabilis* Kunth. (Arecaceae), the Chilean wine palm (C.G. Vosa pers. obs. in the wild and in cultivation). In the case of the palm, this unusual phenomenon has been observed in the leaflets (pinnae) of the fronds in seedlings and fairly young plants. This palm grows preferentially in soft forest soil, rich in peaty humus, and the hooked or coiled leaflet apices aid in seedling dispersal when dragged around by passing animals. This may also be the case in *Prototulbaghia*, considering the muddy, wet, humus-rich soils of its natural habitat.

The fourth micromorphological (anatomical) observation is a family characteristic. We can confirm the presence of laticifers which on damage exude a clear watery liquid in the leaf tissue of *Prototulbaghia*. Similar laticifers are also present in *Tulbaghia* and *Allium* (Mahlberg 1993).

Prototulbaghia has a diploid chromosome complement of $2n = 12$. The karyotype is very similar to that of Group 5 in *Tulbaghia* (Vosa 1975, 2000, 2009), supporting the suggested close relationship between the two genera. This poses the question how such an isolated species, which is not polyploid, could have persisted with such a presumably limited genetic endowment considering that polyploids are generally believed to display increased genetic variability and evolutionary potential (Hunziker & Schaal 1983).

A preliminary assessment of meiosis in *Prototulbaghia*, based on 18 pollen mother cells at metaphase 1, has revealed six regular bivalents with an average of about 20 chiasmata. Apart from the long arms of the longest chromosome of the complement which are unpaired (Figure 19), crossing over appears to be non-localized. This is very unlike the pattern/situation in those species of *Tulbaghia* so far investigated where crossing over is almost exclusively proximal to the centromere (Vosa 1975, 2000). This pattern may indicate a relatively higher degree of recombination than in *Tulbaghia* where the average chiasmata, based on the analyses of six diploid species, is much lower (Vosa 1966). Gender is one of the most important genetic factors affecting recombination frequency (Burt *et al.* 1991). Hence the existence of a two-track heredity in *Tulbaghia* (Vosa 1972) with a higher degree of genetic recombination in ovules than in microspores, may possibly be somewhat enhanced in *Prototulbaghia* as a compensating recombination mechanism. Overall such hypothetical modified genetic inheritance in *Prototulbaghia* may mean that the macromorphological uniformity observed in nature in its populations hides a complex and specialized genomic arrangement. However, these ideas on recombination frequency in *Prototulbaghia* still require testing.

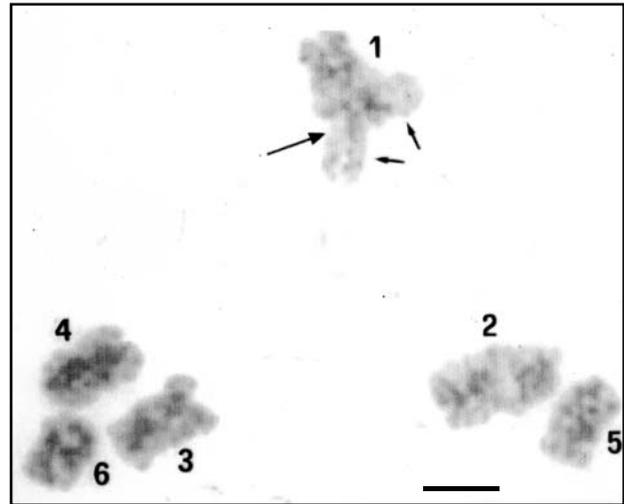


FIGURE 19.—*Prototulbaghia siebertii*. Micrograph of metaphase 1 of meiosis (six bivalents have been numbered in order of decreasing size). Note: in bivalent 1, unpaired segments corresponding to long arms of two involved chromosomes (small arrows). In this metaphase, there are 24 chiasmata, 23 non-localized and one proximal to centromere indicated by larger arrow. Scale bar: 10 μm .

The lack of recombination in the long arms of the longest chromosome of *Prototulbaghia* (Figure 19) may be a parallel evolutionary trend toward the state found in all the species of the closely related *Tulbaghia*. The existing cytological differences between the two genera may be due to the hypothetical need of high recombination in *Prototulbaghia* for the genome as a whole to maintain genetic variability as a narrow endemic with a single population. The paradox is that although it may seem tempting to regard *Prototulbaghia* as an entity with some plesiomorphic features and most probably sister to *Tulbaghia* and intermediate to *Allium*, the existence of the taxon-specific micromorphological and cytological features noted above may also be an indication of its specialized and geographically isolated evolution ('trapped' on its specialized mountain summit habit).

Observations of the cytology discussed above provide evidence of a diagnostic character that provides support for the hypothesis that *Prototulbaghia* may possess features that are ancestral to *Tulbaghia*. Note, however, that in *Tulbaghia* the grouping of species based on karyotype (Vosa 1975, 2000) corresponds only in part to the subdivisions of the genus into sections based on floral morphology (Vosa 2009). Such discrepancies demonstrate that in *Tulbaghia* floral morphology and the karyotype in many cases seem to follow independent evolutionary pathways (Vosa 2009). Despite the similarity in karyotype, differences in floral morphology between *Prototulbaghia* and *Tulbaghia* have also been documented by Vosa (2007) and Siebert *et al.* (2008).

Available molecular phylogenetic analyses of Alliaceae support a close relationship between *Allium* and *Tulbaghia*, although hitherto only two (*T. simmleri* Beauv. and *T. violacea* Harv.) of the 31 species of *Tulbaghia* were considered (Fay & Chase 1996; Friesen *et al.* 2006; Nguyen *et al.* 2008). In such restricted molecular analyses, the potentially misleading effects of poor taxon sampling on phylogenetic analyses and their appli-

cations should be kept in mind (e.g. Heath *et al.* 2008). There is an obvious need for a well-sampled molecular phylogenetic analysis that includes *Prototulbaghia* and more *Tulbaghia* species, together with other members of the Alliaceae, to test proposed generic demarcations and evolutionary trends in the family.

For the description of *Prototulbaghia siebertii*, see Vosa *et al.* 2011. Newly observed features are:

Leaves have a row of tiny translucent teeth adpressed toward the distal abaxial part below the apex; laticifers present. *Spathes* subtend the inflorescence when the flower scape emerges, gradually drying off. *Flower scape* is covered by short, whitish, glandular hairs; keel present towards the base, single, persistent, ridge-like, with translucent teeth. *Flowers* are fugacious. *Perianth* segments are light green in the lower half; inner segments are 4.3 mm wide in the middle. *Gynoecium* has 2–4 ovules per locule. *Capsule* has a withered style on top.

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