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6.1 Taxonomic significance of leaf surface morphology in Aloe section Pictae (Xanthorrhoeaceae)

Taxonomic significance of leaf surface morphology in *Aloe* section *Pictae* (Xanthorrhoeaceae)

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Running title: Leaf surface morphology in *Aloe*. 
Leaf surface morphology is analysed in 32 species representing the maculate species complex (the poorly resolved section *Pictae*) in the genus *Aloe* (Xanthorrhoeaceae). Few comparative morphological data are available for the complex. Leaf surface and stomatal characters observed by SEM show taxonomically significant interspecific variation. Most species are characterised by irregularly-outlined, 4–6-sided epidermal cells, of which the periclinal walls are flat and embellished with micropapillae and the anticlinal walls are indicated by channels on the leaf surface. The outer stomatal pore is typically sunken or plane and surrounded by four lobes on the leaf surface that may overarch the epistomatal chamber. The guard cells have distinct outer and inner stomatal ledges. Two geographical groups, comprising southern and east African species, are distinguishable by their leaf surface morphology. These characters are diagnostic in *A. ellenbeckii*, *A. prinslooi* and *A. suffulta* and support changes in the delimitation of *A. greatheadii*, *A. macrocarpa* and *A. swynnertonii*.


**INTRODUCTION**

*Aloe* L. is a leaf-succulent genus found throughout Africa, the Arabian Peninsula and islands in the western Indian Ocean, and includes numerous endemic species from Madagascar. The genus is traditionally included in Asphodelaceae, a ‘bracketed family’ within the expanded family Xanthorrhoeaceae according to APG II (2003). Besides pharmaceutical and cultural significance, *Aloe* spp. have considerable appeal to succulent collectors and in the horticultural trade. Ever-increasing diversity recognised in the genus necessitated Berger’s original (1908) infrageneric classification in *Das Pflanzenreich* and a subsequent, expanded revision in two volumes (Reynolds, 1950; 1966). The most recent inventory of *Aloe* listed 548 accepted species and infraspecific names and 428 synonyms (Newton, 2001), reflecting new perspectives on diversity and relationships in the genus, increased collecting activity and possible taxonomic exaggeration, a pattern also observed in other popular groups such as Orchidaceae (Pillon & Chase, 2007).

Comparative studies of morphology, anatomy and phytochemistry in several infrageneric groups have led to a revised view of species relationships, including the *A. somaliensis* W.Watson complex (Carter *et al*., 1984) and section *Angualoe* Reynolds (Van Heerden, Viljoen & Van Wyk, 2000). The present study focused on leaf surface morphology in the maculate species complex, a group of ca. 40 species in the poorly resolved section *Pictae* Salm-Dyck (= *Maculatae* Baker; *Saponariae* Berger). The maculate complex is among the most well-defined groups in *Aloe* (Groenewald, 1941). However, distinguishing characters for the group (prominent leaf markings, perianth constricted above the ovary and short stem) are shared with related groups and are likely symplesiomorphic. Species delimitation in the maculate complex is problematic (see Glen & Hardy, 2000; Wabuyele, 2006).
The value of leaf surface morphology in the systematics of alooid genera has been shown in *Aloe*, *Chortolirion* Berger, *Gasteria* Duval, *Haworthia* Duval and *Poellnitzia* Uitewaal (Cutler, 1969, 1972, 1979, 1982; Cutler et al., 1980; Lubbinge, 1971; Newton, 1972; Smith & Van Wyk, 1992). In these genera, leaf surface features are remarkably constant within species and show little geographical variation and environmentally induced plasticity (Cutler & Brandham, 1976; Brandham & Cutler, 1978). Variability has been reported in an unusual species (*A. bowiea* Schult. & J.H.Schult. (Smith & Van Wyk, 1992) and in widely distributed species, in which changes in leaf surface morphology were consistent with observed clinal variation (Cutler et al., 1980). The stomatal arrangement in *Aloe* is of adaptive importance in regulating gas exchange and water loss in habitats experiencing alternating periods of drought and humidity, whereas superficial stomata in *Aloe* have been correlated with humid habitats (Cutler, 1982). Similar stomatal arrangements occur in xeromorphic members of Asparagaceae, such as *Agave* L. (Blunden & Jewers, 1973) and *Dracaena* Vand. ex L. (Klimko & Wiland-Szymańska, 2008).

Wax has possible taxonomic significance in *Aloe* (Cutler, 1979) and protects the leaf surface against microbial infection and herbivory. The cuticular membrane extends throughout the stomatal complex and delimits a series of air-filled chambers (Evert & Eichhorn, 2006). Ledges between the chambers are formed by the tearing of a continuous cutinised layer covering adjacent guard cells (Cutler, 1979). Cuticular lobes on the leaf surface coincide with the position of subsidiary cells, with which they are associated (Cutler, 1979). The lobes and cuticular ledges of the stomatal complex in *Aloe* have received little attention in subsequent studies of the leaf surface.

Leaf markings and surface morphology are of value in the identification of closely related *Aloe* spp. (Cutler, 1972), in particular for sterile specimens. Indeed, the similarity of juvenile plants (Smith, Klopper & Crouch, 2008) and morphology of mature leaves of many species substantiate the regulation of the trade in *Aloe* spp. (with the exception of the intensively farmed *A. vera* L.) by the Convention on International Trade in Endangered Species of Fauna and Flora (CITES). Kenya and South Africa are export hubs for excised leaves, leaf exudate and living plants gathered in the wild and exported mainly to Asia, Europe and the United States (Sachedina & Bodeker, 1999; Oldfield, 2004; Knapp, 2006). Representatives of the maculate complex that have been recorded in trade include *A. ellenbeckii* A.Berger (Oldfield, 2004) and *A. maculata* All. (Pole-Evans, 1919). Many species in the maculate complex are used locally for traditional medicine, dyes and ornamentals (Grace et al., 2009).

In this paper we present an analysis of leaf surface and stomatal characters in the maculate complex in *Aloe*. This is the first systematic survey of leaf surface morphology in the complex and includes new observations for 25 species and further observations for seven species described in previous studies (Newton, 1972; Cutler, 1982). Terminology largely follows that of Cutler (1972; 1979). The taxonomic significance of leaf surface morphology in the maculate complex is discussed.
MATERIALS AND METHODS

PLANT MATERIAL

Leaf surfaces and stomata were examined in 32 species representing the maculate species complex and three other groups in the genus Aloe (Table 1). Species of problematic taxonomic position in the maculate complex (A. suffulta Reynolds and A. wollastonii Rendle) and outgroups A. chabaudii Schönland (section Aethiopicae Berger, a stemless shrub) and A. vanbalenii Pillans (section Arborescentes Berger, a multi-stemmed shrub) were included in the analysis. Plant material was collected from wild populations in South Africa and plants of field provenance grown in glasshouses at the Royal Botanic Gardens, Kew. Voucher specimens were deposited in the National Herbarium of South Africa (PRE) and the herbarium at Kew (K) (Table 1). Living material was fixed in formalin-acetic acid-alcohol (FAA: 70% ethanol, formaldehyde and glacial acetic acid, 85: 10: 5) for at least 48 hours, washed twice in distilled water and transferred to 70% ethanol prior to examination.

ELECTRON MICROSCOPY

Specimens were prepared for examination with a scanning electron microscope (SEM) using a method adapted from Cutler (1972). Leaves were split longitudinally with a sharp blade and mesophyll tissue scraped from the inside of the adaxial leaf surface until only a few cell layers remained attached to the epidermis. A piece (ca. 2 cm²) from the leaf mid-region was dehydrated through an ethanol dilution series (70–100%) over 2.5 hours. Specimens were critical-point dried (Tousimis® Autosamdri® 815B-Series A unit), mounted on aluminium stubs with double-sided tape and coated with gold/palladium alloy in a sputter coater (Emitech K550). Specimens were examined using a Hitachi cold-field emission SEM S4700 at 2 kV over a range of magnifications. Micrographs were recorded digitally.

RESULTS

Observations of leaf surface morphology in representatives of the maculate complex in Aloe are presented in Tables 2 and 3. Micrographs are shown in Figs. 1–37.

Leaf surface characters in Aloe are organised at three levels; the following description refers to maculate species (Tables 2 and 3). Primary sculpturing comprises the outline of epidermal cells in surface view. In the maculate complex, these are usually 4–6-sided and irregular (e.g. A. affinis A. Berge; Fig. 2) or, rarely, uniform (e.g. A. branddraaiensis Groenew.; Fig. 6). Cell shape is discernible by a depression (e.g. A. dewetii Reynolds; Fig. 9) over the anticlinal walls, which may be flanked by ridges on the periclinal walls of one or both neighbouring epidermal cells (e.g. A. pruinosa Reynolds; Fig. 28). Secondary sculpturing refers to the curvature of the periclinal wall in leaf epidermal cells; these are flat (e.g. A. greatheadii var. daviana (Schönland) Glen & D.S.Hardy; Fig. 14), convex (e.g. A. swynnertoni Rendle; Fig. 31) or even domed, as in A. vogtsii Reynolds (Fig. 35). Tertiary sculpturing of the periclinal walls comprises micropapillae variably arranged in a fine, low relief (e.g. A. pruinosa;
Fig. 28) or a coarse and dense arrangement (e.g. *A. greatheadii* Schönland var. *greatheadii*; Fig. 15). In addition to ridges associated with anticlinal walls, micropapillae may aggregate to form ridges on the periclinal walls, particularly near stomata (e.g. *A. petrophila* Pillans; Fig. 26). Occasionally, dense tertiary sculpturing obscures the primary features (e.g. *A. fosteri* Pillans; Fig. 11). The epidermal surface is further covered by a thick cuticle and a layer of wax, which is usually continuous but sometimes deposited as flakes on the leaf surface (e.g. *A. wollastonii*; Fig. 36).

Stomatal frequency is similar on both leaf surfaces in the maculate species complex. The guard cells are always positioned well below the leaf surface and surrounded by four subsidiary cells (Fig. 1). The well-developed cuticle on the leaf surface forms four more or less distinct lobes that surround and partially cover the outer pore of the epistomatal chamber (Fig. 1). In most maculate species, two opposing lobes surrounding this opening are aligned parallel to the longitudinal axis of the leaf and are here termed the ‘L-lobe pair’, whereas the ‘T-lobes’ are aligned parallel to the transverse axis of the leaf (e.g. Fig. 24). The difference in their length, described by a ratio, is of taxonomic significance (Table 3); length refers to the dimension parallel to the respective leaf axis (not to be confused with the length of the lobe projecting over the outer pore of the epistomatal chamber, which is not considered here). The cuticle associated with the guard cell forms an inner and outer cuticular ledge, protruding into the epistomatal and substomatal chambers; the outer cuticular ledge is visible in Fig. 37.

Leaf surface morphology was similar among 88% of maculate species examined (Fig. 2–37), but was conspicuously different in the outgroups *A. chabaudii* (Fig. 8) and *A. vanbalenii* (Fig. 33). In these outgroup species, leaf surface morphology was characterised by dense, rugulose tertiary sculpturing, indistinct anticlinal cell walls, and the outer pore of the epistomatal chamber sunken and surrounded by poorly developed lobes. Limited infraspecific variation was observed in *A. greatheadii* var. *greatheadii* (Fig. 15), *A. immaculata* Pillans (Fig. 17), *A. maculata* (Fig. 22), *A. parvibracteata* Schönland (Fig. 25) and *A. umfoloziensis* Reynolds (Fig. 32), whereas in *A. amudatensis* Reynolds (Fig. 4) there was little variation in the surface features of different leaves from the same individual.

**DISCUSSION**

Patterns in leaf surface morphology support the recognition of maculate species as section *Pictae*. A phytogeographical link is evident between the centres of diversity in the maculate complex. Species from east Africa (*A. lateritia* Engl. var. *graminicala* (Reynolds) S.Carter, Fig. 18; *A. macrocarpa* Tod., Fig. 21; *A. wollastonii*, Figs 36 and 37) are characterised by epidermal cells that are more or less circular in outline, with the periclinal walls convex and micropapillae aggregated into ridges. These characters also occur in *A. maculata* (Fig. 22), *A. swynnertonii* (Fig. 31) and *A. vogtsii* Reynolds (Fig. 35) in southern Africa, and they support the recognition of *A. vogtsii* as a southern form of the widespread *A. swynnertonii* (Glen & Hardy, 2000). Leaf surface features typical of southern African maculate species include epidermal cells that are irregular in outline, with the periclinal walls flat and tertiary sculpturing comprising micropapillae ranging from the distinctly papillate (e.g. *A. burgersfortensis* Reynolds, Fig. 7; *A. parvibracteata*, Fig. 25) to a coarse, dense arrangement (e.g. *A. greatheadii* var. *greatheadii*, Fig. 15; *A. lettyae* Reynolds, Fig. 19).
Our results indicate that leaf surface morphology assists in resolving species concepts among maculate species. In the *A. greatheadii* complex, the present survey found marked similarities in the leaf surface morphology of the closely related species *A. barbertoniae* Pole-Evans (Fig. 5), *A. graciliflora* Groenew. (Fig. 12) and *A. greatheadii* var. *daviana* (Fig. 14), but not *A. longibracteata* Pole-Evans (Fig. 20). The latter shares the distinctive sunken outer pore and dense tertiary sculpturing typical of *A. greatheadii* var. *greatheadii* (Fig. 15). This pattern is also present in *A. immaculata* (Fig. 17), a species considered by Van Wyk & Smith (2005) to be conspecific with *A. greatheadii*. Similarities in leaf surface morphology supports Wabuyele’s (2006) hypothesis for *A. lateritia*. Convex outer periclinal walls, reticulated tertiary sculpturing and poorly developed lobes surrounding the outer pore are common to *A. lateritia* var. *graminicola* (length ratio = 1.2; Fig. 18), *A. wollastonii* (1.2; Figs 36 and 37) and *A. macrocarpa* (1.1; Fig. 21). Leaf surface characters do not, however, support *A. amudatensis* (Fig. 4) as a synonym of *A. ellenbeckii* (Fig. 10). *Aloe ellenbeckii* is morphologically distinct from all other maculate species: the leaf surfaces bear discontinuous elevated ridges, reticulated tertiary sculpturing and poorly developed lobes (length ratio = 1.1). The second maculate taxon with very distinctive leaf surface morphology is *A. prinuslodi* I.Verd. & D.S.Hardy (Fig. 27), in which deep folds are present along the periclinal cell walls, the tertiary sculpturing is densely papillate, and the convex L- and T-lobe pairs enclose a small and almost circular outer pore. The noticeable difference between the papillate tertiary sculpturing of *A. parvibracteata* (Fig. 25) and the reticulate tertiary sculpturing of *A. umfoloziensis* (Fig. 32) supports the recognition of these taxa as distinct species.

Noteworthy differences in leaf surface characters were observed in certain atypical members of the maculate complex. *Aloe monotropa* I.Verd. (Fig. 23), the only maculate taxon consistently found in deep shade and bearing secund flowers, is characterised by copious wax deposits. In *A. suffulta* (Fig. 30), a species of doubtful status in the maculate group due to a peculiarly lax inflorescence and flowers lacking a swollen base and constricted perianth, the anticlinal walls of the epidermal cells are indicated by a prominent ridge in place of the depression present in other species, the outer cuticular ledge is plane, and lobes do not overarch the outer pore of the epistomatal chamber (length ratio = 2.3).

Evidence from the present study indicates that the *A. greatheadii* complex has yet to be resolved, whereas resolution may have been reached for the *A. lateritia* complex. Leaf surface characters are sufficiently distinctive to be diagnostic in *A. ellenbeckii* and *A. prinuslodi*. Morphological similarities in leaf surface characters support the circumscription of the maculate species at the sectional level, add insights into contentious taxonomic hypotheses and highlight the systematic relationships between tropical and subtropical species of *Aloe*.

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REFERENCES


Figure 1. Scanning electron micrograph of stomatal complex on adaxial leaf surface of *Aloe umfoloziensis* in transverse section. l, lobe; ec, epistomatal chamber; sc, substomatal chamber; g, guard cell; s, subsidiary cell; o, outer cuticular ledge; i, inner cuticular ledge. Scale bar 10 μm.
Figures 2–13. Leaf surfaces in *Aloe*; longitudinal axis of leaves orientated ± vertically. Fig 2, *A. affinis*. Fig 3, *A. ammophila*. Fig 4, *A. ammutensis*. Fig 5, *A. barbertonianae*. Fig 6, *A. branddraaiensis*. Fig 7, *A. burgersfortensis*. Fig 8, *A. chabaudii*. Fig 9, *A. dewetii*. Fig 10, *A. ellenbeckii*. Fig 11, *A. fosteri*. Fig 12, *A. grandidentata*. Fig 13, *A. graciliflora*. Scale bars 50 μm.
Figures 14–25. Leaf surfaces in Aloe; longitudinal axis of leaves orientated ± vertically. Fig 14, A. greatheadii var. daviana. Fig 15, A. greatheadii var. greatheadii. Fig 16, A. greenii. Fig 17, A. immaculata. Fig 18, A. lateritia var. graminicola. Fig 19, A. lettyae. Fig 20, A. longibracteata. Fig 21, A. macrocarpa. Fig 22, A. maculata. Fig 23, A. monotropa. Fig 24, A. munenensts. Fig 25, A. parvibracteata. Scale bars 50 µm.
Figures 26–37. Leaf surfaces in Aloë, longitudinal axis of leaves orientated ± vertically. Fig 26, A. petrophila. Fig 27, A. prinsloo. Fig 28, A. pruinosa. Fig 29, A. simii. Fig 30, A. suffulta. Fig 31, A. swynnerdii. Fig 32, A. umfoloziensis. Fig 33, A. vanbalenii. Fig 34, A. vanrooyenii. Fig 35, A. vogtsii. Figs 36, A. wollastoni. Scale bars 50 μm. Fig 37, A. annulatensis. Scale bar 20 μm.