A taxonomic reassessment of the varieties of *Oxalis minuta* (Oxalidaceae) and the change of *O. minuta* var. *callosa* to specific rank as *O. hygrophila*

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*O. minuta* Thunb. var. *callosa* Salter was included in section *Sagittatae* of the genus *Oxalis* L., based on superficial morphological resemblance to *O. minuta* var. *minuta*. We questioned this placement due to the reticulate pollen grains displayed by this taxon, whereas members of section *Sagittatae* have rugulate-reticulate pollen grains. A lack of good herbarium and living specimens prohibited a better taxonomic placement of this taxon. Living plants were recently found in the Pakhuis Pass (Western Cape, South Africa), which enabled this reassessment of its taxonomic affinities. A suite of morphological and palynological characters were identified that differ significantly from both the typical variety and all other members presently included in section *Sagittatae*. These include the ovate to elliptic anthers, the orientation of long and mid-level reproductive organs, the absence of an indumentum on the leaves, the presence of calli on the leaflets, sepals and petals, reticulately deposited epicuticular wax on the leaves, the broadly funnel-shaped corolla tube and the basally fused anthers. We therefore raise *Oxalis minuta* var. *callosa* to specific rank as *O. hygrophila* Dreyer, and transfer it from section *Sagittatae* to section *Latifoliolatae*.

**Introduction**

The genus *Oxalis* L. is well represented in southern Africa by c. 211 species (270 taxa) (Salter 1944, Dreyer and Makwarela 2000). Salter (1944) recognised section *Sagittatae* as one of nine sections present in the region, characterised by the presence of sagittate anthers and the distinctive way in which long and mid-level reproductive floral organs spread on release from the floral tube. This was further supported by the finding that all but two of the taxa in section *Sagittatae* have unique, rugulate-reticulate pollen grains (Dreyer 1996), a pollen type restricted to section *Sagittatae*, and not recorded in any other southern African *Oxalis* taxon. The two taxa with different pollen grains are *O. fibrosa* Bol. f. and *O. minuta* Thunb. var. *callosa* Salter, which have micro-rugulate spine and micro-reticulate pollen grains respectively. The micro-rugulate spine pollen grains present in *O. fibrosa* are, however, related to the rugulate-reticulate pollen typical of the rest of section *Sagittatae* (Dreyer 1996). *O. minuta* var. *callosa*, on the other hand, appears to be palynologically misplaced within section *Sagittatae*. The reticulate pollen grains of this taxon differ markedly from the pollen grains of both the typical variety (*O. minuta* var. *minuta*) and the rest of section *Sagittatae* (Dreyer 1996).

The only available herbarium specimens of *O. minuta* var. *callosa* are two duplicates from the type locality at Groot Kliphuis in the Pakhuis Pass near Clanwilliam (*Leipoldt s.n.* (BOL 9400)). These two sheets are of a poor quality as they lack bulbs and include limited flowering material. We returned to the type locality to collect living material, which enabled a full morphological and palynological comparison between *O. minuta* var. *callosa* and the rest of section *Sagittatae*. The present study thus sets out to re-assess the taxonomic placement of *O. minuta* var. *callosa* within section *Sagittatae*.

**Material and Methods**

The morphological and biogeographical studies were based on herbarium material obtained on loan from the Bolus Herbarium (BOL), Compton Herbarium (NBG) and National Herbarium, Pretoria (PRE). This was supplemented by a comparison of living specimens collected from their natural habitats throughout the flowering season (Table 1).

Morphological characters were studied with a stereomicroscope. Epidermal surfaces of the leaflets were examined with an ABT 60 Scanning Electron Microscope (SEM). Leaflets were dried and mounted onto brass stubs using clear nail varnish as glue, and then sputter-coated with a gold-palladium layer. SEM micrographs of epicuticular wax layers were taken at fixed magnifications.

Pollen grains were collected from both herbarium and living specimens. For the light microscope analysis pollen grains were mounted in glycerin jelly and studied within three days after mounting. For the scanning electron micro-
scope study, unacetylated dry pollen grains were mounted onto brass stubs using double-sided tape. The material was sputter-coated with a gold-palladium layer and studied with the aid of an ABT 60 SEM. SEM micrographs were taken at fixed magnifications to enable comparison.

Results

Morphology

Although the vegetative morphology of *O. minuta* var. *callosa* superficially resembles that of taxa in section *Sagittatae*, a more detailed morphological comparison revealed distinct differences between *O. minuta* var. *callosa* and the rest of section *Sagittatae*. True *Sagittatae* species have sagittate anthers and their long and mid-level reproductive organs spread on release from the corolla tube. They also have hairy leaves (both long simple and multicellular glandular hairs in *O. fibrosa* and unicellular hairs in all the other taxa), always lack calli on their leaflets, sepals or petals, and have epicuticular wax deposited as irregular vertical plates covering both lamina surfaces. Their floral tubes are narrowly funnel-shaped and they possess five free tepal numbers and arrangement are of limited importance. The sexine structure is the main distinguishing character between these three pollen types, while pollen grain dimensions and aperture number and arrangement are of limited importance. The majority of taxa have rugulate-reticulate pollen grains, while *O. fibrosa* produces structurally related micro-rugulate spinate pollen grains. *O. minuta* var. *callosa* is the notable exception, in that it produces micro-reticulate pollen. These pollen types are briefly described below:

**Rugulate-reticulate pollen (Figure 1a)**

Present in *O. eckloniana var. eckloniana*, *O. eckloniana var. hopefieldiana*, *O. eckloniana var. montigena*, *O. eckloniana var. robusta*, *O. eckloniana var. sonderi*, *O. microdonta*, *O. minuta var. minuta*, *O. nidulans var. nidulans* and *O. nidulans var. denticulata*.

Pollen grains pantocolpate with tricolpate and tetracolpate pollen types are briefly described below:

**Palynology**

Three different types of pollen grains were identified among members of section *Sagittatae* (Figure 1). The sexine structure is the main distinguishing character between these three pollen types, while pollen grain dimensions and aperture number and arrangement are of limited importance. The majority of taxa have rugulate-reticulate pollen grains, while *O. fibrosa* produces structurally related micro-rugulate spinate pollen grains. *O. minuta* var. *callosa* is the notable exception, in that it produces micro-reticulate pollen. These pollen types are briefly described below:

| Taxon                  | Specimen (Herbarium)                                                                 |
|------------------------|--------------------------------------------------------------------------------------|
| *O. fibrosa* Bol. f.   | Dreyer 725 (STEU); Salter 2187 (NBG); Bolus s.n. (BOL 13379)                         |
| *O. microdonta* Salter | Salter 1047 (BOL); Salter 2318 (BOL)                                                |
| *O. nidulans* Eckl. & Zeyh. var. *nidulans* | Dreyer 1047 (STEU); Salter 6158 (NBG); Salter 2651 (BOL)                              |
| *O. nidulans* Eckl. & Zeyh. var. *denticulata* (Wolley-Dod) Salter | Dreyer 610 (STEU); Salter 6618 (NBG); Oberlander 7 (STEU)                            |
| *O. minuta* Thunb. var. *minuta* | Dreyer 727 (STEU); Salter 2407 (NBG); Salter 3580 (BOL)                               |
| *O. minuta* Thunb. var. *callosa* Salter | Leipoldt s.n. (BOL 9400); Dreyer & Kumwenda 1 (STEU)                                 |
| *O. eckloniana* Presl. var. *eckloniana* | Salter 2419 (BOL); Salter 3355 (PRE); MacOwan 1814 (PRE)                             |
| *O. eckloniana* Presl. var. *montigena* (Schlfr.) Knuth | Martin 714/37 (BOL); Schlechter 7565 (PRE)                                           |
| *O. eckloniana* Presl. var. *hopefieldiana* Knuth | Salter 789 (NBG); Salter 3373 (NBG); Marloth 642 (PRE)                               |
| *O. eckloniana* Presl. var. *robusta* Salter | Bolus s.n. (BOL 14614); Salter 6617 (BOL); Dreyer 628 (STEU)                         |
Micro-rugulate spinate pollen (Figure 1b)
Present in O. fibrosa.
Pollen grains tricolpate, large grains 38–42µm and smaller grains 30–46µm in diameter. Pollen semi-tectate. Tectum micro-rugulate, heterobrochate. Lumina isodiametrically rounded, oblong to irregularly angular, diminishing in size towards colpi to form a distinct colpus margin. Predominant lumina diameter 0.64–1.58µm. Muri smooth to slightly stratified. Predominant muri thickness 0.44–0.59µm.

Micro-reticulate pollen (Figure 1c)
Present in O. minuta var. callosa.
Pollen grains tricolpate, large grains 35–50µm and smaller grains 30–46µm in diameter. Pollen semi-tectate. Tectum micro-reticulate, heterobrochate. Lumina isodiametrically rounded, oblong to irregularly angular, diminishing in size towards colpi to form a distinct colpus margin. Predominant lumina diameter 0.64–1.58µm. Muri smooth to slightly stratified. Predominant muri thickness 0.44–0.59µm.

Discussion
Based on the combined morphological, palynological and ecological results, it is clear that O. minuta var. callosa is not closely affiliated to the species included in section Sagittatae. This was supported by a preliminary DNA phylogeny based on the non-coding trnL-trnF region of the plastid genome (Oberlander 2003). These results segregated Oxalis minuta var. callosa from other species of section Sagittatae, and placed it in a small, strongly supported clade (92% bootstrap support (Felsenstein 1985)) with O. tomentosa L. and O. oligophylla Salter. O. tomentosa is currently placed in the poorly defined section Angustatae subsection Multifoliolatae, while O. oligophylla resides in the equally questionable section Angustatae subsection Lineares. Although there are some morphological similarities between these three taxa, none of these shared features are unique to this clade.

Based on combined morphological and molecular evidence, we conclude that O. minuta var. callosa should be removed from section Sagittatae. Comparison with all other Oxalis taxa confirms that this taxon is morphologically unique. It does show some resemblance to members of the taxonomically poorly defined section Latifoliolatae. Salter (1944) describes section Latifoliolatae as being artificial, ‘consisting of such species or small groups of species with broad leaflets as do not fall into the more obvious sections’. Within this unnatural assemblage he identified six groups of putatively related taxa. Of these six groups, O. minuta var. callosa shows the greatest morphological resemblance to the group of species that includes O. tenella Jacq., O. aridicola Salter, O. stokoei Weintroub., O. petiolulata Bol. f., O. callosa R. Knuth and O. hirsuta Sond. All of the species in this group, however, display supra-areolate pollen, a pollen type quite different to the micro-reticulate pollen grains of O. minuta var. callosa. Palynologically the latter taxon is more similar to Salter’s (1944) second and the third assemblage of subsection Lineares species, but does not show any strong morphological resemblance to these groups.

O. minuta var. callosa does not share any of the diagnostic characters of any other section or subsection in southern Africa. We thus argue that it is best to elevate O. minuta var. callosa to species status as O. hygrophila Dreyer and to include it in the unnatural section Latifoliolatae as a single-species assemblage, as has recently been done with O. oculifera E.G.H. Oliver (Oliver 1993).
**Taxonomic treatment**

*Oxalis hygrophila* Dreyer nom. nov. et stat. nov.

*O. minuta* Thunb. var. *callosa* Salter in *Journal of South African Botany Supplementary Vol. 1*: 180 (1944). TYPE: Clanwilliam Division, Grootkliphuis, Leipoldt s.n. (BOL. 9400 holo!; SAM, sub SAM 30372!)

Stemless geophyte, 50–70mm tall when in flower. **Bulb** ovoid, acute at apex to form a narrow beak, 9–11mm x 5–7mm; tunics thin, imbricate, light to medium brown, smooth, glabrous. **Rhizome** glabrous, up to 60mm long, with a single, light brown, triangular bract at each node, sessile axillary bulbils present at lower nodes. **Leaves** 10–12 (–20), basally congested, petioles 23–56mm long, glabrous, slightly broadened below the articulation, leaflets 3, lateral leaflets sessile, medial leaflet sessile to very shortly (0.5mm) petiolulate, conduplicate, glabrous, narrowly elliptic to elliptic, 8–19mm x 5–10mm, apices obtuse to retuse, with two prominent round to oblong calli abaxially just below apex, sometimes with 1–6 less conspicuous calli continuing along upper margin, margins entire, reddish. **Inflorescence** one-flowered, subtended by two alternate bracts inserted just below flower, bracts narrowly triangular, 1.5–2mm x 0.8–1.0mm, glabrous, with prominent apical calli. **Flowers** 17–18mm long, white with funnel-shaped yellow tube, **sepals** 5, lanceolate, 6.0mm x 1.0mm, glabrous, apices acute, margins entire, prominently bicallose. **Petals** 5, glabrous, obliquely obovate with a narrow claw, 8–10mm x 6–8mm; apices retuse with a cluster of 5–10 irregularly rounded to oblong orange calli abaxially near apex, claw 3.5–4mm long. Reproductive structures morphologically tristylos, long-level organs 5mm long, mid-level organs 4mm long, short-level organs 2mm long. **Stamens** 10, in 2 whorls of 5 each, filaments glabrous, longer filaments with a prominent toothlike basal appendage reaching up to about half the length of the filament; anthers oval, dorsifixed. **Pollen** tricolpate, micro-reticulate. **Ovary** with 3–5 ovules per locule, narrowly oblong, upper half scattered with simple hairs; **styles** 5, basally united for 1–2mm, sparsely covered with simple and glandular hairs; stigma fimbriate (Figure 2).

![Figure 2](image_url): A dissected flower of *O. hygrophila*. a. Sepals, scale bar represents 3mm. b. Petals, scale bar represents 2.2mm. c. Androecium, scale bar represents 0.8mm. d. Gynoecium, scale bar represents 0.9mm
Diagnostic characters and affinities

*O. hygrophila* is a glabrous, stemless geophyte. It has conduplicate leaflets, each with two prominent round to oblong calli abaxially just below the apex. This species has white flowers with a funnel-shaped yellow tube. All anthers are clearly ovoid. *O. hygrophila* resembles *O. tenella* in flower colour, anther shape and in having similarly shaped, conduplicate leaflets of which the median leaflet is sometimes shortly petiolulate. *O. hygrophila* is, however, stemless and entirely glabrous, with prominent calli on the leaflets, sepals and petals, whereas *O. tenella* has a well-developed stem above ground, pubescent petioles, leaves and sepals and no calli. *O. hygrophila* also resembles *O. callosa* in terms of its stemless habit, leaflet shape and the shared presence of calli on the sepals and petals. *O. callosa* is, however, variously hairy, lacks calli on its leaflets and has rose-coloured petals. *O. tenella* and *O. callosa* also have supra-areolate pollen, while the pollen grains of *O. hygrophila* are micro-reticulate.

Distribution and ecology

*O. hygrophila* is known only from Groot Kliphuis in the Pakhuis Pass near Clanwilliam (Figure 3). It is confined to a distinct seepage band running down the moist western slopes of the Pakhuis Mountain. All of the plants occur fairly low down this slope, in waterlogged shale patches in the full sun. The area appeared to have burnt about two years prior to our visit. In the wild this species flowers during November, although cultivated specimens have flowered during August in 2002 and 2003 in the Stellenbosch University Botanical Gardens, South Africa.

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Figure 3: Geographical distribution of *O. hygrophila*
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