

## An unusual new species of *Oxalis* (Oxalidaceae) from the Knersvlakte, South Africa

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### Abstract

*Oxalis ericifolia* differs from all other southern African *Oxalis* in having massive papillate protrusions on the adaxial leaflet epidermis, broadly swollen peduncular articulations, a wine-red androecium and an extremely short petal claw, less than one fifth the length of the total petal. Other unusual features include the red colour of the claw and the substrate-induced flattening of the bulbs. It was discovered in a unique locality from which two other endemic *Oxalis* species have been described. A phylogenetic analysis of the nuclear ITS region places the new species in the informally recognized *Oxalis flava* clade.

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### 1. Introduction

The taxonomy of southern African *Oxalis* is complicated by the large number of species present in the region (*ca.* 200), and by the presence of many species complexes, which are inconsistently subdivided into varieties and forms (Salter, 1944). In addition, many morphological characters are environmentally plastic, resulting in a single species displaying considerable morphological variation between different microhabitats. These problems complicate the recognition of potentially new taxa. Given the current dearth of knowledge on ecological or reproductive characters of the genus, new taxa are most confidently recognized on the basis of an assemblage of unique morphological characters.

A new taxon collected in June 2008 presented a suite of morphological characters unobserved anywhere else in southern African *Oxalis*. At first glance the filiform petioles and translucent white adaxial leaflet colour were so dissimilar to

any known described species that non-flowering individuals were not immediately recognizable as a member of the genus. Further investigation showed that Salter (1944) had indeed collected this species (Salter 2498), but did not describe it due to a lack of flowering material and the sporadic seasonal above-ground appearance of the plants. Collection of flowering material confirmed that this species is new, and consequently we describe it here as *Oxalis ericifolia* Oberlander & Dreyer.

### 2. Materials and methods

#### 2.1. Morphological assessment

The morphology of the newly collected specimens was studied and compared to all described species (Salter, 1944; Ornduff, 1973; Oliver, 1993; Williamson, 1999; Kumwenda et al., 2004) as well as with all species in the *Oxalis* living collection housed in the Stellenbosch University Botanical Garden. In addition material of the newly-collected specimens was compared to all known and unidentified *Oxalis* species housed in the Stellenbosch University (STEU), Compton (NBG) and Bolus (BOL) herbaria.

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## 2.2. Palynology

Fresh pollen grains were mounted in water for immediate study with the aid of a Nikon YS100 light microscope (LM). Pollen samples were also prepared for Scanning Electron Microscope (SEM) analyses by mounting them onto aluminium stubs using double-sided carbon tape. They were sputter-coated with gold-palladium and studied using a Leo 1430 VP7 scanning electron microscope (SEM).

## 2.3. DNA sequencing and molecular analysis

To determine potential systematic relationships, we performed phylogenetic inference of DNA sequence data obtained from the nuclear Internal Transcribed Spacer region (ITS). DNA extraction and sequencing were performed at the DNA sequencing facility of Stellenbosch University. Following standard cell lysis procedures, DNA extraction used the NucleoSpin (R) 96 Plant genomic DNA extraction kit (Macherey-Nagel). The protocol was performed on a Genesis 200RMP liquid handler (Tecan) using the conditions recommended by the kit manufacturers. PCR, sequencing, contig creation and alignment of the sequence data followed Oberlander et al. (unpublished). Preliminary ITS data suggested a close relationship to the *Oxalis flava* clade (Oberlander et al., unpublished). The ITS sequences of all species within this clade were included in an analysis to more precisely place this new taxon (Table 1). All generated sequences were submitted to the NCBI's GenBank nucleotide database (<http://www.ncbi.nlm.nih.gov>) (Table 1). Parsimony analyses were conducted in PAUP\* v4.0b10 (Swofford, 2003), using heuristic searches to find all most parsimonious trees. Starting trees were generated using random taxon addition, and 1000 TBR branch-swapping replicates, saving ten trees per replicate, were implemented. Support levels for nodes were assessed using nonparametric bootstrap (10 000 replicates) using heuristic searches. Bayesian Inference was conducted on the same data set using MrBayes v3.1.2 (Ronquist and Huelsenbeck, 2003) under the model of sequence evolution as chosen in MrModeltest v2.2 (Nylander, 2004). Five million generations in two separate analyses were run in order to ensure adequate sampling of the posterior distribution.

## 3. Results

### 3.1. Morphological assessment

The new taxon displays many characters not otherwise found in southern African *Oxalis* (Fig. 1). Most distinctively, the petal claw of the flower is much shorter than in any other regional *Oxalis*, never more than one fifth the length of the petal and often much shorter. Although there are other southern African *Oxalis* species with campanulate flowers, this is the only taxon to achieve this by contracting the claw, instead of re-orienting the entire petal. The claw is also wine-red, a character shared by other *Oxalis* such as *O. callosa* Knuth and *O. oculifera* E.G.H. Oliver. The peduncles of this

Table 1

List of all *Oxalis* taxa included in phylogenetic analyses.

Species	Reference number	GenBank accession number
<i>Oxalis adspersa</i> E. & Z.	MO66	EU436936
<i>Oxalis argillacea</i> Bolus f.	MO282	EU436940
<i>Oxalis aurea</i> Schltr	MO267	EU436935
<i>Oxalis campylorrhiza</i> Salter	MO127	EU436947
<i>Oxalis cathara</i> Salter	MO582	EU436931
<i>Oxalis</i> cf. <i>flava</i> L.	MO1101	FJ211169
<i>Oxalis</i> cf. <i>canaliculata</i> manuscript species M. B. Bayer	MO583	EU436946
<i>Oxalis clavifolia</i> Sond.	MO556	EU436951
<i>Oxalis comosa</i> E. Mey. ex Sond.	MO238	EU436954
<i>Oxalis crocea</i> Salter	MO124	EU436950
<i>Oxalis deserticola</i> Salter	MO526	EU436939
<i>Oxalis dines</i> Ornduff	MO653	EU436943
<i>Oxalis disticha</i> Jacq.	MO596	EU436942
<i>Oxalis dregei</i> Sond.	MO796	EU436930
<i>Oxalis ericifolia</i> Oberlander & Dreyer	MO1143	FJ211170
<i>Oxalis fabaefolia</i> Jacq.	MO152	EU436925
<i>Oxalis flava</i> L.	MO25	EU436924
<i>Oxalis flava</i> L.	MO183	FJ211173
<i>Oxalis flava</i> L.	MO414	FJ211174
<i>Oxalis flava</i> L.	MO576	FJ211172
<i>Oxalis flava</i> L.	MO753	FJ211175
<i>Oxalis flaviuscula</i> Salter	MO132	EU436929
<i>Oxalis furcillata</i> Salter	MO228	EU436952
<i>Oxalis inconspicua</i> Salter	MO138	EU436944
<i>Oxalis louisae</i> Salter	MO139	EU436928
<i>Oxalis monophylla</i> L.	MO7	EU436927
<i>Oxalis monophylla</i> L.	MO60	FJ211177
<i>Oxalis monophylla</i> L.	MO795	FJ211176
<i>Oxalis namaquana</i> Sond.	MO144	EU436941
<i>Oxalis obtusa</i> Jacq.	MO194	EU436922
<i>Oxalis pillansiana</i> Salter and Exell	MO549	EU436938
<i>Oxalis purpurea</i> L.	MO300	FJ211171
<i>Oxalis salteri</i> L. Bolus	MO280	EU436932
<i>Oxalis sonderiana</i> (O. Kuntze) Salter	MO140	FJ211178
<i>Oxalis</i> sp. affin. <i>campylorrhiza</i>	MO120	EU436948
<i>Oxalis stenoptera</i> Turcz.	MO553	EU436934
<i>Oxalis suavis</i> R. Knuth	MO385	EU436937
<i>Oxalis uliginosa</i> Schltr	MO394	EU436933
<i>Oxalis viscosa</i> E. Mey. ex Sond.	MO73	EU436949

new taxon are also unique among southern African *Oxalis* species. The basal articulation of the peduncle is so massively swollen that it appears almost gall-like in comparison to the rest of the peduncle. Unlike any other known species, the three leaflets of this species display very large papillae on the adaxial leaflet epidermis. The leaflet margins are rolled upward along the entire length of the leaflet, giving the leaves a distinct linear-ericoid appearance. This makes the plants inconspicuous when not in flower. The shiny white adaxial papillae are so large and dense that they are visible with the naked eye between the rolled leaflet margins. Although many known *Oxalis* species produce conduplicate leaves, no other species has such distinctly involute margins. The new taxon also has wine-red filaments, which is another unusual character for the genus.



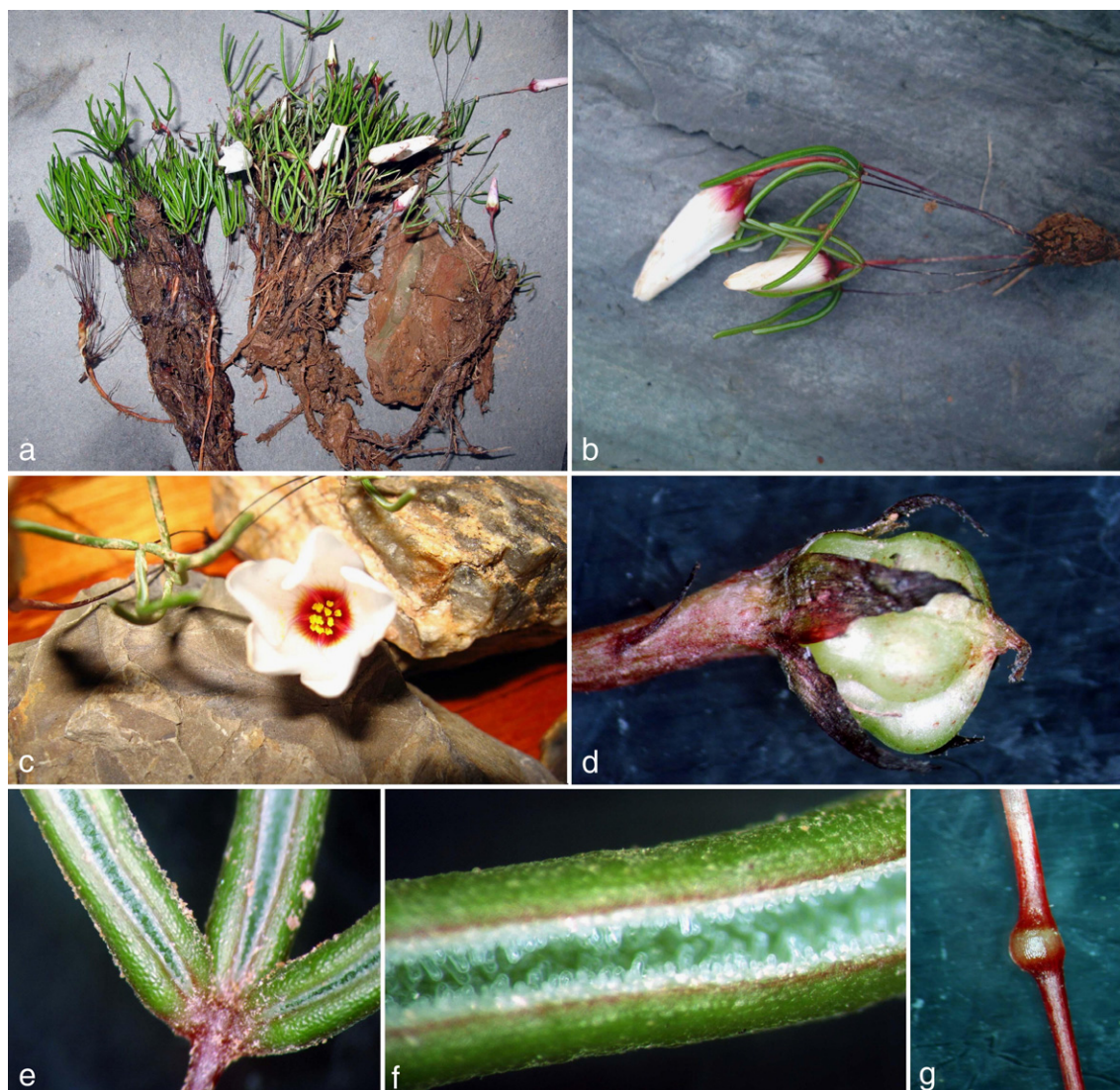


Fig. 1. *Oxalis ericifolia*. (a) Typical clustered habit. (b) side view of immature flower. (c) view of open flower showing wine-red eye at base of corolla. (d) fruit. (e) leaflet articulation. (f) close-up of the adaxial leaf surface showing papillae. (g) swollen basal articulation on peduncle.

### 3.2. DNA sequencing and molecular analysis

Phylogenetic sequence analysis of the new taxon is somewhat equivocal (Fig. 2). Although both parsimony and Bayesian inference agree on the close relationship of this taxon with the *O. flava* clade, parsimony does not support any affinity with the included taxa. Bayesian inference strongly supports a clade containing the new taxon, *O. adspersa* Eckl. & Zeyh., *O. aurea* Schltr. and *O. uliginosa* Schltr. Although Bayesian methods do tend to overinflate support measures (Cummings et al., 2003), it is known that model-based approaches utilize DNA sequence information more efficiently, and thus provide a better estimate of phylogenetic placement. Moreover, the new taxon is not particularly similar to any of these three species.

### 3.3. Palynology

#### 3.3.1. LM

Pollen grains are tricolpate, isopolar and triangularly rounded in polar view and spherical to oblate in equatorial

view (Fig. 3). The apocolpium is reduced, and colpus ends are obtusely rounded.

Pollen grains are semitectate, with a coarsely reticulate, homobrochate tectum. Lumina are irregularly angular, abruptly diminishing in size towards the colpi resulting in a distinct colpus margin with rounded lumina. The average lumina diameter is 2.9  $\mu\text{m}$ . Muri are stratified, and have an average thickness of 0.8  $\mu\text{m}$ . The nexine floor is beset with blunt intraluminary bacula, predominantly clustered along the muri. The colpus membrane is coarsely granular.

The morphology of pollen grains of this species corresponds to pollen grains of *O. tenuipes* var. *tenuipes* (Pollen type C12, Dreyer 1996). This pollen type is very similar to pollen types C10 and C11, in that all three types have open reticulate tectums with intra-luminary bacules clustered along the muri. The only consistent difference between these three pollen types is the average lumina diameter, which is larger in pollen type C12 than in the other two types (Dreyer, 1996). Dreyer (1996) concluded that species with reticulate pollen of these three types are probably closely

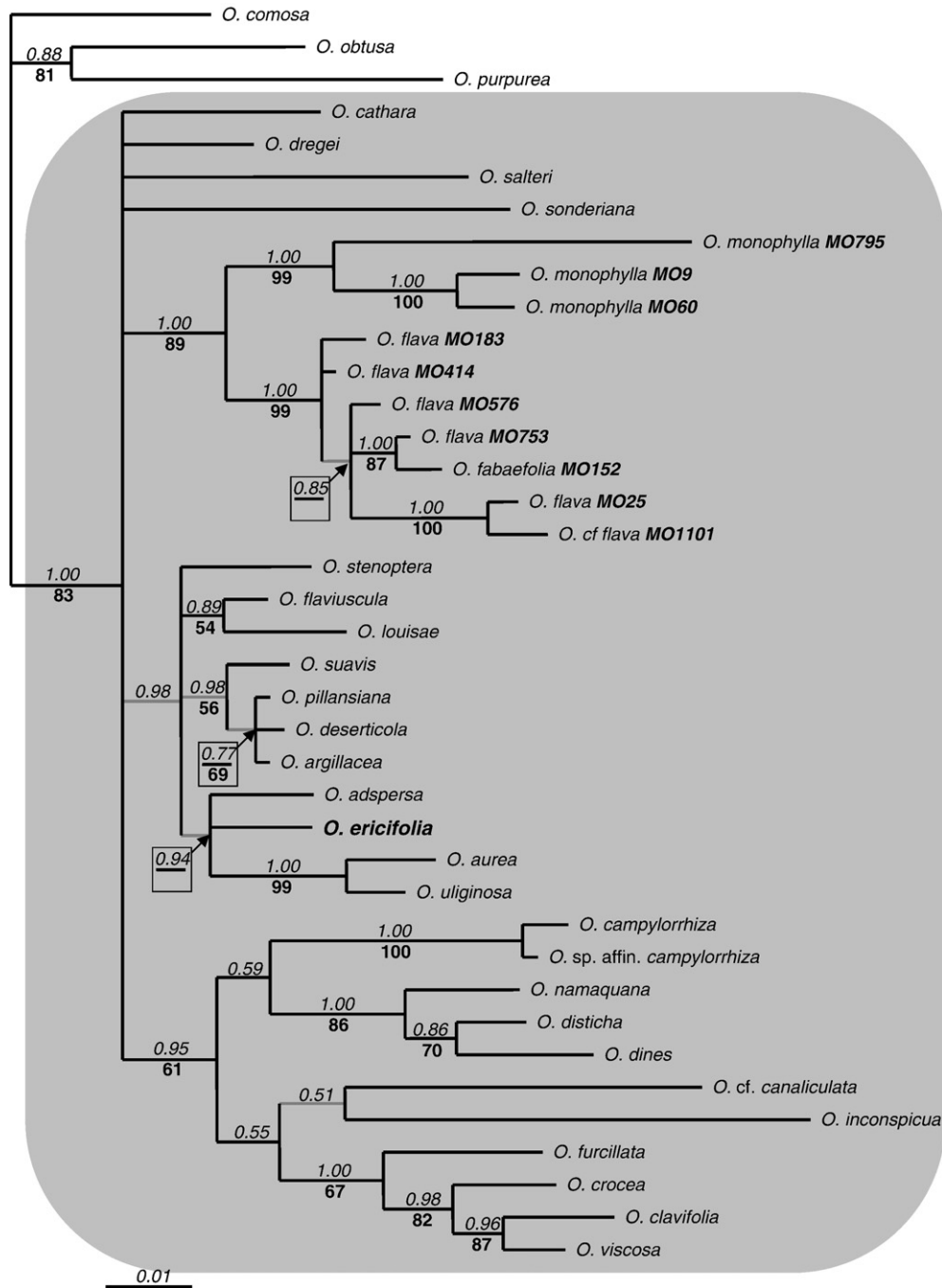


Fig. 2. Bayesian majority-rule consensus tree of the *Oxalis flava* clade (indicated by the grey shading) and outgroups. Branch lengths are average number of changes per site. Numbers above branches are Bayesian posterior probability values; numbers below are parsimony bootstrap. Grey branches indicate nodes that collapse in the parsimony strict consensus tree.

related. Most members of the *O. flava* clade have reticulate pollen of the type C10 or C11.

#### 4. Taxonomy

Morphological data clearly confirm this newly collected taxon to be a distinct new species of *Oxalis*. It is thus newly described as follows:

*Oxalis ericifolia* Oberlander & Dreyer, sp. nov. (Figs. 1 and 4)

Geophytum acaule, bulbis complanatis, petiolis tenuibus filiformibus ad filo metallico similibus, foliolis conduplicatis papillas adaxiales egregie magnas habentibus, pedunculis sub flore crassis et articulis basalibus grandibus, floribus albis centro vinoso-rubris, campanulatis sine tubo florali.

TYPE. —3119 (Calvinia): Western Cape Province, broken shale outcrop 40 km north east of Vanrhynsdorp on road to Nieuwoudtville, 20-06-2008, Oberlander 500 (STEU, holo.; BOL, NBG iso.).



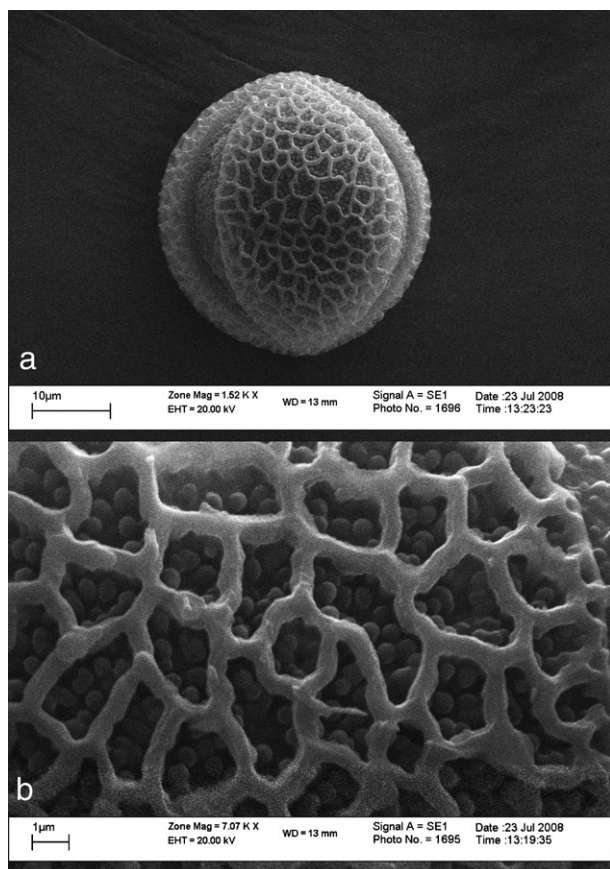


Fig. 3. SEM micrograph of *O. ericifolia* pollen. (a) Whole grain in equatorial view. (b) Mesocolpial portion of the tectum.

Slender, stemless geophyte, 40–70 mm tall, clump-forming, variously covered with hyaline pluricellular hairs, glabrescent with age. *Bulbs* aggregated into complex system of old rhizomes, sheaths, adventitious roots and developing bulbs; with new years growth penetrating through remains of previous years; individual bulbs lanceolate to obovate, flattened due to growth in between layers of substrate, 10–20 mm long, tunics papery, dark brown to black, glabrous. *Rhizome* 45–160 mm long or longer, white to orange, succulent, often very thick, densely glandular pubescent towards apex, glabrescent towards bulb, densely enclosed in fibrous mass of old tunics and bulb remains, leaf bases on upper rhizome imbricated by 3–5 scales, scales sparsely ciliate. *Above-ground stem* absent. *Leaves* apically congested at tip of rhizome, 3–17 per plant, erect, all of similar height; petiole 15–30 mm long, filiform, up to 0.4 mm in diameter, rigid, wiry, red, leaves densely glandular hairy when young, becoming less so with age; leaflets 3, palmate, erect, rigid, sessile to very shortly petiolulate, involute, shortly glandular-pubescent when young, glabrescent with age, 15–28 × 2.0–2.5 mm, apex rounded to slightly emarginate, adaxial epidermis beset with cylindrical, translucent papillae. *Peduncle* 1-flowered, 35–55 mm long, slightly thicker than petioles, swollen apically, basal articulation massively swollen, almost gall-like in appearance, glabrescent with age, shiny. *Sepals* narrowly triangular to lanceolate, 2.0–

3.0 × 1.0–1.5 mm, acute, wine red, glabrescent with glandular hairs along margins; bracts 2, filiform, alternate, variable in position but mostly in middle or upper part, sometimes appressed to calyx, wine red, glabrous. *Corolla* campanulate, white with base of petals wine red, floral tube absent; petals oblanceolate, minutely clawed, 12–16 mm long, apex truncate or rounded, occasionally subacute, ecallose. *Stamens* in 3 series, 2 series per plant, shortest 2.0–3.0 mm long, middle 4.0–5.0 mm long and longest 6.0–7.0 mm long, basally adnate; anthers oblong, yellow; filaments wine red, covered with red glandular hairs; filament teeth oblong to spatulate, adnate to longer filaments, obtuse, glabrous; pollen bright yellow. *Ovary* narrowly ovoid, 1.2–1.4 mm long, glabrous, 5-locular with 2–3 ovules per locule; styles 5, free, sparsely glandular hairy in distal part, in 3 series with 1 series per plant, shortest 1.5 mm long, middle 4.0 mm long, longest 7.0 mm long; stigmas yellow-green, cup-shaped, fimbriate. *Fruit* a 5-locular capsule, globular, not longer than sepals, light green. *Seeds* without endosperm. Pollen tricolporate, triangularly rounded in equatorial view, spherical in polar view, tectum coarsely reticulate with intra-luminary bacules clustered along the muri.

## 5. Distribution and ecology

*O. ericifolia* was discovered in a single locality, growing in association with *O. deserticola* Salter and *O. melanograptia* Salter. It is currently only known from this extremely rocky band on the southwestern face of a broken shale outcrop 40 km north east of Vanrhynsdorp. Despite an apparently restricted distribution range, the plants were found to be locally abundant. Plants were scattered in loose clumps across the broken shale bands, where they grew in full sun. Individuals of all three morph types were present, and numerous individuals bore fertile capsules. *O. ericifolia* flowers in June.

## 6. Diagnosis and relationships

The specific epithet refers to the distinctly ericoid leaflets. Unlike *Erica* leaves, however, the leaflets of *O. ericifolia* are rolled adaxially, and almost completely enclose an unusually papillate adaxial epidermis. Other diagnostic characters of this species include the filiform petioles, the swollen articulation on the peduncle, the wine-red filaments and petal-bases, and the very short claw.

In terms of relationships, several characters suggest that this species belongs in an informal group of species termed the *O. flava* clade (Oberlander et al., submitted for publication). ITS data strongly support a membership in this clade. The pollen types of *O. ericifolia* and other members of the *O. flava* clade are very similar. Another potential character that supports this relationship is the presence of uniseriate pluricellular hairs, which is very common amongst members of the *O. flava* clade. Salter (1944) suggests an affinity with *O. pulvinata* Salter, which differs in being entirely glabrous, in having multifoliolate leaves, and in the well-developed claw of the petal.

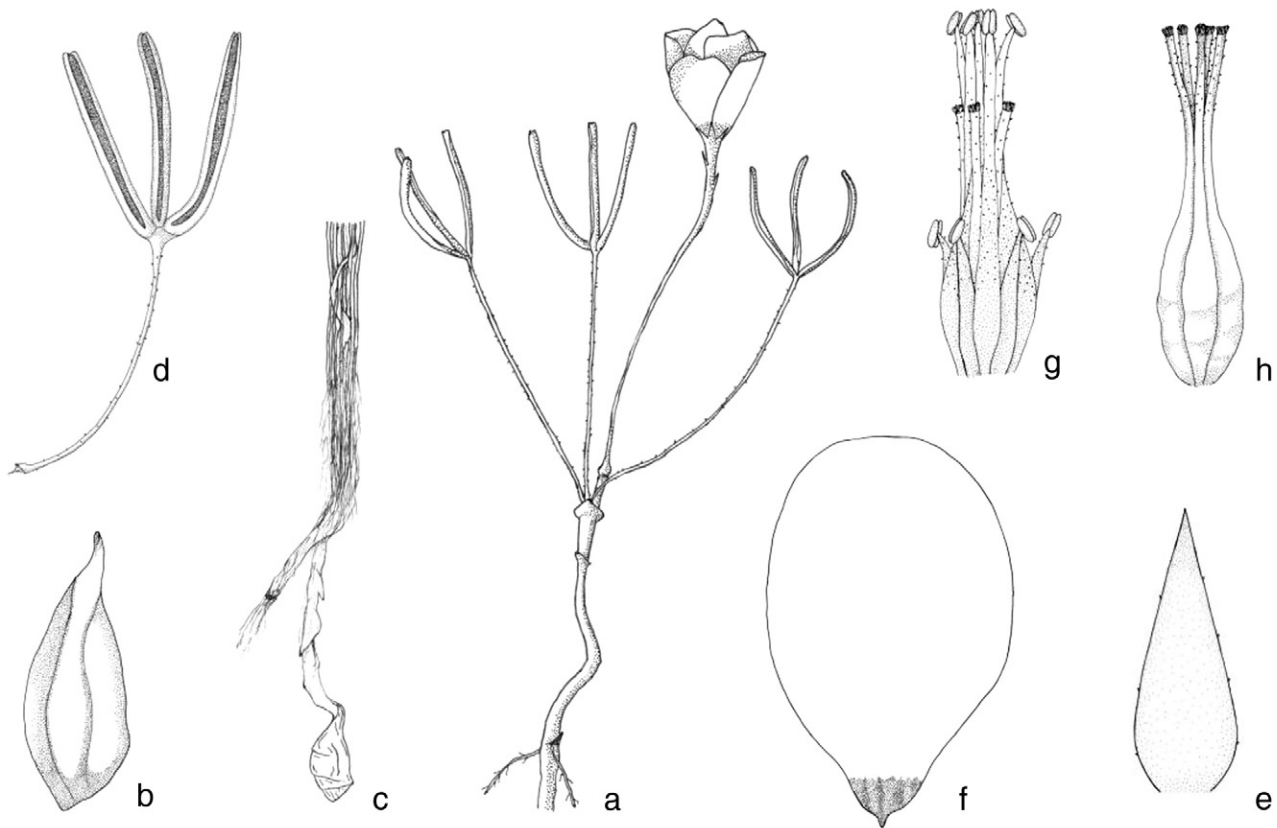


Fig. 4. Line diagram of *O. ericifolia*. (a) above-ground plant and rhizome. (b) bulb. (c) young bulb and underground habit. (d) leaf. (e) sepal. (f) petal. (g) androecium and gynoeceum of the mid morph. (h) gynoeceum.

It would seem surprising that such an unusual species as *O. ericifolia* has not been described in the period between initial discovery and the present, especially given that it is sympatric with *O. deserticola* (described by Salter and Compton; 1935) and *O. melanograptia* (described by Salter and Compton, 1936), two rare species of conservation importance. However, as Salter (1944) mentions, the species appears sporadically, and can go an entire season without appearing above-ground. All three species are currently only known from this outcrop. Another feasible explanation is that *O. ericifolia* flowers slightly earlier than these two species, and may well have finished flowering by the time the other species are in full bloom. When not in flower the plants are inconspicuous, and hard to recognize as a member of the genus *Oxalis* without close observation.

The discovery of *O. ericifolia* brings the number of recognized southern African *Oxalis* species to 201. Due to the artificial nature of the most recent morphological classification (Salter, 1944), this species is not infra-generically placed within one of the nine currently recognized sections. Molecular phylogenetic results also did not conclusively reveal close affinities, but strongly support inclusion of this species within the *O. flava* clade. This is supported by morphological characters such as the pollen type and the presence of uniseriate, multicellular epidermal hairs. Both of these characters are almost exclusively shared by members of the *O. flava* clade.

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