**Oxalis saltusbelli:** A new *Oxalis* (Oxalidaceae) species from the Oorlogskloof Nature Reserve, Nieuwoudtville, South Africa

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Abstract

In South Africa, the genus *Oxalis* includes 200 species inhabiting a wide range of habitats particularly in the winter rainfall areas of the western and southern Cape. Many species are morphologically extremely plastic, which makes species identification and description of new species challenging based solely on morphological criteria. Here we report the discovery of a new species, *Oxalis saltusbelli* Dreyer & Roets, from the Cape Floristic Region of South Africa. Placement of this taxon within the genus was based on DNA sequence-based analyses of the Internal Transcribed Spacer (ITS) region and morphological comparisons with related species. The new species is currently only known from the Oorlogskloof Nature Reserve. It has sticky bulb tunics, multifoliolate leaves, with variable leaflet shapes ranging from oblong to linear, depending on age, concave petioles and lilac flowers. This study brings the number of *Oxalis* species in South Africa to 201 and highlights the urgent need for molecular and morphological re-evaluation of all taxa in this morphologically and ecologically diverse plant lineage.

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1. Introduction

*Oxalis* is represented by 200 species in southern Africa (Salter, 1944; Ornduff, 1973; Oliver, 1993; Williamson, 1999; Kumwenda et al., 2004; Manning and Goldblatt, 2008), of which 120 occur within the Cape Floristic Region (CFR) (Goldblatt and Manning, 2000). Within southern Africa, species in this morphologically and ecologically diverse genus are characterized by having true bulbs that bear seasonal leaves and flowers at the end of a subterranean stem. Most CFR species flower during the wetter and cooler winter months. Within the CFR, *Oxalis* species occur in both Fynbos and Succulent Karoo vegetation, where they occupy habitats ranging from extremely xeric to aquatic (in seasonal pools) across the CFR. As in other heterostylous taxa, flower structure is conservative (Barrett, 1992). Many species also display flower color-polymorphism, making it nearly impossible to distinguish species using floral morphology alone.

The southern African members of the genus were last revised by Salter (1944), with more recent work confined to descriptions of individual species (Salter, 1944; Ornduff, 1973; Bayer, 1992; Oliver, 1993; Williamson, 1999; Kumwenda et al., 2004; Manning and Goldblatt, 2008) and some minor taxonomic changes (Bayer, 1992). It is generally recognized that the current morphological classification is artificial. This can largely be ascribed to considerable vegetative plasticity in the genus. In addition to many morphologically narrowly-defined species, the current taxonomy also includes numerous species complexes that display considerable morphological variation (Salter, 1944). This is, for example, illustrated by the eleven species formally included in section *Angustatae* subsection *Pardales* by Salter (1944). These were recently synonymized under *Oxalis pardalis* Sond. s.l. (Bayer, 1992), despite considerable variation in morphology across this complex. Other well-known, variable and problematic *Oxalis* species complexes include *O. flava* L., *O. purpurea* L., *O. obtusa* Jacq. and *O. hirta* L. The extreme morphological plasticity in *Oxalis* renders the identification and demarcation of new species among southern African members difficult. It is thus often problematic to discern whether a morphologically unique
in order to revise the systematics and taxonomy of Oxalis, we are in the process of completing a DNA-based phylogenetic reconstruction of southern African species. Preliminary results of this study (Oberlander et al., 2004) have enabled us to re-evaluate the taxonomic revision of Salter (1944). These results have also proposed unexpected new relationships between species previously thought to be only distantly related (Salter, 1944). One such example is the O. tomentosa alliance, a well-supported lineage (bootstrap support of 92%; Oberlander et al., 2004) including the species O. oligophylla Salter (section Angustatae subsection Lineares; Salter, 1944), O. hygrophila Dreyer (section Lattifoliolatae; Kumwenda et al., 2004) and O. tomentosa L. (section Angustatae subsection Multifoliolatae; Salter, 1944). Recent research has added O. palmifrons Salter (section Angustatae subsection Multifoliolatae; Salter, 1944) to this alliance. The current taxonomic spread of this alliance is reflected in its very diverse morphology, rendering it hard to identify shared morphological traits between these species. It is within this context that a non-flowering specimen collected from the Oorlogskloof Nature Reserve near Nieuwoudtville, Northern Cape, during July 2007 raised considerable interest. This locality was revisited during June 2008, and flowering and fruiting individuals were obtained.

In this study we evaluate the position of this apparent new taxon based on both DNA and morphological evidence. We also demonstrate how the new species displays a unique combination of characters expressed in other species in the O. tomentosa alliance.

### Table 1

Specimens used in morphological and molecular comparisons

| Species       | Locality                                      | Herbarium (Reference number) | Collector                  | GenBank accession number |
|---------------|-----------------------------------------------|------------------------------|----------------------------|-------------------------|
| O. hygrophila | Groot Kliphuis, Pakhuis Pass near Clanwilliam | STEU (MO230)                 | Dreyer & Kumwenda 1        | EU437024                |
|               | Groot Kliphuis, Pakhuis Pass near Clanwilliam | BOL                          | Leipoldt s.n.              | –                       |
|               | Groot Kliphuis, Pakhuis Pass near Clanwilliam | BOL                          | Bolus s.n. BH 9400         | –                       |
|               | Western slope of Gifberg                     | BOL                          | Dreyer s.n.                | EU437023                |
| O. oligophylla| Gifberg Pass                                  | STEU (MO293)                 | Dreyer s.n.                | EU437023                |
| O. palmifrons | Tweedside                                     | NBG                          | Salter 6053                | –                       |
|               | Wolwerivier, Ceres                            | BOL                          | Salter 6063                | –                       |
|               | South of Middelpos                            | STEU                         | Suda 113                   | –                       |
|               | Ex. Hort.                                     | STEU (MO403)                 | Holmes s.n.                | EU437021                |
|               | Between Sutherland and Matjiesfontein         | STEU (MO824)                 | Oberlander s.n.            | FJ211166                |
| O. saltusbelli| Oorlogskloof Nature Reserve, Nieuwoudtville  | STEU (MO1000)                | Dreyer & Roets 25          | –                       |
|               | Oorlogskloof Nature Reserve, Nieuwoudtville  | STEU (MO1119)                | Dreyer 837                 | FJ211167                |
| O. tomentosa  | Road to Saron, 500 m off R44                  | STEU (MO363)                 | Oberlander 80              | FJ211168                |
|               | Darling                                       | STEU (MO62)                  | Te Roller 10               | EU437022                |
|               | Cape Peninsula                               | BOL                          | Salter 508                 | –                       |
|               | Malmsbury                                     | BOL                          | Salter 2164                | –                       |
| O. bifida     | Jonkershoek                                   | STEU                         | Dreyer s.n.                | EU437017                |
| O. massoniana | Ex Hort.                                      | STEU (MO399)                 | Van Wijk 2968              | EU437015                |
| O. smithiana  | Ex Hort.                                      | STEU (MO322)                 | Bellstedt 699              | EU436964                |
| O. eckloniana | Theronsberg Pass, Ceres                       | STEU (MO39)                  | Dreyer 628                 | EU437025                |
| O. hirta      | Piekensierskloof Pass, Citrusdal              | STEU (MO77)                  | Dreyer 646                 | EU436971                |
| O. fibrosa    | Ladysmith                                     | STEU (MO332)                 | Oberlander 51              | EU436958                |
| O. polyphylla | Silvermine, Cape Peninsula                    | STEU (MO47)                  | Ciliers s.n.               | EU437010                |
| O. engleri ana| Between Caledon and Villiersdorp              | STEU (MO195)                 | Oberlander 10              | EU436961                |
| O. suteroides | Between van Rhyn’s dorp and van Rhyn’s Pass   | STEU (MO527)                 | Oberlander s.n.            | EU436963                |

### 2. Materials and methods

#### 2.1. Morphological assessment

The morphology of the newly collected specimens was studied and compared to fresh material of O. hygrophila, O. oligophylla, O. palmifrons, and O. tomentosa obtained from the field as well as the Oxalis living collection housed in the Stellenbosch University Botanical Garden. This was supplemented by herbarium material from Stellenbosch University (STEU, Stellenbosch, South Africa), Compton (NBG, SANBI, South Africa) and Bolus (BOL, Cape Town) herbaria (Table 1). Both morphological and ecological traits of these species were included in comparative studies. Pollen grains were studied with the aid of a Nikon YS100 light microscope.

#### 2.2. DNA sequencing and molecular analysis

Phylogenetic reconstruction was based on 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. (2004). Due to the uncertain placement of the O. tomentosa alliance within the context of the southern African lineage, a
variety of taxa were included as outgroups (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 branch and bound searches to find all most parsimonious trees, saving all trees. Support levels for nodes were assessed using nonparametric bootstrap (1000 replicates) using branch and bound 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.

2.3. Geographical distribution and habitat preferences

Species geographic distributions were obtained from our own collections, Salter (1944), the National Herbarium Pretoria (PRE) Computerised Information Service database (PRECIS, http://www.sanbi.org/information/databases.htm) and the listed herbaria (Table 1).

3. Results

3.1. Morphological assessment

Morphological data suggested that the newly collected Oorlogskloof specimens are related to the O. tomentosa alliance.

Table 2

| Character                     | O. hygrophila | O. oligophylla | O. palmifrons | O. saltusbelli | O. tomentosa |
|-------------------------------|---------------|----------------|---------------|----------------|--------------|
| Bulb shape                    | Ovate with acute apex | Oval to oval-oblong, often deformed | Ovoid with attenuate-acicular apex | Obovate to globose, with pointed apex | Oblong-ovoid with pointed apex |
| Bulb length                   | 9–11 mm       | 15–30 mm       | up to 40 mm   | 10–60 mm       | 15–20 mm     |
| Bulb tunics colour and texture| Light brown, thin | Black, sticky, hard | Dark brown, rather hard | Blackish-brown, sticky, rather hard | Greyish Brown |
| Rhizome                       | Not enclosed in sheath | Not enclosed in sheath, with adventitious roots extending from upper part | Not enclosed in sheath | Enclosed in tunic sheath, with masses of adventitious roots extending from upper part | Enclosed in tunic sheath |
| Leaf number                   | 10–12         | 1–2 (3)        | Very numerous | 10–12 (20)     | 5–15         |
| Leaf indumentum               | Glabrous      | Adaxially glabrous, abaxially sparsely glandular pilose | Adaxially glabrous, abaxially pubescent | Sparsely covered with long, soft hairs | Pilose on both surfaces |
| Petiole length                | 23–56 mm      | 60–120 mm      | up to 20 mm   | 50–160 mm      | up to 50 mm  |
| Petiole cross-section         | Round         | Round          | Compressed    | Adaxially concave | Round to flattened |
| Leaflet number                | 3             | 3              | 20–29         | (6) 8–11 (13) | 10–20        |
| Leaflet arrangement           | Arranged at 90° angles to one another | Arranged at 90° angles to one another | Palmate | Peltately arranged around swollen pulvinus | Peltately spreading |
| Leaflet shape                 | Narrowly elliptic to elliptic, conduplicate | Narrow linear, conduplicate | Oblong, conduplicate | Linear, oblong to elliptical, open to conduplicate | Oblong-cuneate, often conduplicate |
| Leaflet calli                 | 2, apical, round to oblong, sometime with 1–6 less conspicuous calli along upper margin | Absent | Absent | Absent | Absent |
| Petiolule                     | Sometimes present in medial leaflet | Absent | Absent | Present, 0.3–0.5 mm | Absent |
| Peduncle length               | 25–60 mm      | 120–160 mm     | 5–15 mm       | 75–100 mm      | 50–100 mm    |
| Flower colour                 | White         | White          | White         | Pale lilac     | White |
| Calli on petals               | Present | Absent | Absent | Absent | Absent |

Morphological characters shared by species in this lineage include their acaulescent habit, sticky blackish-brown bulb tunics, and mostly white flowers. With the exception of O. tomentosa, all species are sparse flowerers.

Numerous morphological characters collectively differentiate the newly collected taxon from other species in the O. tomentosa alliance (Table 2). Unique diagnostic characters of this taxon include adaxially concave petioles, the presence of short petiolules on all leaflets and its lilac colored flowers.

3.2. DNA sequencing and molecular analysis

The final alignment featured sixteen taxa and 785 characters, with gaps coded as missing data. Of these characters, 46 (6%) were parsimony-informative. Parsimony branch and bound searches yielded four trees of length 171. The topology of the O. tomentosa alliance was identical in all trees. No tree resolved the strongly supported trichotomy of the newly collected taxon, O. tomentosa and O. palmifrons (Fig. 1).

Bayesian Inference was conducted under the GTR + Γ model of sequence evolution, as selected by MrModeltest. Both independent runs were deemed to have converged on a stable posterior distribution, and the first 1000 sampled generations were discarded as burnin.

Both parsimony and Bayesian Inference show identical topologies for the O. tomentosa alliance. The newly collected taxon is located in a trichotomy with O. palmifrons and...
and *O. tomentosa*. The other two member species of the alliance are strongly supported as sister to these three taxa (Fig. 1).

### 3.3. Geographical distribution and habitat preferences

Biogeographical data of species within the *O. tomentosa* alliance are depicted in Fig. 2. Both *O. tomentosa* and *O. palmifrons* are restricted to shale substrates, with the former species distributed in the low-lying areas of the extreme South-western Cape, while the latter is confined to the Succulent Karoo from Matjiesfontein to the Sutherland district. Both species grow in exposed, sunny positions at low altitudes. *Oxalis hygrophila*, *O. oligophylla* and the newly collected taxon are narrowly endemic sandstone specialists respectively restricted to the Pakhuis Pass, Giftberg and the Bokkeveld Plateau. They all occur at higher elevations of between 400 to 500 m above sea level. *O. hygrophila* prefers seasonally moist seepage bands in full sun, while both *O. oligophylla* and *O. saltusbellii* are closely associated with rocky habitats, where they often occur in shady crevices below rocks.
4. Taxonomy

Morphological data indicate that the newly collected taxon represents a distinct species, and molecular data confirms a close affinity with *O. palmifrons* and *O. tomentosa* in the *O. tomentosa* alliance. It is thus newly described as follows:

*Oxalis saltusbelli* Dreyer & Roets, sp. nov. Figs. 3 and 4.

Bulbi tunicis viscidis atrobrunneis foliolis valde heterophylloides petiolis adaxiale concavis floribus pallide lilacinis distinguatur.

**TYPE.** -3119 (Calvinia): Northern Cape Province, Distr., Oorlogskloof Nature Reserve, along Rock Pigeon hiking trail, ca. 675 m, (-CA), 13-06-2008, Dreyer 837 (STEU, holotype; BOL, NBG isotypes).

Geophyte, 10–250 mm tall, aggregated into clumps. *Bulb* obovate to globose, with pointed to bent, narrow apex, 10–60 mm long, tunics blackish-brown, sticky, indumentum densely glandular hairy when young, glabrescent with age. *Rhizome* vertical, up to 200 mm long, with masses of adventitious roots extending from upper part, upper rhizome nodes with prominent straw-coloured, amplexicaul, broadly triangular scales, abaxially covered in long, soft hairs; sheath present, light brown, glabrescent, glandular hairy along entire length, densely villous at apex. Above-ground stem absent. *Leaves* 3–15 per plant, smaller, robust, semi-succulent and reddish-green in sun plants; larger, more slender, herbaceous and deep green in shade plants; *petioles* adaxially concave to almost U-shaped, 50–160 mm, sparsely covered with long, soft hairs over its entire length; *leaflet* (6) 8–11 (13), very shortly (0.3–0.5 mm) petiolulate, of unequal length, radially arranged around swollen pulvinus, linear and conuplicate in sun plants, oblong to linear and non-conduplicate in shade plants, abaxially purple, elliptical to oblong in young plants, 10–90 × 0.5–3 mm, apex shallowly emarginate, base attenuate, adaxially glabrous, abaxially sparsely covered with long, soft hairs when young, glabrescent with scattered hairs restricted to leaflet margin and mid vein in mature leaves, with two rounded to oblong, reddish-orange apical calli abaxially. *Peduncle* 1-flowered, 75–100 mm long, erect, reddish-green, evenly covered with long soft hairs, bracts 2 on upper part of peduncle, alternate, linear to filiform, up to 0.6 mm long. *Sepals* 5.0 × 1.0–1.5 mm, lanceolate-elliptical, apex acute, abaxially covered with long, soft hairs, green becoming reddish, especially along margins. *Corolla* 18.0–20.0 mm long, light lilac with funnel-shaped yellow tube, tube 8.5–9 mm long, petals obovate to broadly spathulate, abaxially with a few long soft hairs on outer margins, ecallose. *Stamens* in 3 series, 2 series per plant, the shortest level 1.5–2 mm, the middle level 2.5–3.0 mm and the longest level 6.2–6.0 mm long, all basally joined for 1.0–1.5 mm; anthers oblong, yellow; filaments white, glandular hairy over entire length; filament teeth greenish-white, 0.5–2 mm long, apically rounded to obtuse and curved outward. Ovary 1.2–1.5 mm long, ovoid, densely pilose, 5-locules each 1-ovuled; styles 5, separate, in three series with one series per plant, shortest level 1.5 mm long, middle level 3.0 mm long, longest level 6.0 mm long, erect, densely villous; stigmas green, capitate. *Fruit* a globular 5-
locular capsule. Seeds without endosperm. Pollen tricolpate, triagonally rounded in equatorial view, spherical in polar view, tectum finely reticulate.

5. Distribution

*O. saltusbelli* is confined to the Oorlogskloof on the Bokkeveld Escarpment, where it grows along the Rock Pigeon hiking trail and surrounding outcrops (Figs. 2 and 4b–d). It is found in course sand in both exposed, sunny habitats and in moist, shady crevices between sandstone rocks. Plants are found in scattered clusters. Plants flower in June, but flowering is not prolific, with only a few flowers per plant and flowering plants per population.

6. Notes

The specific epithet refers to the restricted distribution of this species to the Oorlogskloof National Park, Nieuwoudtville plateau, Northern Cape Province. Superficially this species (especially the sun forms) bears a very strong resemblance to the sympatric species *O. flava* in general appearance. Closer examination reveals obvious differences between these two species, and they remain distinct in sympatry. *O. saltusbelli* lacks the distinct basal petiolar articulations and papery bracts at the leaf bases that characterize *O. flava*. The sticky, blackish-brown tunics of *O. saltusbelli* are also very different to the papery, pale brown bulb tunics produced by *O. flava*.

7. Discussion

Both the morphological attributes (Table 2) and molecular phylogenetic placement of *O. saltusbelli* confirm a close affinity of this taxon with the *O. tomentosa* alliance, particularly with *O. tomentosa* and *O. palmifrons*. It differs from *O. tomentosa* in having a less hairy indumentum and a grooved petiole. It differs from *O. palmifrons* in having fewer leaves and a more terete petiole. The new species differs from both in the lilac flowers and more linear leaflets, as well as distribution range and substrate preference. *O. saltusbelli* is morphologically most similar to *O. tomentosa* and *O. palmifrons* in terms of leaflet number and arrangement, rhizome length and bulb shape and size. Like all members of this lineage, *O. saltusbelli* produces very distinct heterophyllous juvenile forms which bear a strong morphological similarity to mature plants of *O. tomentosa*. The habitat and substrate of the new species are comparable to both *O. oligophylla* and *O. hygrophylla*. Unique, diagnostic characters of *O. saltusbelli* include the adaxially concave to almost U-shaped petiole and the lilac flowers.

As the current infrageneric classification is artificial, we refrain from placing this taxon in any currently recognised section. Future classification systems should nevertheless reflect this species close relationship with members of the *O. tomentosa* alliance.

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