



Two new species of *Oxalis* (Oxalidaceae) from the Greater Cape Floristic Region

JAN SUDA^{1,2}, JANA KREJČÍKOVÁ^{1,2}, RADKA SUDOVÁ², KENNETH C. OBERLANDER^{2,3} & LÉANNE L. DREYER^{4,5}

¹Department of Botany, Faculty of Science, Charles University in Prague, Benátská 2, Prague, CZ-128 01, Czech Republic

²Institute of Botany, Academy of Sciences of the Czech Republic, Průhonice 1, CZ-252 43, Czech Republic

³Department of Conservation Ecology and Entomology, Stellenbosch University, Private Bag X1, Matieland, 7602, South Africa

⁴Department of Botany and Zoology, Stellenbosch University, Private Bag X1, Matieland, 7602, South Africa

⁵E-mail ld@sun.ac.za

Abstract

Two new multifoliolate species of *Oxalis* L. (Oxalidaceae) from the Hantam Karoo region of South Africa are described and illustrated: *Oxalis carolina* and *O. filifoliolata*. Both species occur in single populations in the extremely geophyte-rich area on the Bokkeveld Plateau in the Northern Cape Province. Morphological characteristics, phylogenetic position, habitat description and conservation status of the new species are provided, in addition to a diagnostic comparison with other phenotypically similar Cape species. Fifteen multifoliolate *Oxalis* species are currently recognized in South Africa, disregarding multifoliolate varieties of otherwise trifoliolate species. A key for multifoliolate *Oxalis* species known from the Bokkeveld Plateau is presented.

Key words: Cape Floristic Region, genome size, Oxalidaceae, phylogeny, South Africa, taxonomy

Introduction

With ± 200 species and many intraspecific taxa, *Oxalis* L. (Oxalidaceae) is the largest geophytic genus in the Greater Cape Floristic Region of South Africa (Dreyer & Makgaka 2003). Several new species have been described recently, including *O. ericifolia* Oberlander & Dreyer (2009: 242) from the Knersvlakte and *O. saltusbelli* Dreyer & Roets (2009: 113), a multifoliolate species from the Bokkeveld Plateau in the Northern Cape Province. Multifoliolate leaves are restricted to a small, but phylogenetically diverse, group of South African *Oxalis* species (Oberlander *et al.* 2011). In his monograph of the genus in South Africa, Salter (1944) recognized 14 multifoliolate species, 12 of which are still recognized (Dreyer & Makgaka 2003, Dreyer *et al.* 2010), in addition to four multifoliolate varieties of otherwise trifoliolate species. Two unusual and very distinct multifoliolate *Oxalis* taxa were discovered north of Nieuwoudtville in the Northern Cape Province during field work collecting species for cytogenetic analyses. We describe these species as *O. carolina* J. Suda & Sudová and *O. filifoliolata* J. Suda & Krejčíková, and evaluate their systematic position based on DNA sequence data, morphology and karyological evidence. In addition, we provide an updated key to all multifoliolate *Oxalis* species reported from the Bokkeveld Plateau, to facilitate identification of this distinct group in the geophyte-rich area.

Material and Methods

Morphological assessment

The new taxa were compared with descriptions of all known southern African species (Salter 1944, Ornduff 1973, Oliver 1993, Williamson 1999, Kumwenda *et al.* 2004, Dreyer *et al.* 2009, Oberlander *et al.* 2009) and with the living collection in the Stellenbosch University Botanical Garden. All named and unnamed *Oxalis* collections from the Stellenbosch University (STEU, Stellenbosch, South Africa) and Compton (NBG, Cape Town, South Africa) herbaria were examined. Although not strictly correct, we use the term “rhizome” when referring to the below-ground stem in order to maintain consistency with previously published taxonomic contributions, including the monograph of South African *Oxalis* (Salter 1944).

DNA sequencing and molecular analysis

The nuclear ribosomal Internal Transcribed Spacer region (ITS; Sun *et al.* 1994) was sequenced for the new taxa following the methods of Oberlander *et al.* (2011). By using morphological characters as a first estimate of phylogenetic placement, we added the sequence of *O. filifoliolata* to the ITS matrix of the *O. flava* Linnaeus (1753: 433) clade sensu Oberlander *et al.* (2009). Similarly, we added *O. carolina* to the ITS matrix of the *O. tomentosa* Linnaeus f. (1782: 244) alliance sensu Dreyer *et al.* (2009). Previous work has shown substantial conflict between nuclear and plastid DNA data (Oberlander *et al.* 2011) regarding the phylogenetic placement of members of the *O. tomentosa* alliance; consequently, we also sequenced the chloroplast non-coding *trnL* intron, 3' *trnL* exon and *trnL-trnF* spacer (*trnL-trnL-trnF*: Taberlet *et al.* 1991) for a taxon set corresponding to the ITS matrix. The chosen outgroups for all analyses were *O. comosa* Meyer ex Harvey & Sonder (1860: 340) and two species of the *O. purpurea* Linnaeus (1753: 433) clade (*O. purpurea* and *O. obtusa* Jacquin (1794: 106)). All generated sequences were submitted to the NCBI database (<http://www.ncbi.nlm.nih.gov>) (see Supplementary material). Parsimony analyses were conducted in PAUP* v4.0b10 (Swofford 2003), using heuristic searches to find most parsimonious trees. Starting trees were generated using random taxon addition, and 1 000 TBR branch-swapping replicates, saving up to 100 trees per replicate. The degree of homoplasy in the data was calculated using the parsimony-based Consistency (CI) and Retention (RI) Indices. Support levels for nodes were assessed using non-parametric bootstrap (BS: 10 000 replicates) using heuristic searches. Bayesian Inference was conducted on the same data set using MrBayes v3.2 (Ronquist *et al.* 2012) under the most complex model of sequence evolution within the 95% confidence interval as estimated by jModelTest v0.1.1 (GTR+G+I in all cases; Posada 2008). Ten million generations in two separate analyses, sampling every 1000th generation, were run in order to ensure adequate sampling of the posterior distribution. Convergence on the posterior was judged using MrBayes' own diagnostics. Posterior probabilities (PP) served as levels of support for nodes. For the two *O. tomentosa* alliance matrices, incongruence between data sets was assessed using the ILD test (Farris *et al.* 1994), with 10 000 replicates and all uninformative characters removed.

Flow Cytometry

Genome size was estimated by propidium iodide flow cytometry using a Partec SL cytometer equipped with 532-nm, 150 mW solid state diode-pumped laser (Cobolt Samba). Sample preparation followed the simplified two-step procedure using Otto's buffers as described in Doležel *et al.* (2007) and Krejčíková *et al.* (2013). *Glycine max* (Linnaeus) Merrill (1917: 274) 'Polanka' (2C = 2.50 pg) was selected as an internal reference standard.

Taxonomy

Oxalis carolina J. Suda & Sudová, *sp. nov.* (Fig. 1)

Species multifoliolata, *O. tomentosae* similis, sed robustior; petiolis longioribus, foliolis paucioribus (numero 9–11) sed maioribus. Corolla cum tuba calva. Sepala cum apicibus retrorsis.

Type:—SOUTH AFRICA, Northern Cape Province, Namakwa District, Hantam Local Municipality: southwest-facing slopes above the road R357, 12.3 km north of Nieuwoudtville, 31°16'52.6''S 19°08'37.2''E, 550 m, 9 June 2010, J. Suda & R. Sudová J628 (holotype NBG!, isotypes PRC!, STEU!).



FIGURE 1. *Oxalis carolina*. a. flowering individual in spring; b. bulb; c. sterile plant in autumn; d. pollen.

Robust geophyte, stemless or rarely with shortly (< 12 mm) exerted above-ground stem. *Bulbs* solitary, ovate with prominent beak, up to 25 mm long, outer tunics dark brown, glabrous, inner tunics reddish-brown; young bulbs lanceolate, much narrower (usually less than 5 mm broad) than mature bulbs, with paler tunics. *Rhizome* up to 30 cm long, often with numerous, prominently beaked bulbils. *Leaves* congested at apex of rhizome or above-ground stem, 3–12 per plant, erect, increasing in size after flowering; petiole 3–13 cm at flowering, elongating up to 25 cm later in the season, densely pilose with long (exceeding the diameter of the petiole), patent, stout, grey trichomes; leaflets (7–)9–11(–12), peltately spreading, oblong to narrowly obovate, 10–40 mm \times 1–8 mm (larger in full shade), slightly conduplicate at flowering, but flat or weakly involute at fruiting stage, tomentose with grey, stout hairs, more densely so abaxially, mid-vein dilated and prominent beneath in proximal half of leaflet, apex emarginate, abaxial surface with scattered, round, orange calli that became larger and elongated towards apex. *Peduncle* 1-flowered, 3–14 cm long, usually only slightly longer than leaves, pilose with long grey hairs; bracts alternate, enclosing calyx, filiform to narrowly lanceolate, 2.0–3.0 mm \times 0.2–0.3 mm, sparsely pilose. *Sepals* narrowly to broadly lanceolate, 4–6 mm \times 1–2 mm, tips distinctly recurved, densely pilose abaxially (especially near base), glabrous adaxially, margins and

sometimes surface brownish red, with two inconspicuous, orange calli near apex. *Flowers* tristylous, corolla white to pale lilac, with narrowly funnel-shaped, white to yellow, glabrous tube. *Petals* broadly obovate, 2.0–3.5 cm × 0.8–1.2 cm, with distinct claw ± as long as or slightly shorter than lamina. *Stamens* 3-seriate with 2 series per plant, shortest series 2.5–3.5 mm, middle 4.0–5.0 mm, longest 8.0–10.0 mm long, filaments sparsely pilose, dilating towards base, with distinct, glabrous, papery teeth on the longer filaments, ± 2.5 × 0.2 mm; pollen tricolpate, tectum reticulate, with prominent blunt, scattered intra-luminary bacules. *Ovary* ovoid, densely pilose in distal half; styles 5, pilose, in three series with one series per plant, reciprocally herkogamous to stamen series, shortest ± 3 mm long, middle 5–6 mm, longest 8–10 mm long; stigmas yellow, fimbriate. *Fruit* not seen. *Seeds* not seen.

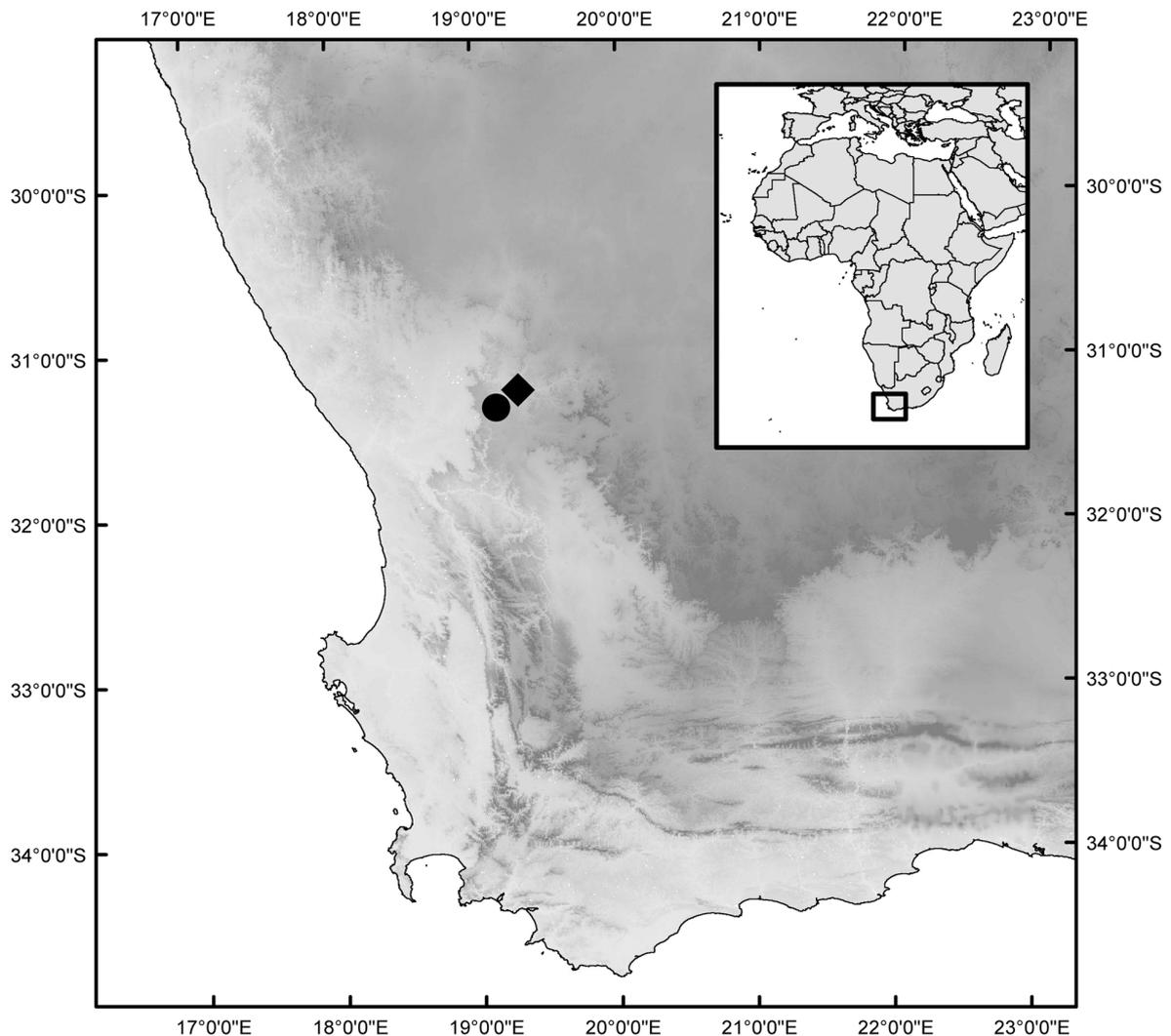


FIGURE 2. Geographic distribution of *Oxalis carolina* (circle) and *O. filifoliolata* (diamond).

Diagnostic characters:—*Oxalis carolina* is morphologically most similar to *O. tomentosa* from the SW Cape. In addition to a clear geographic gap between the distributional ranges of these two species, *O. carolina* is a more robust plant, with longer petioles and fewer, but larger leaflets (mostly 9–11 compared to 10–20 in *O. tomentosa*). Another distinct feature of *O. carolina* is the presence of recurved sepal tips (Table 1, Fig. 1). In addition, *O. tomentosa* has a pubescent corolla tube, while the corolla tube of *O. carolina* is glabrous. Despite morphological similarities, *O. carolina* can hardly be regarded as a recent autopolyploid derivative of the diploid *O. tomentosa*, because their monoploid genome sizes differ by ca. 10% (1Cx-values for *O. carolina* and *O. tomentosa* are 0.29 pg and 0.32 pg, respectively). Nonetheless, detailed molecular work is required to accurately identify progenitors of the new species. *Oxalis palmifrons* T.M. Salter (1936: 161) and

O. saltusbelli are two other multifoliolate species found in the Northern Cape Province. The former is easily recognized by very numerous leaves with more than 20 leaflets and markedly compressed petioles. The recently described *O. saltusbelli* from the Nieuwoudtville area has uniquely grooved petioles, its mature leaflets are narrower and indumentum is sparser and softer than that of *O. carolina* (Table 1). Other potentially sympatric multifoliolate species (e.g., *O. zeyheri* Sonder in Harvey & Sonder (1860: 347)) are prominently caulescent and thus easy to distinguish from *O. carolina*.

Distribution and ecology:—*Oxalis carolina* grows on southwest-facing slopes above the road R357 north of Nieuwoudtville, in open Hantam Karoo vegetation (Fig. 2). Other *Oxalis* species recorded at this locality include *O. callosa* R. Knuth (1927: 15), *O. flava*, *O. obtusa* and *O. suteroides* T.M. Salter (1935: 116). The slope is very stony and the long rhizome of *O. carolina* appears to be an adaptation to these conditions. Bulbs are deeply-seated in loamy soil and were often found below boulders or in crevices between stones. A regular sympatric occurrence of mosses indicates enough moisture during the growth season. *Oxalis carolina* generally favours shaded microsites, sheltered either by boulders or by surrounding shrubby vegetation, and the species is exposed to direct sunlight only occasionally and for short periods. The size of the population was estimated at a few hundred individuals. Plants often grow aggregated into clumps. Only a small proportion of individuals were flowering (and flowers were seldom fully open), while most plants produced only leaves. The palmately compound leaves superficially resemble some multifoliolate *Lupinus* (Fabaceae) species. Vegetative reproduction via bulbils seems to be the prevailing mode of propagation; bulbils are especially common along the rhizome in autumn. Although it is possible that other populations of *O. carolina* occur in the area, our repeated searches during 2011–2012 in localities with similar ecological conditions were not successful.

Phenology:—The species flowers from mid-May to late June.

Phylogenetic analysis:—The ITS data set consisted of 20 taxa and 783 characters, of which 58 were parsimony-informative. The *trnL-trnL-trnF* data set consisted of 20 taxa and 937 characters, of which 16 were parsimony-informative. Parsimony analyses of ITS and *trnL-trnL-trnF* found 15 trees of length 221 (CI: 0.760; RI: 0.694), and 78 600 trees of length 81 (CI: 0.951; RI: 0.909), respectively. Bayesian analyses provided 15 002 trees after burn-in (harmonic mean log likelihood ITS: -2 437.37, *trnL-trnL-trnF*: -1 854.72). All diagnostics indicated that all runs for each marker had converged on the same stable posterior distributions. Parsimony and Bayesian trees were very similar, and similar to previous analyses using these markers (Oberlander *et al.* 2009, 2011, Dreyer *et al.* 2010). The *O. tomentosa* alliance is strongly supported as a monophyletic group in the ITS data set (Fig. 3), with *O. carolina* and two accessions of *O. palmifrons* resolving with the multifoliolate members of the alliance (PP: 1.00; BS: 98%). In contrast, *O. palmifrons* does not group with the remainder of the alliance in *trnL-trnL-trnF* trees, and *O. carolina* is only weakly supported (PP: 0.98; BS: 53%) as sister to the remaining species of the alliance. The ILD test in PAUP* showed that the ITS and *trnL-trnL-trnF* data sets were barely congruent with one another ($P = 0.067$). Although clearly associated with members of the *O. tomentosa* alliance in both analyses, neither nuclear nor plastid data support a close relationship of *O. carolina* to any particular member species.

Genome size:—The mean 2C-value was estimated to be 1.16 pg, showing the new species to be tetraploid. The amount of nuclear DNA is very different from the closest relatives of *O. carolina* (i.e., *O. oligophylla* T.M. Salter (1938: 114), *O. palmifrons*, *O. saltusbelli* and *O. tomentosa*), all of which are uniformly diploid according to flow cytometric analysis (Fig. 5B); another related species, *O. hygrophila* Dreyer (2004: 262), seems to be hexaploid on the basis of genome size data.

Conservation status:—The species is known from only one very localized population consisting of a few hundred individuals. Because the population occurs close to the road verge, it may be threatened by possible future road construction. This species would qualify as Vulnerable under South African Red List Criteria (Raimondo *et al.* 2009), due to a geographic range of less than 20 km² and only one known population (Criterion D).

Etymology:—The specific epithet honours the Charles University in Prague (Universitas Carolina Pragensis), which is the oldest university in Central Europe and *Alma Mater* of the first three authors.

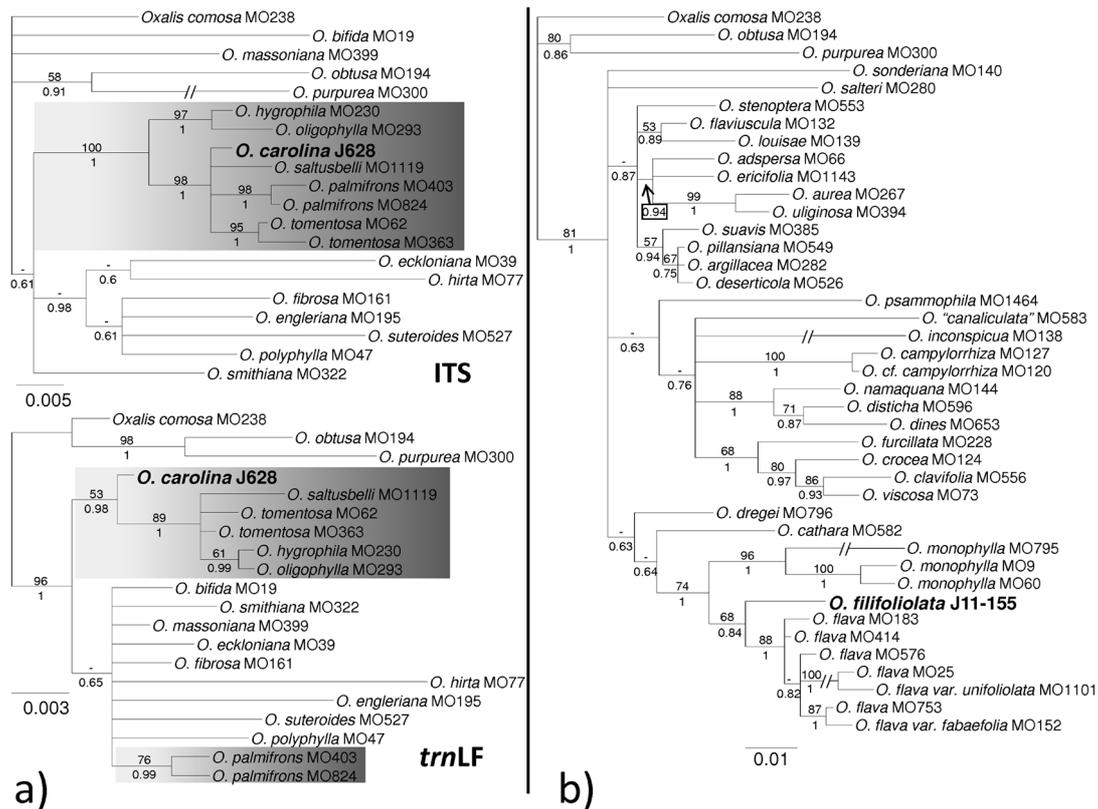


FIGURE 3. Phylogenetic placement of a) *Oxalis carolina* based on ITS and *trnL-trnL-trnF* sequence data; and b) *O. filifoliolata* based on ITS sequence data. All trees presented are Bayesian 50% majority-rule consensus trees. Numbers below the branches indicate Bayesian Posterior Probability values, numbers above the branches refer to Parsimony Bootstrap values. The new taxa are highlighted in larger, bold font. The shading in a) indicates the *O. tomentosa* alliance.

***Oxalis filifoliolata* J. Suda & Krejčíková, sp. nov. (Fig. 4)**

Species gracillima, multifoliolata. Pedunculi et item petioli tenues, basi articulati. Foliola saepissime 4-7, callosa. Petala coloris arancii, infra albo-marginata, basis viridi-flava, ostium coloris rubri lateris. Filamenta calva, edentata.

Type:—SOUTH AFRICA, Northern Cape Province, Namakwa District, Hantam Local Municipality: Gannabos north of Nieuwoudtville, 31°13'41.0"S 19°15'33.9"E, 420 m, 24 May 2011, J. Suda & J. Raichová J11-155 (holotype NBG!, isotypes BOL!, PRC!, STEU!).

Stemless, slender geophyte, 40–55 mm tall. *Bulbs* solitary, fusiform to narrowly ovate, 20–30 mm long, outer tunics dark brown, glabrous, inner tunics reddish-brown, papery. *Rhizome* 40–100 mm long, covered by glabrous, dark brown sheaths, with large, semi-amplexicaul scales at nodes in distal half, with 2–4 prominent apical scales surrounding bases of petioles and peduncles, apical scales apiculate, reddish-brown, glabrous. *Leaves* congested at apex of rhizome, 1–8 per plant, erect; petiole 35–50 mm long, with prominent basal articulation, white and slightly dilated below articulation, rigid, wiry, black, filiform above basal articulation, ± 0.4 mm in diameter; leaflets 4–7, peltately spreading, linear, conduplicate, glabrous, 7–15 mm × 1.0–1.5 mm, apex emarginate with two distinct, round, orange calli. *Peduncle* 1-flowered, 40–55 mm long, filiform but slightly thicker than petioles, apically swollen, glabrous, black, shiny, basal articulation prominent, with portion below basal articulation slightly swollen and completely surrounded by scales; bracts 2, inserted on distal part of peduncle, alternate, filiform, black. *Sepals* triangular to lanceolate, 2.5–3.0 mm × 1.0–1.5 mm, ecallose, acute, brown, streaked and blotched with dark brown markings abaxially, especially around margins. *Flowers* tristylous, corolla bright orange with brick-red to brownish throat and greenish-yellow, narrowly

funnel-shaped tube, conspicuously whitish-edged on outer margin beneath. *Petals* adhering for $\pm 3\text{--}5$ mm to form broadly funnel-shaped tube, oblanceolate, truncate to rounded, glabrous. *Stamens* 3-seriate with 2 series per plant, the shortest 2.0–2.5 mm, the middle 3.5–4.0 mm, the longest 5.0–6.0 mm long and exerted ± 2 mm from tube; filaments pale yellow, glabrous, edentate; pollen yellow, tricolpate, tectum reticulate, with prominent blunt, scattered intra-luminary bacules. *Ovary* 1.2–1.4 mm long, ovoid, glabrous, 5-locular with 2–3 ovules per locule; styles 5, glabrous, in three series with one series per plant, reciprocally herkogamous to stamen series, shortest ± 2.5 mm long, middle ± 3.5 mm long, longest ± 7.0 mm long; stigmas orange, fimbriate. *Fruit* not seen. *Seeds* not seen.

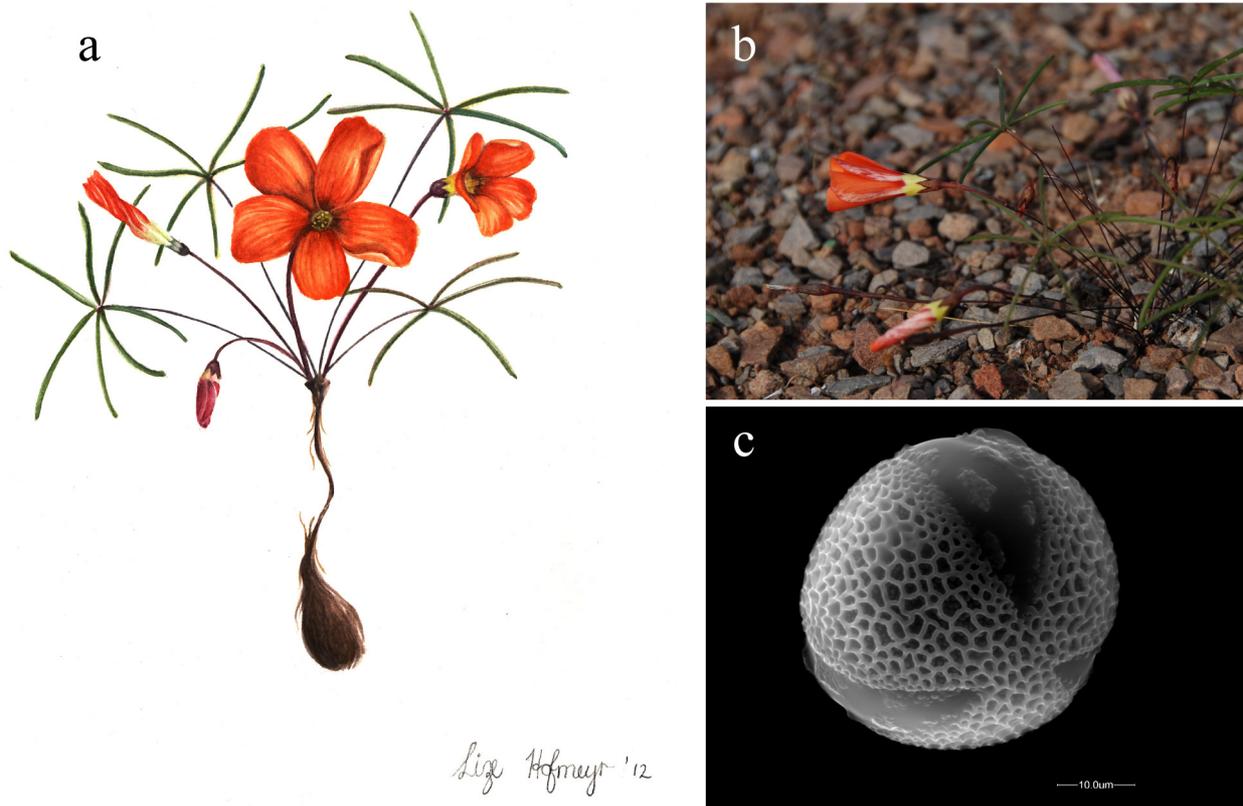


FIGURE 4. *Oxalis filifoliolata*. a. illustration; b. flowering individual in its natural habitat; c. pollen.

Diagnostic characters:—In its delicate habit, filiform petioles and linear leaflets, the new taxon superficially resembles the recently described *O. ericifolia*. However, peduncles of the new taxon lack the large swelling surrounding basal articulation that is characteristic of *O. ericifolia*, while the leaflets lack the large papillae on the adaxial leaflet epidermis. The very short petal claws of *O. ericifolia* result in open, campanulate flowers without a floral tube. This is very different to *O. filifoliolata*, which has much longer claws and a well-developed floral tube. The petal claws of *O. ericifolia* are red, with white petal lobes, while the claws of *O. filifoliolata* are greenish-yellow, the throat brick-red to brownish and the petal lobes bright orange. The filaments of *O. ericifolia* are wine-red, while those of *O. filifoliolata* are pale yellow (Table 1). *Oxalis filifoliolata* also shows numerous morphological similarities to members of the highly variable *O. flava* clade (Oberlander *et al.* 2011), and has the same type of pollen as all members of this clade (Dreyer 1996). Similarities between *O. filifoliolata* and some of the multifoliolate forms of *O. flava* include the acaulescent to near acaulescent habit, glabrous leaves, distinct basal articulations on the petioles, with the petiole somewhat dilated below this articulation, and the multifoliolate leaves with a peltate leaflet arrangement. However, *O. filifoliolata* is substantially more delicate than any of the known forms of *O. flava*. It has dark brown outer bulb tunics, while the bulb tunics of *O. flava* are usually light brown. Each leaflet has two distinct orange apical calli, which are absent from the leaflets of *O. flava*. *Oxalis flava* usually has orange calli on its sepals,

while the sepals of the new taxon are ecallose. Flower colours known from the *O. flava* complex include white, yellow and light pink, while the new taxon has orange flowers with a brick-red to brownish throat and greenish-yellow tube. The new taxon has glabrous, edentate filaments, while the filaments of *O. flava* are minutely glandular-pilose with obtuse teeth (Table 1).

Oxalis pulvinata Salter (1940: 170) is another multifoliolate member of the *O. flava* clade (Oberlander *et al.* 2011) with filiform petioles, which occurs in the same geographic region as *O. filifoliolata* and may be confused with it. *Oxalis pulvinata* differs from *O. filifoliolata* in having pale brown bulb tunics, pale rose or yellow petals and abruptly swollen, pulvinate lower and upper petiole articulations. Both its leaflets and sepals are ecallose, and the often blood red filaments bear distinct teeth.

Distribution and ecology:—*Oxalis filifoliolata* is known from a single population at Gannabos north of Nieuwoudtville *en route* to Loeriesfontein (Fig. 2). The population is large, consisting of several thousand individuals spread over approximately two kilometres along the route. It is more abundant in the lower lying flat areas, but some individuals do occur on the slopes and even summits of the surrounding hills. The substrate is dry and very stony. Plants usually grow in open places unassociated with surrounding vegetation and are mostly exposed to full sun.

Phenology:—The species flowers from mid-May to late June.

Phylogenetic analysis:—The ITS data set consisted of 41 taxa and 800 characters, of which 110 were parsimony-informative. Parsimony analyses of ITS found 353 trees of length 407 (CI: 0.568; RI: 0.657). Bayesian analyses provided 15 002 trees after burn-in (harmonic mean log likelihood: -3 566.6). All diagnostics indicated that both runs had converged on the same stable posterior distribution. Parsimony and Bayesian trees were very similar to each other and to trees in previous analyses using this marker (Oberlander *et al.* 2009, 2011, Dreyer *et al.* 2010) (Fig. 3). Although the *O. flava* clade is well-supported (PP: 1.00; BS: 81%), internal relationships are generally poorly resolved. The well-supported sister relationship between *O. flava* and *O. monophylla* Linnaeus (1771: 241) is complicated here by the new taxon, which resolves as sister to *O. flava* with only moderate support (PP: 0.84; BS: 68%). Although we also sequenced a non-coding plastid marker for the new taxon (the *trnS-trnG* spacer), more limited sampling for the *O. flava* clade compared to ITS precluded any conclusive findings beyond a well-supported relationship with two sequences of *O. flava*, although further relationships within this group were unresolved (data not shown).

Genome size:—The mean 2C-value was estimated to be 0.64 pg, showing the new species to be diploid. The monoploid genome size of *O. filifoliolata* is much smaller than the values determined for other members of the *O. flava* clade (Fig. 5A).

Conservation status:—The species is known from only one rather large population. Due to its restricted geographic area, *O. filifoliolata* deserves attention of conservation authorities. This plant would qualify as Vulnerable under South African Red List criteria (Raimondo *et al.* 2009), due to a known range smaller than 20 km² and less than five known populations (Criterion D).

Etymology:—The specific epithet refers to the long, thin leaflets.

Note:—Non-flowering and partially dry material of *O. filifoliolata* was first observed in the *Aloe dichotoma* Masson (1776: 310) forest at Gannabos in September 2009, but was considered to be a multifoliolate form of the superficially similar *O. ericifolia*. Subsequent visits in May and June 2011, however, found the population in full flower, and the striking orange flowers clearly distinguished this species from *O. ericifolia*. It is very surprising that *O. filifoliolata* was only discovered now, given that it grows in sympatry with the charismatic and easily visible *Aloe dichotoma*. *Oxalis filifoliolata* produces bright orange flowers during May/June, which coincides with the flowering time of *A. dichotoma*, arguably a phenomenon that would attract many visitors to the site. The most plausible explanation for this late discovery of *O. filifoliolata* is its very diminutive size, and the inconspicuous leaves and leaflets. An unusual variety of *O. flava* var. *unifoliolata* Dreyer & Oberlander (2010: 258) was recently described from this same locality (Dreyer *et al.* 2010).

TABLE 1. Comparison of the morphological characters of *Oxalis carolina*, *O. filifoliolata* and the phenotypically similar South African *Oxalis* species.

Species / Diagnostic character	<i>O. carolina</i>	<i>O. tomentosa</i>	<i>O. saltusbelli</i>	<i>O. palmifrons</i>	<i>O. filifoliolata</i>	<i>O. ericifolia</i>	<i>O. flava</i>	<i>O. pulvinata</i>
Bulb tunic	dark brown	greyish-brown	blackish-brown	dark brown	dark brown	dark brown	pale brown	pale brown
Petioles	stout, long (up to 25 cm), ±rounded, densely pilose	stout, rather short (up to 5 cm), ±rounded, densely pilose	long (up to 16 cm), grooved, sparsely covered with long soft hairs	short (c. 2 cm), compressed	filiform, slightly swollen at the base	filiform, with large swollen basal articulation	linear, not or slightly swollen at the base	filiform, with large swollen basal articulation
Leaflets	peltately spreading, (7–)9–11(–12), up to 4 cm long, with distinct calli on abaxial side	peltately spreading, 10–20, not longer than 2 cm, ecallose	peltate, (6–)8–11(–13), up to 9 cm long, with 2 apical calli	palmate, above 20 in number, ecallose	4–7, filiform, with 2 orange apical calli, glabrous	3, filiform, ecallose, papillose on the adaxial side	(1–) 2–12, linear-oblong, ecallose, glabrous	7–13, linear, ecallose, glabrous
Sepals	with 2 orange calli, tips distinctly recurved	ecallose	ecallose	ecallose	ecallose	ecallose	usually with orange calli	ecallose
Petals	white to pale violet, tube glabrous	white to pale violet, tube pubescent	pale lilac, tube glabrous	white or yellow	bright orange, throat brick-red, base greenish-yellow	white with wine red base	white, yellow, pale pink	pale rose or yellow
Filaments	toothed	toothed	toothed	toothed	edentate, pale yellow	toothed, wine red	toothed	toothed

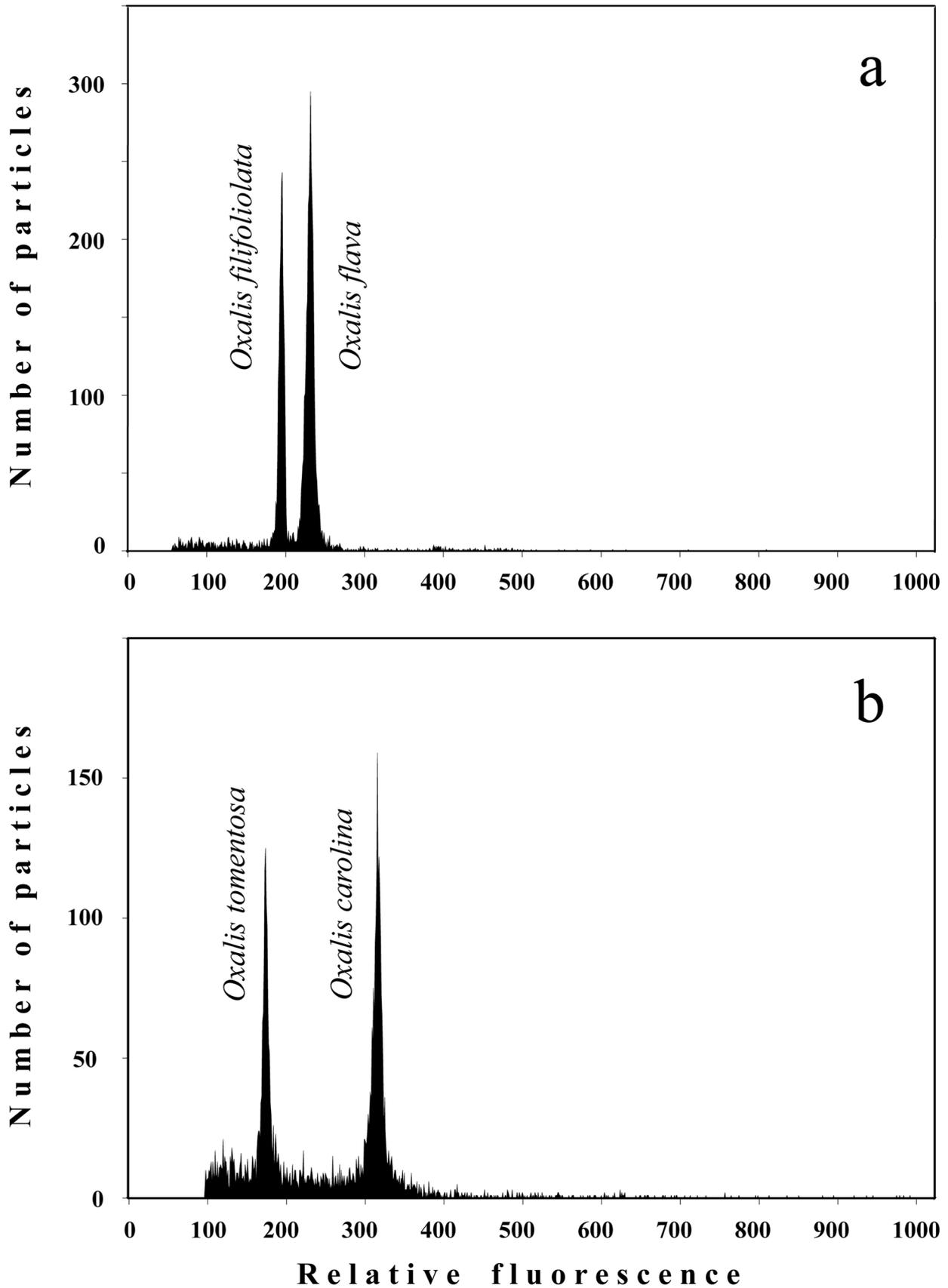


FIGURE 5. Fluorescence histograms showing simultaneous analysis of a) *O. filifoliolata* and its closest relative *O. flava* (differing ~20% in their genome sizes); and b) *O. carolina* and morphologically most similar *O. tomentosa* (differing ~80% in their genome sizes).

Key to the multifoliolate species of *Oxalis* from the Bokkeveld Plateau, South Africa

1. Well-developed above-ground stem (exserted at least 12 mm above ground) *O. zeyheri*
 - Stemless or with very short above-ground stem (less than 12 mm above ground) 2
2. Leaflets glabrous 3
 - Leaflets pubescent to hirsute 5
3. Plant semi-succulent, leaflets oblong to linear-oblong, petiole fleshy to winged, sepals callose *O. flava*
 - Plant wiry to delicate, leaflets linear, conduplicate or U-shaped in cross-section, petioles filiform, sepals ecallose
..... 4
4. Petiole base and apex distinctly swollen, petals with two swollen basal papillate swellings, filaments toothed
..... *O. pulvinata*
 - Petiole base and apex slightly swollen, petals non-papillate, filaments edentate *O. filifoliolata*
5. Petioles sparsely pilose, grooved, leaflets with two apical calli, sepals ecallose *O. saltusbelli*
 - Petioles densely pilose, terete, leaflets with multiple abaxial calli, sepals distinctly callose, recurved *O. carolina*

Acknowledgements

We thank J. Krejčík, M. Lučanová and P. Trávníček for their help in the field. Jiří Machač provided SEM photos of pollen, and Pavel Zavadil assisted with the Latin diagnosis. Department of Environment and Nature Conservation, Northern Cape is thanked for issuing collection and transport permits (nos. ODB 669 2011 FLORA 033 2011, ODB 670 2011 FLORA 034 2011 and ODB 1792 2011 FLORA 077 2011). The work was supported by the Czech Science Foundation (project P506/10/0643 awarded to JS). Additional support was provided by the Grant Agency of the Charles University (project no. 410111), Academy of Science of the Czech Republic (long-term research development project no. RVO 67985939) and institutional resources of Ministry of Education, Youth and Sports of the Czech Republic for the support of science and research. This research was further supported by a NRF grant (GUN nr. 2053585) awarded to LD. KCO was supported by project CZ.1.07/2.3.00/30.0048 of the European Social Fund in the Czech Republic (through the operational programme Education for Competitiveness).

References

- Doležel, J., Greilhuber, J. & Suda, J. (2007) Estimation of nuclear DNA content in plants using flow cytometry. *Nature Protocols* 2: 2233–2244.
<http://dx.doi.org/10.1038/nprot.2007.310>
- Dreyer, L.L. (1996) *A palynological review of Oxalis (Oxalidaceae) in Southern Africa*. Ph.D. thesis, University of Pretoria, 190 pp.
- Dreyer, L.L. & Makgakga, M.C. (2003) *Oxalis* L. In: Germishuizen, G. & Meyer, N.L. (eds.) *Plants of southern Africa: an annotated checklist*. *Strelitzia* 14: 762–770.
- Dreyer, L.L., Roets, F. & Oberlander, K.C. (2009) *Oxalis saltusbelli*: a new *Oxalis* (Oxalidaceae) species from the Oorlogskloof Nature Reserve, Nieuwoudtville, South Africa. *South African Journal of Botany* 75: 110–116.
<http://dx.doi.org/10.1016/j.sajb.2008.08.004>
- Dreyer, L.L., Oberlander, K.C. & Roets, F. (2010) Reassessment of the taxonomic status of *Oxalis fabaefolia* (Oxalidaceae) and the description of a unique variety of *Oxalis flava* from the Northern Cape Province of South Africa. *Blumea* 55: 253–258.
<http://dx.doi.org/10.3767/000651910X545004>
- Farris, J.S., Källersjö, M., Kluge, A.G. & Bult, C. (1994) Testing significance of incongruence. *Cladistics* 10: 315–319.
<http://dx.doi.org/10.1111/j.1096-0031.1994.tb00181.x>
- Harvey, W.H. & Sonder, O.W. (1860) *Flora Capensis*. Volume 1. Hodges, Smith, and Co., Dublin, 547 pp.
- Jacquin, N.J. (1794) *Oxalis. Monographia, Iconibus Illustrata*. White & Sons, London, 119 pp.
- Knuth, R. (1927) Species novae africanae generis *Oxalis*. *Botanische Jahrbücher* 61: 8–28.
- Krejčíková, J., Sudová, R., Lučanová, M., Trávníček, P., Urfus, T., Vít, P., Weiss-Schneeweiss, H., Kolano, B., Oberlander, K., Dreyer, L.L. & Suda, J. (2013) High ploidy diversity and distinct patterns of cytotype distribution in

- a widespread species of *Oxalis* in the Greater Cape Floristic Region. *Annals of Botany* 111: 641–649.
<http://dx.doi.org/10.1093/aob/mct030>
- Kumwenda, M.W., Dreyer, L.L. & Marais, E.M. (2004) A taxonomic reassessment of the varieties of *Oxalis minuta* (Oxalidaceae) and the change of *O. minuta* var. *callosa* to specific rank as *O. hygrophila*. *South African Journal of Botany* 70: 259–264.
- Linnaeus, C. (1753) *Species Plantarum*. Volume 1. Impensis Laurentii Salvii, Stockholm, 560 pp.
- Linnaeus, C. (1771) *Mantissa Plantarum Altera*. Volume 2. Holmiae, Stockholm, 587 pp.
- Linnaeus, C. fil. (1782) *Supplementum Plantarum*. Impensis Orphanotrophei, Brunswick, 467 pp.
- Masson, F. (1776) An account of three journeys from the Cape Town into the southern parts of Africa; Undertaken for the discovery of new plants, towards the improvement of the Royal Botanical Gardens at Kew. By Mr. Francis Masson, one of His Majesty's gardeners. Addressed to Sir John Pringle, Bart. P. R. S. *Philosophical Transactions of the Royal Society of London* 66: 268–317.
<http://dx.doi.org/10.1098/rstl.1776.0017>
- Merrill, E.D. (1917) *An Interpretation of Rumphius's Herbarium Amboinense*. Bureau of Printing, Manila, 595 pp.
- Oberlander, K.C., Dreyer, L.L. & Curran, H. (2009) An unusual new species of *Oxalis* (Oxalidaceae) from the Knersvlakte, South Africa. *South African Journal of Botany* 75: 239–245.
<http://dx.doi.org/10.1016/j.sajb.2008.11.004>
- Oberlander, K.C., Dreyer, L.L. & Bellstedt, D.U. (2011) Molecular phylogenetics and origins of southern African *Oxalis*. *Taxon* 60: 1667–1677.
- Oliver, E.G.H. (1993) A new species of *Oxalis* from the Western Cape. *Bothalia* 23: 72–74.
- Ornduff, R. (1973) *Oxalis dines*, a new species from the western Cape. *Journal of South African Botany* 39: 201–203.
- Posada, D. (2008) Selection of models of DNA evolution with jModelTest. pp. 93–112. In: Posada, D. (ed.) *Bioinformatics for DNA sequence analysis*. Humana Press, Totowa, USA. Available from <http://darwin.uvigo.es/software/jmodeltest.html>.
- Raimondo, D., von Staden, L., Foden, W., Victor, J.E., Helme, N.A., Turner, R.C., Kamundi, D.A. & Manyama, P.A. (2009) Red List of South African Plants. *Strelitzia* 25: 1–668.
- Ronquist, F., Teslenko, M., van der Mark, P., Ayres, D., Darling, A., Höhna, S., Larget, B., Liu, L., Suchard, M.A. & Huelsenbeck, J.P. (2012) MrBayes 3.2: efficient Bayesian MCMC inference and model choice across a large model space. *Systematic Biology* 61: 539–542.
<http://dx.doi.org/10.1093/sysbio/sys029>
- Salter, T.M. (1935) *Plantae Novae Africanae series III*. *Journal of South African Botany* 1: 111–125.
- Salter, T.M. (1936) *Plantae Novae Africanae series VII*. *Journal of South African Botany* 2: 145–169.
- Salter, T.M. (1938) *Plantae Novae Africanae series X*. *Journal of South African Botany* 4: 109–119.
- Salter, T.M. (1940) *Plantae Novae Africanae series XV*. *Journal of South African Botany* 6: 170–175.
- Salter, T.M. (1944) *The genus Oxalis in South Africa: a taxonomic revision*. The Journal of South African Botany. Supplemental Edition, Vol. 1. Cape Times Limited, Cape Town.
- Sun, Y., Skinner, D., Liang, G. & Hulbert, S. (1994) Phylogenetic analysis of *Sorghum* and related taxa using Internal Transcribed Spacers of nuclear ribosomal DNA. *Theoretical and Applied Genetics* 89: 26–32.
<http://dx.doi.org/10.1007/BF00226978>
- Swofford, D.L. (2003) *PAUP*: Phylogenetic Analysis Using Parsimony (* and Other Methods), Version 4.0b10*. Sinauer, Sunderland.
- Taberlet, P., Gielly, L., Pautou, G. & Bouvet, J. (1991) Universal primers for amplification of three non-coding regions of chloroplast DNA. *Plant Molecular Biology* 17: 1105–1109.
<http://dx.doi.org/10.1007/BF00037152>
- Williamson, G. (1999) A new succulent *Oxalis* (Oxalidaceae) from the Richtersveld. *Aloe* 36: 68–70.