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RESEARCH ARTICLE

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Two new *Oxalis* species (*Oxalidaceae*) from the Ai-Ais / Richtersveld Transfrontier Park, South Africa

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Key words

Oxalidaceae
Oxalis petricola
O. rubricallosa
phylogeny
taxonomy

Abstract South Africa has a rich, but scantily surveyed, desert flora. Documenting annual and geophytic species in this biome is challenging, as they usually only flower after adequate precipitation, which is characteristically erratic and infrequent. Recent floristic surveys in the Ai-Ais / Richtersveld Transfrontier Park were conducted after abnormally high precipitation and revealed the presence of numerous potentially new plant taxa, including two new members of the genus *Oxalis*. These taxa are clearly morphologically distinct from any known species. Here we explore the relationships of these species to other southern African species based on analyses of Internal Transcribed Spacer (ITS) and *trn*S-*trn*G DNA sequence data. Molecular data and morphological comparisons show that these collections represent new species closely allied to *Oxalis pes-caprae*, *O. compressa*, *O. copiosa* and *O. haedulipes*. Accordingly we describe them here as *O. petricola* and *O. rubricallosa*. *Oxalis petricola* is known from one isolated population of fewer than 500 individuals and is probably of special conservation concern. *Oxalis rubricallosa* is known from two small populations. The belated discovery of a large and showy species such as *O. rubricallosa* highlights the urgent need for more extensive floristic surveys of southern African desert regions.

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INTRODUCTION

The desert biome of South Africa stretches in a narrow band, less than 30 km wide, from the mouth of the Orange River on the Atlantic coast eastwards along the Orange River for c. 300 km (Mucina & Rutherford 2006). It contains one of the richest desert floras in the world with a high percentage of endemic species (Cowling et al. 1998). A large portion of this biome is formally protected within the Ai-Ais / Richtersveld Transfrontier Park: six of the fifteen distinct vegetation units of the South African desert biome fall within the Park boundaries (Mucina & Rutherford 2006). However, within-Park pressure on natural resources due to semi-nomadic pastoral land use and mining pose threats to some of these fragile habitats. In addition, many species endemic to this biome have been shown to be at risk from climate change (Rutherford et al. 1999a, b). Areas with high levels of endemism are thought to house most undiscovered plant species (Joppa et al. 2011). When these areas coincide with areas at risk from habitat loss, high extinction rates may result. It is thus a matter of urgency to document species and their distributions in under-collected areas in order to make informed management decisions. For that reason, floristic surveys were conducted in the Ai-Ais / Richtersveld Transfrontier Park during July 2011 after above-average precipitation in the preceding months. These surveys revealed the presence of several clearly new taxa of the genus *Oxalis* L.

Oxalis is well-represented in South Africa with more than 200 species (Salter 1944, Ornduff 1973, Bayer 1992, Oliver 1993, Williamson 1999, Kumwenda et al. 2004, Manning & Goldblatt 2008, Dreyer et al. 2009, 2010, Oberlander et al. 2009). The vast majority are confined to the winter-rainfall western regions including the arid Succulent Karoo and Desert biomes. All South African *Oxalis* species are bulbous and bear leaves and flowers at the apex of a subterranean stem that emerges above-ground during the wetter months of the year. Many species are morphologically extremely plastic, which makes species delineation challenging based solely on morphological criteria. At higher taxonomic levels this plasticity is also reflected in incongruence between morphological classifications (e.g. Salter 1944) and those based on pollen (Dreyer 1996) and molecular phylogenetic (Oberlander et al. 2011) studies. For example, in the most recent morphological treatment of the genus, species were divided into 9 sections (Salter 1944). Section *Cernuae* includes 23 species that share the 'primitive' character of forming umbellate inflorescences. A phylogeny published by Oberlander et al. (2011) based on molecular data indicates that some members of sect. *Cernuae* are sister to the majority of South African taxa. However, other taxa traditionally grouped in this section are more closely related to species with single-flowered inflorescences (e.g. the *O. stellata* Eckl. & Zeyh. clade).

The two undescribed *Oxalis* taxa show morphological similarities to members of sect. *Cernuae* in having umbellate inflorescences and longitudinally grooved bulbs. In particular they resemble species in the *O. pes-caprae* L. and *O. livida* Jacq. clades of Oberlander et al. (2011). The habitat preferences of these species are similar to two other desert-endemic species from central Namibia (*O. purpurascens* T.M.Salter and *O. pseudocernua* R.Knuth) which suggests close affinity to these and other species in the *O. pes-caprae* clade. Consequently, we evaluate the phylogenetic placement of these newly collected taxa using comparative morphology and DNA sequence data of the nuclear Internal Transcribed Spacer (ITS) and plastid non-coding *trn*S-*trn*G (*trn*SG) regions. We discuss the major differences between the two new taxa and their closest relatives and conclude that they represent new species, here described as *O. petricola* and *O. rubricallosa*.

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MATERIALS AND METHODS

Specimens of the two new *Oxalis* taxa were collected during field surveys of the South African section of the Ai-Ais / Richtersveld Transfrontier Park of southern Africa during July 2011. *Oxalis petricola* was collected from a small population of less than 500 individuals on a south-facing cliff in the Richtersberg Mountain Desert vegetation unit (Mucina & Rutherford 2006), while *O. rubricallosa* was represented by two isolated populations on south-facing slopes in Western Gariep Hills Desert vegetation. Leaf samples of these collections were preserved in silica gel for use in DNA-based phylogenetic studies.

To determine potential systematic relationships, phylogenetic analyses of DNA sequence data from the ITS and *trn*SG regions were performed. DNA extraction from dried leaf material followed a modified 2×CTAB procedure as used in Oberlander et al. (2004). Amplification of the ITS region was performed using the primers AB101 and AB102 (Sun et al. 1994), while the *trn*SG region was amplified using primers *trn*S and *trn*G (Hamilton 1999). PCR, sequencing, contig creation and alignment of the sequence data followed Oberlander et al. (2011).

Preliminary *trn*SG data showed a close relationship between the new taxa and members of the *O. pes-caprae* clade (Oberlander et al. 2011), but was too poorly resolved to suggest closest relatives. The *trn*SG and ITS sequences of all described representatives of this clade as well as representative species from the other two major southern African *Oxalis* clades were used to more precisely place these species (Table 1). *Oxalis acetosella* L., *O. pachyrrhiza* Wedd. and two members of the New World sect. *Ionoxalis*, *O. latifolia* Kunth and *O. perdicaria* (Molina) Bertero, were used as outgroups. A list of sampled taxa, taxonomic details and GenBank accession numbers are provided in Table 1. An Incongruence Length Difference (ILD) test (Farris et al. 1995) was performed in PAUP* v. 4.0b10 (Swofford 2002) (1 000 replicates) to assess the combinability of the two separate datasets. Parsimony analyses were performed in PAUP* using heuristic searches, with random-taxon-addition starting trees and TBR branch-swapping and saving ten trees per replicate, with all other options kept as default. Gaps were coded as missing data. Support levels for nodes were assessed using 1 000 bootstrap replicates (BS), using the same settings except for simple sequence addition. Bayesian inference used MrBayes v. 3.2 (Ronquist et al. 2012) in separate and partitioned analyses conducted under the GTR+G+I model of sequence evolution for ITS and GTR+G+I for *trn*SG, as chosen in jModeltest v. 0.1.1 (Posada 2008). Two analyses of ten million generations each were performed, sampling every 1 000 generations, with a burnin of 25 % and convergence on a stable posterior distribution judged using diagnostics in the MrBayes program. Support levels for clades were judged by the associated posterior probabilities (PP).

We compared our new collections morphologically with all known species (Salter 1944, Ornduff 1973, Oliver 1993, Williamson 1999, Kumwenda et al. 2004, Manning & Goldblatt 2008, Oberlander et al. 2009, Dreyer et al. 2009, 2010) of sect. *Cernuae*, and to material of unidentified *Oxalis* specimens housed in the Stellenbosch University (STEU), Compton (NBG) and Bolus (BOL) herbaria. Specimens of the new species are deposited in STEU, NBG and the Kimberley South African National Parks Herbarium (KSAN).

Table 1 *Oxalis* species used in this study, with GenBank accession numbers, voucher specimens and classification *sensu* Salter (1944) for southern African taxa.

Species	Current section	Current subsection	Ref. number	Voucher specimen	Accession	
					ITS	*trn*SG
O. acetosella L.	–	–	MO665	J. Walter 7182	EU436870	EU437159
O. bifida Thunb.	*Oppositae*	*Bifurcatae*	MO19	L. Dreyer 608	EU437017	EU437303
O. capillacea E.Mey. ex Sond.	*Angustatae*	*Pardales*	MO36	L. Dreyer 625	EU437013	EU437299
O. caprina L.	*Cernuae*	*Stellatae*	MO7	L. Dreyer & Oberlander 0001	EU436892	EU437180
O. commutata Sond.	*Latifoliolatae*	–	MO17	L. Dreyer 606	EU436898	EU437186
O. compressa L.f.	*Cernuae*	*Eu-cernuae*	MO519	Bayer & Oberlander 0009	EU436883	EU437172
O. copiosa F.Bolus	*Cernuae*	*Eu-cernuae*	MO227	K. Oberlander 0021	EU436887	EU437176
O. dentata Jacq.	*Cernuae*	*Lividae*	MO49	C. Cilliers 0004	EU436888	EU437177
O. eckloniana C.Presl	*Sagittatae*	–	MO39	L. Dreyer 628	EU437025	EU437311
O. engleriana Schltr.	*Angustatae*	*Multifoliolatae*	MO195	K. Oberlander 0010	EU436961	EU437247
O. fibrosa F.Bolus	*Sagittatae*	–	MO332	K. Oberlander 0051	EU436958	EU437244
O. flava L.	*Crassulae*	–	MO25	L. Dreyer 614	EU436924	EU437211
O. cf. *haedulipes* T.M.Salter	*Cernuae*	*Eu-cernuae*	MO434	L. Mucina 180903/9	EU436886	EU437175
	Cernuae	*Eu-cernuae*	MO1459	Dreyer, Roets & Oberlander 2011/7-08	JQ716440	JQ716447
O. heterophylla D.C.	*Oppositae*	*Bifurcatae*	MO518	Bayer & Oberlander 0008	EU437018	EU437304
O. imbricata Eckl. & Zeyh.	*Oppositae*	*Subintegrae*	MO345	K. Oberlander 0062	EU436896	EU437184
O. incarnata L.	*Oppositae*	*Subintegrae*	MO346	K. Oberlander 0063	EU436957	EU437243
O. knuthiana T.M.Salter	*Cernuae*	*Eu-cernuae*	MO153	L. Dreyer 721	EU436884	EU437173
O. latifolia Knuth	–	–	MO254	K. Oberlander 0023	EU436875	EU437164
O. melanosticta Sond.	*Stictophyllae*	–	MO33	L. Dreyer 622	EU436901	EU437189
O. monophylla L.f.	unplaced	–	MO9	Dreyer & Oberlander 0003	EU436927	EU437214
O. oculifera E.G.H.Oliv.	*Latifoliolatae*	–	MO295	L. Dreyer 799	EU436953	EU437239
O. orbicularis T.M.Salter	*Oppositae*	*Subintegrae*	MO366	K. Oberlander 0066	EU436899	EU437187
O. pachyrrhiza Wedd.	–	–	MO460	L. Dreyer 809	EU436871	EU437160
O. perdicaria (Molina) Bertero	–	–	MO647	Till Botrand 0002	EU436878	EU437167
O. pes-caprae L.	*Cernuae*	*Eu-cernuae*	MO93	L. Dreyer 662	EU436882	EU437171
O. petricola Dreyer, Roets & Oberl.	unplaced	unplaced	MO1472	Dreyer, Roets & Oberlander 2011/07-21	JQ716439	JQ716442
O. polyphylla Jacq.	*Angustatae*	*Lineares*	MO47	C. Cilliers 0002	EU437010	EU437296
O. pseudo-cernua R.Knuth	*Cernuae*	*Costatae*	MO614	P. Craven 4781	EU436880	EU437169
O. pulchella Jacq.	*Foveolatae*	–	MO23	L. Dreyer 612	EU436907	EU437195
O. purpurascens T.M.Salter	*Cernuae*	*Costatae*	MO51	L. Dreyer 634	EU436881	EU437170
O. purpurata Jacq.	*Cernuae*	*Purpuratae*	MO1118	L. Dreyer 836	FJ426283	N/A
O. rubricallosa Oberl., Dreyer & Roets	unplaced	unplaced	MO1461	Dreyer, Roets & Oberlander 2011/07-10	JQ716438	JQ716441
O. smithiana Eckl. & Zeyh.	*Oppositae*	*Bifurcatae*	MO322	DUB 699	EU436964	EU437250
O. tenella Jacq.	*Latifoliolatae*	–	MO70	L. Dreyer 639	EU436978	EU437264
O. tomentosa L.f.	*Angustatae*	*Multifoliolatae*	MO62	Te Roller 0006	EU437022	EU437308

RESULTS AND DISCUSSION

The aligned ITS and *trn*SG datasets included 35 and 34 (a *trn*SG sequence for *O. purpurata* was unavailable) taxa, and 828 and 1 013 characters, respectively. Parsimony analysis yielded 14 trees of length 687 for ITS (CI 0.590, RI 0.580) and 892 trees of length 288 for *trn*SG (CI 0.774, RI 0.731). The Bayesian 50 % majority-rule consensus trees showed similar patterns of relationships (harmonic mean log likelihood for ITS: -4835.00; harmonic mean log likelihood for *trn*SG: -3095.73. Trees available from KCO). Although the ITS and *trn*SG trees showed some differences, primarily in the placement of *O. pseudo-cernua* and *O. knuthiana*, the ILD test did not reject the combinability of the two datasets (P = 0.065). The final dataset included 35 taxa and 1 841 characters of which 226 (12.3 %) were parsimony-informative, and yielded 64 trees of length 992 (CI 0.633, RI 0.602). The Bayesian inference trees had very similar topologies (harmonic mean log likelihood -7974.99, Fig. 1).

Based on plastid and nuclear DNA data, *O. petricola* and *O. rubricallosa* were retrieved as members of the group of taxa informally named the *O. pes-caprae* clade *sensu* Oberlander et al. (2011). The new species were strongly supported as sister taxa in combined analyses (BS 85 %, PP 0.99) and were in turn sister to the highly branched species *O. knuthiana* (BS 67 %, PP 0.99). Both relationships were primarily supported

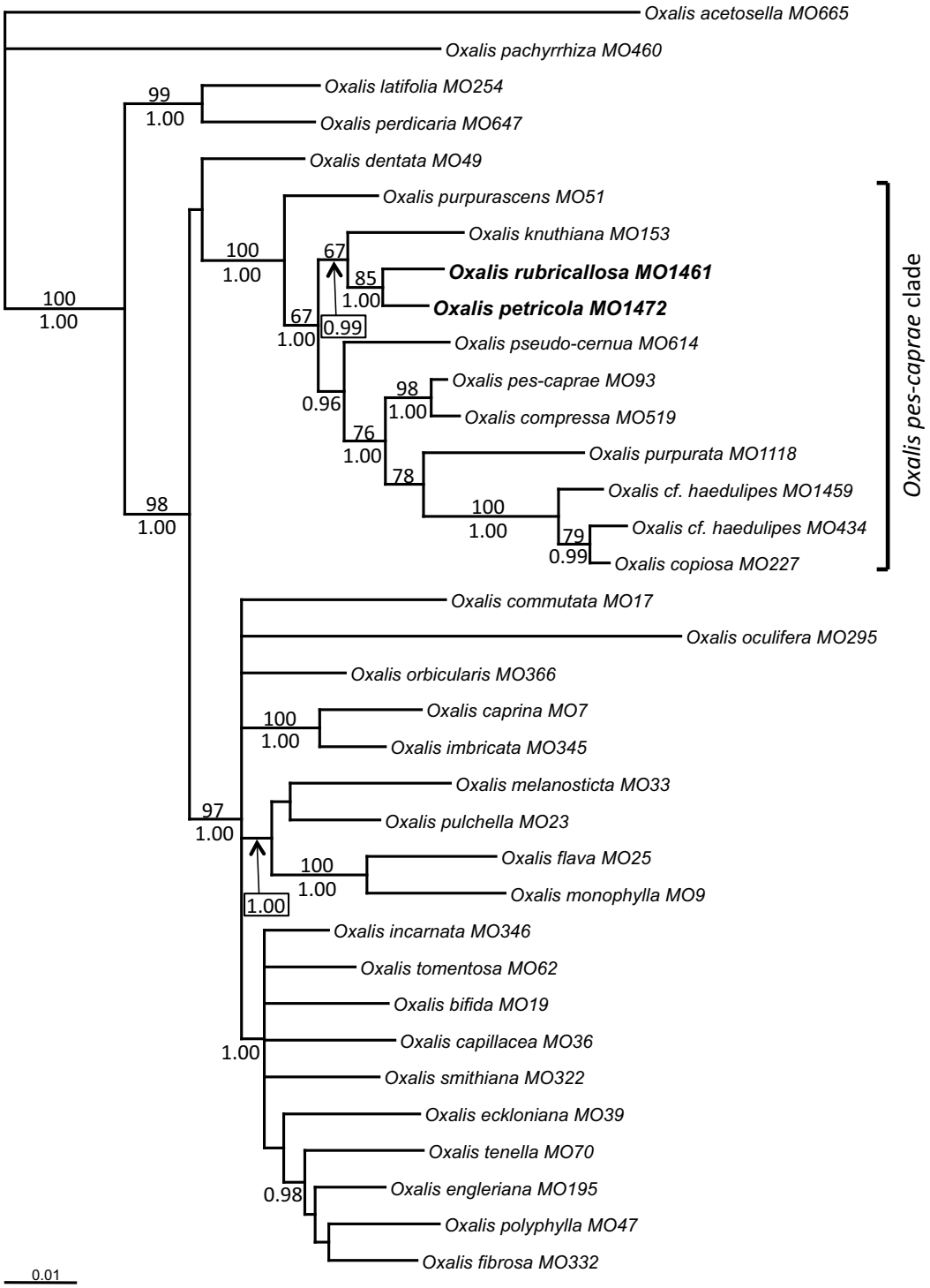

Fig 1 Bayesian 50 % majority-rule consensus tree of the combined dataset. Numbers below the branches indicate Bayesian Posterior Probability values > 0.95, numbers above the branches refer to Parsimony Bootstrap values > 65 %. The clade corresponding to the *Oxalis pes-caprae* clade is indicated. The new taxa are indicated in **bold**. The bar below the tree indicates branch lengths in expected number of substitutions per site.

Fig 2 a. Desert vegetation in the Ai-Ais / Richtersveld Transfrontier Park after good rains. — b–d. *Oxalis petricola*. b. Habitus; c. bulb; d. flowers. — e–g. *O. rubricallosa*. e. Habitus; f. bulb (bottom) and rhizome covered by old bulb tunics (top); g. flowers; insert to g. side-view of flowers showing prominent calli at tips of sepals (arrows). — Scale bars = 50 mm; insert = 5 mm.

Fig. 3 *Oxalis petricola* and close-up of sticky bulb. — Scale bars = 25 mm.

Fig. 4 *Oxalis rubricallosa*. — Scale bar = 50 mm.

by ITS data. Trees derived from *trn*SG did not conflict with the sister relationship of the two new species, but placed *O. knuthiana* in a different position as sister to the clade containing *O.* cf. *haedulipes* T.M.Salter and *O. copiosa* Bolus (BS 96 %, PP 1.00). This may indicate hybridisation or incomplete lineage sorting in the ancestry of this lineage. However, such potentially complex ancestries do not influence the validity of the species described here, as under either scenario the many morphological differences between these and currently described species are sufficient to warrant specific status.

The morphology of *O. petricola* and *O. rubricallosa* is consistent with placement in the *O. pes-caprae* clade (Oberlander et al. 2011). Both have umbellate inflorescences and prominently veined to grooved bulb tunics similar to other members of this lineage, although neither of these characters are confined to this clade. In fact, to date no non-DNA synapomorphic characters have been identified for this morphologically variable clade. The distinctive branched habit of both new species is rare among southern African *Oxalis* and is shared by only two other members of sect. *Cernuae sensu* Salter (1944): *O. knuthiana* T.M.Salter of the *O. pes-caprae* clade and the poorly known *O. schaeferi* Knuth. However, *O. petricola* and *O. rubricallosa* can be distinguished from these species by the white or pink corolla (yellow in *O. knuthiana* and *O. schaeferi*) and the much larger leaflet size. The new species can be easily distinguished from one another by the production of gummy bulb tunics in *O. petricola* and the presence of large orange to red coloured calli at the sepal tips in *O. rubricallosa*.

TAXONOMIC TREATMENT

1. ***Oxalis petricola*** Dreyer, Roets & Oberl., *sp. nov.* — Fig. 2b–d, 3

Typus. Dreyer, Roets & Oberlander 2011/07-21 (holo STEU; iso KSAN, NBG), South Africa, Northern Cape, Ai-Ais / Richtersveld Transfrontier Park, *en route* to Richtersberg camp site between road markers R8 and R12, 2817 AB, July 2011.

Geophyte up to 200 mm tall with branched stems. *Bulb* contorted narrow-ovoid to ovoid, tapering to the apex, 20–30 by 30–70 mm, usually producing a contractile root; tunics dark brown to black, glabrous, distinctly longitudinally ridged, irregularly splitting, gummy with soil and stone particles embedded. *Rhizome* white, up to 100 mm long, glabrous to sparsely minutely glandular hairy, with light brown alternating scales with sparse glandular hairs, sometimes enveloped along most of length by bulb remains of multiple previous years; adventitious roots glandular-pilose. *Above-ground stem* glabrous, glaucous, maroon or green, fleshy, c. 2 mm diam at the base and rather brittle, with 1–3 light brown papery scales on basal part, branched; branches alternate, the lower arising from leaf scales and the upper from leaf axils. *Leaves* petiolate, trifoliolate, the lower widely spreading, alternate or occasionally in pseudo-whorls, the upper in apically congested pseudo-whorls of 3–7; petioles 35–60 mm long, glabrous, glaucous; leaflets 3, 6–13 by 6–13 mm with distinct 0.5 mm long reddish petiolules, broadly cuneate-obcordate to obcordate, lobes rounded at the apex, incised to at most 1/4 of length, abaxially and adaxially

glabrous, adaxially green, abaxially maroon-green. *Peduncles* arising from apical pseudo-whorls, occasionally cauline, as long as to almost twice the length of petioles, 60–95 mm long, 2–6-flowered, green, glabrous. *Bracts* small, 2–3 mm long, linear, with 2 large linear orange calli merging at the apex. *Pedicels* slender, 8–22 mm long. *Sepals* 5, 4–5.5 mm long, lanceolate, acute to acuminate, glabrous, ecallose, base green and tip pink. *Corolla* 12–16 mm long, white, with broad funnel-shaped yellow tube. *Petals* 5, 6–7 mm wide, glabrous, ecallose, abaxial border light pink, sparsely to moderately hairy, lamina broadly rounded, claw a 1/3 to a 1/2 of petal length. *Stamens* 10, in 3 whorls; 2 whorls per plant, lower whorl 2 mm long, middle whorl 4 mm long, longest whorl 7 mm long; filaments sparsely hairy; anthers oval, yellow. *Ovary* narrowly ovoid, 2 mm long, 5-loculed, yellow, glabrous; styles 5, separate, reciprocally herkogamous with two stamen whorls, short-whorled styles curving outwards between filaments, mid- and long-whorled styles erect, glabrous; stigmas green, fimbriate. *Fruit* unknown. *Seed* unknown.

Diagnostic characters — Multi-branched semi-succulent geophyte with glaucous stems and widely spreading leaves. Bulbs tunics dark brown to black, gummy. Flowers white with light pink, hairy abaxial petal border and ecallose sepals.

Distribution — Only known from the type locality.

Note — *Oxalis petricola* is superficially similar to *O. rubricallosa*. The morphological characters that differentiate the two species are discussed under the latter species. In addition to morphology, the two species differ in their habitat requirements as is evident from the different veld types they occupy.

2. *Oxalis rubricallosa* Oberl., Dreyer & Roets, *sp. nov.* — Fig. 2e–g, 4

Typus. *Dreyer, Roets & Oberlander 2011/07-10* (holo STEU; iso KSAN, NBG), South Africa, Northern Cape, Ai-Ais / Richtersveld Transfrontier Park, Akkedis Pass, 2817 AA, July 2011.

Geophyte up to 500 mm tall with branched stems. *Bulb* narrow-oblong, up to 80 by 30 mm, current bulb imbricated by previous years bulb remains through the action of the contractile root, such that most of rhizome is encased by successively older bulb remains; tunics dark brown, papery, glabrous and prominently veined, often conspicuously undulate, giving the bulbs a frilly appearance. *Rhizome* up to 500 mm long, glabrous, with light brown scales, for most of length thickly sheathed by old tunics. Adventitious roots glandular-pilose. *Above-ground stem* apple green, fleshy, glabrous, c. 2 mm diam at the base, with 2–3 light brown papery scales on basal part, branched; branches alternate, the lower arising from leaf scales and/or leaf axils, the upper from leaf axils. *Leaves* petiolate, trifoliolate, oriented at acute angles to the stem; lower cauline or in abortive tufts of 2–5 at nodes and the upper apically congested into whorls of 9–17; petioles 20–80 mm long, green, glabrous, base sparsely ciliate; leaflets 3, abaxial surface sparsely ciliate when young, glabrous when mature, 6–17 by 6–16 mm, broadly obcordate, incised, green, ecallose; petiolules distinct, 0.5 mm long, reddish, wrinkled, ciliate. *Peduncles* 2–5(–7)-flowered, same length as petioles, green, glabrous. *Bracts* small, linear, with two large orange calli merging at the apex, 3 mm long. *Pedicels* slender, green, 5–13 mm long. *Sepals* 5, 1–2 by 6–8 mm, oblong, obtuse, abaxially and adaxially glabrous, with very prominent orange to red calli at the tips. *Corolla* 16–17 mm long, pink (fading to white in dried specimens) with short, funnel-shaped yellow tube. *Petals* 5, glabrous, ecallose, claw slightly shorter than lamina. *Stamens* 10, in 3 whorls, 2 whorls per plant, lower whorl 2 mm long, middle whorl 4 mm long, longest whorl 6 mm long; filaments glabrous, toothed; anthers oval, yellow. *Ovary* ovoid, 2 mm long, 5-loculed, translucent, glabrous at the base and hairy above the middle. *Styles* 5, separate, hairy, reciprocally herkogamous with two stamen whorls, short-whorled styles curving outwards between filaments, mid- and long-whorled styles erect; stigmas yellow, fimbriate. *Fruit* unknown. *Seed* unknown.

Diagnostic characters — Multi-branched semi-succulent geophyte with apple green stems and leaves oriented at acute angles to the stem. Bulbs more than 20 cm deep when mature, tunics undulate giving them a frilly appearance, dark brown. Flowers light pink with prominently callose sepals.

Distribution — Two populations of *O. rubricallosa* have been recorded, both in the Ai-Ais / Richtersveld Transfrontier Park, one at Akkedis Pass and another close to the Domorogh Pass. Both populations were on south-facing slopes in Western Gariep Hills Desert vegetation.

Note — *Oxalis rubricallosa* can be distinguished from *O. petricola* by the more robust habit, the prominent calli on the sepals, a greater number of leaves per pseudo-whorl, the apple green stems and the much deeper-seated, non-gummy bulbs. Except for hairs on the filaments and the abaxial petal edges, the above-ground parts of *O. petricola* are almost entirely glabrous, while *O. petricola* has ciliate petiole bases, petiolules, ovary, styles and young abaxial leaflet surfaces. In addition, the filaments of *O. rubricallosa* have prominent teeth, while those of *O. petricola* are edentate.

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