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Thecaphora capensis sp. nov., an unusual new anther smut on *Oxalis* in South Africa

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Key words

anther-smut
Cape Floristic Region
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Abstract The smut genus *Thecaphora* contains plant parasitic microfungi that typically infect very specific plant organs. In this study, we describe a new species of *Thecaphora* from *Oxalis lanata* var. *rosea* (Oxalidaceae) in the Cape Floristic Region of South Africa. Molecular phylogenetic reconstructions based on large subunit ribosomal DNA sequence data confirmed the generic placement of the fungus and confirmed that it represents an undescribed species for which the name *T. capensis* sp. nov. is provided. The closest known sister species of the new taxon is *T. oxalidis* that infects the fruits of *Oxalis* spp. in Europe, Asia and the Americas. In contrast, *T. capensis* produces teliospores within the anthers of its host. This is the first documented case of an anther-smut from an African species of *Oxalis* and the first *Thecaphora* species described from Africa.

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INTRODUCTION

The smut genus *Thecaphora* resides in the Glomosporiaceae (Bauer et al. 2001, Vánky et al. 2008) and includes c. 60 described species. Species of *Thecaphora* produce sori within various plant organs including seeds, flowers, leaves, stems and roots. The host range of these fungi is broad and includes various monocotyledonous and dicotyledonous families (Vánky et al. 2008). Only a single species, *T. oxalidis* is known on members of the Oxalidaceae. Hosts of *T. oxalidis* include the species *Oxalis corniculata*, *O. dillenii*, *O. fontana* and *O. stricta* (all in sect. *Corniculatae*) and *O. laxa* (sect. *Alpinae*) (Lourteig 1994, 1995, 2000) from Europe, Asia and the Americas.

Oxalis is the largest genus within the Oxalidaceae, and the c. 500 included species are concentrated mainly in South and Central America and southern Africa (Salter 1944, Lourteig 1994, 1995, 2000). The New World represents the larger centre of species diversity (c. 250 species) for *Oxalis* (Lourteig 1994, 1995, 2000), where the plants display diverse growth forms including geophytes (underground storage organ), annuals, stem succulent perennials and small trees. The majority of southern African species are confined to the winter rainfall region of the Western Cape Province (Oberlander et al. 2002). This area, known as the Cape Floristic Region (CFR, Goldblatt & Manning 2000), displays an exceptionally rich floristic diversity, and is considered as one of six global Floral Kingdoms (Good 1947, Takhtajan 1986). *Oxalis* is the seventh largest genus in the CFR (Goldblatt & Manning 2000) and the largest bulbous genus in the region. The CFR *Oxalis* spp. flower during the wet winter months (April to August), and escape the drier summer months below ground, only to emerge again at the onset of the next rainy season (Dreyer et al. 2006).

Thecaphora oxalidis is known only from Europe, Asia and the Americas. Although this species is well defined, its generic placement has been problematic. When it was first described, the fungus was placed in *Ustilago* based on morphology (Ellis & Tracy 1890). More recent advances in fungal taxonomy have shown that *Ustilago* includes only species associated with members of the plant family Poaceae (Bauer et al. 1997, 2001, Vánky 1999). Members of *Ustilago* associated with other host families were thus transferred to new genera (e.g. *Bauerago* for species associated with Cyperaceae; *Vankya* for species associated with the Liliaceae; and *Microbotryum* for species with violet spores commonly associated with the Caryophyllaceae) (Ershad 2000, Vánky 1998, 1999). *Ustilago oxalidis* was transferred to the Glomosporiaceae (Bauer et al. 2001) in the monotypic genus *Kochmania* (Piątek 2005). More recently, ultrastructural and DNA sequence data showed that *Kochmania* resides in the genus *Thecaphora* (Vánky et al. 2008). This taxonomic placement is followed in the present study.

The reddish brown teliospore masses of *T. oxalidis* are formed within the seeds of its hosts, while the anamorph stage resides within host anthers (Ellis & Tracy 1890). We are unaware of any described *Thecaphora* sp. that forms teliospores in the anthers of its hosts. During recent surveys, a smut fungus infecting the anthers of *Oxalis lanata* var. *rosea* in the CFR of South Africa was discovered (Fig. 2). The aim of this study was to identify the fungus and to consider its taxonomic placement based on morphology and phylogenetic reconstructions obtained from large subunit ribosomal DNA gene sequence data (LSU).

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MATERIALS AND METHODS

Specimens

Individuals of *O. lanata* var. *rosea* infected with an unidentified smut fungus were collected from the Jonkershoek Forestry Reserve (Assegaaibos area), Stellenbosch, South Africa during the course of botanical surveys in July and August 2007. Infections of anther smut were found on four specimens of *O. lanata* var. *rosea*. To obtain fresh material for analysis, whole plants were collected, potted and maintained under nursery conditions (reference number MO211) in the Stellenbosch Botanical Garden, University of Stellenbosch, Stellenbosch, South Africa. Herbarium specimens of the teliospores of the unknown fungus were deposited in the herbarium of the National Collection of Fungi, Pretoria, South Africa (PREM) and Herbarium Ustilaginales Vánky (HUV), Tübingen, Germany (Table 1).

Table 1 List of isolates and LSU sequences used in this phylogenetic study with GenBank accession numbers. Isolates obtained in the present study are indicated in **bold**.

Species	Voucher[a]	GenBank acc. no.	Reference
Doassansiopsis deformans	MP 2066	AF009849	Begerow et al. 1997
Sporisorium sorghi	MP 2036a	AF009872	Begerow et al. 1997
Thecaphora affinis	TUB 015855	EF647747	Vánky et al. 2008
T. alsinearum	HUV 10535	EF200057	Vánky & Lutz 2007
	HUV 11533	EF200058	Vánky & Lutz 2007
T. amaranthi	HUV 15882	AF009873	Begerow et al. 1997
	HUV 20727	EF200038	Vánky & Lutz 2007
T. capensis	**HUV 21531**		
	PREM 60075	**EU660478**	**This study**
	PREM 60076	**EU660479**	
	PREM 60077	**EU660480**	
	PREM 60078	**EU660481**	
T. haumanii	HUV 19965	EF647749	Vánky et al. 2008
T. hedysari	HUV 13620	EF647750	Vánky et al. 2008
T. hennenea	HUV 14434	EF200039	Vánky & Lutz 2007
T. italica	HUV 20344	EF200050	Vánky & Lutz 2007
	HUV 20345	EF200051	Vánky & Lutz 2007
T. lathyri	HUV 11020	EF647748	Vánky et al. 2008
T. leptidium	HUV 5916	EF647745	Vánky et al. 2008
T. melandrii	HUV 13273	EF200048	Vánky & Lutz 2007
	HUV 12677	EF200049	Vánky & Lutz 2007
T. oxalidis	TUB 015854	EF647746	Vánky et al. 2008
T. polymniae	HUV 17240	EF647751	Vánky et al. 2008
T. saponariae	HUV 15015	EF200042	Vánky & Lutz 2007
	TUB 012794	EF200041	Vánky & Lutz 2007
T. schwartzmaniana	HUV 21117	EF647752	Vánky et al. 2008
T. seminis-convolvuli	GD 1391	AF009874	Begerow et al. 1997
T. solani	TS 5	AY344049	Andrade et al. unpubl.
	TS 22	AY344054	Andrade et al. unpubl.
T. spilanthis	HUV 21043	EF647753	Vánky et al. 2008
	AFTOL 1913	DQ832241	Matheny et al. 2006
T. thlaspeos	TUB 015857	EF647754	Vánky et al. 2008
Urocystis colchici	AFTOL 1647	DQ838576	Matheny et al. 2006

[a] Acronyms: AFTOL = Assembling the Fungal Tree Of Life, http://aftol.org; GD = G. Dem; HUV = Herbarium Ustilaginales Vánky, Tübingen, Germany; MP = M. Piepenbring; TS = Instituto de Investigaciones Agropecuarias, Centro Regional de Investigación Carillanca, Chile; TUB = Herbarium of the Spezielle Botanik/Mykologie, Eberhard-Karls-Universität Tübingen, Germany; PREM = National Collection of Fungi, Pretoria, South Africa.

DNA phylogeny

Teliospores of the unknown fungus did not germinate on artificial media and DNA isolations were made directly from naturally infected tissue. Genomic DNA was extracted from fungal teliospores using a Sigma GenElute™ plant genomic DNA miniprep kit (Sigma-Aldrich Chemie CMBH, Steinheim, Germany) according to the manufacturer's protocol. The primers LROR and LR5 (www.biology.duke.edu/fungi/mycolab/primers.htm) were used to amplify the nuclear LSU rDNA gene region. PCR reaction volumes (50 µL) consisted of: 32.5 µL ddH_2O, 1 µL DNA, 5 µL (10×) reaction buffer (Super-Therm, JMR Holdings, USA), 5 µL $MgCl_2$, 5 µL dNTP (10 mM of each nucleotide), 0.5 µL (10 mM) of each primer and 0.5 µL Super-Therm Taq polymerase (JMR Holdings, USA). DNA fragments were amplified using a Gene Amp®, PCR System 2700 thermal cycler (Applied Biosystems, Foster City, USA). PCR reaction conditions were: an initial denaturation step of 2 min at 95 °C followed by 35 cycles of 30 s denaturation at 95 °C, 30 s annealing at 55 °C and 1 min elongation at 72 °C. The PCR process terminated with a final elongation step of 8 min at 72 °C. Amplified PCR products were cleaned using the Wizard® SV gel and PCR clean-up system (Promega, Madison, Wisconsin, USA) following the manufacturer's protocols. Purified fragments were sequenced using a Big Dye™ Terminator v. 3.0 cycle sequencing premix kit (Applied Biosystems). The fragments were analysed on an ABI PRISIM™ 3100 Genetic Analyser (Applied Biosystems).

The sequence data obtained were compared with accessions acquired from the NCBI's GenBank nucleotide database (www.ncbi.nlm.nih.gov) using a parsimony, likelihood and Bayesian approach (Table 1). The species *Doassansiopsis deformans*, *Sporisorium sorghi* and *Urocystis colchici* were chosen as outgroup based on results of previous analyses (Vánky et al. 2008). Sequences were automatically aligned using the Clustal X (1.81) software package. For parsimony, a heuristic search (5 000 random addition sequence replicates) using the Phylogenetic Analysis Using Parsimony (PAUP), v. 4.0 beta 10 software package (Swofford 2000) was performed with tree-bisection-reconnection (TBR) branch swapping and characters treated as unordered and equally weighted. Starting trees were obtained through step-wise addition. All most parsimonious trees were combined into a consensus tree. One tree was saved per replicate to facilitate an optimal search of tree space. A total of 5 000 bootstrap replicates (Felsenstein 1985) were performed with the simple-stepwise addition option in order to estimate confidence levels.

For maximum likelihood analysis, likelihood settings were set to the GTR+I+G model as determined by Akaike Information Criteria (AIC) in Modeltest 3.06 (Posada & Crandall 1998). The data were analysed using a genetic algorithm to find the trees with the highest likelihood in the software program GARLI v. 0.951 (Zwickl 2006) using default values. Confidence values were estimated using bootstrap analysis (100 replicates), which were summarized as a 50 % majority rule consensus tree in PAUP.

Bayesian phylogenetic inference was implemented using the GTR+I+G (shape parameter with 4 rate categories) model and the Markov Chain Monte Carlo technique in the software package MrBayes v. 3.1.2 (Ronquist & Huelsenbeck 2003). Two

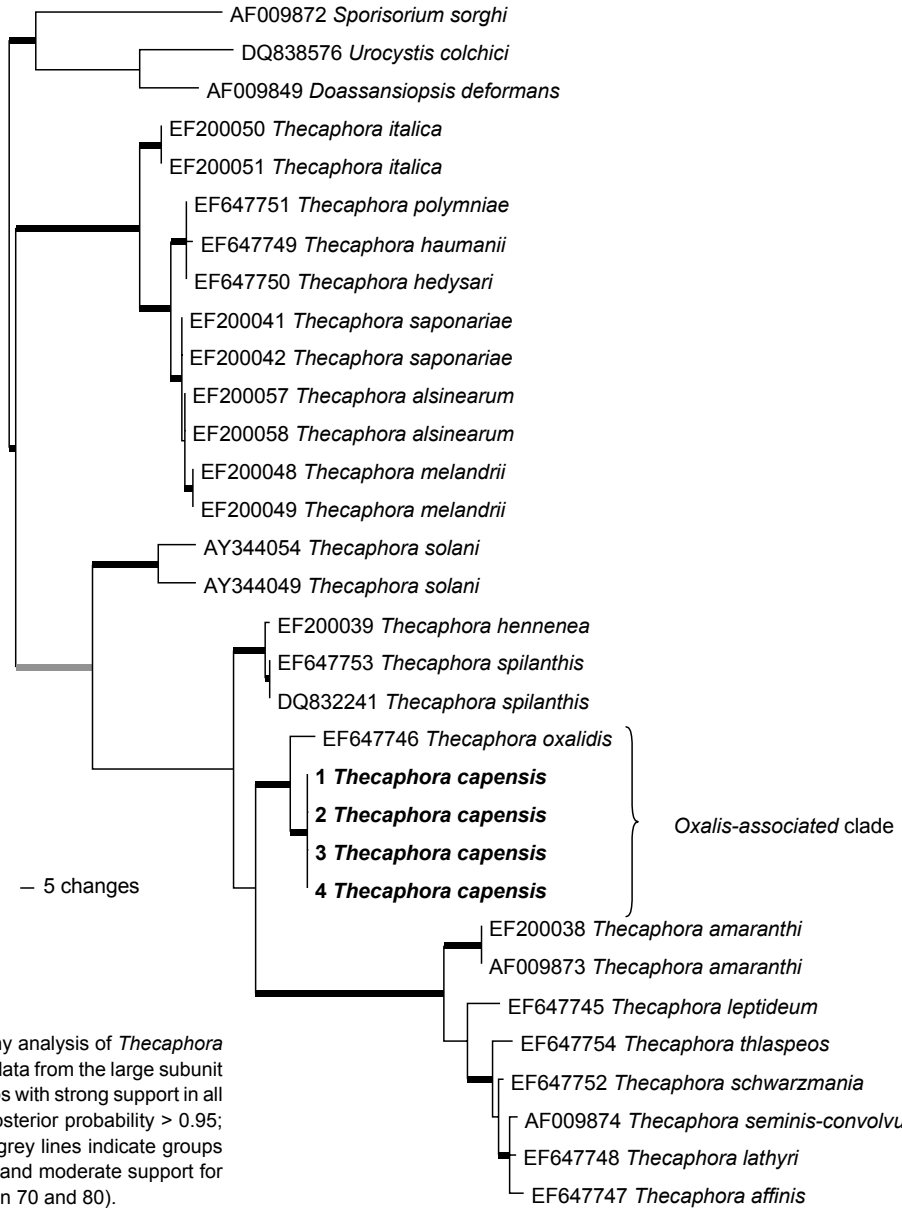

Fig. 1 One of four trees resulting from parsimony analysis of *Thecaphora* and closely related species, based on sequence data from the large subunit rDNA region. Thickened black lines indicate groups with strong support in all analysis (parsimony bootstrap > 80; Bayesian posterior probability > 0.95; Maximum likelihood bootstrap > 80). Thickened grey lines indicate groups with strong support using model based methods and moderate support for parsimony analysis (parsimony bootstrap between 70 and 80).

independent Markov chains of 5 000 000 generations each (sample frequency of 500) were initiated from a random starting tree. The first 500 000 generations were discarded as burnin and the remaining trees were pooled into a 50 % majority rule consensus tree. Bayesian analyses were repeated five times for improved sampling of tree space and to guard against local optima in searches.

Morphology

Teliospores of the unknown fungus and *T. oxalidis* (Herbarium of Dominik Begerow, reference number 684) were collected from infected plant organs and mounted in lactophenol on microscope slides and studied using a Nikon Eclipse E600 light microscope (Nikon Corporation, Tokyo, Japan) with differential interference contrast. Photographic images were captured using a Nikon DXM1200 digital camera (Nikon Corporation, Tokyo, Japan). In addition, spores were studied with a Leo 1430 VP7 scanning electron microscope (SEM, Leo Electronic Systems, Cambridge, UK). For SEM, spores were mounted on brass stubs using double-sided carbon tape, sputter coated with gold-palladium and viewed using standard methods. Measurements (n = 50) of all taxonomically informative characters were made.

RESULTS

DNA phylogeny

The aligned LSU rDNA sequence data matrix contained 32 taxa (including outgroups) and 1034 characters. Of these, 144 characters were parsimony informative and 137 were parsimony

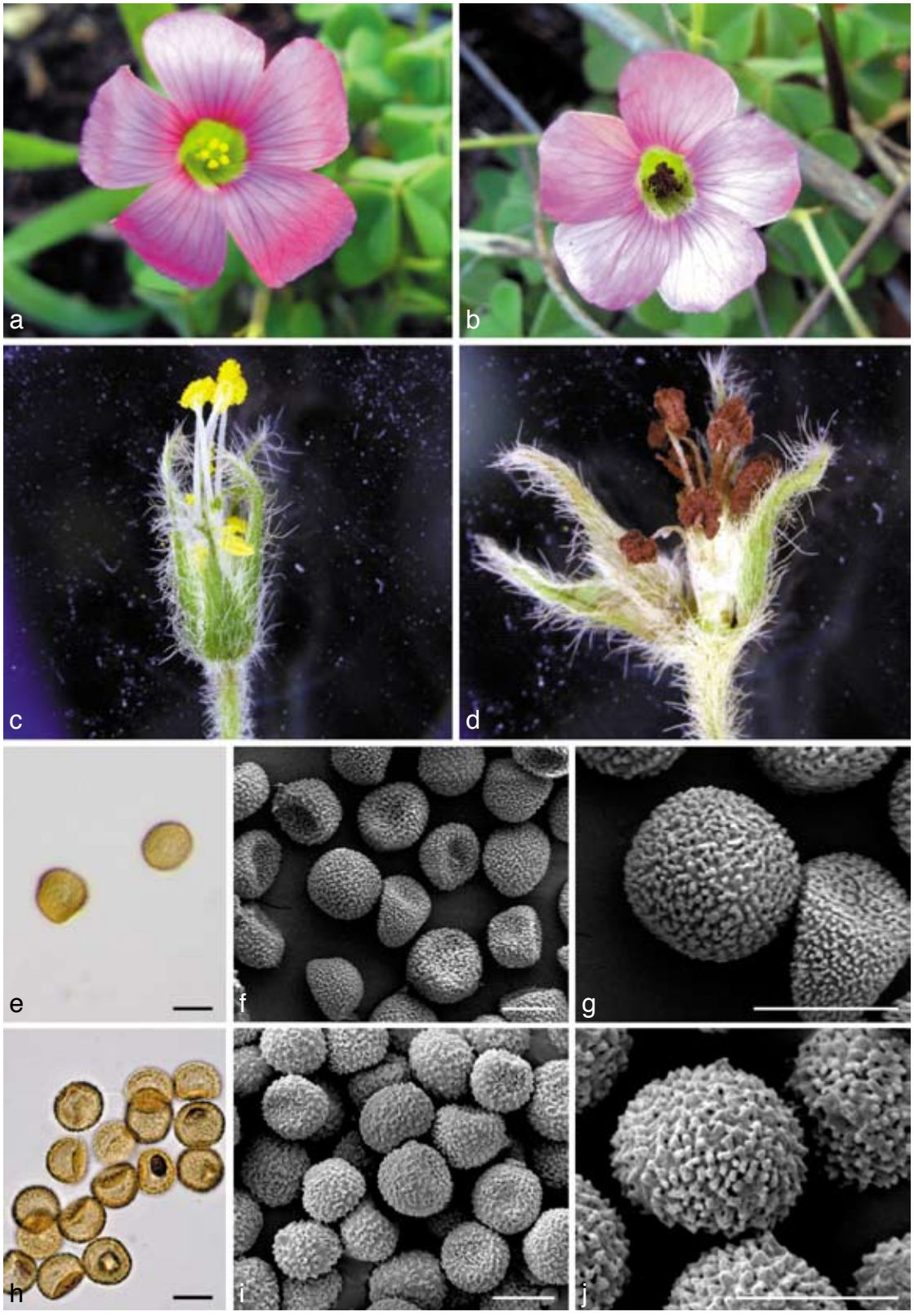

Fig. 2 Light and electron micrographs of *Thecaphora capensis* and *T. oxalidis* on *Oxalis* sp. a. Healthy *O. lanata* var. *rosea* flower; b. *Oxalis lanata* flower showing mass of *T. capensis* teliospores replacing pollen in anthers; c. close-up of healthy anthers (petals removed); d. close-up of infected anthers (petals removed); e. light micrograph of *T. capensis* teliospores mounted in lactophenol; f, g. scanning electron micrographs of *T. capensis* teliospores; h. light micrograph of *T. oxalidis* teliospores mounted in lactophenol; i, j. scanning electron micrographs of *T. oxalidis* teliospores. — Scale bars = 10 µm.

uninformative while the rest were constant. Parsimony analysis of the dataset resulted in four trees of 466 steps long, one of these was chosen for presentation (Fig. 1). The consistency- and retention indices were 0.7446 and 0.851, respectively, indicating low homoplasy. The trees resulting from the different analyses were very similar and did not differ markedly from the tree presented by Vánky et al. (2008). Aligned sequences have been deposited in TreeBase (accession number S2201).

All included South African specimens had identical LSU rDNA sequences. In analysis the samples from the South African *Oxalis* specimens clustered together with strong support (Fig. 1). These samples grouped as sister to *T. oxalidis* within a strongly supported clade. This *Oxalis*-associated clade is strongly supported as a derived group within *Thecaphora* in all analyses (Fig. 1).

Taxonomy

The external morphology of *Oxalis lanata* specimens infected with this anther-smut did not differ significantly from apparently healthy specimens. Rather than presenting anthers at two different levels as in healthy plants (Salter 1944, Fig. 2c), all anthers of infected plants were carried at approximately the same height (Fig. 2d). The most conspicuous external symptom of infection was the reddish brown teliospore masses (Fig. 2b, d) which were distinct from the normally yellow, pollen-filled anthers. Flowers of infected plants appeared to live longer than those that were healthy.

Light- and scanning-electron microscope studies showed that sori lacked a peridium, columella and sterile cells. Spores were light yellowish brown in colour (Fig. 2e) and were produced singly rather than in spore-balls. The spore surfaces were finely verruculose (Fig. 2f, g). These morphological characteristics are typical of species of *Thecaphora* and they were similar to those of *T. oxalidis* (Fig. 2h–j).

Morphological comparisons and analyses of phylogenetic data provided strong support for the view that the specimens from *Oxalis* anthers in South Africa represent an undescribed species of *Thecaphora*. The fungus is, therefore, described as follows:

Thecaphora capensis Roets & L.L. Dreyer, *sp. nov*. — MycoBank MB508255; Fig. 2

Sori in antheris, vice pollinis massa sporarum pulveracea porphyrea. Sporae unicae globosae 14–17 × 16–18 µm, crocueae nec violaceae, dense irregulatimque subtiliterque microreticulatae, verruculosae, verrucis ad 0.75 µm altis, saepe ad basibus anastomosis. Anamorpha non visa.

Etymology. Name refers to the Cape region of South Africa.

Sori in anthers, replacing pollen with reddish brown powdery mass of spores. *Spores* single, globose, 14–17 × 16–18 µm, pale yellowish brown (Fig. 2e), lacking violet tints, surface densely, irregularly and finely micro-reticulate, verruculose, warts up to 0.75 µm high, often anastomosing at the bases (Fig. 2f, g). *Anamorph* not seen.

Specimens examined. South Africa, Western Cape Province, Jonkershoek forestry station (Assegaaibos), on flowers of *Oxalis lanata* var. *rosea*, July 2007, *F. Roets & L.L. Dreyer*, PREM 60075 holotype; HUV 21532 isotype; PREM 60076 paratype; PREM 60077 paratype; PREM 60078 paratype.

DISCUSSION

This study records the first *Thecaphora* species to have been discovered in Africa. The smut was shown to represent an undescribed species for which the name *T. capensis* has been provided. *Thecaphora capensis* is closely related to *T. oxalidis* which is also found on *Oxalis* species but is known only from Asia, Europe and the Americas. The two fungi are similar but they are morphologically distinct and are unlikely to be confused.

Although teliospore size-ranges of *T. capensis* and *T. oxalidis* overlap, these species can be readily be distinguished by their teliospore surface ornamentation. The teliospore surface-warts in *T. oxalidis* are much larger than those in *T. capensis*, resulting in teliospores of *T. oxalidis* having a rougher surface sculpture. The most distinct difference between these two species, however, is that the teliospores of *T. oxalidis* are formed within the fruits of its hosts (anamorph in anthers), while those of *T. capensis* are formed in the anthers. This study thus introduces the first known *Thecaphora* species to produce teliospores within the anthers of its host.

Interestingly, there are some groups of smut fungi, where a switch in organ specificity can be observed. *Microbotryum* is the most prominent example with multiple origins of sporulation in anthers (Kemler et al. 2006), but *Antherospora* was recently described with the same evolutionary trend and is closely related to *Urocystis* (Bauer et al. 2008). *Thecaphora capensis* represents only the second species in the genus associated with *Oxalis* and only the third species that produce solitary spores rather than spore balls. While *T. capensis* appears to be confined to *O. lanata* var. *rosea* in South Africa, *T. oxalidis* has been found on various *Oxalis* spp. and is distributed globally. This global distribution can be ascribed to the wide distribution (e.g. *O. stricta*) and/or the weedy nature (e.g. *O. corniculata*) of some of its host plants. It is also very likely that this species is overlooked in other countries in which these hosts occur.

The phylogenetic relationship between the hosts of *T. oxalidis* and *T. capensis* is interesting. *Thecaphora oxalidis* has been reported from various hosts in section *Corniculatae* (Oberlander pers. comm.), as well as from *O. laxa* in sect. *Alpinae*, which is not a close relative of species in sect. *Corniculatae*. In molecular phylogenetic reconstructions, the southern African species of *Oxalis* resolve together in a clade with strong support, and the hosts of *T. oxalidis* and *T. capensis* are thus phylogenetically distantly related (Oberlander pers. comm.). The *O. lanata* host of *T. capensis* resolves within a well-supported subclade of the southern African clade, characterised by the presence of well-developed above-ground stems. The remaining species in this clade have not been carefully inspected for infections by *Thecaphora* spp., but it seems probable that they would include additional hosts of the smut.

An interesting observation in this study was that all flowers of infected plants had anthers where the pollen was completely replaced by teliospores. This suggests that the fungus grows endophytically in infected plants after infection. All native South African *Oxalis* spp. are bulbous (Salter 1944) and they only produce stems and leaves during the rainy season (winter in winter rainfall species; summer in summer rainfall species).

It would therefore be interesting to know whether this fungus also survives within these bulbs during the hot and dry summer months. If this is true, pollen and consequently seed production in infected populations of *O. lanata* would be compromised, as infected plants are rendered permanently sterile.

Propagules of *T. capensis* are most likely vectored between hosts by *Oxalis* pollinating-insects, as is true for various anther-infecting smut fungi such as *Microbotryum violaceum* (Roy 1994). Although the pollination biology of native South African *Oxalis* species is poorly documented, they are mostly believed to follow generalist pollination strategies (Dreyer, pers. obs.). The possible permanent infection of the host, coupled with the apparent ease of spore dispersal in *T. capensis* could severely limit the fitness and survival of *O. lanata* plants and will have a large role to play in the ecology of infected populations.

More than 200 species of *Oxalis* are endemic to South Africa (Salter 1944). Most of these are confined to the CFR of the Western Cape Province (Oberlander et al. 2002), a region that has been largely under-collected for fungi. It is thus possible that many more *Thecaphora* species and/or hosts await discovery in this region. Future studies should focus on elucidating these associations and consider the effect that these fungi have on host plant population dynamics.

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