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A dynamic, web-based resource to identify rust fungi (Pucciniales) in southern Africa

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Abstract

Rust fungi (Pucciniales) are some of the most important plant pathogens that cause diseases of agricultural and tree crops. There are approximately 8,000 described species worldwide. The rust fungi of South Africa were extensively studied by Ethel M. Doidge (1887 – 1965), who listed 468 species. Many nomenclatural and taxonomic changes, together with the discovery of new species and incursions of exotic species, have subsequently outdated Doidge's monograph. To address this problem, we have developed an interactive Lucid key for the identification of 50 species of rust fungi in 17 genera from countries in southern Africa. The key is dynamic and may be updated in real-time. The Lucid key provides a platform to progressively provide descriptions and images for all rust fungi in southern Africa. Plant pathologists and mycologists are invited to participate in the development of this resource.

Keywords

cybertaxonomy, key, Lucid, morphology, *Puccinia porri*, rust fungi, taxonomy, Uredinales

Introduction

Rust fungi (Pucciniomycotina, Pucciniales) are highly specialized obligate plant pathogens. They cause some of the most important diseases of plants used for agriculture and forestry, as well as for natural ecosystems globally. There are approximately 550 species of rust recorded from southern Africa (Berndt 2008b). Some introduced taxa have severely impacted agriculture, forestry and endangered native plants, including *Austropuccinia psidii* (Roux et al. 2016), *Melampsora medusae* (Trench et al. 1988), *Puccinia graminis* (Pretorius et al. 2015; Visser et al. 2011), and *Uromycladium acaciae* (Little and Payn 2016). Rust fungi have also been intentionally introduced to South Africa as biological control agents, such as *Uromycladium tepperianum*, to control the weedy and exotic *Acacia saligna* (Wood and Morris 2007).

Doidge (1950) listed the rust fungi in southern Africa, and included information on host, location and specimens examined (available at: http://www.westerdijkinstitute.nl/BioloMICSNews.aspx?Rec=5637). This work covered 468 species, of which 128 were either described or re-combined in a series of earlier papers (Doidge 1926; 1928; 1939; 1941; 1948a; b). Doidge (1950) remains an essential reference for the identification of rust fungi in southern Africa, including Angola, Botswana, Lesotho, Malawi, Mozambique, Namibia, South Africa, Swaziland, Tanzania, Zambia and Zimbabwe.

Subsequent to Doidge (1950), there have been 43 new species described, 48 new introductions recorded, and 18 name changes in 27 scientific papers that treat rust fungi in South Africa. Most of these changes and additions were summarized by Berndt (2008b), who studied the biodiversity of rust fungi in southern Africa and estimated the potential species richness in relation to the diversity of plant species. Berndt (2008b) also published a species list that updated new reports, species descriptions and taxonomy of all rust fungi in Botswana, Namibia and South Africa.

Lists of species have two weaknesses for identification of taxa. Firstly, a list is outdated after any taxonomic change or new discovery. For example, since Berndt (2008b) compiled the latest list of rust fungi for southern Africa, at least four new species were described (Berndt 2008a; Maier et al. 2015; Martin et al. 2017; Wood et al. 2014), several new species reported (Berndt 2009; McTaggart et al. 2015b; Mostert et al. 2008; Mostert et al. 2010; Roux et al. 2013) and names changed (Beenken and Wood 2015; Berndt 2008a). Secondly, lists often do not provide information on morphology or how to identify taxa. Rust fungi are generally host specific and morphologically distinct, which helps identify species from a list. However, identification of a species from a list of names may be problematic in cases where either (i) the host identity is uncertain, (ii) there are more than one species of rust on one host, (iii) two hosts are required for the completion of the lifecycle (heteroecious rusts), or (iv) good quality drawings or images are not provided. To complicate matters, rust fungi, which have up to five stages in their life cycles, were often classified in anamorphic genera, e.g. *Aecidium* and *Uredo*, if their teliospores were not known.

Accurate identification of plant pathogenic fungi and the discovery of cryptic species has been advanced by molecular data (Crous et al. 2015). However, rust fungi are obligate pathogens and challenging for molecular work. Possibly for this reason, there

are few reference sequences of rust fungi publically available. For example, of the 4,000 described species of *Puccinia*, approximately 200 (0.05%) of these have either an ITS or LSU sequence on GenBank (Marin-Felix et al. 2017). Molecular identification from a barcode marker is more common for well-studied species of rust fungi.

A web-based resource to identify rust fungi by host and morphology in southern Africa is introduced in the present study. The resource is based around a Lucid key, freely available to all users. The key is dynamic, and can be updated according to taxonomic changes or the discovery of new taxa. The scientific community is invited to contribute specimens and images to the development of this key.

Methods

Taxon selection and identification

The first 50 species of rust that accompany the release of this key were collected from the Gauteng, KwaZulu-Natal, Limpopo and Mpumalanga provinces in South Africa, and from Botswana and Swaziland. Specimens were usually collected during field surveys of forestry plantations as well as in adjacent native or farmed vegetation. These 50 species are commonly encountered or important pathogens of trees in natural and planted forests, including *Austropuccinia psidii*, *Phakopsora myrtacearum*, *Ravenelia macowaniana*, *Uromyces aloës* and *Uromycladium acaciae*. Specimens were identified on the basis of their host and morphology of spores. In some cases molecular barcodes were used for identification. This is described below for the identification of *Puccinia porri*, and was published for identifications made in prior studies (Maier et al. 2015; McTaggart et al. 2015b; Roux et al. 2013).

Morphology and image capture

Spore stages, such as aeciospores, urediniospores and teliospores, were removed from host material with a scalpel, then mounted in clear lactic acid (100% v/v) on a microscope slide and gently heated. Slides were examined with a Leica DM 2500 compound microscope using differential interference microscopy and images were taken with a Leica DFC550 camera. Measurements of each examined spore stage were made from a minimum of 20 spores per specimen.

The approach to stacking multiple images follows that of Shivas et al. (2014). Composite images were made with image stacking software Helicon Focus (Helicon Soft, Kharkov). For example, teliospores shown on the website are montaged from two to four images taken through different focal planes. Images of spore stages with ornamented walls were captured in two focal planes, one through the equator of the spores, and the other through the upper surface of the wall. The roll-over Java Script used by Shivas et al. (2014), to simulate focusing through a microscope, was incorporated for spore stages with ornamented walls.

Host symptoms were photographed with hand-held digital cameras, for example a Coolpix Nikon S9300. Host symptoms of fresh leaf material were scanned on an Epson Perfection V700 flatbed scanner with a minimum resolution of 300 dpi. Images that were finally used for the website were selected based on their quality and diagnostic potential.

Key development

An interactive key, the *Rust Fungi of Southern Africa*, was built using Lucid 3.5.32 (http://www.lucidcentral.org). The dataset used for rust fungi had 93 features and 320 character states, which included the morphological features of all spore stages present on the examined specimens.

Results

The key has been made publicly available at the following URL: http://collections.daf.qld.gov.au/web/key/africarust.

There are 50 taxa uploaded to the website. Two of these are species of *Ravenelia* that may represent new taxa. There are 18 genera on the website, of which *Aecidium* and *Uredo* are anamorphic genera used for species with unknown telial stages that have uncertain phylogenetic positions. The website contains 190 images, of which 38 are field shots, 48 are scanned host symptoms and 104 are spore stages taken from a light microscope.

A comprehensive list of rust fungi reported in southern Africa since Doidge (1950) is included in the 'references and records' page of the website. The list includes references that have described or reported new taxa, and changed taxonomic names of rust fungi in southern Africa.

One taxon included in the *Rust Fungi of Southern Africa* is a new addition for the region. *Puccinia porri*, which was taxonomically resolved by McTaggart et al. (2016a), was found on *Allium porrum* in South Africa. This identification was confirmed by an ITS-LSU sequence that had 100% identity over 1646 characters to specimens on GenBank identified as *P. porri* by McTaggart et al. (2016a). This sequence has been deposited in GenBank (KY849820) and the specimen can be viewed on the *Rust Fungi of Southern Africa* (collections.daff.qld.gov.au/web/key/africarust/Media/Html/pucciniaporri.html). Rust fungi on species of *Allium* in South Africa were previously identified as *P. allii* (Doidge 1950), which is a species complex.

Discussion

Identification of rust fungi is challenging for a number of reasons, including their complex lifecycles, multiple species on one host, multiple hosts and the fact that there

are few contemporary resources with information about their biology and morphology. Furthermore, identification based on a molecular barcode is not always possible, as many species have not been sequenced. The *Rust Fungi of Southern Africa* is a web-based, interactive resource that allows users to identify taxa based on host range and morphology. The identification is supported by comparison to images of symptoms and spore stages made from reference specimens. It further acts as a real-time list of rust fungi in southern Africa.

Berndt (2008b) recorded about 546 species of rust fungi in Botswana, Namibia and South Africa. The literature indicates that there are 572 species of rust fungi in southern Africa, which we have listed in the *Rust Fungi of Southern Africa*. Many of these species will certainly represent the same organism, for example, independently described aecial or uredinial stages of teleomorphic species (Berndt 2008b). There are 90 species of *Aecidium* and 53 species of *Uredo* in the list of taxa, and these will likely belong to other genera such as *Puccinia* (discussed by McTaggart and Shivas in Marin-Felix et al. 2017).

Further diversity may be expected from cryptic species, which have been found in multiple genera of rust fungi on hosts in the Annonaceae (Beenken 2014), Fabaceae (Doungsa-ard et al. 2015; McTaggart et al. 2015a) and Poaceae (Demers et al. 2017; Liu and Hambleton 2013). Doidge (1950) recorded one species of rust, *Uromyces aloës*, on 18 different host species, and this may represent a taxon with cryptic diversity.

Two rust fungi were recently described in southern Africa from agricultural and forestry hosts, namely *Macruropyxis fulva* on *Saccharum* and *Phakopsora myrtacearum* on *Eucalyptus* (Maier et al. 2015; Martin et al. 2017). It is interesting that two new rusts were found on introduced, well-studied plants in southern Africa. Host jumps were found to be one of the main drivers of speciation for rust fungi (McTaggart et al. 2016b), and host shifts or jumps from native plant species in South Africa to introduced species may explain the observed new taxa on exotic, well-studied hosts.

The Rust Fungi of Southern Africa is the second publicly released Lucid key to identify rust fungi. The Rust Fungi of Australia (available at: http://collections.daff.qld.gov.au/web/key/rustfungi) currently contains 122 species (Shivas et al. 2014). The broader scientific and non-scientific communities are invited to contribute images and specimens to the authors and help build these resources. Submissions for the resource will be acknowledged as a contribution on the home page of the website.

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