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ARTICLE

Online identification guides for Australian smut fungi (*Ustilaginomycotina*) and rust fungi (*Pucciniales*)

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Abstract: Interactive identification keys for Australian smut fungi (*Ustilaginomycotina* and *Pucciniomycotina*, *Microbotryales*) and rust fungi (*Pucciniomycotina*, *Pucciniales*) are available online at http://collections.daff.qld.gov.au. The keys were built using Lucid software, and facilitate the identification of all known Australian smut fungi (317 species in 37 genera) and 100 rust fungi (from approximately 360 species in 37 genera). The smut and rust keys are illustrated with over 1,600 and 570 images respectively. The keys are designed to assist a wide range of end-users including mycologists, plant health diagnosticians, biosecurity scientists, plant pathologists, and university students. The keys are dynamic and will be regularly updated to include taxonomic changes and incorporate new detections, taxa, distributions and images. Researchers working with Australian smut and rust fungi are encouraged to participate in the on-going development and improvement of these keys.

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INTRODUCTION

The smut fungi (*Ustilaginomycotina* and *Pucciniomycotina*, *Microbotryales*) and rust fungi (*Pucciniomycotina*, *Pucciniales*) in the *Basidiomycota*, together represent the most economically important and largest group of plant pathogens (Cummins & Hiratsuka 2003, Vánky 2011). A great diversity of smut and rust fungi occur on both agricultural and environmental species. Worldwide, there are about 1650 species of smut fungi (Vánky 2011) and almost 8000 species of rust fungi (Kirk *et al*. 2008). Economically important smut and rust species in Australia include wheat common bunt (*Tilletia caries*), sugarcane smut (*Sporisorium scitamineum*), wheat stem rust (*Puccinia graminis*), wheat stripe rust (*P. striiformis*), and barley leaf rust (*P. hordei*). *Puccinia psidii* appeared in Australia in 2010, and now threatens many susceptible, native species of *Myrtaceae* in natural environments (Pegg *et al*. 2014). Several species of smut and rust fungi that are absent from Australia pose serious biosecurity threats to agriculture if introduced, e.g. karnal bunt of wheat (*T. indica*), pine-gall rust (*Endocronartium harknessii*), coffee leaf rust (*Hemileia vastatrix*) and grapevine leaf rust (*Phakopsora euvitis*). Several rust fungi have been introduced to Australia as biological control agents of environmental weeds, with some having considerable success, e.g. *Puccinia chondrillina* on skeleton weed (Cullen *et al*. 1973), and *Maravalia cryptostegiae* on rubbervine (Tomley & Evans 2004).

Vánky & Shivas (2008) revised the Australian smut fungi, and a separate interactive Lucid key to 296 species with over 1000 images was developed to accompany the revision (Shivas *et al*. 2008). Despite the importance of rust fungi in Australia, the most recent monograph is over a century old and considered about 160 species (McAlpine 1906). The number of known smut and rust fungi in Australia has increased since these revisions through discoveries of new taxa, new incursions, and introductions of biological control agents.

The identification of smut and rust fungi has mostly been based on morphology and knowledge of the host species. Morphological identification of smut fungi is reliant on differences between sori and teliospores (McTaggart *et al*. 2012a, Vánky 2013). Rust fungi have up to five spore stages in their life cycles (Hennen & Hennen 2000, Cummins & Hiratsuka 2003). Morphological characters of the teliospore and urediniospore stages, such as size, apex shape and wall thickness, ornamentation, and germ pore position and number, are useful for species identification. Identification of a rust fungus may be challenging if a life cycle stage is absent on a specimen. Molecular identification based on sequence data from the Large Subunit (LSU) region or Internal Transcribed Spacer (ITS) region of nuclear ribosomal DNA, may identify species or genera of smut and rust fungi in cases where the host is unknown or morphology is inconclusive (Schoch *et al*. 2012). This approach is limited to fungi that have an available reference sequence on a public, nucleotide database.

Fig. 1. Fact sheet for *Anthracocystis heteropogonicola* from the *Smut Fungi of Australia* Lucid key.

Approximately 3% of rust fungi (310 LSU sequences and 210 ITS sequences) and 21% of smut fungi (346 ITS sequences) had reference sequences on GenBank when accessed on 18 March 2014.

We have developed interactive keys as contemporary online resources to aid the identification of Australian smut and rust fungi. The keys are freely available, dynamic, and will be upgraded to accommodate untreated or newly discovered taxa in Australia, as well as changes in taxonomy, host range and distribution.

Methods

Taxon selection

The taxa covered by the keys include all 317 known Australian smut fungi and 100 rust fungi. The initial 100 rust fungi were chosen from species that are agriculturally important, recently introduced, or endemic. All specimens have been lodged in the Plant Pathology Herbarium, Biosecurity Queensland (BRIP) or other herbaria. Specimens were also borrowed from Australian herbaria (DAR, MEL, PERTH and VPRI) for examination. Biological and distribution data for many of these specimens can be accessed at http://collections.daff.qld.gov.au

Morphology and image capture

Spores of smut or rust fungi were removed from host material with a scalpel and mounted in clear lactic acid (100 % v/v) on a microscope slide and gently heated to boiling. Slide preparations were examined with a Leica DM 2500 compound microscope using differential interference microscopy and images taken with a Leica DFC550 or DFC500 camera. Measurements of spore dimensions were made from a minimum of 20 spores, with values expressed as ranges and outliers given in parentheses. Composite images were constructed with image stacking software Helicon Focus (Helicon Soft, Kharkov).

Some of the images of spores were captured in two focal planes, one through the equator of the spores, and the other through the upper surface of the spores. A simple JavaScript was employed to allow users to toggle between

Fig. 2. Fact sheet for *Puccinia stylidii* from the *Rust Fungi of Australia* Lucid key.

the two different focal planes when viewing these spore images in the key. Host symptoms were captured in the field using an array of compact digital cameras and digital SLR cameras. In the laboratory, host symptoms were scanned using Epson Perfection V700 flatbed scanners, with a minimum resolution of 300 dpi. Close-up images of sori, especially aecia, uredinia and telia, were captured with a Leica DFC550 camera mounted on a Leica M165C stereo microscope. Images were selected based on quality and diagnostic potential.

Key development

Lucid 3.5.16 (www.lucidcentral.org) software was used to produce two interactive, multi-access keys, one for the Australian smut fungi, and the other for the Australian rust fungi. The key to smut fungi uses 53 readily observable features (characters) and 334 character states that include host, distribution and morphology of the sori and spores. The Lucid guide for the rust fungi uses 92 features and 512 character states that also includes host, distribution and morphological features of all of the spore stages known to occur in Australia.

Lucid software was used to automatically generate natural language descriptions for all taxa included in the key to Australian rust fungi. Scores for the 92 features were used to provide uniform description formats for all species in the key. Fact sheets for each of the smut and rust taxa were authored using Adobe Dreamweaver CS5.5 software.

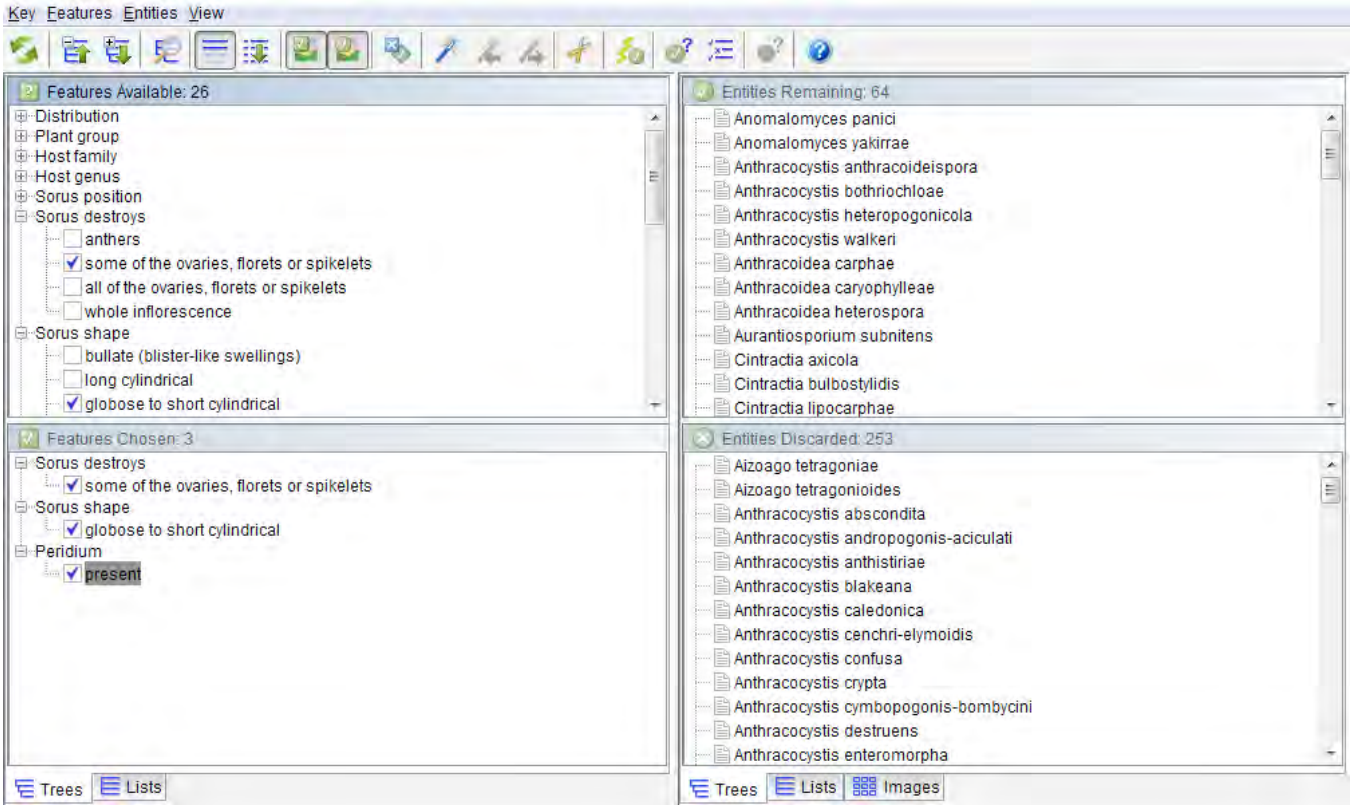

Fig. 3. Screenshot of the Lucid matrix key from *Smut Fungi of Australia*.

Table 1. New records of smut fungi in Australia since 2008.

Species	Host species	Reference
Aizoago tetragoniae	*Tetragonia diptera*	Vánky & Shivas (2013)
Aizoago tetragonioides	*Tetragonia tetragonioides*	Vánky & Shivas (2013)
Anomalomyces yakirrae	*Yakirra majuscula*	Shivas *et al*. (2013)
Aurantiosporium subnitens	*Scleria novae-hollandiae*	This publication
Entyloma ageratinae	*Ageratina riparia*	Morin (2012)
Eriocortex eriocauli	*Eriocaulon scullionii*	Vánky *et al*. (2013)
Macalpinomyces mackinlayi	*Eulalia mackinlayi*	McTaggart & Shivas (2009a)
Moreaua actinoschoeni	*Actinoschoenus* sp.	Shivas *et al*. (2011)
Moreaua chrysitricis	*Chrysitrix distigmatosa*	Shivas *et al*. (2011)
Moreaua peckii	*Schoenus cruentus*	Vánky (2009)
Sporisorium andrewmitchellii	*Enneapogon* aff. *lindleyanus*	Crous *et al*. (2012)
Sporisorium warambiense	*Xerochloae laniflora*	Piątek & Shivas (2011)
Tilletia challinoriae	*Panicum trachyrhachis*	McTaggart & Shivas (2009b)
Tilletia geeringii	*Eriachne festucacea*	Li *et al*. (2014)
Tilletia mactaggartii	*Eriachne burkittii*	Li *et al*. (2014)
Tilletia majuscula	*Yakirra majuscula*	Shivas & McTaggart (2009)
Tilletia marjaniae	*Eriachne pulchella* subsp. *dominii*	Li *et al*. (2014)
Tilletia micrairae	*Micraira dunlopii*	Barrett *et al*. (2009)
Tilletia pseudoraphidis	*Pseudoraphis spinescens*	Shivas & McTaggart (2009)
Tilletia sehimicola	*Sehima nervosum*	Shivas & McTaggart (2009)
Ustanciosporium appendiculatum	*Rhynchospora exserta*	Shivas *et al*. (2010)

Table 2. New species of rust fungi described from host plants in Australia since 1906.

Species†	Host species (family)	Type location	Reference
Aecidium acanthocarpi	*Acanthocarpus verticillatus* (*Lomandraceae*)	Varanus Island, WA	Walker & van der Merwe (2009)
Aecidium brachycomes	*Brachycome* sp. (*Asteraceae*)	Mt Kosciusko, NSW	Petrak (1953)
Aecidium gaubae	*Arthrocnemum arbuscula* (*Amaranthaceae*)	Ourjen, Vic.	Petrak (1953)
Atelocauda shivasii	*Ormosia ormondii* (*Fabaceae*)	Noah Beach, Qld	Walker (2001)
Bibulocystis gloriosa	*Caesalpinia scortechinii* (*Fabaceae*)	Mt Glorious, Qld	Walker & Shivas (2009)
Bibulocystis pulcherrima	*Daviesia latifolia* (*Fabaceae*)	McKinnon's Corner, Vic.	Walker *et al*. (2006)
Cystopsora notelaeae	*Notelaea longifolia* (*Oleaceae*)	Pittwater, NSW	Sydow (1937b)
Endoraecium parvum	*Acacia* spp. (*Fabaceae*)	Caloundra, Qld	Berndt (2011)
Endoraecium tierneyi	*Acacia harpophylla* (*Fabaceae*)	Tambo, Qld	Walker (2001), Scholler & Aime (2006)
Endoraecium violae-faustiae	*Acacia* spp. (*Fabaceae*)	Kuranda, Qld	Berndt (2011)
Endoraecium walkerianum	*Acacia* spp. (*Fabaceae*)	Yass, NSW	Berndt (2011)
Maravalia limoniformis	*Austrosteenisia blackii* (*Fabaceae*)	Cooktown, Qld	McTaggart *et al*. (2008)
Nyssopsora citriobati	*Pittosporum multiflorum* (*Pittosporaceae*)	Salisbury, NSW	Sydow (1938)
Puccinia argophyllae	*Argophyllum nullumense* (*Argophyllaceae*)	Lost World Valley, Qld	Teakle (1959)
Puccinia arthrocnemi	*Arthrocnemum halocnemoides* (*Amaranthaceae*)	Eucolo Gorge, SA	Hansford (1954)
Puccinia bassiae	*Bassia* spp. (*Amaranthaceae*)	NSW, SA	Samuel (1924)
Puccinia cygnorum	*Kunzea glabrescens* (*Myrtaceae*)	Perth, WA	Shivas & Walker (1994), Makinson & Butcher (2014)
Puccinia gastrolobii	*Gastrolobium calycinum* (*Fabaceae*)	WA	Dietel (1922)
Puccinia grevilleae	*Grevillea mimosoides* (*Proteaceae*)	Almaden, Qld	McTaggart & Shivas (2008)
Puccinia kenmorensis	*Bothriochloa decipiens* (*Poaceae*)	Brisbane, Qld	Cummins (1945)
Puccinia orellana	*Senecio dryadens* (*Asteraceae*)	Brown Mountain, NSW	Sydow (1937a)
Puccinia osbornii	*Olearia rudis* (*Asteraceae*)	Kangaroo Island, SA	Sydow (1937a)
Puccinia paspalina	*Paspalum orbiculare* (*Poaceae*)	Brisbane, Qld	Cummins (1945)
Puccinia scaevolincola	*Scaevola aemula* (*Goodeniaceae*)	Weethalle, NSW	Petrak (1953)
Puccinia sclerolaenae	*Bassia biflora* (*Amaranthaceae*)	Roma, Qld	Massee (1910)
Puccinia semibarbatae	*Bulbine semibarbata* (*Liliaceae*)	Eyre Peninsula, SA	Osborn & Samuel (1922)
Puccinia ursiniae	*Ursinia anthemoides* (Asteraceae)	Perth, WA	Shivas (1991)
Puccinia visci	*Viscum angulatum* (*Santalaceae*)	Brisbane, Qld	Cribb (1955)
Sphaerophragmium quadricellulare	*Acacia pennata* (*Fabaceae*)	Cape York Peninsula, Qld	Alcorn & Walker (1996)
Uredo xanthostemonis	*Xanthostemon paradoxus* (Myrtaceae)	East Alligator River, NT	Walker (1983)
Uredopeltis chevalieri	*Grewia breviflora* (*Tiliaceae*)	Beverley Springs, WA	Walker & Shivas (2004)
Uredopeltis euphaeus	*Hypoxis glabella* (*Hypoxidaceae*)	Wiseman's Ferry, NSW	Sydow (1937b)
Uromyces gaubae	*Caltha introloba* (*Ranunculaceae*)	Mt Kosciusko, NSW	Petrak (1953)
Uromyces lomandracearum	*Lomandra longifolia* (Lomandraceae)	Northmead, NSW	Walker & van der Merwe (2009)
Uromycladium naracoortensis	*Acacia* spp. (Fabaceae)	Naracoorte Caves, SA	Berndt (2010)

† does not include subspecific taxa

RESULTS

Both keys have been compiled for use on the internet, and are freely available at the following URLs:

- collections.daff.qld.gov.au/web/key/smutfungi
- collections.daff.qld.gov.au/web/key/rustfungi

The keys are wrapped in a web page, along with the applet version of the Lucid Player (Fig. 3). To access the keys, users require a web browser and the Java Runtime Environment, which can be downloaded for free from www.java.com. Each taxon has its own web page (Figs 1–2), which is accompanied by additional resources including host index, glossary, references, and a tutorial on how to use the key.

Since the revision of Australian smut fungi by Vánky & Shivas (2008), a further 21 species of smut fungi have been described or recorded as new records (Table 1). Since the revision of Australian rust fungi by McAlpine (1906), 35 new species of rust fungi have been described in Australia (Table 2). There are also about 115 new records of rust fungi in Australia (including introduced biological control agents) that are provided in a comprehensive list, with references, on the website. At least another 50 undescribed species are represented in Australian herbaria (unpublished). Our current estimate of the number of Australian rust fungi is approximately 360 species.

DISCUSSION

The Lucid guides to the Australian smut fungi and rust fungi simplify and promote accurate identification of taxa (genera and species), as the identification is made on available characters selected by the user. These characters are morphology, host range and distribution. Confirmation of specimen identity is enhanced by comparison with high quality images of authentic reference specimens taken in the field and in the laboratory.

Since the last revision of smut fungi in Australia (Vánky & Shivas 2008), six new generic names with type species from Australia have been established: *Aizoago* (Vánky & Shivas 2013), *Langdonia* (McTaggart *et al.* 2012b), *Shivasia* (Lutz *et al.* 2012), *Stollia* (McTaggart *et al.* 2012b), *Triodiomyces* (McTaggart *et al.* 2012b), and *Tubisorus* (Vánky & Lutz 2011). The genus *Anthracosystis* was resurrected (McTaggart *et al.* 2012b), and the first representative of *Aurantiosporium* from Australia was collected (Table 1).

McAlpine (1906) treated ten genera in the *Rusts of Australia*, and this number has more than tripled since his revision. McAlpine (1906) included several taxa that were later combined into new genera, such as *Ceratocoma* (*Cronartium*) *jacksoniae* (Cummins & Hiratsuka 2003), *Endoraecium* (*Uromyces*) *digitatum* (Scholler & Aime 2006), *Hamaspora* (*Phragmidium*) *acutissima* (*as longissima*), *Tranzschelia* (*Puccinia*) *discolor* and *Skierka* (*Uromyces*) *diploglottidis*. There are several additional genera that have been reported from Australia: *Atelocauda* (Walker 2001), *Bibulocystis* (Walker *et al.* 2006), *Cerotelium* (Simmonds 1966), *Coleosporium* (Anon. 2014), *Cystopsora* (Sydow 1937b), *Dasturella* (Johnson 1985), *Diabole* (Burrows *et al.* 2012), *Goplana* (Langdon & Herbert 1944), *Kernkampella* (Walker *et al.* 2006), *Maravalia* (Tomley & Evans 2004), *Masseeëlla* (Liberato *et al.* 2014), *Miyagia* (Cooke & Dube 1989), *Nyssopsora* (Sydow 1938), *Olivea* (Daly *et al*. 2006), *Phakopsora* (Weinert *et al*. 2003), *Prospodium* (Thomas *et al.* 2006), *Pucciniastrum* (Shivas 1989), *Ravenelia* (Walker 1983), *Sphaerophragmium* (Alcorn & Walker 1996), *Thekopsora* (McTaggart *et al.* 2013), and *Uredopeltis* (Walker & Shivas 2004).

The keys to the smut and rust fungi of Australia are the first online, taxonomically focused diagnostic tools created for the identification of Australian plant pathogenic fungi. A Lucid based identification key for smut fungi of Thailand has been released at the URL collections.daff.qld.gov.au/web/key/thaismutfungi. A similar key for the rust fungi of south-east Asia is under development. We invite ustilaginologists and uredinologists to contribute to the construction of these keys. Protocols for the submission of images, morphological data, and specimens will be developed. In the future a BLAST database, based on vouchered herbarium specimens will be added to all keys to enable accurate molecular identification of taxa.

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