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 ongoing development and improvement of these keys.

Key words: Australia, Key, Lucid, Morphology, Uredinales, Taxonomy, Ustilaginales

Article info: Submitted: 27 June 2014; Accepted: 31 October 2014; Published: 11 November 2014.

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*) and rust species in Australia include wheat common bunt (*Puccinia graminis*), wheat stem rust (*P. striiformis*), wheat stripe rust (*P. striiformis*), and barley leaf rust (*Phakopsora euvitis*). Several 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.

Vánky & Shivasi (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.

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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. 1. Fact sheet for Anthracocystis heteropogonica from the Smut 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*.

| 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 ericauli*      | *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 andrewmitchelli* | *Enneapogon aff. lindleyanus* | Crous et al. (2012)         |
| *Sporisorium warambiense*   | *Xerochloa laniflora*         | Piątek & Shivas (2011)      |
| *Tilletia chailinoniae*     | *Panicum trachyrhachis*       | McTaggart & Shivas (2009b)  |
| *Tilletia geeringii*        | *Eriachne festucaea*          | Li et al. (2014)            |
| *Tilletia macitaggartii*    | *Eriachne burkittii*          | Li et al. (2014)            |
| *Tilletia majuscula*        | *Yakirra majuscula*           | Shivas & McTaggart (2009)   |
| *Tilletia marjaniae*        | *Eriachne pulchella subsp. dominii* | Li et al. (2014)         |
| *Tilletia micraiare*        | *Micraira dunlopii*           | Barrett et al. (2009)       |
| *Tilletia pseudoraphidis*   | *Pseudoraphis spinescens*     | Shivas & McTaggart (2009)   |
| *Tilletia sehmicola*        | *Sehima nervosum*             | Shivas & McTaggart (2009)   |
| *Ustanciosporium appendiculatum* | *Rhynchospora exsreta*         | 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 gauvae* | *Arthronemum arbucula* (Amaranthaceae) | O'urtjen, Vic. | Petrak (1953) |
| *Atelocauda shivasii* | *Ormosia ormondi* (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) |
| *Cystospora 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 harpophylla* (Fabaceae) | Tambo, Qld | Berndt (2011) |
| *Maravalia limoniformis* | *Austrostoepea 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 | Hansford (1954) |
| *Puccinia arthrocnemi* | *Arthronemum halocnemoides* (Amaranthaceae) | Eucolo Gorge, SA | Samuel (1924) |
| *Puccinia bassiae* | *Bassia spp.* (Amaranthaceae) | NSW, SA | Osborn & Samuel (1922) |
| *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 oreiliana* | *Senecio drydens* (Asteraceae) | Brown Mountain, NSW | Sydow (1937a) |
| *Puccinia osbornii* | *Olearia rudis* (Asteraceae) | Kangaroo Island, SA | Sydow (1937a) |
| *Puccinia pascalina* | *Paspalum orbiculae* (Poaceae) | Brisbane, Qld | Cummins (1945) |
| *Puccinia scaveolincola* | *Scaevola aemula* (Goodeniaceae) | Weethalle, NSW | Petrik (1953) |
| *Puccinia sclerolaenae* | *Bassia biflora* (Amaranthaceae) | Roma, Qld | Massie (1910) |
| *Puccinia semibarbatae* | *Bulbine semibarbata* (Liliaceae) | Eyre Peninsula, SA | Osborn & Samuel (1922) |
| *Puccinia urinosiae* | *Ursinia anthemoideas* (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* | *Hyposis glabella* (Hyposidaceae) | Wiseman’s Ferry, NSW | Sydow (1937b) |
| *Uromyces gaubae* | *Calidium introloba* (Ranunculaceae) | Mt Kosciusko, NSW | Petrik (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 (genus 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), Triodionymycetes (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) dipligosolidis. 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 (Synod 1937b), Dasturella (Johnson 1985), Diabole (Burrows et al. 2012), Gopiana (Langdon & Herbert 1944), Kernkampella (Walker et al. 2006), Maravalia (Tomley & Evans 2004), Masseeellia (Liberato et al. 2014), Miyagia (Cooke & Dube 1989), Nysospora (Sydow 1938), Olivea (Daly et al. 2006), Phakopsora (Weinert et al. 2003), Prosopodium (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.

ACKNOWLEDGEMENTS

This work was partly funded by the Australian Biological Resources Study, grant number RFL212-33.

REFERENCES

Alcorn JL, Walker J (1996) Sphaerophragmium quadriceellulare sp. nov. (Mycota, Uredinales) on Acacia in Australia, with brief notes on some related rust genera. Mycotaxon 58: 325–330.

Anon. (2014) Fangipani rust. http://www.daff.qld.gov.au/plants/lifestyle-horticulture/nursery/frangipani-rust.

Barrett MD, Barrett RL, Shivas RG, McTaggart AR (2009) Tilletia micrairae. Persoonia 22: 170–171.

Berndt R (2010) Uromycladium naracoortensis, a new species of rust fungi (Uredinales) from Australia, with new observations on described Uromycladium species. Polish Botanical Journal 55: 299–308.

Berndt R (2011) Taxonomic revision of Enderaecium digitatum (rust fungi, Uredinales) with description of four new species from Australia and Hawaii. Mycological Progress 10: 497–517.

Burrows NJ, Lukitsch BV, Liberato JR (2012) Rediscovery of the rust Diabole cubensis, released as a classical biological control agent against the invasive weed Mimosa pigra in Australia. Australasian Plant Disease Notes 7: 171–175.

Cooke RP, Dubé AJ (1989). Host-Pathogen Index of Plant Diseases in South Australia. Adelaide: South Australian Department of Agriculture.

Cribb JW (1955) Puccinia visci sp. n. on Viscum in Queensland. Papers, University of Queensland Department of Botany 3: 75–76.

Crous PW, Summerell BA, Shivas RG, Burgess TI, Decock CA, et al. (2012) Fungal Planet description sheets: 107–127. Persoonia 28: 138–182.
Teakle DS (1959) Puccinia argophylli sp. n. on Argophyllum nullumense in Queensland. Queensland Journal of Agricultural and Animal Sciences 16: 91–92.

Thomas SE, Ellison CA, Tomley AJ (2006) Studies on the rust Prospodium tuberculatum, a new classical biological control agent released against the invasive alien weed Lantana camara in Australia. 2. Host range. Australasian Plant Pathology 35: 321–328.

Tomley AJ, Evans HC (2004) Establishment of, and preliminary impact studies on, the rust, Maravalia cryptostegiae, of the invasive alien weed, Cryptostegia grandiflora in Queensland. Queensland Journal of Agricultural and Animal Sciences 16: 91–92.

Thomas SE, Ellison CA, Tomley AJ (2006) Studies on the rust Prospodium tuberculatum, a new classical biological control agent released against the invasive alien weed Lantana camara in Australia. 2. Host range. Australasian Plant Pathology 35: 321–328.

Walker J (1983) Pacific mycogeography: deficiencies and irregularities in the distribution of plant parasitic fungi. Australian Journal of Botany Supplementary series 13: 89–136.

Walker J (2001) A revision of the genus Atelocauda (Uredinales) and description of Racospermyces gen. nov. for some rusts of Acacia. Australasian Mycologist 20: 3–28.

Walker J, Beilharz V, Pascoe IG, Priest MJ (2006) Bibulocystis gen. nov. (Uredinales) on Daviesia (Fabaceae) and Albizia (Mimosaceae) in Australasia, with comments on the genera Cystomyces and Dicheiinia and rust genera with teliospore cysts. Australasian Plant Pathology 35: 1–22.

Walker J, Shivas RG (2004) Uredopeltis chevalieri sp. nov., the rust of Grewia (Tiliaceae) formerly known as Phakopsora (or Dasturella) grewiae, its first record in Australia and a summary of the known rusts of Grewia. Australasian Plant Pathology 33: 41–47.

Walker J, Shivas RG (2009) Bibulocystis gloriosa sp. nov. (Pucciniales) on Caesalpinia scortechinii in Queensland, with comments on Spumula caesalpiniae. Australasian Plant Pathology 38: 29–35.

Walker J, van der Merwe M (2009) Two previously undescribed rusts of Acanthocarpus and Lomandra (Lomandraceae) in Australia. Australasian Plant Pathology 38: 525–532.

Weinert MP, Shivas RG, Pitkethley RN, Daly AM (2003) First record of grapevine leaf rust in the Northern Territory, Australia. Australasian Plant Pathology 32: 117–118.