First report of *Puccinia vincae* in Australia

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Abstract

The rust fungus *Puccinia vincae* was found for the first time in Australia on blue periwinkle (*Vinca major*). *Puccinia vincae* was confirmed by comparison of the ITS2-LSU region to reference sequences from Europe as well as by morphology.

Keywords Apocynaceae · Host specificity · Introduced rust

Blue periwinkle (*Vinca major*, Apocynaceae) is a garden ornamental and an invasive perennial weed in Australia, where it is regarded as a significant environmental weed in Victoria, South Australia, Western Australia, New South Wales, the ACT and Tasmania (Anon 2008; Weeds of Australia, accessed 9 Dec 2019, <http://keyserver.lucidcentral.org:8080/weeds/>). There are three species of rust fungi (Pucciniales) that infect *Vinca* in its native range in Europe, namely *Puccinia vincae* on *Vinca major*, *P. cribrata* on *V. minor* and *P. anatolica* on *V. herbacea* (Klenke and Scholler 2015).

Three specimens of rust on *V. major* were collected from urban gardens and roadsides in South Australia in the spring of 2018 (Table 1). Infected plants of BRIP.

68423 and BRIP 68436 had conspicuous brown, powdery uredinia and telia on the abaxial surfaces of shoots of young leaves that were smaller, thicker and lighter than healthy

leaves (Fig. 1, a–c). Urediniospores were ellipsoid, oval or globose, echinulate, 28–35 × 18–26 µm (Fig. 1, d & e, central). Teliospores were 2-celled, with longitudinal stripes and warts, 37–46 × 21–25 µm (Fig. 1, d & e). The collection from Stirling (BRIP 70683) showed conspicuous shoots with small spermatogonia on the abaxial surface of smaller and lighter green leaves (Fig. 1, f). Other plants had small yellow spots (Fig. 1 g) on healthy looking leaves with dark brown powdery telia on the abaxial surface. The rust was morphologically identical to *Puccinia vincae* (Hernandez et al. 2002). All specimens were deposited in the Queensland Plant Pathology Herbarium (BRIP).

DNA was extracted from single pustules of four specimens (Table 1, excluding BRIP 70683) using the DNeasy Ultra Clean Microbial Kit (Qiagen, Hilden). One pustule from each specimen was placed in a 2 mL plastic reaction tube and homogenized in a Tissue Lyser (Qiagen, Hilden) with glass beads (0.5–6.35 mm diam.) at 25 Hz for 90 s. The ITS2 to large subunit (LSU) region of ribosomal DNA (rDNA) was amplified by PCR with high fidelity Phusion® DNA Polymerase (New England Biolabs, MA, USA), using the primers Rust2INV (Aime 2006) and LR6 (Vilgalys and Hester 1990), and annealing conditions of Aime (2006). Unpurified PCR product was cleaned and sequenced by the Australian Genome Research Facility (AGRF, Brisbane). The LSU sequences were deposited in GenBank (Table 1).

We used a phylogenetic species hypothesis to compare Australian rust specimens on *Vinca major* to sequences of *P. vincae* in GenBank and a specimen of *P. cribrata* collected from Germany (BRIP 69907). LSU sequences that represented three monophyletic groups of *Puccinia* (Dixon et al. 2010) were downloaded from GenBank (Table 2) and aligned using MAFFT v.7 (Katoh and Standley 2013). The most likely tree

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Table 1 Specimen details for species of *Puccinia* on *Vinca* examined in this study

Taxon	Accession No.	Host	Location	Lat	Long	Alt.	Date	Collector	GenBankAccession
<i>Puccinia vincae</i>	BRIP 68423	<i>Vinca major</i>	Australia, SA, Adelaide	34°55'49"S	138°37'0"E	50 m	24/9/18	J. Kruse	MN322826
	BRIP 68436		Australia, SA, Belair	34°59'50"S	138°37'57"E	~325 m	25/9/18	J. Kruse	MN322828
	BRIP 70683		Australia, SA, Stirling	35°0'0"S	138°25'12"E	~525 m	15/10/18	J. Randles	—
	BRIP 69750	<i>Vinca minor</i>	Italy, Puglia, Martina Franka	40°39'17"N	17°18'36"E	~400 m	22/04/16	J. Kruse	MN322827
	BRIP 69907		Germany, Bavaria, Coburg	50°16'43"N	10°55'30"E	~350 m	17/06/17	J. Kruse	MN322825

Fig. 1 Phylogram obtained from a maximum likelihood search in IQ Tree v.1.7 beta of the large subunit region of ribosomal DNA. aRLT values (≥ 0.9) and ultrafast bootstrap values ($\geq 95\%$) from 10,000 replicates above nodes

Table 2 Specimen and sequence data of taxa used in phylogenetic analyses

Taxon	Source	Herbarium number	GenBank LSU
<i>Austropuccinia psidii</i>	Pegg et al. (2014)	BRIP 57793	KF318449
<i>Ceratocoma jacksoniae</i>	McTaggart et al. (2016b)	BRIP 57762	KT199394
<i>Miyagia pseudosphaeria</i>	Marin-Felix et al. (2017)	BRIP 59245	KX999863
<i>Puccinia abrupta</i>	Marin-Felix et al. (2017)	BRIP 59295	KX999864
<i>Puccinia carduorum</i>	Marin-Felix et al. (2017)	BRIP 60127	KX999869
<i>Puccinia chrysanthemi</i>	Pedley (2009)	NA	EU816926
<i>Puccinia cnici</i>	Marin-Felix et al. (2017)	BRIP 60111	KX999872
<i>Puccinia convolvuli</i>	Aime (2006)	BPI 871465	DQ354512
<i>Puccinia dichondrae</i>	Marin-Felix et al. (2017)	BRIP 60027	KX999874
<i>Puccinia geitonoplesii</i>	McTaggart et al. (2016a)	BRIP 57603	KM249861
<i>Puccinia gnaphaliicola</i>	McTaggart et al. (2014a)	BRIP 58451	KF690703
<i>Puccinia graminis</i> f. sp. <i>tritici</i>	Duplessis et al. (2011)	CRL 75–36–700-3	NW_003526581.1*
<i>Puccinia hemerocallidis</i>	McTaggart et al. (2016a)	BRIP 53476	KM249855
<i>Puccinia iridis</i>	McTaggart et al. (2016a)	BRIP 56852	KM249853
<i>Puccinia kuehnii</i>	McTaggart et al. (2016a)	BRIP 59200	KU296887
<i>Puccinia lagenophorae</i>	McTaggart et al. (2014)	BRIP 57563	KF690696
<i>Puccinia magnusiana</i>	Dixon et al. (2010)	BPI 879281	GU058000
<i>Puccinia malvacearum</i>	Matheny PB and Hibbett DS (unpublished)	PBM 2572	EF561641
<i>Puccinia melanocephala</i>	Dixon et al. (2010)	BPI 878929	GU058001
<i>Puccinia menthae</i>	Aime (2006)	BPI 871110	DQ354513 [#]
<i>Puccinia merrilliana</i>	Marin-Felix et al. (2017)	BRIP 56913	KX999885
<i>Puccinia myrsiphylli</i>	McTaggart et al. (2016a)	BRIP 57782	KM249854
<i>Puccinia polysora</i>	Crouch and Szabo (2011)	HSZ1879	HQ189433
<i>Puccinia purpurea</i>	Marin-Felix et al. (2017)	BRIP 57994	KX999889
<i>Puccinia rhagodiae</i>	Marin-Felix et al. (2017)	BRIP 60078	KX999890
<i>Puccinia scleriae</i>	Marin-Felix et al. (2017)	BRIP 56911	KX999893
<i>Puccinia striiformis</i>	Jin et al. (2010)	HSZ1834	GQ457306
<i>Puccinia ursinae</i>	McTaggart et al. (2014a)	BRIP 57993	KF690705
<i>Sphaerophragmium acaciae</i>	McTaggart et al. (2015)	BRIP 56910	KJ862350
<i>Uromyces appendiculatus</i>	McTaggart et al. (2014b)	BRIP 60020	KM249870
<i>Uromyces lomandracearum</i>	McTaggart et al. (2016b)	BRIP 59022	KM249862
<i>Uromyces pisi-sativi</i>	Marin-Felix et al. (2017)	BRIP 60151	KX999900
<i>Uromyces setariae-italicae</i>	Marin-Felix et al. (2017)	BRIP 56931	KX999902
<i>Uromyces striatus</i>	Marin-Felix et al. (2017)	BRIP 61228	KX999903
<i>Uromyces tenuiculis</i>	Marin-Felix et al. (2017)	BRIP 60012	KX999904
<i>Uromyces viciae-fabae</i>	McTaggart et al. (2016a)	BRIP 59246	KM249865
<i>Uromyces wedeliae</i>	Marin-Felix et al. (2017)	BRIP 60000	KX999907

*whole genome sequence

was searched in IQ Tree v.1.7 beta (Nguyen et al. 2015) with a model test (command -m TEST), 10,000 ultrafast bootstraps (Hoang et al. 2018) and an approximate likelihood ratio test with 10,000 replicates (Guindon et al. 2010). The rust fungi on *Vinca* occurred in a monophyletic group within a clade of rust fungi on hosts in the Poaceae (Clade II sensu Dixon et al. 2010). Based on a phylogenetic species concept, the

Australian sequences on *Vinca major* were conspecific with *P. vincae*, which supported the morphological identification. All sequences were identical. *Puccinia cribrata* (on *V. minor*) was sister to *P. vincae* (Fig. 2).

This is the first report of *P. vincae* from Australia. Infected periwinkle plants have since been observed at several sites in South Australia between Adelaide and Bridgewater. It is not

Fig. 2 Symptoms of *Puccinia vincae* on *Vinca major* in Australia: **a** infected shoots, **b** and **c** uredinia on the abaxial leaf surface, **d** and **e** equatorial and surface view of urediniospores and teliospores (BRIP 68436), **f** infected shoots with spermogonia, **g** yellow spots of telia on leaves (BRIP 70683). Bar, 10 μ m

known how and when *P. vincae* was introduced to Australia. *Puccinia vincae* is widespread on *Vinca* spp. in Europe and the USA (Hernandez et al. 2002). The rust joins a list of over 100 exotic rusts established in Australia (Shivas et al. 2014).

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References

- Aime MC (2006) Toward resolving family-level relationships in rust fungi (Uredinales). *Mycoscience* 47:112–122
- Anon (2008) Weed management guide, managing weeds for biodiversity, Periwinkle (*Vinca major*). CRC for Australian Weed Management. ISBN: 978-1-920932-71-8 www.weedsrc.org.au
- Crouch JA, Szabo LJ (2011) Real-time PCR detection and discrimination of the southern and common corn rust pathogens *Puccinia polysora* and *Puccinia sorghi*. *Plant Dis* 95:624–632
- Dixon LJ, Castlebury LA, Aime MC, Glynn NC, Comstock JC (2010) Phylogenetic relationships of sugarcane rust fungi. *Mycol Prog* 9: 459–468
- Duplessis S, Cuomo CA, Lin Y-C, Aerts A, Tisserant E, Veneault-Fourrey C, Joly DL, Hacquard S, Amselem J, Cantarel BL, Chiu R, Coutinho PM, Feau N, Field M, Frey P, Gelhaye E, Goldberg J, Grabherr MG, Kodira CD, Kohler A, Kües U, Lindquist EA, Lucas SM, Mago R, Mauceli E, Morin E, Murat C, Pangilinan JL, Park R, Pearson M, Quesneville H, Rouhier N, Sakthikumar S, Salamov AA, Schmutz J, Selles B, Shapiro H, Tanguay P, Tuskan GA, Henrissat B, Van de Peer Y, Rouzé P, Ellis JG, Dodds PN, Schein JE, Zhong S, Hamelin RC, Grigoriev IV, Szabo LJ, Martin F (2011) Obligate biotrophy features unraveled by the genomic analysis of rust fungi. *Proc Natl Acad Sci* 108:9166–9171
- Guindon S, Dufayard JF, Lefort V, Anisimova M, Hordijk W, Gascuel O (2010) New algorithms and methods to estimate maximum-likelihood phylogenies: assessing the performance of PhyML 3.0. *Syst Biol* 59:307–321
- Hernandez JR, Palm-Hernandez ME, Tidwell T (2002) First report of *Puccinia vincae* on *Vinca* spp. in California. *Plant Dis* 86:75
- Hoang DT, Chernomor O, von Haeseler A, Minh BQ, Vinh LS (2018) UFBoot2: improving the ultrafast bootstrap approximation. *Mol Biol Evol* 35:518–522
- Jin Y, Szabo LJ, Carson M (2010) Century-old mystery of *Puccinia striiformis* life history solved with the identification of *Berberis* as an alternate host. *Phytopathology* 100:432–435
- Katoh K, Standley DM (2013) MAFFT multiple sequence alignment software version 7: improvements in performance and usability. *Mol Biol Evol* 30:772–780
- Klenke F, Scholler M (2015) Pflanzenparasitische Kleinpilze. Bestimmungsbuch für Brand-, Rost-, Mehltau-, Flagellatenpilze und Wucherlingsverwandte in Deutschland, Österreich, der Schweiz und Südtirol. Springer Berlin Heidelberg, p 1172
- Marin-Felix Y, Groenewald JZ, Cai L, Chen Q, Marinowitz S, Barnes I, Bensch K, Braun U, Camporesi E, Damm U, De Beer ZW, Dissanayake A, Edwards J, Giraldo A, Hernández-Restrepo M, Hyde KD, Jayawardena RS, Lombard L, Luangsa-ard J, McTaggart AR, Rossman AY, Sandoval-Denis M, Shen M, Shivas RG, Tan YP, van der Linde EJ, Wingfield MJ, Wood AR, Zhang JQ, Zhang Y, Crous PW (2017) Genera of phytopathogenic fungi: GOPHY 1. *Stud Mycol* 86:99–216
- McTaggart AR, Geering ADW, Shivas RG (2014a) The rusts on Goodeniaceae and Stylidiaceae. *Mycol Prog* 13:1017–1025

- McTaggart AR, Geering ADW, Shivas RG (2014b) *Uredinopsis pteridis* and *Desmella aneimiae*, the first rust fungi (Pucciniales) reported on ferns (Pteridophyta) in Australia. Aust Plant Dis Notes 9:149
- McTaggart AR, Doungsa-ard C, Geering ADW, Aime MC, Shivas RG (2015) A co-evolutionary relationship exists between *Endoraecium* (Pucciniales) and its *Acacia* hosts in Australia. Persoonia 35:50–62
- McTaggart AR, Shivas RG, Doungsa-ard C, Weese TL, Beasley DR, Hall BH, Metcalf DA, Geering ADW (2016a) Identification of rust fungi (Pucciniales) on species of *Allium* in Australia. Australas Plant Pathol 45:581–592
- McTaggart AR, Shivas RG, van der Nest MA, Roux J, Wingfield BD, Wingfield MJ (2016b) Host jumps shaped the diversity of extant rust fungi (Pucciniales). New Phytol 209:1149–1158
- Nguyen L-T, Schmidt HA, von Haeseler A, Minh BQ (2015) IQ-TREE: a fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies. Mol Biol Evol 32:268–274
- Pedley KF (2009) PCR-based assays for the detection of *Puccinia horiana* on chrysanthemums. Plant Dis 93:1252–1258
- Pegg GS, Giblin FR, McTaggart AR, Guymier GP, Taylor H, Ireland KB, Shivas RG, Perry S (2014) *Puccinia psidii* in Queensland, Australia: disease symptoms, distribution and impact. Plant Pathol 63:1005–1021
- Shivas RG, Beasley DR, McTaggart AR (2014) Online identification guides for Australian smut fungi (Ustilaginomycotina) and rust fungi (Pucciniales). IMA Fungus 5:195–202
- Vilgalys R, Hester M (1990) Rapid genetic identification and mapping of enzymatically amplified ribosomal DNA from several *Cryptococcus* species. J Bacteriol 172:4239–4246