

Identification of rust fungi (Pucciniales) on species of *Allium* in Australia

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Abstract

Collections of rust fungi (Pucciniales) on species of *Allium* in Australia have previously been identified as *Puccinia allii*. These identifications are unsatisfactory as *P. allii* is a species complex that has yet to be taxonomically resolved. Some of the cryptic species in this complex may represent new introductions and have potential biosecurity consequences for Australia. A combined morphological and molecular approach has been

used to identify the species of rust on *Allium* collected in Australia. Three different species of rust, *Puccinia alli sensu lato*, *P. allii sensu* Gäumann (1959) and *P. mixta*, were identified on species of *Allium* in Australia, based on the morphology of urediniospores and teliospores, and analyses of the internal transcribed spacer and large subunit regions of nuclear ribosomal DNA. One of these species, *P. mixta*, may not be established in Australia. The taxonomy of rusts on *Allium* is discussed.

Keywords: *Allium cepa*, *A. sativum*, *Puccinia porri*, scoring indels, taxonomy, Uredinales

Introduction

Allium (Amaryllidaceae) contains approximately 780 species, many of which are agriculturally important, such as bulb onion (*A. cepa* var. *cepa*), shallot (*A. cepa* var. *aggregatum*), spring onion (*A. fistulosum*), leek (*A. porrum*), garlic (*A. sativum*) and chives (*A. schoenoprasum*) (Friesen et al. 2006). There are at least ten valid names for species of *Puccinia* and *Uromyces* (Pucciniaceae, Pucciniales) that have been reported on species of *Allium* worldwide (Table 1). Many of these rusts were described in the 1800s, and the application of correct names is challenging for several reasons, including that their morphologies were not described in sufficient detail, type specimens were not lodged, and the hosts were sometimes not identified to species level. Gäumann (1959) accepted three autoecious species of rust on *Allium*, namely *P. allii* with mostly two-celled teliospores, *P. porri* with equal numbers of one- and two-celled teliospores, and *U. ambiguus* with one-celled teliospores. Other studies have concluded that the rusts on *Allium* cannot be

Table 1. Basionyms for autoecious species of *Puccinia* and *Uromyces* described on species of *Allium*.

Name	Host	Year	Country	Reference
<i>Uredo porri</i> Sowerby	<i>Allium porrum</i>	1809	England	(Sowerby 1809)
<i>Xyloma allii</i> DC	<i>A. multiflorum</i>	1815	France	(de Candolle 1815)
<i>Uredo alliorum</i> DC	<i>A. vineale</i>	1815	France	(de Candolle 1815)
	<i>A. porri</i>			
	<i>A. multiflorum</i>			
	<i>Allium</i> sp.			
<i>Uredo ambigua</i> DC	<i>Allium</i> sp.	1815	France	(de Candolle 1815)
<i>Puccinia allii</i> F. Rudolphi	<i>A. oleraceum</i>	1829	Italy	(Rudolphi 1829)
	<i>A. vineale</i>			
<i>Puccinia alliorum</i> Corda	<i>Nothoscordum</i>	1840	Portugal	(Corda 1840)
	<i>gracile</i> var. <i>gracile</i>			
	(as <i>A. fragrans</i>)			
<i>Puccinia mixta</i> Fuckel	<i>A. schoenoprasum</i>	1870	Germany	(Fuckel 1870)
<i>Uromyces bicolor</i> Ellis	<i>A. validum</i>	1893	USA	(Coville 1893)
<i>Puccinia blasdalei</i> Dietel & Holw.	<i>A. serratum</i>	1893	USA	(Dietel 1893)
<i>Uromyces aterrimus</i> Dietel & Holw.	<i>A. validum</i>	1895	USA	(Dietel 1895)
	<i>A. unifolii</i>			
<i>Puccinia granulispota</i> Ellis & Gall.	<i>A. cernuum</i>	1895	USA	(Ellis and Everhart 1895)
<i>Puccinia allii-japonici</i> Dietel	<i>A. japonicum</i>	1903	Japan	(Dietel 1903)
<i>Uromyces durus</i> Dietel	<i>A. nipponicum</i>	1907	Japan	(Dietel 1907)
<i>Puccinia allii-cepulae</i> Mundk. & Thirum.	<i>A. cepa</i>	1946	India	(Mundkur and Thirumalachar 1946)

adequately differentiated by spore morphology (Goto 1935; Jennings et al. 1990), host range (Jennings et al. 1990) or morphology of the infection structures (Niks and Butler 1993). Consequently, many collections of rust on *Allium* have been identified as *P. allii* (Laundon and Watenton 1964), which has resulted in a species complex.

Jennings et al. (1990) and Niks and Butler (1993) showed there were at least three morphological species of rust on *Allium* in Europe, with spring onions and garlic each susceptible to multiple species of rust. These authors noted that the identification of rusts on *Allium* was challenging if teliospores were absent. Jennings et al. (1990) provided three features for differentiation of rust on *Allium*, namely (i) whether the rust infected leek, chives or wild leek (*A. ampeloprasum* as '*A. babingtonii*'), (ii) the ratio of one- and two-celled teliospores, and (iii) the density of echinulations on the surface of urediniospores. This latter feature was not considered reliable in a subsequent study (Anikster et al. 2004). Jennings et al. (1990) identified the rust on leek as *P. allii* with mostly two-celled teliospores; the rust on chives as *P. mixta* with about 40 % two-celled teliospores in mature telia, and the rust on wild leek as *Uromyces ambiguus* with one-celled teliospores. Niks and Butler (1993) further differentiated the rusts on leek and chives by morphology of the infection structure.

Koike et al. (2001) and Anikster et al. (2004) determined that rust on garlic and chives in the USA was a different species to that on wild leek, leek and garlic in Europe, as leek was not susceptible to infection by the American rust following experimental inoculations. Anikster et al. (2004) recovered *P. allii* in two clades that correlated with geographical origins from the

USA or Europe in a molecular phylogenetic analysis based on the internal transcribed spacer (ITS) region of ribosomal DNA (rDNA). In addition, they found the two clades differed in the number of nuclei in their basidiospores, and by differences in the size of their teliospores (Anikster et al. 2004). The rust from the USA that has a majority of two-celled teliospores, and infects garlic and chives, but not leek, is hereafter referred to as *P. allii sensu* Koike et al. (2001).

There are an estimated 360 species of rust (Pucciniales) in Australia (Shivas et al. 2014), with only one species recorded on *Allium*, which is an introduced genus. *Puccinia allii* was first reported as the cause of rust on garlic, chives and spring onion in Australia (Rochecouste 1984). Metcalf (2002) showed in pathogenicity tests that an isolate of *Puccinia allii* from Tasmania could infect chives, garlic, bulb onions, spring onions and shallot but not leek. Until very recently, *P. allii* was only recorded from the eastern states of mainland Australia and Tasmania. However, in October 2013, rust on garlic was observed for the first time in South Australia (Fig. 1). It was not known whether *P. allii* from eastern Australia caused this outbreak, or if it was caused by an introduced species.

The primary purpose of this study was to investigate the diversity of rusts on species of *Allium* in Australia collected over a 30-year period. A secondary aim was to resolve the taxonomy of the *P. allii* species complex and apply meaningful names to the rusts on *Allium*. A phylogenetic species concept of rusts on *Allium* was determined from the ITS and large



Fig. 1. Field symptoms of rust on species of *Allium*. **a** Uredinia of *Puccinia porri* on *A. ampeloprasum*, BRIP 61575; **b** Telia of *P. porri* on *A. porrum*, BRIP 61576; **c** Uredinia (orange) and telia (black) of *P. allii sensu lato* on *A. sativum* from South Australia, BRIP 59724; **d** Uredinia (orange) and telia (black) of *P. mixta* on *A. schoenoprasum*, BRIP 61556

subunit (LSU) regions of rDNA, and morphological characters were inferred for differentiation of these species.

Materials and Methods

Taxon selection

Specimens of rust on *Allium* spp. from Australia and overseas were obtained from the Australian reference herbaria (BRIP, DAR and VPRI) (Shivas et al. 2006) (Table 2). Fresh collections of rust on *A. sativum* were obtained from South Australia and Tasmania. Species of *Puccinia* and *Uromyces* from hosts other than *Allium* were included to represent phylogenetic groups in the Pucciniaceae as determined by Dixon et al. (2010).

DNA extraction, amplification and phylogenetic analyses

Fungal tissue was selectively removed from fresh leaf material with a filtered pipette tip attached to a vacuum pump. DNA was extracted using the UltraClean Microbial DNA Isolation Kit (MoBio Laboratories, Solana Beach, CA, USA). The ITS region was amplified with ITS1F/ITS4B (Gardes and Bruns 1993) and the LSU region was amplified with Rust 2INV/LR6 (Vilgalys and Hester 1990; Aime 2006). PCRs were done with high fidelity Phusion DNA polymerase (New England Biolabs Inc.) using the following thermocycler conditions: initial denaturation step of 1 min at 98 °C; 35 cycles of 10 s at 98 °C, 30 s at 62 °C and 45 s at 72 °C; a final extension step for 5 min at 72 °C. Amplified products were purified and sequenced by Macrogen Korea, Seoul. Sequences were deposited in GenBank with the accession codes provided in Table 2.

Table 2. Host, isolate number, country and GenBank numbers of taxa included in the phylogenetic analyses. GenBank numbers obtained for this study in **bold** font.

Species	Isolate no.	Country	Host	Host Family	ITS/LSU GenBank accession
<i>Ceratocoma jacksoniae</i>	BRIP 57762	Australia	<i>Daviesia</i> sp.	Fabaceae	NA/KT199394 ¹
<i>P. allii</i> s. Gäumann (1959)	BRIP 61619	Australia	<i>A. cepa</i> var. <i>aggregatum</i>	Amaryllidaceae	KU296866/ KU296866
<i>P. allii</i> s. Gäumann (1959)	BRIP 61620	Australia	<i>A. cepa</i> var. <i>aggregatum</i>	Amaryllidaceae	KU296867/ KU296867
<i>P. allii</i> s. Gäumann (1959)	DAR 25710	Australia	<i>A. cepa</i> var. <i>aggregatum</i>	Amaryllidaceae	KU296868/NA
<i>P. allii</i> s. Gäumann (1959)	DAR 67439	Australia	<i>A. fistulosum</i>	Amaryllidaceae	KU296869/ KU296869
<i>P. allii</i> s. Gäumann (1959)	BRIP 61618	Australia	<i>A. sativum</i>	Amaryllidaceae	KU296870/ KU296870
<i>P. allii</i> s. Gäumann (1959)	BRIP 62277	Australia	<i>A. sativum</i>	Amaryllidaceae	KU296871/ KU296871
<i>P. allii</i> s. Koike et al. (2001)	HSZ0162	USA	<i>A. sativum</i>	Amaryllidaceae	AF511077/AF511077 ²
<i>P. allii</i> s. Koike et al. (2001)	HSZ0343	USA	<i>A. sativum</i>	Amaryllidaceae	AF511076/AF511076 ²
<i>P. allii</i> s. Koike et al. (2001)	HSZ0344	USA	<i>A. sativum</i>	Amaryllidaceae	AF511078/AF511078 ²
<i>P. allii</i> s. Koike et al. (2001)	HSZ0508	USA	<i>A. sativum</i>	Amaryllidaceae	AF511075/AF511075 ²
<i>P. allii</i> s. Koike et al. (2001)	HSZ0341	USA	<i>A. schoenoprasum</i>	Amaryllidaceae	AF511080/AF511080 ²
<i>P. allii</i> s. lat.	BRIP 62279	Australia	<i>A. fistulosum</i>	Amaryllidaceae	KU296872/ KU296872
<i>P. allii</i> s. lat.	BRIP 62010	China	<i>A. fistulosum</i>	Amaryllidaceae	KU296873/ KU296873
<i>P. allii</i> s. lat.	BRIP 62011	China	<i>A. fistulosum</i>	Amaryllidaceae	KU296874/NA
<i>P. allii</i> s. lat.	BRIP 62269	China	<i>A. fistulosum</i>	Amaryllidaceae	KU296875/ KU296875
<i>P. allii</i> s. lat.	BRIP 62271	China	<i>A. fistulosum</i>	Amaryllidaceae	KU296876/ KU296876
<i>P. allii</i> s. lat.	BRIP 62272	China	<i>A. fistulosum</i>	Amaryllidaceae	KU296877/ KU296877
<i>P. allii</i> s. lat.	BRIP 62273	China	<i>A. fistulosum</i>	Amaryllidaceae	KU296878/ KU296878
<i>P. allii</i> s. lat.	BRIP 59595	Australia	<i>A. sativum</i>	Amaryllidaceae	KM249849/KM249849
<i>P. allii</i> s. lat.	BRIP 59655	Australia	<i>A. sativum</i>	Amaryllidaceae	KM249850/KM249850
<i>P. allii</i> s. lat.	BRIP 59724	Australia	<i>A. sativum</i>	Amaryllidaceae	KM249851/KM249851
<i>P. allii</i> s. lat.	BRIP 61815	Australia	<i>A. sativum</i>	Amaryllidaceae	KU296879/ KU296879
<i>P. allii</i> s. lat.	BRIP 61962	Australia	<i>A. sativum</i>	Amaryllidaceae	KU296880/ KU296880
<i>P. allii</i> s. lat.	BRIP 61966	Australia	<i>A. sativum</i>	Amaryllidaceae	KU296881/ KU296881
<i>P. allii</i> s. lat.	VPRI 41965	Australia	<i>A. sativum</i>	Amaryllidaceae	KU296882/ KU296882
<i>P. allii</i> s. lat.	BRIP 62270	China	<i>A. sativum</i>	Amaryllidaceae	KU296883/ KU296883

<i>P. boroniae</i>	BRIP 57783	Australia	<i>Chorilaena quercifolia</i>	Rutaceae	NA/KU296884
<i>P. cyperi</i>	BRIP 60997	Australia	<i>Cyperus iria</i>	Cyperaceae	NA/KU296885
<i>P. graminis</i>	BRIP 60137	Australia	<i>Glyceria maxima</i>	Poaceae	NA/KM249852
<i>P. haemodorii</i>	BRIP 57767	Australia	<i>Anigozanthus</i> sp.	Haemodoraceae	KF690675/KF690693 ³
<i>P. hemerocallidis</i>	BRIP 53476	Australia	<i>Hemerocallis</i> sp.	Hemerocallidaceae	KM249855/KM249855
<i>P. heterospora</i>	BRIP 60937	Australia	<i>Abutilon</i> sp.	Malvaceae	NA/KU296886
<i>P. iridis</i>	BRIP 56852	Australia	<i>Iris</i> sp.	Iridaceae	NA/KM249853
<i>P. kuehni</i>	BRIP 59200	Australia	<i>Saccharum officinarum</i>	Poaceae	NA/KU296887
<i>P. lagenophorae</i>	BRIP 57563	Australia	<i>Emilia sonchifolia</i>	Asteraceae	NA/KF690696 ³
<i>P. malvacearum</i>	BRIP 57522	Australia	<i>Malva parviflora</i>	Malvaceae	NA/KU296888
<i>P. melanocephala</i>	BRIP 59199	Australia	<i>Saccharum officinarum</i>	Poaceae	NA/KU296889
<i>P. menthae</i>	BRIP 59667	Australia	<i>Mentha spicata</i>	Lamiaceae	NA/KU296890
<i>P. mixta</i>	DAR 38182	Australia	<i>A. schoenoprasum</i>	Amaryllidaceae	KU296891/NA
<i>P. mixta</i>	BRIP 61581	England	<i>A. schoenoprasum</i>	Amaryllidaceae	KU296892/KU296892
<i>P. mixta</i>	BRIP 61576	Germany	<i>A. schoenoprasum</i>	Amaryllidaceae	KU296893/KU296893
<i>P. mixta</i>	BRIP 61577	Germany	<i>A. schoenoprasum</i>	Amaryllidaceae	KU296894/KU296894
<i>P. mixta</i>	BRIP 61589	Germany	<i>A. schoenoprasum</i>	Amaryllidaceae	KU296895/KU296895
<i>P. mixta</i>	BRIP 61556	Netherlands	<i>A. schoenoprasum</i>	Amaryllidaceae	KU296896/KU296896
<i>P. mixta</i>	HSZ0509	USA	<i>A. schoenoprasum</i>	Amaryllidaceae	AF511087/AF511087 ²
<i>P. myrsiphylli</i>	BRIP 57782	Australia	<i>Asparagus asparagoides</i>	Asparagaceae	NA/KM249854
<i>P. porri</i>	BRIP 61586	England	<i>A. ampeloprasum</i>	Amaryllidaceae	KU296897/KU296897
<i>P. porri</i>	BRIP 61575	Germany	<i>A. ampeloprasum</i>	Amaryllidaceae	KU296898/KU296898
<i>P. porri</i>	BRIP 61588	England	<i>A. fistulosum</i>	Amaryllidaceae	KU296899/KU296899
<i>P. porri</i>	BRIP 61590	Germany	<i>A. fistulosum</i>	Amaryllidaceae	KU296900/KU296900
<i>P. porri</i>	BRIP 61570	Albania	<i>A. porrum</i>	Amaryllidaceae	KU296901/KU296901
<i>P. porri</i>	BRIP 61579	England	<i>A. porrum</i>	Amaryllidaceae	KU296902/KU296902
<i>P. porri</i>	BRIP 61580	England	<i>A. porrum</i>	Amaryllidaceae	KU296903/KU296903
<i>P. porri</i>	BRIP 61583	England	<i>A. porrum</i>	Amaryllidaceae	KU296904/KU296904
<i>P. porri</i>	MS165/92	Germany	<i>A. porrum</i>	Amaryllidaceae	AY187090/AY187090 ²
<i>P. porri</i>	BRIP 61573	Germany	<i>A. porrum</i>	Amaryllidaceae	KU296905/KU296905
<i>P. porri</i>	BRIP 61591	Germany	<i>A. porrum</i>	Amaryllidaceae	KU296906/KU296906
<i>P. porri</i>	BRIP 61592	Germany	<i>A. porrum</i>	Amaryllidaceae	KU296907/KU296907

<i>P. porri</i>	BRIP 61567	Greece	<i>A. porrum</i>	Amaryllidaceae	KU296908/KU296908
<i>P. porri</i>	BRIP 61571	Montenegro	<i>A. porrum</i>	Amaryllidaceae	KU296909/KU296909
<i>P. porri</i>	HSZ0004	England	<i>A. sativum</i>	Amaryllidaceae	AF511079/AF511079 ²
<i>P. porri</i>	YA8799	Turkey	<i>A. sativum</i>	Amaryllidaceae	AF511073/AF511073 ²
<i>P. porri</i>	YA8827	Israel	<i>Allium ampeloprasum</i>	Amaryllidaceae	AF511074/AF511074 ²
<i>P. psidii</i>	BRIP 58517	Australia	<i>Myrtus communis</i>	Myrtaceae	KF318430/KF318447 ⁴
<i>P. stylidii</i>	BRIP 60107	Australia	<i>Stylidium armeria</i>	Stylidiaceae	NA/KJ622215 ³
<i>P. ursiniae</i>	BRIP 57993	Australia	<i>Ursinia anthemoides</i>	Asteraceae	NA/KF690705 ³
<i>Puccinia</i> sp.	BRIP 62274	Thailand	<i>A. tuberosum</i>	Amaryllidaceae	KU296910/KU296910
<i>Puccinia</i> sp.	BRIP 61458	Philippines	<i>Allium tuberosum</i>	Amaryllidaceae	KM249856/KM249856
<i>Puccinia</i> sp.	BRIP 56915	Australia	<i>Smilax australis</i>	Smilacaceae	KM249857/KM249857
<i>Puccinia</i> sp.	BRIP 59643	Australia	<i>Smilax australis</i>	Smilacaceae	KM249858/KM249858
<i>Uredo dianellae</i>	BRIP 57433	Philippines	<i>Dianella javanica</i>	Hemerocallidaceae	NA/ KM249859
<i>Uredo geitonoplesii</i>	BRIP 57603	Australia	<i>Geitonoplesium cymosum</i>	Hemerocallidaceae	NA/ KM249861
<i>Uromyces appendiculatus</i>	BRIP 60020	Australia	<i>Phaesolus vulgaris</i>	Fabaceae	NA/KM249870 ⁵
<i>Uro. appendiculatus</i> var. <i>crassitunicatus</i>	BRIP 60929	Australia	<i>Macroptilium atropurpureum</i>	Fabaceae	NA/ KU296911
<i>Uro. lomandracearum</i>	BRIP 59022	Australia	<i>Lomandra</i> sp.	Asparagaceae	NA/ KM249862
<i>Uro. transversalis</i>	BRIP 59244	Australia	<i>Gladiolus</i> sp.	Iridaceae	NA/ KM249864
<i>Uro. vicia-fabae</i>	BRIP 59246	Australia	<i>Vicia faba</i>	Fabaceae	NA/ KM249865

N/A not applicable

¹McTaggart et al. (2015)

²Anikster et al. (2004)

³McTaggart et al. (2014a)

⁴Pegg et al. (2013)

⁵McTaggart et al. (2014b)

LSU sequences of rusts on *Allium* and species of *Puccinia* and *Uromyces* representative of the Pucciniaceae were aligned using the MAFFT algorithm (Kato et al. 2009) in SATe (Liu et al. 2012). The alignment was examined with two phylogenetic criteria, Bayesian inference and maximum likelihood. MrBayes was used to conduct a Markov Chain Monte Carlo (MCMC) search with Bayesian inference (Ronquist and Huelsenbeck 2003). Four runs, each consisting of four chains, were implemented for 10 million generations. The cold chain was heated at a temperature of 0.25. Substitution model parameters were sampled every 1000 generations and trees were saved every 1000 generations. Convergence of the Bayesian analyses was confirmed using the cumulative and compare functions in AWTY (Nylander et al. 2008) (available at: ceb.csit.fsu.edu/awty/) and 30,000 trees were summarized. Maximum likelihood (ML) was implemented as a search criterion in RAxML (Stamatakis 2014). GTRGAMMA was specified as the model of evolution in both criteria. The RAxML analyses were run with a rapid Bootstrap analysis (command -f a) using a random starting tree and 1000 maximum likelihood bootstrap replicates.

Further phylogenetic analyses were made with an alignment of the ITS and LSU regions. Isolates without these data were excluded from the analyses, except for BRIP 62011, 62277 and DAR 38182. Partitions were set for the ITS and LSU regions, and run under Bayesian inference and maximum likelihood. Indels of the ITS and LSU regions were scored as present or absent characters that corresponded to 20 homologous sites in the alignment. The indels were deleted and run as standard characters in Bayesian inference and multistate characters in

RAxML analyses as a third partition. The same parameters as above were used for MrBayes and RAxML. The ML analysis included a Markov model for the multistate characters (command -K MK).

Morphological examination

Fungal structures were scraped from leaf material, mounted in lactic acid and gently heated to boiling. Preparations were examined with a Leica DMLB microscope and digital images were taken with a Leica DFC500 camera. Measurements were made from the imaged spores.

Results

Phylogenetic analyses

The rusts on species of *Allium* examined in this study were polyphyletic in the Pucciniaceae based on the LSU region (Fig. 2). Most of the rusts on *Allium*, including all of the Australian specimens, formed a well-supported clade within Pucciniaceae group 2 *sensu* Dixon et al. (2010). These rusts occurred in five clades, which were recovered in identical topologies by ML and Bayesian inference based on the ITS and LSU regions of rDNA (Fig. 3). These five clades corresponded to (i) rusts on *A. porrum*, *A. ampeloprasum* and *A. fistulosum* from Europe, considered *Puccinia porri*; (ii) rusts on *A. fistulosum* and *A. sativum* in Australia and China, considered *P. allii sensu lato*; (iii) rusts on several species of *Allium* used in the study of Metcalf (2002), considered *P. allii sensu* Gäumann (1959); (iv) specimens from the USA on *A. sativum* and *A. schoenoprasum* included in the study by Anikster et al. (2004), considered *Puccinia allii sensu* Koike et al. (2001), and (v) rusts on *A. schoenoprasum* from

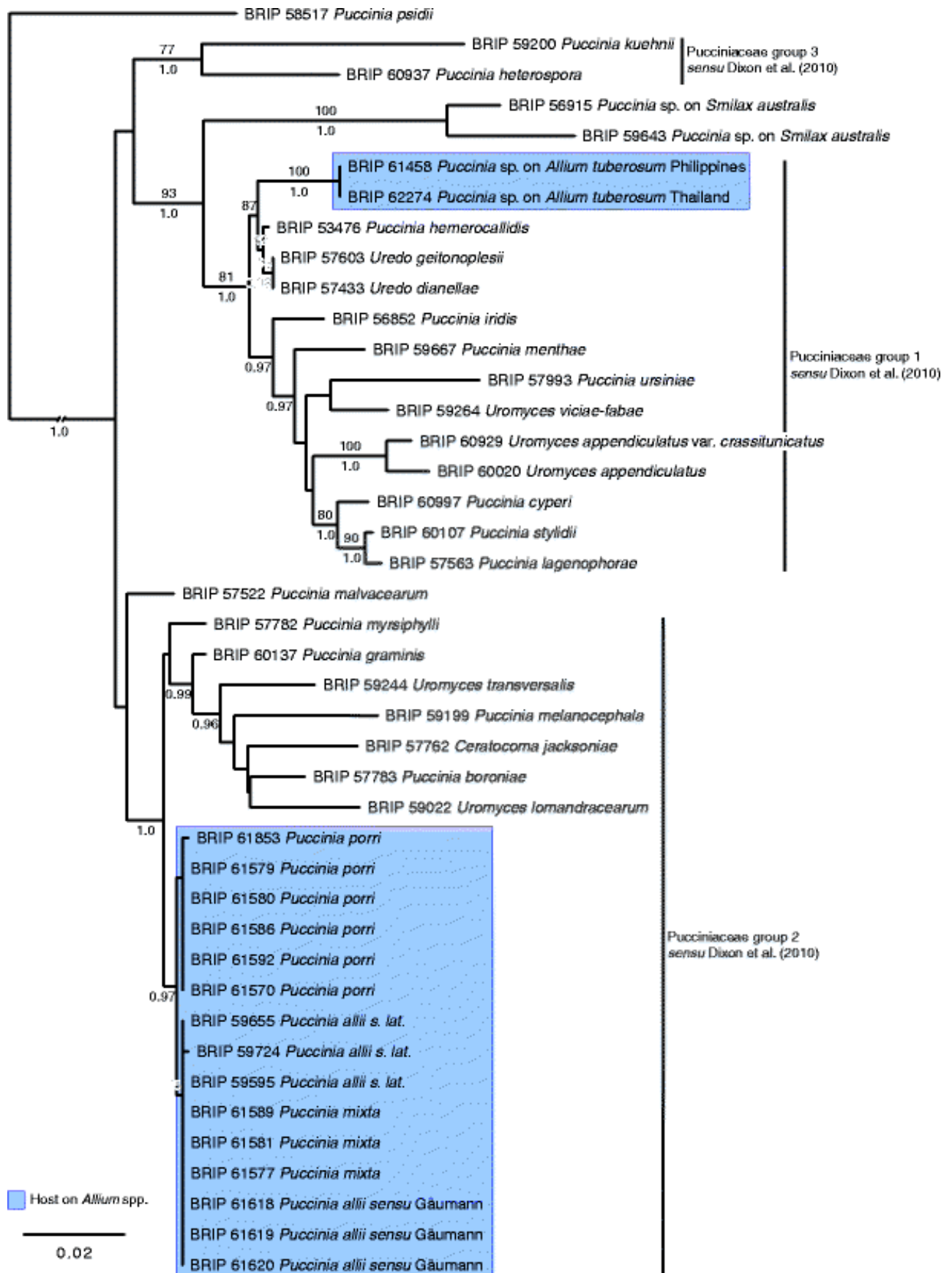


Fig. 2. Phylogram obtained from a Bayesian analysis of the LSU region of rDNA in MrBayes. Posterior probabilities (≥ 0.95) summarized from 30,000 trees shown above nodes. Bootstrap values ($\geq 70\%$) from 1000 replicates in a maximum likelihood search with RAxML shown below nodes.

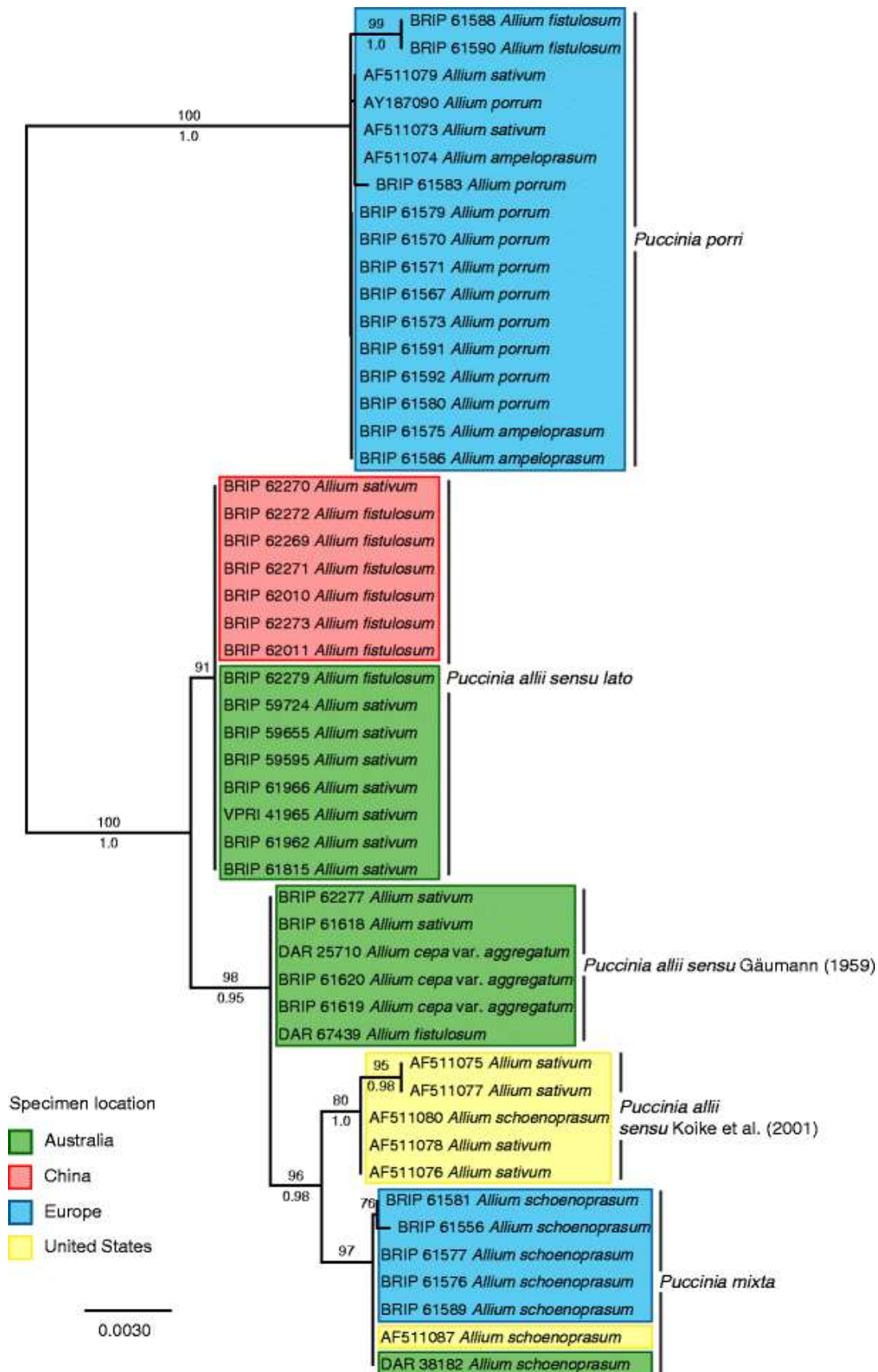


Fig. 3. Phylogram obtained from a maximum likelihood search of the ITS and LSU regions of rDNA, and indels from these regions scored as standard characters in RAxML. Bootstrap values ($\geq 70\%$) from 1000 replicates in a maximum likelihood search shown above nodes. Posterior probabilities (≥ 0.95) summarized from 30,000 trees shown below nodes.

Europe and one specimen from Australia, considered *Puccinia mixta*. With the exception of *Puccinia porri*, these rusts were differentiated by indels in the ITS2 region and were identical in the LSU region.

The remaining two specimens of *Puccinia* sp. on *A. tuberosum* from the Philippines and Thailand, formed a highly supported monophyletic clade with three rusts, *Puccinia hemerocallidis*, *Uredo dianellae* and *Uredo geitonoplesii* on host species in the subfamily Hemerocallidoideae (Xanthorrhoeaceae). These rusts were recovered in a larger well-supported clade that contained rusts in Pucciniaceae group 1 *sensu* Dixon et al. (2010).

Morphology and taxonomy of specimens examined

Three morphologically distinct spore types were found among Australian specimens. The most important morphological characters for species identification are summarised in Table 3. Taxonomic descriptions are provided for five of the species studied from Australia and Europe. Species names were applied if they could be linked to the original concept of the type. *Puccinia allii sensu lato* was used for phylogenetic species that could not be associated with a known rust on *Allium*.

Puccinia allii sensu lato (Fig. 1C; 4A, B, C)

Uredinia on both leaf surfaces, subepidermal, erumpent, round, yellow, peridium present.

Urediniospores ellipsoidal, obovoid or oval, subhyaline to yellow, $26\text{--}34 \times 21\text{--}27 \mu\text{m}$; wall $1.0\text{--}2.5 \mu\text{m}$ thick, apex not thickened, echinulate, 21–33 spines per $10 \mu\text{m}^2$, with 7–10

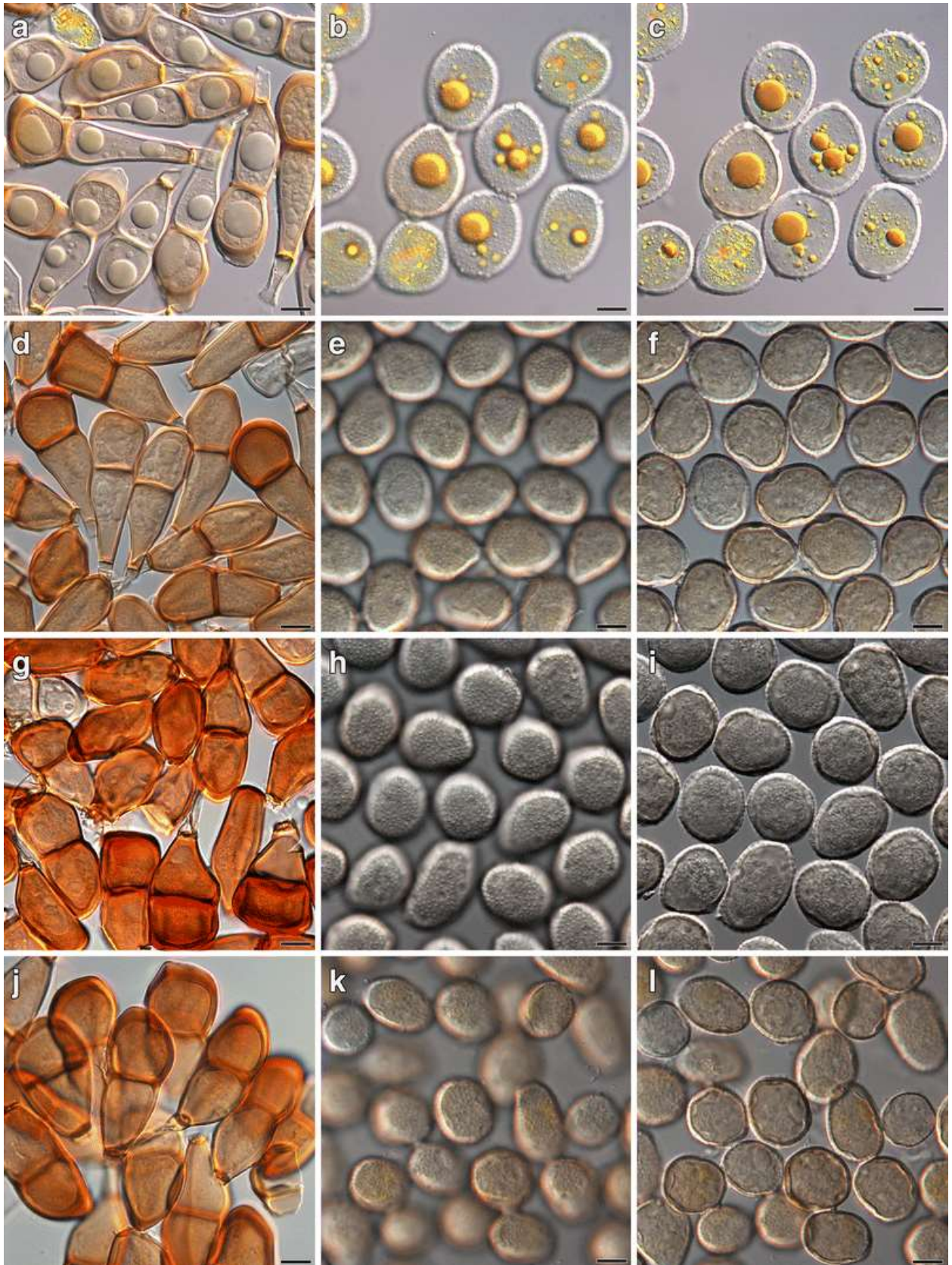


Fig. 4. *Puccinia* spp. on *Allium* in Australia and Europe. (a-c) Teliospores, and surface and equatorial planes of urediniospores of *P. allii* sensu lato BRIP 59724; (d-f) Teliospores, and surface and equatorial planes of urediniospores of *P. allii* sensu Gäumann (1959) BRIP 62277; (g-i) Teliospores, and surface and equatorial planes of urediniospores of *P. mixta* BRIP 61577; (j-l) Teliospores, and surface and equatorial planes of urediniospores of *Puccinia porri* BRIP 61575. Scale =10 μ m

Table 3. Host and morphological characters for differentiation of the five taxa recovered in the present study.

Species	Host	Location	Percentage of 2-celled teliospores	Teliospore apex
<i>Puccinia allii s. lat.</i>	<i>Allium fistulosum</i> , <i>A. sativum</i>	Australia (QLD, SA, TAS, VIC), China	90–95 %	Usually thickened, up to 7 µm
<i>Puccinia allii sensu</i> Koike et al. (2001) ¹	<i>A. sativum</i> , <i>A.</i> <i>schoenoprasum</i>	USA	97–99 % (on garlic) 62–95 % (on chives)	Not thickened
<i>P. allii sensu</i> Gäumann (1959)	<i>Allium</i> spp.	Australia (QLD, TAS)	50–60 %	Slightly thickened up to 4.5 µm
<i>P. mixta</i>	<i>A. schoenoprasum</i> <i>A. ampeloprasum</i> ,	Europe	80–90 %	Not thickened
<i>P. porri</i>	<i>A. fistulosum</i> , <i>A.</i> <i>porrum</i>	Europe	90–95 %	Usually thickened, up to 9 µm

¹Data taken from Anikster et al. (2004)

scattered germ pores. *Telia* on both leaf surfaces, subepidermal, linear, black. *Teliospores* clavate, apex rounded to truncate, mostly two-celled spores (~90–95%), yellowish brown, constricted at septum smooth walled; one-celled spores oval to clavate, $35\text{--}58 \times 16\text{--}17 \mu\text{m}$; two-celled spores $42\text{--}65 \times 16\text{--}20 \mu\text{m}$, wall $1.5\text{--}2.0 \mu\text{m}$ thick at sides, $3\text{--}7 \mu\text{m}$ at apex, upper cell ellipsoidal to doliiform, $19\text{--}29 \times 16\text{--}20 \mu\text{m}$, lower cell clavate, paler than the upper cell, $26\text{--}38 \times 11\text{--}15 \mu\text{m}$, gradually tapered to a narrow base $4\text{--}6 \mu\text{m}$.

Specimens examined on *Allium sativum*: AUSTRALIA. Queensland: Stanthorpe, on *Allium sativum*, 20 Nov. 2014, C.E. Gambley, BRIP 61815, GenBank ITS-LSU: KU296879. South Australia: Croydon Park, on *A. sativum*, 01 Oct. 2013, G. Fundak, BRIP 59595, GenBank ITS-LSU: KM249849; *loc. id.*, on *A. sativum*, 01 Oct. 2013, G. Fundak, BRIP 59655, GenBank ITS-LSU: KM249850; *loc. id.*, 23 Oct. 2013, I. Campbell, BRIP 59724, GenBank ITS-LSU: KM249851; Waterloo Corner, on *A. sativum*, 13 Nov. 2013, I. Campbell, BRIP 60168; *loc. id.*, on *A. sativum*, 20 Nov. 2013, G. Fundak, BRIP 60166; Virginia, on *A. sativum*, 20 Nov. 2013, G. Fundak, BRIP 60167. Tasmania: Channel Highway, on *A. sativum*, 07 Jan. 2015, A.D.W. Geering, D. Metcalf, M.D.E. & R.G. Shivas, BRIP 61962, GenBank ITS-LSU: KU296880; Richmond, on *A. fistulosum*, 07 Jan. 2015, A.D.W. Geering, D. Metcalf, M.D.E. & R.G. Shivas, BRIP 62279, GenBank ITS-LSU: KU296872. Victoria: Mildura, on *A. sativum*, Jan. 2015, D. Metcalf, BRIP 61966, GenBank ITS-LSU: KU296881; Bayles, on *A. sativum*, 09 Nov. 2011, VPRI 41965, GenBank ITS-LSU: KU296882. CHINA. Yunnan, Yi Liang, on *A. sativum*, 16 Nov. 2014, S. Hongsanan, BRIP 62270, GenBank ITS-LSU: KU296883; Yunnan, LuFeng, on *A. fistulosum*, 15 Nov. 2014, S. Hongsanan, BRIP

62269, GenBank ITS-LSU: KU296875; Dali, on *A. fistulosum*, 22 Oct. 2014, *K.D. Hyde*, BRIP 62271, GenBank ITS-LSU: KU296876; Kunming, Ciba, on *A. fistulosum*, 08 Nov. 2014, *S. Hongsanan*, BRIP 62272, GenBank ITS-LSU: KU296877; 29 Nov. 2014, *J. Monkai*, BRIP 62273, GenBank ITS-LSU: KU296878; Shandong, on *A. fistulosum*, 25 Nov. 2014, *Y.M. Li*, BRIP 62010, GenBank ITS-LSU: KU296873; Beijing, on *A. fistulosum*, 30 Nov. 2014, *Y.M. Li*, BRIP 62011, GenBank ITS-LSU: KU296874.

Hosts — *Allium fistulosum*, *A. sativum*

Notes — This rust may be a recent introduction to Australia. The rusts collected in Australia before 2013, and included in the phylogenetic analyses, were not recovered in this clade. This rust can be identified from other species on *Allium* by the teliospores that are mostly two-celled with a thickened apex, and a basal cell that tapers towards the pedicel. The present study indicates that this rust also occurs in China on the same hosts.

Puccinia allii sensu Gäumann (1959) *Die Rostpilze Mitteleuropas* (Germany), 12: 435. 1959. (Fig. 4D, E, F)

Uredinia on leaves, subepidermal, erumpent, linear. *Urediniospores* globose, subglobose, ellipsoidal or obovoid, subhyaline, $26\text{--}32(-36) \times 22\text{--}26(-28) \mu\text{m}$; wall $1.5\text{--}2.5 \mu\text{m}$ thick, echinulate, 20–29 spines per $10 \mu\text{m}^2$, with 6–12 scattered germ pores. *Telia* on leaves, subepidermal, not erumpent, ellipsoidal to linear, often confluent, black. *Teliospores* cylindrical or clavate, apex rounded to truncate, approximately equal proportions of one and

two-celled spores (40–60%), reddish brown, smooth walled; one-celled spores ovoid to obovoid or irregular $23\text{--}45 \times 12\text{--}24 \mu\text{m}$, wall $1.5\text{--}3.0 \mu\text{m}$ thick at sides, $2.5\text{--}4.5 \mu\text{m}$ thick at apex; two-celled spores cylindrical to obovoid $36\text{--}53 \times 18\text{--}26 \mu\text{m}$, upper cell similar in length or slightly shorter than lower cell, wall $1.5\text{--}3.0 \mu\text{m}$ thick at sides, apex slightly thickened $2.5\text{--}4.5 \mu\text{m}$; remnants of pedicel persistent.

Specimens examined: AUSTRALIA. New South Wales: Richmond, on *Allium cepa* var. *aggregatum*, 1975, *N. Nair*. DAR 25710, GenBank ITS: KU296868; Queensland, Blenheim, on *A. sativum*, 26 June 1980, *G. Haines*, BRIP 13201. Queensland: Kalbar, on *A. sativum*, Sep. 1982, *A. Psesser*, BRIP 13806; *loc. id.*, on *A. sativum*, 04 Oct. 1982, *G. Haines*, BRIP 13826; Toowoomba, on *A. sativum*, 14 Oct. 1992, *J. McPherson*, BRIP 20442. Tasmania: Kingston, on *A. fistulosum*, Sep. 1992, *S. Grice*, DAR 67439, GenBank ITS-LSU: KU296869; Huonville, on *A. sativum*, 18 Oct. 2004, *S. Smith*, BRIP 61618, GenBank ITS-LSU: KU296870; Richmond, on *Allium cepa* var. *aggregatum*, 2000, *D. Metcalf*, BRIP 61619, GenBank ITS-LSU: KU296866; on *A. cepa* var. *aggregatum*, 2000, *D. Metcalf*, BRIP 61620, GenBank ITS-LSU: KU296867; Richmond, on *A. sativum*, 07 Jan 2015, *A.D.W. Geering & R.G. Shivas*, BRIP 62277, GenBank ITS-LSU: KU296871.

Hosts — *Allium cepa* var. *aggregatum*, *A. fistulosum* *A. sativum*

Notes — *Puccinia allii sensu* Gäumann (1959) has approximately equal numbers of one- and two-celled teliospores, which are not thickened at the apex. This rust matches the concept of

P. allii described by Gäumann (1959), as firstly, the host range includes *A. fistulosum*, *A. sativum* and *A. cepa* var. *aggregatum* (as *A. ascalonicum*), but not *A. ampeloprasum*, *A. porrum* and *A. schoenoprasum*, and secondly, up to 50 % of teliospores are one-celled. It is not known whether *P. allii sensu* Gäumann (1959) is comparable to the original concept of *P. allii* by de Candolle (1815) and Rudolphi (1829) based on European specimens. A specimen of this rust from Europe was not included in our study. DNA was not successfully amplified and sequenced from the Queensland specimens.

Puccinia mixta Fuckel, *Jb. nassau. Ver. Naturk.* 23–24: 58. 1870. (Fig. 1D; 4G, H, I)
Uredinia on leaves, subepidermal, erumpent, linear. *Urediniospores* globose, subglobose, ellipsoidal or obovoid, subhyaline, $26\text{--}32(-36) \times 22\text{--}26(-28)$ μm ; wall 1.5–2.5 μm thick, echinulate, 22–31 spines per 10 μm^2 , with 6–12 scattered germ pores. *Telia* on leaves, subepidermal, not erumpent, ellipsoidal to linear, confluent, black. *Teliospores* cylindrical or clavate, apex rounded to truncate, mostly two-celled (80–90%) with remainder one-celled, reddish brown, smooth walled; one-celled spores ovoid to obovoid or irregular $24\text{--}32 \times 16\text{--}21$ μm , wall 1.5–3.0 μm thick at sides, apex not thickened; two-celled spores cylindrical to obovoid $31\text{--}57 \times 15\text{--}23$ μm , upper cell similar in length or slightly shorter than lower cell, wall 1.5–3.0 μm thick at sides, not thickened or slightly thickened at apex.

Specimens examined: AUSTRALIA. New South Wales, East Lindfield, on *Allium schoenoprasum*, 1971, *D.L.G. Smith*, DAR 38182, GenBank ITS: KU296891; ENGLAND. Oxfordshire, Oxford, on *A. schoenoprasum*, 04 Sept. 2014, *L.S. Shuey*, BRIP 61581,

GenBank ITS-LSU: KU296892. GERMANY. Bochum, on *A. schoenoprasum*, 29 Aug. 2014, A.R. McTaggart & L.S. Shuey, BRIP 61576, GenBank ITS-LSU: KU296893; Bochum, on *A. schoenoprasum*, 29 Aug. 2014, A.R. McTaggart & L.S. Shuey, BRIP 61577, GenBank ITS-LSU: KU296894; Waldring, on *A. schoenoprasum*, 09 Sept. 2014, A.R. McTaggart & L.S. Shuey, BRIP 61589, GenBank ITS-LSU: KU296895. NETHERLANDS. Nieuwenhoorn, on *A. schoenoprasum*, 10 Aug. 2014, L.S. Shuey, D.O.C. Harteveld & A.R. McTaggart, BRIP 61556, GenBank ITS-LSU: KU296896.

Host — *Allium schoenoprasum*

Notes — *Puccinia mixta* was described by Fuckel (1870) on *A. schoenoprasum* from Germany. This was the first species of rust described from chives, and is an appropriate name to apply to this clade, which included specimens from Germany. The concept of *P. mixta* was refined by Jennings et al. (1990), who found that 60 % of teliospores in mature telia were one-celled. The results from the present study indicated that two-celled teliospores were more common, based on specimens from England, Germany and the Netherlands. The important characters that distinguish *P. mixta* are (i) teliospores are not thickened at the apex, and (ii) *A. schoenoprasum* is the only known host species. This rust was first collected in Australia in 1972, but no further specimens have been confirmed as *P. mixta*.

Puccinia porri (Sowerby) G. Winter, *Rabenh. Krypt.-Fl., Edn 2* (Leipzig) 1.1: 200. 1881.
(Fig. 1A, B; 4J, K, L)

Basionym: *Uredo porii* Sowerby, *Col. fig. Engl. Fung. Mushr.* (London) 3: pl. 411. 1810.

Uredinia on leaves and peduncles, subepidermal, erumpent, linear. *Urediniospores* globose, subglobose, ellipsoidal to obovoid, pale brown, $20\text{--}32 \times 17\text{--}24 \mu\text{m}$; wall $1.5\text{--}3.0 \mu\text{m}$ thick, echinulate, $20\text{--}31$ spines per $10 \mu\text{m}^2$, with $7\text{--}12$ scattered germ pores. *Telia* on leaves and peduncles, subepidermal, not erumpent, ellipsoidal to linear, confluent, black. *Teliospores* cylindrical or clavate, apex rounded to truncate, mostly two-celled ($\sim 90\text{--}95\%$) with remainder one-celled, reddish brown, smooth walled; one-celled spores ellipsoid or oval to irregular $31\text{--}41 \times 14\text{--}26 \mu\text{m}$, wall $1.5\text{--}3.0 \mu\text{m}$ thick at sides, apex not thickened or thickened up to $5 \mu\text{m}$; two-celled spores cylindrical to obovoid $36\text{--}51 \times (18\text{--})21\text{--}25(\text{--}28) \mu\text{m}$, wall $1.5\text{--}3.0 \mu\text{m}$ thick at sides, apex usually thickened $3\text{--}9 \mu\text{m}$, with persistent pedicel, up to $25 \mu\text{m}$.

Specimens examined: ALBANIA. Tirana, on *A. porrum*, 21 Aug. 2014, A.R. McTaggart & L.S. Shuey, BRIP 61570, GenBank ITS-LSU: KU296901. ENGLAND. Hampshire, Lyndhurst, on *A. porrum*, 05 Sept. 2014, L.S. Shuey, BRIP 61583, ITS-LSU: KU296904; London, Richmond, on *A. ampeloprasum*, 07 Sept. 2014, L.S. Shuey, BRIP 61586, ITS-LSU: KU296897; London, Richmond, on *A. fistulosum*, 07 Sept. 2014, L.S. Shuey, BRIP 61588, GenBank ITS-LSU: KU296899; Shepherd's Bush, on *A. porrum*, 03 Sept. 2014, L.S. Shuey, BRIP 61579, GenBank ITS-LSU: KU296902; Oxfordshire, Oxford, on *A. porrum*, 04 Sept. 2014, L.S. Shuey, BRIP 61580, GenBank ITS-LSU: KU296903. GERMANY. Bochum, on *A. ampeloprasum*, 29 Aug. 2014, A.R. McTaggart & L.S. Shuey, BRIP 61575, GenBank ITS-LSU: KU296898; Bochum, on *A. porrum*, 29 Aug. 2104, A.R. McTaggart & L.S. Shuey, BRIP 61573, GenBank ITS-LSU: KU296905; Bochum, on *A. porrum*, 09 Sept. 2014, A.R.

McTaggart & L.S. Shuey, BRIP 61592, GenBank ITS-LSU: KU296907; *loc. id.*, on *A. porrum*, 09 Sept. 2014, *A.R. McTaggart & L.S. Shuey*, BRIP 61591; *loc. id.*, on *A. fistulosum*, 09 Sept. 2014, *A.R. McTaggart & L.S. Shuey*, BRIP 61590. GREECE. Thessaloniki, on *A. porrum*, 17 Aug. 2014, *A.R. McTaggart & L.S. Shuey*, BRIP 61567, GenBank ITS-LSU: KU296908. MONTENEGRO. Budvar, on *A. porrum*, 23 Aug. 2014, *A.R. McTaggart & L.S. Shuey*, BRIP 61571, GenBank ITS-LSU: KU296909.

Hosts — *Allium ampeloprasum*, *A. fistulosum*, *A. porrum*, *A. sativum* (Anikster et al. 2004)

Notes — Sowerby (1809) described *Uredo porri* on leek from England as “1000th of an inch in diameter containing apparently bright orange seeds”. Winter (1884) transferred this rust to *Puccinia*, described the aecial and telial stages, and increased the host range to 14 species of *Allium*, including *A. cepa*, *A. fistulosum*, *A. sativum* and *A. schoenoprasum*. The rusts on *A. ampeloprasum*, *A. fistulosum* and *A. porrum* from Europe formed a monophyletic group and most likely correspond to the specimen described on leek in England by Sowerby (1809). Anikster et al. (2004) determined that this rust could also infect garlic. *Puccinia porri* can be differentiated from other rusts as it infects leek, and has mostly two-celled teliospores with thickened apices. This rust is not known to occur in Australia.

Discussion

The species of *Puccinia* that infect *Allium* were recovered in six clades that were treated as distinct species based on morphology, host range, geographic range, and presence of indels in

the ITS region. Three species, *Puccinia alli sensu lato*, *P. allii sensu* Gäumann (1959) and *P. mixta*, were identified on species of *Allium* in Australia. One of these, *P. mixta* on *A. schoenoprasum*, has not been collected since 1972 (DAR 38182), which indicates that this rust may not have established in Australia. *Puccinia allii sensu lato* on *A. fistulosum* and *A. sativum*, may represent a recent incursion in Australia as the earliest collection of this rust was made in 2013. The earliest detection of rust on *Allium* in Australia dates back to 1975. This rust, herein referred to as *Puccinia allii sensu* Gäumann (1959), may represent the first introduction of rust on *Allium* in Australia.

Puccinia mixta and *P. porri* are taxonomically distinct from the *P. allii* species complex. They were differentiated from other rusts on *Allium* by host species, morphology and a phylogenetic species concept based on the ITS and LSU regions of rDNA. Application of the names *P. mixta* and *P. porri* was straightforward, as the hosts and locations were given in the original type descriptions. However, it is more difficult to apply names for other rust fungi described on *Allium* in Europe, as the hosts were not identified to species level in these descriptions, for example, *Uredo alliorum* and *Uredo ambigua* (de Candolle 1815). The rusts examined in this study were mostly host specific on *Allium* spp. and this may be an important character to consider with application of other names. A greater diversity of rusts on *Allium* in their native ranges is expected, for example, four rusts have been described on native hosts in the USA, namely *P. blasdalei*, *P. granulisporea*, *Uromyces aterrimus* and *U. bicolor* (Table 1).

The species of rust on *A. tuberosum* from the Philippines and Thailand may be undescribed as there are no rust fungi described specifically on *A. tuberosum*, a plant native to Asia. It is not unusual for rusts in the Pucciniaceae that infect the same host species to be polyphyletic, as has been observed for the rusts on sugar cane, *P. kuehnii* and *P. melanocephala* (Dixon et al. 2010).

This study has identified three rusts, *P. porri* (on leek in Europe), *P. mixta* (on chives in Europe) and *P. allii* s. lat. sensu Koike et al. (2001) (on garlic and chives in the U.S.A.) that are not known to occur in Australia. Their identification will help devise effective quarantine protocols to protect the onion, leek and garlic industries in Australia against exotic threats. Resolution of the *P. allii* complex will require further sampling of rusts on other species of *Allium* that can be linked to earlier described species.

Acknowledgements

Funding provided by Horticulture Innovation Australia Limited project number VN13001 is gratefully acknowledged. This work was partly funded by the Australian Biological Resources Study, grant number RFL212-33.

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