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Plasmopara sphagneticolae sp. nov. (Peronosporales) on *Sphagneticola* (Asteraceae) in Australia

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Abstract A specimen of downy mildew on leaves of *Sphagneticola trilobata* found in northern Queensland was identified by a systematic approach as a novel species of *Plasmopara*. A new species, *Plasmopara sphagneticolae*, is proposed for this specimen, which differs from other species of *Plasmopara* by morphology, host range, and sequence data from nuclear-ribosomal DNA and mitochondrial DNA. *Plasmopara sphagneticolae*, together with *P. halstedii*, are downy mildews found on host species in the tribe Heliantheae (Asteraceae). *Plasmopara halstedii* causes downy mildew on *Helianthus annuus*, and is not present on sunflower in Australia. Phylogenetic analysis of the large subunit region of ribosomal DNA showed that *P. sphagneticolae* was sister to *P. halstedii* on sunflower.

Keywords Invasive weed · *Peronosporales* · Molecular phylogeny · Taxonomy

Species of *Plasmopara* (Peronosporales) cause downy mildew on hosts in several plant families, namely Acanthaceae, Asteraceae, Balsaminaceae, Geraniaceae, Malvaceae, Onagraceae, Orobanchaceae, Violaceae and Vitaceae (Voglmayr et al. 2004). Some species cause economically important diseases, including *P. viticola* on grape, *P. geranii* on geranium and *P. halstedii* on sunflower. The genus *Plasmopara* is non-monophyletic, and the type species, *P. pygmaea* on Ranunculaceae, has a close relationship to genera such as *Bremia*, *Paraperonospora* and *Basidiophora*. Species of *Plasmopara* have ellipsoid to pyriform haustoria with monopodial sphorangiophores (Göker et al. 2003; Voglmayr et al. 2004).

In Australia, one species of downy mildew, *Plasmopara majewskii*, has been reported on hosts in Asteraceae (Constantinescu and Thines 2010). It was discovered in Australia and New Zealand on two weedy species, *Arctotis* × *hybrida* and *Arctotheca calendula* (tribe Arctoteae), yet is unknown from the native host range in South Africa. *Plasmopara majewskii* was described based on its morphology, unique hosts, and a phylogenetic analysis of the Large Subunit (LSU) region of nuclear-ribosomal DNA (rDNA) (Constantinescu and Thines 2010).

In 2014, one specimen of downy mildew was collected in northern Queensland on *Sphagneticola trilobata* (Heliantheae, Asteraceae), a native of tropical America that is an environmental weed in Australia (Anon. 2013). This specimen was lodged in the Brisbane Plant Pathology Herbarium as BRIP 61010. We used a systematic approach with two molecular loci from rDNA and mitochondrial DNA, to identify the downy mildew on *Sphagneticola trilobata* in Australia.

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 images were taken with a Leica DFC500 camera. Measurements were made digitally from photographed spores.

Fungal tissue was selectively removed from fresh leaf material and DNA extracted using the UltraClean Microbial DNA Isolation Kit (MoBio Laboratories, Solana Beach, CA, USA). The LSU region was amplified with LROR/LR7 (Vilgalys and Hester 1990) and the Cytochrome c oxidase subunit 2 (CO2) region of mitochondrial DNA with the primers designed by Hudspeth et al. (2000). All PCRs were done with high fidelity Phusion enzyme (New England Biolabs Inc.) according to the manufacturer's

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Table 1 Hosts and GenBank numbers of taxa included in the phylogenetic analyses. New species described in bold face

Species	Host genus/family	LSU GenBank accession
Bremia lactucae s. str.	*Lactuca*/Asteraceae	EF553478[a]
Novotelnova scorzonerae	*Scorzonera*/Asteraceae	EF553477[a]
Paraperonospora leptosperma	*Anthemis*/Asteraceae	EF553472[a]
Paraperonospora tanaceti	*Tanacetum*/Asteraceae	EF553473[a]
Peronospora rumicis	*Rumex*/Polygonaceae	AY035476[b]
Peronospora trivialis	*Cerastium*/Caryophyllaceae	AY035471[b]
Phytophthora arecae	*Cocos*/Arecaceae	AY035530[b]
Phytophthora litchii	*Litchi*/Sapindaceae	AY035531[b]
Plasmopara baudysii	*Berula*/Apiaceae	AY035517[b]
Plasmopara centaureae-mollis	*Centaurea*/Asteraceae	EF553476[a]
Plasmopara densa	*Rhinanthus*/Orobanchaceae	EF553463[a]
Plasmopara euphrasiae	*Euphrasia*/Orobanchaceae	EF553468[a]
Plasmopara halstedii	*Flaveria*/Asteraceae	AY178534[c]
Plasmopara halstedii	*Rudbeckia*/Asteraceae	KF927152[d]
Plasmopara halstedii	*Rudbeckia*/Asteraceae	KF927154[d]
Plasmopara halstedii	*Helianthus*/Asteraceae	EF553469[a]
Plasmopara majewskii	*Arctotis*/Asteraceae	HQ402932[e]
Plasmopara megasperma	*Viola*/Violaceae	AY035516[b]
Plasmopara nivea	*Aegopodium*/Apiaceae	AF119604[f]
Plasmopara obducens	*Impatiens.*/Balsaminaceae	EF196869[g]
Plasmopara pimpinellae	*Pimpinella*/Apiaceae	AY035519[b]
Plasmopara pusilla	*Geranium*/Geraniaceae	AY035521[b]
Plasmopara sphagneticolae	***Sphagneticola*/Asteraceae**	**KM085176**
Plasmopara viticola	*Vitis*/Vitaceae	AY035524[2]
Plasmoverna anemones-ranunculoidis	*Anemone*/Ranunculaceae	EF553471[1]
Protobremia sphaerosperma	*Tragopogon*/Asteraceae	EF553480[1]

[a] Voglmayr and Constantinescu (2008)
[b] Riethmüller et al. (2002)
[c] Spring et al. (2003)
[d] Rivera et al. (2014)
[e] Constantinescu and Thines (2010)
[f] Riethmüller et al. (1999)
[g] Voglmayr and Thines (2007)

recommendations. Annealing temperatures were 60 °C for the LSU reaction and 62 °C for the COX2 reaction. Amplified products were purified and sequenced by Macrogen Korea. The LSU and CO2 sequences of BRIP 61010 were deposited in GenBank as KM085176 and KM085175, respectively.

The LSU sequence was added to a subset of the alignment deposited by Voglmayr and Constantinescu (2008) (TreeBASE accession S1909) (Table 1). The sequences were aligned with MAFFT (Katoh et al. 2009) and run using two phylogenetic criteria. MrBayes was used to conduct a Markov Chain Monte Carlo (MCMC) search with Bayesian inference (Ronquist and Huelsenbeck 2003) and maximum likelihood (ML) was implemented as a search criterion in RAxML (Stamatakis 2006). GTRGAMMA was specified as the model of evolution for both criteria.

In BLAST searches, *Plasmopara* on *Sphagneticola* was similar to other isolates in the *P. halstedii* complex. The nuclear LSU region was 99 % identical (1396/1415 identities) to *P. halstedii* on *Rudbeckia fulgida* (KF927152), 99 % identical (1216/1230 identities) to *P. halstedii* on *Helianthus annuus* (EF553469), and 94 % identical (612/646 identities) to *P. halstedii* on *Flaveria bidentis* (AY178534). The CO2 region was 94 % identical (536/573 identities) to *P. halstedii* on *Gerbera jamesonii* (KC690148). The topologies recovered by maximum likelihood and Bayesian inference based on the LSU region were identical (Fig. 1). *Plasmopara* on

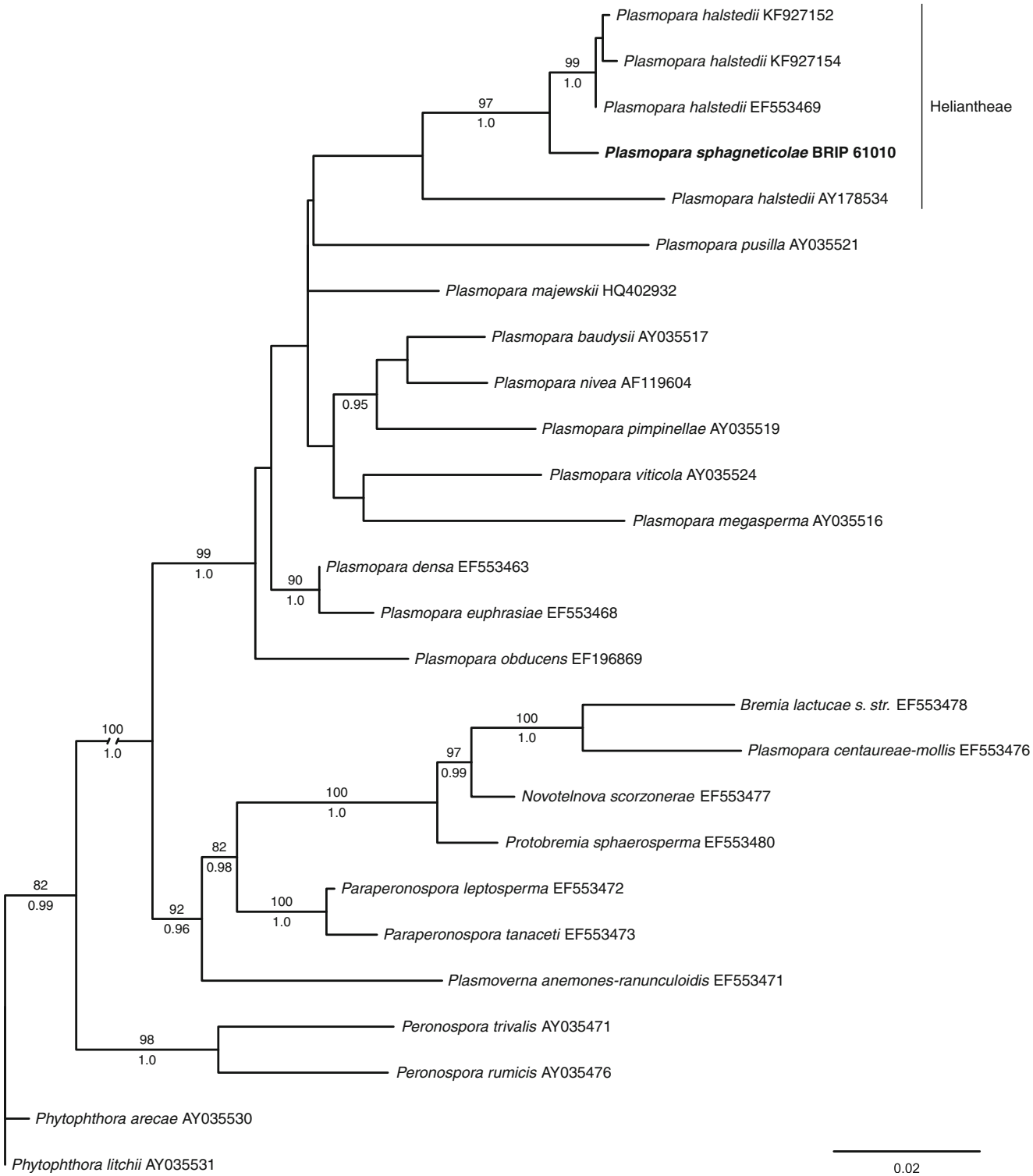

Fig. 1 Phylogram obtained from a maximum likelihood analysis of the LSU region in RaxML. Bootstrap values (≥70 %) from 1,000 replicates in a ML (command –f a) search shown above nodes. Posterior probabilities (≥0.95) from a Bayesian search and summarized from 23,200 trees shown below nodes

Sphagneticola was sister to *P. halstedii* on *Helianthus* and *Rudbeckia*, and formed a well-supported (bootstrap 97 %, posterior probability 1.0) monophyletic group of species of *Plasmopara* on host species in the tribe Heliantheae (Asteraceae) (Fig. 1). *Plasmopara halstedii* on *Flaveria*, which is also in the Heliantheae, was sister to these taxa, a relationship recovered in similar studies (Choi et al. 2009; Spring et al. 2003).

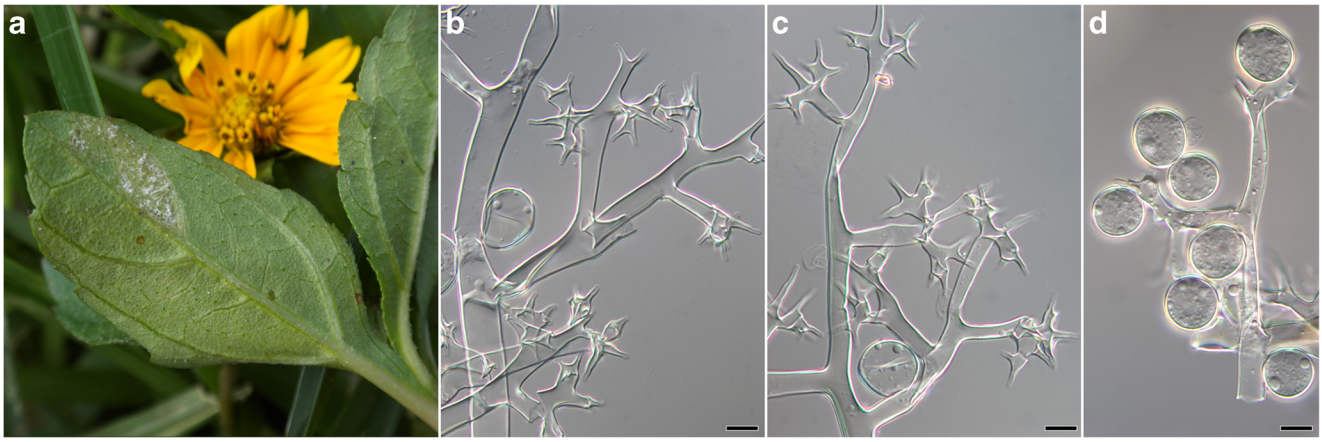

Fig. 2 *Plasmopara sphagneticolae* on *Sphagneticola triolobata* (BRIP 61010). **a**. Host symptoms; **b-c**. Sporangiophores; **d**. Sporangia. Scale=10 μm

A phylogenetic species concept of *Plasmopara* differentiates *Plasmopara* on *Sphagneticola* from *P. halstedii* on *Helianthus* by LSU sequence data, host range and morphology. *Plasmopara sphagneticolae* is described as a new species to represent this taxon.

Plasmopara sphagneticolae McTaggart & R.G. Shivas, **sp. nov.** (Fig. 2)

MycoBank no.: MB 809437

Etymology: Named after the host genus

On leaves, hypophyllous on irregular leaf spots, often vein limited, corresponding upper surface chlorotic becoming necrotic, up to 1 cm wide. Sporangiophores abaxial, forming a white downy mass, scattered, slender, straight, 300–500 μm long, 7–13 μm wide, basal end slightly bulbous, callose plugs present . Branches arborescent, branching 3 times and terminating in a group of sporangium-bearing denticles; primary branches alternate, arising 70–90° to the main axis, up to 125 μm long; secondary branches, up to 60 μm long, alternate or opposite; tertiary branches, up to 10 μm long, with up to three denticles 4–15 μm×1.5–4.0 μm with truncate tips. Sporangia globose, subglobose to ellipsoid, 15–27×14–20 μm, broadest sub-median or median, base round, tip round or slightly apiculate; wall 0.5–1.0 μm thick.

Host plant: *Sphagneticola trilobata* (Asteraceae)

Distribution: Queensland, Australia

Holotype: AUSTRALIA, Queensland, Babinda, Boulders Road (−17.3471, 145.8864), on *Sphagneticola trilobata*, 05 Apr. 2014, *A.R. McTaggart & S.G. McKenna*, BRIP 61010, GenBank LSU: KM085176, CO2: KM085175.

Diagnosis: *Plasmopara sphagneticolae* has globose to subglobose sporangia and occurs on *Sphagneticola* in the tribe Heliantheae (Asteraceae). It differs from descriptions of *P. halstedii* on sunflower, which has ovate to oblong sporangia 27–40×12–16 μm, and sporangiophores from 400 to 700 μm in length (Farlow 1882; Spring et al. 2003).

Currently *Plasmopara halstedii* represents a species complex. It was originally reported from *Eupatorium*, and then described on five further genera of Asteraceae, namely, *Ambrosia*, *Bidens*, *Helianthus*, *Rudbeckia* and *Silphium* (Farlow 1882). Farlow (1882) noted there were morphological differences between the specimens on these different hosts. Species of downy mildew on more than 35 host genera in Asteraceae were since considered *Plasmopara halstedii s. lat.* (Choi et al. 2009). Novotel'nova (1966) proposed a taxonomy for *Plasmopara* on Asteraceae based on morphology and host range. *Plasmopara halstedii* was limited to the tribe Eupatorieae under this taxonomy, and three species, *P. helianthi*, *P. affinis* and *P. angustiterminalis*, were designated for hosts in the Heliantheae (Novotel'nova 1966). This was not widely adopted, and Leppik (1966, cited in Choi et al. 2009) considered morphology inadequate to differentiate species.

Systematic studies based on nuclear rDNA and mitochondrial DNA have subsequently begun to delimit taxa in the *P. halstedii* species complex (Choi et al. 2009; Constantinescu and Thines 2010; Duarte et al. 2014; Spring et al. 2003; Voglmayr and Constantinescu 2008; Voglmayr et al. 2004). Species of *Plasmopara* on Asteraceae can be defined by their host range and morphology under a phylogenetic species concept. This study confirms the hypothesis that species of downy mildew on Asteraceae show greater diversity than the broad concept applied to *P. halstedii* (Choi et al. 2009; Constantinescu and Thines 2010; Duarte et al. 2014; Spring et al. 2003).

Plasmopara does not occur on *Helianthus* in Australia or New Zealand, and these countries remain the last sunflower growing regions free of downy mildew (Constantinescu and Thines 2010). It is not known whether *P. sphagneticolae* is the same species reported as *P. halstedii* on *Sphagneticola* from its natural host range in America (Mattos et al. 2006).

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