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PLASMOPARA HALSTEDII IS ABSENT FROM AUSTRALIA AND NEW ZEALAND*

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Abstract. *Plasmopara halstedii* (Farl.) Berl. & de Toni is among the most important species hampering commercial sunflower production in many countries. Downy mildew on *Arctotheca* and *Arctotis* collected in Australia and New Zealand has been attributed to *Plasmopara halstedii*, although it has never been reported on sunflower in those two countries. Potentially this makes it difficult for Australia and New Zealand to claim to be free of sunflower downy mildew; this has implications for quarantine and trade. Here we present morphological and molecular analyses of specimens of *Plasmopara* on *Arctotis* and *Arctotheca* collected in Australia and New Zealand. Our results demonstrate that these plants are not attacked by *Plasmopara halstedii* but by a new species which we formally describe as *Plasmopara majewskii* sp. nov. in this study. Consequently, quarantine regulations for *P. halstedii* need to be enforced in order to protect the commercial sunflower industry in Australia and New Zealand.

Key words: *Arctotis, Arctotheca*, downy mildew, *Plasmopara halstedii*, new species, sunflower, Australia, New Zealand

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INTRODUCTION

Plasmopara halstedii (Farl.) Berl. & de Toni, the causal agent of sunflower downy mildew, is the most important pathogenic oomycete affecting this crop (Nishimura 1922, 1926; Novotel'nova 1966; Sackston 1981; Hall 1989a; Tourvieille de Labrouhe *et al*. 2002). This oomycete has also been reported from other Asteraceae (Novotel'nova 1962; Leppik 1966a, b; Kenneth & Palti 1984) and has an almost worldwide distribution (Anonymous 1988; Anonymous 1998). However, recent molecular phylogenetic investigations (Spring *et al*. 2003, 2006) cast doubt on the broad species concept for *Plasmopara* species on Asteraceae. In Australasia, *P. halstedii* was first reported on two hosts introduced from South Africa, namely the garden ornamental *Arctotis* × *hybrida* (African daisy) in New Zealand (Hall 1989b; McKenzie & Dingley 1996) and later the invasive weed *Arctotheca calendula* (capeweed) in Australia (Brown 1997: 54). Because *P. halstedii* is an important quarantine pathogen in Australia and New Zealand which has not yet been found on sunflower in these countries (Anonymous 1997), we saw the need to examine specimens reported and/or collected from these countries.

MATERIAL AND METHODS

The examined specimens are listed under holotype and additional specimens. For morphological analysis we used the methods described in Voglmayr *et al.* (2006 and references therein). DNA extraction and PCR followed the methods in Telle and Thines (2008). PCR reactions for *nrLSU* amplification were adapted as described in Riethmüller *et al*. (2002), using the primers given therein. Figure 2 lists the GenBank accession numbers. The alignments can be requested from marco.thines@senckenberg.de. Sequences of additional downy mildew taxa were downloaded from GenBank

* This paper is dedicated to Professor Tomasz Majewski on the occasion of his 70th birthday.

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(http://www.ncbi.nlm.nih.gov/genbank/index.html). Alignments were done using mafft (Katoh *et al*. 2002), version 6 (Katoh & Toh 2008a) using the Q-INS-i algorithm (Katoh & Toh 2008b); we used default values for all other parameters. Phylogenetic reconstructions were done using the RAxML webserver (Stamatakis *et al*. 2008) at Cipres (http://8ball.sdsc.edu:8889/cipres-web/Home.do) for maximum likelihood inference (Felsenstein 1981). Analyses for 100 bootstrap (Felsenstein 1985) replicates were repeated five times and bootstrap values were averaged over the five replicates. Minimum evolution (ME) analysis was done using MEGA 4.0 (Tamura *et al*. 2007), using the Tamura-Nei substitution model. All other parameters were set to default values. For the ME analysis, the robustness of the phylogenetic reconstruction was tested with 1000 bootstrap replicates.

Taxonomy

Plasmopara majewskii Constantinescu & Thines, ***sp. nov.*** Figs 1 & 2

Plasmopara halstedii *preacipue differt apicibus extremitatibus ramis non tumidis*.

Type: on *Arctotis* ×*hybrida*, AUSTRALIA, Victoria, French Island, Woodlyn Nurseries, Five Ways, 38°18′S 145°18′E, 11 May 1994, *leg*. *A. Sivapalan*, *det. J. H. Cunnington* (17 July 2003 as *Plasmopara* sp.) (holotype: UPS F-180508; isotype: VPRI 20080a). Ex-type sequence for partial *nrLSU* has been deposited in GenBank, accession number HQ402932.

Etymology. Dedicated to Professor Tomasz Majewski to honor his important contribution to Peronosporales taxonomy.

Mostly on leaves, producing clearly defined epiphyllous spots. Spots first yellowish, later becoming light brown, polyangular, 2 mm diam., with distinct margin, rarely coalescing; infected tissues become necrotic. Sporangiophores hypophyllous (mostly obscured by leaf trichomes), forming a white to yellowish down en masse, scattered, slender, straight, 360–650 µm long; basal end slightly bulbous, up to 13 µm wide; trunk 160–350 µm long, of uniform width or gradually tapering or enlarging upwards, 7–12 µm wide at base, 7–11 µm wide below first branch; callose plugs present. Branches arborescent, branching many times and terminating in a group of sporangium-bearing branchlets; branching monopodial, in two stages; primary branches alternate, arising *ca* 80° to the main axis, (30–)50–90 µm long, uniform in width or distally broadening, not or slightly constricted at base, callose plugs present; secondary branches alternate, 20–40 µm long, uniform in width to distally broadened, not constricted at base. Sporangium-bearing branchlets (2–)3 at the end of each branch, diverging *ca* 50–90°, arising from a common base which is either not modified or slightly swollen, rarely differentiated into axial and abaxial long-conical to almost cylindrical, of variable length, 7–21 µm long, (2–)3–4(–5) µm wide at base, 1–2 µm wide just below tip, tip often round and slightly inflated, rarely blunt or cup-like. Sporangia ovoid, subglobose to broadly ellipsoidal, (15–)20–27(–32) × (13–) 16–22(–26) µm, L/B (1.09–)1.14–1.33(–1.66), n = 103, broadest sub-median or median, base round, tip round or slightly apiculate; wall *ca* 0.5 µm thick; pore 3–5 µm diam., covered by a lenticular or outwardly convex papilla 0.5–1.5(–2.0) µm thick; pedicel sometimes present, 0.5–1.0 long, 1(–2) µm wide in young sporangia, translucent and obscured or visible as a flat scar in mature sporangia. Haustoria pyriform to vesicular, 4–5 µm wide, surrounded by a sheath *ca* 1 µm thick. Resting organs not seen.

Additional specimens examined. On *Arctotis* sp.: NEW ZEALAND, Auckland, Aug. 1988, *leg. C. F. Hill, det. G. Hall* (IMI 329031); ditto, Nov. 1988, *leg. G. Hall, det. C. F. Hill* (PDD 55844); South Auckland, Papakura, 37°03′S 174°57′E, June 1989, *leg. C. F. Hill* (IMI 333133). On *Arctotheca calendula* (L.) Levyns: AUSTRALIA, NSW, Coleambally, 13 June 1973, *leg. L. R. Fraser, det. J. Walker* (DAR 23092); Castle Hill, between Church St. and Tuckwell Rd., 2 Oct. 1967, *J. Walker 67/142* (DAR 16592); NEW ZEALAND, Auckland, MARC, 21 Feb. 1992, *leg. E. H. C. McKenzie* (PDD 60083; GenBank HQ402931).

Results and discussion

The first report of *P. halstedii* on *Arctotheca calendula* (under *Arctotis calendulacea*) is from Portugal (Câmara *et al.* 1936: 200). This fungus/host/place combination was later mentioned by Leppik

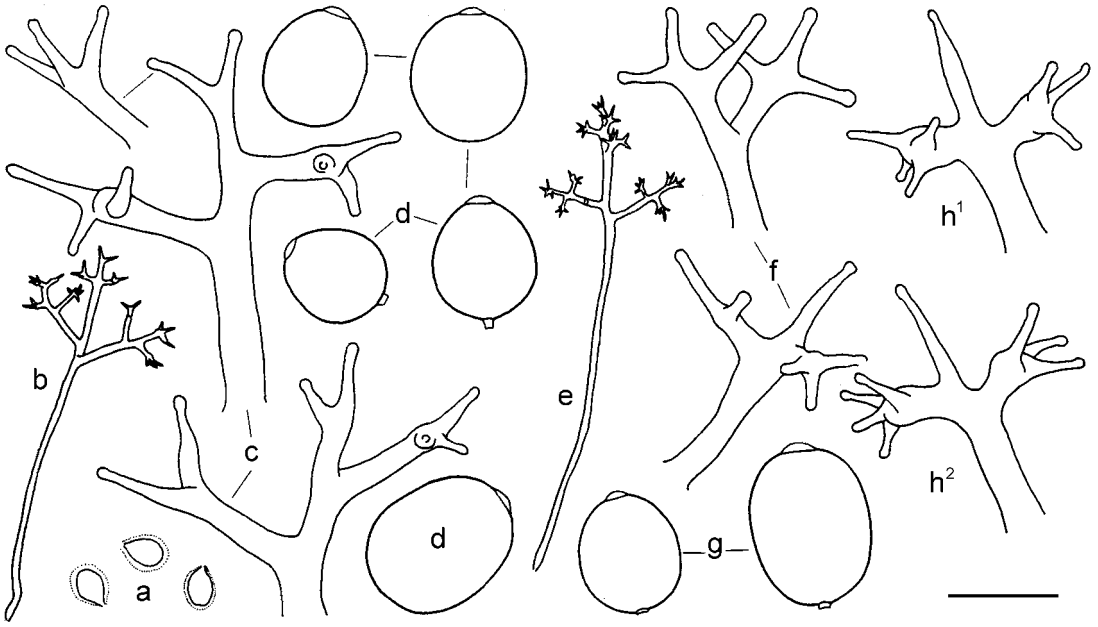

Fig. 1. *Plasmopara* spp. (a – haustoria, b & e – sporangiophores, c, f & h – apex of branches, d & g – sporangia). a–d – *P. majewskii* Constant. & Thines *sp. nov.* on *Arctotis hybrida* (from holotype), e–g – on *Arctotheca calendulae* (from PDD 60083). h – Apex of branches in *P. halstedii* (Farl.) Berl. & de Toni on *Helianthus annuus* (h^1 – from holotype of *P. helianthi* in LEP, h^2 – from lectotype of *P. halstedii* in FH). Scale bar = 125 μm for b & e, 20 μm for others.

(1966a) and Kenneth and Palti (1984), but omitted by authors dealing with the oomycetes of Portugal (Câmara & Oliveira 1944; Lucas & Dias 1976). *Bremia lactucae* was also reported in Portugal on the same host (Lucas *et al*. 1982); however, one of the voucher specimens (LISE 88932) was found to be a *Plasmopara* but not *P. halstedii* (García-Blázquez *et al*. 2007).

Both the morphological and molecular analyses of specimens of Asteraceae collected in Australia and New Zealand and reported as infected by *P. halstedii* show that the oomycete is indeed a member of the genus *Plasmopara*, yet distinct from *P. halstedii*. The most characteristic morphological feature of *P. halstedii* is that almost all branches of the sporangiophores terminate in a long, subulate tip with two opposite, shorter extensions (branches) inflated at the distal part and bearing branchlets on which the sporangia are formed (Fig. 1h^1, h^2).

Phylogenetic analyses revealed that *Plasmopara majewskii* is a highly distinct species (Fig. 2) with unresolved phylogenetic placement within the genus *Plasmopara*. Specimens from *Arctotis* are separated from those from *Arctotheca* with moderate support, the distance being comparable to that between *Plasmopara densa* and *P. euphrasiae*. It is possible that downy mildew on *Arctotheca* is caused by a closely related yet distinct species of *Plasmopara*. Further investigations based on a broad sampling of downy mildews from both *Arctotis* and *Arctotheca*, and sequencing of more variable genes, are needed to resolve this question. The genes *cox2* and *nrITS* have been successfully used to distinguish closely related downy mildew species (Voglmayr & Constantinescu 2008; Choi *et al*. 2009; Thines *et al*. 2009) and could yield the necessary resolution.

Numerous Asteraceae other than sunflower were reported as hosts of *P. halstedii* in the nineteenth century (e.g., Farlow 1883, 1884; Berlese & De Toni 1888; Halsted 1888) and more recently (e.g., Novotel'nova 1962; Leppik 1966a; Sackston 1981; Constantinescu & Negrean 1983; Farr *et al*. 1989; Romero & Carrion 1998). Many of these studies were based on literature records and not on specimen

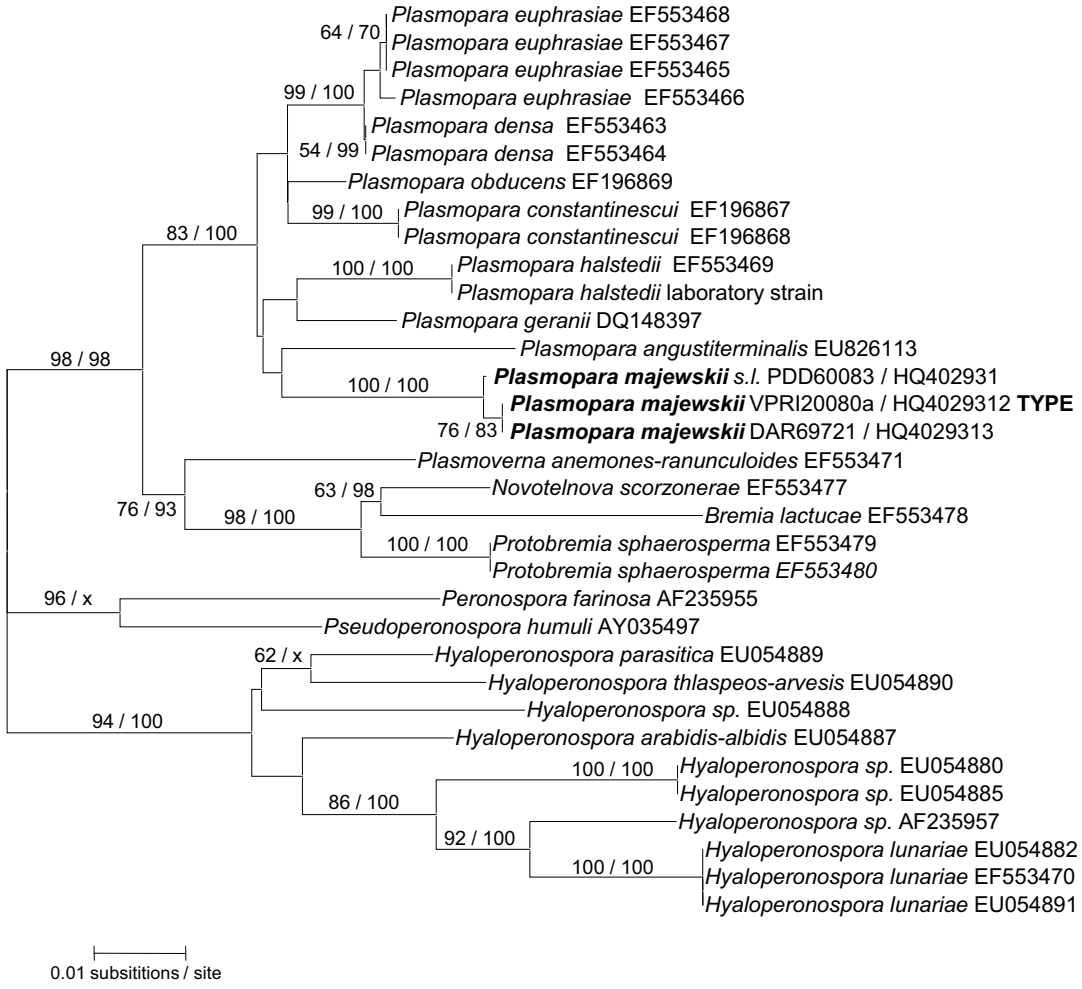

Fig. 2. Phylogenetic tree (minimum evolution) for selected downy mildews based on partial *nrLSU* sequences. *Plasmopara* accessions from *Arctotis* and *Arctotheca* are bolded. Bootstrap support values from minimum evolution and maximum likelihood analysis are given in this order on the respective branches. Only support values greater than 50 are given.

examination. Our studies in progress show that several of these hosts do not harbor *P. halstedii*.

Conclusion

Both *Arctotis* and *Arctotheca* are native to southern Africa but naturalized in Australia and New Zealand. They are not attacked by *Plasmopara halstedii*, the causal agent of sunflower downy mildew, but by a previously overlooked species, *Plasmopara majewskii*, which is morphologically and phylogenetically distinct. Interestingly, *Arctotis* and *Arctotheca* are not reported as hosts of any oomycete in South Africa (Crous *et al.* 2000). *Plasmopara halstedii* was reported from South Africa some 25 years ago (Keetch 1994; Viljoen *et al.* 1997, 1998; Viljoen & Gulya 1998), but according to Crous *et al.* (2000) it is no longer present in that country. It is possible that *P. halstedii* could not withstand the typical dry summers of southern Africa. As *P. halstedii* is absent from Australia and New Zealand, strict quarantine regulations for sunflower seeds introduced into Australia and New Zealand are warranted.

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