



Single host plant species may harbour more than one species of *Peronospora* – a case study on *Peronospora* infecting *Plantago*

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Key words

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Abstract The genus *Peronospora* is the largest genus of the oomycetes, fungus-like members of the kingdom *Straminipila* that also contains amoeboid (e.g., *Leukarachnion*) and plant-like (e.g., *Laminaria*) lifeforms. *Peronospora* species are obligate biotrophic plant pathogens, causing high economic losses in various crops and ornamentals, including *Plantago* species. Several species of *Plantago* are used as speciality crops and medicinal plants. In this study, *Peronospora* species parasitic on *Plantago* were investigated based on morphology and phylogenetic analyses using two nuclear (ITS, nrLSU) loci and one mitochondrial (*cox2*) locus. As a result of these investigations, 10 new species are added to the already known *Peronospora* species on *Plantago*. Interestingly, it was found that four independent species are parasitic to *Plantago major*, highlighting that the reliance on the host plant for pathogen determination can be misleading in *Peronospora*. Taking this into account, morphological and phylogenetic analyses should be conducted as a prerequisite for effective quarantine regulations and phytosanitary measures.

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INTRODUCTION

Peronospora is the most species-rich genus of the *Oomycota*, with more than 400 species described in the genus (Constantinescu 1991, Thines & Choi 2016). There are reports of downy mildew from various species of the genus *Plantago*, including some crops and medicinal plants (Farr & Rossmann 2023), but so far only six species of *Peronospora* on *Plantago* have been validly described: *Peronospora akatsukae* on *Plantago asiatica* from Japan (Ito & Murayama 1943), *Peronospora alta* on *Plantago major* from Europe, Asia, North America and South America (Fuckel 1870, De-Souza et al. 2008), *Peronospora canescens* on *Plantago canescens* from Russia (Jaczewski & Jaczewski 1931), *Peronospora gaponenkoae* on *Plantago lanceolata* from Germany (Davis et al. 2021), *Peronospora plantaginis* on *Plantago aristata* from the western USA (Underwood 1897), and *Peronospora kuewa* on *Plantago princeps* from Hawai'i (USA) (Davis et al. 2021). Of these species, *P. alta* and *P. plantaginis* have been reported from several unrelated hosts and throughout a broad geographic range (Mandal et al. 2010, Farr & Rossmann 2023). Considering that *Peronospora* species are generally highly host-specific (García-Blázquez et

al. 2008, Voglmayr et al. 2014a, Hoffmeister et al. 2020, Ploch et al. 2022), it seems warranted to review reports of *P. alta* and *P. plantaginis* from hosts other than the type hosts, as these might have been erroneously attributed to these species. This is due to the application of a broad species concept, like that of Yerkes & Shaw (1959), which has proven obsolete in studies of several species complexes (García-Blázquez et al. 2008, Choi et al. 2015a, Thines et al. 2019). As some members of *Plantago*, such as *Plantago ovata* and *Plantago lanceolata*, are commercially used, the species delimitation of *Peronospora* species parasitic on *Plantago* is also essential for appropriate phytosanitary and quarantine regulation. Therefore, it was the aim of this study to test the hypothesis that *Peronospora* on *Plantago* might be more species-rich than assumed so far, by detailed phylogenetic and morphological investigations.

MATERIALS AND METHODS

Plant and oomycete material

Dried specimens of *Peronospora* from various hosts in the genus *Plantago* were analysed in this study. Most specimens are deposited in the Fungarium Senckenbergianum at the Senckenberg Museum für Naturkunde in Görlitz (GLM) and Frankfurt am Main (FR). Information on the fungarium samples investigated is provided in Table 1. Where possible, observations were made from at least three specimens to capture the variability of the species (see Additional specimens examined in the Taxonomy section).

Morphology

Infected leaves were scrutinized for pathogen structures under a dissecting microscope (Discovery V8, Carl Zeiss, Göttingen,

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Table 1 GenBank accession numbers of the *cox2* mtDNA, ITS, and LSU sequences used for the phylogenetic study.

Species	Herb. ^a No.	Host	Year	Country	GenBank accession number				Reference		
					<i>cox2</i>	ITS	LSU	<i>trnL-F</i> (host)		ITS (host)	
<i>Peronospora a</i>	GLM-F62908	<i>Plantago uliginosa</i>	2004	Germany	ON130140	ON130168	ON130192	–	OR238546	This study	
	GLM-F69086	<i>Plantago uliginosa</i>	1995	Germany	ON230138	ON130166	ON130190	OR240093	–	OR238546	This study
	GLM-F91154	<i>Plantago major</i>	2008	Germany	ON230139	ON130167	ON130191	–	–	OR238545	This study
<i>Peronospora</i> aff. <i>akatsukae</i>	GLM-F73317	<i>Plantago major</i>	2002	Germany	ON230129	ON130157	ON130181	–	–	OR238569	This study
	GLM-F73347	<i>Plantago major</i>	2003	Germany	ON230130	ON130158	ON130182	–	–	OR238570	This study
	GLM-F73353	<i>Plantago major</i>	2003	Germany	ON230131	ON130159	ON130183	–	–	OR238571	This study
	GLM-F73358	<i>Plantago major</i>	2003	Germany	ON230132	ON130160	OR240106	–	–	OR238572	This study
	GLM-F74492	<i>Plantago major</i>	2003	Germany	ON230133	ON130161	ON130185	OR240107	–	OR238573	This study
	GLM-F74880	<i>Plantago major</i>	2003	Germany	ON230134	ON130162	ON130186	–	–	OR238574	This study
	KUS-F20033	<i>Plantago asiatica</i>	2003	Korea	–	OR400967	–	–	–	This study	
	KUS-F20057	<i>Plantago asiatica</i>	2003	Korea	–	OR400968	–	–	–	This study	
	KUS-F20241	<i>Plantago major</i>	2004	Korea	OR405309	OR400966	–	–	–	This study	
	FR39 (isotype)	<i>Plantago major</i>	1863	Germany	OR250010	OR238580	–	–	–	This study	
<i>Peronospora alta</i>	GLM-F86432	<i>Plantago major</i>	2006	Germany	KJ654234	ON130150	ON130196	–	OR238547	This study	
	WSP13842	<i>Plantago major</i>	1942	USA	OR250011	OR238581	–	–	OR238548	This study	
	GLM-F135033	<i>Plantago amplexicaulis</i>	2015	Spain	ON230135	ON130163	ON130187	OR240094	–	This study	
<i>Peronospora gaponenkoae</i>	GLM-F74725	<i>Plantago lanceolata</i>	2006	Germany	MT644993	MT644704	ON130173	OR240095	–	Davis et al. (2021), this study	
	GLM-F74749	<i>Plantago lanceolata</i>	2006	Germany	ON230123	ON130151	ON130175	–	OR238549	This study	
	GLM-F86433	<i>Plantago lanceolata</i>	2006	Germany	MT644994	MT644703	ON130174	OR240096	–	Davis et al. (2021), this study	
	GLM-F135029	<i>Plantago lanceolata</i>	2015	Germany	ON230124	ON130152	ON130176	–	OR238550	This study	
	GLM-F135030	<i>Plantago lanceolata</i>	2015	Germany	ON230125	ON130153	ON130177	–	OR238551	This study	
	GLM-F135031	<i>Plantago lagopus</i>	2016	Greece	ON230126	ON130154	ON130178	OR240097	–	This study	
	WSP25727	<i>Plantago patagonica</i>	1943	USA	OR250012	OR238582	–	–	OR238553	This study	
	WSP36618	<i>Plantago patagonica</i>	1942	USA	OR250013	OR238583	OR238576	–	OR238554	This study	
	GLM-F135028	<i>Plantago afra</i>	2015	Spain	ON230136	ON130164	ON130188	OR240099	OR238557	This study	
	BP1910918	<i>Plantago princeps</i>	2019	USA	MN068037	MN061374	MN061375	OR240100	–	Davis et al. (2021), this study	
<i>Peronospora kuewa</i> (= <i>P. gaponenkoae</i>)	GLM-F74548	<i>Plantago arenaria</i>	2004	Germany	ON230137	ON130165	ON130189	OR240101	OR238558	This study	
	GLM-F135032	<i>Plantago media</i>	2015	Germany	ON230141	ON130169	ON130193	OR240102	OR238559	This study	
<i>Peronospora lata</i>	RD1544	<i>Plantago myosuroides</i>	1999	Argentina	ON230143	ON130170	ON130195	–	OR238561	This study	
	RD1951	<i>Plantago major</i>	2004	Argentina	ON230142	ON130171	ON130194	–	OR238560	This study	
<i>Peronospora mahi</i>	WSP36575	<i>Plantago rhodosperma</i>	1952	USA	OR250016	OR238586	–	–	OR238562	This study	
	WSP13762	<i>Plantago aristata</i>	1966	USA	OR250017	OR238587	OR238577	–	OR238563	This study	
<i>Peronospora plantaginis</i>	WSP31671	<i>Plantago aristata</i>	1917	USA	OR250018	OR238588	–	–	OR238564	This study	
	WSP35628	<i>Plantago aristata</i>	1918	USA	–	OR238589	OR238578	–	OR238565	This study	
<i>Peronospora plantaginis</i> (= <i>P. psyllicola</i>)	–	<i>Plantago ovata</i> s.l.	–	India	–	KC153428	–	–	–	Mandal (2006)	
	GLM-F135034	<i>Plantago boissieri</i>	2015	Spain	ON230127	ON130155	ON130179	OR240103	OR238566	This study	
<i>Peronospora psyllicola</i>	GLM-F135035	<i>Plantago boissieri</i>	2015	Spain	ON230128	ON130156	ON130180	OR240104	OR238567	This study	
	RD1446	<i>Plantago pachyneura</i>	2000	Argentina	OQ680628	OQ676406	OQ676436	OR240105	OR238568	This study	
<i>Peronospora subglobosa</i>	WSP36626	<i>Plantago virginica</i>	1928	USA	OR250014	OR238584	–	OR240098	OR238555	This study	
	WSP36627	<i>Plantago virginica</i>	1951	USA	OR250015	OR238585	–	–	OR238556	This study	
<i>Peronospora wawawaiana</i>	WSP36704	<i>Plantago patagonica</i>	1894	USA	OR250019	OR238590	OR238579	–	OR238575	This study	
	GLM-F49006	<i>Cucumis</i> sp.	1997	Germany	MH730773	MH730807	MH730848	–	–	Thines et al. (2019)	

Bold is the newly generated sequences in this study.

^a Acronyms of herbarium collections: GLM, Senckenberg Gesellschaft für Naturforschung; Senckenberg Museum für Naturkunde Görlitz, Görlitz, Germany.

Table 2 Morphological comparison of species of *Peronospora* infecting species of *Plantago*.

Species	Host	Conidiophores (n = 50)		Trunks (n = 50)		Conidiophores/ trunks ratio	Branching orders
		Length (µm)	Base (µm)	Length (µm)	Width (µm)		
<i>Peronospora a</i>	<i>Plantago lagopus</i>	(244–272–420–568(–599))	6–10–13	(78–)141–291–441(–476)	(5.0–)5.2–7.4–9.5(–10.3)	(1.2–)1.3–1.6–1.8(–1.9)	4–6
<i>Peronospora</i> aff. <i>akatsukae</i>	<i>Plantago major</i>	(204–)216–389–562(–684)	12–15–17	(118–)171–336–501(–554)	(6.4–)8.5–10.3–12.1(–13.3)	(1.2–)1.3–1.6–1.8(–2.1)	5–7
<i>Peronospora alta</i>	<i>Plantago major</i>	(194–)221–400–579(–587)	10–13–16	(142–)150–292–433(–455)	(6.6–)7.0–8.7–10.3(–10.5)	(1.2–)1.3–1.7–2.1(–2.2)	6–8
<i>Peronospora bicolor</i>	<i>Plantago amplexicaulis</i>	(250–)290–409–528(–538)	9–10–11	(137–)150–278–406(–422)	(5.2–)5.6–7.9–10.2(–10.3)	(1.2–)1.3–1.6–1.9(–2.0)	4–6
<i>Peronospora gaponenkoae</i>	<i>Plantago lanceolata</i>	(309–)349–600–851(–881)	9–13–17	(213–)233–419–604(–614)	(7.5–)7.6–8.5–9.4(–10.3)	(1.3–)1.4–1.5–1.6(–1.7)	6–7
<i>Peronospora greenii</i>	<i>Plantago patagonica</i>	(278–)295–425–555(–635)	10–13–16	(127–)152–282–412(–503)	(5.6–)6.7–9.5–12.2(–13.9)	(1.2–)1.3–1.6–1.9(–2.2)	3–7
<i>Peronospora krungthepopsis</i>	<i>Plantago afra</i>	(212–)251–420–588(–666)	9–10–11	(147–)155–304–452(–510)	(6.3–)6.6–8.2–9.8(–10.0)	(1.3–)1.4–1.6–1.7(–1.8)	4–7
<i>Peronospora lacrimioidea</i>	<i>Plantago arenaria</i>	(307–)323–403–483(–494)	10–12–14	(159–)164–256–348(–381)	(8.1–)8.4–10.4–12.4(–13.3)	(1.5–)1.6–1.8–1.9(–2.0)	4–6
<i>Peronospora lata</i>	<i>Plantago media</i>	(229–)262–336–410(–411)	10–12–14	(119–)141–182–222(–230)	(7.5–)7.7–9.0–10.2(–11.1)	(1.5–)1.6–1.8–1.9(–2.0)	4–6
<i>Peronospora mahi</i>	<i>Plantago rhodosperma</i>	(204–)272–388–504(–639)	9–11–13	(112–)115–250–344(–435)	(5.6–)6.5–7.7–8.9(–10.2)	(1.3–)1.4–1.6–1.9(–2.5)	4–7
<i>Peronospora plantaginis</i> (isotype)	<i>Plantago aristata</i>	(176–)227–363–498(–514)	9–11–13	(89–)133–238–343(–347)	(7.0–)7.6–8.8–9.9(–10.6)	(1.2–)1.3–1.6–1.9(–2.1)	5–6
<i>Peronospora psylliicola</i>	<i>Plantago boissieri</i>	(251–)319–471–623(–681)	7–10–13	(147–)180–308–436(–518)	(5.9–)7.7–9.8–11.9(–12.4)	(1.3–)1.4–1.6–1.8(–2.0)	4–6
<i>Peronospora subglobosa</i>	<i>Plantago virginica</i>	(234–)273–366–458(–547)	8–11–14	(143–)145–217–288(–370)	(5.3–)6.0–8.4–10.7(–13.2)	(1.4–)1.5–1.7–2.0(–2.1)	4–7
<i>Peronospora wawawaiana</i>	<i>Plantago patagonica</i>	(198–)260–334–408(–502)	11–13–14	(126–)148–193–238(–297)	(5.4–)7.6–9.8–12.0(–13.6)	(1.1–)1.4–1.8–2.2(–2.7)	4–6
Ultimate Branchlets (UB) (n = 100)							
		Length of the longer UB (µm)		Length of the shorter UB (µm)		Longer/shorter ratio	
<i>Peronospora a</i>		(7.14–)7.50–17.58–27.66(–27.86)	(4.62–)5.92–9.92–13.91(–14.27)	(1.03–)1.17–1.88–2.59(–2.69)			
<i>Peronospora</i> aff. <i>akatsukae</i>		(4.01–)6.15–12.93–19.71(–20.03)	(3.06–)3.46–7.40–11.34(–11.70)	(1.31–)1.89–2.37–2.85(–2.88)			
<i>Peronospora alta</i>		(8.83–)9.81–13.06–16.30(–17.49)	(5.83–)6.09–8.77–11.45(–12.45)	(1.38–)1.44–1.59–1.73(–1.88)			
<i>Peronospora bicolor</i>		(5.54–)5.69–12.38–19.07(–20.14)	(3.76–)3.78–7.95–12.12(–13.31)	(1.19–)1.22–1.56–1.90(–2.25)			
<i>Peronospora gaponenkoae</i>		(9.24–)10.20–12.90–15.59(–17.29)	(5.01–)5.18–7.89–10.59(–11.59)	(1.36–)1.47–1.72–1.97(–2.04)			
<i>Peronospora greenii</i>		(9.78–)11.39–13.94–16.48(–21.43)	(6.71–)8.21–10.25–12.28(–15.49)	(1.10–)1.23–1.37–1.51(–1.82)			
<i>Peronospora krungthepopsis</i>		(4.36–)4.39–11.93–19.46(–21.73)	(2.31–)3.76–6.53–9.29(–10.31)	(1.03–)1.06–1.91–2.76(–2.83)			
<i>Peronospora lacrimioidea</i>		(6.12–)6.38–13.40–20.42(–21.32)	(3.67–)3.86–9.37–14.88(–15.28)	(1.14–)1.21–1.65–2.09(–2.70)			
<i>Peronospora lata</i>		(11.53–)11.55–18.38–25.20(–26.66)	(5.38–)6.75–10.88–15.01(–15.81)	(1.11–)1.12–1.95–2.77(–2.82)			
<i>Peronospora mahi</i>		(9.07–)11.09–13.86–16.62(–19.35)	(5.62–)7.60–8.92–10.23(–10.93)	(1.07–)1.34–1.56–1.77(–1.95)			
<i>Peronospora plantaginis</i> (isotype)		(6.03–)8.29–11.84–15.38(–17.15)	(3.34–)5.39–7.88–10.38(–11.23)	(1.22–)1.31–1.53–1.75(–2.20)			
<i>Peronospora psylliicola</i>		(4.78–)6.90–12.51–18.13(–21.05)	(3.34–)4.06–7.11–10.15(–13.49)	(1.13–)1.35–1.76–2.18(–2.55)			
<i>Peronospora subglobosa</i>		(9.73–)12.11–14.11–16.12(–17.90)	(7.11–)8.31–10.46–12.62(–14.64)	(1.06–)1.18–1.38–1.58(–1.70)			
<i>Peronospora wawawaiana</i>		(6.91–)9.73–12.93–16.13(–21.05)	(4.77–)7.17–9.54–11.92(–11.83)	(1.01–)1.15–1.37–1.59(–2.02)			
Conidia (n = 100)							
		Length (µm)		Length/width		Oospores (µm)	
<i>Peronospora a</i>		(21.21–)21.56–26.17–30.77(–31.01)	(15.16–)15.35–18.81–22.27(–23.57)	(1.07–)1.14–1.47–1.80(–1.94)	–	–	–
<i>Peronospora</i> aff. <i>akatsukae</i>		(24.01–)24.24–32.18–40.11(–40.38)	(15.15–)16.01–18.55–21.08(–22.50)	(1.19–)1.22–1.83–2.44(–2.50)	25–31–37	–	–
<i>Peronospora alta</i>		(25.15–)25.86–28.13–30.39(–31.98)	(19.10–)19.23–20.74–22.25(–22.51)	(1.15–)1.16–1.41–1.66(–1.82)	–	–	–
<i>Peronospora bicolor</i>		(29.87–)32.72–37.97–43.22(–43.56)	(15.51–)17.16–20.51–23.86(–25.46)	(1.58–)1.59–1.89–2.19(–2.32)	–	–	–
<i>Peronospora gaponenkoae</i>		(16.17–)18.74–21.02–23.30(–24.53)	(15.20–)15.75–17.32–18.88(–20.40)	(1.03–)1.14–1.24–1.34(–1.35)	–	–	–
<i>Peronospora greenii</i>		(31.54–)33.12–35.25–37.38(–39.80)	(20.36–)21.23–23.17–25.10(–26.16)	(1.34–)1.42–1.53–1.63(–1.69)	–	–	–
<i>Peronospora krungthepopsis</i>		(31.19–)35.20–38.54–41.87(–43.78)	(14.06–)15.40–17.87–20.35(–23.74)	(1.70–)1.93–2.18–2.43(–2.74)	–	–	–
<i>Peronospora lacrimioidea</i>		(31.38–)33.54–37.92–42.30(–45.25)	(20.32–)21.12–22.05–22.97(–23.86)	(1.37–)1.51–1.72–1.94(–2.12)	–	–	–
<i>Peronospora lata</i>		(23.73–)24.02–27.97–31.91(–33.69)	(18.17–)19.30–22.67–26.03(–26.59)	(1.11–)1.12–1.30–1.47(–1.51)	–	–	–
<i>Peronospora mahi</i>		(21.59–)22.08–23.64–25.19(–27.95)	(15.44–)17.92–19.84–21.77(–23.60)	(1.04–)1.09–1.20–1.31(–1.46)	29–34–38	–	–
<i>Peronospora plantaginis</i> (isotype)		(35.24–)38.87–41.50–44.14(–45.48)	(19.00–)21.31–23.09–24.87(–26.42)	(1.52–)1.62–1.81–1.99(–2.24)	21–33–45	–	–
<i>Peronospora psylliicola</i>		(20.30–)26.88–31.36–35.84(–41.12)	(17.88–)19.53–21.18–22.84(–24.35)	(1.14–)1.33–1.48–1.62(–1.75)	–	–	–
<i>Peronospora subglobosa</i>		(22.90–)24.32–26.79–29.25(–31.98)	(20.45–)20.57–22.16–23.75(–25.67)	(1.07–)1.10–1.21–1.32(–1.44)	–	–	–
<i>Peronospora wawawaiana</i>		(27.49–)29.63–32.78–35.93(–39.40)	(16.44–)18.57–20.65–22.74(–27.16)	(1.27–)1.41–1.60–1.79(–2.14)	26–31–36	–	–

Germany). Pathogen structures were mounted onto glass slides in drops of 70 % aqueous lactic acid solution, briefly heated using an alcohol lamp, and covered with a coverslip. Afterwards, the morphological characteristics were recorded using a compound light microscope (Imager M2, Carl Zeiss Göttingen, Germany) equipped with DIC, and photographs were taken at 100× or 200× for conidiophores, and 400× or 1000× for conidia and ultimate branchlets using a Zeiss Axiocam MRc5 camera (Carl Zeiss, Göttingen, Germany). Measurements were done in Axiovision Rel. v. 4.8 (Zeiss, Oberkochen, Germany) on the pictures taken. The breadth of the conidiophores was measured at the middle between the base and the first ramification. Measurements are reported as (minimum–)mean minus standard deviation–mean plus standard deviation(–maximum) and shown in Table 2. In the taxonomic descriptions, the typical ranges (the range of the standard deviation, rounded to the nearest 0.5 μm) are given. So all ranges there should be read in a way that most measurements would fall within that range.

DNA extraction, PCR, and sequencing

For each specimen about 1 mm² of infected plant tissue was excised using a sterile razor blade, transferred to a 2 mL reaction tube with three iron beads of each 3 mm and 1 mm diam per sample and disrupted using a mixer mill (MM2, Retsch, Germany) by shaking the tubes twice at 50 Hz for 90 s. Genomic DNA was extracted using the innuPREP plant DNA kit (Analyticjena, Germany) according to the instructions of the manufacturer. PCR amplification was performed with the primers ITS1-O (Bachofer 2004) and LR-0 (Moncalvo et al. 1995) for the internal transcribed spacer (ITS) regions. The D1-3 region of the 28S nuclear large ribosomal subunit (LSU) was amplified with the primers LR0R (Stamatakis 2006) and LR6-O (Kumar et al. 2016). The mitochondrial cytochrome oxidase subunit 2 (*cox2*) gene was amplified with the primers *cox2*-F (Hudspeth et al. 2000) and *cox2*-RC4 (Choi et al. 2015b). For the host, PCR amplification was performed with the primers *trnL*-c and *trnF*-F (Taberlet et al. 1991) for the *trnL*-F regions, and the primers ITS1 and ITS4 (White et al. 1990) for the ITS regions. PCR conditions for ITS and *cox2* amplifications were as outlined in Choi et al. (2015a), PCR conditions for LSU amplifications

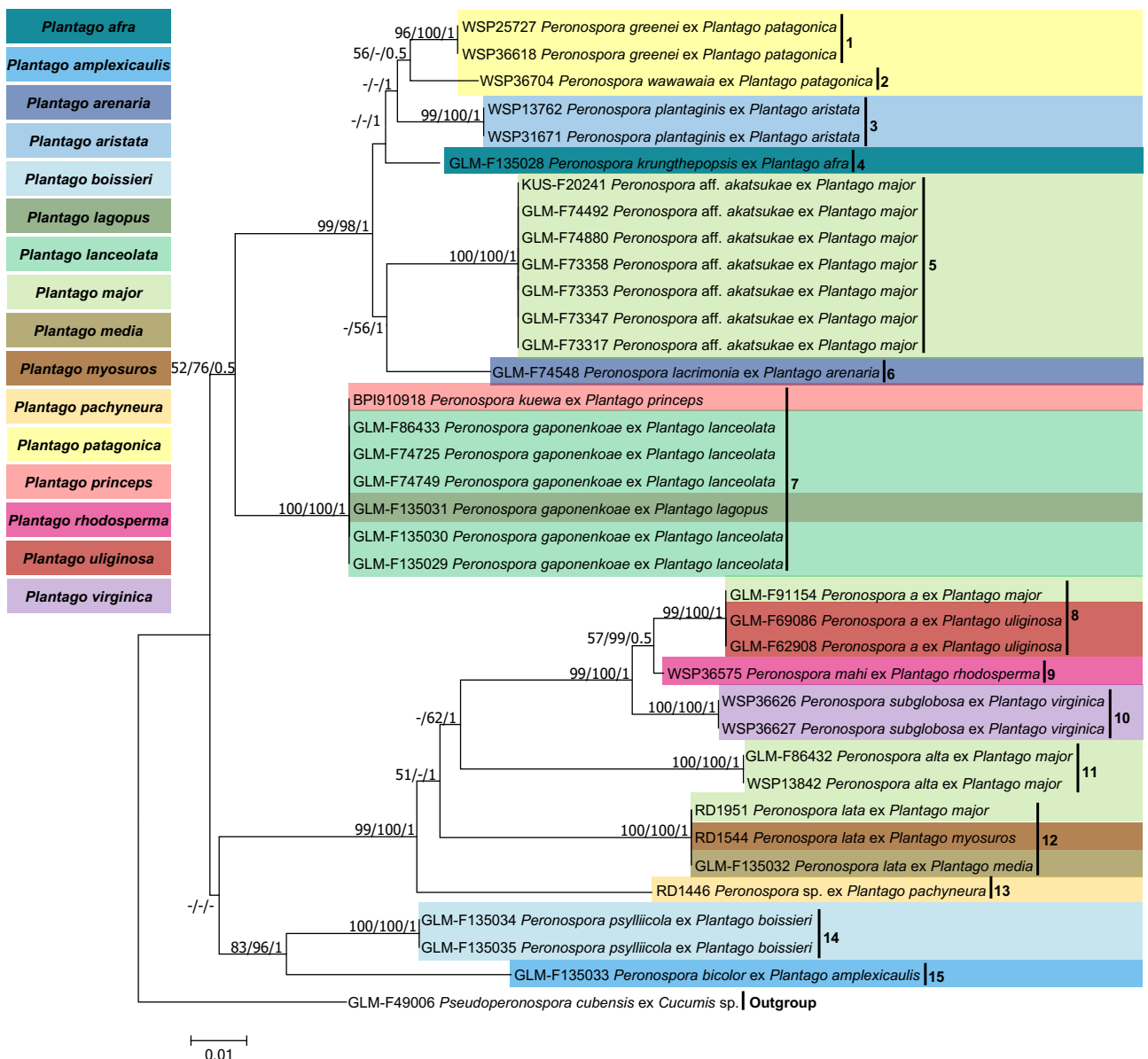


Fig. 1 Maximum likelihood phylogenetic inference for *Peronospora* species on *Plantago* based on the *cox2* mitochondrial DNA sequences with *Pseudoperonospora cubensis* as outgroup, bootstrap support values of Maximum Likelihood and Minimum Evolution methods (BS ≥ 50 %) and Bayesian posterior probabilities (PP ≥ 0.50) are indicated along branches. A dash denotes lower support for the presented topology or an alternate topology. The scale bar denotes the number of nucleotide substitutions per site.

were as outlined in Davis et al. (2021), PCR conditions for *trnL-F* and ITS amplifications were as outlined in Ali et al. (2016). However, for the amplifications, MangoTaq (Bioline GmbH, Luckenwalde, Germany) was used according to the instructions of the manufacturer. Amplicons were bidirectionally sequenced at the Biodiversity and Climate Research Centre (BiK-F, Frankfurt, Germany) laboratory using primers identical to those used for amplifications.

Phylogenetic analyses

The resulting sequences were edited and assembled using Geneious (v. 5.6, Biomatters, Auckland, New Zealand). An alignment of each locus was performed using MAFFT v. 7 (Kato & Standley 2013) employing the Q-INS-i algorithm (Kato & Toh 2008). The alignment is available at TreeBASE (Accession 30250 for ITS, 30252 for nrLSU, 30253 for *cox2*).

Three different tree construction methods were used, namely Minimum Evolution (ME), Maximum Likelihood (ML), and Bayesian Inference (BI). Minimum Evolution analysis was done using MEGA7 (Kumar et al. 2016), with the default settings of the program except using the Tamura-Nei model and pairwise deletion; support for internal nodes was estimated by 1000 bootstrap replicates (Felsenstein 1985). For ML analyses, 1000 rounds of random addition of sequences as well as 1000 fast bootstrap replicates were performed using RAXML v. 8.2.7 (Stamatakis 2014) applying the GTRGAMMA substitution default model. For BI analyses, the Akaike Information Criterion (AIC) was used to determine the best fitting model of sequence evolution as implemented in MrModeltest v. 2.3 (Nylander 2004) and computation was done using MrBayes v. 3.1.6 (Ronquist et al. 2012), applying GTR+G as substitution model both for ITS and nrLSU with six gamma categories, and HKY+I+G as

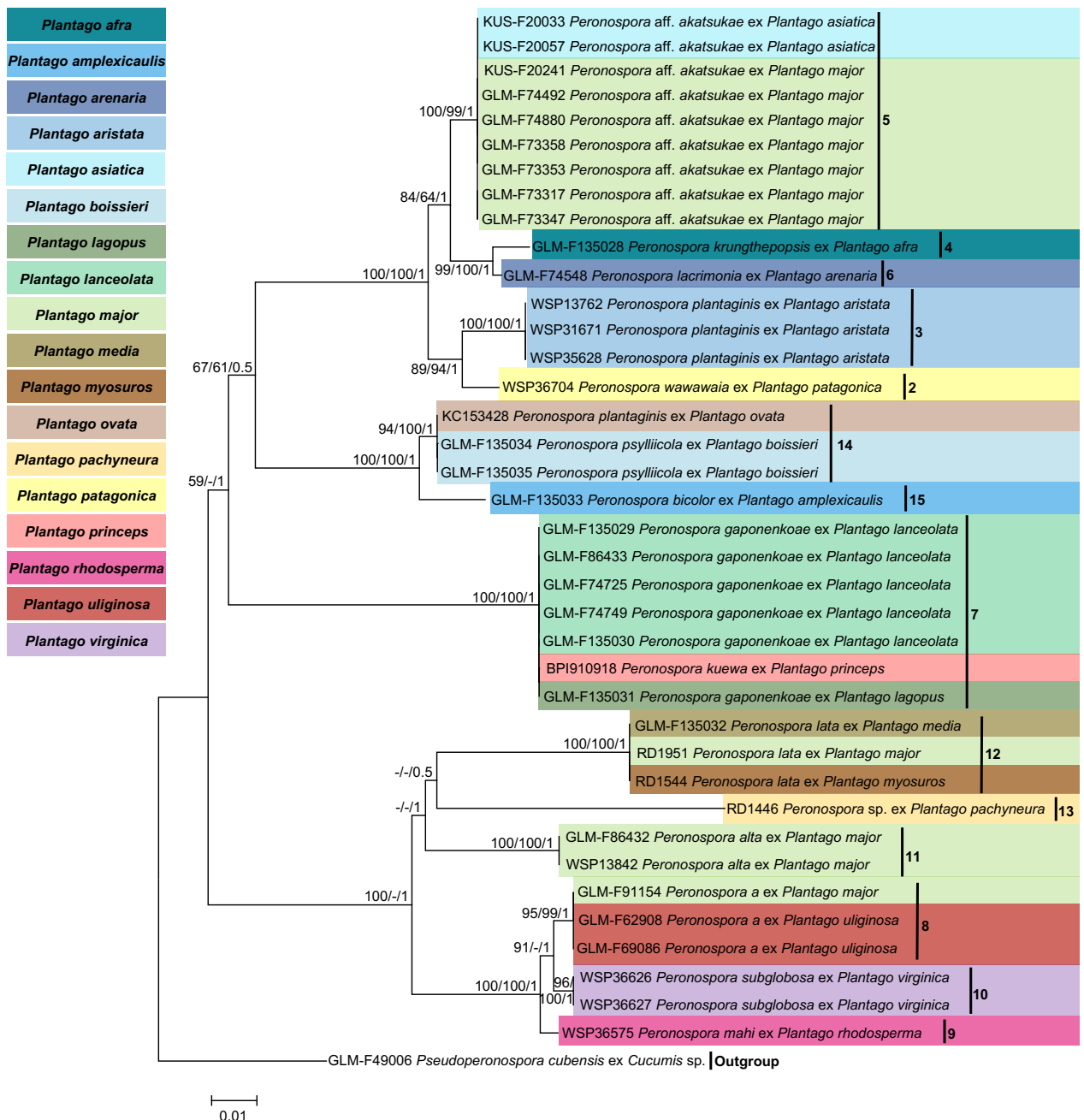


Fig. 2 Maximum likelihood phylogenetic reconstruction for *Peronospora* species on *Plantago* based on the internal transcribed spacer (ITS) sequences with *Pseudoperonospora cubensis* as outgroup, bootstrap support values of Maximum Likelihood and Minimum Evolution methods (BS $\geq 50\%$) and Bayesian posterior probabilities (PP ≥ 0.50) are indicated along branches. A dash denotes lower support for the presented topology or an alternate topology. The scale bar denotes the number of nucleotide substitutions per site.

the substitution model for *cox2* with two invgamma categories. Analyses were run for 5 million generations, with every 10 000th tree sampled and discarding the first 30 % of the trees before calculating posterior probabilities, to ensure sampling from the stationary phase. *Pseudoperonospora cubensis* was used as an outgroup in all analyses.

RESULTS

Morphology

Measurements of morphological characteristics of *Peronospora* species on *Plantago* from 14 different species are summarized in Table 2. The main morphological characters differentiating *Peronospora* were the shape of conidia, the ratio of the length-and-breadth of conidia, the length of conidiophores, and the length of the ultimate branchlets. Regarding the 10 new species found in this study, *Peronospora* sp. on *Plantago amplexicaulis* has a unique combination of submonopodial branching, large conidia (38 μm long on average) with a length-to-breadth ratio of 1.89 ± 0.3 (Fig. 7). *Peronospora* sp. on *Plantago afra* has the most elongate conidia, length-to-breadth ratio of 2.18 ± 0.2 (Fig. 10). *Peronospora* sp. on *Plantago major*, *P. media*, and *P. myosuroides* has the longest ultimate branchlets (the longer ones 18 μm long on average, the shorter ones 11 μm long on average) and shortest trunks (av. 182 μm long) (Fig. 12). *Peronospora* sp. on *Plantago major* and *Plantago uliginosa* has the narrowest trunks (av. 7 μm broad) and paired or three ultimate branchlets (Fig. 4). *Peronospora* sp. on *Plantago arenaria* has the broadest trunks (av. 10 μm broad) (Fig. 11). There are two species parasitic on *Plantago patagonica*:

Peronospora sp. collected from Montana and Wisconsin has the broadest conidia (av. 23 μm) (Fig. 9) and *Peronospora* sp. collected from Washington has the shortest conidiophores (av. 334 μm) and the highest conidiophores/trunk ratio (up to 2.7, Fig. 17). *Peronospora* sp. on *Plantago rhodosperma* and *Peronospora* sp. on *Plantago virginica* have the most globose conidia, with a length-to-breadth ratio of 1.2 ± 0.1 on average (Fig. 13, 16). *Peronospora* sp. on *Plantago boissieri* and *Plantago ovata* has ellipsoid to ovoid conidia with a dirty grey to tainted red colour (Fig. 15). *Peronospora* aff. *akatsukae* on *Plantago major* and *Plantago asiatica* has the broadest conidiophore bases (av. 15 μm broad) (Fig. 5). Of the five known species, *P. alta* and *P. akatsukae* are characterised by subdichotomous branching in up to eight orders (Ito & Murayama 1943), but *P. alta* has shorter ultimate branchlets (av. 13 μm) (Fig. 6). *Peronospora canescens* has comparatively broad conidia (up to 26 μm ; Jaczewski & Jaczewski 1931). *Peronospora gaponenkoae* is the most easily distinguished species, as it has the longest conidiophores (av. 600 μm long), trunks (av. 419 μm long), as well as the second-smallest conidia ($21 \times 17 \mu\text{m}$, Fig. 8). *Peronospora plantaginis* has the largest conidia ($42 \times 23 \mu\text{m}$ on average, Fig. 14). Further details for the morphological comparison between closely related species are given in the taxonomy section and in Table 2.

Phylogenetic analyses

In the course of this study we obtained 33 *cox2* sequences, 37 ITS sequences, and 29 LSU sequences for *Peronospora* on *Plantago*, which includes ITS and *cox2* sequence data from the type specimen of *P. alta* collected by Fuckel more than 150 years ago (Fuckel 1870). Additional sequences from GenBank

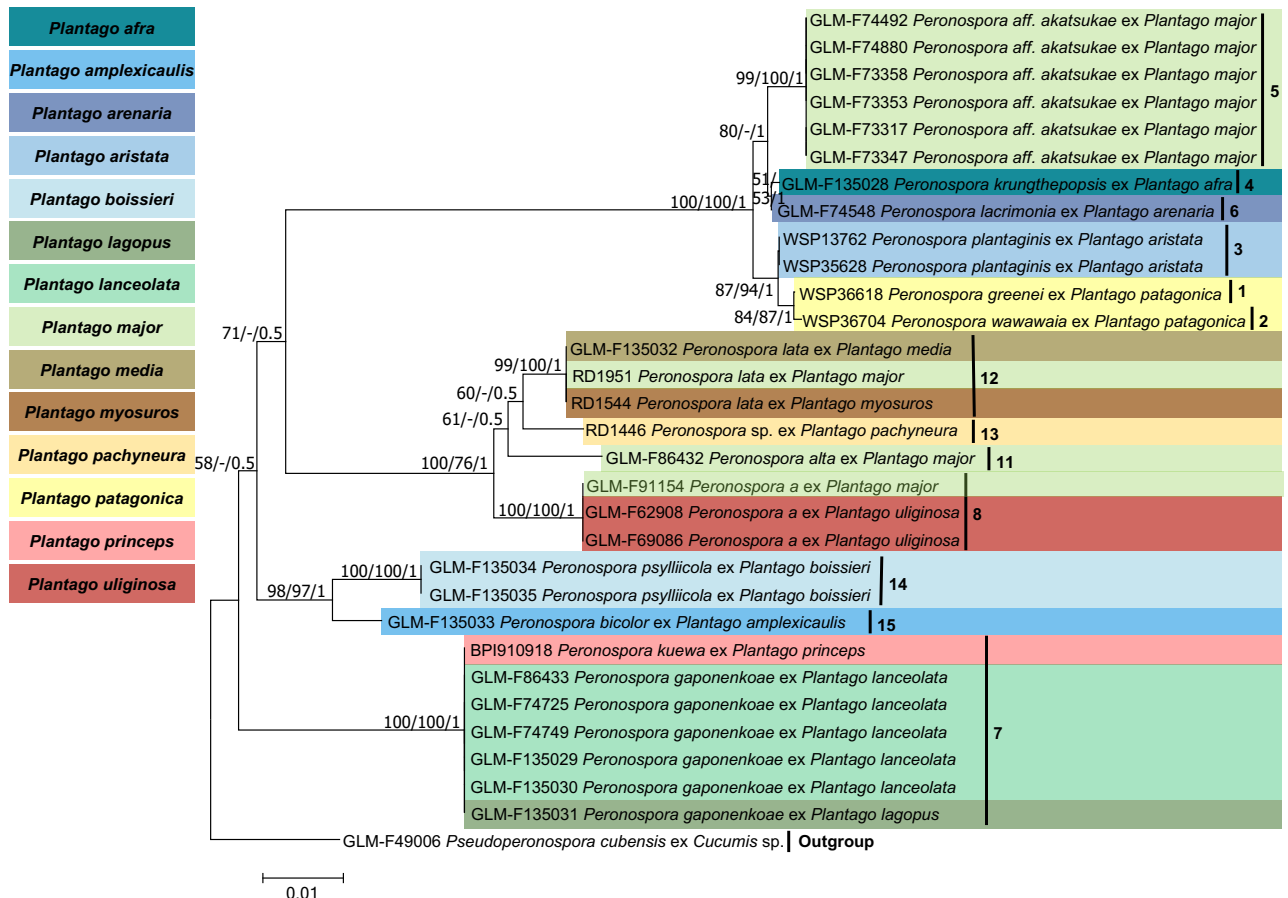


Fig. 3 Maximum likelihood phylogenetic reconstruction for *Peronospora* species on *Plantago* based on the nLSU sequences with *Pseudoperonospora cubensis* as outgroup, bootstrap support values of Maximum Likelihood and Minimum Evolution methods (BS $\geq 50\%$) and Bayesian posterior probabilities (PP ≥ 0.50) are indicated along branches. A dash denotes lower support for the presented topology or an alternate topology. The scale bar denotes the number of nucleotide substitutions per site.

(<https://www.ncbi.nlm.nih.gov/genbank/>) were added to form a complete dataset with all loci included for 42 specimens. The alignments contained 625, 963, and 346 aligned sites, respectively. We also obtained 15 *trnL-F* sequences and 31 ITS sequences for confirming the identity of the host plants. For the ITS phylogenetic analysis, the shorter ITS sequences of the type specimen of *P. alta* (*Fungi Rhenani Exsiccati* 39) and *P. greenei* (a new species described in this study) were not included to avoid the interference of leading and trailing gaps with distance calculations. However, in the 308 nucleotides that could be obtained for the type of *P. alta*, the sequence was identical with sequences from more recent collections of the species, but different from all other species investigated. Similarly, the *cox2* sequence of FR39 was also short, as it had to be obtained by semi-nested PCR, resulting in a sequence of 284 nucleotides for the *cox2* gene that separated *P. alta* from all other species of the study by at least 18 SNPs but was identical in sequence as compared to more recent collections of the species. The two sequences from the type specimen were deposited in GenBank (OR250010 for *cox2*, OR238580 for ITS). The tree topologies of the reconstructions based on single loci did not show conflicts at nodes highly supported in all analyses, and the phylogenetic reconstructions of ITS, nrLSU, and *cox2* using ME, ML, and BI on this dataset were almost identical, and only minor differences in support were observed. The cor-

responding phylogenetic trees for the different loci, ITS, nrLSU, and *cox2* are presented in Fig. 1–3, respectively. In total, 15 phylogenetic lineages of *Peronospora* on *Plantago* were included. These were mostly parasitic to a single host species. *Peronospora* parasitic to *Plantago major* was found in four different clades, namely lineages 5, 8, 11, and 12. Similarly, there were two clearly separated sister lineages (1 and 2), which both parasitise *Plantago patagonica*. Most clades were resolved as monophyletic with maximum support. Only a few higher-level relationships could be resolved. However, a monophyletic group consisting of lineages 1–6 was inferred, which is characterised by having elongated conidia with a length-to-breadth ratio higher than 1.5. Within this clade, lineages 4–6 formed a sub-clade in ITS and LSU, characterised by a length-to-breadth ratio of the conidia higher than 1.7. Similarly, lineages 8–12 formed a monophyletic clade, which members are characterised by subglobose to broadly oval conidia with a length-to-breadth ratio lower than 1.5. Lineages 14–15 were forming a monophyletic clade of species characterised by large conidia (higher than $37 \times 20 \mu\text{m}$ on average). The recently described species, *P. kuewa*, differed in one gap from *P. gaponenkoae* in the ITS sequence, but was otherwise identical in all nucleotide sequences investigated in this study. Consequently, no support for the recognition of *P. kuewa* as a species independent from *P. gaponenkoae* was obtained.



Fig. 4 Symptoms and morphology of *Peronospora* a parasitic on *Plantago uliginosa*. a. Down on the leaf surface; b–d. conidia; e–g. ultimate branchlets; h–j. conidiophores. — Scale bars: b–g = 20 μm ; h–j = 50 μm .

TAXONOMY

Based on phylogenetic relationships and morphological differences, 10 new species of *Peronospora* on *Plantago* are described here, and one species is relegated to synonymy with *Peronospora japonenkoae*. In addition, details are given for other known species of *Peronospora* parasitic to the genus *Plantago*.

Peronospora a M. Mu & Thines, *sp. nov.* — MycoBank MB 849681; Fig. 4

Etymology. A from Latin 'a' an astonished interjection, referring to the astonishment that so many species of *Peronospora* are parasitic to species of the *Plantago major* species complex.

Typus. GERMANY, Saxony, Elbe-Elster lowland, Döbrichau northwest, a path to Kleinesee, disturbed grassland, on living leaves of *Plantago uliginosa*, 24 July 1995, H. Jage (GLM-F69086, holotype), GenBank: *cox2* = ON230138; ITS = ON130166; LSU = ON130190.

Diagnosis — Differs from *P. mahi* and *P. subglobosa* in having more elongated conidia, as well as paired or three, and longer ultimate branchlets.

Lesions on leaves yellow-brown and slightly reddish, diffuse to vein-delimited. *Down* present on the lower leaf surface, purplish or beige, consisting of scattered to dense felt-like conidiophore outgrowth. *Conidiophores* hyaline, straight, thin-walled, 272–568 µm long, av. 420 µm; trunk mostly 141–441 µm long, av. 291 µm, 5–9.5 µm broad, sometimes slightly swollen to up to 13 µm at the base, ratio of the total length to trunk length 1.3–1.8, callose plugs absent. *Branching* subdichotomous in 4–6 orders, with almost straight to curved branches, gradually attenuate, often at acute angles. *Ultimate branchlets* curved, rarely straight, mostly S-shaped, paired or sometimes three branchlets differing in length; with the longer ones 7.5–27.5 µm long, the shorter ones 6–14 µm long, and with a ratio of the longer to the shorter ultimate branchlet of 1.2–2.6, base 2–3.5 µm broad, apex obtuse. *Conidia* spherical to obovate with an orange-brown to pink-brown colour, 21.5–31 µm long, 15.5–22.5 µm broad, length-to-breadth ratio 1.1–1.8. *Oospores* not observed.

Habitat — Living leaves of *Plantago major* and *Plantago uliginosa* (*Plantaginaceae*).

Known distribution — Germany.

Additional specimens examined. GERMANY, Saxony, Lausitzer Grenzwall, Weißwasser, Waldhausstraße, roadside of the restaurant 'Waldhaus am Braunsteich', on living leaves of *Plantago major*, 12 Sept. 2008, H. Jage, GLM-F91154, GenBank: *cox2* = ON230139; ITS = ON130167; LSU = ON130191; Saxony-Anhalt, White Elster Valley, Maßlau West, a meadow on living leaves of *Plantago uliginosa*, 8 Aug. 2004, H. Jage, GLM-F62908, GenBank: *cox2* = ON230140; ITS = ON130168; LSU = ON130192.

Notes — *Plantago uliginosa* has often been seen as con-specific with *Plantago major*, and placed in the subspecies *P. major* subsp. *intermedia*, until morphological and genetic investigations have shown that the species can be clearly differentiated based on variable markers, leaf morphology, and the number of seeds per capsule (Morgan-Richards & Wolff 1999). In addition, the ecological preferences of the two species differ. *Peronospora a* is related to *P. subglobosa* and *P. mahi*, forming a clade in both the *cox2* and ITS phylogenetic trees. These species share similar characteristics, including the presence of round conidia, but *P. a* has narrower conidia (av. 19 µm broad), paired or three and longer ultimate branchlets (av. 18 µm long) compared to *P. mahi* and to *P. subglobosa*.

Peronospora aff. akatsukae — Fig. 5

Representative specimen. GERMANY, Saxony-Anhalt, Fürstenmoor, Kolonnenweg, on living leaves of *Plantago major*, 21 Sept. 2003, H. Jage, GLM-F74492, GenBank: *cox2* = ON230133; ITS = ON130161; LSU = ON130185.

Lesions on leaves first yellow to brown, later dark brown, diffuse to vein-delimited. *Down* present on the lower leaf surface, consisting of scattered to dense felt-like conidiophore outgrowth. *Conidiophores* hyaline, straight, thin-walled, 216–562 µm long, av. 389 µm; trunk mostly 171–501 µm long, av. 336 µm, 8.5–12 µm broad, sometimes slightly swollen to up to 17 µm at the base, ratio of the total length to trunk length 1.3–1.8, callose plugs absent. *Branching* subdichotomous in 5–7 orders, with sub-straight to curved branches, gradually attenuate, often at acute angles. *Ultimate branchlets* almost straight to curved, mostly paired or sometimes three branchlets differing in length, with the longer ones 6–19.5 µm long, the shorter ones 3.5–11.5 µm long, and with a ratio of the longer to the shorter ultimate branchlet of 1.9–2.9, base 2–3 µm broad, apex obtuse. *Conidia* ellipsoid to elongate with a dirty grey to tawny colour, 24–40 µm long, 16–21 µm broad, length-to-breadth ratio 1.2–2.4, basal part of the conidia mostly protruding. *Oospores* 25–37 µm.

Habitat — *Plantago asiatica*, *Plantago major*.

Known distribution — Germany, South Korea.

Additional specimens examined. GERMANY, Saxony, Dresden Elbe Valley Widening, Coswig (Saxony)-Kötitz, Gauernitz Elbe Island, bank mud, on living leaves of *Plantago major*, 17 Aug. 2003, H. Jage, GLM-F73358, GenBank: *cox2* = ON230132; ITS = ON130160; LSU = ON130184; Saxonian Suisse, Königstein N, opposite Königstein, Elblache ('Hafen') near OT Halbestadt on living leaves of *Plantago major*, 15 Aug. 2003, GLM-F73347, GenBank: *cox2* = ON230130; ITS = ON130158; LSU = ON130182; *ibid.*, GLM-F73353, GenBank: *cox2* = ON230131; ITS = ON130159; LSU = ON130183; Saxony-Anhalt, Helme-Unstrut Buntsandsteinland, Dorndorf, exit to Gleina, roadside on living leaves of *Plantago major*, 1 June 2003, H. Jage, GLM-F74880, GenBank: *cox2* = ON230134; ITS = ON130162; LSU = ON130186; Western Altmarkplatten, Kraatz N, wasteland on living leaves of *Plantago major*, 7 Aug. 2002, H. Jage, GLM-F73317, GenBank: *cox2* = ON230129; ITS = ON130157; LSU = ON130181.

Additional specimens sequenced. SOUTH KOREA, Jeju, Jeju National University, on living leaves of *Plantago asiatica*, 12 Nov. 2003, Y. Choi, KUS-F20057, GenBank: ITS = OR400968; Yangpyeong, practice forest on living leaves of *Plantago major*, 26 May 2004, Y. Choi, KUS-F20241, GenBank: *cox2* = OR405309; ITS = OR400966; *ibid.*, on living leaves of *Plantago asiatica*, 4 Nov. 2003, Y. Choi, KUS-F20033, GenBank: ITS = OR400967.

Notes — *Peronospora aff. akatsukae*, like *P. a*, *P. alta*, and *P. lata*, can infect *Plantago major*, but it is more closely related to *P. lacrimoidea* and *P. krungthepopsis*, not only in terms of phylogenetic relationships but also in terms of the morphology, as *P. aff. akatsukae*, *P. lacrimoidea*, and *P. krungthepopsis* all have elongated conidia with a length-to-breadth ratio higher than 1.7. Two specimens of *P. aff. akatsukae* were found parasitizing *Plantago asiatica* from Korea. Considering that *Peronospora* species are highly host-specific and the lack of detailed measurement data for the type specimen of *P. akatsukae* on *Plantago asiatica* from Japan (Ito & Murayama 1943), this species is temporarily referred to as *P. aff. akatsukae*.

Peronospora alta Fuckel, Fungi Rhenani Exsiccati Fasc. 1: 39. 1863 — Fig. 6

Typus. GERMANY, Hesse, Eltville am Rhein, Hattenheim, on living leaves of *Plantago major*, 1863, Fuckel, Fungi Rhenani Exsiccati 39, specimen deposited in the herbarium WIES (Lectotype designated here, MycoBank typification number MBT 10013906), GenBank: *cox2* = OR250010; ITS = OR238580.

Lesions on leaves and petioles first yellow spots, later yellow-brown, diffuse to vein-delimited. *Down* present on the lower leaf surface, consisting of scattered to dense felt-like conidiophore outgrowth. *Conidiophores* hyaline, straight, thin-walled,

221–579 μm long, av. 400 μm ; trunk mostly 150–433 μm long, av. 292 μm , 7–10.5 μm broad, sometimes slightly swollen to up to 16 μm at the base, ratio of the total length to trunk length 1.3–2.1, callose plugs absent. *Branching* subdichotomous in 6–8 orders, with sub-straight to curved branches, gradually attenuate, often at acute angles. *Ultimate branchlets* almost straight to curved, rarely straight, paired branchlets differing in length, with the longer ones 9.8–16.3 μm long, the shorter ones 6.1–11.5 μm long, with a ratio of the longer to the shorter ultimate branchlet of 1.4–1.7, base 2.5–3.0 μm broad, apex slightly sharp. *Conidia* subglobose to obovate with a pale brown to violet colour, 25.9–30.4 μm long, 19.2–22.2 μm broad, length-to-breadth ratio 1.2–1.7. *Oospores* not observed.

Habitat — *Plantago major*.

Known distribution — Europe, USA.

Additional specimens examined. GERMANY, Saxony, Mulde-Hillsite, Kriebstein, Castle Kriebstein, coarse gravel near Zschopau, on living leaves of *Plantago major*, 18 June 2006, H. Jage, GLM-F86432, GenBank: ITS = ON130150; LSU = ON130196; *cox2* = KJ654234. – USA, Wisconsin, Dodge County, on living leaves of *Plantago major*, 23 June 1942, C.G. Shaw, WSP13842, GenBank: *cox2* = OR250011, ITS = OR238581.

Notes — *Peronospora alta* is a common species and has been reported – often erroneously because of the application of a broad species concept – from Europe, Asia, North America, and South America (Gäumann 1923, Yu 1998, De-Souza et al. 2008). This species is parasitic on the leaves of the plant *Plantago major*, native to Europe. However, as several species of *Peronospora* were found parasitic to *Plantago major*, these reports require a re-evaluation. In addition, the various reports made on hosts other than *Plantago major* should be considered uncertain in light of the high host specificity found for most spe-

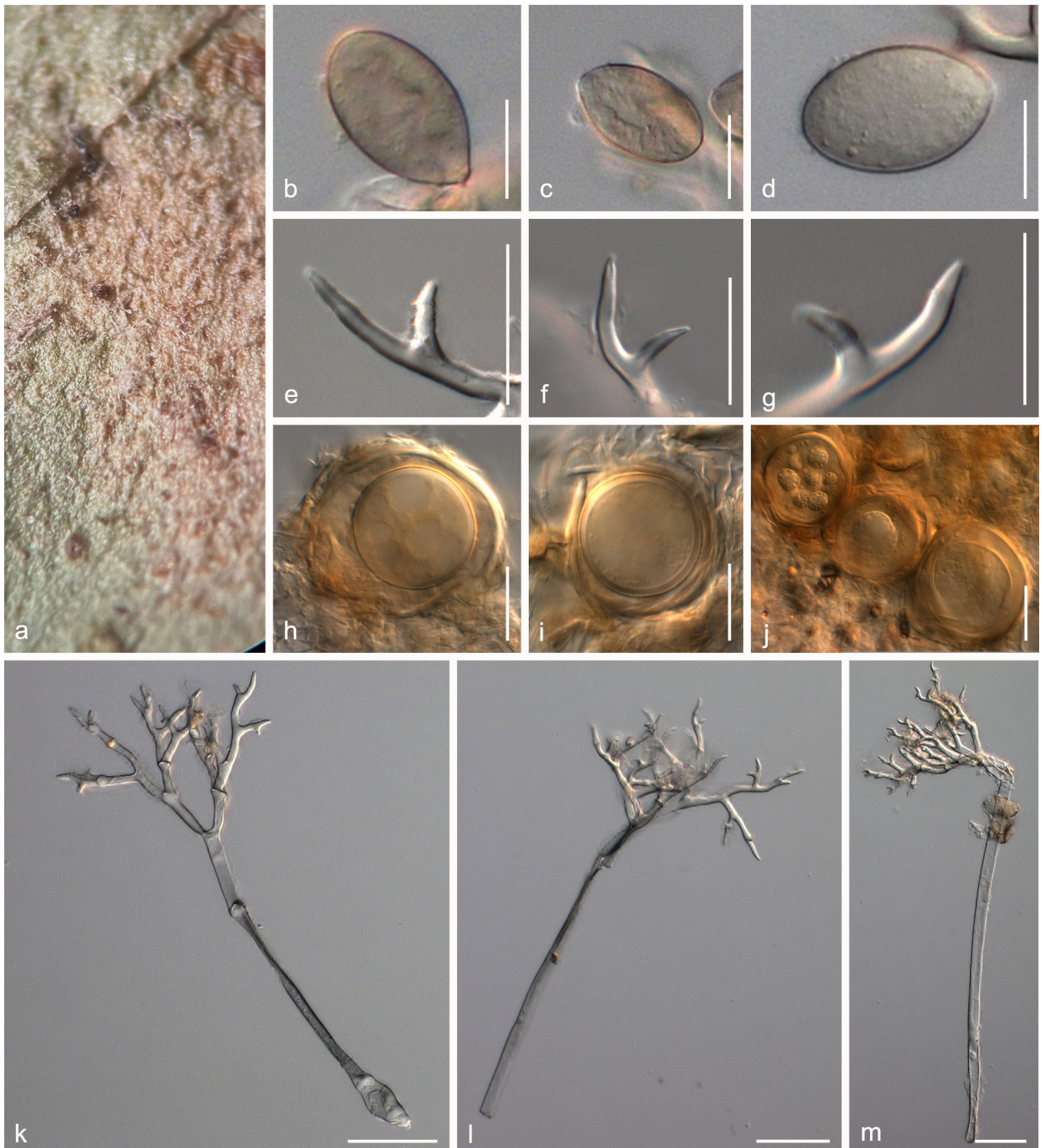


Fig. 5 Symptoms and morphology of *Peronospora* aff. *akatsukae* parasitic on *Plantago major*. a. Down on the leaf surface; b–d. conidia; e–g. ultimate branchlets; h–j. oospores; k–m. conidiophores. — Scale bars: b–j = 20 μm ; k–m = 50 μm .

cies in this study. In order to avoid misidentification regarding *P. alta* on *Plantago major* and the species on the same host found in this study, we re-measured and -sequenced the type specimen of *P. alta*, collected more than 150 years ago. Sequences that could be obtained from the type specimen were identical with GLM-F86432 in both ITS and *cox2* sequence stretches (KJ654234 for *cox2*, ON130150 for ITS), but different from all other species of *Peronospora* on *Plantago* species investigated. The morphology of the type of *P. alta* as well as that of GLM-F86432 resembled Fuckel's original description. It is noteworthy that *P. alta* has conidiophores branching subdichotomously in up to eight orders, rendering it one of the most complex downy mildew species in this respect. As a branching with up to eight orders has also been reported for *P. akatsukae*, and both species are similar also in respect to the pale violet conidia produced, De-Souza et al. (2008) hypothesised that *P. akatsukae* might be conspecific with *P. alta*. However,

the conidia of *P. alta* can only reach about 32 μm in length according to our morphological results, while *P. akatsukae* has been reported to produce conidia up to 47 μm long and with a different length-to-breadth ratio. Thus, it seems more likely that they represent independent species. Unfortunately, this could not be tested, as no authentic *P. akatsukae* specimens were available for the study.

***Peronospora bicolor* M. Mu, J. Kruse & Thines, sp. nov.** — MycoBank MB 849682; Fig. 7

Etymology. Specific epithet is derived from the colour of the conidia, which is often lighter towards the pedicel.

Typus. SPAIN, Andalucia, Malaga, north-east of Nerja, Sierra de Tejada Almiaray Alhama, hiking trail, open space on living leaves of *Plantago amplexicaulis*, 28 Apr. 2015, J. Kruse (GLM-F 135033, holotype), GenBank: *cox2* = ON230135; ITS = ON130163; LSU = ON130187.



Fig. 6 Symptoms and morphology of *Peronospora alta* parasitic on *Plantago major*. a. Down on the leaf surface; b–d. conidia; e–g. ultimate branchlets; h–i. conidiophores. — Scale bars: b–g = 20 μm ; h–i = 50 μm .

Diagnosis — Differs from *P. psylliicola* in having more elongated conidia, submonopodial branching, as well as shorter conidiophores and trunks.

Lesions on leaves first yellowish white, later brown, diffuse to vein-delimited. Down present on the lower leaf surface, consisting of scattered to dense felt-like conidiophore outgrowth. Conidiophores hyaline, straight, thin-walled, 290–528 μm long, av. 409 μm ; trunk mostly 150–410 μm long, av. 278 μm , 5.5–10 μm broad, sometimes slightly swollen to up to 11 μm at the base, ratio of the total length to trunk length 1.3–1.9, callose plugs absent. Branching submonopodial in 4–6 orders, with sub-straight to curved branches, gradually attenuate, often at acute angles. Ultimate branchlets almost straight to curved, rarely straight, paired branchlets differing in length, with the longer ones 5.5–19 μm long, the shorter ones 4–12 μm long, with a ratio of the longer to the shorter ultimate branchlet of 1.2–1.9, base 2.5–3.5 μm broad, apex obtuse. Conidia narrowly olivaceous to limoniform with a caramel colour, often lighter to almost hyaline towards the pedicel, 32.5–43 μm

broad, length-to-breadth ratio 1.6–2.2, basal part of the conidia mostly protruding. Oospores not observed.

Habitat — *Plantago amplexicaulis*.

Known distribution — Spain.

Notes — *Plantago amplexicaulis* is widely distributed around the Mediterranean Sea and the Nile Delta (Täckholm 1974), and it seems possible that the pathogen has a much wider distribution than confirmed in this study. *Peronospora bicolor* on *Plantago amplexicaulis* has a sister species, *P. psylliicola* on *Plantago boissieri*, which is clustering with it in the phylogenetic tree based on *cox2*, ITS, and LSU (Fig. 2; Table 2). However, there are differences in the shape and length-to-breadth ratio of conidia, which in *P. bicolor* is 1.89 ± 0.2 and, thus, more elongated than in *P. psylliicola* with 1.48 ± 0.1 . These differences require detailed measurements, but while it is not effortless to distinguish the two species based on morphology, they are readily distinguishable on the basis of sequences for any of the three loci used, as well as the host plant parasitised.

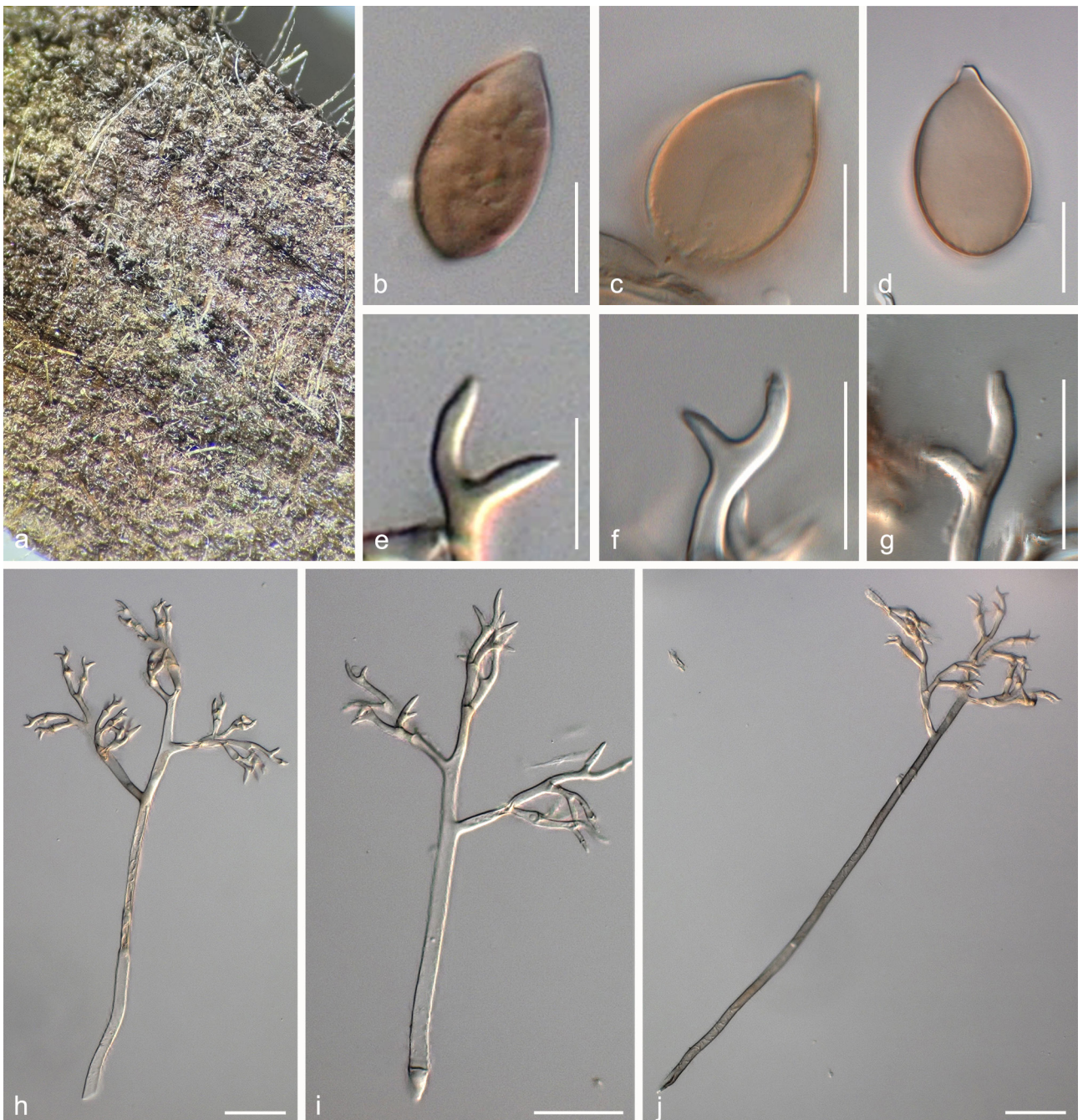


Fig. 7 Symptoms and morphology of *Peronospora bicolor* parasitic on *Plantago amplexicaulis*. a. Down on the leaf surface; b–d. conidium; e–g. ultimate branchlets; h–j. conidiophores. — Scale bars: b–g = 20 μm ; h–j = 50 μm .

Peronospora gaponenkoae Thines, Mycologia 113: 647 — 2021; Fig. 8

Synonym. *Peronospora kuewa* W.J. Davis et al., Mycologia 113: 648. 2021. *Invalidly described synonym.* *Peronospora lanceolatae* Gapon., Semejstvo Peronosporaceae Srednej Azii i Yuzhnogo Kazakhstana. 308. 1972, *nom. inval.*

Typus. GERMANY, Saxony, Kollm, Hauptstraße, roadside near the entry of 'Zum Weinberg', on living leaves of *Plantago lanceolata*, 11 July 2006, H. Boyle (GLM-F74725, holotype), GenBank: *cox2* = MT644993; ITS = MT644704; LSU = ON130173.

Lesions on leaves chlorotic, sometimes with violet anthocyanin colour on the upper surface, diffuse to vein-delimited. *Down* present on the lower leaf surface, consisting of scattered to dense felt-like conidiophore outgrowth. *Conidiophores* hyaline, straight, thin-walled, 349–851 μm long, av. 600 μm ; trunk mostly 233–604 μm long, av. 419 μm , 7.5–9.5 μm broad, sometimes slightly swollen to up to 17 μm at the base, ratio of the total length to trunk length 1.4–1.6, callose plugs absent. *Branching* subdichotomous in 6–7 orders, with sub-straight to curved branches, gradually attenuate, often at right angles. *Ultimate branchlets* usually slightly curved to curved, rarely straight, paired branchlets differing in length, with the longer ones 10–15.5 μm long, the shorter ones 5–10.5 μm long, with a ratio of the longer to the shorter ultimate branchlet of 1.5–2, base mostly 3 μm broad, apex obtuse. *Conidia* ellipsoid to obovate with a grey to brown colour, 18.5–23.5 μm long,

16–19 μm broad, length-to-breadth ratio 1.1–1.3. *Oospores* not observed.

Habitat — *Plantago lagopus*, *Plantago lanceolata*, *Plantago princeps*.

Known distribution — Germany, Greece, USA (Davis et al. 2021).

Additional specimens examined. GERMANY, Baden-Württemberg, Main-Tauber-district, Tauberbischofsheim West, Main-Tauber area hiking trail, Semi-arid grassland, on living leaves of *Plantago lanceolata*, 4 June 2015, J. Kruse, GLM-F135030, GenBank: *cox2* = ON230125; ITS = ON130153; LSU = ON130177; Hessen, High Meissner, Meissner plateau, Fulda-Werra-Bergland, Werra-Meissner-Kreis, Hausener Hute, mountain meadow below Naturfreundehaus, summit plateau, on living leaves of *Plantago lanceolata*, 10 June 2015, J. Kruse, GLM-F135029, GenBank: *cox2* = ON230124; ITS = ON130152; LSU = ON130176; Saxony, Zittau Mountains, Lückendorf, Hochwaldstraße, the embankment on the west side, on living leaves of *Plantago lanceolata*, 3 Aug. 2006, H. Boyle, GLM-F74749, GenBank: *cox2* = ON230123; ITS = ON130151; LSU = ON130175. — GREECE, Rhodes, southern Aegean, eastern coast, Kalathos, Masari, on living leaves of *Plantago lagopus*, 20 Mar. 2016, J. Kruse, GLM-F135031, GenBank: *cox2* = ON230126; ITS = ON130154; LSU = ON130178.

Notes — Based on molecular phylogenetic analyses, Thines (2019a) hypothesised that *P. kuewa* (BPI 910918) is probably conspecific with *P. gaponenkoae*, which is supported in this study. Additional analyses of the data available indicated that *P. gaponenkoae* and *P. kuewa* are identical in both *cox2* and LSU and only have a gap differentiating them in the ITS. Even though infection trials reported in Davis et al. (2021) did not

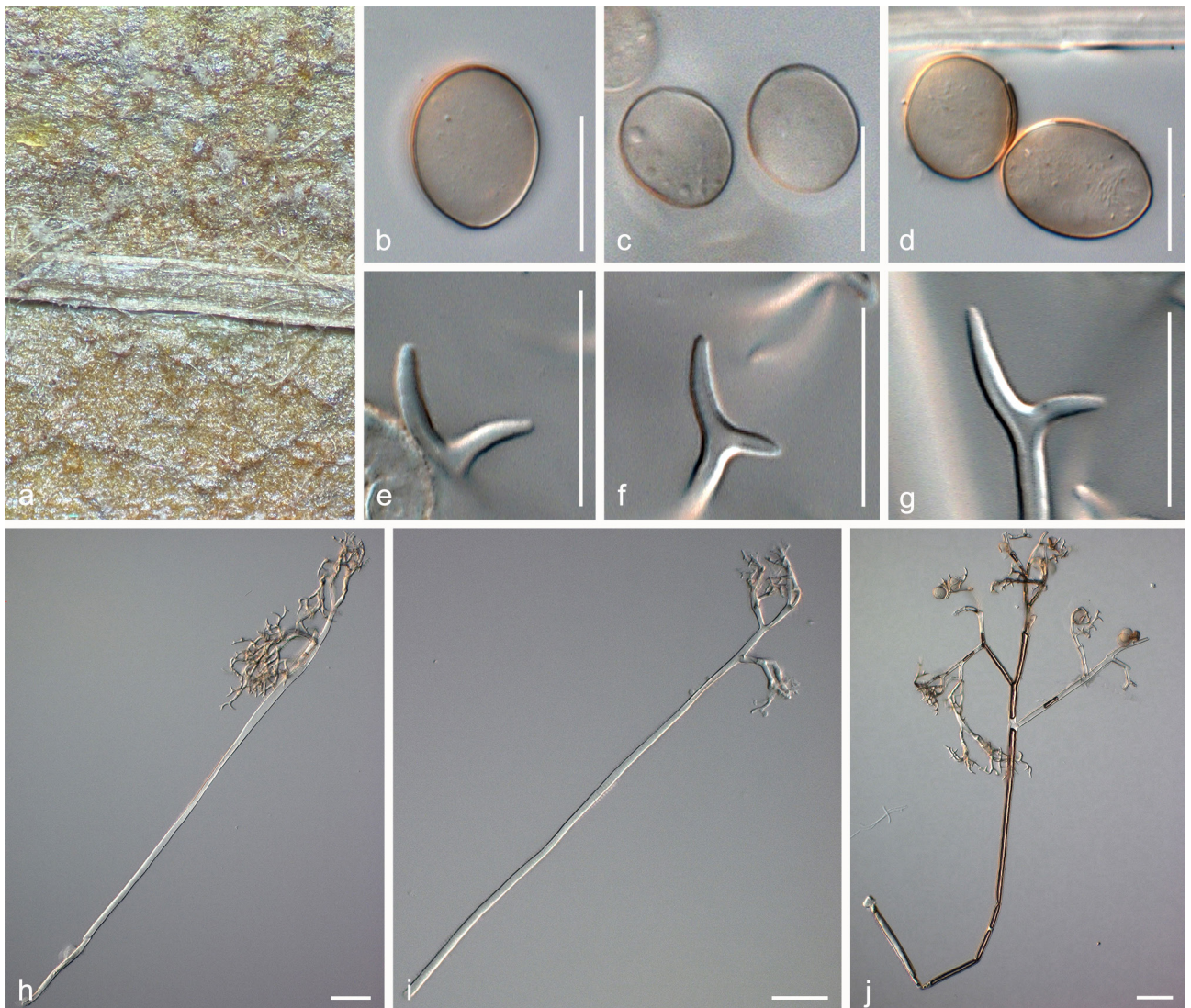


Fig. 8 Symptoms and morphology of *Peronospora gaponenkoae* parasitic on *Plantago lanceolata*. a. Down on the leaf surface; b–d. conidia; e–g. ultimate branchlets; h–j. conidiophores. — Scale bars: b–g = 20 μm ; h–j = 50 μm .

show a successful transfer to *Plantago lanceolata*, we speculate that this might have been due to different infection conditions required or partial resistance in local populations of *Plantago lanceolata*. We assume that the most plausible explanation for the sequence identity of BPI 910918 is that infections on the native species *Plantago princeps* have originated from the invasive plant *Plantago lanceolata*, also considering the remote location of Hawai'i and the high frequency at which *P. gaponenkoae* can be found on *Plantago lanceolata*. Thus, *P. gaponenkoae* is distributed throughout the native and invasive range of its host, with confirmed reports from the USA (Davis et al. 2021), Germany, Greece, and countries of the former USSR (Gaponenko 1972). *Peronospora gaponenkoae* is usually parasitic on the leaves of *Plantago lanceolata*, but can also be found on the closely related species *Plantago lagopus* and it apparently also shifted to *Plantago princeps* in Hawai'i (Davis et al. 2021). It is noteworthy that *P. gaponenkoae* has the longest conidiophores (600 μm on average) and trunks (419 μm on average), and it also has the second smallest and the roundest conidia of all *Peronospora* species parasitising *Plantago*, with conidia of av. $21 \times 17 \mu\text{m}$ and the length-to-breadth ratio of 1.24 ± 0.1 , rendering it relatively easy to identify.

Peronospora greenei M. Mu & Thines, *sp. nov.* — MycoBank MB 849683; Fig. 9

Etymology. Named to commemorate Henry Campbell Greene, acknowledging his outstanding contributions to the knowledge of lower prairie ecosystems and the plant pathogenic fungi and oomycetes inhabiting their plants, including the collection of numerous well-preserved specimens.

Typus. USA, Wisconsin, Dane County, Madison, on living leaves of *Plantago patagonica*, 21 June 1943, H.C. Greene (WSP25727, holotype), GenBank: *cox2* = OR250012; *ITS* = OR238582.

Diagnosis — Differs from *P. wawawaiana* in having larger and rounder conidia, as well as longer conidiophores and trunks.

Lesions on leaves yellowish white to grey, diffuse to vein-delimited. **Down** present on the lower leaf surface, consisting of scattered to dense felt-like conidiophore outgrowth. **Conidiophores** hyaline, straight, thin-walled, 295–555 μm long, av. 425 μm ; trunk mostly 152–412 μm long, av. 282 μm , 6.5–12 μm broad, sometimes slightly swollen to up to 16 μm at the base, ratio of the total length to trunk length 1.3–1.9, callose plugs absent. **Branching** subdichotomous in 3–7 orders, with almost straight to curved branches, gradually attenuate, often at acute angles.

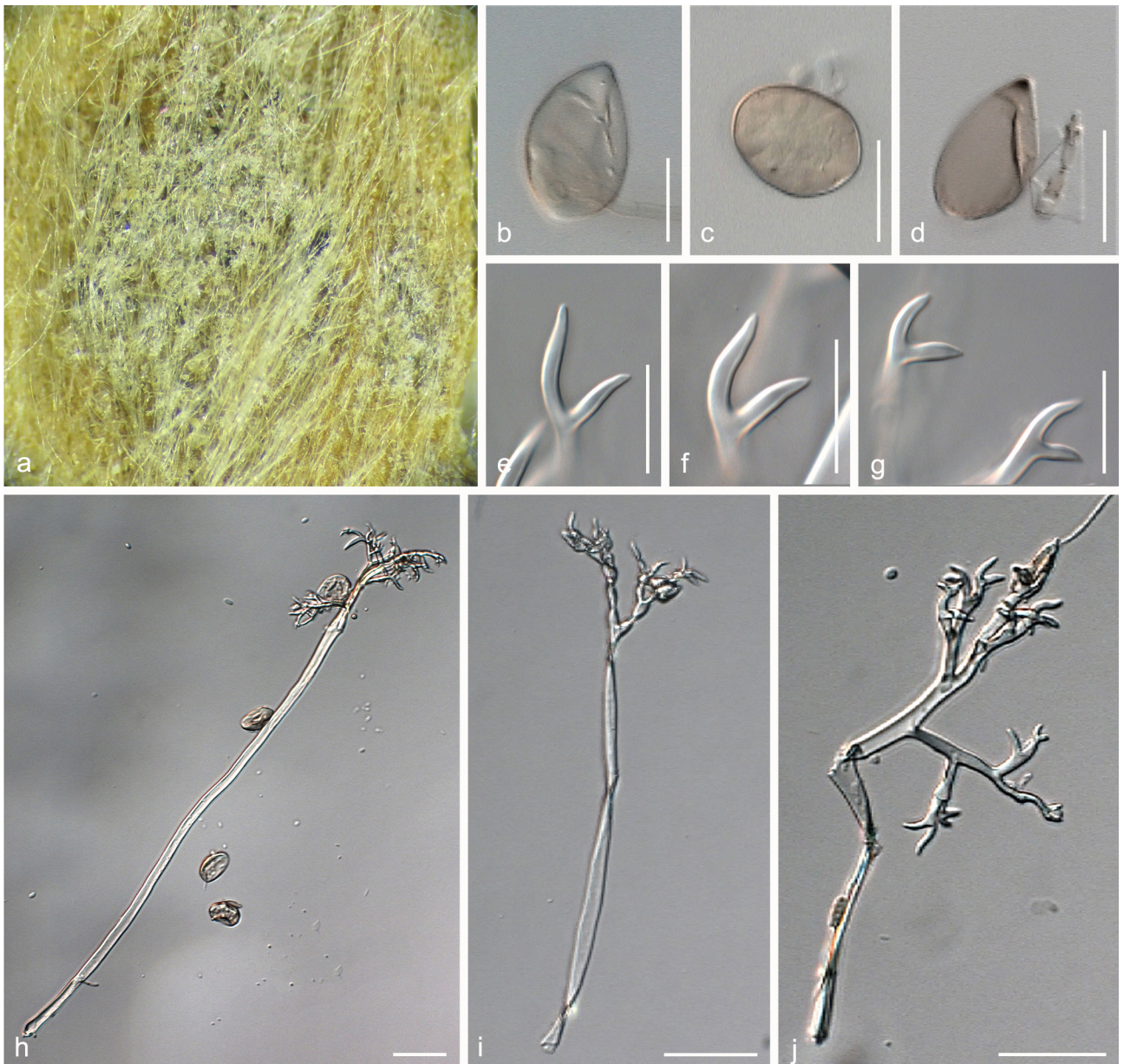


Fig. 9 Symptoms and morphology of *Peronospora greenei* parasitic on *Plantago patagonica* (syn. *Plantago spinulosa*). a. Down on the leaf surface; b–d. conidia; e–g. ultimate branchlets; h–j. conidiophores. — Scale bars: b–g = 20 μm ; h–j = 50 μm .

Ultimate branchlets almost straight to curved, paired branchlets differing in length, with the longer ones 11.5–16.5 μm long, the shorter ones 8–12.5 μm long, with a ratio of the longer to the shorter ultimate branchlet of 1.2–1.5, base 2.5–3.5 μm broad, apex obtuse. *Conidia* broadly ellipsoid to ovoid with an ochre to greyish sepia colour, 33–37.5 μm long, 21–25 μm broad, length-to-breadth ratio 1.4–1.6, basal part of the conidia mostly protruding. *Oospores* not observed.

Habitat — *Plantago patagonica* (syn. *Plantago spinulosa*).
Known distribution — USA.

Additional specimen examined. USA, Montana, Lake County, Elmo, grassland, on living leaves of *Plantago patagonica*, 5 Aug. 1942, H.T. Rogers & J.M. Rogers, WSP36618, GenBank: *cox2* = OR250013; ITS = OR238583; LSU = OR238576.

Notes — *Plantago patagonica* is native to much of North America and parts of southern South America (Bernal et al. 2013). *Peronospora greenei* is not the only pathogen that can infect *Plantago patagonica*, as *P. wawawaiiana* also can affect this plant species. *Peronospora wawawaiiana* is sister to *P. greenei*, as indicated by the *cox2* phylogenetic tree. They share a single base difference in the LSU sequence but exhibit

three base differences in the ITS sequence and nine base differences in the *cox2* sequence. This is in line with that *cox2* is a good marker for phylogenetic species delimitation among closely related *Peronospora* species, and the ITS region of the nuclear rDNA has been demonstrated to be a suitable choice for phylogenetic analysis at the genus level in *Peronospora* (Voglmayr 2003, Göker et al. 2004, Choi et al. 2015a, b). Some downy mildew species may require multiple loci for accurate molecular identification (Choi et al. 2015b, Thines et al. 2019), but already based on *cox2* and ITS, it is evident that *P. greenei* and *P. wawawaiiana* are distinct species, probably owing to the geographic separation between the central plains of North America and the western Rocky Mountains. Although they are similar in terms of phylogenetics, they exhibit noticeable differences in their morphology, as *P. greenei* possesses larger (av. 35 μm long, 23 μm broad) and rounder (length-to-breadth ratio of 1.53 ± 0.1) conidia, as well as longer conidiophores (av. 425 μm long) and trunks (av. 282 μm long).



Fig. 10 Symptoms and morphology of *Peronospora krungthepopsis* parasitic on *Plantago afra*. a–c. Conidia; d–f. ultimate branchlets; g–i. conidiophores. — Scale bars: a–f = 20 μm ; g–i = 50 μm .

Peronospora krungthepopsis M. Mu, J. Kruse & Thines, *sp. nov.* — MycoBank MB 849684; Fig. 10

Etymology. The name is derived from Krung Thep (Bangkok), referring to the reminiscence of the crowded roads on multiple layers in Bangkok, as reflected by the often-systemic nature of the pathogen, sporulating densely on leaves arranged in several layers.

Typus. SPAIN, Andalusia, Almería, SE Sorbas, A-1102, parking Cuevas de Sorbas, barren area, on living leaves of *Plantago afra*, 21 Apr. 2015, J. Kruse (GLM-F 135028, holotype), GenBank: *cox2* = ON230136; ITS = ON130164; LSU = ON130188.

Diagnosis — Differs from *P. lacrimoidea* in having narrower and more elongated conidia, shorter ultimate branchlets, longer conidiophores and trunks, as well as narrower trunks and conidiophore bases.

Lesions on leaves yellow-grey, diffuse to vein-delimited if not systemic. **Down** present on the lower leaf surface, consisting of scattered to dense felt-like conidiophore outgrowth. **Conidiophores** hyaline, straight, thin-walled, 251–588 μm long, av. 420 μm ; trunk mostly 155–452 μm long, av. 304 μm , 6.5–10 μm broad, slightly swollen to up to 11 μm at the base, ratio of the total length to trunk length 1.4–1.7, callose plugs absent. **Branching** subdichotomous in 4–7 orders, with sub-straight to curved branches, gradually attenuate, often at acute angles. **Ultimate branchlets** almost straight to curved, paired branchlets

differing in length, with the longer ones 4.5–19.5 μm long, the shorter ones 4–9.5 μm long, with a ratio of the longer to the shorter ultimate branchlet of 1.1–2.8, base 2–3 μm broad, apex obtuse. **Conidia** narrowly ellipsoid to elongate ellipsoid with a dirty violet to greyish russet colour, 35–42 μm long, 15.5–20.5 μm broad, length-to-breadth ratio 1.9–2.4, basal part of the conidia mostly protruding. **Oospores** not observed.

Habitat — *Plantago afra*.

Known distribution — Spain.

Notes — *Plantago afra* has a broad geographic distribution, including the western Mediterranean region, West Asia, and North Africa (Kazmi 1974), it is widely used and cultivated for its medicinal properties in India, Pakistan, and Iran (Izadi-Darbandi & Mehdikhani 2018). *Peronospora krungthepopsis* formed a clade with *P. lacrimoidea* on *Plantago arenaria* based on ITS and LSU sequences. Although they only have two base differences in LSU, they can be well differentiated by ITS and *cox2* indicating that they are distinct species. In terms of morphology, *P. krungthepopsis* exhibits narrower (av. 18 μm broad) and more elongated conidia (length-to-breadth ratio of 2.18 ± 0.2), shorter ultimate branchlets (av. 12 μm long), longer conidiophores (av. 420 μm long) and trunks (av. 304 μm long), as well as narrower trunks (av. 8 μm broad) and conidiophore bases (av. 10 μm broad).

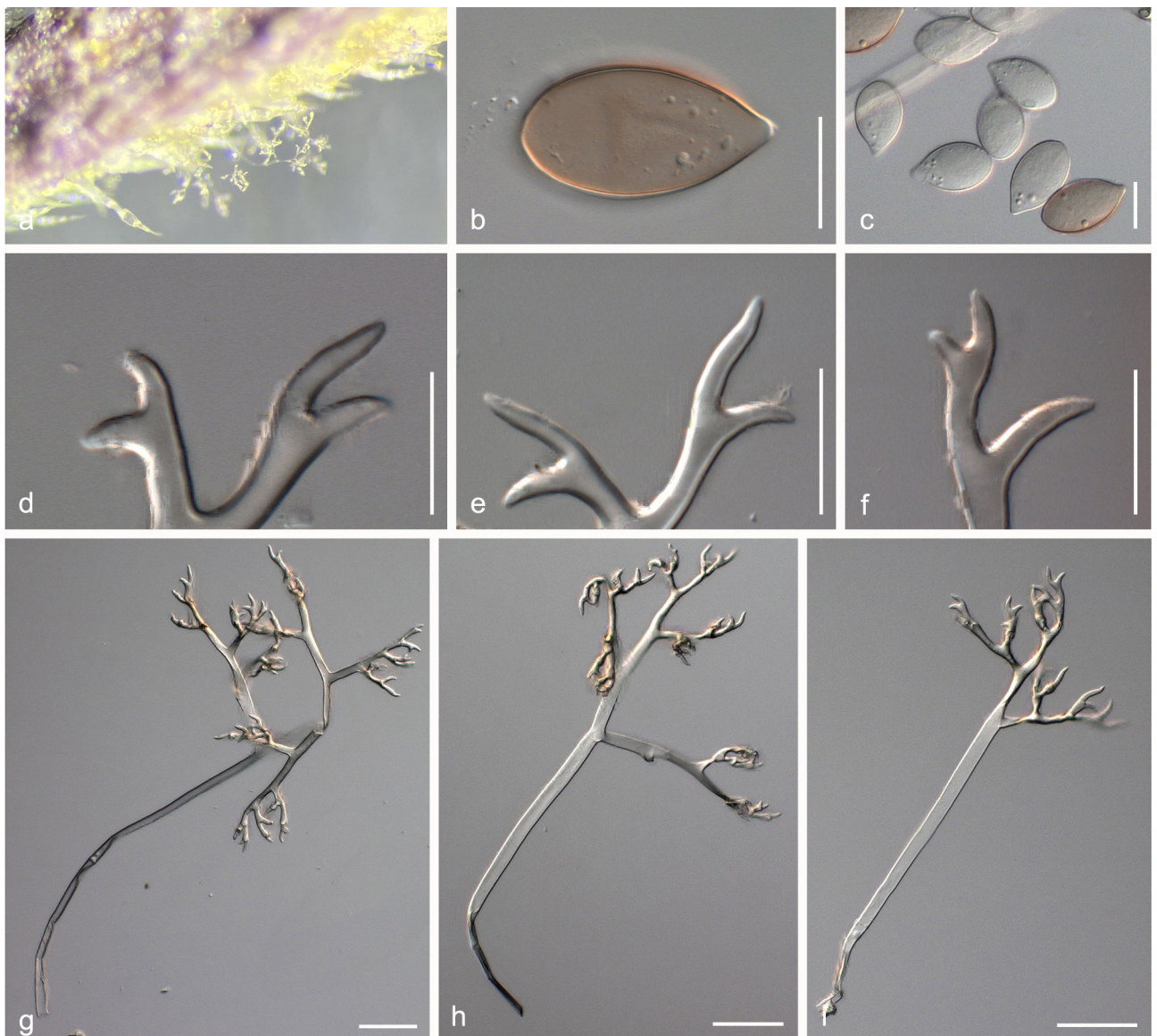


Fig. 11 Symptoms and morphology of *Peronospora lacrimoidea* parasitic on *Plantago arenaria*. a. Down on the leaf surface; b–c. conidia; d–f. ultimate branchlets; g–i. conidiophores. — Scale bars: b–f = 20 μm ; g–i = 50 μm .

Peronospora lacrimoidea, M. Mu & Thines, *sp. nov.* — MycoBank MB 849685; Fig. 11

Etymology. Named after the lacrimoid conidia.

Typus. GERMANY, Saxony-Anhalt, Fläming, Holzdorf E, sandy wasteland, near airfield fence, former sports facilities, on living leaves of *Plantago arenaria*, 22 Aug. 2004, H. Jage (GLM-F74548, holotype), GenBank: *cox2* = ON230137; ITS = ON130165; LSU = ON130189.

Diagnosis — Differs from *P. krungthepopsis* in having broader and rounder conidia, longer ultimate branchlets, shorter conidiophores and trunks, as well as broader trunks and conidiophore bases.

Lesions on leaves yellow to dark brown, diffuse to vein-delimited. *Down* present on the lower leaf surface, purplish or beige, consisting of scattered to dense felt-like conidiophore outgrowth. *Conidiophores* hyaline, slightly curved, thin-walled, 323–483 µm long, av. 403 µm; trunk mostly 164–348 µm long, av. 256 µm, 8.5–12.5 µm broad, sometimes slightly swollen to up to 14 µm at the base, ratio of the total length to trunk length 1.3–1.9, callose plugs absent. *Branching* sub-dichotomous in 4–6 orders, with sub-straight to curved branches, gradually attenuate, often at acute angles. *Ultimate branchlets* almost straight to curved, paired branchlets differing in length, with the longer ones 6.5–20.5 µm long, the shorter ones 4–15 µm long, and with a ratio of the longer to the shorter

ultimate branchlet of 1.2–2.1 µm, base 2.5–3.5 µm broad, apex obtuse. *Conidia* ovoid to lacrimoid with a light grey to brown colour, 33.5–42.5 µm long, 21–23 µm broad, length-to-breadth ratio 1.5–2, basal part of the conidia mostly protruding. *Oospores* not observed.

Habitat — *Plantago arenaria*.

Known distribution — Germany.

Notes — *Plantago arenaria* is widely distributed in grasslands throughout the temperate world (Stewart 1996). *Peronospora lacrimoidea* is the first *Peronospora* species on *Plantago arenaria* discovered. Interestingly, *P. lacrimoidea* formed a clade of species with elongated conidia (length-to-breadth ratio of 1.72) together with *P. krungthepopsis* (length-to-breadth ratio of 2.18) on *Plantago afra*.

Peronospora lata M. Mu, J. Kruse & Thines, *sp. nov.* — MycoBank MB 849686; Fig. 12

Etymology. 'Lata' from Latin, meaning 'broad, referring to the broad conidia.

Typus. GERMANY, Hesse, Meißnervorland, Fulda-Werra-Bergland, Berkatal municipality, Werra-Meißner district, northwest of Frankershausen, hollows on Zechstein limestone and gypsum, dry slope, on living leaves of *Plantago media*, 13 June 2015, J. Kruse (GLM-F135032, holotype), GenBank: *cox2* = ON230141; ITS = ON130169; LSU = ON130193.



Fig. 12 Symptoms and morphology of *Peronospora lata* parasitic on *Plantago media*. a. Down on the leaf surface; b–d. conidia; e–g. ultimate branchlets; h–j. conidiophores. — Scale bars: b–g = 20 µm; h–j = 50 µm.

Diagnosis — Differs from *P. a* in having broader and rounder conidia, longer ultimate branchlets, shorter conidiophores and trunks, as well as broader trunks. Differs from *P. alta* in having broader and rounder conidia, paired or three and longer ultimate branchlets, as well as shorter conidiophores and trunks. Differs from *P. mahi* in having larger and more elongated conidia, paired or three and longer ultimate branchlets, as well as shorter conidiophores and trunks. Differs from *P. subglobosa* in having more elongated conidia, paired or three and longer ultimate branchlets, as well as shorter conidiophores and trunks.

Lesions on leaves first chlorotic, later dark brown, diffuse to vein-delimited. **Down** present on the lower leaf surface, with a grey colour, consisting of scattered to dense felt-like conidiophore outgrowth. **Conidiophores** hyaline, straight to curved, thin-walled, 262–410 µm long, av. 336 µm; trunk mostly 141–222 µm long, av. 182 µm, 7.5–10 µm broad, sometimes slightly swollen to up to 14 µm at the base, ratio of the total length to trunk length 1.6–1.9, callose plugs absent. **Branching** subdichotomous in 4–6 orders, with sub-straight to curved branches, gradually attenuate, often at acute angles. **Ultimate branchlets** slightly curved to curved, mostly paired or sometimes three branchlets differing in length, with the longer ones 11.5–25 µm long, the shorter ones 7–15 µm long, with a ratio of the longer to the shorter ultimate branchlet of 1.1–2.8, base 2.5–3.5 µm broad, apex obtuse. **Conidia** subglobose to broadly ellipsoid with cinnamon to dirty mauve colour, 24–32 µm long,

19.5–26 µm broad, length-to-breadth ratio 1.1–1.5. **Oospores** not observed.

Habitat — *Plantago major*, *Plantago media*, *Plantago myosuuros*.

Known distribution — Argentina, Germany.

Additional specimens examined. ARGENTINA, on living leaves of *Plantago major*, 2004, RD1951, GenBank: *cox2* = ON230142; ITS = ON130171; LSU = ON130194; same location, on living leaves of *Plantago myosuuros*, 1999, RD1544, GenBank: *cox2* = ON230143; ITS = ON130170; LSU = ON130195.

Notes — This species has apparently also been introduced to other continents alongside its host. Thus, it seems likely that *Peronospora* species on *Plantago* can also be seed-borne, similar to other species of *Peronospora* (Pathak et al. 1978, Inaba et al. 1983, Rathore et al. 1987, Roongruangsree et al. 1988, Danielsen et al. 2004, Scott et al. 2004, Feng et al. 2014, Testen et al. 2014). *Peronospora lata* formed a monophyletic clade with *P. a*, *P. alta*, *P. mahi*, and *P. subglobosa*. The conidial shape of all of these species is characterised by a length-to-breadth ratio of the conidia lower than 1.5.

Peronospora mahi M. Mu & Thines, *sp. nov.* — MycoBank MB 849687; Fig. 13

Etymology. From the Kanza word for herbaceous plants, which form the great prairies of the central USA.

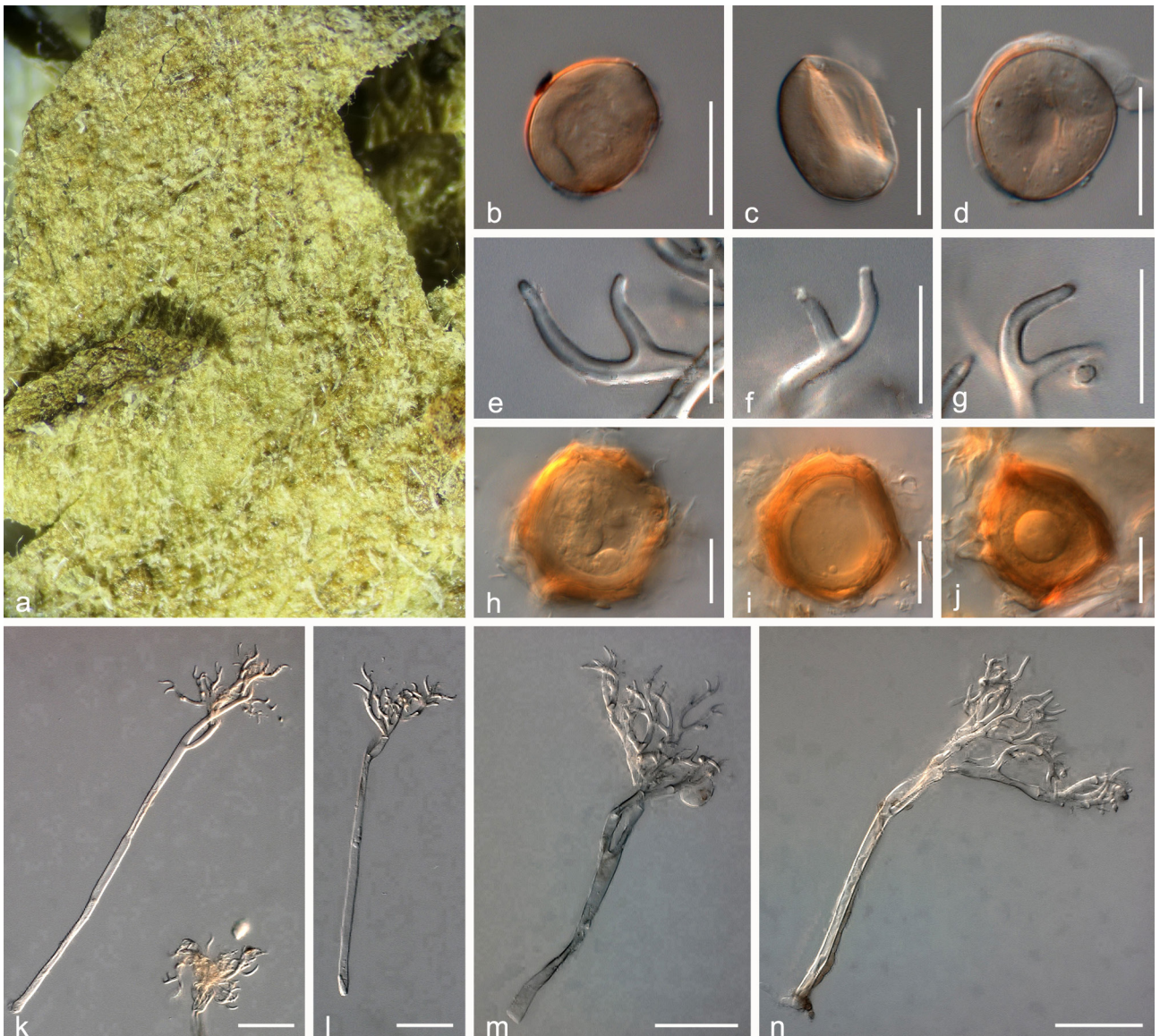


Fig. 13 Symptoms and morphology of *Peronospora mahi* parasitic on *Plantago rhodosperma*. a. Down on the leaf surface; b–d. conidia; e–g. ultimate branchlets; h–j. oospores; k–n. conidiophores. — Scale bars: b–j = 20 µm; k–n = 50 µm.

Typus. USA, Kansas, Riley County, Prairie south of Manhattan, on living leaves of *Plantago rhodosperma*, 9 June 1952, C.T. Rogerson (WSP36575, holotype), GenBank: *cox2* = OR250016; ITS = OR238586.

Diagnosis — Differs from *P. a* in having rounder conidia, as well as paired and shorter ultimate branchlets. Differs from *P. subglobosa* in having smaller conidia.

Lesions on leaves yellow-brown to dark brown, diffuse to vein-delimited. **Down** present on the lower leaf surface, consisting of scattered to dense felt-like conidiophore outgrowth. **Conidiophores** hyaline, straight, thin-walled, 272–504 μm long, av. 388 μm ; trunk mostly 115–344 μm long, av. 250 μm , 6.5–9 μm broad, sometimes slightly swollen to up to 13 μm at the base, ratio of the total length to trunk length 1.4–1.9, callose plugs absent. **Branching** subdichotomous in 4–8 orders, with sub-straight to curved branches, gradually attenuate, often at acute angles. **Ultimate branchlets** almost straight to curved, paired branchlets differing in length, with the longer ones 11–16.5 μm long, the shorter ones 7.5–10 μm long, with a ratio of the longer to the shorter ultimate branchlet of 1.3–1.8, base 2–3 μm broad, apex obtuse. **Conidia** globose to subglobose with a grey to amber colour, 22–25 μm long, 18–22 μm broad,

length-to-breadth ratio 1.1–1.3, basal part of the conidia mostly protruding. **Oospores** 29–38 μm .

Habitat — *Plantago rhodosperma*.

Known distribution — USA.

Notes — *Plantago rhodosperma* is native to the United States and mainly distributed in North America (McGregor et al. 1986, Villaseñor 2016). *Peronospora mahi* forms a clade with *P. a* and *P. subglobosa* in the phylogenetic tree characterised by round conidia, however *P. mahi* has larger conidia (av. 31 μm long) compared to *P. a* and *P. subglobosa*.

Peronospora plantaginis Underw., Bull. Torrey Bot. Club 24: 83. 1897 — Fig. 14

Synonym. *Pseudoperonospora plantaginis* (Underw.) M.P. Sharma & Pushpedra, J. Mycol. Plant Pathol. 28: 74. 1998.

Typus. USA, Alabama, Auburn on living leaves of *Plantago aristata*, 24 May 1896, F.S. Earle (WSP36558, isotype).

Lesions on leaves yellow to brown, diffuse to vein-delimited. **Down** present on the lower leaf surface, consisting of scat-

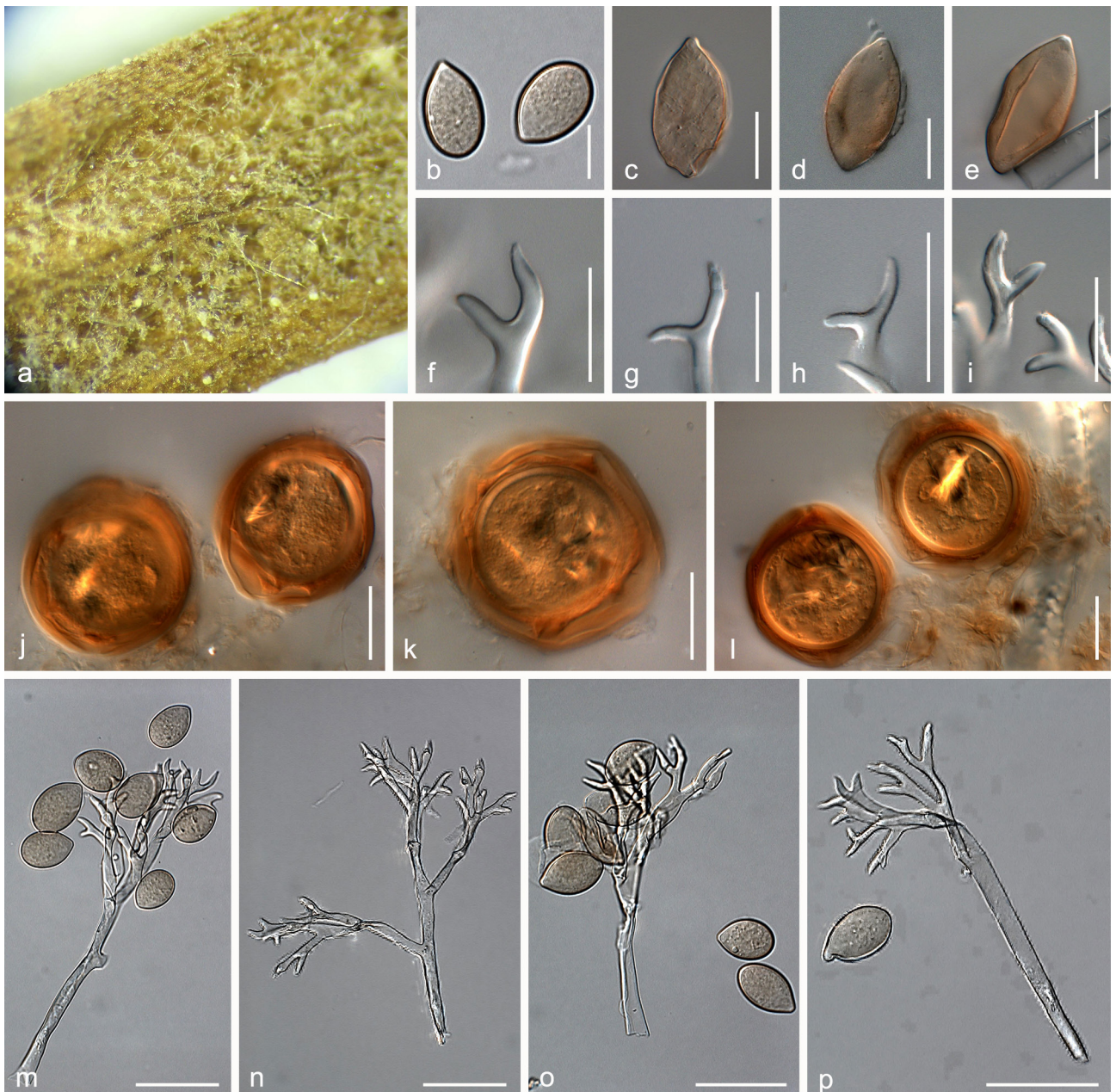


Fig. 14 Symptoms and morphology of *Peronospora plantaginis* (isotype WSP36558) parasitic on *Plantago aristata*. a. Down on the leaf surface; b–e. conidia; f–i. ultimate branchlets; j–l. oospores; m–p. conidiophores. — Scale bars: b–l = 20 μm ; m–p = 50 μm .

tered to dense felt-like conidiophore outgrowth. *Conidiophores* hyaline, straight to curved, thin-walled, 227–498 μm long, av. 363 μm ; trunk mostly 133–343 μm , av. 238 μm , 7.5–10 μm broad, sometimes slightly swollen to up to 13 μm at the base, ratio of the total length to trunk length 1.3–1.9, callose plugs absent. *Branching* subdichotomous in 5–6 orders, with sub-straight to curved branches, gradually attenuate, often at acute angles. *Ultimate branchlets* almost straight to curved, paired branchlets differing in length, with the longer ones 8.5–15.5 μm long, the shorter ones 5.5–10.5 μm long, and with a ratio of the longer to the shorter ultimate branchlet of 1.3–1.8, base 2–4 μm broad, apex obtuse. *Conidia* narrowly oval or lemon-shaped with a brown to russet colour, often somewhat lighter towards the pedicel, 39–44 μm long, 21.5–25 μm broad, length-to-breadth ratio 1.6–2, basal part of the conidia mostly protruding. *Oospores* 21–45 μm .

Habitat — *Plantago aristata* (Underwood 1897).

Known distribution — USA.

Additional specimens examined. USA, Alabama, Auburn, on living leaves of *Plantago aristata*, 20 Apr. 1917, F.C. Stakman, WSP31671, GenBank: *cox2* = OR250018; ITS = OR238588; Kansas, Lawrence, on living leaves

of *Plantago aristata*, 10 June 1918, G.W. Wilson, WSP35628, GenBank: ITS = OR238589; LSU = OR238578; Wisconsin, Dane County, Basco, on living leaves of *Plantago aristata*, 16 June 1966, H.C. Greene, WSP13762, GenBank: *cox2* = OR250017; ITS = OR238587; LSU = OR238577.

Notes — The host of *Peronospora plantaginis*, *Plantago aristata*, is primarily distributed in North America (GBIF 2023). However, due to a lack of molecular research on the type specimen of *P. plantaginis* on *Plantago aristata* (Underwood 1897), *P. plantaginis* has been reported on eight species of *Plantago* from North America and Eurasia, applying a broad species concept (Farr & Rossman 2023). To address this confusion, we sequenced *cox2*, ITS, and nrLSU gene fragments for *P. plantaginis* on *Plantago aristata* and found that the *P. plantaginis* isotype on *Plantago aristata* differs from an ITS sequence in GenBank, KC153428 on *Plantago ovata* s.lat., which had been erroneously identified as *P. plantaginis*. However, KC153428 belongs to the new species *P. psyllicola* (see below). Additionally, we also re-measured the isotype specimen of *P. plantaginis*, and the results agree with Underwood's measurements from 1897 (Underwood 1897; Table 2). In the LSU phylogenetic tree, we observed that *P. plantaginis* is closely related to *P. greenei*

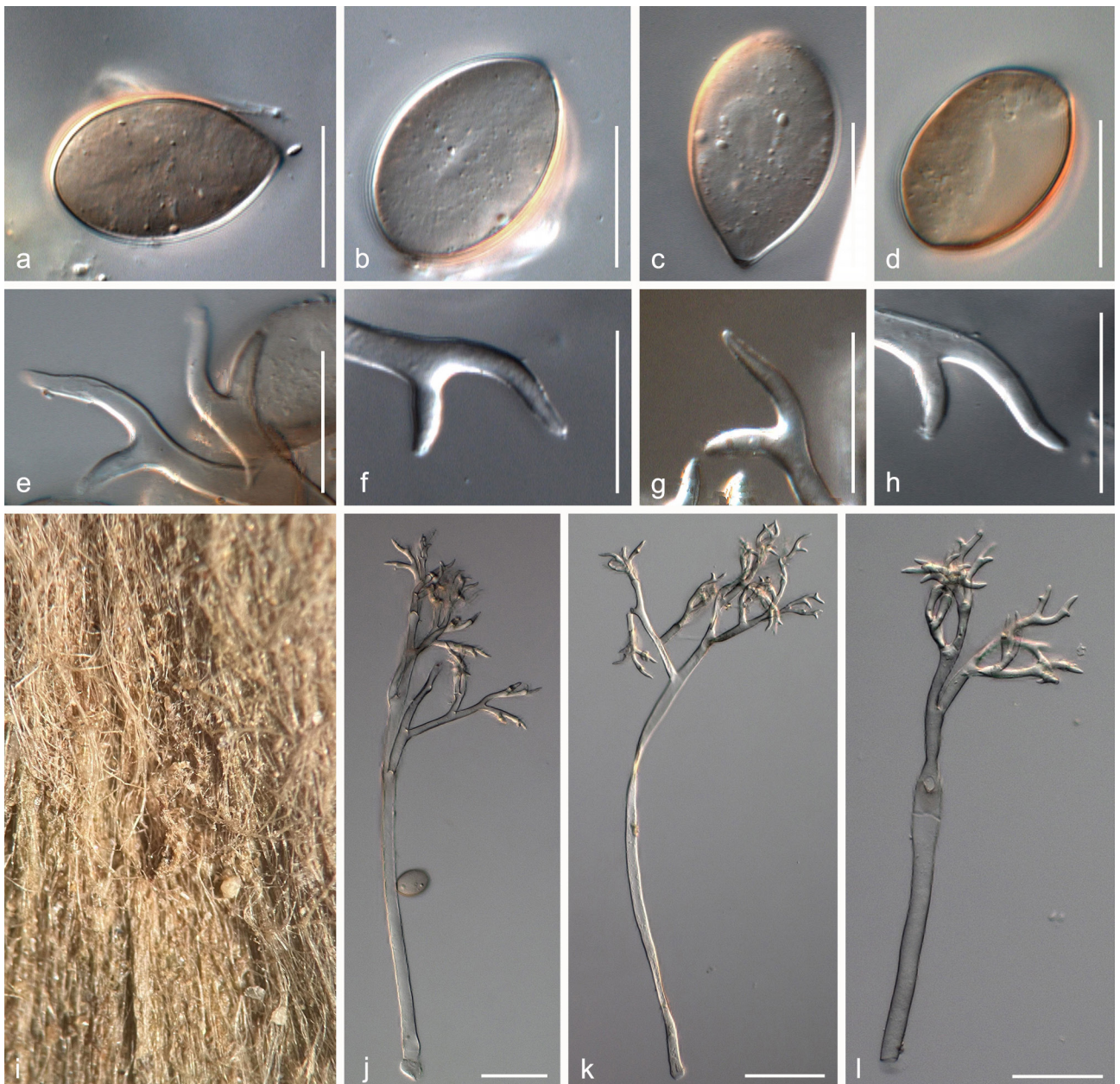


Fig. 15 Symptoms and morphology of *Peronospora psyllicola* parasitic on *Plantago boissieri*. a–d. Conidia; e–h. ultimate branchlets; i. down on the leaf surface; j–l. conidiophores. — Scale bars: a–h = 20 μm ; i–l = 50 μm .

and *P. wawawaiana*, with only three base differences from *P. greenei* and two base differences from *P. wawawaiana*. However, these three species can be clearly differentiated using ITS and *cox2* sequences. This further verifies that in some cases LSU is not as effective as ITS and *cox2* as a molecular barcode. In comparison to *P. greenei* and *P. wawawaiana*, *P. plantaginis* has larger (av. $42 \times 23 \mu\text{m}$) and more elongated (length-to-breadth ratio of 1.81 ± 0.2) conidia, shorter ultimate branchlets (av. $12 \mu\text{m}$ long), as well as narrower conidiophore bases (av. $11 \mu\text{m}$ broad) and trunks (av. $9 \mu\text{m}$ broad).

Peronospora psyllicola M. Mu, J. Kruse & Thines, *sp. nov.* — MycoBank MB 849688; Fig. 15

Etymology. From the Latin word *psyllion*, meaning a plantain crop, in reference to the commercial use of one of the hosts of the species, namely *Plantago ovata*.

Typus. SPAIN, Andalucia, Almeria, SE Sorbas, A-1102, parking Cuevas de Sorbas, barren area, on living leaves of *Plantago boissieri*, 21 Apr. 2015, J. Kruse (GLM-F135034, holotype), GenBank: *cox2* = ON230127; ITS = ON130155; LSU = ON130179.

Diagnosis — Differs from *P. bicolor*, apart from the colour of the conidia, in having rounder conidia, subdichotomous branching, as well as longer conidiophores and trunks.

Lesions on leaves dark brown, diffuse to vein-delimited. **Down** present on the lower leaf surface, consisting of scattered to dense felt-like conidiophore outgrowth. **Conidiophores** hyaline, straight, thin-walled, $319\text{--}623 \mu\text{m}$ long, av. $471 \mu\text{m}$; trunk mostly $180\text{--}436 \mu\text{m}$ long, av. $308 \mu\text{m}$, $7.5\text{--}12 \mu\text{m}$ broad, sometimes slightly swollen to up to $13 \mu\text{m}$ at the base, ratio of the total length to trunk length $1.4\text{--}1.8$, callose plugs absent. **Branching** subdichotomous in 4–7 orders, with sub-straight to curved branches, gradually attenuate, often at acute angles. **Ultimate branchlets** almost straight to curved, paired branchlets differing in length, with the longer ones $7\text{--}18 \mu\text{m}$ long, the shorter ones $4\text{--}10 \mu\text{m}$ long, with a ratio of the longer to the shorter ultimate branchlet of $1.4\text{--}2.2$, base $2\text{--}4 \mu\text{m}$ broad, apex obtuse. **Conidia** ellipsoid to ovoid with a dirty grey to rust colour, $27\text{--}36 \mu\text{m}$ long, $19.5\text{--}23 \mu\text{m}$ broad, length-to-breadth ratio $1.3\text{--}1.6$, basal part of the conidia mostly protruding. **Oospores** not observed.

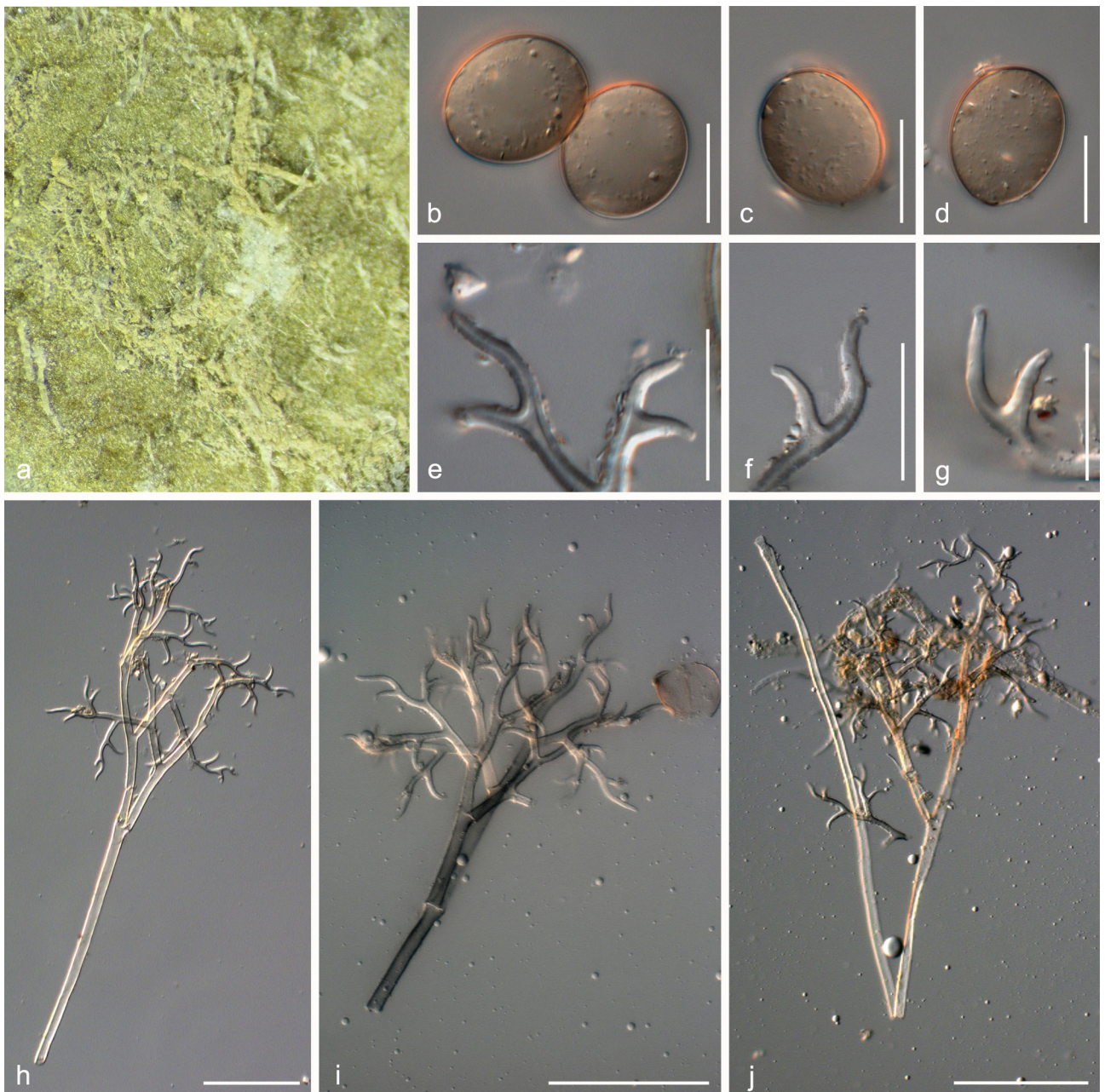


Fig. 16 Symptoms and morphology of *Peronospora subglobosa* parasitic on *Plantago virginica*. a. Down on the leaf surface; b–d. conidia; e–g. ultimate branchlets; h–j. conidiophores. — Scale bars: b–g = $20 \mu\text{m}$; h–j = $50 \mu\text{m}$.

Habitat — *Plantago boissieri*, *Plantago ovata* s.lat. (Mandal 2006).

Known distribution — India (Mandal 2006), Spain.

Additional specimen examined. SPAIN, Valencia, Torrebaja, barren area and arable fallow land on Rio Ebron, on living leaves of *Plantago boissieri*, 28 Apr. 2015, J. Kruse, GLM-F135035, GenBank: *cox2* = ON230128; ITS = ON130156; LSU = ON130180.

Notes — *Plantago boissieri*, one host of *P. psylliicola*, is predominantly reported from the regions surrounding the Persian Gulf, with some additional reports from Afghanistan (GBIF 2023), and is also distributed in Spain (Böhling & Eckstein 2009, this study). There is one ITS sequence labelled as *P. plantaginis* from GenBank (KC153428) derived from infected *Plantago ovata* s.lat., which is an important crop species grown for its seeds, e.g., in India and Pakistan. The downy mildew pathogen of *Plantago ovata* has usually been reported as *P. plantaginis* (Mandal 2006), but that species could only be confirmed from its type host, *Plantago aristata*. From the ITS phylogenetic tree, it

is apparent that KC153428 is not conspecific with *P. plantaginis* on *Plantago aristata* but with the new species *P. psylliicola*, as their sequences are identical. *Peronospora psylliicola* clusters with *P. bicolor* with strong support in the phylogenies based on *cox2*, ITS, and LSU (Fig. 1–3). These two species share some similarities in morphology, e.g., both have large conidia (larger than $37 \times 20 \mu\text{m}$ on average, according to the measurements of Mandal (2006)) (Fig. 7, 15, Table 2), but, apart from the colour, there are differences in the shape of conidia and the length-to-breadth ratio, as the conidia of *P. psylliicola* (length-to-breadth ratio of 1.48 ± 0.1) are rounder than those of *P. bicolor* (length-to-breadth ratio of 1.89 ± 0.2).

Peronospora subglobosa M. Mu & Thines, *sp. nov.* — MycoBank MB 849689; Fig. 16

Etymology. From the Latin 'subglobosa' meaning almost round, referring to the subglobose conidia.

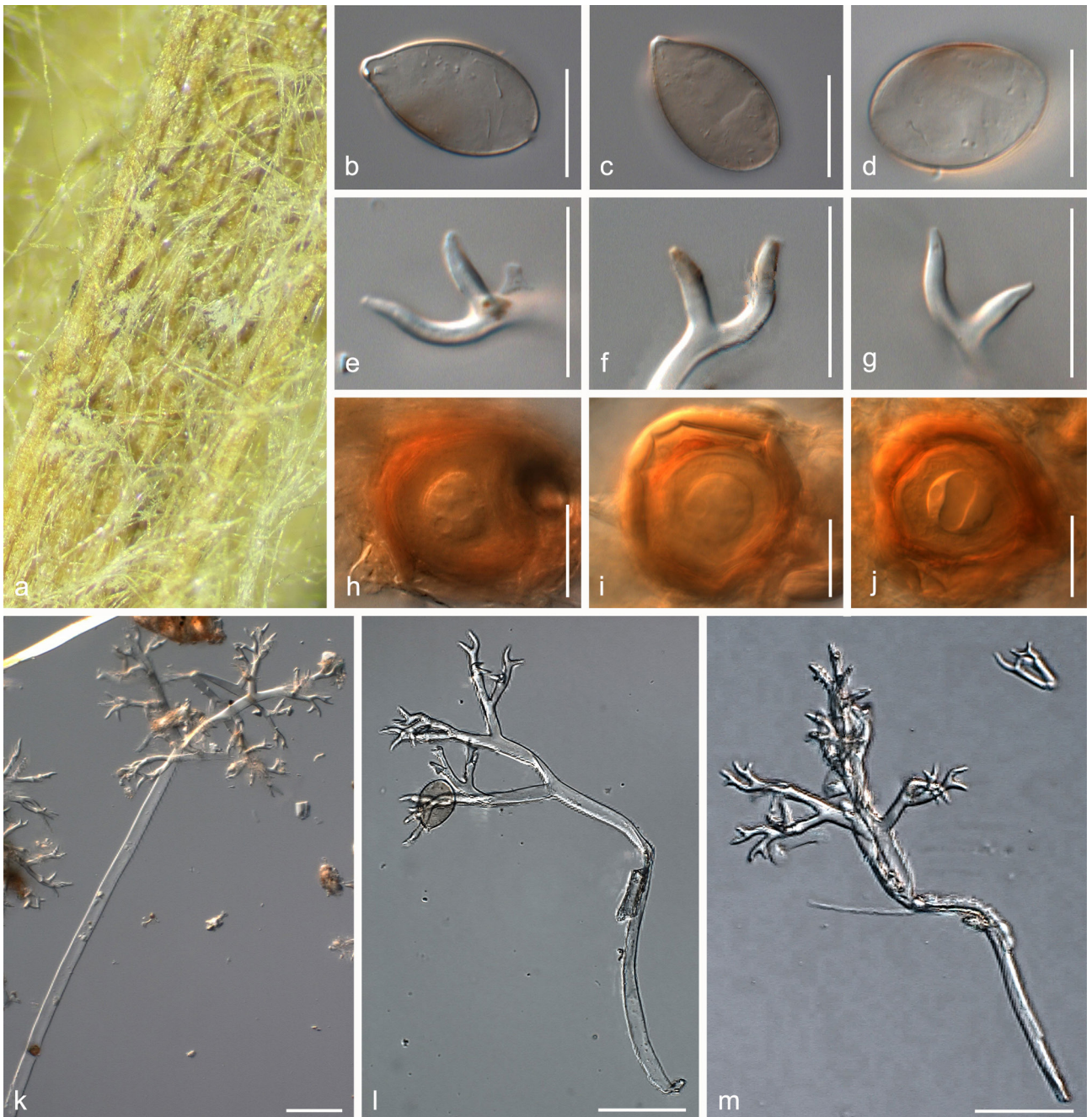


Fig. 17 Symptoms and morphology of *Peronospora wawawaiana* parasitic on *Plantago patagonica*. a. Down on the leaf surface; b–d. conidia; e–g. ultimate branchlets; h–j. oospores; k–m. conidiophores. — Scale bars: b–j = 20 μm ; k–m = 50 μm .

Typus. USA, Pennsylvania, Juniata County, southwest of Ease Waterford, Tuscarora Creek, on living leaves of *Plantago virginica*, 21 May 1951, H. Wahl (WSP36627, holotype), GenBank: *cox2* = OR250015; ITS = OR238585.

Diagnosis — Differs from *P. a* in having broader and rounder conidia, as well as paired and shorter ultimate branchlets. Differs from *P. mahi* in having larger conidia.

Lesions on leaves chlorotic, and yellow-brown, diffuse to vein-delimited. *Down* present on the lower leaf surface, consisting of scattered to dense felt-like conidiophore outgrowth. *Conidiophores* hyaline, straight, thin-walled, 273–458 µm long, av. 366 µm; trunk mostly 145–288 µm long, av. 217 µm, 6–10.5 µm broad, sometimes slightly swollen to up to 14 µm at the base, ratio of the total length to trunk length 1.5–2, callose plugs absent. *Branching* subdichotomous in 4–7 orders, with sub-straight to curved branches, gradually attenuate, often at acute angles. *Ultimate branchlets* almost straight to curved, rarely straight, paired branchlets differing in length, with the longer ones 12–16 µm long, the shorter ones 8.5–12.5 µm long, with a ratio of the longer to the shorter ultimate branchlet of 1.2–1.6, base 2–3 µm broad, apex obtuse. *Conidia* subglobose or globose with a smoke grey to dirty orange colour, 24.5–29.5 µm long, 20.5–24 µm broad, length-to-breadth ratio 1.1–1.3. *Oospores* not observed.

Habitat — *Plantago virginica*.

Known distribution — USA.

Additional specimen examined. USA, Virginia, Arlington County, Clarendon, old garden, on living leaves of *Plantago virginica*, 13 May 1928, S.F. Blake WSP36626, GenBank: *cox2* = OR250014; ITS = OR238584.

Notes — *Plantago virginica* is a native plant species of North America, but it has also been introduced into Asia (Luo et al. 2019, Knapp & Naczi 2021). *Peronospora subglobosa* forms a clade with *P. a* and *P. mahi* in *cox2* and ITS phylogenies, characterised by species with round conidia. However, *P. subglobosa* has broader conidia (av. 22 µm broad) compared to both *P. a* and *P. mahi*.

Peronospora wawawaiana M. Mu & Thines, *sp. nov.* — MycoBank MB 849690; Fig. 17

Etymology. Named after Wawawai where the type specimen has been found.

Typus. USA, Washington, Whitman County, Wawawai, on living leaves of *Plantago patagonica*, 9 June 1894, C.V. Piper (WSP36704, holotype), GenBank: *cox2* = OR250019; ITS = OR238590; LSU = OR238579.

Diagnosis — Differs from *P. greenei* in having smaller and more elongated conidia, as well as shorter conidiophores and trunks.

Lesions on leaves beige to light brown, diffuse to vein-delimited. *Down* present on the lower leaf surface, consisting of scattered to dense felt-like conidiophore outgrowth. *Conidiophores* hyaline, straight, thin-walled, 260–408 µm long, av. 334 µm; trunk mostly 148–238 µm long, av. 193 µm, 7.5–12 µm broad, sometimes slightly swollen to up to 14 µm at the base, ratio of the total length to trunk length 1.4–2.2, callose plugs absent. *Branching* subdichotomous in 4–6 orders, with sub-straight to curved branches, gradually attenuate, often at acute angles. *Ultimate branchlets* almost straight to curved, paired branchlets differing in length, with the longer ones 9.5–16 µm long, the shorter ones 7–12 µm long, with a ratio of the longer to the shorter ultimate branchlet of 1.2–1.6, base 2–3 µm broad, apex obtuse. *Conidia* ovoid to ellipsoid with a smoke grey to grey-brown colour, 29.5–36 µm long, 18.5–23 µm broad, length-to-breadth ratio 1.4–1.8, basal part of the conidia mostly protruding. *Oospores* 26–36 µm.

Habitat — *Plantago patagonica*.

Known distribution — USA.

Notes — *Peronospora wawawaiana* and *P. greenei* can be both parasitise on *Plantago patagonica*, and are sister species in the phylogenetic reconstructions, but *P. wawawaiana* has smaller (av. 33 µm long, 20 µm broad), and more elongated (length-to-breadth ratio of 1.6 ± 0.2) conidia, as well as shorter conidiophores (av. 334 µm long) and trunks (av. 193 µm long).

KEY TO THE SPECIES OF PERONOSPORA ON PLANTAGO

Because of the lack of detailed measurements available from the original descriptions, *Peronospora akatsukae* and *P. canescens* are excluded from this key.

1. Length/breadth ratio of the conidia higher than 1.5 . . . 2
1. Length/breadth ratio of the conidia lower than 1.5 3
2. Ratio of the longer/shorter ultimate branchlet length lower than 1.5 4
2. Ratio of the longer/shorter ultimate branchlet length higher than 1.5 7
3. Ratio of the longer/shorter ultimate branchlet length lower than 1.5 10
3. Ratio of the longer/shorter ultimate branchlet length higher than 1.5 14
4. Conidia mostly shorter than 35 µm 5
4. Conidia mostly longer than 35 µm 6
5. Conidiophore trunk mostly shorter than 250 µm *P. wawawaiana*
5. Conidiophore trunk mostly longer than 250 µm 12
6. Conidia frequently narrower than 19 µm *P. bicolor*
6. Conidia rarely narrower than 20 µm *P. plantaginis*
7. Conidia mostly shorter than 35 µm 8
7. Conidia mostly longer than 35 µm 20
8. Length/breadth ratio of the conidia mostly higher than 1.6 *P. psylliicola*
8. Length/breadth ratio of the conidia mostly lower than 1.5 9
9. Longer ultimate branchlet frequently longer than 20 µm *Peronospora a*
9. Longer ultimate branchlet rarely longer than 20 µm . . . 19
10. Conidia mostly shorter than 25 µm *P. mahi*
10. Conidia mostly longer than 25 µm 11
11. Length/breadth ratio of the conidia lower than 1.3 *P. subglobosa*
11. Length/breadth ratio of the conidia higher than 1.3 . . 12
12. Conidia mostly shorter than 30 µm *P. alta*
12. Conidia mostly longer than 30 µm 13
13. Conidiophores frequently longer than 500 µm *P. greenei*
13. Conidiophores frequently shorter than 300 µm *P. wawawaiana*
14. Longer ultimate branchlet on average longer than 15 µm 15
14. Longer ultimate branchlet on average shorter than 15 µm 16
15. Conidiophore trunks shorter than 250 µm *P. lata*
15. Conidiophore trunks mostly longer than 250 µm *P. a*
16. Conidia mostly shorter than 25 µm 17
16. Conidia mostly longer than 25 µm 18
17. Conidiophores mostly shorter than 450 µm *P. mahi*
17. Conidiophores mostly longer than 450 µm (on *Plantago lanceolata*) *P. gaponenkoeae*
18. Conidia frequently longer than 32 µm *P. psylliicola*
18. Conidia rarely longer than 32 µm *P. alta*

19. Length/breadth ratio of the conidia frequently higher than 1.8 *P. aff. akatsukae*
 19. Length/breadth ratio of the conidia rarely higher than 1.8 *P. alta*
 20. Conidia rarely broader than 22 μm *P. krungthepopsis*
 20. Conidia frequently broader than 22 μm 21
 21. Conidia broader than 20 μm *P. lacrimoidea*
 21. Conidia frequently narrower than 20 μm *P. bicolor*

DISCUSSION

Approximately 400 species have been described in the genus *Peronospora* (Constantinescu 1991), making it the largest genus of oomycetes. When the first molecular phylogenetic trees for the genus were published (Riethmüller et al. 2002, Voglmayr 2003) it was realised that the narrow species concept of Gäumann (1923) was more fitting than the broad species concept of Yerkes & Shaw (1959). This led to a reappraisal of various species names, including pathogens of some economically important crops, such as *P. effusa* (Choi et al. 2007, 2015b). At the same time, several economically important new species were described, both in *Peronospora* (Thines et al. 2009, Voglmayr et al. 2014b, Hoffmeister et al. 2020) and *Hyaloperonospora* (Choi et al. 2020), as well as in *Plasmopara* (Thines 2011, Görg et al. 2017).

In this study, the species of downy mildew on *Plantago* were revisited, as a recent study, in which two new species were described, namely *P. gaponenkoae* and *P. kuewa* (Davis et al. 2021) suggested the need to further studies in this group. As a result of these investigations, a surprisingly high number of new species were found – ten new *Peronospora* species infecting *Plantago* were discovered and described in the present manuscript. It is noteworthy that the diversity of *Peronospora* pathogens infecting *Plantago* is high, with distinct species on the two most widely used medicinal species, *Plantago lanceolata* and *Plantago ovata* s.lat. (*P. gaponenkoae* and *P. psylliicola*, respectively). All *Peronospora* species on *Plantago* can be distinguished morphologically, even though sometimes a combination of several characters needs to be considered.

Interestingly, species that are in well-supported clusters share some morphological similarities, and, sometimes also parasitise closely related hosts, a pattern that is phylogenetically confirmed for the first time for *Peronospora* species affecting a single plant genus. Lineages 1–6 (*P. greenei* on *Plantago patagonica*, *P. wawawaiana* on *Plantago patagonica*, *P. plantaginis* on *Plantago aristata*, *P. krungthepopsis* on *Plantago afra*, *P. aff. akatsukae* on *Plantago major* and *Plantago asiatica*, and *P. lacrimoidea* on *Plantago arenaria*) all have elongated conidia with a length-to-breadth ratio higher than 1.5. Similarly, lineages 4–6 form a monophyletic sub-clade of species that all have very elongated conidia with a length-to-breadth ratio higher than 1.7. Another monophyletic group containing five species (lineages 8–12, *P. a* on *Plantago uliginosa* and *Plantago major*, *P. mahi* on *Plantago rhodosperma*, *P. subglobosa* on *Plantago virginica*, *P. alta* on *Plantago major*, as well as *P. lata* on *Plantago media*, *Plantago major* and *Plantago myosuroides*) also has shared morphological characteristics, as all have roundish oval conidia with a length-to-breadth ratio lower than 1.5. The clade with lineages 14 and 15 (*P. bicolor* on *Plantago amplexicaulis*, as well as *P. psylliicola* on *Plantago boissieri* and *Plantago ovata* s.lat.) are forming a monophyletic clade of species with very large conidia (larger than $37 \times 20 \mu\text{m}$ on average). In addition, they have similar conidiophore shapes, branching orders, and ultimate branchlet lengths. This suggests that the morphological properties of *Peronospora* species might be more conserved than the host range, in line with the multiple

host jumps observed in *Peronospora* (Thines & Kummer 2013, Ploch et al. 2022).

As an exception for biotrophic oomycetes, four pathogen species of the same genus were found to be parasitic to the same host species, namely *P. a*, *P. alta*, *P. lata*, and *P. aff. akatsukae*, all parasitic to *Plantago major* and its closely related sister species, *Plantago uliginosa*. Also, on *Plantago patagonica*, two pathogen species are able to co-exist, albeit geographically separated, namely *P. greenei* and *P. wawawaiana*. This is noteworthy, as for pathogens, the main ecological envelope (i.e., the set ecological factors determining the fitness) will be factors pertaining to the host plant (Thines 2019a). If also the arguably second most important ecological envelope, the climate, is similar, it remains unclear, how the species are differentiated in their niches in a way that allows co-existence. It might be possible, however, that the infection process itself differs, i.e., that there are different infection efficiencies with the three main infection strategies – transmission by seeds, transmission by airborne conidia, and transmission by soilborne oospores. To elucidate this, both field observations and experimental approaches would be needed, so it will be up to future studies to elucidate niche differentiation in downy mildews. Such ecological studies might also help to understand how different oomycete genera are able to co-exist on the same host species, e.g., *Perofascia lepidii* and *Hyaloperonospora lepidii-sativi* on *Lepidium sativum* (Constantinescu & Fatehi 2002) or *Plasmopara australis* and *Pseudoperonospora cubensis* on some North American *Cucurbitaceae*, e.g., *Luffa cylindrica* (Wallace et al. 2016, Thomas et al. 2017).

It is also noteworthy that *P. gaponenkoae* can parasitise three species of host plants: the closely related species *Plantago lanceolata* and *Plantago lagopus*, as well as *Plantago princeps* in its introduced range (Thines 2019a, Davis et al. 2021). While Thines (2019a) already speculated that the downy mildew Hawaiian *Plantago* species could be conspecific with the invalidly published species, *Peronospora lanceolatae*, two species names were published in Davis et al. (2021), namely *P. gaponenkoae* and *P. kuewa* on *Plantago lanceolata* and *Plantago princeps*, respectively. In this study, we found that the sequences for both species are identical for all three loci investigated, aside from a one base-pair indel in ITS, supporting the hypothesis that the endemic *Plantago* species from Hawai'i contracted the downy mildew from the introduced population of *Plantago lanceolata*. Given also the broad overlap in morphology, we conclude that the two species are not distinct and relegate *P. kuewa* into synonymy with *P. gaponenkoae*. Thus, the case of the downy mildew on *Plantago princeps* might be a good example for a host shift after range expansions to a host species that was previously naïve to downy mildew infections (Thines 2019a).

Interestingly, *P. akatsukae* might also be able to infect two related species, *Plantago asiatica* and *Plantago major* (Shipunov et al. 2021), as the measurements for *P. aff. akatsukae* from *Plantago major* agree well with those from *Plantago asiatica* reported from Japan by Ito & Maruyama (1943), especially considering the elongated conidia.

It seems likely that also *P. canescens*, described from *Plantago canescens* in Russia is distinct from the species investigated in this study. The host is related to *Plantago major* (Shipunov et al. 2021), but the larger conidia reported for *P. canescens* (Jaczewski & Jaczewski 1931), as compared to the species found on *Plantago major* in this study, strongly suggest that it is an independent species. Considering the high species-richness of the genus *Plantago* (Shipunov et al. 2021), it seems likely that there will be additional species of *Peronospora* on *Plantago* awaiting discovery. Given the economic importance of some

Plantago species, it seems wanted to investigate the species boundaries of *Peronospora* species on *Plantago* further and also to conduct infection experiments to evaluate the potential host range of the different species. This is crucial for a risk assessment with respect to the introduction of alien *Peronospora* species to regions in which *Plantago* species are cultivated for their medicinal and dietary properties.

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