Mycological Progress (2024) 23:51
https://doi.org/10.1007/s11557-024-01984-y

DGfM

ORIGINAL ARTICLE

Peronospora species on *Myosotis* – a showcase for the uncharted diversity of a highly diverse and specialised downy mildew genus

Man Mu[1,2] · **Young-Joon Choi**[3] · **Marco Thines**[1,2]

Received: 14 June 2024 / Revised: 27 June 2024 / Accepted: 29 June 2024 / Published online: 19 August 2024

Abstract

Peronospora is the largest genus of obligate biotrophic oomycetes, causing downy mildew disease on various cultivated and ornamental plants worldwide. Despite the known high host specificity in *Peronospora*, many host–pathogen combinations have not been thoroughly investigated with respect to their phylogenetic relationships. This is especially the case for groups in which only one species has been described on a host genus. In this study, we highlight the fact that *Peronospora* is still vastly underexplored by investigating 48 specimens of *Peronospora* parasitic on the ornamental plant genus *Myosotis* (*Boraginaceae*). Morphology and multigene phylogenetic relationships revealed six new *Peronospora* species parasitic on *Myosotis*, with one species parasitising the widely cultivated species *Myosotis sylvatica*, providing further evidence that *Peronospora* species are generally highly host-specific. As a consequence, *Peronospora* occurrences on the several hundred plant species that are not type host species for a described *Peronospora* species should be re-examined, as they might represent species overlooked in previous studies.

Keywords *Boraginaceae* · Downy mildew · Evolution · Phylogeny · Six new taxa

Introduction

The genus *Peronospora* is the most species-rich genus of oomycetes, with more than 400 described species (Constantinescu 1991). However, there are still many host/pathogen associations for which the species identity is unclear (Thines and Choi 2016). This also applies to species of culinary herbs and ornamentals, such as basil, sage, and poppy (Choi et al. 2009; Thines et al. 2009, 2019; Voglmayr et al. 2014; Hoffmeister et al. 2020). The downy mildew species parasitic to *Myosotis* are of particular interest in this respect, as some species of the genus are common and widespread ornamentals, e.g. *Myosotis sylvatica*. Downy mildew is a common disease of ornamental *Myosotis* species in several countries. However, so far the disease has been attributed to a single species, *Peronospora myosotidis*. This pathogen reportedly parasitises e.g. *Myosotis arenaria*, *M. arvensis* (syn. *M. intermedia*, the type host of *P. myosotidis*), *M. caespitosa*, *M. hispida*, *M. nemorosa*, *M. paulstris*, *M. sparsiflora*, *M. sylvatica*, *M. stricta*, *M. verna*, *M. versicolor*, as well as species of the genera *Amsinckia* and *Brunnera* (de Bary 1863; Stevenson 1926; Rogerson 1958; Jafar 1962; Gjaerum 1980; Pantone et al. 1985; Adamska 2001; Dietrich and Müller 2001; Lane et al. 2006). Some taxa previously recognised as specialised forms of *P. myosotidis* on genera of *Boraginaceae* (Constantinescu 1991) other than the ones mentioned above are now considered distinct species, namely *Peronospora pulmonariae* (syn. *Peronospora myosotidis* f. *pulmonariae*) on *Pulmonaria angustifolia* and *Pulmonaria officinalis* (Klenke and Scholler 2015); *Peronospora echii* (syn. *Peronospora myosotidis* var. *echii*) on *Echium vulgare* (Klenke and Scholler 2015); and *Peronospora lithospermi* (syn. *Peronospora myosotidis* f. *lithospermi*) on *Lithospermum arvense* (Gäumann 1923).

Section Editor: Tanay Bose.

✉ Young-Joon Choi
yjchoi@kunsan.ac.kr

✉ Marco Thines
m.thines@thines-lab.eu

[1] Institute of Ecology. Evolution and Diversity, Department of Biological Sciences, Goethe University, Max-Von-Laue-Str, 13, Frankfurt Am Main 60439, Germany

[2] Senckenberg Biodiversity and Climate Research Centre, Senckenberganlage 25, Frankfurt Am Main 60325, Germany

[3] Department of Biological Science, Kunsan National University, Gunsan 54150, Korea

To disentangle species complexes, multigene sequence analyses are often necessary for downy mildews (Choi et al. 2015b), in addition to detailed analyses of the morphology of the species. The aim of the current study is to use this combination of phylogenetic and morphological analyses to evaluate the occurrences of *Peronospora* on *Myosotis*. Because of their economic relevance, the diversity and specialisation of downy mildew species on these hosts have immediate relevance also for phytosanitary measures with the aim of the prevention of introducing new plant diseases.

Materials and methods

Plant and oomycete material

In total, 48 *Peronospora* specimens originating from *Myosotis* from different sites in Germany, and 1 *Peronospora* specimen originating from *Myosotis* from Iceland, were examined and 22 were selected for multigene-based molecular phylogenetic analyses. The specimens were deposited in the Herbarium Senckenbergianum at the Senckenberg Museum für Naturkunde in Görlitz (GLM) and Frankfurt am Main (FR). Details of the specimens used in this study are provided in Table 1.

DNA extraction, PCR, sequencing

From each herbarium specimen, small amounts of infected leaf tissue were excised and placed into a 2 ml reaction tube with two steel beads with a diameter of 3 mm and five steel beads with a diameter of 1 mm per sample and disrupted using a mixer mill (MM200, Retsch, Germany) by milling twice (inverting the tubes in between) at 25 Hz for 15 min. Genomic DNA was extracted using the BioSprint 96 DNA Plant Kit (Qiagen, Venlo, Netherlands) on a KingFisher Flex (Thermo Scientific, Waltham, MS, USA) automated extraction instrument, according to the protocol provided by the manufacturer. Two nuclear loci (ITS, nrLSU) and four mitochondrial loci (*COX*2, *COX*1, *NAD1*, and *RPS10*) were amplified using oomycete-specific primers, as previously outlined by Choi et al. (2015b). In addition, two regions of LSU rDNA, D1–5 and D6–8, were amplified using two primer sets, LR0R (Vilgalys and Hester 1990) and LR6-O (Riethmüller et al. 2002) and LR6-OR2C (Thines et al. 2019) and LR9 (Hopple and Vilgalys 1999), respectively. In some cases, due to weak amplification, the *COX*2 locus and the spacer region had to be amplified separately using two primer sets, cox2-F (Hudspeth et al. 2000) and cox2-RC4 (Choi et al. 2015a) for the gene, and FM79 & FMPh-10b (Martin et al. 2004) for the spacer region. For confirmation of the host identity, PCR amplification was performed with the primers matK-xf (Ford et al. 2009) and matK-MALP (Dunning and Savolainen 2010) for the *matK* regions, and the primers ITS1-Plant and ITS4 (Navarro et al. 2003; White et al. 1990) for the ITS regions. Amplicons were bidirectionally sequenced at the Biodiversity and Climate Research Centre (BiK-F, Frankfurt, Germany) laboratory using the same primers as those used for amplifications.

Phylogenetic analyses

Sequences were edited and assembled using Geneious version 5.6 (Biomatters, Auckland, New Zealand). An alignment of each locus was performed using MAFFT version 7 (Katoh and Standley 2013) employing the Q-INS-i algorithm (Katoh and Toh 2008). SequenceMatrix version 1.7.8 (Vaidya et al. 2011) was used for concatenating individual gene sequences and for checking unusually similar or divergent sequences. The TrEase webserver (Mishra et al. 2023) was used for calculating some initial phylogenetic trees. After making sure that no conflicting support was present in individual loci, a concatenated alignment of nuclear (ITS, nrLSU) and mitochondrial (*COX*2, *COX*1, *NAD1*, *RPS10*), loci was constructed. The concatenated alignment is available on Zenodo (https://doi.org/10.5281/zenodo.10850909). Phylogenetic inference was performed using three different tree construction methods, Minimum Evolution (ME), Maximum Likelihood (ML) and Bayesian inference (BI). ME analyses were done using MEGA version 7.0 (Kumar et al. 2016), with the default settings of the program except using the Tamura-Nei model and pairwise deletion, and the support for internal nodes was estimated by 1,000 bootstrap replicates (Felsenstein 1985). For ML analyses, 1,000 rounds of random addition of sequences as well as 1,000 fast bootstrap replicates were performed using RAxML version 8.2.7 (Stamatakis 2014) with 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 version 2.3 (Nylander 2004) and computation was done using MrBayes version 3.1.6 (Ronquist et al. 2012), applying GTR + I + G as the substitution model for the six-locus tree. Analyses were run for 4 million generations, with sampling every 10,000th tree. The first 30% of the trees were discarded and the remaining trees were used for calculating posterior probabilities, to ensure sampling from the stationary phase. *Pseudoperonospora cubensis* was used as outgroup in all analyses. Information on the GenBank accessions for the specimens used in this study is shown in Table 1.

Table 1 Information on downy mildew specimens used in this study

Species	Herb[a] No.	Host	Year	Country	Genbank Accession Number									Reference
					18 s + ITS	**LSU D1-3**	**LSU D6-8**	***COX*1**	***COX*2**	***NAD1***	***RPS10***	**ITS (host)**	***matK* (host)**	
Peronospora asperuginis	WU 22884	*Asperugo procumbens*	1999	Austria	AY198264	—	—	—	—	—	—	—	—	Voglmayr 2003
Peronospora globosa	GLM-F69974	*Myosotis persoonii*	2015	Germany	PP528446	PP528469	PP531588	PP544723	PP544745	PP544702	PP544681	PP763515	PP783618	This study
Peronospora globosa	GLM-F75954	*Myosotis persoonii*	2005	Germany	PP528447	PP528470	PP531589	PP544724	PP544746	PP544703	PP544682	PP763516	PP783619	This study
Peronospora globosa	GLM-F78099	*Myosotis persoonii*	2002	Germany	PP528448	PP528471	PP531590	PP544725	PP544747	PP544704	PP544683	PP763517	PP783620	This study
Peronospora globosa	GLM-F74181	*Myosotis persoonii*	2001	Germany	PP776903	—	—	—	—	—	—	PP763518	PP783621	This study
Peronospora globosa	GLM-F74465	*Myosotis persoonii*	2003	Germany	PP776904	—	—	—	—	—	—	PP763519	PP783622	This study
Peronospora globosa	GLM-F77850	*Myosotis persoonii*	1999	Germany	PP776905	—	—	—	—	—	—	PP763520	—	This study
Peronospora laura	GLM-F67407	*Myosotis scorpioides*	1987	Germany	PP528449	PP528472	PP531591	PP544726	PP544748	PP544705	PP544684	PP763521	PP783623	This study
Peronospora laura	GLM-F68376	*Myosotis scorpioides*	1985	Germany	PP528450	PP528473	PP531592	PP544727	PP544749	PP544706	PP544685	PP763522	PP783624	This study
Peronospora laura	GLM-F69866	*Myosotis scorpioides*	1980	Germany	PP528451	PP528474	PP531593	PP544728	PP544750	PP544707	PP544686	PP763523	PP783625	This study
Peronospora mira	GLM-F73835	*Myosotis sylvatica*	2001	Germany	PP528452	PP528475	PP531594	PP544729	PP544751	PP544708	PP544687	PP763524	PP783626	This study
Peronospora mira	GLM-F74149	*Myosotis sylvatica*	2001	Germany	PP528453	PP528476	PP531595	PP544730	PP544752	PP544709	PP544688	PP763525	PP783627	This study
Peronospora mira	GLM-F78289	*Myosotis sylvatica*	1999	Germany	PP528454	PP528477	PP531596	PP544731	PP544753	PP544710	PP544689	PP763526	PP783628	This study
Peronospora myosotidis	GLM-F73872	*Myosotis arvensis*	1998	Germany	PP528455	PP528478	PP531597	PP544732	PP544754	PP544711	PP544690	PP763527	PP783629	This study
Peronospora myosotidis	GLM-F76288	*Myosotis arvensis*	2002	Germany	PP528456	PP528479	PP531598	PP544733	PP544755	PP544712	PP544691	PP763528	PP783630	This study
Peronospora myosotidis	GLM-F78074	*Myosotis arvensis*	2002	Germany	PP528457	PP528480	PP531599	PP544734	PP544756	PP544713	PP544692	PP763529	PP783631	This study
Peronospora myosotidis	IC79–2	*Myosotis arvensis*	2023	Iceland	PP528458	PP528481	—	PP544735	PP544757	—	—	PP763530	—	This study

Table 1 (continued)

Species	Herb[a] No.	Host	Year	Country	Genbank Accession Number									Reference
					18 s + ITS	**LSU D1-3**	**LSU D6-8**	***COX*1**	***COX*2**	***NAD1***	***RPS10***	**ITS (host)**	***matK* (host)**	
Peronospora myosotidis	GLM-F48345	*Myosotis arvensis*	2000	Germany	PP776906	—	—	—	—	—	—	PP763531	PP783632	This study
Peronospora myosotidis	GLM-F73375	*Myosotis arvensis*	2003	Germany	PP776907	—	—	—	—	—	—	PP763532	PP783633	This study
Peronospora myosotidis	GLM-F75125	*Myosotis arvensis*	1997	Germany	PP776908	—	—	—	—	—	—	PP763533	—	This study
Peronospora myosotidis	GLM-F78086	*Myosotis arvensis*	2002	Germany	PP776909	—	—	—	—	—	—	PP763534	PP783634	This study
Peronospora myosotidis	GLM-F63054	*Myosotis arvensis*	2004	Germany	PP776910	—	—	KJ654079	KJ654228	—	—	PP763535	—	Choi et al. 2015a
Peronospora myosotidis	WU22912	*Myosotis arvensis*	1999	Austria	AY198263	—	—	—	—	—	—	—	—	Voglmayr 2003
Peronospora noli-me-oblivisci	GLM-F68067	*Myosotis discolor*	1993	Germany	PP528459	PP528482	PP531600	PP544736	PP544758	PP544714	PP544693	PP763536	—	This study
Peronospora noli-me-oblivisci	GLM-F73687	*Myosotis discolor*	2001	Germany	PP528460	PP528483	PP531601	PP544737	PP544759	PP544715	PP544694	PP763537	—	This study
Peronospora noli-me-oblivisci	GLM-F77872	*Myosotis discolor*	1999	Germany	PP528461	PP528484	PP531602	PP544738	PP544760	PP544716	PP544695	PP763538	—	This study
Peronospora oranien-baumensis	GLM-F73470	*Myosotis sparsiflora*	2003	Germany	PP528462	PP528485	PP531603	PP544739	PP544761	PP544717	PP544696	PP763539	PP783635	This study
Peronospora oranien-baumensis	GLM-F73919	*Myosotis sparsiflora*	1998	Germany	PP528463	PP528486	PP531604	PP544740	PP544762	PP544718	PP544697	PP763540	PP783636	This study
Peronospora oranien-baumensis	GLM-F74307	*Myosotis sparsiflora*	2003	Germany	PP528464	PP528487	PP531605	PP544741	PP544763	PP544719	PP544698	PP763541	PP783637	This study
Peronospora oranien-baumensis	GLM-F69949	*Myosotis sparsiflora*	2004	Germany	PP776911	—	—	—	—	—	—	PP763542	PP783638	This study
Peronospora oranien-baumensis	GLM-F73478	*Myosotis sparsiflora*	2003	Germany	PP776912	—	—	—	—	—	—	PP763543	PP783639	This study
Peronospora oranien-baumensis	GLM-F73483	*Myosotis sparsiflora*	2003	Germany	PP776913	—	—	—	—	—	—	PP763544	PP783640	This study

Table 1 (continued)

Species	Herb[a] No.	Host	Year	Country	Genbank Accession Number									Reference
					18 s + ITS	**LSU D1-3**	**LSU D6-8**	***COX*1**	***COX*2**	***NAD1***	***RPS10***	**ITS (host)**	***matK* (host)**	
Peronospora oranien-baumensis	GLM-F75652	*Myosotis sparsiflora*	2005	Germany	PP776914	—	—	—	—	—	—	PP763545	PP783641	This study
Peronospora oranien-baumensis	GLM-F76865	*Myosotis sparsiflora*	2000	Germany	PP776915	—	—	—	—	—	—	PP763546	PP783642	This study
Peronospora oranien-baumensis	GLM-F76867	*Myosotis sparsiflora*	2000	Germany	PP776916	—	—	—	—	—	—	PP763547	PP783643	This study
Peronospora oranien-baumensis	GLM-F77823	*Myosotis sparsiflora*	1999	Germany	PP776917	—	—	—	—	—	—	PP763548	—	This study
Peronospora oranien-baumensis	GLM-F78105	*Myosotis sparsiflora*	2002	Germany	PP776918	—	—	—	—	—	—	PP763549	PP783644	This study
Peronospora quantula	GLM-F64083	*Myosotis ramosis-sima*	2004	Germany	PP528465	PP528488	PP531606	PP544742	PP544764	PP544720	PP544699	PP763550	PP783645	This study
Peronospora quantula	GLM-F64090	*Myosotis ramosis-sima*	2004	Germany	PP528466	PP528489	PP531607	PP544743	PP544765	PP544721	PP544700	PP763551	PP783646	This study
Peronospora quantula	GLM-F64114	*Myosotis ramosis-sima*	2004	Germany	PP528467	PP528490	PP531608	PP544744	PP544766	PP544722	PP544701	PP763552	PP783647	This study
Peronospora quantula	GLM-F67983	*Myosotis ramosis-sima*	1995	Germany	PP776919	—	—	—	—	—	—	PP763553	PP783648	This study
Peronospora quantula	GLM-F68131	*Myosotis ramosis-sima*	1994	Germany	PP776920	—	—	—	—	—	—	PP763554	—	This study
Peronospora quantula	GLM-F73675	*Myosotis ramosis-sima*	2001	Germany	PP776921	—	—	—	—	—	—	PP763555	—	This study
Peronospora quantula	GLM-F73729	*Myosotis ramosis-sima*	2001	Germany	PP776922	—	—	—	—	—	—	PP763556	PP783649	This study
Peronospora quantula	GLM-F74249	*Myosotis ramosis-sima*	2001	Germany	PP776923	—	—	—	—	—	—	PP763557	PP783650	This study

Table 1 (continued)

Species	Herb[a] No.	Host	Year	Country	Genbank Accession Number									Reference
					18 s + ITS	**LSU D1-3**	**LSU D6-8**	***COX*1**	***COX*2**	***NAD1***	***RPS10***	**ITS (host)**	***matK* (host)**	
Peronospora quantula	GLM-F75691	*Myosotis ramosissima*	2005	Germany	PP776924	—	—	—	—	—	—	PP763558	PP783651	This study
Peronospora quantula	GLM-F76870	*Myosotis ramosissima*	2000	Germany	PP776925	—	—	—	—	—	—	PP763559	—	This study
Peronospora quantula	GLM-F77851	*Myosotis ramosissima*	1999	Germany	PP776926	—	—	—	—	—	—	PP763560	PP783652	This study
Peronospora quantula	GLM-F78027	*Myosotis ramosissima*	2002	Germany	PP776927	—	—	—	—	—	—	PP763561	PP783653	This study
Peronospora quantula	GLM-F78097	*Myosotis ramosissima*	2002	Germany	PP776928	—	—	—	—	—	—	PP763562	PP783654	This study
Peronospora trigonotidis	SMK19287	*Trigonotis peduncularis*	2002	Korea	AY608611	—	—	—	—	—	—	—	—	Choi et al. 2005
Peronospora violacea	WU 22942	*Knautia arvensis*	2000	Austria	AY198297	—	—	—	—	—	—	—	—	Voglmayr 2003
Pseudoperonospora cubensis	GLM-F49006	*Cucumis sp.*	1997	Germany	MH730807	MH730848	MH730828	MH730767	MH730773	MH730869	MH730893	—	—	Thines et al. 2019

Holotype of new species introduced in this study are printed in bold

[a]Acronyms of herbarium collections: GLM, Senckenberg Gesellschaft für Naturforschung: Senckenberg Museum für Naturkunde Görlitz, Görlitz, Germany; IC, Blávík Arctic Research Station, Blávík on Fáskrúðsfjörður, Fjarðabyggð, Iceland

Morphology

Morphological characteristics of conidiophores, conidia, and oospores were investigated using dried herbarium specimens, as described in Mu et al. (2024). Briefly, conidiophores were scratched from dried herbarium specimens into lactic acid solution, heated, and covered with coverslips for observation using a Zeiss Imager M2 (Zeiss, Oberkochen, Germany) microscope equipped with differential interference contrast. Measurements are reported as (minimum)standard deviation towards the minimum–mean–standard deviation towards the maximum(maximum).

Results

Phylogenetic analyses

The phylogenetic relationships were based on a concatenated alignment that did not show a strongly supported conflicting topology as compared to individual gene trees from two nuclear loci (ITS, nrLSU) and four mitochondrial loci (*COX*2, *COX*1, *NAD1*, and *RPS10*). Therefore, only the phylogeny based on the concatenation of all loci is shown in Fig. 1. The final concatenated alignment contained a total of 21 specimens that had all loci amplified and included,

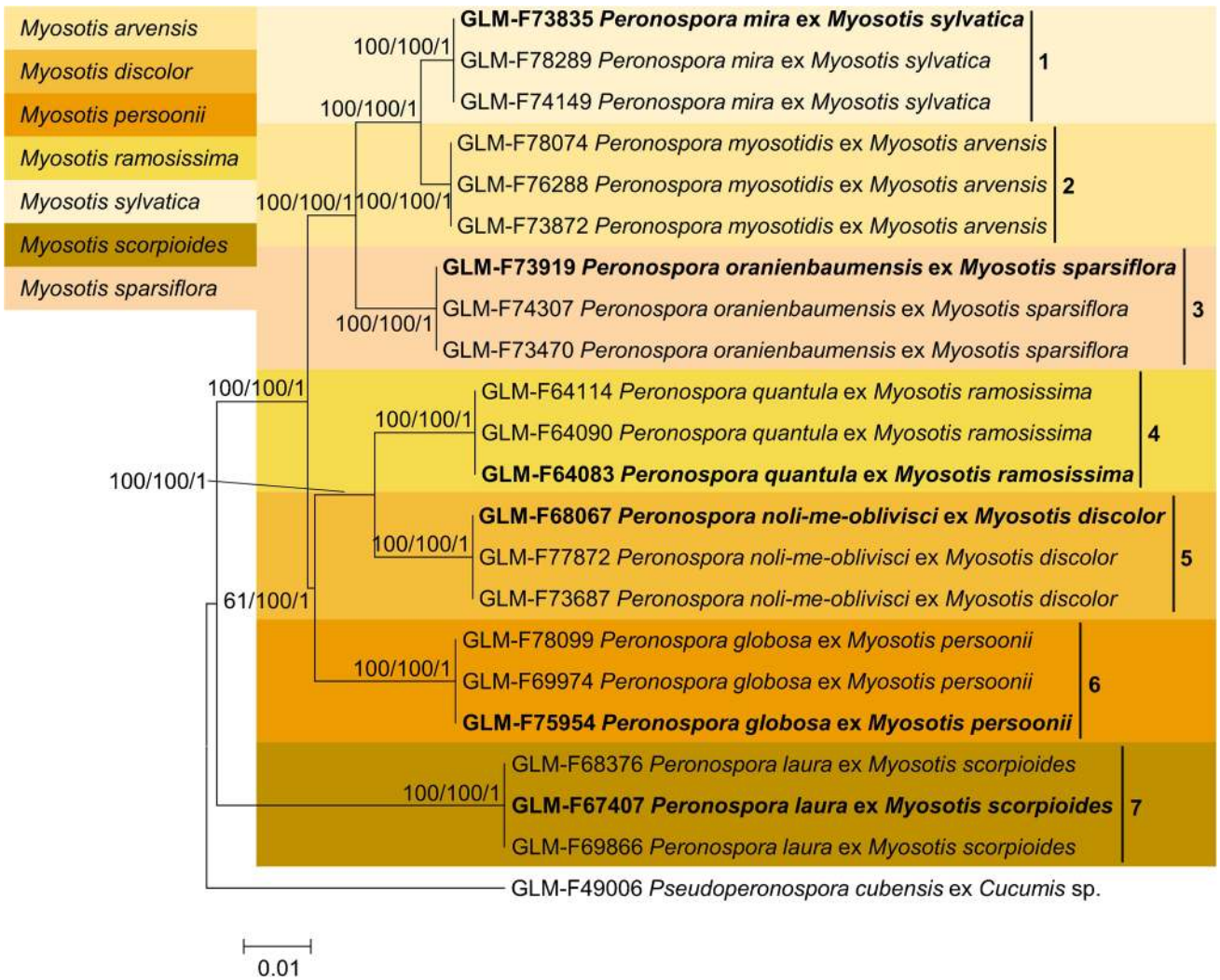

Fig. 1 Maximum likelihood tree of *Peronospora* species on *Myosotis* based on a concatenated alignment of two nuclear (ITS, nrLSU) and four mitochondrial loci (cytochrome c oxidase subunit II and I, NADH dehydrogenase subunit I, ribosomal protein S10) with *Pseudoperonospora cubensis* as the outgroup. Bootstrapping support values of Maximum Likelihood and Minimum Evolution methods (BP ≥ 60%) and Bayesian posterior probabilities (PP ≥ 0.90) are indicated along branches. The scale bar equals the number of nucleotide substitutions per site. Ex-holotype sequences of the new species described in this study are printed in bold type

with an overall alignment length of 5278 aligned sites. The phylogenetic reconstructions in ME, ML and BI using this dataset showed no significant conflicts in the topologies, and the tree from the ML inference is presented in Fig. 1 with highly supported values from all analyses. In addition, separate phylogenetic trees based on these ITS, LSU D1-3, LSU D 6–8, *COX*2, *COX*1, *NAD1*, and *RPS10* loci were constructed to showcase the phylogenetic resolution of these loci (Fig. 2, S1–S6). In total, 7 phylogenetic lineages of *Peronospora* on *Myosotis* were observed, each forming a well-supported group in all phylogenetic trees, corresponding to a particular host. Interestingly, Lineages 1–6 formed a monophyletic clade with high supported values, but Lineage 7 (*Peronospora* sp. on *Myosotis scorpioides*) is closely related to *Peronospora trigonotidis* on *Trigonotis* also belonging to the family *Boraginaceae*. The host identity was confirmed using ITS sequencing and database comparisons.

Morphology

Measurements of morphological characteristics of *Peronospora* species on *Myosotis* are summarized in Table 2. The main morphological characteristics differentiating *Peronospora* were the shape of conidia, as well as the length, breadth, and length-to-breadth ratio of conidia, the length of conidiophores and trunks, as well as the length of the ultimate branchlets. The most prominent features that can be used for the differentiation of individual lineages are given in the following text, but for unequivocal determination, a combination of several characteristics needs to be considered.

Peronospora sp. on *Myosotis persoonii* has the broadest conidia (up to 19.1 µm broad, av. 17.4 µm broad) and the roundest conidia (length-to-breadth ratio of 1.1 on average), as well as the broadest trunks (9.9 µm broad on average) and conidiophore bases (up to 18 µm) (Fig. 3); *Peronospora* sp. on *Myosotis scorpioides* has the shortest conidiophores (449 µm long on average) and the narrowest trunks (7.1 µm broad on average), as well as the longest ultimate branchlets with the longer ones 10.1 µm long on average (Fig. 4); *Peronospora* sp. on *Myosotis sylvatica* has rather narrow conidiophore bases (10 µm broad on average), but can only be distinguished from other species when considering the full set of characteristics (Fig. 5); *Peronospora myosotidis* on *Myosotis arvensis* (syn. *M. intermedia*) has the longest conidiophores (633 µm long on average) and trunks (435 µm long on average) (Fig. 6); *Peronospora* sp. on *Myosotis discolor* has the longest conidia (up to 22.8 µm long) and the highest length-to-breadth aspect ratio of conidia (up to 1.49, av. 1.28) (Fig. 7); *Peronospora* sp. on *Myosotis sparsiflora* has the second longest conidiophores (617 µm long on average) and trunks (412 µm long on average), but can only be distinguished from *Peronospora* sp. on *Myosotis discolor* when considering the full set of characteristics (Fig. 8); *Peronospora* sp. on *Myosotis ramosissima* has the smallest conidia (16.1 × 14.3 µm on average), the shortest ultimate branchlets (the longer ones 7.2 µm long on average, the shorter ones 5.0 µm long on average) (Fig. 9).

Taxonomy

Based on phylogenetic reconstructions and morphological differences, one known species and six new species of *Peronospora* on *Myosotis* are detailed here.

Peronospora globosa M. Mu & Thines, sp. nov. (Fig. 3).

MycoBank: MB 853058.

Etymology: From the Latin *globosus* meaning spherical, referring to the globose conidia.

Description: *Lesions* on leaves yellow–brown to dark grey, diffuse to vein-delimited in *Myosotis persoonii*. *Down* present on the lower leaf surface, consisting of scattered to dense felt-like conidiophore outgrowth. *Conidiophores* hyaline, straight to curved, thin-walled, 383–538–694 µm long, av. 538 µm long; trunk 236–464 µm long, av. 350 µm long, 8.3–11.4 µm broad, sometimes slightly swollen to up to 18 µm at the base, ratio of the total length to trunk length 1.4–1.7, $n = 50$, callose plugs absent. *Branching* subdichotomous in 4–8 orders, with sub-straight to curved branches, gradually attenuate, often at right angles. *Ultimate branchlets* straight to substraight, rarely curved, paired branchlets differing in length, with the longer ones 6.5–11.5 µm long, the shorter ones 4.63–8.49 µm long, with a ratio of the longer to the shorter ultimate branchlets 1.1–1.7, $n = 100$, base 1.9–2.5 µm broad, apex slightly sharp. *Conidia* subglobose to ellipsoid with a pale grey tint, 18.6–20.4 µm long, 16.4–18.3 µm broad, length-to-breadth ratio 1.06–1.19, $n = 100$. *Oospores* not observed.

Diagnosis: Differs from *P. laura* in having larger conidia, longer conidiophores and trunks, broader trunks and conidiophore bases, as well as shorter ultimate branchlets. Differs from *P. mira* in having larger conidia, shorter conidiophores and trunks, broader trunks and conidiophore bases, as well as paired and longer ultimate branchlets. Differs from *P. myosotidis* in having larger conidia, shorter conidiophores and trunks, as well as broader trunks and conidiophore bases. Differs from *P. noli-me-oblivisci* in having rounder conidia, longer conidiophores and trunks, as well as broader trunks and conidiophore bases. Differs from *P. oranienbaumensis* in having larger conidia, shorter conidiophores and trunks, as well as broader trunks and conidiophore bases. Differs from *P. quantula* in having larger conidia, longer conidiophores and trunks, broader trunks and conidiophore bases, as well as longer ultimate branchlets.

Habitat: Living leaves of *Myosotis persoonii*.

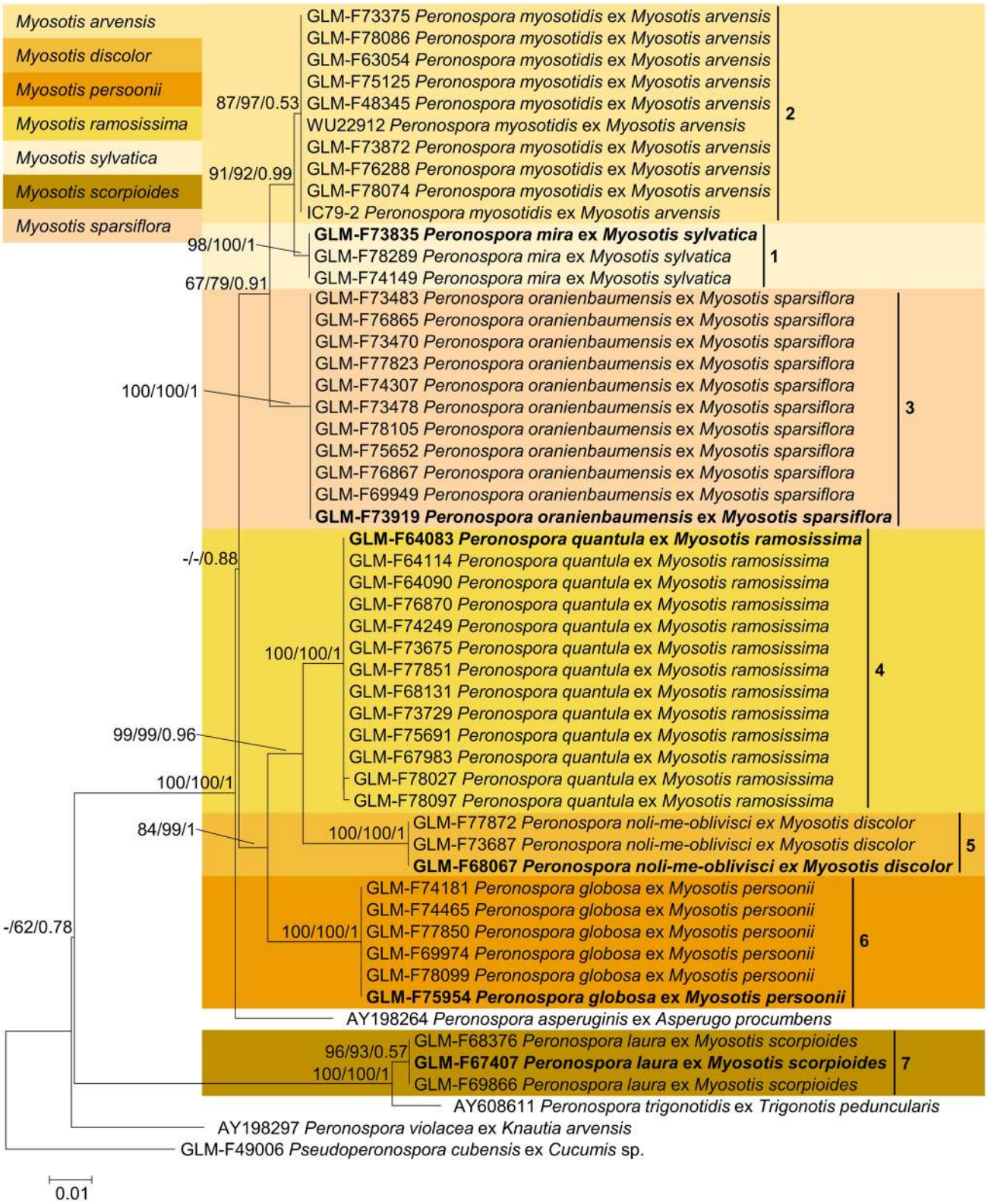

Fig. 2 Maximum likelihood tree of *Peronospora* species on *Myosotis* based on ITS with *Pseudoperonospora cubensis* as outgroup. Bootstrapping support values from Maximum Likelihood and Minimum Evolution analyses ≥ 50%, and Bayesian posterior probabilities ≥ 0.90 are indicated along branches in the respective order. The scale bar equals the number of nucleotide substitutions per site. A dash "–" denotes less than 50% bootstrap support for the presented topology in ME and ML. Ex-holotype sequences of the new species described in this study are printed in bold type

Table 2 Morphological comparison of species of *Peronospora* infecting species of *Myosotis*

Species	Host	Conidiophores ($n = 50$)		Trunks ($n = 50$)	
		Length (µm)	Base (µm)	Length (µm)	Breadth (µm)
Peronospora globosa	*Myosotis persoonii*	(315)383–538–694(944)	11–14–18	(209)236–350–464(719)	(7.8)8.3–9.9–11.4(13.2)
Peronospora laura	*Myosotis scorpioides*	(300)328–449–569(688)	9–12–15	(165)207–301–395(478)	(5.7)6.0–7.1–8.3(9.8)
Peronospora mira	*Myosotis sylvatica*	(324)405–565–725(858)	7–10–13	(176)249–394–539(676)	(6.5)7.2–8.7–10.2(11.4)
Peronospora myosotidis	*Myosotis arvensis*	(369)489–633–777(905)	7–11–15	(211)324–435–547(683)	(5.1)6.4–7.6–8.7(10.3)
Peronospora noli-me-oblivisci	*Myosotis discolor*	(252)350–474–599(707)	7–10–12	(139)211–326–442(557)	(5.9)6.5–7.8–9.1(10.8)
Peronospora oranienbaumensis	*Myosotis sparsiflora*	(328)421–617–813(1047)	7–12–17	(205)262–412–562(732)	(5.1)5.7–8.4–11.0(14.0)
Peronospora quantula	*Myosotis ramosissima*	(154)331–499–668(808)	6–11–15	(178)219–327–434(509)	(6.5)7.6–9.3–11.0(13.4)

Species	Conidiophores/trunks	Branching orders	Ultimate Branchlets (UB) ($n = 100$)		
			Length of the longer UB (µm)	Length of the shorter UB (µm)	Longer/shorter ratio
Peronospora globosa	(1.2)1.4–1.6–1.7(2.0)	4–8	(4.22)6.51–9.03–11.54(14.30)	(2.91)4.63–6.56–8.49(10.77)	(1.00)1.13–1.41–1.68(1.99)
Peronospora laura	(1.3)1.4–1.5–1.7(1.8)	4–7	(5.89)7.44–10.06–12.68(16.65)	(3.75)4.31–6.37–8.43(12.50)	(1.00)1.28–1.64–2.00(2.69)
Peronospora mira	(1.2)1.3–1.5–1.7(2.0)	4–9	(4.35)5.35–7.83–10.32(13.33)	(2.49)3.73–5.26–6.79(9.91)	(1.02)1.22–1.50–1.79(2.16)
Peronospora myosotidis	(1.2)1.3–1.5–1.6(1.9)	5–8	(4.80)6.99–9.60–12.20(14.21)	(3.51)4.63–6.73–8.84(10.79)	(1.01)1.17–1.47–1.78(2.37)
Peronospora noli-me-oblivisci	(1.2)1.3–1.6–1.8(2.1)	4–8	(4.49)6.38–8.76–11.14(14.30)	(3.46)4.73–6.39–8.05(10.11)	(1.00)1.17–1.39–1.61(1.89)
Peronospora oranienbaumensis	(1.3)1.4–1.5–1.7(2.0)	5–8	(5.92)7.42–9.62–11.83(14.41)	(3.82)4.77–6.21–7.66(9.82)	(1.11)1.29–1.57–1.85(2.49)
Peronospora quantula	(1.4)1.5–1.6–1.8(1.9)	6–9	(4.11)5.56–7.20–8.84(11.04)	(2.55)3.73–4.96–6.19(8.79)	(1.01)1.19–1.49–1.79(2.13)

Species	Conidia ($n = 100$)			Oospores (µm)	Reference
	Length (µm)	Breadth (µm)	Length-to-breadth ratio		
Peronospora globosa	(17.43)18.55–19.46–20.37(21.13)	(15.57)16.41–17.37–18.33(19.13)	(1.02)1.06–1.12–1.19(1.26)	—	This study
Peronospora laura	(15.38)16.45–17.70–18.95(20.35)	(12.50)14.21–15.35–16.48(17.45)	(1.03)1.08–1.16–1.23(1.39)	—	This study
Peronospora mira	(12.97)15.79–17.11–18.42(19.52)	(12.22)13.38–14.35–15.31(16.94)	(1.01)1.11–1.19–1.28(1.45)	—	This study
Peronospora myosotidis	(13.77)15.74–17.27–18.79(20.52)	(12.96)13.86–14.93–15.99(17.03)	(1.01)1.08–1.16–1.23(1.35)	—	This study
Peronospora noli-me-oblivisci	(16.57)19.32–20.64–21.97(22.75)	(13.93)15.20–16.17–17.14(18.64)	(1.05)1.18–1.28–1.38(1.49)	—	This study
Peronospora oranienbaumensis	(14.67)16.11–17.70–19.28(20.57)	(13.27)14.05–15.44–16.84(18.23)	(1.06)1.08–1.15–1.22(1.30)	27.5–30.2–32.8	This study
Peronospora quantula	(14.30)15.00–16.09–17.18(18.67)	(11.68)13.27–14.30–15.32(15.89)	(1.03)1.05–1.13–1.21(1.35)	—	This study

Distribution: Germany.

Typus: Germany, Saxony-Anhalt, Helme-Unstrut Buntsandsteinland, on living leaves of *Myosotis persoonii*, 5 Nov. 2005, H. Jage, holotype GLM-F75954.

Additional specimens examined: Germany, Brandenburg, Naundorfer Morl, on living leaves of *Myosotis persoonii*, 28 Apr. 2015, H. Jage, GLM-F69974; Saxony-Anhalt, Annaburger Heide, on living leaves of *Myosotis persoonii*, 23 May 1999, H. Jage, GLM-F77850; Dessau Elbetal, on living leaves of *Myosotis persoonii*, 9 May 2002, H. Jage, GLM-F78099; Dübener Heide, on living leaves of *Myosotis persoonii*, 13 May 2001, H. Jage, GLM-F74181; Roßlau-Wittenberger Vorfläming, on living leaves of *Myosotis persoonii*, 5 Nov. 2003, H. Jage, GLM-F74465.

Note: *Myosotis persoonii* has a distribution range that includes the western Mediterranean, Spain, Portugal, and the border area between Spain and France (Tutin et al. 1972). In the phylogenetic tree, *Peronospora globosa* forms a highly supported clade with *P. quantula* and *P. noli-me-oblivisci*. They share a common characteristic of shorter conidiophores and trunks, with conidiophores measuring shorter than 550 µm on average and trunks shorter than 350 µm. However, *P. globosa* differs from *P. quantula* and *P. noli-me-oblivisci* in terms of its broader and rounder conidia, with an average breadth of 17.37 µm and a length-to-breadth ratio of 1.12 ± 0.1.

Peronospora laura M. Mu & Thines, sp. nov. (Fig. 4).

MycoBank: MB 853059.

Etymology: Refers to Latin *laura* (*Ruta angustifolia*) to which inflorescences the sporangiophores bear similarity.

Description: *Lesions* on leaves initially chlorotic, later often turning yellowish brown to dark brown when dried, diffuse to vein-delimited in *Myosotis scorpioides*. *Down* present on the lower leaf surface, consisting of scattered to dense felt-like conidiophore outgrowth. *Conidiophores* hyaline, straight to curved, thin-walled, 328–569 µm long, av. 449 µm long; trunk 207–395 µm long, av. 301 µm long, 6.0–8.3 µm broad, sometimes slightly swollen to up to 15 µm at the base, ratio of the total length to trunk length 1.4–1.7, $n = 50$, callose plugs absent. *Branching* subdichotomous in 4–7 orders, with sub-straight to curved branches, gradually attenuate, often at right angles. *Ultimate branchlets* substraight to curved, paired branchlets differing in length, with the longer ones 7.4–12.7 µm long, the shorter ones 4.3–8.4 µm long, with a ratio of the longer to the shorter ultimate branchlet of 1.3–2.0, $n = 100$, base 1.7–2.2 µm broad, apex slightly sharp. *Conidia* ellipsoid to obovate with a pale yellow–brown to brown tint, 16.5–19.0 µm long, 14.2–16.5 µm broad, length-to-breadth ratio 1.1–1.2, $n = 100$, basal part of the conidia mostly protruding. *Oospores* not observed.

Diagnosis: Differs from *P. mira* in having shorter and conidiophores and trunks, narrower trunks, as well as paired and longer ultimate branchlets. Differs from *P. myosotidis* in having larger conidia, shorter conidiophores and trunks, as well as narrower trunks and conidiophore bases. Differs from *P. noli-me-oblivisci* in having smaller conidia, shorter conidiophores and trunks, as well as narrower trunks. Differs from *P. oranienbaumensis* in having shorter conidiophores and trunks, narrower trunks, as well as longer ultimate branchlets. Differs from *P. quantula* in having larger conidia, shorter conidiophores and trunks, as well as narrower trunks. Differs from *P. globosa* in having smaller conidia, shorter conidiophores and trunks, as well as narrower trunks and conidiophore bases.

Habitat: Living leaves of *Myosotis scorpioides* (syn. *Myosotis palustris*).

Distribution: Germany.

Typus: Germany, Saxony, Tellerhäuser, on living leaves of *Myosotis scorpioides*, 11 Aug. 1987, H. Jage, holotype GLM-F67407.

Additional specimens examined: Germany, Saxony, Oberwiesenthal, on living leaves of *Myosotis scorpioides*, 15 Aug. 1985, H. Jage, GLM-F68376; Saxony-Anhalt, Gaditz, on living leaves of *Myosotis scorpioides*, 22 Sep. 1980, H. Jage, GLM-F69866.

Note: *Myosotis scorpioides* is widely distributed in the Northern Hemisphere (GBIF 2024). In the phylogenetic tree, *Peronospora laura* did not cluster with the other six *Peronospora* species on *Myosotis*. Interestingly, it is closely related to *Peronospora trigonotidis* on *Trigonotis peduncularis* within the same family, *Boraginaceae*, but apart from being phylogenetically distinct, *P. trigonotidis* has longer conidiophores (up to 800 µm long), as well as larger conidia (averaging 22 × 19 µm). Morphologically, compared to all *Peronospora* species on *Myosotis*, *Peronospora laura* exhibits the shortest conidiophores (449 µm long on average) and narrowest trunks (7.1 µm broad on average).

Peronospora mira M. Mu & Thines, sp. nov. (Fig. 5).

MycoBank: MB 853060.

Etymology: From Latin *mira* (remarkable, wonderful) highlighting the fact that it is a miracle that this species has been overlooked despite its widespread occurrence.

Description: *Lesions* on leaves initially chlorotic, later turning yellowish-brown or reddening, finally often dark brown upon drying, sometimes vein-delimited in *Myosotis sylvatica*. *Down* present on the lower leaf surface, consisting of scattered to dense felt-like conidiophore outgrowth. *Conidiophores* hyaline, straight to curved, thin-walled, 405–725 µm long, av. 565 µm long; trunk mostly 249–539 µm long, av. 394 µm long, 7.2–10.2 µm broad, sometimes slightly swollen to up to 13 µm at the base,

Fig. 3 Symptoms and morphology of *Peronospora globosa* parasitic on *Myosotis persoonii*. **a** Down on the leaf surface. **b–d** Conidium. **e–g** Ultimate branchlets. **h–k** Conidiophores. Scale bars: b–g = 20 µm; h–k = 50 µm

ratio of the total length to trunk length1.3–1.7, $n = 50$, callose plugs absent. *Branching* subdichotomous in 4–9 orders, with sub-straight to curved branches, gradually attenuate, often at right angles. *Ultimate branchlets* sub-straight to curved, paired branchlets differing in length, with the longer ones 5.4–10.3 µm long, the shorter ones 3.7–6.8 µm long, with a ratio of the longer to the shorter ultimate branchlets 1.2–1.8, $n = 100$, base 1.8–2.6 µm broad, apex slightly sharp. *Conidia* subglobose to ellipsoid with a pale brown-violet to grey tint, 15.8–18.4 µm long, 13.4–15.3 µm broad, length-to-breadth ratio 1.1–1.3, $n = 100$, basal part of the conidia mostly protruding. *Oospores* not observed.

Diagnosis: Differs from *P. laura* in having longer conidiophores and trunks, as well as shorter ultimate branchlets. Differs from *P. myosotidis* in having shorter conidiophores and trunks, as well as shorter ultimate branchlets. Differs from *P. noli-me-oblivisci* in having smaller conidia, longer conidiophores and trunks, as well as shorter ultimate branchlets. Differs from *P. oranienbaumensis* in having smaller conidia, shorter conidiophores and trunks, as well as shorter ultimate branchlets. Differs from *P. quantula* in having longer conidiophores and trunks, narrower trunks and conidiophore bases, as well as longer ultimate branchlets. Differs from *P. globosa* in having smaller conidia, narrower trunks and conidiophore bases, as well as shorter ultimate branchlets.

Habitat: Living leaves of *Myosotis sylvatica*.

Distribution: Germany.

Typus: Germany, Saxony-Anhalt, Bad Schmiedeberg, on living leaves of *Myosotis sylvatica*, 29 Sep. 2001, H. Jage, holotype GLM-F73835.

Additional specimens examined: Germany, Saxony-Anhalt, Selbitz, on living leaves of *Myosotis sylvatica*, 9 Jun 1999, H. Jage, GLM-F78289; Uthausen, Mark Pannewitz, on living leaves of *Myosotis sylvatica*, 8 May 2001, H. Jage, GLM-F74149.

Note: *Myosotis sylvatica* is mainly distributed in North America and Europe (Kew Science 2024).

Fig. 4 Symptoms and morphology of *Peronospora laura* parasitic on *Myosotis scorpioides*. **a** Down on the leaf surface. **b** Conidium. **c–e** Ultimate branchlets. **f–h** Conidiophores. Scale bars: b–e = 20 µm; f–h = 50 µm

Peronospora mira shows a close relationship with *P. myosotidis* in the phylogenetic tree. Moreover, they exhibit similarities in morphology, making it challenging to distinguish between them. However, there are still some discernible differences between them. *Peronospora mira* has shorter conidiophores and trunks (with an average length of 565 µm and 394 µm, respectively) as well as shorter ultimate branchlets.

Peronospora myosotidis de Bary, Annls Sci. Nat., Bot., sér. 4 20: 112. 1863 (Fig. 6).

= *Peronospora myosotidis* var. *minor* Benua, in Jaczewski & Jaczewski, Opredelitel' Gribov. Sovershennye Griby (Diploidnye Stadii). I. Fikomitsety (Moscow): 129. 1931.

Description: *Lesions* on leaves first chlorotic, later yellow–brown or reddening, sometimes dark grey when dried, diffuse to vein-delimited in *Myosotis arvensis*.

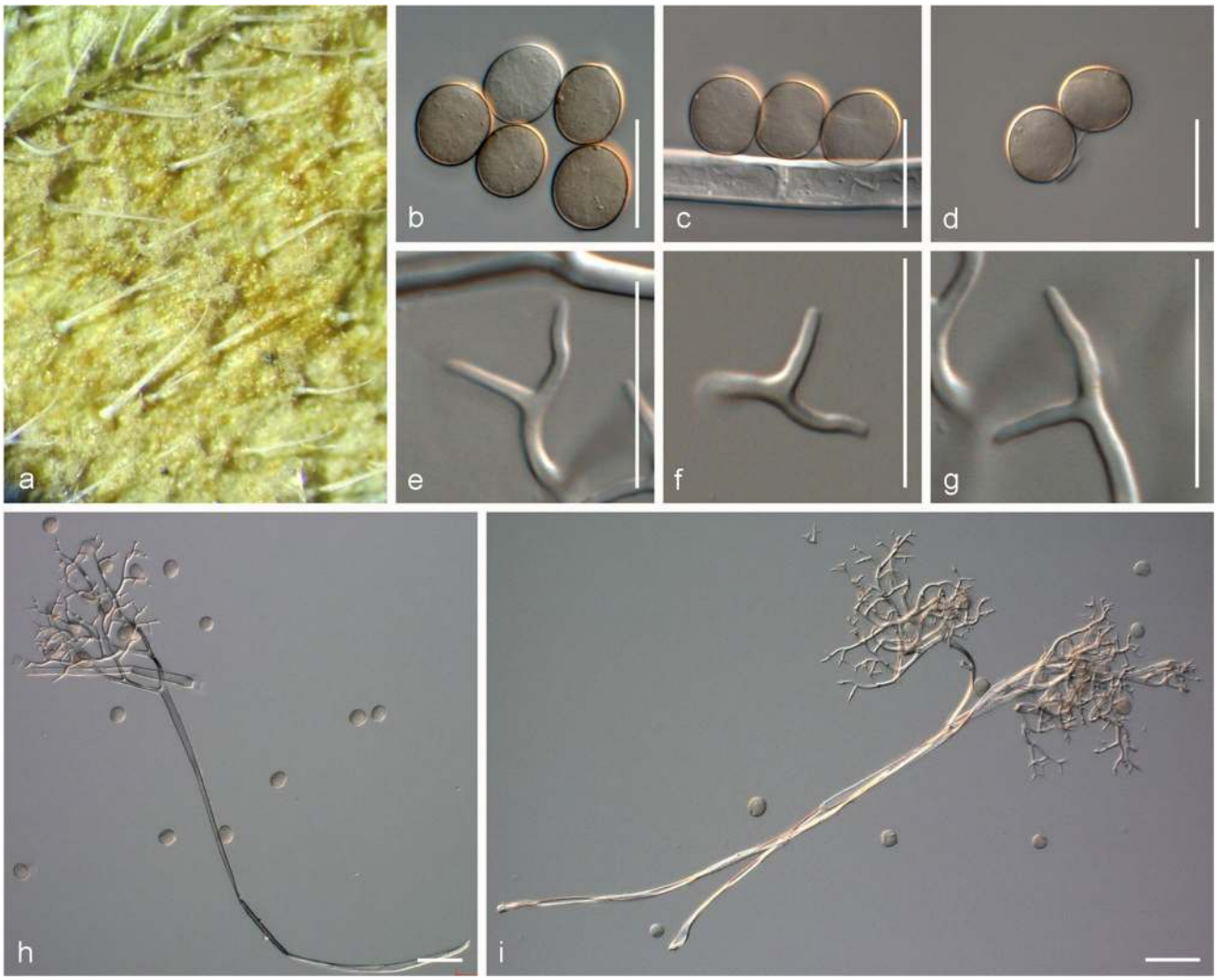

Fig. 5 Symptoms and morphology of *Peronospora mira* parasitic on *Myosotis sylvatica*. **a** Down on the leaf surface. **b–d** Conidium. **e–g** Ultimate branchlets. **h–i** Conidiophores. Scale bars: b–g = 20 µm; h–i = 50 µm

Down present on the lower leaf surface, consisting of scattered to dense felt-like conidiophore outgrowth. *Conidiophores* hyaline, straight to curved, thin-walled, 489–777 µm long, av. 633 µm long; trunk 324–547 µm long, av. 435 µm long, 6.4–8.7 µm broad, sometimes slightly swollen to up to 15 µm at the base, ratio of the total length to trunk length 1.3–1.6, $n = 50$, callose plugs absent. *Branching* subdichotomous in 5–8 orders, with sub-straight to curved branches, gradually attenuate, often at right angles. *Ultimate branchlets* straight to substraight, rarely curved, paired branchlets differing in length, with the longer ones 7.0–12.2 µm long, the shorter ones 4.6–8.8 µm long, with a ratio of the longer to the shorter ultimate branchlets 1.2–1.9, $n = 100$, base 1.7–2.4 µm broad, apex slightly sharp. *Conidia* subglobose to ellipsoid with a yellow–brown to pale grey tint, 15.7–18.8 µm long, 13.9–16.0 µm broad, length-to-breadth ratio 1.1–1.2, $n = 100$, basal part of the conidia mostly protruding. *Oospores* not observed**.**

Type host: *Myosotis arvensis* (given under the synonym *M. intermedia* in the original description; de Bary 1863).

Confirmed habitat: Living leaves of *Myosotis arvensis*.

Reported hosts: *M. arvensis* (= *M. intermedia*; de Bary 1863; Stevenson 1926; Voglmayr 2003; Choi et al. 2015a), *Myosotis verna* (Rogerson 1958), *M. arenaria*, *M. hispida*, *M. paulstris*, *M. sylvatica*, *M. sparsiflora*, *M. stricta*, *M. versicolor* (Stevenson 1926), *M. caespitosa* (Adamska 2001), *M. nemorosa* (Dietrich and Müller 2001), *Amsinckia* (fiddlenecks) (Pantone et al. 1985) and *Brunnera macrophylla* (Lane et al. 2006) of *Boraginaceae*.

Reported distribution: Germany (de Bary 1863; Gäumann 1923), Switzerland (de Bary 1863), UK (Gäumann 1923; Lane et al. 2006), Austria (Gäumann 1923; Voglmayr 2003), Denmark, Norway, Russia, Sweden (Gäumann 1923),

Fig. 6 Symptoms and morphology of *Peronospora myosotidis* parasitic on *Myosotis arvensis*. **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

New Zealand (Jafar 1962; Gjaerum 1980), Czech Republic (Dietrich and Müller 2001).

Additional specimens examined: Germany, Hessen, Burghaun, on living leaves of *Myosotis arvensis*, 22 Jul. 2000, H. Jage, GLM-F48345; Saxony-Anhalt, Düben-Dahlener Heiden, on living leaves of *Myosotis arvensis*, 6 Sep. 1997, *H. Jage,* GLM-F75125; Dübener Heide, on living leaves of *Myosotis arvensis*, 18 May 2002, H. Jage, GLM-F78074; Helme-Unstrut Buntsandsteinland, Loderleben, Quernetal, on living leaves of *Myosotis arvensis*, 9 Apr. 2004, H. Jage, GLM-F63054; Ilm-Saale-Muschelkalkplatten, on living leaves of *Myosotis arvensis*, 18 Jun. 2003, *H. Jage,* GLM-F73375; Ländchen, Hohenkamern, on living leaves of *Myosotis arvensis*, 3 Jul. 2002, H. Jage, GLM-F76288; Magdeburger Börde, Calbe, on living leaves of *Myosotis arvensis*, 23 May 1998, *H. Jage,* GLM-F73872; Zerbster Ackerland, on living leaves of *Myosotis arvensis*, 15 May 2002, *H. Jage,* GLM-F78086; Iceland, Múlaþing, Hallormsstaður, near Kliftjörn, on living leaves of *Myosotis arvensis*, 05. Aug. 2023, M. Thines, IC79–2.

Note: *Peronospora myosotidis* is widely distributed in Europe (de Bary 1863; Gäumann 1923; Stevenson 1926; Dietrich and Müller 2001; Voglmayr 2003; Lane et al. 2006; Choi et al. 2015a) and in Oceania (Jafar 1962; Gjaerum 1980). It has been assumed to be parasitic to various *Myosotis* species (de Bary 1863; Stevenson 1926; Rogerson 1958; Adamska 2001; Dietrich and Müller 2001; this study), and two related genera, *Brunnera* and *Amsinckia* of *Boraginaceae*

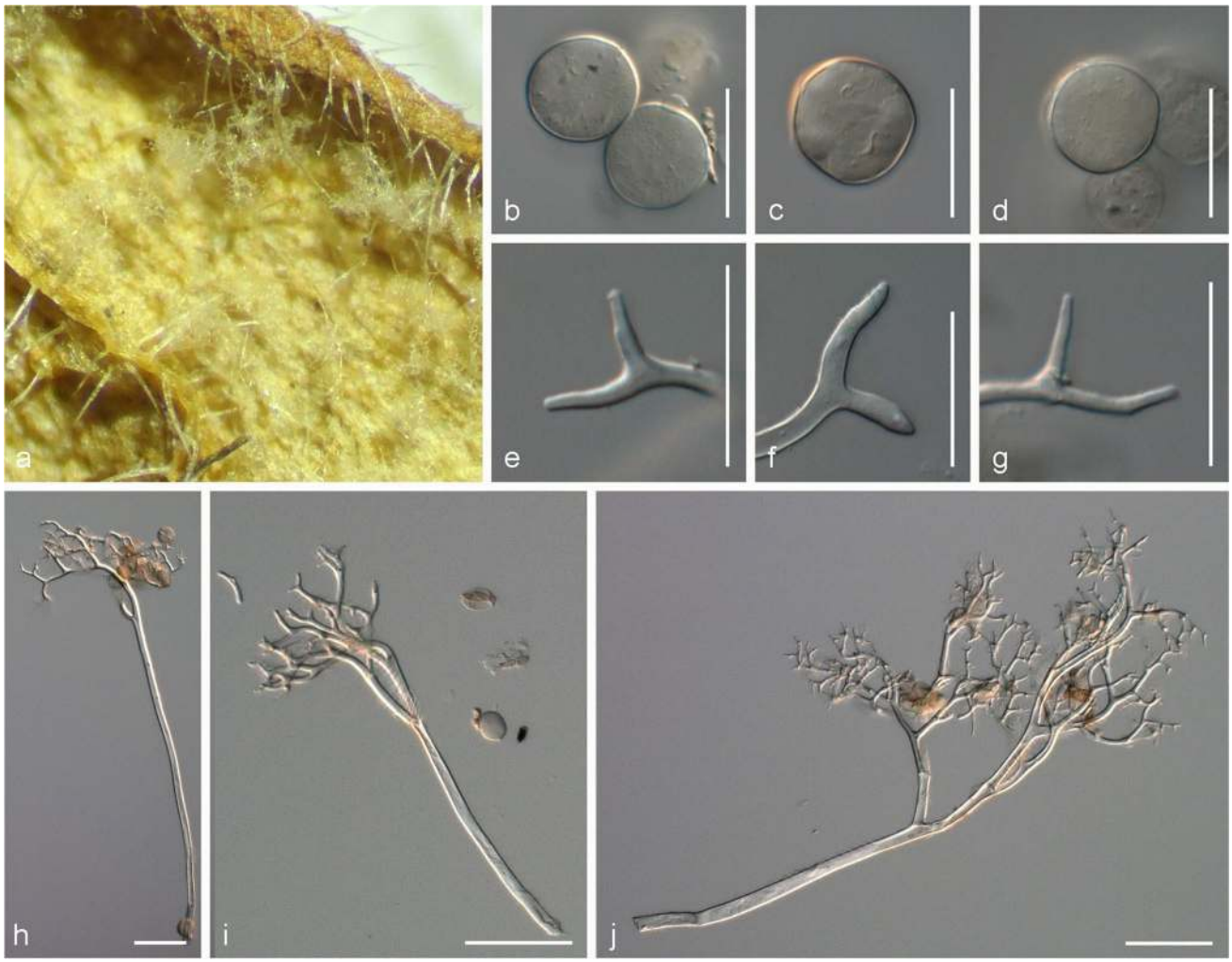

Fig. 7 Symptoms and morphology of *Peronospora noli-me-oblivisci* parasitic on *Myosotis discolor*. **a** Down on the leaf surface. **b–d** Conidia. **e–g** Ultimate branchlets. **h–j** Conidiophores. Scale bars: b–g = 20 µm; h–j = 50 µm

(Pantone et al. 1985; Lane et al. 2006). According to the ITS sequence identity of AY198263 from specimen WU22912 infecting *Myosotis arvensis* (Fig. 2), and the *COX*1 sequence KJ654079 and *COX*2 sequence KJ654228 from specimen GLM-F63054 also infecting *Myosotis arvensis* in Fig. S3–4 (Voglmayr 2003; Choi et al. 2015a), and the large number of SNPs when compared with *Peronospora* from other hosts in the genus *Myosotis*, it can be assumed that *P. myosotidis* might be restricted to infect *Myosotis arvensis*. The morphology in this study is almost identical to the description of de Bary and Lane (de Bary 1863; Lane et al. 2006), with comparative details of the measurements shown in Table 2. In the phylogenetic tree, *P. myosotidis* clustered together in a highly supported clade with *P. mira*. Despite their close relationship in both phylogeny and morphology, *P. myosotidis* can be distinguished by having longer conidiophores and trunks (average 633 µm long and 435 µm long, respectively), as well as paired and shorter ultimate branchlets.

Peronospora noli-me-oblivisci M. Mu & Thines, sp. nov. (Fig. 7).

MycoBank: MB 853061.

Etymology: Named after the literal translation of the host genus in some German languages.

Description: *Lesions* on leaves yellow–brown to dark brown, often vein-delimited in *Myosotis discolor*. *Down* present on the lower leaf surface, consisting of scattered to dense felt-like conidiophore outgrowth. *Conidiophores* hyaline, straight to curved, thin-walled, 350–599 µm long, av. 474 µm long; trunk 211–442 µm long, av. 326 µm long, 6.5–9.1 µm broad, sometimes slightly swollen to up to 12 µm at the base, ratio of the total length to trunk length 1.3–1.8, $n = 50$, callose plugs absent. *Branching* subdichotomous in 4–8 orders, with sub-straight to curved branches, gradually attenuate, often at right angles. *Ultimate branchlets* straight to substraight, paired branchlets differing in length, with the longer ones 6.4–11.1 µm long, the shorter ones

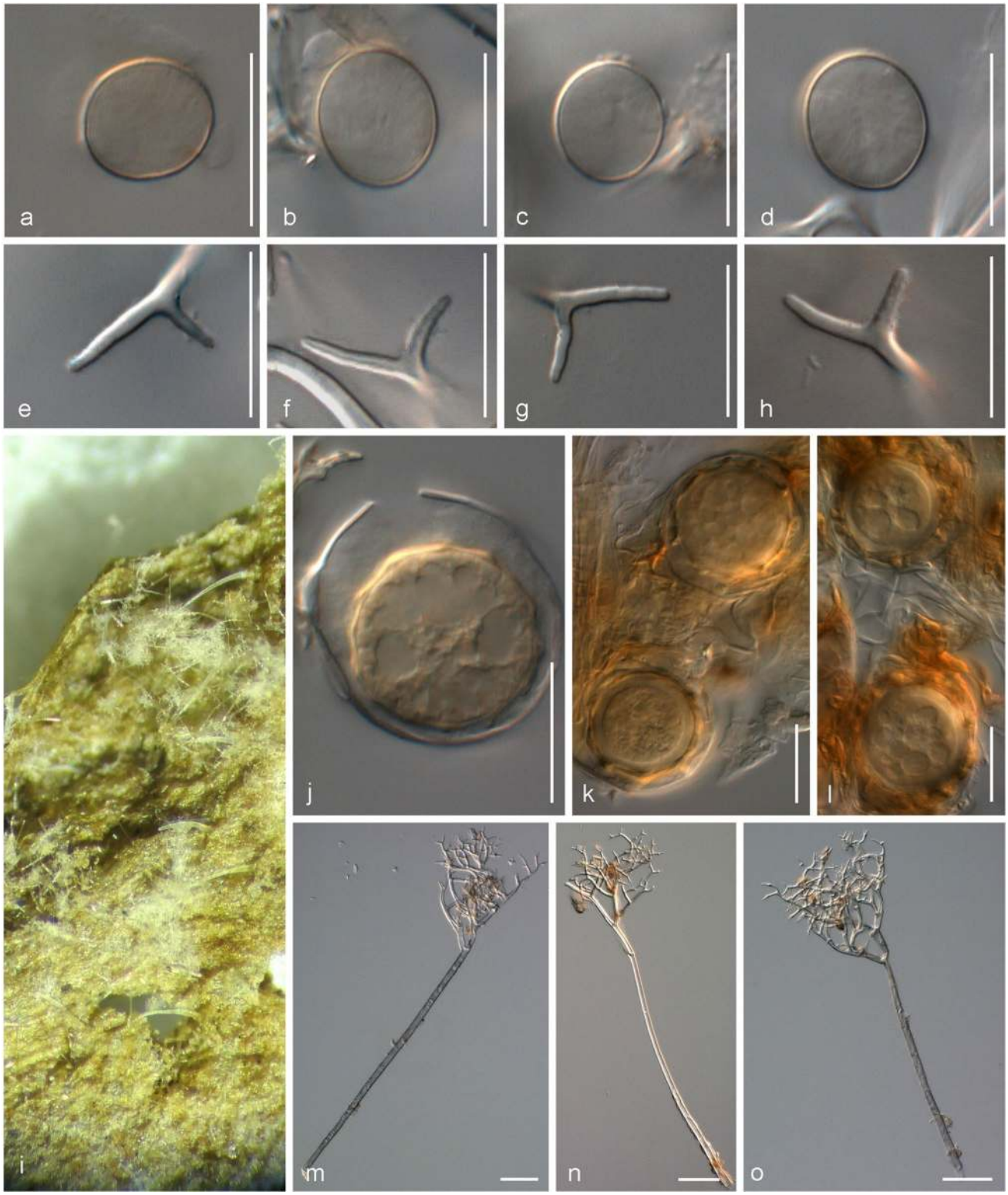

Fig. 8 Symptoms and morphology of *Peronospora oranienbaumensis* parasitic on *Myosotis sparsiflora*. **a–d** Conidium. **e–h** Ultimate branchlets. **i** Down on the leaf surface. **j–l** Oospores. **m–o** Conidiophores. Scale bars: a–h, j–l = 20 µm; h–j = 50 µm

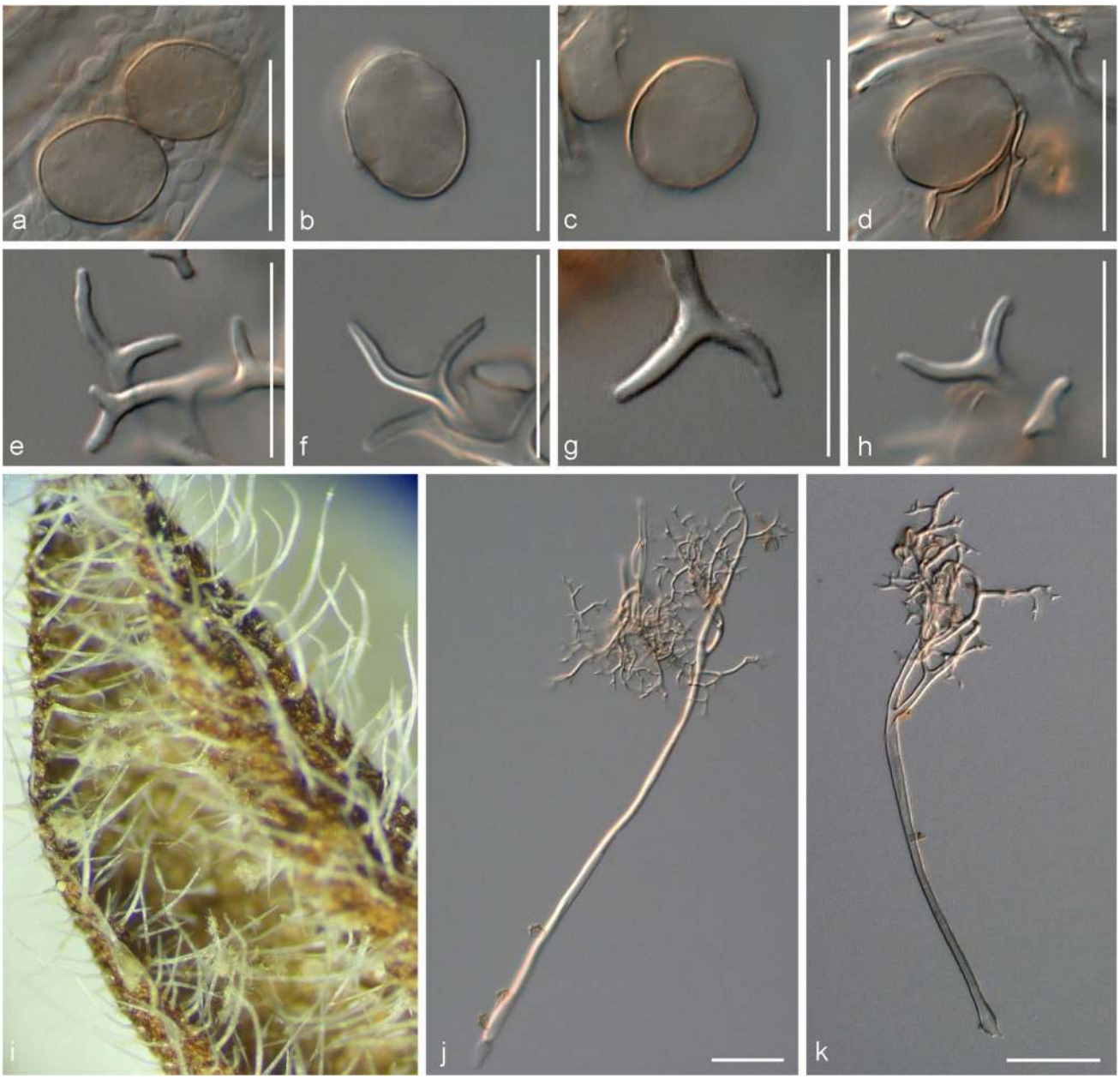

Fig. 9 Symptoms and morphology of *Peronospora quantula* parasitic on *Myosotis ramosissima*. **A–D** Conidium. **E–H** Ultimate branchlets. **I** Down on the leaf surface. **J–K** Conidiophores. Scale bars: a–h = 20 µm; j–k = 50 µm

4.7–8.1 µm long, with a ratio of the longer to the shorter ultimate branchlet 1.2–1.6, $n = 100$, base 1.8–2.4 µm broad, apex slightly sharp. *Conidia* ellipsoid to obovate with a pale grey tint, 19.32–22.0 µm long, 15.2–17.1 µm broad, length-to-breadth ratio 1.2–1.4, $n = 100$. *Oospores* not observed.

Diagnosis: Differs from *P. laura* in having larger and more elongated conidia, as well as longer conidiophores and trunks. Differs from *P. mira* in having larger and more elongated conidia, shorter conidiophores and trunks, as well as paired and longer ultimate branchlets. Differs from *P. myosotidis* in having larger and more elongated conidia, shorter conidiophores and trunks, as well as shorter ultimate branchlets. Differs from *P. oranienbaumensis* in having larger and more elongated conidia, shorter conidiophores and trunks, as well as narrower trunks and conidiophore bases. Differs from *P. quantula* in having larger and more elongated conidia, narrower trunks and conidiophore bases, as well as longer ultimate branchlets. Differs from *P. globosa* in having more elongated conidia, shorter conidiophores and trunks, as well as narrower trunks and conidiophore bases.

Habitat: Living leaves of *Myosotis discolor*.

Distribution: Germany.

Typus: Germany, Saxony-Anhalt, Zahna, on living leaves of *Myosotis discolor*, 15 Apr. 1993, H. Jage, holotype GLM-F68067.

Additional specimens examined: Germany, Saxony-Anhalt, Schiedsberg, on living leaves of *Myosotis discolor*, 18 May 2001, H. Jage, GLM-F73687; Zahna, Roßlau-Wittenberger Vorfläming, on living leaves of *Myosotis discolor*, 1 May 1999, H. Jage, GLM-F77872.

Note: *Myosotis discolor* is primarily distributed in Europe and North America (GBIF 2024). *Peronospora noli-me-oblivisci* clusters closely with *P. quantula*, demonstrating strong support for their relationship in the phylogenetic trees. They also exhibit similarities in morphological features. However, *P. noli-me-oblivisci* distinguishes itself by having more elongated conidia, with an average length of 20.6 µm and a length-to-breadth ratio of conidia of 1.28 ± 0.1.

Peronospora oranienbaumensis M. Mu & Thines, sp. nov. (Fig. 8).

MycoBank: MB 853062.

Etymology: Specific epithet refers to the town in which the type specimen has been found and in which the host plant occurs frequently.

Description: *Lesions* on leaves yellow–brown to dark, diffuse to vein-delimited in *Myosotis sparsiflora*. *Down* present on the lower leaf surface, consisting of scattered to dense felt-like conidiophore outgrowth. *Conidiophores* hyaline, straight to curved, thin-walled, 421–813 µm long, av. 617 µm long; trunk 262–562 µm long, av. 412 µm long, 5.7–11.0 µm broad, sometimes slightly swollen to up to 17 µm at the base, ratio of the total length to trunk length 1.4–1.7, $n = 50$, callose plugs absent. *Branching* subdichotomous in 5–8 orders, with sub-straight to curved branches, gradually attenuate, often at right angles. *Ultimate branchlets* straight to substraight, rarely curved, paired branchlets differing in length, with the longer ones 7.4–11.8 µm long, the shorter ones 4.8–7.7 µm long, with a ratio of the longer to the shorter ultimate branchlet 1.3–1.9, $n = 100$, base 1.8–2.4 µm broad, apex slightly sharp. *Conidia* subglobose to ellipsoid with a pale brown tint, 16.1–19.3 µm long, 14.1–16.8 µm broad, length-to-breadth ratio 1.1–1.3, $n = 100$. *Oospores* 28–33 µm, $n = 10$.

Diagnosis: Differs from *P. laura* in having longer conidiophores and trunks, broader trunks, as well as shorter ultimate branchlets. Differs from *P. mira* in having larger conidia, longer conidiophores and trunks, as well as paired and longer ultimate branchlets. Differs from *P. myosotidis* in having larger conidia, shorter conidiophores and trunks, as well as broader trunks and conidiophore bases. Differs from *P. noli-me-oblivisci* in having smaller conidia, longer conidiophores and trunks, as well as narrower trunks and conidiophore bases. Differs from *P. quantula* in having larger conidia, longer conidiophores and trunks, as well as longer ultimate branchlets. Differs from *P. globosa* in having smaller conidia, longer conidiophores and trunks, as well as narrower trunks and conidiophore bases.

Habitat: Living leaves of *Myosotis sparsiflora*.

Distribution: Germany.

Typus: Germany, Saxony-Anhalt, Oranienbaum, on living leaves of *Myosotis sparsiflora*, 30 Mar. 1998, H. Jage, holotype GLM-F73919.

Additional specimens examined: Germany, Saxony-Anhalt, Dessauer Elbetal, on living leaves of *Myosotis sparsiflora*, 13 Oct. 2004, H. Jage, GLM-F69949; Hallesches Ackerland, on living leaves of *Myosotis sparsiflora*, 11 Apr. 1999, H. Jage, GLM-F77823; Lobitzsch, on living leaves of *Myosotis sparsiflora*, 6 May 2003, H. Jage, GLM-F73470; Lützen-Hohenmölsener Platte, on living leaves of *Myosotis sparsiflora*, 9 May 2003, H. Jage, GLM-F73483; Magdeburger Börde, on living leaves of *Myosotis sparsiflora*, 8 Apr. 2000, H. Jage, GLM-F76865; Querfurter Platte, on living leaves of *Myosotis sparsiflora*, 7 May 2003, H. Jage, GLM-F73478; Roßlau-Wittenberger Vorfläming, on living leaves of *Myosotis sparsiflora*, 23 May 2002, H. Jage, GLM-F78105; Tangermünder Elbetal, on living leaves of *Myosotis sparsiflora*, 31 May 2005, H. Jage, GLM-F75652; Wörlitz northeast, Auengehölz, on living leaves of *Myosotis sparsiflora*, 20 May 2003, H. Jage, GLM-F74307; Zerbster Ackerland, on living leaves of *Myosotis sparsiflora*, 9 Apr. 2000, H. Jage, GLM-F76867.

Note: *Myosotis sparsiflora* primarily inhabits Eurasia (GBIF 2024). It often has a limp stem and is fragile, with very sparse hair and many branches. *Peronospora oranienbaumensis* clusters closely with *P. myosotidis* and *P. mira*, showing strong support for their relationship in the phylogenetic trees. They form a distinct clade characterized by conidiophores and trunks, with conidiophores measuring longer than 550 µm on average and trunks longer than 390 µm. However, *P. oranienbaumensis* differs from *P. myosotidis* and *P. mira* due to its broader conidia, measuring an average of 15.4 µm in breadth.

Peronospora quantula M. Mu & Thines, sp. nov. (Fig. 9).

MycoBank: MB 853063.

Etymology: Specific epithet refers to the Latin *quantulus* (so small), referring to the small conidia.

Description: *Lesions* dark brown to grey, often vein-delimited in *Myosotis ramosissima*. *Down* present on the lower leaf surface, consisting of scattered to dense felt-like conidiophore outgrowth. *Conidiophores* hyaline, straight to curved, thin-walled, 331–668 µm long, av. 499 µm long; trunk 219–434 µm long, av. 327 µm long, 7.6–11.0 µm broad, sometimes slightly swollen to up to 15 µm at the base, ratio of the total length to trunk length 1.5–1.8, $n = 50$, callose plugs absent. *Branching* subdichotomous in 6–9 orders,

with sub-straight to curved branches, gradually attenuate, often at right angles. *Ultimate branchlets* straight to sub-straight, rarely curved, paired branchlets differing in length, with the longer ones 5.6–8.8 µm long, the shorter ones 3.7–6.2 µm long, with a ratio of the longer to the shorter ultimate branchlet 1.2–1.8, $n = 100$, base 1.7–2.2 µm broad, apex slightly sharp. *Conidia* subglobose to ellipsoid with a pale brown tint, 15.0–17.2 µm long, 13.3–15.3 µm broad, length-to-breadth ratio 1.05–1.21, $n = 100$. *Oospores* not observed.

Diagnosis: Differs from *P. laura* in having smaller conidia, longer conidiophores and trunks, as well as shorter ultimate branchlets. Differs from *P. mira* in having smaller conidia, shorter conidiophores and trunks, as well as paired and shorter ultimate branchlets. Differs from *P. myosotidis* in having smaller conidia, shorter conidiophores and trunks, as well as shorter ultimate branchlets. Differs from *P. noli-me-oblivisci* in having smaller conidia, broader trunks and conidiophore bases, as well as shorter ultimate branchlets. Differs from *P. oranienbaumensis* in having smaller conidia, shorter conidiophores and trunks, as well as shorter ultimate branchlets. Differs from *P. globosa* in having smaller conidia, shorter conidiophores and trunks, narrower trunks and conidiophore bases, as well as shorter ultimate branchlets.

Habitat: Living leaves of *Myosotis ramosissima*.

Distribution: Germany.

Typus: Germany, Saxony-Anhalt, Petersberg, on living leaves of *Myosotis ramosissima*, 8 May 2004, H. Jage, holotype GLM-F64083.

Additional specimens examined: Germany, Saxony, Elbe-Elster-Niederung, on living leaves of *Myosotis ramosissima*, 16 May 2001, H. Jage, GLM-F73675; Saxony-Anhalt, Annaburger Heide, on living leaves of *Myosotis ramosissima*, 23 May 1999, H. Jage, GLM-F77851; Dessauer Elbetal, Kleinzerbster Busch, on living leaves of *Myosotis ramosissima*, 2 Apr. 2000, H. Jage, GLM-F76870; Dessauer Elbetal, Schützberg, on living leaves of *Myosotis ramosissima*, 22 May 1994, H. Jage, GLM-F68131; Derenburg, on living leaves of *Myosotis ramosissima*, 15 May 2004, H. Jage, GLM-F64114; Dübener Heide, on living leaves of *Myosotis ramosissima*, 15 May 2001, H. Jage, GLM-F73729; Helme-Unstrut Buntsandsteinland, on living leaves of *Myosotis ramosissima*, 24 May 2005, H. Jage, GLM-F75691; Magdeburger Elbtal, on living leaves of *Myosotis ramosissima*, 15 May 2002, H. Jage, GLM-F78027; Nordöstliches Harzvorland, Cochstedt, on living leaves of *Myosotis ramosissima*, 21 Apr. 2001, H. Jage, GLM-F74249; Tagebauregion Bitterfeld, on living leaves of *Myosotis ramosissima*, 11 May 2002, H. Jage, GLM-F78097; Unteres Saaletal, on living leaves of *Myosotis ramosissima*, 8 May 2004, H. Jage, GLM-F64090; Zerbster Ackerland, on living leaves of *Myosotis ramosissima*, 1 May 1995, H. Jage, GLM-F67983.

Note: *Myosotis ramosissima* is primarily distributed in Europe and around the Mediterranean (GBIF 2024). *Peronospora quantula* clusters closely with *P. noli-me-oblivisci* in all phylogenetic trees, exhibiting strong support for their relationship. Although they share similarities in morphological features, *P. quantula* differentiates itself by having rounder conidia, characterized by an average length-to-breadth ratio of 1.13 ± 0.1.

Discussion

The vast majority of species in *Peronospora* remain to be discovered, even though more than 400 species have already been described in this genus (Thines and Choi 2016). There are still many morphologically similar species of downy mildew, especially within the genus *Peronospora*, which makes it challenging to accurately identify *Peronospora* species (Spring and Thines 2004). Additionally, research on the diversity of *Peronospora* is still limited, and many previous identifications of *Peronospora* species are based on a broad concept of the species (see Yerkes and Shaw 1959). Based on this broad concept of species, some species have been misidentified, merged or synonymized (Choi et al. 2015b), and even the sequence references in GenBank are questionable.

Considering the occasional similarity of downy mildew species at certain genetic loci (Choi et al. 2017; Thines et al. 2019), therefore, in this study, we employ multiple genetic loci for analysis: two nuclear loci (ITS and nrLSU) and four mitochondrial loci (*COX*2, *COX*1, *NAD1,* and *RPS10*).

Prior to this study, there was only one validly published species of *Peronospora* that infects species of *Myosotis*. Six new *Peronospora* species from *Myosotis* have been identified based on morphology and phylogenetic analyses, all *Peronospora* species on *Myosotis* species formed distinct clades according to each specific host species and could also be distinguished morphologically. It is noteworthy that similar to *Peronospora* on *Plantago* (Mu et al. 2024), phylogenetically related species share some morphological traits. *Peronospora mira*, *P. myosotidis*, and *P. oranienbaumensis* cluster together with strong support and form a clade characterized by long conidiophores and trunks, with conidiophores measuring more than 550 µm on average, as well as trunks longer than 390 µm. The second multi-species clade, containing *P. noli-me-oblivisci*, *P. quantula*, and *P. globosa* has shorter conidiophores and trunks, with conidiophores measuring less than 550 µm on average and trunks shorter than 350 µm. As the earlier diverging branch, *P. laura* displays the shortest conidiophores (average 449 µm long) and trunks (average

301 µm long) among all *Peronospora* species on *Myosotis*. However, it should be noted that under varying environmental conditions, these figures might be different (see Dudka et al. 2007) and can only be seen as representative within the areas from which specimens were obtained in this study.

The only *Peronospora* species validly published on *Myosotis*, *Peronospora myosotidis* has been thought to be widely distributed throughout the world and was reported from many host species of *Myosotis* and even from *Amsinckia* and *Brunnera* of *Boraginaceae*, based on a broad species concept (de Bary 1863; Gäumann 1923; Stevenson 1926; Jafar 1962; Gjaerum 1980; Dietrich and Müller 2001; Voglmayr 2003; Lane et al. 2006; Choi et al. 2015a). Considering that the phylogenetic analyses of this study indicate that *P. myosotidis* is seemingly restricted to infecting only *Myosotis arvensis*, it can be expected that many more species infecting *Myosotis* will need to be described in the future.

For example, a downy mildew has been documented on *Myosotis laxa* in Wisconsin, USA (Shaw 1951) and attributed to *P. myositidis*, but this species can be distinguished from all *Peronospora* species by its characteristic features of having monopodially branching conidiophores and having 2–4 ultimate branchlets of equal length at each ultimate ramification. Thus, there are considerable challenges in accurately identifying *Peronospora* species based solely on morphology, and it is crucial to perform a molecular identification of both pathogen species and host plants to accurately determine highly host-specific pathogen species and ensure that quarantine regulations for species with a potentially negative economic impact can be implemented efficiently. Taking into consideration that there are 15 species of *Myosotis* in Germany and adjacent territories (Müller et al. 2021) that harbour already 7 species of *Peronospora*, it is a conservative estimate that the about 100 species of the genus (Müller et al. 2021) are parasitised by more than 20 distinct species of *Peronospora*. Similarly, there are 11 species of *Plantago* in Germany and adjacent territories, which have been found to be parasitised by 6 distinct species of *Peronospora* (Mu et al. 2024). Similarly, the 10 *Papaver* species found in the same area are parasitised by 5 distinct *Peronospora* species (Voglmayr et al. 2014). Conservatively assuming that about one third of the species described in these genera could be infected by a distinct species of *Peronospora*, about100 new species could be expected on these genera alone. Taking into consideration that the situation seems to be similar in other groups, such as *Chenopodiaceae* (Choi et al. 2015a, b), *Ranunculus* (Ploch et al. 2022), and *Trifolium* (García-Blázques et al. 2008), and considering their species richness (Müller et al. 2021), this would add a conservative estimate of another 750 undiscovered *Peronospora* species. However, these groups are only exemplary for other families of flowering plants affected by *Peronospora* species. Extrapolating this to the additional genera summarised by Klenke and Scholler (2015), this would lead to an estimate of more than 2500 species of *Peronospora* to be discovered. Even though this rough estimate needs refinement by upcoming phylogenetic investigations in the genus, it is likely an underestimate, considering that there are many genera of herbaceous plants that can be infected by downy mildew pathogens, but are not listed in Klenke and Scholler (2015), as they do not grow in Germany and adjacent territories. Therefore, a worldwide survey is needed before number of species to be expected in *Peronospora* can be estimated with confidence.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s11557-024-01984-y.

Acknowledgements We thank the curator of GLM at the Senckenberg Institute in Görlitz for allowing access to specimens deposited in the herbarium. This study was partly performed at the Blávík Arctic Research Station in Blávík on Fáskrúðsfjörður, Fjarðabyggð, Iceland.

Authors' contributions MT and YJC conceived the study. MM and YJC conducted laboratory work. MM performed all morphological investigations. MM analysed the data with contributions from MT. MM wrote a first draft, which MT revised, with contributions of YJC. All authors agreed with the submission of the manuscript.

Funding Open Access funding enabled and organized by Projekt DEAL. A special gratitude is extended to the China Scholarship Council (CSC) for supporting the first author through a doctoral fellowship in Germany (No. 202108530140).

Data availability Specimens are deposited in the Herbarium Senckenbergianum at the Senckenberg Museum für Naturkunde in Görlitz (GLM) and Frankfurt am Main (FR). Sequence data are submitted to GenBank. The concatenated alignment has been submitted to Zenodo (https://doi.org/10.5281/zenodo.10850909). The names of *Peronospora* species have been submitted to MycoBank.

Declarations

Ethics approval and consent to participate Not applicable.

Consent for publication Not applicable.

Competing interests MT is the Editor-in-Chief of Mycological Progress, but was not involved in the editorial processes associated with handling this manuscript.

References

Adamska I (2001) Microscopic fungus-like organisms and fungi of the Słowiński National Park. II. (NW Poland). Act Mycol 36:31–65. https://doi.org/10.5586/am.2001.005

Choi Y-J, Hong S-B, Shin H-D (2005) A re-consideration of *Pseudoperonospora cubensis* and *P. humuli* based on molecular and morphological data. Mycol Res 109:841–848. https://doi.org/10.1017/S0953756205002534

Choi Y-J, Shin H-D, Thines M (2009) Two novel *Peronospora* species are associated with recent reports of downy mildew on sages. Mycol Res 113(12):1340–1350. https://doi.org/10.1016/j.mycres.2009.08.010

Choi Y-J, Beakes G, Glockling S, Kruse J, Nam B, Nigrelli L, Ploch S, Shin HD, Shivas RG, Telle S, Voglmayr H, Thines M (2015a) Towards a universal barcode of oomycetes – A comparison of the *cox*1 and *cox*2 loci. Mol Ecol Res 15:1275–1288. https://doi.org/10.1111/1755-0998.12398

Choi Y-J, Klosterman SJ, Kummer V, Voglmayr H, Shin HD, Thines M (2015b) Multi-locus tree and species tree approaches toward resolving a complex clade of downy mildews (*Straminipila*, *Oomycota*), including pathogens of beet and spinach. Mol Phylogen Evol 86:24–34. https://doi.org/10.1016/j.ympev.2015.03.003

Choi Y-J, Wong J, Runge F, Mishra B, Michelmore R, Thines M (2017) BrRxLR11—a new phylogenetic marker with high resolution in the downy mildew genus *Bremia* and related genera. Mycol Prog 16:185–190. https://doi.org/10.1007/s11557-016-1258-9

Constantinescu O (1991) An annotated list of *Peronospora* names. Thunbergia 15:1–110

de Bary A (1863) Recherches sur le développement de quelques champignons parasites. Ann Sci Nat, Bot Ser 4(20):5–148

Dietrich W, Müller J (2001) Die Rost-, Brand- und Falschen Mehltaupilze des tschechischen Teiles des Erzgebirges (Krušné hory). Czech Mycol 53:89–118

Dudka IO, Anishchenko IM, Terent'eva NG (2007) The variability of *Peronospora alta* Fuckel conidia in dependence on the ecological conditions. In: Lebeda A, Spencer-Phillips PTN (eds). Advances in Downy Mildew Research (vol. 3). Kostelec na Hané: Palacký University in Olomouc and JOLA. pp. 39–46

Dunning LT, Savolainen V (2010) Broad-scale amplification of matK for DNA barcoding plants, a technical note. Bot J Linn Soc 164:1–9. https://doi.org/10.1111/j.1095-8339.2010.01071.x

Felsenstein J (1985) Confidence limits on phylogenies: an approach using the bootstrap. Evol 39:783–791. https://doi.org/10.1111/j.1558-5646.1985.tb00420.x

Ford CS, Ayres KL, Toomey N, Haider N, Stahl JVA, Kelly LJ, Wikström N, Hollingsworth PM, Duff RJ, Hoot SB, Cowan RS, Chase MW, Wilkinson MJ (2009) Selection of candidate coding DNA barcoding regions for use on land plants. Bot J Linn Soc 159:1–11. https://doi.org/10.1111/j.1095-8339.2008.00938.x

García-Blázquez G, Göker M, Voglmayr H, Martín MP, Tellería MT, Oberwinkler F (2008) Phylogeny of *Peronospora*, parasitic on *Fabaceae*, based on ITS sequences. Mycol Res 112:502–512

Gäumann E (1923) Beiträge zu einer Monographie der Gattung *Peronospora* Corda. Beiträge Zur Kryptogamenflora Der Schweiz 5:1–360

GBIF (2024) Global Biodiversity Information Facility. Free and Open Access to Biodiversity Data. https://www.gbif.org/. Accessed 6 March 2024

Gjaerum HB (1980) Additions to the *Peronosporales* in the Canary Islands. Bot Macarones 7:39–42

Hoffmeister M, Ashrafi S, Thines M, Maier W (2020) Two new species of the *Peronospora belbahrii* species complex, *Pe. choii* sp. nov. and *Pe. salviae-pratensis* sp. nov., and a new host for *Pe. salviae-officinalis*. FUSE 6:38–52. https://doi.org/10.3114/fuse.2020.06.03

Hopple JS, Vilgalys R (1999) Phylogenetic relationships in the mushroom genus *Coprinus* and dark-spored allies based on sequence data from the nuclear gene coding for the large ribosomal subunit RNA: Divergent domains, outgroups, and monophyly. Mol Phyl Evol 13:1–19. https://doi.org/10.1006/mpev.1999.0634

Hudspeth DSS, Nadler S, Hudspeth MES (2000) A *COX*2 molecular phylogeny of the *Peronosporomycetes*. Mycologia 92:674–684. https://doi.org/10.1080/00275514.2000.12061208

Jaczewski AA, Jaczewski PA (1931) Opredelitel' Gribov. Sovershennye Griby (Diploidnye Stadii). I. Fikomitsety, Moscow: Selkolkhozgiz

Jafar H (1962) Some new records in *Peronosporaceae* in New Zealand. New Zeal J Agr Res 5:512–515. https://doi.org/10.1080/00288233.1962.10419950

Katoh K, Standley DM (2013) MAFFT multiple sequence alignment software version 7: improvements in performance and usability. Mol Biol Evol 30:772–780. https://doi.org/10.1093/molbev/mst010

Katoh K, Toh H (2008) Improved accuracy of multiple ncRNA alignment by incorporating structural information into a MAFFT-based framework. BMC Bioinf 9:1–13. https://doi.org/10.1186/1471-2105-9-212

Kew Science (2024) Plants of the World Online. Retrieved March 20, 2024, from https://powo.science.kew.org/

Klenke F, Scholler M (2015) Pflanzenparasitische Kleinpilze. Springer, Berlin, Heidelberg

Kumar S, Stecher G, Tamura K (2016) MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. Mol Biol Evol 33:1870–1874. https://doi.org/10.1093/molbev/msw054

Lane CR, Scrace JM, Hutchinson D (2006) First record of the downy mildew *Peronospora myosotidis* on *Brunnera macrophylla* in the UK. Pl Pathol 55:574–574. https://doi.org/10.1111/j.1365-3059.2006.01370.x

Martin FN, Tooley PW, Blomquist C (2004) Molecular detection of *Phytophthora ramorum*, the causal agent of sudden oak death in California, and two additional species commonly recovered from diseased plant material. Phytopathol 94:621–631. https://doi.org/10.1094/PHYTO.2004.94.6.621

Mishra B, Ploch S, Weiland C (2023) Thines M (2023) The TrEase web service: inferring phylogenetic trees with ease. Mycol Prog 22:84. https://doi.org/10.1007/s11557-023-01931-3

Mu M, Choi YJ, Kruse J, Crouch JA, Ploch S, Thines M (2024) Single host plant species may harbour more than one species of *Peronospora* – a case study on *Peronospora* infecting *Plantago*. Persoonia 52:94–118. https://doi.org/10.3767/persoonia.2024.52.04. . (MüllerF,RitzCM,WelkE,WescheK(eds)(2021)Rothmaler-ExkursionsfloravonDeutschland.Gefäßpflanzen:Grundband.Springer,Berlin)

Müller F, Ritz CM, Welk E, Wesche K (eds) (2021) Rothmaler-Exkursions flora von Deutschland. Gefäßpflanzen: Grundband. Springer, Berlin

Navarro E, Bousquet J, Moiroud A, Munive A, Piou D, Normand P. (2003) Molecular phylogeny of *Alnus* (*Betulaceae*), inferred from nuclear ribosomal DNA ITS sequences. In Frankia Symbiosis: Proceedings of the 12th Meeting on Frankia and Actinorhizal Plants, Carry-le-Rouet, France, June 2001, Springer Netherlands 2003: 207–217. https://doi.org/10.1007/978-94-017-1601-7_22

Nylander JA (2004) Bayesian phylogenetics and the evolution of gall wasps. Doctoral dissertation, Acta Universitatis Upsaliensis

Pantone D, Brown S, Womersley C (1985) Biological Control of Fiddleneck Californ Agr 39:4–5

Ploch S, Kruse J, Choi YJ, Thiel H, Thines M (2022) Ancestral state reconstruction in *Peronospora* provides further evidence for host jumping as a key element in the diversification of obligate parasites. Mol Phyl Evol 166:107321. https://doi.org/10.1016/j.ympev.2021.107321

Riethmüller A, Voglmayr H, Göker M, Weiß M, Oberwinkler F (2002) Phylogenetic relationships of the downy mildews (*Peronosporales*) and related groups based on nuclear large subunit ribosomal DNA sequences. Mycologia 94(5):834–849. https://doi.org/10.1080/15572536.2003.11833177

Rogerson CT (1958) Kansas Mycological Notes: 1957. Tr Kansas Ac Sci (1903–) 61:262–272. https://doi.org/10.2307/3626445

Ronquist F, Teslenko M, Mark PVD, Ayres DL, Höhna S, Larget B, Liu L, Suchard MA, Huelsenbeck JP (2012) MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. Syst Biol 61:539–542. https://doi.org/10.1093/sysbio/sys029

Shaw CG (1951) New species of the *Peronosporaceae*. Mycologia 43:445–455. https://doi.org/10.1080/00275514.1951.12024146

Spring O, Thines M (2004) On the necessity of new characters for classification and systematics of biotrophic *Peronosporomycetes*. Planta 219:910–914. https://doi.org/10.1007/s00425-004-1341-3

Stamatakis A (2014) RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. Bioinf 30:1312–1313. https://doi.org/10.1093/bioinformatics/btu033

Stevenson JA (1926) Foreign plant diseases: a manual of economic plant diseases which are new to or not widely distributed in the United States. US Government Printing Office, Washington DC

Thines M, Choi Y-J (2016) Evolution, diversity, and taxonomy of the *Peronosporaceae*, with focus on the genus *Peronospora*. Phytopathol 106:6–18. https://doi.org/10.1094/PHYTO-05-15-0127-RVW

Thines M, Telle S, Ploch S, Runge F (2009) Identity of the downy mildew pathogens of basil, coleus, and sage with implications for quarantine measures. Mycol Res 113(5):532–540. https://doi.org/10.1016/j.mycres.2008.12.005

Thines M, Denton GJ, Beal EJ, Kilty A, Denton JO, Shin H-D, Choi Y-J (2019) *Peronospora aquilegiicola* sp. nov., the downy mildew affecting columbines in the UK is an invasive species from East Asia. Eur J Pl Pathol 155:515–525. https://doi.org/10.1007/s10658-019-01787-y

Tutin TG, Heywood VH, Burges NA, Moore DM, Valentine DH, Walters SM, Webb DA (1972) Flora Europaea. Vol. 3. *Diapensiaceae* to *Myoporaceae*. Cambridge University Press Fl Eur 3:1–370

Vaidya G, Lohman DJ, Meier R (2011) SequenceMatrix: concatenation software for the fast assembly of multi-gene datasets with character set and codon information. Cladist 27:171–180. https://doi.org/10.1111/j.1096-0031.2010.00329.x

Vilgalys R, Hester M (1990) Rapid genetic identification and mapping of enzymatically amplified ribosomal DNA from several *Cryptococcus* species. J Bact 172:4238–4246. https://doi.org/10.1128/jb.172.8.4238-4246.1990

Voglmayr H (2003) Phylogenetic relationships of *Peronospora* and related genera based on nuclear ribosomal ITS sequences. Mycol Res 107:1132–1142. https://doi.org/10.1017/S0953756203008438

Voglmayr H, Montes-Borrego M, Landa BB (2014) Disentangling *Peronospora* on *Papaver*: phylogenetics, taxonomy, nomenclature and host range of downy mildew of opium poppy (*Papaver somniferum*) and related species. PLoS ONE 9:96838. https://doi.org/10.1371/journal.pone.0096838

White TJ, Bruns T, Lee S, Taylor JW (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ (eds) PCR Protocols: A Guide to Methods and Applications. Academic Press, New York, pp 315–322

Yerkes WD, Shaw CG (1959) Taxonomy of the *Peronospora* species on Cruci-ferae and Chenopodiaceae. Phytopathol 49:499–507