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Multi-locus tree and species tree approaches toward resolving a complex clade of downy mildews (Straminipila, Oomycota), including pathogens of beet and spinach

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Abstract

Accurate species determination of plant pathogens is a prerequisite for their control and quarantine, and further for assessing their potential threat to crops. The family Peronosporaceae (Straminipila; Oomycota) consists of obligate biotrophic pathogens that cause downy mildew disease on angiosperms, including a large number of cultivated plants. In the largest downy mildew genus *Peronospora*, a phylogenetically complex clade includes the economically important downy mildew pathogens of spinach and beet, as well as the type species of the genus *Peronospora*. To resolve this complex clade at the species level and to infer evolutionary relationships among them, we used multi-locus phylogenetic analysis and species tree estimation.

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Both approaches discriminated all nine currently accepted species and revealed four previously unrecognized lineages, which are specific to a host genus or species. This is in line with a narrow species concept, i.e. that a downy mildew species is associated with only a particular host plant genus or species. Instead of applying the dubious name *Peronospora farinosa*, which has been proposed for formal rejection, our results provide strong evidence that *Peronospora schachtii* is an independent species from lineages on *Atriplex* and apparently occurs exclusively on *Beta vulgaris*. The members of the clade investigated, the *Peronospora rumicis* clade, associate with three different host plant families, Amaranthaceae, Caryophyllaceae, and Polygonaceae, suggesting that they may have speciated following at least two recent inter-family host shifts, rather than contemporary cospeciation with the host plants.

Keywords

Cospeciation; Host shift; Multi-locus phylogeny; Oomycetes; *Peronospora farinosa*; Species tree

1 Introduction

Downy mildew is one of the most important oomycete plant diseases and causes serious damage to a variety of cultivated and ornamental plants. In the cultivation of sugar beet (*Beta vulgaris*) and spinach (*Spinacia oleracea*), downy mildew is probably the most widespread and a potentially destructive disease worldwide (Byford, 1981). Although occurrences of this disease have been reported in most countries where these crops are cultivated, a proper nomenclature and the identity of these pathogens has remained controversial (Francis and Byford, 1983; Brandenberger et al., 1991), reflected by the plethora of taxonomic changes that have previously been proposed (Yerkes and Shaw, 1959; Byford, 1967). Fifty-three *Peronospora* names were validly published for species occurring on particular genera or species of plants included in the family Chenopodiaceae s. str. (Constantinescu, 1991), including the species occurring on *Beta* and *Spinacia*. Since Yerkes and Shaw (1959) advocated the synonymization of all of these names under a single species, *Peronospora farinosa* (Fr.) Fr., the oldest available name thought to be attributable to a *Peronospora* species on Chenopodiaceae (but see Choi and Thines (2014) for a discussion of its status), *P. farinosa* has often been regarded as the sole parasitic fungus causing downy mildew disease on the host family. However, this view has been challenged with the advent of phylogenetic approaches, which revealed that *Peronospora* samples from various chenopodiaceous hosts represent phylogenetically distinct lineages according to the specific host genera or host species and together even do not form a monophyletic group (Voglmayr, 2003; Choi et al., 2007c, 2008a, 2010). This is in line with the narrow species concept of Gäumann (1919, 1923). As a result, Choi et al. (2007c) have reinstated the name *P. effusa* (Grev.) Rabenh. for *Peronospora* specimens on *S. oleracea*, and Choi et al. (2008a) noted the existence of host-specific *Peronospora* species infecting *Chenopodium*, that are distinct from lineages occurring on *Atriplex*, the type host genus for *P. farinosa*. Importantly, the Latin description provided by Fries (1832) for *P. farinosa*, for which apparently no type material is extant, has insufficient detail for the name to be associated with any particular species of *Peronospora*. In addition, it describes several features that are atypical for a *Peronospora* species (Choi and Thines, 2014). Therefore, the name *Botrytis farinosa* Fr. (*Peronospora*

farinosa) has recently been proposed to be rejected to enable a detailed taxonomic revision of the more than fifty species of downy mildew pathogens to which the name *P. farinosa* has often been misapplied (Choi and Thines, 2014).

Beet downy mildew (BDM; *Peronospora* strains parasitic to sugar beet and other varieties of *Beta vulgaris*) occurs in almost every country where sugar beet and other varieties of *Beta vulgaris* are cultivated. However, the nomenclature of BDM has not yet been settled with respect to species designation, being mostly still designated as *P. farinosa* f. sp. *betae* Byford. This is again due to the two conflicting views regarding species differentiation in downy mildews as referred to above. The name *Peronospora schachtii* Fuckel was first introduced to accommodate the pathogen on *Beta vulgaris* (Fuckel, 1866). Adopting this narrow species concept, most monographs reported the causal agent of BDM as *P. schachtii* (Gustavsson, 1959; Kochman and Majewski, 1970; Ul'yanishchev et al., 1985; Vanev et al., 1993; Mazelaitis and Staneviciene, 1995). Conversely, Yerkes and Shaw (1959) proposed the synonymization of *P. schachtii* under *P. farinosa*, which was widely accepted by plant pathologists, but also some taxonomists. As a consequence, most studies erroneously referred to BDM as *P. farinosa* (or *P. farinosa* f. sp. *betae*) (Byford, 1981; Constantinescu and Negrean, 1983; Francis and Byford, 1983; Francis and Waterhouse, 1988; Yu et al., 1998; García-Blázquez et al., 2006; Kim et al., 2010). Hence, the latter name is widely used when referring to BDM (11,500 hits in Google using the search string "*Peronospora farinosa*" AND beet, 3510 hits in Google using the search string "*Peronospora schachtii*" AND beet, at 18 Aug 2014).

In a previous ITS rDNA sequence-based study (Choi et al., 2007c), BDM isolates formed a monophyletic cluster with three other *Peronospora* species, *P. effusa* parasitic to spinach (*Spinacia oleracea*), *P. rumicis* Corda (the type species of the genus *Peronospora*) parasitic to *Rumex* spp., and *P. obovata* Bonord. parasitic to *Spergula arvensis*. Additionally, five other *Peronospora* species were also placed within the same clade in a preliminary survey; *P. lepigoni* Fuckel parasitic to *Spergularia rubra*, *P. polycarponis* Mayor & Vienn.-Bourg. to *Polycarpon diphylum*, *P. minor* (Casp.) Gäum. to *Atriplex patula*, *P. atriplicis-hastatae* S. vul. & Rayss to *A. prostrata*, and *P. littoralis* Gäum. to *A. littoralis*. The close phylogenetic relationship among these nine species is not surprising. Morphologically, these species share several similarities: all show monopodial branching pattern in the conidiophores, all have straight to sub-straight branches, and all produce ellipsoidal to broadly ellipsoidal and brownish conidia. The morphological similarity between BDM and *P. effusa* has been previously described (Berlese and De Toni, 1888; Wilson, 1914). Choi et al. (2007c) also recognized this similarity, although they also found a slight difference in the length to width ratio of the conidia. Nonetheless, a decision regarding the conspecificity or distinctiveness of the species could not be reached, as too few specimens and loci were included. However, it became apparent that at least *P. effusa* was separate from all of the closely related species (Choi et al., 2007c).

For the usefulness in identification, taxonomy, and phylogeny of oomycetes and fungi, the ITS rDNA region has become the standard nuclear DNA barcode marker (Schoch et al., 2012). However, the lack of ITS variability does not always automatically confirm their conspecificity; there are several cases like the one investigated here (Choi et al., 2007c) where

the ITS region exhibits insufficient variability for phylogenetic distinction in closely related species, e.g. in *Peronospora* (Voglmayr et al., 2014b), *Pseudoperonospora* (Choi et al., 2005) and *Phytophthora* (Goodwin et al., 1999; Cooke et al., 2000; Jung and Burgess, 2009). Due to the limitations of the ITS region, further multi-locus phylogenies are required. Therefore, in the current study concatenation-based phylogenetic analyses of two nuclear (ITS, heat shock protein 90 [*hsp90*]) and five mitochondrial loci (cytochrome *c* oxidase subunit II and I [*cox2* and *cox1*], a spacer region between *cox2* and *cox1* genes [*coxS*], NADH dehydrogenase subunit I [*nad1*], ribosomal protein S10 and its flanking region [*rps10*]) were conducted. As there are concerns about the accuracy of species relationships inferred from concatenation method (Edwards et al., 2007; Kubatko and Degnan, 2007; Edwards, 2009; Blair et al., 2012), a coalescent-based approach to estimates gene trees and species tree topologies simultaneously (Heled and Drummond, 2010; Bouckaert et al., 2014) was additionally performed. The aim of the present study was to resolve one of the most complex phylogenetic clades of downy mildews using multi-locus sequence data, and to develop evolutionary hypotheses related to speciation and host shifts in this group.

2 Materials and methods

2.1 Oomycete specimens

In total 90 specimens of *Peronospora* originating from three host families, Amaranthaceae (including Chenopodiaceae), Caryophyllaceae, and Polygonaceae, which all belong to the order Caryophyllales, were included in the present study. On the Amaranthaceae, 12 beet (*Beta vulgaris* var. *cicla* and var. *saccharifera*) specimens, originating from Asia, Europe, North and South America, and 24 spinach (*Spinacia oleracea*) specimens from Asia, Europe, North America, and Oceania were analyzed, along with eight samples occurring on *Atriplex*. In addition, three species parasitic on the Caryophyllaceae, *P. polycarponis*, *P. lepigoni*, and *P. obovata*, and the *P. rumicis* complex parasitic on the Polygonaceae were included for phylogenetic study. *Peronospora boni-henrici* Gäum. (parasitic to *Chenopodium bonus-henricus*) was used as an outgroup. The host plants and geographic samplings of downy mildew specimens were summarized in Table S1, in addition with taxonomic changes of *Peronospora* names by this study. The samples were either collected by the authors or loaned from herbaria BPI, DM, GLM, HMAS, HMR, KUS-F, UPS, VPRI, and WU. Herbarium abbreviations are according to Thiers (2014). Acronyms of private collections include those of the following: DML, from the USDA ARS laboratory of Steven Klosterman in Salinas California, USA; RD, R. Delhey in the Phytopathology Lab of Bahía Blanca, Argentina; JK-F, Julia Kruse in Biodiversity and Climate Research Centre (BiK-F), Frankfurt am Main, Germany; VK, Volker Kummer in University Potsdam, Germany. Information on the specimens sequenced in this study is shown in Table 1.

2.2 DNA extraction, PCR amplification, and sequencing

In total, 5–20 mg of infected plant tissue from herbarium specimens were disrupted in a mixer mill (MM2, Retsch, Germany), using three iron beads of each 3 mm and 1 mm diameters per sample and shaking at maximum speed for 3 min. Genomic DNA was extracted using the BioSprint 96 DNA Plant Kit (Qiagen) on a KingFisher Flex (Thermo Scientific) robot or using the NucleoSpin® Plant II kit (Machery-Nagel). Five mitochondrial

and two nuclear markers were amplified by PCR using combinations of Oomycete-specific primers (Table S2); primers ITS1-O (Bachofer, 2004) and LR-0 (reverse complementary to LR-OR (Moncalvo et al., 1995)) for the complete ITS rDNA (amplicon length of 854 bp), HSP90-F1 (Blair et al., 2008) and HSP90-R1C (designed here: ACMCCCTTGACRAASGABAGRTAC) for *hsp90* nDNA (926 bp), *cox2*-F (Hudspeth et al., 2000) and FMPH-10b (Martin et al., 2004) for *cox2* mtDNA and the spacer region between *cox2* and *cox1* genes (1015–1089 bp), OomCox1-levup and OomCox1-levlo (Robideau et al., 2011) for the *cox1* mtDNA (680 bp), Prv9-F and Prv9-R (Blair et al., 2012) for *rps10* mtDNA (approx. 601 bp), NADHF3C (designed here: AGGWGCDTTAAGATCAACDGCWCA) and NADHR1 (Kroon et al., 2004) for *nad1* mtDNA (548 bp). In some cases, the *cox2* gene and the spacer region had to be separately amplified with two primer sets, *cox2*-F & *cox2*-R (Hudspeth et al., 2000) and FM79 & FMPH-10b (Martin et al., 2004). Amplification reactions were carried out in 25 μ L with genomic DNA from 1 to 3 ng, 1 \times Mango PCR Buffer, 0.2 mM dNTPs, 2 mM MgCl₂, 0.8 mg/mL BSA, 0.4 μ M forward and reverse primers, and 0.5 Unit Mango Taq Polymerase (Bioline GmbH, Luckenwalde, Germany). PCR conditions were as follows: an initial denaturation step of 95 $^{\circ}$ C for 4 min; 36 cycles of 95 $^{\circ}$ C for 40 s, primer set specific annealing temperature for 40 s (see Table S2), 72 $^{\circ}$ C extension for 60 s; final extension of 5 min at 72 $^{\circ}$ C. For *hsp90* gene and a spanning region of *cox2* and *cox2*-1 spacer, the extension time was extended to 90 s. For *rps10* gene, the extension time was shortened to 40 s, and the cycle numbers were increased to 40 cycles. Amplicons were sequenced at the Biodiversity and Climate Research Centre (BiK-F) laboratory using primers identical to those used for amplifications.

2.3 Phylogenetic analysis

Sequences were edited using the DNASTar software package (DNASTar, Inc., Madison, Wis., USA), version 5.05. An alignment of each locus was performed using MAFFT 7 (Katoh and Standley, 2013) employing the Q-INS-i algorithm (Katoh and Toh, 2008). SequenceMatrix 1.7.8 (Vaidya et al., 2011) was used for concatenating individual gene sequences and for checking unusually similar or divergent sequences. We made concatenation alignments for each nuclear (ITS and *hsp90*), mitochondrial (*cox2*, *coxS*, *cox1*, *nad1*, *rps10*), and all seven loci. To assess the relative stability of trees with respect to different inference methods, we used four different tree construction methods: Maximum Likelihood (ML), Minimum Evolution (ME), Maximum Parsimony (MP), and Bayesian inference (MCMC). The best-fit substitution models were identified for each locus in MEGA 6.0 (Tamura et al., 2013); these were the general time reversible (GTR) model for mitochondrial loci and the Tamura-Nei (TN) for nuclear loci. Evolutionary rates were estimated at a value of 1.0. For ML analyses, 1000 rounds of random addition of sequences as well as 1000 fast bootstrap replicates were performed using RAxML 7.0.3 (Stamatakis, 2006) as implemented in raxmlGUI 1.3 (Silvestro and Michalak, 2012) using the GTRCAT variant. ME analysis was done using MEGA 6.0, with the default settings of the program, except for using the TN model instead of the maximum composite likelihood model. MP analysis was performed in PAUP 4.0b10 (Swofford, 2002), using 1000 replicates of heuristic search with random addition of sequences and subsequent TBR branch swapping. All sites were treated as unordered and unweighted, with gaps treated as missing data. The reproducibility of the internal nodes of

the resulting trees was tested by bootstrap analysis using 1000 replications. MCMC analysis was performed using MrBayes, version 3.2 (Ronquist et al., 2012), with the GTR model and gamma-distributed substitution rates. Four incrementally heated simultaneous Markov chains were run for 10 M generations, with a tree saved every 1000th generation. We assessed convergence and the effective sample size exceeding 200 for each parameter every run, as implemented in Tracer v.1.5 (Rambaut and Drummond, 2009). The AWTY software (Nylander et al., 2008) was also used to confirm the convergence graphically. After checking for convergence and ESS, we discarded the first 10% of trees as a burn-in.

2.4 Species tree estimation

Uncertainty in phylogenetic relationships resulting from the multi-locus concatenation was estimated with *BEAST 2 (Heled and Drummond, 2010). Individual Bayesian trees were inferred for each locus in three datasets (nuclear, mitochondrial, and all seven loci), and relationships based on the combined dataset were estimated using Bayesian analysis. BEAUTi 2 (Bouckaert et al., 2014) was used to create the XML-formatted input files for *BEAST 2. Individual assignments to taxonomic units followed the specific host plant, as well as the clades inferred from the concatenation analysis, under the Taxon sets tab. For the dataset partitioned by locus, the best-fit substitution models and evolutionary rates were identical with ones of the above phylogenetic analysis. The topology and support values were compared under two tree priors (Yule versus birth–death process) and molecular clock models (strict versus relaxed). All datasets were run for 100 M generations, sampling every 5000th generation. Topological convergence and adequate ESS were checked using Tracer and AWTY, as outlined above. After removing the first 25% of trees, a maximum clade credibility tree with the 95% highest probability density was produced by using TreeAnnotator version 2.1.2 (Rambaut and Drummond, 2010), and visualized in FigTree (<http://tree.bio.ed.ac.uk/software/figtree/>). We used DensiTree (Bouckaert, 2010) to jointly visualize the posterior distribution of the species trees from *BEAST 2. As this program uses transparent lines, thus in areas where many of the trees agree with respect to topology and branch length, there will be many lines drawn and the screen will show a darker color for the respective branch.

3 Results

3.1 ITS rDNA phylogeny

The phylogenetic relationship of ITS sequences was inferred using ML, ME, MP, and MCMC analyses. Since all four analyses did not show support for divergent topologies, only the ML tree is presented in Fig. S1, with the addition of the support values of the other analyses. The *Peronospora* isolates originating from the genera *Beta*, *Emex*, *Rumex*, *Spergula*, *Spergularia*, *Polycarpon*, and *Spinacia* grouped together with high support values in all analyses. Within this groups, most sequences were identical, thus relationships in this group could not be resolved. Even though ITS sequence showed a sufficient resolution to distinguish *P. effusa* isolates ex *Spinacia oleracea* from other isolates with varying support, discrimination between *Peronospora* specimens from *Polycarpon*, *Rumex*, *Spergula*, and *Spergularia* was not possible. Although a group including all BDM specimens was obtained, it also included specimens from *Emex spinosa*. The two groups representing *P. effusa* and

BDM (including *P. sp. ex Emex*) revealed a unique single-base substitution in the ITS2 region and the 5.8S gene, respectively. On *Atriplex*, two distinct lineages representing *P. litoralis* and *P. minor/P. atriplicis-hastatae* were found, which were sister to all other groups.

3.2 Multi-locus phylogeny

Trees based on each concatenated alignment for five mitochondrial and two nuclear loci (Fig. S2) showed no strong conflicting support with respect to a phylogeny based on the concatenated alignment of mitochondrial and nuclear loci (fig 1). A concatenated alignment of all mitochondrial and nuclear loci had 4378 total characters, including 311 variable characters, 123 of which were parsimony informative; *cox2* (708 characters, 65 variable, 30 parsimony informative), the spacer region between *cox2* and *cox1* (272, 22, 9), *cox1* (701, 58, 28), *nad1* (502, 47, 22), *rps10* (541, 19, 4), ITS (799, 41, 10), and *hsp90* (855, 59, 20). The final matrix was deposited in TreeBASE (<http://www.treebase.org>) and is available under <http://purl.org/phylo/treebase/phyloids/study/TB2:S16896>.

Since the concatenated dataset for all mitochondrial and nuclear loci revealed no significant conflicts in the tree topologies derived from ML, ME, MP, and MCMC analyses, only the tree from ML inference is shown in Fig. 1. The multi-gene phylogeny showed a high resolution, enabling differentiation between all closely related species of the complex clade, and resolving the uncertainties found in the ITS tree (Fig. S1). The discrimination of the lineages parasitic to specific host genera or species received high to maximum support in all phylogenetic analyses. In the multi-locus tree, all BDM sequences together formed a distinct group, with high support values of 100/100/99/100 in four analyses. All sequences, excluding one specimen 'CAL03' (Table 1), were identical to each other, demonstrating the genetic homogeneity of the pathogen causing BDM disease in several regions worldwide. BDM was further distinguishable from *Peronospora* specimens from *Emex spinosa*, which had identical ITS sequences. All sequences of *P. effusa* grouped together with maximum support in all analyses. Also the resolution for *Peronospora* specimens from caryophyllaceous plants was much improved in the concatenated dataset, compared to the ITS tree. All *Peronospora* specimens from the Caryophyllaceae, *Polycarpon*, *Spergula*, and *Spergularia*, grouped together with the high support of 88/100/99/100. They further split into three subgroups, corresponding to a particular host plant; *P. polycarponis* from *Polycarpon diphyllum*, *P. obovata* from *Spergula arvensis*, and *P. lepigoni* from *Spergularia rubra*. A fourth subgroup exists with a previously undescribed lineage of *Peronospora* occurring on *Spergularia marina*. All specimens from *Rumex* species formed a monophyletic group with high support values. This group was further subdivided corresponding to particular host species. The type species of *Peronospora*, *P. rumicis*, is likely restricted to *R. acetosella*, as specimens from both *R. thyrsiflorus* and the other from *R. acetosa* (including *R. arifolius*) were highly distinct from *P. rumicis* from *R. acetosella*. *Peronospora rumicis* and *Peronospora* specimens from *R. thyrsiflorus* grouped together with maximum support in all analyses. Within specimens identified as *R. thyrsiflorus*, two distinct groups were observed, but based on the specimens investigated in this study, no morphological differences could be found in preliminary screens. The *Peronospora* isolates from *Emex spinosa* and *Spergularia marina* have respectively been considered to be *P. rumicis* and *P. lepigoni*, but are distinct from those species on the basis of the multi-locus phylogeny. *Peronospora* isolates parasitic

to *Atriplex* were consistently found to be the sister group to all other lineages in the *Peronospora* clade under investigation. The monophyly of this group received low support values in all analyses, probably due to the slightly different placements of *P. littoralis* in nuclear (Fig. S2a) and mitochondrial datasets (Fig. S2b). This group was further divided into three clades corresponding to a particular *Atriplex* species, *A. littoralis*, *A. prostrata*, and *A. patula*, with the latter two clades grouping together with strong support (100/99/98/100).

3.3 Species tree estimation

In order to evaluate the results from the concatenated dataset, the species trees of the mitochondrial and nuclear loci were generated by *BEAST, and summarized in a maximum clade credibility tree (fig 2A) and cloudogram (Fig 2B). With an identical tree topology, which was robust to changes in the tree prior (Yule versus birth–death process) and molecular clock model (strict versus relaxed) tested, the species trees showed largely congruent results with the concatenated dataset, except for the grouping of the *Peronospora* species occurring on members of *Atriplex*. The species trees strongly supported a grouping of the *Peronospora* species from *Atriplex*, with a posterior probability of 1.00, while it was not significantly supported by the concatenated dataset (Fig 1), in which support values were low in all analyses (ML, ME, MP, and MCMC). In both species trees, *P. effusa* occupied a basal position to specimens from Caryophyllaceae, Polygonaceae and *Beta vulgaris*, like in the concatenated tree, but the posterior probability is low (0.68) in the maximum clade credibility tree (Fig 2A), due to a slightly different placement of *P. effusa* and *Peronospora* species from *Atriplex* in mitochondrial loci (Fig. S3b). The species tree estimations confirmed that the four *Peronospora* lineages from Caryophyllaceae form a monophyletic cluster, but along with the concatenation analysis, the evolutionary relationships within this cluster were unclear with regard to none or only low posterior probabilities.

4 Discussion

Despite ITS rDNA being recognized as a potential barcoding marker for identification of Oomycetes (Robideau et al., 2011) and Fungi (Schoch et al., 2012), ITS sequences in some cases may be unsuitable to resolve the phylogenetic relationships among closely related species. In the largest genus of oomycetes, *Peronospora*, there are a few complex clades containing several distinct species, which were mostly not distinguishable based on ITS sequences, e.g. the *Peronospora arborescens* complex (Voglmayr et al., 2014b), the *P. effusa-rumicis-schachtii* complex (Choi et al., 2007c), the *P. sparsa* complex parasitic to Rosaceae (Cooke et al., 2002; Voglmayr, 2003; Choi et al., 2007a), and the *P. viciae* complex parasitic to Fabaceae (García-Blázquez et al., 2008). To determine the previously unresolved relationships within the *Peronospora effusa-rumicis-schachtii* complex (Voglmayr, 2003; Choi et al., 2007c), the present study used multi-locus analyses based on a concatenated dataset and species tree estimations. The nearly identical topologies inferred from the concatenated and coalescent-based analyses illustrate the distinctiveness of all *Peronospora* species included in this study and correspondence to the specific host genus or species that they infect. Additionally, this study revealed some previously unnoticed, host-specific lineages of *Peronospora* and that the type species of this genus is likely restricted to *R. acetosella*. These results support the view that a narrow species concept reflects the

evolutionary history of downy mildews much better than a broad species concept, which is in line with recent studies for other species complexes, such as *Bremia lactucae* (Voglmayr et al., 2004; Choi et al., 2007b, 2011c), *Hyaloperonospora parasitica* (Choi et al., 2003, 2011b; Göker et al., 2004, 2009; Voglmayr and Göker, 2011; Voglmayr et al., 2014a), *Peronospora arborescens* (Voglmayr et al., 2014b), *Peronospora lamii* (Belbahri et al., 2005; Choi et al., 2009c; Thines et al., 2009b), and *Plasmopara halstedii* (Spring et al., 2003; Komjáti et al., 2007; Choi et al., 2009a).

The taxonomic status of the causal agent for beet downy mildews (BDM) remained obscure due to previous uncertainties on species delimitation of downy mildews. This is due to two conflicting views regarding species differentiation in downy mildews. As a result, BDM is often referred to as either *P. schachtii* (narrow host range) or *P. farinosa* (broad host range). The present phylogenetic study has revealed the distinctiveness of BDM from all other *Peronospora* species. The independence of BDM agrees with previous works about host specialization as several authors were unable to cross-inoculate between BDM and *Peronospora* isolates from other chenopodiaceous hosts, suggesting a strict host specificity (Leach, 1931; Richards, 1939; Darpoux and Durgéat, 1962; Byford, 1967; Klosterman et al., 2014). Consequently, we suggest that the name *P. schachtii* should be used for BDM. In addition, *P. farinosa* is a name that cannot be associated with any type specimen and the description contains insufficient detail to ascertain the pathogen and host species; it is even unclear if the original description refers to a downy mildew. Thus, the name *Botrytis farinosa* (the basionym of *P. farinosa*) has been proposed for rejection (Choi and Thines, 2014). None of the species described from *Atriplex* (the type host of the dubious taxon *P. farinosa*) is closely related to *P. schachtii*.

In spite of the economic importance of beet downy mildew, the genetic diversity of *P. schachtii* has not yet been investigated. In this study, all but one specimens originating from geographically diverse countries (Argentina, China, France, Germany, Korea, and the USA) were identical to each other in ITS, *cox2*, *cox1*, *nad1*, *rps10*, and *hsp90* loci. The one strain (CAL03) that was not identical had only a single base substitution in the spacer region between *cox2* and *cox1*. The *Peronospora* samples of the two varieties of *Beta vulgaris*, namely, var. *saccharifera* (sugar beet) and var. *cicla* (Swiss chard) did thus not show genetic differentiation from each other in the loci investigated. The beet crop is thought to be native to the Mediterranean (De Bock, 1985; Hanelt, 2001), and has been domesticated over thousands of years from a wild form without swollen roots. Its potential as a source of sugar was not discovered until 1747, and only in the early part of the 19th century when its breeding reached high sugar levels, the beet became a popular vegetable in Europe (Rolph, 1917). In other parts of the world, cultivation has started only recently. This suggests that *P. schachtii* may have spread together with the crop, possibly starting from an originally very small pathogen population. The resulting comparatively short period of time the crop is grown worldwide might explain the genetic homogeneity of the *P. schachtii* isolates.

The present study consistently supports a monophyletic grouping of specimens occurring on spinach, but *P. effusa* isolates appear to be genetically much more heterogeneous compared to *P. schachtii*. Spinach originated from the Southwest Asian region and has cultivated longer cultivation history outside of its native area. It has become an established vegetable in

Europe in the early part of the 16th century, although it has been grown in China since the 7th or 8th century (Sturtevant, 1890). Consequently, the downy mildew pathogens have undergone a recent and major expansion in geographic range throughout the past centuries, possibly also starting from a small area.

The present study also sheds some light on the phylogeny of a taxonomically important species, *P. rumicis*, the type species of the genus *Peronospora*. Although *Peronospora* specimens from *Rumex* species formed a monophyletic group in all phylogenetic analyses, they are differentiated into several distinct, highly supported lineages. It is noteworthy that the *Peronospora* specimens on *R. acetosa* s.l. are genetically distinct from *P. rumicis* on the type host, *R. acetosella*. This suggests that the name *P. rumicis* should not be applied to downy mildew pathogens other than from *R. acetosella*, even though it has commonly been used to refer to downy mildew pathogens of members of the genus *Rumex* and closely related genera (Hall, 1994; Constantinescu and Fatehi, 2002).

The multilocus phylogeny and species tree estimations provide additional evidence that distinct species of downy mildews can generally infect only a narrow range of closely related host plants or, sometimes, only a particular host species. However, there is increasing evidence that this is not a curious exception in obligate biotrophic fungi. As these organisms live in intimate and specific associations with their host, their phylogeny often mirrors the phylogeny of their hosts (Fahrenholz, 1913), which has sometimes been referred to as “Fungi as plant taxonomists” (Hijwegen, 1979). Similarly, for the white blister rusts (Albuginaceae), another obligate biotrophic family of oomycetes, several distinct species of *Albugo* and *Pustula* were observed to exist on specific host species of the Brassicaceae (Choi et al., 2006, 2007d, 2008b; Thines et al., 2009a; Ploch et al., 2010; Choi et al., 2011a) and Asteridae (Ploch et al., 2011; Rost and Thines, 2012), respectively. Also, in several well-known obligate biotrophic fungi such as rusts (Uredinales), smuts (Ustilaginales), and powdery mildews (Erysiphales), specific associations have been widely recorded, resulting in a high species diversity of about 7000, 1600, and 900 species, respectively (Ainsworth, 2008; Braun and Cook, 2012; Vánky, 2012), which by far surpasses facultative or necrotrophic pathogens. However, there are also a few exceptions from this, such as *Pseudoperonospora cubensis* in case of the downy mildews (Runge et al., 2011) or *Albugo candida* in case of the white blister rusts (Choi et al., 2007d, 2008b, 2009b; Thines et al., 2009a; Ploch et al., 2010).

The macro-evolutionary patterns in downy mildews and other biotrophic pathogens are still vastly unexplored. But from the various hosts parasitized by some closely related downy mildew species, e.g. in *Peronospora* (Voglmayr, 2003) or *Hyaloperonospora* (Göker et al., 2004), it becomes apparent that downy mildews and their hosts cannot have co-diverged on larger evolutionary scales. In this study, the molecular affinities among *Peronospora* species roughly mirror that their host plants, but the congruence of downy mildew and host phylogenies is mostly biased to the terminal branches. This phenomenon can probably be explained by the recent radiation and speciation of downy mildews following at least two independent host shifts from Amaranthaceae to two other families, Caryophyllaceae and Polygonaceae, rather than contemporary cospeciation with host plants. This pattern of host shifts and subsequent radiation has also been observed in the downy mildew genus *Bremia*

(Choi & Thines, unpublished). These results suggest that host shifts and radiation are the main evolutionary driving forces for the speciation of downy mildews. Mostly, host shifts occur between closely related species and genera within a host family, which can at first sight be misinterpreted as cospeciation. However, also within the parasites of a specific host family, host and parasite phylogenies usually do not match completely, providing clear evidence for radiation after host shifts in a range of related hosts. Similarly, in white blister rusts (Choi et al., 2007d, 2008b, 2011d; Thines et al., 2009a), powdery mildews (Matsuda and Takamatsu, 2003; Inuma et al., 2007), rusts (Savile, 1979; Van der Merwe et al., 2008) and smuts (Antonovics et al., 2002; Lopez-Villavicencio et al., 2005), it was shown that host shifts may occur frequently throughout the evolutionary history of plant pathogens, although the underlying mechanisms still remain obscure. In summary, it can be assumed that the downy mildews and probably also other biotrophic pathogens evolve by (1) host shifts that expand their host ranges to taxonomically closely related but more rarely to distantly related plants, and (2) subsequent radiation and speciation in a group of closely related hosts that eventually lead to specialization and dependence on a specific host genus or even a single species. This pattern of repeated rare major host jumps followed by radiation might enable biotrophic pathogens to persist in their hosts groups, as this helps to evade effector-triggered immunity. Arguably, this type of immunity becomes more pronounced with increasing time of host-pathogen co-existence, and if the pathogen has a negative effect on plant fitness, it could eventually drive most pathogenic species to extinction. This might explain, why most of the deep-rooted downy mildew genera exhibit broad host ranges, like in *Peronospora* or *Plasmopara*, or consist of only a single or few species, like in the case of *Benua* or *Plasmoverna*, respectively. Further studies are required to evaluate if this pattern also holds up for other biotrophic pathogens, such as powdery mildew, rust, or smut fungi.

Supplementary Material

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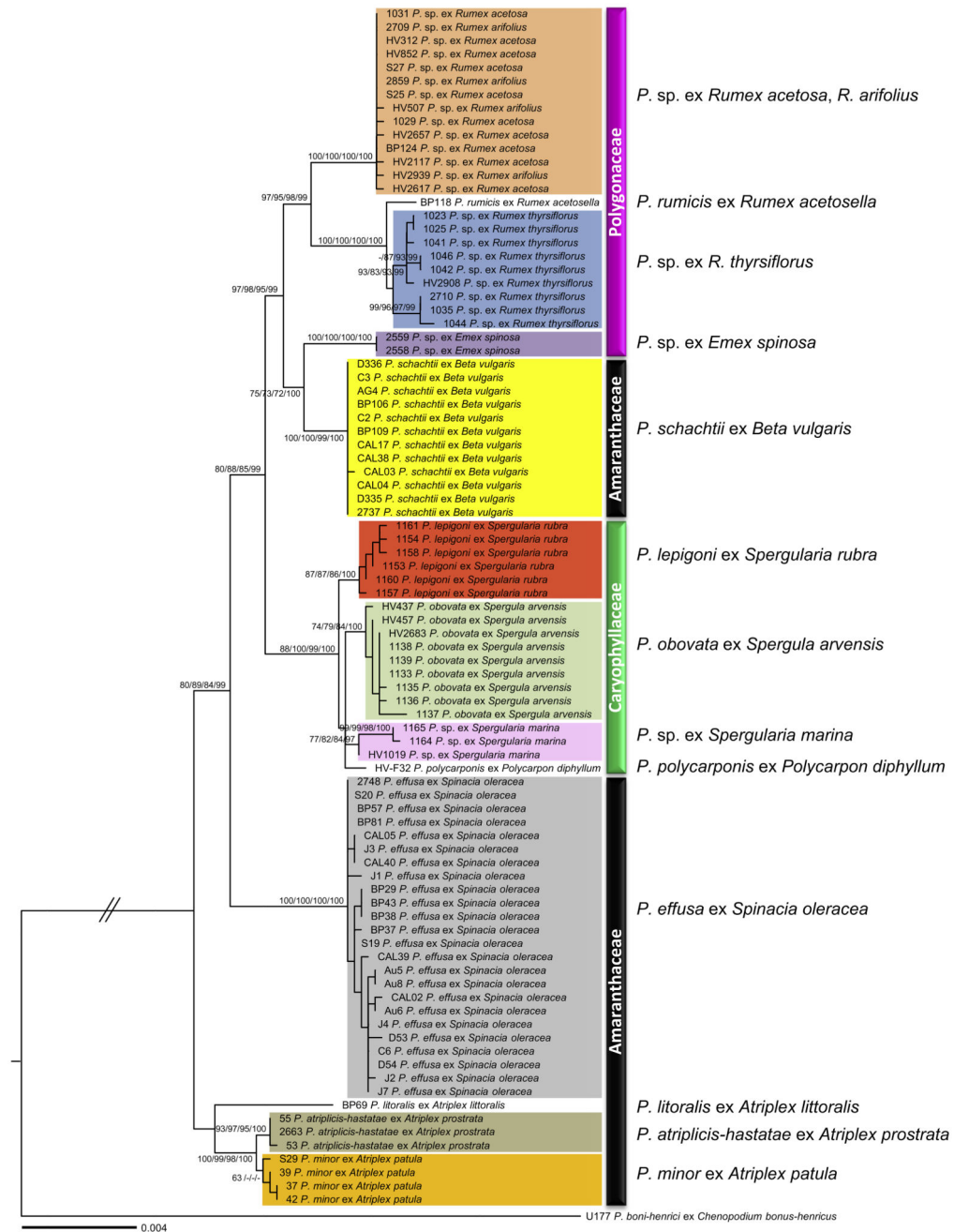


Fig. 1. Phylogenetic tree inferred from Maximum Likelihood analysis of a concatenated alignment (4378 nucleotides with 123 parsimony informative sites) of five mitochondrial (*cox2*, *coxS*, *cox1*, *nad1*, *rps10*) and two nuclear loci (ITS and *hsp90*). Support values (ML BS/ME BS/MP BS/MCMC PP) higher than 60% are given above or below the branches. The scale bar equals the number of nucleotide substitutions per site. Specimens originating from different host families are marked with black (Amaranthaceae), pink (Polygonaceae), and green (Caryophyllaceae) bars. ML: Maximum Likelihood, ME: Minimum Evolution, MP:

Maximum Parsimony, MCMC: Bayesian inference, BS: Bootstrap support, PP: Posterior Probabilities. (For interpretation of thereferences to color in this figure legend, the reader is referred to the web version of this article.)

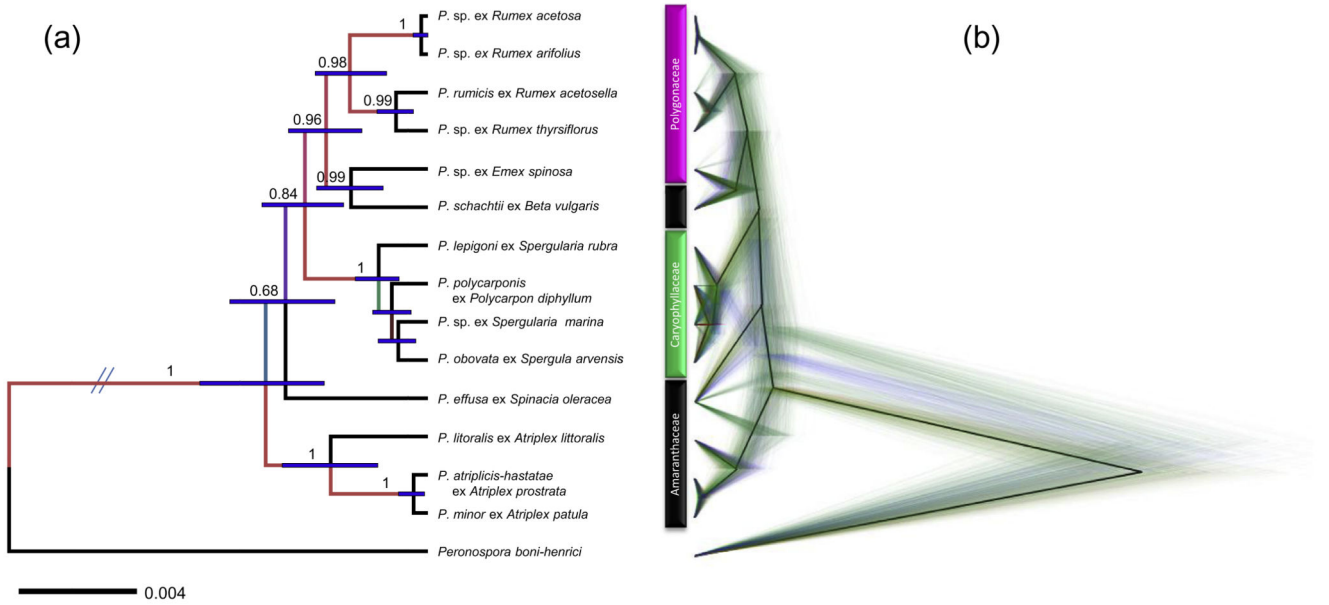


Fig. 2.

Species tree estimation from five mitochondrial (*cox2*, *coxS*, *cox1*, *nad1*, *rps10*) and two nuclear loci (ITS and *hsp90*) using *BEAST. (a) Maximum clade credibility tree visualized by FigTree. Bars correspond to the 95% highest posterior density range, and posterior probabilities higher than 0.60 are shown above branches. (b) Cloudogram of all trees of the MCMC visualized by DensiTree (Bouckaert, 2010). Each possible topology is shown in green with branch lengths averaged among all trees showing that particular topology. Four alternative interpretations are shown in black for the consensus tree, blue for the most frequently occurring topology, red for second, and yellow for third most frequently occurring topology. Higher levels of uncertainty are represented by lower densities of the lines. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Table 1

Information on downy mildew specimens used in this study.

Taxon	Host plant	Geographic origin	Year collected	Herbarium voucher	DNA accession	GenBank accession number				
						ITS	hsp90	cox2-spacer-cox1a	nad1	rps10
<i>Peronospora litoralis</i>	<i>Atriplex litoralis</i>	Sweden, Oland	1959	BPI789214	BP69	KP330778	KP330688	KP330598	KP330868	KP330958
<i>P. minor</i>	<i>Atriplex patula</i>	Sweden, Västergötland	1962	UPS	S29	KP330779	KP330689	KP330599	KP330869	KP330959
<i>P. minor</i>	<i>Atriplex patula</i>	Germany, Sachsen	1993	GLM68024	39	KP330780	KP330690	KP330600	KP330870	KP330960
<i>P. minor</i>	<i>Atriplex patula</i>	Germany, Sachsen	1999	GLM77764	42	KP330781	KP330691	KP330601	KP330871	KP330961
<i>P. minor</i>	<i>Atriplex patula</i>	Germany, Sachsen-Anhalt	2003	GLM73462	37	KP330782	KP330692	KP330602	KP330872	KP330962
<i>P. atriplicis-hastatae</i>	<i>Atriplex prostrata</i>	Germany, Schleswig-Holstein	2012	JK-F0359	2663	KP330783	KP330693	KP330603	KP330873	KP330963
<i>P. atriplicis-hastatae</i>	<i>Atriplex prostrata</i>	Germany, Sachsen-Anhalt	2003	GLM73408	55	KP330784	KP330694	KP330604	KP330874	KP330964
<i>P. atriplicis-hastatae</i>	<i>Atriplex prostrata</i>	Germany, Sachsen-Anhalt	1990	GLM67010	53	KP330785	KP330695	KP330605	KP330875	KP330965
<i>P. schachtii</i>	<i>Beta vulgaris</i> var. <i>cicla</i>	Korea, Namyangju	2007	KUS-F23163	2737	KP330786	KP330696	KP330606	KP330876	KP330966
<i>P. schachtii</i>	<i>Beta vulgaris</i> var. <i>cicla</i>	Korea, Yongin	2007	KUS-F23152	D335	KP330787	KP330697	KP330607	KP330877	KP330967
<i>P. schachtii</i>	<i>Beta vulgaris</i> var. <i>cicla</i>	Korea, Yongin	2007	KUS-F23153	D336	KP330788	KP330698	KP330608	KP330878	KP330968
<i>P. schachtii</i>	<i>Beta vulgaris</i> var. <i>cicla</i>	USA, California	2012	DML3	CAL3	KP330789	KP330699	KP330609	KP330879	KP330969
<i>P. schachtii</i>	<i>Beta vulgaris</i> var. <i>cicla</i>	USA, California	2012	DML4	CAL17	KP330790	KP330700	KP330610	KP330880	KP330970
<i>P. schachtii</i>	<i>Beta vulgaris</i> var. <i>cicla</i>	USA, California	2012	DML38	CAL38	KP330791	KP330701	KP330611	KP330881	KP330971
<i>P. schachtii</i>	<i>Beta vulgaris</i> var. <i>saccharifera</i>	Argentina, Bahía Blanca	1991	RD864	AG4	KP330792	KP330702	KP330612	KP330882	KP330972
<i>P. schachtii</i>	<i>Beta vulgaris</i> var. <i>saccharifera</i>	China, Sichuan	1979	HMAS57030	C3	KP330793	KP330703	KP330613	KP330883	KP330973
<i>P. schachtii</i>	<i>Beta vulgaris</i> var. <i>saccharifera</i>	China, Yunnan	1979	HMAS57033	C2	KP330794	KP330704	KP330614	KP330884	KP330974
<i>P. schachtii</i>	<i>Beta vulgaris</i> var. <i>saccharifera</i>	Germany, Nossen	1888	BPI790777	BP106	KP330795	KP330705	KP330615	KP330885	KP330975
<i>P. schachtii</i>	<i>Beta vulgaris</i> var. <i>saccharifera</i>	USA, California	2012	DML17	CAL4	KP330796	KP330706	KP330616	KP330886	KP330976
<i>P. schachtii</i>	<i>Beta vulgaris</i> var. <i>saccharifera</i>	Unknown	1931	BPI790781	BP109	KP330797	KP330707	KP330617	KP330887	KP330977
<i>P. sp. (formerly as P. runitcis)</i>	<i>Emex spinosa</i>	Greece, Tsambika	2011	VK 0471/1	2558	KP330798	KP330708	KP330618	KP330888	KP330978
<i>P. sp. (formerly as P. runitcis)</i>	<i>Emex spinosa</i>	Greece, Tsambika	2012	VK 0471/2	2559	KP330799	KP330709	KP330619	KP330889	KP330979
<i>P. polycarponis</i>	<i>Polycarpon diphyllum</i>	France, Montpellier	2001	WU33676	HVF32.2	KP330800	KP330710	KP330620	KP330890	KP330980

Taxon	Host plant	Geographic origin	Year collected	Herbarium voucher	DNA accession	ITS	GenBank accession number	<i>hsp90</i>	<i>cox2-spacer-cox1a</i>	<i>nad1</i>	<i>rps10</i>
<i>P. sp.</i> (formerly as <i>P. runicis</i>)	<i>Rumex acetosa</i>	Austria, Oberösterreich	2011	WU34310	HV2617	KP330801	KP330711	KP330621	KP330891	KP330981	KP330981
<i>P. sp.</i> (formerly as <i>P. runicis</i>)	<i>Rumex acetosa</i>	Austria, Oberösterreich	2001	WU22925	HV0312	KP330802	KP330712	KP330622	KP330892	KP330982	KP330982
<i>P. sp.</i> (formerly as <i>P. runicis</i>)	<i>Rumex acetosa</i>	Austria, Wien	2004	WU33899	HV2117	KP330803	KP330713	KP330623	KP330893	KP330983	KP330983
<i>P. sp.</i> (formerly as <i>P. runicis</i>)	<i>Rumex acetosa</i>	England, South Yorkshire	2011	WU34347	HV2657	KP330804	KP330714	KP330624	KP330894	KP330984	KP330984
<i>P. sp.</i> (formerly as <i>P. runicis</i>)	<i>Rumex acetosa</i>	Finland, Nylandia	1960	UPS	S27	KP330805	KP330715	KP330625	KP330895	KP330985	KP330985
<i>P. sp.</i> (formerly as <i>P. runicis</i>)	<i>Rumex acetosa</i>	Finland, Nylandia	1970	BPI790739	BP124	KP330806	KP330716	KP330626	KP330896	KP330986	KP330986
<i>P. sp.</i> (formerly as <i>P. runicis</i>)	<i>Rumex acetosa</i>	Germany,Sachsen-Anhalt	2004	GLM62941	1029	KP330807	KP330717	KP330627	KP330897	KP330987	KP330987
<i>P. sp.</i> (formerly as <i>P. runicis</i>)	<i>Rumex acetosa</i>	Germany, Baden-Württemberg	2001	WU33730	HV0852	KP330808	KP330718	KP330628	KP330898	KP330988	KP330988
<i>P. sp.</i> (formerly as <i>P. runicis</i>)	<i>Rumex acetosa</i>	Germany, Sachsen-Anhalt	2005	GLM75948	1031	KP330809	KP330719	KP330629	KP330899	KP330989	KP330989
<i>P. sp.</i> (formerly as <i>P. runicis</i>)	<i>Rumex acetosa</i>	Sweden, Härjedalen	1975	UPS	S25	KP330810	KP330720	KP330630	KP330900	KP330990	KP330990
<i>P. runicis</i>	<i>Rumex acetosella</i>	Denmark, Ulvshale	1913	BPI790753	BP118	KP330811	KP330721	KP330631	KP330901	KP330991	KP330991
<i>P. sp.</i> (formerly as <i>P. runicis</i>)	<i>Rumex arifolius</i>	Austria, Kärnten	2000	WU32786	HV0507	KP330812	KP330722	KP330632	KP330902	KP330992	KP330992
<i>P. sp.</i> (formerly as <i>P. runicis</i>)	<i>Rumex arifolius</i>	Italy, Lombardia	2012	WU35780	HV2939	KP330813	KP330723	KP330633	KP330903	KP330993	KP330993
<i>P. sp.</i> (formerly as <i>P. runicis</i>)	<i>Rumex arifolius</i>	Italy, Valle d'Aosta	2013	JK-F0513	2709	KP330814	KP330724	KP330634	KP330904	KP330994	KP330994
<i>P. sp.</i> (formerly as <i>P. runicis</i>)	<i>Rumex arifolius</i>	Romania, Harghita	1980	HMR2940	2859	KP330815	KP330725	KP330635	KP330905	KP330995	KP330995
<i>P. sp.</i> (formerly as <i>P. runicis</i>)	<i>Rumex thyrsiflorus</i>	Austria, Niederösterreich	2012	WU35760	HV2908	KP330816	KP330726	KP330636	KP330906	KP330996	KP330996
<i>P. sp.</i> (formerly as <i>P. runicis</i>)	<i>Rumex thyrsiflorus</i>	Germany, Niedersachsen	2009	JK-F0051	2710	KP330817	KP330727	KP330637	KP330907	KP330997	KP330997
<i>P. sp.</i> (formerly as <i>P. runicis</i>)	<i>Rumex thyrsiflorus</i>	Germany, Sachsen	2008	GLM90812	1023	KP330818	KP330728	KP330638	KP330908	KP330998	KP330998
<i>P. sp.</i> (formerly as <i>P. runicis</i>)	<i>Rumex thyrsiflorus</i>	Germany, Sachsen	2008	GLM90995	1025	KP330819	KP330729	KP330639	KP330909	KP330999	KP330999

Taxon	Host plant	Geographic origin	Year collected	Herbarium voucher	DNA accession	ITS	GenBank accession number	<i>hsp90</i>	<i>cox2-spacer-cox1a</i>	<i>mad1</i>	<i>rps10</i>
<i>P. sp.</i> (formerly as <i>P. runicis</i>)	<i>Rumex thyrsiflorus</i>	Germany, Sachsen	2003	GLM74313	1035	KP330820	KP330730	KP330640	KP330910	KP331000	KP331000
<i>P. sp.</i> (formerly as <i>P. runicis</i>)	<i>Rumex thyrsiflorus</i>	Germany, Sachsen-Anhalt	2004	GLM64100	1042	KP330821	KP330731	KP330641	KP330911	KP331001	KP331001
<i>P. sp.</i> (formerly as <i>P. runicis</i>)	<i>Rumex thyrsiflorus</i>	Germany, Sachsen-Anhalt	2003	GLM73428	1046	KP330822	KP330732	KP330642	KP330912	KP331002	KP331002
<i>P. sp.</i> (formerly as <i>P. runicis</i>)	<i>Rumex thyrsiflorus</i>	Germany, Sachsen-Anhalt	2003	GLM73434	1041	KP330823	KP330733	KP330643	KP330913	KP331003	KP331003
<i>P. sp.</i> (formerly as <i>P. runicis</i>)	<i>Rumex thyrsiflorus</i>	Germany, Sachsen-Anhalt	2003	GLM74510	1044	KP330824	KP330734	KP330644	KP330914	KP331004	KP331004
<i>P. obovata</i>	<i>Spergularia arvensis</i>	Austria, Niederösterreich	2011	WU34372	HV2683	KP330825	KP330735	KP330645	KP330915	KP331005	KP331005
<i>P. obovata</i>	<i>Spergularia arvensis</i>	Austria, Oberösterreich	2000	WU32858	HV0437	KP330826	KP330736	KP330646	KP330916	KP331006	KP331006
<i>P. obovata</i>	<i>Spergularia arvensis</i>	Austria, Oberösterreich	2000	WU22914	HV0457	KP330827	KP330737	KP330647	KP330917	KP331007	KP331007
<i>P. obovata</i>	<i>Spergularia arvensis</i>	Germany, Sachsen-Anhalt	2003	GLM73399	1135	KP330828	KP330738	KP330648	KP330918	KP331008	KP331008
<i>P. obovata</i>	<i>Spergularia arvensis</i>	Germany, Sachsen-Anhalt	2001	GLM73791	1138	KP330829	KP330739	KP330649	KP330919	KP331009	KP331009
<i>P. obovata</i>	<i>Spergularia arvensis</i>	Germany, Sachsen-Anhalt	2005	GLM75937	1136	KP330830	KP330740	KP330650	KP330920	KP331010	KP331010
<i>P. obovata</i>	<i>Spergularia arvensis</i>	Germany, Sachsen-Anhalt	2002	GLM76294	1137	KP330831	KP330741	KP330651	KP330921	KP331011	KP331011
<i>P. obovata</i>	<i>Spergularia arvensis</i>	Germany, Sachsen-Anhalt	2002	GLM76717	1139	KP330832	KP330742	KP330652	KP330922	KP331012	KP331012
<i>P. obovata</i>	<i>Spergularia arvensis</i>	Germany, Sachsen-Anhalt	2000	GLM76830	1133	KP330833	KP330743	KP330653	KP330923	KP331013	KP331013
<i>P. sp.</i> (formerly as <i>P. lepigoni</i>)	<i>Spergularia marina</i>	Austria, Burgenland	2001	WU33808	HV1019	KP330834	KP330744	KP330654	KP330924	KP331014	KP331014
<i>P. sp.</i> (formerly as <i>P. lepigoni</i>)	<i>Spergularia marina</i>	Germany, Sachsen-Anhalt	2001	GLM73809	1165	KP330835	KP330745	KP330655	KP330925	KP331015	KP331015
<i>P. sp.</i> (formerly as <i>P. lepigoni</i>)	<i>Spergularia marina</i>	Germany, Sachsen-Anhalt	2005	GLM75894	1164	KP330836	KP330746	KP330656	KP330926	KP331016	KP331016
<i>P. lepigoni</i>	<i>Spergularia rubra</i>	Germany, Sachsen	2005	GLM75718	1153	KP330837	KP330747	KP330657	KP330927	KP331017	KP331017
<i>P. lepigoni</i>	<i>Spergularia rubra</i>	Germany, Sachsen	1998	GLM77927	1154	KP330838	KP330748	KP330658	KP330928	KP331018	KP331018
<i>P. lepigoni</i>	<i>Spergularia rubra</i>	Germany, Sachsen-Anhalt	2004	GLM64180	1160	KP330839	KP330749	KP330659	KP330929	KP331019	KP331019
<i>P. lepigoni</i>	<i>Spergularia rubra</i>	Germany, Sachsen-Anhalt	1991	GLM68688	1161	KP330840	KP330750	KP330660	KP330930	KP331020	KP331020
<i>P. lepigoni</i>	<i>Spergularia rubra</i>	Germany, Sachsen-Anhalt	2001	GLM73623	1157	KP330841	KP330751	KP330661	KP330931	KP331021	KP331021
<i>P. lepigoni</i>	<i>Spergularia rubra</i>	Germany, Sachsen-Anhalt	2002	GLM76298	1158	KP330842	KP330752	KP330662	KP330932	KP331022	KP331022
<i>P. effusa</i>	<i>Spinacia oleracea</i>	Australia, Leppington	2002	VPRI30202	Au6	KP330843	KP330753	KP330663	KP330933	KP331023	KP331023
<i>P. effusa</i>	<i>Spinacia oleracea</i>	Australia, Turna	2000	VPRI22523	Au5	KP330844	KP330754	KP330664	KP330934	KP331024	KP331024
<i>P. effusa</i>	<i>Spinacia oleracea</i>	Australia, V.I.C.	2003	VPRI31625	Au8	KP330845	KP330755	KP330665	KP330935	KP331025	KP331025

Taxon	Host plant	Geographic origin	Year collected	Herbarium voucher	DNA accession	GenBank accession number	<i>hsp90</i>	<i>cox2-spacer-cox1a</i>	<i>matI</i>	<i>rps10</i>
<i>P. effusa</i>	<i>Spinacia oleracea</i>	China, Sichuan	1958	HMAS57074	C6	KP330846	KP330756	KP330666	KP330936	KP331026
<i>P. effusa</i>	<i>Spinacia oleracea</i>	Japan, Fukui	2000	DM68	J4	KP330847	KP330757	KP330667	KP330937	KP331027
<i>P. effusa</i>	<i>Spinacia oleracea</i>	Japan, Gifu	Unknown	DM22	J1	KP330848	KP330758	KP330668	KP330938	KP331028
<i>P. effusa</i>	<i>Spinacia oleracea</i>	Japan, Hokkaido	2004	DM81	J7	KP330849	KP330759	KP330669	KP330939	KP331029
<i>P. effusa</i>	<i>Spinacia oleracea</i>	Japan, Shiga	Unknown	DM34	J2	KP330850	KP330760	KP330670	KP330940	KP331030
<i>P. effusa</i>	<i>Spinacia oleracea</i>	Japan, Shiga	2000	DM65	J3	KP330851	KP330761	KP330671	KP330941	KP331031
<i>P. effusa</i>	<i>Spinacia oleracea</i>	Korea, Namyangju	1999	KUS-F15680	2748	KP330852	KP330762	KP330672	KP330942	KP331032
<i>P. effusa</i>	<i>Spinacia oleracea</i>	Korea, Seoul	2001	KUS-F18808	D53	KP330853	KP330763	KP330673	KP330943	KP331033
<i>P. effusa</i>	<i>Spinacia oleracea</i>	Korea, Sinan	2001	KUS-F18809	D54	KP330854	KP330764	KP330674	KP330944	KP331034
<i>P. effusa</i>	<i>Spinacia oleracea</i>	Mexico, Nogales	1953	BPI788308	BP37	KP330855	KP330765	KP330675	KP330945	KP331035
<i>P. effusa</i>	<i>Spinacia oleracea</i>	Mexico, Nogales	1949	BPI788314	BP43	KP330856	KP330766	KP330676	KP330946	KP331036
<i>P. effusa</i>	<i>Spinacia oleracea</i>	Sweden, Gästrikland	1942	UPS	S19	KP330857	KP330767	KP330677	KP330947	KP331037
<i>P. effusa</i>	<i>Spinacia oleracea</i>	USA, California	2012	DML2	CAL2	KP330858	KP330768	KP330678	KP330948	KP331038
<i>P. effusa</i>	<i>Spinacia oleracea</i>	USA, California	2012	DML39	CAL39	KP330859	KP330769	KP330679	KP330949	KP331039
<i>P. effusa</i>	<i>Spinacia oleracea</i>	USA, California	2012	DML40	CAL40	KP330860	KP330770	KP330680	KP330950	KP331040
<i>P. effusa</i>	<i>Spinacia oleracea</i>	USA, California	2012	DML5	CAL5	KP330861	KP330771	KP330681	KP330951	KP331041
<i>P. effusa</i>	<i>Spinacia oleracea</i>	USA, Kingston	1896	UPS	S20	KP330862	KP330772	KP330682	KP330952	KP331042
<i>P. effusa</i>	<i>Spinacia oleracea</i>	USA, Maryland	1958	BPI788300	BP29	KP330863	KP330773	KP330683	KP330953	KP331043
<i>P. effusa</i>	<i>Spinacia oleracea</i>	USA, Maryland	1951	BPI788309	BP38	KP330864	KP330774	KP330684	KP330954	KP331044
<i>P. effusa</i>	<i>Spinacia oleracea</i>	USA, Oklahoma	1943	BPI791055	BP81	KP330865	KP330775	KP330685	KP330955	KP331045
<i>P. effusa</i>	<i>Spinacia oleracea</i>	USA, Virginia	1932	BPI788361	BP57	KP330866	KP330776	KP330686	KP330956	KP331046
<i>P. boni-henrici</i>	<i>Chenopodium bonus-henricus</i>	Austria, Tirol	1991	GLM51002	177	KP330867	KP330777	KP330687	KP330957	KP331047

^aIncluding *cox2*, *cox1*, and the spacer region between *cox2* and *cox1*.