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Phylogenetic investigations in the downy mildew genus *Bremia* reveal several distinct lineages and a species with a presumably exceptional wide host range

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Abstract *Bremia lactucae* is one of the most devastating and widespread pathogens in lettuce production worldwide. Despite its economical importance, uncertainty prevails about the species delimitation in the genus *Bremia*. Commonly, *Bremia* is considered to be monotypic, containing only *Bremia lactucae*, while taxonomists have described additional species, and molecular phylogenetic studies have shown significant sequence divergence between accessions from different hosts. Here, we report that several previously described species are genetically highly distinct from *Bremia lactucae* parasitic to *Lactuca sativa*. These include *Bremia lapsanae*, *Bremia sonchicola*, and *Bremia taraxaci*. In addition to these host-specific species, a plurivorous species is revealed, which infects hosts from three different tribes in the Asteraceae subfamilies Asteroideae and Carduoideae. The broad host range of clade 1 is exceptional for downy mildews and only paralleled by *Pseudoperonospora cubensis*, which infects a broad range of Cucurbitaceae. The taxonomic status of *Bremia cirsii* and of *Bremia centaureae* remains unresolved, as the accessions from *Cirsium* and *Centaurea*, respectively, did not form a monophylum but were partly contained in the plurivorous clade 1. *Bremia lactucae* was found to be restricted to *Lactuca sativa* and *Lactuca serriola*. Thus, it can be assumed that *Bremia* infections on weeds apart from *Lactuca* species do not pose a significant risk for lettuce production. However, it is unlikely that breeding resistance genes from *Lactuca serriola* into *Lactuca sativa* will result in durable resistance of lettuce to downy mildew disease, because the current study provides additional evidence that *Bremia* accessions from both hosts form a population continuum.

Keywords Lettuce downy mildew · Peronosporaceae · Species concepts · Molecular phylogenetics · cox2 · nrLSU · Resistance

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Introduction

The genus *Bremia* (Regel 1843) contains pathogens parasitic to a wide range of Asteraceae and is widespread in temperate and subtropical regions of the northern hemisphere. Before the advent of molecular phylogenetic studies, two species, *Bremia graminicola* and *Bremia lactucae*, were broadly recognised, while other Asteraceae-infecting species were mostly considered to be conspecific with *Bremia lactucae*. As the former species was shown to be unrelated to the type species, *Bremia lactucae*, Thines et al. (2006) introduced the genus *Graminivora* to accommodate *B. graminicola*, leaving *Bremia* apparently monotypic. However, about 20 species and several varieties of *Bremia* have been described as parasites of Asteraceae, often on the basis of rather subtle morphological differences. Because of the difficulties of morphological species delimitation in downy mildews, the broad species concept advocated by Yerkes and Shaw (1959) was usually applied. This view was supported by Skidmore and Ingram (1985), who claimed that morphological characters were too variable to distinguish the host-specific *Bremia* lineages they found, and therefore treated them as *formae speciales* rather than as distinct species. However, molecular phylogenetic studies revealed that a considerable genetic diversity exists within the genus *Bremia* (Voglmayr et al. 2004; Voglmayr and Constantinescu 2008; Choi et al. 2007a) and thus the existence of different species in the genus *Bremia* should be reconsidered. The so far most extensive phylogenetic study for *Bremia* (Voglmayr et al. 2004) contained accessions from numerous asteraceous hosts from Europe, including from all eleven host specific groups of Skidmore and Ingram (1985). However, only limited conclusions could be drawn as the study was mostly based on single accessions per host species and on only moderately variable nrLSU rDNA sequences. In Voglmayr and Constantinescu (2008), taxon sampling was restricted to clarify the phylogenetic relationships of *Plasmopara centaureae-mollis*, which was unequivocally placed in the genus *Bremia*. Hence, the presence of multiple species in European populations of *Bremia lactucae* could not yet been investigated in detail. It was the aim of this study to investigate, whether it is possible to differentiate several *Bremia* species, described from or present in Europe in a combined analysis of the highly variable *cox2* gene and moderately variable nrLSU sequences.

Materials and methods

Oomycete material

The oomycete material used in this study is listed in Table 1. Herbarium acronyms are according to Thiers (2009). The nomenclature of the hosts is derived from Angiosperm Phylogeny Group (2009) for taxonomic ranks above and Funk et al. (2005) for taxonomic ranks below the family level.

DNA-extraction, PCR and sequencing

DNA was extracted using the Innupure Plant DNA extraction kit, with the modifications for herbarised material described in Telle and Thines (2008). For amplification of the mitochondrial *cox2* gene, the primers described by Hudspeth et al. (2000) were employed as described previously (Choi et al. 2007b), but using the MangoTaq Polymerase (Bioline, Luckenwalde, Germany), and with the addition of BSA to a final concentration of 8 μg/μl in the PCR reaction mix. The amplification of the nrLSU D1-D3 regions was carried out as described in Riethmüller et al. (2002), again with MangoTaq DNA polymerase and the addition of BSA to a final concentration of 8 μg/μl. Sequencing was carried out by a commercial sequencing company (GATC Biotech, Konstanz, Germany) with the primers used for PCR. As resolution with LSU sequencing did not significantly improve when sequencing the full-length amplicon, sequencing was only carried out using the primer LR6 described by Moncalvo et al. (1995). GenBank accession numbers of all sequences are listed in Table 1.

Alignments and phylogenetic inference

Alignments wee done using mafft (Katoh et al. 2005), version 6.717 applying the Q-INS-i algorithm, independently for *cox2* and nrLSU sequences. Alignments were then concatenated for phylogenetic inference. Maximum Likelihood (ML) analysis was carried out

Table 1 Oomycete material used in this study

Pathogen	Host	DNA accession number	year	Country	GenBank accession number *cox2*	GenBank accession number nrLSU
Bremia tulasnei	*Senecio vulgaris*	HUH890	2006	Germany	GU361007	GU361022
Bremia tulasnei	*Senecio vulgaris*	HUH982	2008	Germany	GU361008	GU361021
Bremia cf. *centaureae*	*Centarea jacea*	HV528	2000	Austria	GU360998	GU361043
Bremia cf. *centaureae*	*Centaurea jacea*	HV2243	2006	Austria	GU360999	GU361040
Bremia cf. *cirsii*	*Cirsium arvense*	HUH513[a]	2002	Germany	GU360992	GU361013
Bremia cf. *cirsii*	*Cirsium arvense*	HUH746[a]	2005	Germany	GU360993	GU361026
Bremia cf. *cirsii*	*Cirsium arvense*	HV2242	2006	Austria	GU360995	GU361033
Bremia cf. *cirsii*	*Cirsium arvense*	HV777	2000	Austria	GU360994	GU361018
Bremia cf. *cirsii*	*Cirsium oleraceum*	HV1001	2001	Austria	GU360996	GU361029
Bremia cf. *cirsii*	*Cirsium oleraceum*	HV2236	2006	Austria	GU360997	GU361025
Bremia lactucae	*Lactuca sativa*	HUH429[a]	2001	Germany	GU360979	GU361012
Bremia lactucae	*Lactuca sativa*	HUH473[a]	2002	Germany	GU361003	GU361014
Bremia lactucae	*Lactuca sativa*	HUH521[a]	2002	Germany	GU360981	GU361016
Bremia lactucae	*Lactuca sativa*	HV1036	2001	Austria	GU361004	GU361020
Bremia lactucae	*Lactuca serriola*	HUH497[a]	2002	Germany	GU360980	GU361015
Bremia lactucae	*Lactuca serriola*	HUH756[a]	2005	Germany	GU360986	GU361017
Bremia lapsanae	*Lapsana communis*	HV393	2000	Austria	GU361005	GU361031
Bremia sonchicola	*Sonchus oleraceus*	MG2153	2003	Germany	GU361009	GU361042
Bremia sp.	*Arctium* sp.	HV2235	2006	Austria	GU360988	GU361027
Bremia sp.	*Arctium* sp.	KR12281[b]	2003	Germany	GU360989	GU361041
Bremia sp.	*Carlina acaulis*	HV483	2000	Austria	GU360990	GU361034
Bremia sp.	*Cirsium acaule*	MG1836	2002	Austria	GU360991	GU361036
Bremia sp.	*Helichrysum* sp.	Heli01	n.a.	U.S.A.	GU361000	GU361028
Bremia sp.	*Hieracium murorum*	HV515	2000	Austria	GU361001	GU361035
Bremia sp.	*Hieracium murorum*	HV629	2000	Austria	GU361002	GU361032
Bremia sp.	*Leontodon hispidus*	HV2244	2006	Austria	GU361006	GU361024
Bremia taraxaci	*Taraxacum officinale*	HV2241	2006	Austria	GU361011	GU361023
Bremia taraxaci	*Taraxacum officinale*	HV772	2000	Austria	GU361010	GU361019
Plasmoverna anemones-ranunculoidis	*Anemone ranunculoidis*	HUH574[a]	2004	Germany	GU360983	GU361038
Plasmoverna anemones-ranunculoidis	*Anemone ranunculoidis*	HUH583[a]	2004	Germany	GU360984	GU361037
Plasmoverna pygmea	*Anemone nemorosa*	HUH573[a]	2004	Germany	GU360982	GU361039
Protobremia sphaerosperma	*Tragopogon pratensis*	HUH720[a]	2005	Germany	GU360985	GU361030
Protobremia sphaerosperma	*Tragopogon pratensis*	HUH921[a]	2007	Germany	GU360987	GU361044

[a] deposited in HOH

[b] deposited in KR

n.a = not available.

using RAxML (Stamatakis 2006) on the webserver at CIPRIS (http://8ball.sdsc.edu:8889/cipres-web/Home.do) with likelihood search and estimation of invariable sites. All other parameters were set to default values. As the rapid bootstrapping algorithm (Stamatakis et al. 2008) can lead to deviation in the range of several percent, the analysis was repeated five times with 100 bootstrap replicates each and the bootstrap values obtained were averaged. Minimum Evolution (ME) analysis was done using Mega 4.0

(Tamura et al. 2007), using the Tamura-Nei substitution model. All other parameters were set to default values. For testing tree robustness, 1,000 bootstrap replicates were carried out. Maximum Parsimony (MP) analysis was carried out using Mega 4.0, with all parameters set to default values, again performing 1,000 bootstrap replicates. For Bayesian analysis (BA), MrBAYES (Huelsenbeck and Ronquist 2001), version 3.1.2 was used. Four incrementally heated chains were run for 10 million generations implementing the GTR-I-G substitution model and 4 gamma categories, with a sampling frequency of 1,000. The first 5,000 trees were discarded, and the remaining 5,000 trees were used to compute a majority rule consensus tree to reveal the posterior probabilities. For testing reproducibility of the results and deviation between individual runs, three additional parallel runs were carried out for 2 million generations as described above, and the first 1,000 trees sampled were discarded. The average standard deviation for the remaining 1,000 trees was calculated at 0.007 and was only moderately decreasing through the last 500 trees sampled. Nonetheless, the first 5 million generations were discarded from the first analysis, for ensuring optimal results.

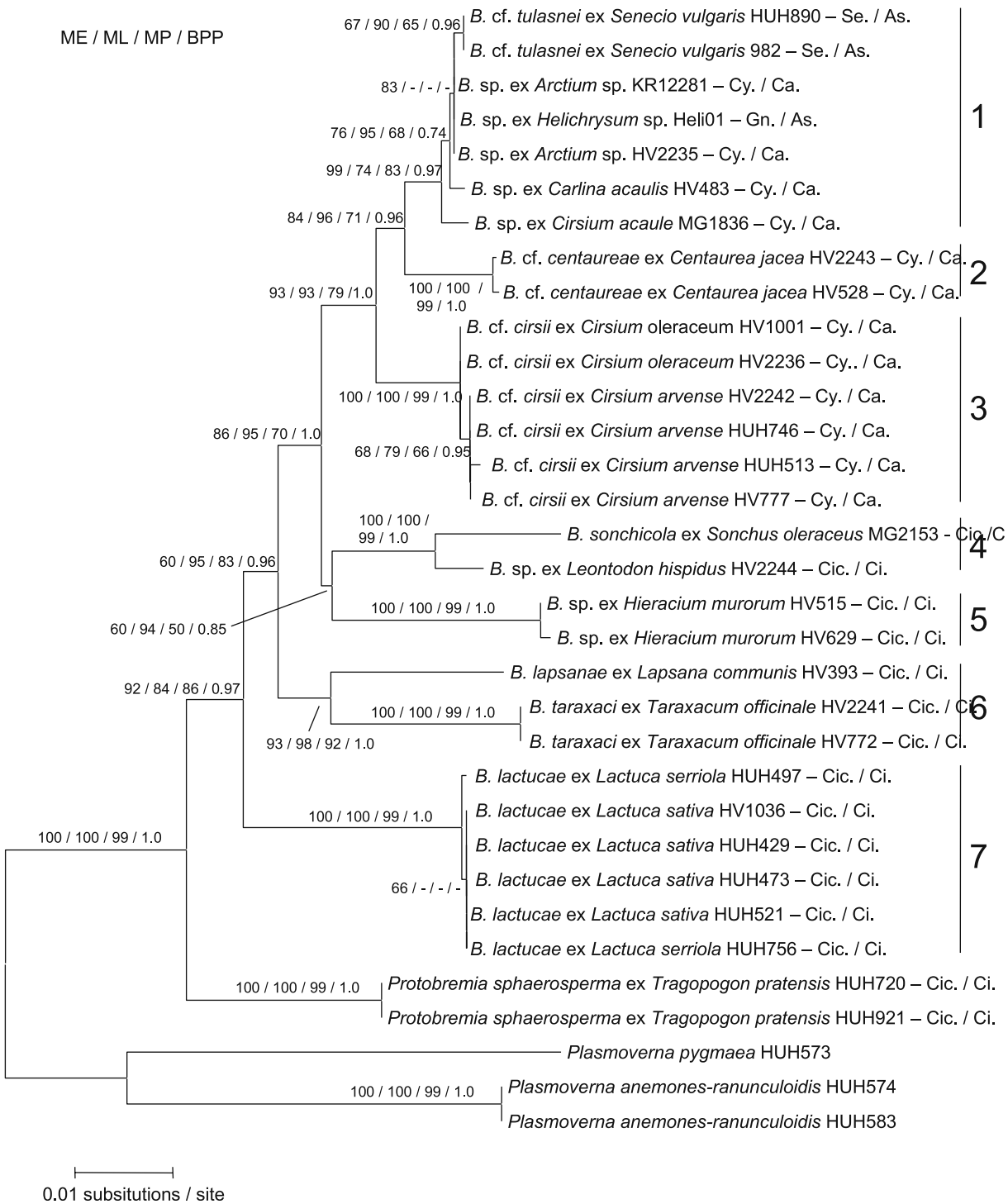
Results

All phylogenetic analyses yielded similar topologies, and no significantly supported inconsistencies were observed. The best tree from the Minimum Evolution analysis, with the support values from all phylogenetic analyses (ME, ML, MP, and BA), is given in Fig. 1.

Protobremia sphaerosperma was consistently placed as sister group to a monophyletic *Bremia* clade with moderate to high support. Within *Bremia*, nine distinct phylogenetic lineages were observed, which correspond to parasites of a single respective genus, except for one lineage (corresponding to clade 1), which comprised several hosts from two of the three subfamilies commonly present in Europe and presumably included *B. tulasnei* from *Senecio vulgaris*. This lineage was placed as sister group to the *Centaurea*-infecting lineage (clade 2), and these two lineages formed a highly (ME, ML, and BA analyses) or moderately (MP analysis) supported clade together with the *Cirsium*-infecting lineage (clade 3). This clade contained all specimens of *Bremia* infective to species inside the tribe Cynareae of the Asteraceae subfamily Carduoideae. All lineages referred to below are comprised solely of parasites of the tribe Cichorieae of the Cichorioideae. *Bremia sonchicola* and *Bremia* from *Leontodon hispidus* were grouped together in clade 4 showing an unsupported sister-group relationship to *Bremia* from *Hieracium murorum* (clade 5). The latter three phylogenetic species were the closest relatives of the clade containing the Cynareae-infecting lineages. The monophyly of this assemblage was moderately supported in ME, weakly supported in MP, but highly supported in both ML and BA analyses. The highly supported clade 6, containing *B. taraxaci* and *B. lapsanae*, was sister group to all previously discussed lineages with weak support in ME, moderate support in MP, and high support in ML and BA analyses.

Bremia lactucae s. str. was consistently found to be the most basal lineage of *Bremia*. *Bremia* samples from *Lactuca serriola* and from *Lactuca sativa* were not discriminated in the analysis, although one of the specimens of *Bremia* from *Lactuca serriola* was placed basal to the other specimens in this clade with weak support in ME analysis. The monophyly of the seven clades which contained the nine distinct phylogenetic lineages of Bremia was highly supported in all analyses, except for the plurivorous lineage forming clade 1 which contained specimens from several hosts of two subfamilies of the Asteraceae, which was weakly supported in ML, moderately supported in MP, but highly supported in ME and BA analyses. The two species of the outgroup taxon, *Plasmoverna*, were genetically distinct.

Fig. 1 Phylogenetic tree for the downy mildew genus *Bremia* based on Minimum Evolution analysis of the concatenated sequence alignment from partial cox2 and nrLSU sequences. Numbers on braches denote statistical support in Minimum Evolution, Maximum Likelihood, Maximum Parsimony and Bayesian Analyses, respectively. Numbers on vertical lines are clade numbers referred to in the manuscript. The bar indicates genetic distances in substitutions per site. DNA accession numbers are given after the host taxon names, followed by abbreviated host tribe and host subfamily designations for the pathogens of Asteraceae. Host tribes were abbreviated as follows—Cic. = Cichorieae, Cy. = Cynareae, Gn. = Gnaphalieae, Se. = Senecioneae. For host subfamilies, the following abbreviations were used—As. = Asteroideae, Ca. = Carduoideae, Ci. = Cichorioideae


ME / ML / MP / BPP
67 / 90 / 65 / 0.96
B. cf. tulasnei ex Senecio vulgaris HUH890 – Se. / As.
B. cf. tulasnei ex Senecio vulgaris 982 – Se. / As.
83 / - / - / -
B. sp. ex Arctium sp. KR12281 – Cy. / Ca.
B. sp. ex Helichrysum sp. Heli01 – Gn. / As.
76 / 95 / 68 / 0.74
B. sp. ex Arctium sp. HV2235 – Cy. / Ca.
99 / 74 / 83 / 0.97
B. sp. ex Carlina acaulis HV483 – Cy. / Ca.
84 / 96 / 71 / 0.96
B. sp. ex Cirsium acaule MG1836 – Cy. / Ca.
1
B. cf. centaureae ex Centaurea jacea HV2243 – Cy. / Ca.
100 / 100 / 99 / 1.0
B. cf. centaureae ex Centaurea jacea HV528 – Cy. / Ca.
2
93 / 93 / 79 /1.0
B. cf. cirsii ex Cirsium oleraceum HV1001 – Cy. / Ca.
B. cf. cirsii ex Cirsium oleraceum HV2236 – Cy.. / Ca.
100 / 100 / 99 / 1.0
B. cf. cirsii ex Cirsium arvense HV2242 – Cy. / Ca.
B. cf. cirsii ex Cirsium arvense HUH746 – Cy. / Ca.
3
86 / 95 / 70 / 1.0
68 / 79 / 66 / 0.95
B. cf. cirsii ex Cirsium arvense HUH513 – Cy. / Ca.
B. cf. cirsii ex Cirsium arvense HV777 – Cy. / Ca.
100 / 100 / 99 / 1.0
B. sonchicola ex Sonchus oleraceus MG2153 - Cic./C
4
60 / 95 / 83 / 0.96
B. sp. ex Leontodon hispidus HV2244 – Cic. / Ci.
100 / 100 / 99 / 1.0
B. sp. ex Hieracium murorum HV515 – Cic. / Ci.
5
60 / 94 / 50 / 0.85
B. sp. ex Hieracium murorum HV629 – Cic. / Ci.
B. lapsanae ex Lapsana communis HV393 – Cic. / Ci.
92 / 84 / 86 / 0.97
100 / 100 / 99 / 1.0
B. taraxaci ex Taraxacum officinale HV2241 – Cic. / Ci.
6
93 / 98 / 92 / 1.0
B. taraxaci ex Taraxacum officinale HV772 – Cic. / Ci.
B. lactucae ex Lactuca serriola HUH497 – Cic. / Ci.
100 / 100 / 99 / 1.0
B. lactucae ex Lactuca sativa HV1036 – Cic. / Ci.
100 / 100 / 99 / 1.0
B. lactucae ex Lactuca sativa HUH429 – Cic. / Ci.
B. lactucae ex Lactuca sativa HUH473 – Cic. / Ci.
7
66 / - / - / -
B. lactucae ex Lactuca sativa HUH521 – Cic. / Ci.
B. lactucae ex Lactuca serriola HUH756 – Cic. / Ci.
100 / 100 / 99 / 1.0
Protobremia sphaerosperma ex Tragopogon pratensis HUH720 – Cic. / Ci.
Protobremia sphaerosperma ex Tragopogon pratensis HUH921 – Cic. / Ci.
Plasmoverna pygmaea HUH573
100 / 100 / 99 / 1.0
Plasmoverna anemones-ranunculoidis HUH574
Plasmoverna anemones-ranunculoidis HUH583
0.01 subsitutions / site

Discussion

The resolution of infrageneric phylogenetic relationships of *Bremia* accessions from Europe is significantly improved in comparison to previous studies including a variety of *Bremia* specimens (Voglmayr et al. 2004; Voglmayr and Constantinescu 2008). This is most likely due to the highly informative *cox2* gene, which has been proven to be suitable for inferring subgeneric to suprafamiliar phylogenetic relationships in previous studies (Hudspeth et al. 2000, 2003; Choi et al. 2007b, 2008, 2009a, b, c; Thines et al. 2008, 2009a, b). Although tree topology is partly different from previously inferred topologies in *Bremia* (Voglmayr et al. 2004; Voglmayr and Constantinescu 2008), all comparable highly supported clades from the corresponding studies were also observed in the present study. Only minor topological differences were observed when comparing *cox2* and nrLSU trees (data not shown), mainly related to the crown group of *Bremia* (clade 1) consisting of specimens from various tribes of the Asteraceae.

Evolution and radiation of *Bremia* has most likely started in the Asteraceae subfamily Cichorioideae, and more specifically in the tribe Cichorieae, which is in line with the conclusions of Voglmayr et al. (2004). Evidence for this comes from the fact that both the sister genus of *Bremia*, *Protobremia*, and the two basal clades of *Bremia* (clades 6 and 7) are parasites of this tribe. After the divergence of these lineages, a host jump occurred to Carduoideae, possibly to the tribe Cynareae. However, to ascertain this hypothesis, a more extensive sampling of *Bremia* from this subfamily is necessary. Interestingly, multiple host jumps have occurred in evolutionarily recent times in clade 1, which comprises hosts in the tribes Cynareae, Gnaphalieae, and Senecioneae, and the taxonomic status of which will be discussed later. As genetic distances are comparatively short within this clade, gene flow may commonly occur within this apparently rapidly radiating lineage, which thus might constitute a single species with a wide host range, covering hosts from at least two subfamilies of Asteraceae. This wide host range is exceptional for downy mildews, in which the commonly applied broad species concept has been refuted by several recent molecular phylogenetic studies (Göker et al. 2004, 2009; García-Blázquez et al. 2008; Choi et al. 2007c, 2009b, c; Thines et al. 2009a). However, comparably broad host ranges have been reported for *Pseudoperonospora cubensis* (Choi et al. 2005) and for *Albugo candida* (Voglmayr and Riethmüller 2006; Choi et al. 2006, 2007b, 2008, 2009a; Thines et al. 2009b). The basis of host specificity versus broad host ranges remains obscure, but it can be expected that those pathogens with broad host ranges have effectors targeting pathways basal in pathogen defence networks that are highly conserved (for discussion see Thines et al. 2009b).

Several of the nine distinct phylogenetic lineages correspond to previously described species from Europe, as well to the specialised forms observed and described by Skidmore and Ingram (1985). In addition to *B. lactucae*, *B. lapsanae*, *B. sonchicola*, and *B. taraxaci*, apparently two additional yet undescribed species were revealed, one parasitic to *Hieracium murorum*, and another parasitic to *Leontodon hispidus*. It is likely that they correspond to the infection groups C and K of Skidmore and Ingram (1985), classified as *B. lactucae*, *formae specialis hieracii* and *leontodi*, respectively. As stated previously, morphological discrimination of species within *Bremia* is difficult (Syrovátko et al. 1984, Skidmore and Ingram 1985, Voglmayr et al. 2004), and only minor differences were observed between specimens of the undescribed species and those of most other species investigated in this study (data not shown). As a higher number of specimens should be investigated for accessing the morphological variability within the new species, we refrain from introducing new species names in this work. Whether it will be possible to distinguish all phylogenetic species of *Bremia* morphologically is unclear and remains to be investigated in future studies. It will also be important to scrutinize the type specimens of *B. centaureae* and *B. cirsii* thoroughly to assess their taxonomic status, and specimens from the type hosts need to be included in phylogenetic analyses. This is of crucial importance, as a specimen from *Cirsium acaule* was placed into the plurivorous clade 1, apart from clade 3 containing the other *Bremia* accessions from *Cirsium*. Also one specimen from *Centaurea jacea* was placed in clade 1, apart from the other two accessions, which were placed in clade 2, based on *cox2* sequence data. As unfortunately no nrLSU sequences could be obtained for this specimen, it is not included in the phylogenetic analyses (Fig. 1). Therefore, also the taxonomic status of *B. centaureae* remains unclear,

because conspecificity with *B. tulasnei* cannot be ruled out at present, and no specimen from the type host (*Centaurea montana*) has been included. In addition to the examination of the type specimens of both *B. centaureae* and *B. cirsii* it will be necessary to conduct a broad sampling of Bremia from the corresponding host genera for estimating the host range of the phylogenetic species revealed in these hosts. Although the *B. tulasnei* specimens from *Senecio vulgaris* formed a distinct lineage within the plurivorous clade 1 with weak bootstrap support in ME and MP, and strong support in ML and BA, an extended sampling of specimens, multigene analyses, and cross-infection tests might be necessary for clarifying whether they are distinct from the other *Bremia* accessions contained clade 1. However, increased sampling and multigene analyses are preferable over cross-inoculation experiments, as for example Komjáti et al. (2007) reported that *Plasmopara angustiterminalis* can infect some sunflower lines, albeit the pathogen is highly distinct from *P. halstedii* based on both morphological (Novotel'nova 1963) and molecular phylogenetic data (Voglmayr et al. 2004, Komjáti et al. 2007). For the *Bremia* clade 3, exclusively found on *Cirsium*, it is noteworthy that accessions from *Cirsium arvense* and *Cirsium oleraceum* are separated with weak support in ME and MP, moderate support in ML and high support in BA. This could indicate some degree of host specialisation and genetic diversification on the various *Cirsium* species, which should be investigated by a more intense sampling of this group and by including additional genes with high phylogenetic resolution, like ITS (Choi et al. 2007a; Thines 2007). Considering the results of Voglmayr et al. (2004), it is likely that extended sampling from other hosts, investigated with highly variable genes, will reveal additional phylogenetically distinct lineages that should be considered independent species.

The finding that *B. lactucae* forms a genetically distinct clade restricted to *Lactuca sativa* and *Lactuca serriola* provides additional evidence that weeds from other genera than *Lactuca* are unlikely to serve as an inoculum for lettuce crop production, which is in line with previous conclusions based on infection studies (Lebeda and Syrovátko 1988, Lebeda et al. 2002, 2008). However, the position of the isolate HOH HUH756 from *Lactuca serriola* within the *Lactuca sativa* isolates and the short genetic distance to the other isolate from *Lactuca serriola*, HOH HUH497, indicates that gene flow is likely to occur between *Bremia* accessions from the respective hosts (Lebeda 2002), which is in line with previous inoculation studies, e.g. Lebeda and Syrovátko (1988); Lebeda and Petrzelova (2004). Considering the efforts for breading *Bremia* resistance genes from *Lactuca serriola* into *Lactuca sativa* this finding is important, as it seems possible that these efforts will prove futile in the light of gene flow between pathogens from these hosts. Also Lebeda et al. (2008) reported that while the occurrence of virulence and resistance factors was mostly unique for each host-pathogen system, there was some overlap between the populations that show the presumably high potential for the evolution of new strains overcoming resistance in either pathosystem. It can thus be expected that due to recombination events any resistance bred in from *Lactuca serriola* might be quickly overcome, unless the respective genes have proven to provide protection against a broad range of isolates of *B. lactucae* from *Lactuca serriola*. Also the rare *Bremia* infections in *Lactuca saligna* will in this context be crucial to test with respect to their phylogenetic relationship with isolates from *Lactuca sativa* and *Lactuca serriola*. Based on the phylogenetic results of this study, non-host resistance genes against *Bremia lactucae*, which might be present in weeds commonly associated with lettuce production, could possibly become a source of more durable resistance against downy mildew in lettuce production.

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