Introduction

The genus *Papaver* (*Papaveraceae*) comprises about 80 annual, biennial and perennial herbs distributed in Central and southwestern Asia, Central and Southern Europe and North Africa [1]. The most well-known species is opium or oilseed poppy (*P. somniferum*), an ancient crop and medicinal plant cultivated for its edible seed as well as for the production of opium, the source for important pharmaceutical drugs including morphine, thebaine, codeine, papaverine, and noscapine [2].

One of the most important diseases of *P. somniferum* is downy mildew caused by *Peronospora* spp, which is responsible for substantial crop losses world-wide (c.f. [2–6]). Several *Papaver* species have been reported to be hosts of *Peronospora* [7], and four species have been described from various *Papaver* species [8]. However, their taxonomic status, synonymy as well as host range have been much disputed, leading to substantial confusion in the literature about the number of species present on *Papaver* and their correct naming.

The most well-known species of *Peronospora* on *Papaver* and closely related host genera is *P. arborescens*, which was originally described from *Papaver rhoes* by Berkeley [9]. Subsequently it has been also reported from numerous other hosts like Argemone mexicana [10], several *Meconopsis* spp. including *M. betonicifolia*, *M. cambrica*, *M. latifolia*, *M. napaulensis*, *M. palyathomensis* and *M. simplicifolia* [7,11–14], and *Papaver* spp. including *P. alpinum*, *P. argemone*, *P. caucasicum*, *P. dubium*, *P. hybridum*, *P. lecoqii*, *P. nudicaule*, *P. orientale*, *P. papaveraceum*, *P. pinnatum*, *P. somniferum* [3,4,7,11–13,15–23].

*Peronospora cristata*, the second species described from *Papaver*, was reported to infect *P. argemone*, *P. hybridum*, *P. rhoes* and *P. somniferum* [5,8,13,14,17], but also *Meconopsis betonicifolia* [24] and *M. cambrica* [14]. Remarkably, *P. cristata* has only been reported on host species that are also recorded hosts of *P. arborescens*, raising the question about correct species identification and whether one or two species are involved. In the description of *P. cristata*, Tranzschel [25] reported verrucose oospores which are remarkably distinct from the smooth oospores of *P. arborescens*, but following Reid [14] who did not mention the oospore characteristics this important
character has been largely ignored, and accessions from various *Papaver* and *Meconopsis* species were attributed to *P. cristata* primarily on conidial sizes that are distinctly larger than those of *P. arborescens*.

From *M. cambrica*, a third species, *P. meconopidis*, has been described [26], which, however, has not received much attention in the plant pathology literature and has been commonly synonymised with *P. cristata* due to conidia of similar size, or ignored, following Reid [14] who did not even mention *P. meconopidis*. The fourth species, *P. argemone*, was described from *Papaver argemone* and has conidial sizes in the range of *P. cristata* and *P. meconopidis*. Therefore, Reid [14] synonymised it with *P. cristata*, ignoring the fact that the oospores of *P. argemone* were described as smooth, in contrast to the verrucose oospores of *P. cristata*. Following the approach of Reid [14], two *Peronospora* species, *P. arborescens* and *P. cristata*, have been accepted on *Papaver* until recently, which were primarily distinguished on their different conidial sizes, and more recently, on distinct ITS sequences [5,20].

For risk assessment of infections of the economically important opium poppy (*Papaver somniferum*) crop, it is crucial to clarify the host ranges of the pathogens involved. Furthermore, since high numbers of wild *Papaver* spp. are coincident with the phenology of the cultivated opium poppy, if there is a host overlap, those *Papaver* spp. could act as alternative hosts and be potential sources of primary inoculum for the disease contributing to disseminating the pathogen within opium poppy crops. In the current study, we report the results of extensive molecular and morphological investigations on *Peronospora* accessions from various *Papaver* species and from *Meconopsis cambrica* to clarify nomenclature, species boundaries and host ranges of the species involved.

Materials and Methods

Morphological Analysis

Conidiophores and conidia were removed from the underneath of infected leaves, transferred to a drop of anhydrous lactic acid on a slide, carefully torn apart using forceps and needles, shortly heated using an alcohol burner and covered with a cover slip. For oogonia, host tissue was soaked in 2% KOH on a slide, carefully torn apart using forceps and needles, shortly heated using a Zeiss Axio Imager.A1 (Zeiss, Jena, Germany) microscope equipped with a Zeiss AxioCam ICC3 digital camera. Measurements are reported as maxima and minima in parentheses and the mean plus and minus the standard deviation of a number of measurements given in parentheses.

Sample Sources

Information on the samples used for sequencing and phylogenetic analyses is given in Table 1. Details on the specimens used for morphological analysis are given in the description of the species. The herbarium acronyms are given according to Thiers [27].

DNA Extraction, PCR and Sequencing

For DNA extraction, infected dry host tissue was placed in 2 ml reaction tubes together with six sterile 2 mm glass beads and ground in a Retsch 200 mixer mill for 10 min. Alternatively, conidiophores were scraped off the leaf surface of the hosts, put in 1.5 μl reaction tubes and ground with sterile quartz sand and a conical micropestle. DNA was extracted using the modified CTAB protocol described in Rüdiger et al. [28] or using the Macherey-Nagel NucleoSpin Plant II extraction kit according to the manufacturer’s instructions.

A ca. 2200 long fragment containing partial nuSSU-ITS-LSU rDNA was amplified using primers DC6 [29] and LR6-O [20] or LR6-O1 (designed here; 5′ CGCAGTGCCAGAGCGAGCC 3′). In cases where no product could be obtained, the ITS was amplified using primers DC6 and ITS4 [30]. For cycle sequencing, primers ITS5-P (designed here; 5′ GGAGAGGTGAAGTCGGTTCGTGGTCAACAAGG 3′), ITS4, LR0R [31] and LR6-O were used. For the mitochondrial cytochrome c oxidase subunit I (*cox1*) sequences, primers Oom-CoxI-lev-up and Oom-CoxI-lev-lo [32] were used for amplification and cycle sequencing; the cytochrome c oxidase subunit II (*cox2*) was amplified and cycle-sequenced with the forward and reverse primers of Huseth et al. [33]. The PCR products were purified using an enzymatic PCR cleanup [34] according to the protocol of Voglmayr and Jaklitsch [35]. DNA was cycle-sequenced using the ABI PRISM Big Dye Terminator Cycle Sequencing Ready Reaction Kit v. 3.1 (Applied Biosystems, Warrington) and an automated DNA sequencer (AB 3730xl Genetic Analyzer, Applied Biosystems).

Phylogenetic Analysis

All alignments were produced with Muscle version 3.6 [36]. For evaluation of species status, a combined analysis of *cox1* and *cox2* was performed, adding a representative selection of *Peronospora* species according to the phylogenetic tree of Göker et al. [37], with two *Pseudoperonospora* species as outgroup to root the tree according to the phylogenies of Göker et al. [38]. Because the ITS-LSU rDNA did not show much phylogenetic information to separate closely related species, it was not included in the phylogenetic analyses but the sequences were deposited in GenBank (Table 1); in addition, for complete reference the GenBank accession numbers of ITS sequences of *Peronospora* accessions included in the present study that have already been deposited by the authors in the course of previous studies [4,39] are also listed in Table 1.

Maximum parsimony (MP) analysis was performed with PAUP* version 4.0 b10 [40], using 1000 replicates of heuristic search with random addition of sequences and subsequent TBR branch swapping (MULTREES option in effect, COLLAPSE = MAXBR-LEN, steepest descent option not in effect). All molecular characters were unordered and given equal weight; analyses were performed with gaps treated as missing data. Bootstrap analysis with 1000 replicates was performed in the same way, but using 5 rounds of random sequence addition and subsequent branch swapping during each bootstrap replicate.

For maximum likelihood (ML) and Bayesian analyses, the HKY substitution model [41] was selected for both *cox1* and *cox2* by Modeltest 3.6 [42] using the hierarchical likelihood ratio tests, with invariant sites and gamma distribution for the remaining sites (HKY+I+G). In the combined analyses, substitution parameters were estimated separately for each region. For ML analyses, 10 runs with 100 thorough bootstrap replicates each were computed with RAxML [43] as implemented in raxmlGUI 1.3.1 [44] using the GTRCAT substitution model. For Bayesian analyses using MrBayes version 3.1.2 [45], three parallel runs of four incrementally heated simultaneous Markov chains were performed over 1 million generations from which every 100th tree was sampled in each run, implementing the HKY+I+G substitution model.

Nomenclature

The electronic version of this article in Portable Document Format (PDF) in a work with an ISSN or ISBN will represent a publication work according to the International Code of Nomenclature for algae, fungi, and plants, and hence the new names contained in the electronic publication of a PLOS ONE article are
| Taxon | Host | Geographic origins | Collector | Accession | Voucher | ITS | cox1 | cox2 |
|-------|-------|-------------------|-----------|-----------|---------|-----|------|------|
| P. alsinearum | Stellaria media | Austria, Niederösterreich, Prellenkirchen | H. Voglmayr | HV2572 | WU 32433 | KJ651340 KJ651341 |
| P. apula | Papaver apulum | Croatia, Istrija, Bale, Mandriol | H. Voglmayr | HV2388 | WU 32408 | KJ651342 |
| P. arborescens | Papaver rhoeas | Austria, Burgenland, Kittsee | H. Voglmayr | HV17 | WU 22880 | KJ651344 |
| P. arborescens | Papaver rhoeas | Austria, Niederösterreich, Bad Vöslau | H. Voglmayr | HV2604 | WU 32411 | KJ651345 |
| P. arborescens | Papaver rhoeas | Austria, Wien, Leopoldstadt, Praterspitz | H. Voglmayr | HV2823 | WU 32412 | KJ651346 |
| P. arborescens | Papaver rhoeas | Croatia, Istrija, Peroj | H. Voglmayr | HV2917 | WU 32413 | KJ651347 |
| P. arborescens | Papaver rhoeas | France, Drôme, Karrière des Balmes | H. Voglmayr | HV24 | WU 32414 | KJ651348 |
| P. arborescens | Papaver rhoeas | Germany, Baden-Württemberg, Tübingen | H. Voglmayr | HV37 | WU 32415 | KJ651349 |
| P. arborescens | Papaver rhoeas | Hungary, Hajdu-Bihar, Hajduboroszló | H. Voglmayr | HV2427 | WU 32416 | KJ651350 |
| P. arborescens | Papaver rhoeas | Italy, Lombardia, Brescia, Arzenate | G. Negresan | 9164 | MA-Fungi 7684 | KJ651351 |
| P. arborescens | Papaver rhoeas | Spain, Toledo, Hormigas, Malpica de Tajo | B. B. Landa & M. Montes | R1 | MA-Fungi 7685 | KJ651352 |
| P. arborescens | Papaver rhoeas | Spain, Ponte de Cávadas | A. Guzmán | MA-Fungi 7686 | GLM 6048 | KJ651353 |
| P. arborescens | Papaver rhoeas | Spain, Ponte de Córdoba | A. Guzmán | MA-Fungi 7687 | GLM 6049 | KJ651354 |
| P. arborescens | Papaver rhoeas | Spain, Malaga, Antequera, Arzénate | F. Juge | 9298 | WU 32434 | KJ651355 |
| P. arborescens | Papaver rhoeas | Spain, Casillas, Santa Ana, Arzénate | F. Juge | 9398 | WU 32435 | KJ651356 |
| P. arborescens | Papaver rhoeas | Spain, Malaga, Antequera, Lavedo | F. Juge | 9397 | WU 32436 | KJ651357 |
| P. chrysosplenii | Chrysosplenium alternifolium | Austria, Oberösterreich, St. Willibald | H. Voglmayr | HV58 | WU 22892 | KJ651358 |
| P. conglomerata | Geranium molle | Austria, Niederösterreich, Brunn an der Wild | H. Voglmayr | HV2678 | WU 32437 | KJ651359 |
| P. corydalis | Corydalis solida | Austria, Niederösterreich, Mannersdorf-Leibnitz | H. Voglmayr | HV2157 | WU 32438 | KJ651360 |
| P. cristata | Papaver hybridum | Spain, Cardona, Coll de PAM | B. B. Landa & M. Montes | BL-C00 | BL-C01 | KJ651361 |
| P. cristata | Papaver hybridum | Spain, Cardona, ARA | B. B. Landa & M. Montes | BL-C02 | BL-C03 | KJ651362 |
| P. cristata | Papaver hybridum | Spain, Cardona, ARA | D. E. L. Cooke | P24 | DQ885375 | KJ651363 |
| P. meconopsidis | Meconopsis cambrica | Austria, Steiermark, Graz | H. Voglmayr | HV2010 | WU 32439 | KJ651364 |
| P. meconopsidis | Meconopsis cambrica | UK, London, Kew Gardens | D. E. L. Cooke | P24 | KJ651365 | KJ651366 |
| P. meconopsidis | Papaver pavoninum | Turkmenistan, Kordon Kepelya | V. A. Melnik | HV2965 | KJ651367 | KJ651368 |
| Taxon                  | Host                  | Geographic origins    | Collector               | Accession   | Voucher   | ITS       |
|-----------------------|-----------------------|-----------------------|-------------------------|-------------|-----------|-----------|
| *P. meconopsidis*     | *Papaver somniferum*  | Australia, Tasmania   | P.J. Cotterill          | BL-Cot1     | KJ651311  | KJ651312  |
|                       |                       |                       |                         | BL-Cot2     | KJ651313  | KJ651314  |
|                       |                       |                       |                         | BL-Cot3     | KJ651315  | KJ651316  |
|                       |                       | Australia, Tasmania   | P.J. Cotterill          | BL-Cot1     | KJ651317  | KJ651318  |
|                       |                       |                       |                         | BL-Cot2     | KJ651319  | KJ651320  |
|                       |                       |                       |                         | BL-Cot3     | KJ651321  | KJ651322  |
|                       |                       | Afghanistan, Jalalabad| M. A. Ghani             | HV2963      | KJ651323  | KJ651324  |
|                       |                       | Austria, Niederösterreich, Zrendorf| | H. Voglmayr | KJ651325  | KJ651326  |
|                       |                       |                       |                         | HV2976      | KJ651327  | KJ651328  |
|                       |                       | Austria, Oberösterreich, St. Willibald| | H. Voglmayr | KJ651329  | KJ651330  |
|                       |                       |                       |                         | HV2987      | KJ651331  | KJ651332  |
|                       |                       |                       |                         | HV2988      | KJ651333  | KJ651334  |
|                       |                       |                       |                         | HV2989      | KJ651335  | KJ651336  |
|                       |                       |                       |                         | HV2990      | KJ651337  | KJ651338  |
|                       |                       |                       |                         | HV2991      | KJ651339  | KJ651340  |
|                       |                       |                       |                         | HV2992      | KJ651341  | KJ651342  |
|                       |                       |                       |                         | HV2993      | KJ651343  | KJ651344  |
|                       |                       |                       |                         | HV2994      | KJ651345  | KJ651346  |
|                       |                       |                       |                         | HV2995      | KJ651347  | KJ651348  |
|                       |                       |                       |                         | HV2996      | KJ651349  | KJ651350  |
|                       |                       |                       |                         | HV2997      | KJ651351  | KJ651352  |
|                       |                       |                       |                         | HV2998      | KJ651353  | KJ651354  |
|                       |                       |                       |                         | HV2999      | KJ651355  | KJ651356  |
|                       |                       |                       |                         | HV3000      | KJ651357  | KJ651358  |
|                       |                       |                       |                         | HV3001      | KJ651359  | KJ651360  |
|                       |                       |                       |                         | HV3002      | KJ651361  | KJ651362  |
|                       |                       |                       |                         | HV3003      | KJ651363  | KJ651364  |
|                       |                       |                       |                         | HV3004      | KJ651365  | KJ651366  |
|                       |                       |                       |                         | HV3005      | KJ651367  | KJ651368  |
|                       |                       |                       |                         | HV3006      | KJ651369  | KJ651370  |
|                       |                       |                       |                         | HV3007      | KJ651371  | KJ651372  |
|                       |                       |                       |                         | HV3008      | KJ651373  | KJ651374  |
|                       |                       |                       |                         | HV3009      | KJ651375  | KJ651376  |
|                       |                       |                       |                         | HV3010      | KJ651377  | KJ651378  |
|                       |                       |                       |                         | HV3011      | KJ651379  | KJ651380  |
|                       |                       |                       |                         | HV3012      | KJ651381  | KJ651382  |
|                       |                       |                       |                         | HV3013      | KJ651383  | KJ651384  |
|                       |                       |                       |                         | HV3014      | KJ651385  | KJ651386  |
|                       |                       |                       |                         | HV3015      | KJ651387  | KJ651388  |
|                       |                       |                       |                         | HV3016      | KJ651389  | KJ651390  |
|                       |                       |                       |                         | HV3017      | KJ651391  | KJ651392  |
|                       |                       |                       |                         | HV3018      | KJ651393  | KJ651394  |
|                       |                       |                       |                         | HV3019      | KJ651395  | KJ651396  |
|                       |                       |                       |                         | HV3020      | KJ651397  | KJ651398  |
|                       |                       |                       |                         | HV3021      | KJ651399  | KJ651400  |
|                       |                       |                       |                         | HV3022      | KJ651401  | KJ651402  |

For institution codes of herbarium vouchers see Thiers [27]; asterisks (*) denote ITS sequences published in Landa et al. [4] and Montes-Borrego et al. [39]; all others were newly generated in the present study (formatted in bold).
Results

The final matrix was deposited in TreeBASE (http://www.treebase.org) and is available under http://purl.org/phylo/treebase/phylo/study/TB2:S15609.

Of the 1262 characters of the combined cox1 - cox2 alignment, 254 were parsimony informative (120 in cox1, 114 in cox2). MP analyses revealed 16 MP trees 1035 steps long, one of which was selected and presented in Figure 1, with MP and ML bootstrap support above 50% and posterior probabilities above 90% given at first, second and third position, respectively, above/below the branches. Topologies of the MP trees slightly differed in the phylogenetic positions of P. sordida, P. corydalis and P. chrysophlebi. The three Bayesian runs revealed almost identical posterior probabilities (PP) and were fully compatible with the MP strict consensus tree.

The Peronospora-accessions from Papaver/Meconopsis were contained within three highly supported clades, one comprising Peronospora cristata from Papaver hybridum (clade 1 in Figure 1); a second clade containing P. apula from Papaver alpinum, P. argemones from Papaver argemones and P. mephanides from Meconopsis cambrica, Papaver parvum and P. somniferum (clade 2 in Figure 1); and a third clade with P. arborescens from Papaver rheas, P. somniferi from Papaver somniferum, P. sp. 1 from Papaver dubium and P. sp. 2 from Papaver sp. (clade 3 in Figure 1). Whereas within the Peronospora arborescens clade the ITS did not resolve accessions from the different hosts, in the cox1 - cox2 trees the accessions from Papaver dubium, P. rheas and P. somniferum were placed in three distinct monophyletic clades, the former two with high and the latter with medium to high support (Figure 1).

Within the Peronospora arborescens sensu lato clade (clade 3 in Figure 1), Peronospora accessions from Papaver somniferum consistently exhibited 11 and 10 common sequence substitutions in cox1 and cox2, respectively, if compared to sequences of P. arborescens from Papaver rheas, rendering them molecularly clearly distinct. Peronospora accessions from Papaver somniferum also differed morphologically from those from P. rheas in larger conidia (mean 21.1 × 17.7 μm vs. 18.3 × 16.1 μm).

Taxonomy

As a result of the morphological and molecular phylogenetic investigations, six taxa are here recognised as occurring on Papaver, two of which are described as new. In addition, detailed descriptions of the other four already described species are provided. All type specimens cited were morphologically investigated in the present study.

Peronospora Apula Voglmayr, sp. nov. Figure 2

MycoBank: MB 808433.

Description. Infection systemic or localized, when systemic whole plants or leaves stunted, stems strongly distorted, sinuous. Down mostly hypophyllous, greyish, consisting of dense and felt-like conidiophores. Conidiophores hyaline, straight to slightly sinuous, (170–270–340–500) μm long; trunk straight or curved, (60–)150–280–(360) μm long (n = 25), variable in width, 4–10.5 μm wide; callose plugs absent; upper part monopodially or sub dichotomously branched 5–6 times. Branches curved, sinuous. Ultimate branches in pairs, straight to slightly curved, (3–)5.5–11.5–(19.5) μm long, 2–2.5 μm wide at the base (n = 349), apex obtuse. Conidia subhyaline to pale brown, subglobose, ellipsoidal to obovate, (14–)16.5–20–(22) μm long, (12–)14.5–17–19 μm wide, mean 18.3 × 13.8 μm, l/w ratio (1.04–)1.07–1.25–1.45 (n = 114), greatest width median, base and tip round; pedicel absent in most conidia but a scar visible at the point of attachment; producing germ tubes. Oogonia globose, subglobose to irregular, yellow brown to dark reddish brown, (29–)40–48–(54) μm diam., wall smooth, 1.5–2.7 μm thick (n = 88). Oospores distinctly aplerotic, globose, (21–)25–30–(34) μm diam., wall 1.9–4.5 μm thick (n = 88), smooth.

Molecular diagnosis. Peronospora apula differs from its closest phylogenetic neighbour, P. argemones, by unique fixed alleles in two tree loci (cox1, cox2) based on alignments of the separate loci deposited in TreeBASE as study S15609: cox1 positions 343: A; 49, 205, 346, 349, 425, 640: C; 148, 610, 664: G; 193, 217, 340, 652: T; cox2 positions 154, 232, 370, 565: A; 295, 469: C; 65, 109, 187, 372, 373, 449: G; 105, 133, 262: T.

Etymology. Referring to its host, Papaver alpinum Ten.

Habitat. On living leaves and stems of Papaver alpinum.

Holotype. CROATIA, Istria, SE Rovinj, ca. 300 m ESE Kamp Večtar, field, 18 May 2012, H. Voglmayr HV2911 (WU 32410).

Additional specimens examined. CROATIA, Istrija, N Rovinj, E Valalta, 17 May 2012, H. Voglmayr HV2911 (WU 32409). Istrija, Mandriol ESE Bale, 14 May 2010, H. Voglmayr HV2338 (WU 32408).

Comments. Peronospora apula appears to be confined to Papaver alpinum. It is closely related to P. argemones which differs by larger conidia, its host Papaver argemones and by different ITS (10 substitutions), cox1 (14 substitutions) and cox2 (15 substitutions) sequences.

Peronospora arborescens (Berk.) de Bary, Monatsh. Königl. Preuss. Akad. Wiss. Berlin: 308-333 (1855) Figure 3.

Basionym: Botrytis arborescens Berk., J. hort. Soc., London 1:31 (1846).

Description. Infection commonly systemic, more rarely localized, when systemic whole plants or leaves stunted, strongly distorted, chlorotic, dwarfed, when localized producing polyan- guous, greyish, consisting of dense and felt-like conidiophores. Conidiophores hyaline, straight to slightly sinuous, (290–360–600–720) μm long; trunk straight or curved, (110–)190–380–(460) μm long (n = 23), variable in width, 5.5–13 μm wide; callose plugs absent; upper part monopodially or sub dichotomously branched 5–7 times. Branches distinctly curved, sinuous. Ultimate branches in pairs, slightly to strongly curved, (2–)4.5–9.5(–18) μm long, 2–2.7 μm wide at the base (n = 511), apex obtuse. Conidia subhyaline to pale brown, subglobose, ellipsoidal to obovate, (14–)16.5–20–(24) μm long, (12.5–)15–17.5–(20) μm wide, mean 18.3 × 16.1 μm, l/w ratio (1.00–)1.07–1.21–1.45 (n = 413), greatest width median, base and tip round; pedicel absent in most conidia but a scar visible at the point of attachment; producing germ tubes. Oogonia globose, subglobose to irregular, yellow brown to dark reddish brown, (37–)43–51–(59) μm diam., wall commonly wrinkled, smooth, ca. 1 μm thick (n = 193). Oospores distinctly aplerotic, globose, (21–)26–30–(33) μm diam., wall 1.5–2.7 μm thick (n = 193), smooth.
Phylogenetics and Taxonomy of Peronospora on Papaver

clade 1 P. cristata

- P. cristata Papaver hybridum BL-C25
- P. cristata Papaver hybridum BL-C75
- P. cristata Papaver hybridum BL-B25
- P. cristata Papaver hybridum BL-C90

- P. conglomerata Geranium molle HV2678
- P. lamii Lamium purpureum HV2619

- P. sordida Scrophularia nodosa HV2395
  - Pseudoperonospora cannabina Cannabis sativa HV2740
  - Pseudoperonospora cubensis Echinocystis lobata HV2776

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clade 2

- P. bulbocephali Corydalis cava HV7
- P. alsinearum Stellaria media HV2572
- P. boni-henrici Chenopodium bonus-henricus HV639
- P. arthuri Oenothera biennis agg. HV2298
- P. holostei Holosteum umbellatum HV2439
- P. ranunculi Ranunculus repens HV2293
- P. trivalis Cerastium holosteoides HV2518
- P. chrysosplenii Chrysosplenium alternifolium HV58
- P. corydalis Corydalis solida HV2157

- P. meconopsis Meconopsis cambrica HV2010
- P. meconopsis Meconopsis cambrica HV2360
- P. meconopsis Meconopsis cambrica HV2440
- P. meconopsis Papaver somniferum HV190
- P. meconopsis Papaver somniferum HV2728
- P. meconopsis Papaver somniferum HV2749
- P. meconopsis Papaver somniferum HV2963
- P. meconopsis Papaver somniferum HV2966
- P. meconopsis Papaver somniferum BL-Cot3
- P. meconopsis Papaver somniferum BL-Cot1
- P. meconopsis Papaver somniferum BL-Cot2
- P. meconopsis Papaver pavoninum HV2965
- P. apula Papaver apulum HV2909
- P. apula Papaver apulum HV2911
- P. apula Papaver apulum HV2388
- P. argemone Papaver argemone HV2427

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clade 3 P. arborescens s.l.

- P. arborescens Papaver rhoeas HV17
- P. arborescens Papaver rhoeas HV2462
- P. arborescens Papaver rhoeas HV2604
- P. arborescens Papaver rhoeas HV2823
- P. arborescens Papaver rhoeas HV2917
- P. arborescens Papaver rhoeas HV2942
- P. arborescens Papaver rhoeas HV815
- P. arborescens Papaver rhoeas HV-F24
- P. arborescens Papaver rhoeas HV-F57
- P. arborescens Papaver rhoeas MA-Fungi 27844
- P. arborescens Papaver rhoeas MA-Fungi 27843
- P. arborescens Papaver rhoeas MA-Fungi 9164
- P. arborescens Papaver rhoeas R1

- P. sp. 1 Papaver dubium HV2961
- P. sp. 1 Papaver dubium MA-Fungi 27841

- P. sp. 2 Papaver sp. HV2962

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Figure 1. Phylogram showing phylogenetic relationships of *Peronospora* accessions from *Papaver* and *Meconopsis*. One of 16 most parsimonious trees 1035 steps long inferred from the combined cox1-cox2 sequence data matrix; parsimony and likelihood bootstrap support above 50% and posterior probabilities above 90% are given at first, second and third position, respectively, above/below the branches. The tree was rooted with two species of *Pseudoperonospora* according to Göker et al. [38].
doi:10.1371/journal.pone.0096838.g001

**Habitat.** On living leaves and stems of *Papaver rhoes* L.

**Typification.** UK, King’s Cliffe, without date and collector, ex Herb. Berkeley (K/M 178950, holotype).

**Selected additional specimens examined.** AUSTRIA, Burgenland, Neusiedl/See, Kittsee, E Großer Raubwald near Edelstal, 27 Mar 1999, H. Voglmayr HV17 (WU 22880). Niederösterreich, Baden, Bad Vöslau, 19 Apr 2011, H. Voglmayr HV2604 (WU 32411). Wien, Leopoldstadt, Praterspitz, 4 Apr 2012, H. Voglmayr HV2823 (WU 32412). CROATIA, Istrija, E Peroj, 20 May 2012, H. Voglmayr HV2917 (WU 32413).

Figure 2. Morphological features of *Peronospora apula*. a, b conidiophores; c ultimate branchlets; d–h conidia; i–k oogonia and oospores. Sources: a, e–k WU 32410, holotype; b WU 32408; c, d WU 32409. Scale bars a, b 50 μm, c-k 20 μm.
doi:10.1371/journal.pone.0096838.g002
Comments. *Peronospora arborescens* appears to be confined to *Papaver rhoeas*, on which it is very common. Its disease symptoms are highly distinctive, infected plants being distorted and showing light yellowish green discolouration. The various accessions included in our molecular phylogenetic analyses covered most of Europe (Austria, Croatia, France, Germany, Hungary, Italy, Romania, Spain), and were molecularly highly homogeneous. Accessions from other *Papaver* species were placed in distinct clades and represent genetically distinct lineages. The accessions from Spain were partly sampled within cultivated opium poppy fields.

**Figure 3. Morphological features of *Peronospora arborescens*.** a, b conidiophores; c ultimate branchlets; d–h conidia; i–k oogonia and oospores. Sources: a WU 32416; b, k WU 32411; c, f, g WU 32412; d, h WU 32418; e WU 32413. Scale bars a 100 μm, b 50 μm, c–k 20 μm. doi:10.1371/journal.pone.0096838.g003
**Peronospora argemones** Gäm., **Beitr. Kryptfl. Schweiz** 5(no. 4): 72 (1923) **Figure 4.**

**Description.** Infection commonly systemic, whole plants or leaves stunted, slightly to strongly distorted. **Down hypophyllous,** greyish, consisting of dense and felt-like conidiophores. **Conidiophores** hyaline, straight to slightly sinuous, (220–)290–490(–590) µm long; trunk straight to slightly curved, (80–)150–300(–390) µm long (n = 35), variable in width, 4–13 µm wide; callose plugs absent; upper part monopodially or subdichotomously branched 5–6 times. **Branches** tightly intertwined, curved, sinuous. **Ultimate branchlets** in pairs, slightly curved, (2.5–)6–14(–23) µm long, 1.8–2.8 µm wide at the base (n = 468), apex obtuse. **Conidia** subhyaline to pale brown, subglobose, ellipsoidal to obovate, (16.5–)19–23.5(–26) µm long, (14.5–)16.5–20(–23) µm wide, mean 21.1 × 18.1 µm, l/w ratio (1.02–)1.1–1.23(–1.37) (n = 181), greatest width median, base and tip round; pedicel absent in most conidia but a scar visible at the point of attachment; producing germ tubes. **Oogonia** globose, subglobose to irregular, light to dark reddish brown, (42–
Peronospora meconopsidis

**Typification.** GERMANY, Berlin, Lichtenfels, June 1896, P. Sydow, Phys. Prot. Prot. 5 (K(M) 181196), lectotype here designated, MBT177702; WU s.n., isotype.

**Additional specimen examined.** GERMANY, Sachsen-Anhalt, Bitterfeld, SW Friedersdorf, Muldeaue, 8 May 2004, H. Jage (GLM 64084).

**Comments.** Peronospora meconopsidis appears to be confined to *Papaver argemone*. Gaumann [13] separated *P. argemone* from *P. arborescens* based on larger conidia. Our measurements fit well those reported by Gaumann [13] (21.1 ± 18.1 vs. 21 ± 18.6). *P. argemone* is not closely related to *P. arborescens* but to *P. meconopsidis* which has larger conidia. Its closest relative, however, is *P. apula*, which differs by slightly smaller conidia and its host *Papaver apium*.

*Peronospora cristata* thenchzell, Trav. Mus. Bot. Acad. Sci. St. Petersburg 1:49 (1902) Figure 5.

**Description.** Infection commonly systemic, more rarely localized, when systemic, whole plants or leaves stunted, slightly distorted, chlorotic. Down hypophyllous, greyish, consisting of dense and felt-like conidiophores. *Conidiophores* hyaline, straight or slightly sinusoidally curved, (200–240–330–380) μm long; trunk straight or curved, (95–130–220–280) μm long (n = 32), variable in width, 4.5–10.5 μm wide; callose plugs absent; upper part monopodially or subdichotomously branched 3–5 times. Branches straight to slightly sinusoidally curved. *Ultimate branchlets* in pairs, slightly to distinctly curved, (2.5–)5–10.5–19) μm long, 2–5.6 μm wide at the base (n = 263), apex obtuse. *Conidia* pale brown to brown, broadly ellipsoidal, ellipsoidal to obovate, (21.5–)25–28(–39) μm long, greatest width 26.5–19.6 μm, l/w ratio (1.35–)1.27–1.43(–1.53) (n = 122), greatest width median, base and tip round; pedicel absent in most conidia but a scar visible at the point of attachment; producing germ tubes. *Oogonia* mostly globose, rarely subglobose to irregular, reddish brown, (29–40–48–54) μm diam., wall smooth, 1.5–1.5 μm thick (n = 88). *Oospores* distinctly aplerotic, globose, (21–25–30–34) μm diam., wall 1.7–3 μm thick (n = 88), smooth.

**Habitat.** On living leaves of *Meconopsis cambrica* Vig., Papaver pavoninum C.A. Mey. and *P. somniferum* L.

**Typification.** SWITZERLAND, Neuchâtel, Val-de-Travers, Mòtiers, Gorges de la Poite Raaia, on *Meconopsis cambrica*, 31 July 1945, 21 Sep 1946, 17 Sep 1947, 17 Aug 1949, 14 July 1952, 14 July 1952, E. Mayor (NEU, lectotype here designated, MBT177701). ibid., 17 Sep 1947, E. Mayor (NEU, isotype). Neuchâtel, Vallée des Ponts, Combe Varin, garden, 27 June 1920, E. Mayor (NEU, syntype).

**Additional selected specimens examined.** On *Meconopsis cambrica*: AUSTRIA, Styria, Graz, Botanical Garden, 14 Sep 2002, H. Voglmayr HV2010 (WU 32422), UK, London, Kew Gardens, 14 Nov 2008, H. Voglmayr HV2360 (WU 32423). On *Papaver pavoninum*: RUSSIA, Moskva, Principal Botanical Garden an SSSR, 1 June 1959, E. Protosenko (K(M) 179239). On *Papaver somniferum*: AFGHANISTAN, Jalalabad, 14 Mar 1973, M.A. Ghani (K(M)179245). AUSTRIA, Niederoesterreich, Gansendorf, Weiden an der March, Dornparz ESE Zwerndorf, 6 July 2010, H. Voglmayr HV2749 (WU 32424). Austria, Oberoesterreich, Schärding, St. Willibald, 31 July 2005, H. Voglmayr HV2190 (WU 32425).

**Comments.** *Peronospora meconopsidis* is commonly observed on *Meconopsis cambrica* and *Papaver somniferum*, but its disease symptoms are usually rather inconspicuous and localized compared to most other *Peronospora* species from *Papaver*. Although it has not been recorded from *Papaver somniferum* in Europe, it is apparently common and widespread on that host according to own...
observations and upon examination of herbarium specimens. It has commonly been misidentified as *Peronospora arborescens*, which mainly differs by conspicuous disease symptoms relating to mostly systemic infection which has never been observed for *P. meconopsidis*. Based on similar conidial sizes, *P. meconopsidis* has been referred to as *Peronospora cristata* in recent literature, which goes back to Reid [14] who classified accessions from *Meconopsis cambrica* under that species. However, *P. cristata* is easily distinguishable by its verrucose oospores, which are unique in *Peronospora* on *Papaver*. The Australia records of *Peronospora cristata* from *Papaver somniferum* [5] therefore actually represent *Peronospora meconopsidis*, which is also corroborated by sequence data (Figure 1).

Oospores of *Peronospora meconopsidis* are reported here for the first time, and they have only been found in young infected plants of

Figure 5. Morphological features of *Peronospora cristata*. a-c conidiophores; d ultimate branchlets; e–i conidia; j–l oogonia and oospores. Sources: a, b, d, e, j, k LE 185561, holotype; c, h WU 32419; f, g, i, l WU 32421. Scale bars a-c 50 μm, d-l 20 μm. doi:10.1371/journal.pone.0096838.g005
Papaver somniferum collected in Asia. No oospores were found in specimens of Papaver somniferum from Europe or from Meconopsis cambrica despite thorough investigations.

The species was first described as Peronospora gaeumannii by Mayor [46]. However, because this is a younger homonym of P. gaeumannii Mundk., Mayor [26] proposed the new name P. meconopsidis. Three authentic specimens mentioned in the original description of P. gaeumannii Mayor are present at NEU, of which the largest, best developed and preserved is here selected as lectotype. This folder also contains the original drawings and spore statistics published in Mayor [46]. According to the herbarium label, the lectotype specimen consists of several collections from the same place collected from 1945 to 1952, which were subsequently mixed and cannot be separated any more. The mean spore sizes recorded by Mayor [46] agree well with those of the current study (23.5 ± 2.1 vs. 24.8 ± 2.0 μm).

**Peronospora somniferae** Voglmayr, sp. nov. Figure 7. Mycobank MB 808434.

**Description.** Infection systemic or localized, when systemic whole plants or leaves stunted, stems strongly distorted, sinuous.
Down mostly hypophyllous, greyish, consisting of dense and felt-like conidiophores. Conidiophores hyaline, straight to slightly sinuous, (280–)320–510(–660) μm long; trunk straight or curved, (100–)140–330(–490) μm long (n = 33), variable in width, 5.5–17 μm wide; callose plugs absent; upper part monopodially or subdichotomously branched 4–7 times. Branches straight to sinuously curved. Ultimate branchlets in pairs, straight to slightly curved, (2–)4.5–10(–18.5) μm long, 1.9–3.2 μm wide at the base (n = 550), apex obtuse. Conidia subhyaline to pale brown, subglobose, ellipsoidal to obovate, (15.5–)19–23(–28) μm long, (14.5–)16.5–19(–22.5) μm wide, mean 21.1 ± 17.7 μm, l/w ratio (1.01–)1.11–1.28(–1.48) (n = 927), greatest width median, base and tip round; pedicel absent in most conidia but a scar visible at the point of attachment; producing germ tubes. Oogonia globose, subglobose to irregular, yellow brown to dark reddish brown, (31–)40–46(–57) μm diam., wall smooth, ca. 1 μm thick (n = 157).

Figure 7. Morphological features of *Peronospora somniferi*. a, b conidiophores; c ultimate branchlets; d–h conidia; i–k oogonia and oospores. Sources: a, g WU 32432; b, d, i–k WU 32428, holotype; c MA 65574; e, f WU 32429; h WU 32430. Scale bars a, b 50 μm, c–k 20 μm. doi:10.1371/journal.pone.0096838.g007
Oospora distinctly aplerotic, globose, (19–)24–28(–34) μm diam., wall 1.6–2.9 μm thick (n = 157), smooth.

**Molecular diagnosis.** *Peronospora somniferi* differs from its closest phylogenetic neighbour, *P. arborescens*, by unique fixed alleles in two tree loci (*cox1*, *cox2*) based on alignments of the separate loci deposited in TreeBASE as study S15609: *cox1* positions 205, 337, 418, 491, 646; A; 195; C; 229, 331, 499, 557, 589; T; *cox2* positions 313, 370, 430, 523; A; 493; C; 382, 541; G; 220, 253, 427; T.

**Etymology.** Referring to its host, *Papaver somniferum* L.

**Habitat.** On living leaves and stems of *Papaver somniferum*.

**Holotype.** CZECH REPUBLIC, Morava, Hranice, between Teplice nad Bečvou and Černotín, 25 June 2011, H. Voglmayr HV2726 (WU 32428).

**Additional specimens examined.** AUSTRIA, Oberösterreich, Linz Land, Kronsdorf, Schieferegg, 25 May 2004, G. Bedlan 721 (WU 32427). IRAN, Neelabad, 10 Apr 1973, M.A. Ghani 3 (K(M) 179246). SPAIN, Albacete, Casa Arriba los Llanos, 10 June 2005, B. Landa (WU 32429). Sevilla, Écija, Casilla San José, 29 Apr 2004, B. Landa (WU 32430). Sevilla, Écija, San Rafael, 29 Apr 2004, B. Landa (WU 32431). Sevilla, Écija, Vacas, without collector and date (MA-Fungi 65574). Malaga, Antequera, Monteluna, without collector and date (MA-Fungi 65590). Marchena, Cortijo del Rio, 4 Apr 2004, B. Landa (WU 32432).

**Comments.** *Peronospora somniferi* appears to be confined to *Papaver somniferum*. It is closely related to *P. arborescens* which differs by smaller conidia and a different host, *Papaver rhoes*. *Peronospora somniferi* cannot be reliably distinguished from *P. arborescens* by ITS data alone because there is only a single consistent nucleotide difference at the beginning of the ITS1, but *cox1* and *cox2* are distinctive and good barcode markers for the species (11 and 10 diagnostic substitutions, respectively). *Peronospora somniferi* has been reported as an economically important pathogen of *Papaver somniferum* throughout Europe [4]. *Peronospora meconopsidis*, which also occurs on *Papaver somniferum*, differs mainly by a non-systemic infection which is characterised by typical polyangular spots (see Figure 8).

**Key to known Species of Peronospora on Papaver and Meconopsis**

Note: Morphological identification requires well developed material as well as knowledge of the host. For conidial measurements, it is crucial that a sufficient number of mature conidia are included. Because some species are highly similar, they often cannot be unequivocally identified by morphology alone, and sequence data (*cox1* or *cox2*) are essential in case of poorly developed specimens, if the host species is unknown or represents a genetically distinct entity may turn up in the future.

1 Oospora wall irregularly verrucose, only known from *Papaver hybridum*.................................................................................................P. cristata
   Oospora wall smooth........................................................................................................2

2(1) Conidia in mean longer than 20 μm..................................................3
   Conidia in mean shorter than 20 μm.................................................................5

3(2) Infection local, conidia in mean longer than 22 μm, confirmed from *Meconopsis cambrica*, *Papaver pavoninum*, *P. somniferum*..................................................................................................................P. meconopsidis
   Infection systemic, conidia in mean shorter than 22 μm..........................4

4(1) On *Papaver argemone*........................................................................P. argemone
   On *Papaver somniferum*.................................................................P. somniferi

5(2) On *Papaver apulum*, conidiophores (170–)270–430(–500) μm high.......................................................................................P. apula
   On *Papaver rhoes*, conidiophores (290–)360–600(–720) μm high................................................................................................................P. arborescens

**Discussion**

**Molecular Phylogenetic Investigations**

The current investigations clearly show that the biodiversity of *Peronospora on Papaver* is higher than previously perceived, which is in line with other investigations on *Peronosporaceae* (e.g. [37,47–63]), demonstrating that high biodiversity is commonly the result of high host specificity. *Peronospora from Papaver* are distributed amongst three clades (Figure 1), of which *P. cristata* from clade 1 is phylogenetically isolated from the other *Peronospora* species from *Papaver*, but clades 2 and 3 appear to be closely related (Figure 1). Of special interest is clade 3, the species of which were formerly classified under *Peronospora arborescens*, and which is here referred to as *P. arborescens* sensu lato clade. Whereas the ITS data are highly similar within this clade and therefore do not allow for unequivocal distinction (data not shown), the *cox1* and *cox2* data are highly distinctive for the accessions from various hosts, in which each form genetically homogenous lineages irrespective of the geographic origins. This is evident for the *Peronospora* accessions from *Papaver rhoes* and *P. somniferum* which were sampled from various regions all over Europe, and which form distinct uniform genetic lineages. In addition, also differences in conidial sizes could be documented for both lineages. Therefore, the accessions from *Papaver somniferum*, previously classified under *P. arborescens*, are here described as a distinct species, *P. somniferi*. Apart from these two lineages, another two genetically distinct entities were present within the *P. arborescens* s.l. clade (Figure 1). *Peronospora* sp. 1 from *Papaver dубium*, and *Peronospora* sp. 2 from an unidentified *Papaver* species. However, because only few accessions were available for these, we currently refrain from describing them as new taxa. Considering the numerous additional *Papaver* species for which *Peronospora* have been recorded (see [4]) but for which no accessions were available for molecular phylogenetic investigations, additional genetically distinct entities may turn up in the future.

**Molecular Barcoding**

*cox1*, chosen as barcoding locus for higher animals and considered to be the primary barcoding marker for organisms unless shown to be unsuitable (http://www.barcodedlife.org), has been demonstrated to be an appropriate barcoding locus for oomycetes [32], which is confirmed in the current study. However, *cox2* shows similarly good resolution and consequently is an equally good barcoding marker; it even has some advantages over *cox1*, as it usually amplifies better especially in cases of low DNA quantity or old herbarium samples [as also shown in [64]], and thus *cox2* sequences are available for many more species in *Peronosporales*. The current study provides additional data for equally good discriminative power of *cox1* and *cox2*; e.g. *Peronospora somniferi* is distinct from *Peronospora arborescens* by shared 11 and 10 substitutions in *cox1* and *cox2*, respectively, clearly showing a similar significant barcode gap between both species in both markers. Therefore, both *cox1* and *cox2* are considered good markers for reliable identification of *Peronospora* species on *Papaver*, and it is recommended to sequence both loci in studies of *Peronosporales* whenever possible to obtain representative robust data for molecular barcoding. On the other hand, the ITS region does not resolve closely related species like *P. arborescens* and *P. somniferi*, which has been observed also in other groups of *Peronosporaceae* (e.g. [47,54]).
Nomenclature of *Peronospora* on *Papaver*

In the recent literature, accessions infecting *Papaver* were mainly classified as *Peronospora arborescens*. However, as the molecular data clearly show, there are several species involved, six of which are recognized and treated in the current publication. However, nomenclature of these species is partly complex mainly due to misleading species concepts of the past, as shall be outlined below.

In a phytopathological perspective, an important result of recent molecular phylogenetic investigations was that downy mildew disease of the crop *Papaver somniferum* is caused by two distinct species, which were classified as *Papaver arborescens* and *Papaver cristata* [4,5]. However, the current detailed investigations show that this classification cannot be retained and has to be substantially modified.

Starting from Gaumann [13], *Peronospora* accessions from *Papaver somniferum* have been classified as *Peronospora arborescens*, which has been consistently followed. However, the current molecular phylogenetic investigations show that these accessions are genetically distinct from accessions from the type host, *Papaver rhoeas*, and there are also differences in conidial sizes. Consequently, the *Peronospora* accessions from *Papaver somniferum* belonging to the *Peronospora arborescens* sensu lato clade (clade 3; Figure 1) are here classified as a distinct species, *P. somniferi*.

Recently, the name *Peronospora cristata* was used for a second *Peronospora* species on *Papaver somniferum* that was first recorded from that host by Scott et al. [5] from Tasmania. This name was applied because their ITS sequences matched an English accession from *Meconopsis cambrica* that had previously been deposited in GenBank (DQ885375) under *Peronospora cristata*. This naming apparently goes back to Reid [14], who compared conidial measurements of *Peronospora* from *Meconopsis cambrica* with those from *Papaver argemone* and considered them to be conspecific due to similar size. In addition, because conidial sizes were also similar to those reported for *Peronospora cristata* from *Papaver hybridum*.
considered accessions from these three hosts to be conspecific, and thus classified them under *P. cristata* due to priority. However, he ignored that the oospores of *Peronospora cristata* are distinctly verrucose, being significantly different from the smooth oospores of *Peronospora argemone*. These substantial morphological differences are also mirrored by a distant phylogenetic position of *P. cristata* (Figure 1), confirming that *P. cristata* is a clearly distinct species confined to *Papaver hybridum*.

In addition, also the accessions from *Papaver argemone* and *Meconopsis cambrica* are phylogenetically distinct (Figure 1) and therefore have to be classified under *P. argemone* and *P. meconopsis*, respectively. Consequently, as the *Peronospora of M. cambrica* and the second *Peronospora* species from *P. somniferum* are conspecific, the correct name to be applied for this species is *P. meconopsis*.

### Host-parasite Relationships

The phylogenetic relationships of *Peronospora on Papaver* and those of their hosts are only partly congruent. For instance, within *Papaver* sect. *Argemonium*, which form a closely related group [1], the *Peronospora* species from *Papaver argemone* and *P. apalum* (*Peronospora argemone* and *P. apalum*) are closest relatives, whereas the *Peronospora* from the third species of the section, *Papaver paeonii*, falls within *P. meconopsis* which is sister group to the former two species (Figure 1). However, *Peronospora cristata*, parasitising the fourth species of sect. *Argemonium*, *Papaver hybridum*, is phylogenetically and morphologically distinct from all other *Peronospora* species on *Papaver*.

Remarkably, *Peronospora meconopsis* infects hosts from two genera, *Meconopsis* and *Papaver*. However, molecular phylogenetic investigations show that *Meconopsis* is polyphyletic and embedded within *Papaver*, *M. cambrica* being unrelated to the other *Meconopsis* species [1]. Whereas the two main hosts of *Peronospora meconopsis*, *M. cambrica* and *Papaver somniferum* are contained within the same clade but not closely related, the third host species, *P. paeonii*, is member of the phylogenetically isolated *Papaver* section *Argemonium* [1]. This indicates that phylogenetic radiation of *Peronospora on Papaver* is rather effected by host jumps.

Finally, from a pytopathological point of view the results from this study showed that wild *Papaver* spp. cannot play any role as primary inoculum for downy mildew epidemics in cultivated opium poppy crops since there is high host specificity. This result was corroborated when different *Peronospora* specimens (*P. somniferi*, *P. cristata* and *P. arborescens*) were sampled from the same fields of cultivated *Papaver somniferum* where *Papaver hybridum* and *Papaver rhoes* were growing as weeds. Consequently, to avoid development of downy mildew epidemics in cultivated opium poppy efforts should be placed in controlling airborne *P. somniferi* sporangia from diseased opium poppy plants as well as avoiding the use infected seed lots [22].

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### Author Contributions

Conceived and designed the experiments: HV MMB BBL. Performed the experiments: HV MMB BBL. Analyzed the data: HV MMB BBL. Contributed reagents/materials/analysis tools: HV MMB BBL. Wrote the paper: HV.

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12. Alcock NL (1933) *Peronospora arborescens* is polyphyletic and embedded within *Papaver*, *Meconopsis cambrica* being unrelated to the other *Meconopsis* species [1]. Whereas the two main hosts of *Peronospora meconopsis*, *M. cambrica* and *Papaver somniferum* are contained within the same clade but not closely related, the third host species, *P. paeonii*, is member of the phylogenetically isolated *Papaver* section *Argemonium* [1]. This indicates that phylogenetic radiation of *Peronospora on Papaver* is rather effected by host jumps.

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