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Morphological and Molecular Analyses Support the Existence of Host-specific *Peronospora* Species Infecting *Chenopodium*

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Abstract About 20 species of *Peronospora* have been reported to cause downy mildew on *Chenopodium*, but, particularly in plant pathology literature, only one species, *P. farinosa*, is considered to be involved. We performed sequence analysis of the ITS rDNA to reveal the phylogenetic relationships of *Peronospora* specimens from five species of *Chenopodium*, viz. *C. album*, *C. ambrosioides*, *C. bonus-henricus*, *C. hybridum*, and *C. polyspermum*. The five clades corresponded to particular *Chenopodium* species, and showed a high level of sequence divergence. Differences in the morphology of the conidia and ultimate branchlets also supported the separation of the five groups at the host species level. These results suggest that the names *P. variabilis*, *P. boni-henrici*, *P. chenopodii*, and *P. chenopodii-polyspermi* should be used for the four downy mildew pathogens specific to *C. album*, *C. bonus-henricus*, *C. hybridum*, and *C. polyspermum*, respectively. The *Peronospora* on *C. ambrosioides* was found to be an independent species.

Keywords Chenopodiaceae · Evolution · Host specificity · ITS rDNA · Phylogenetic analysis

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Introduction

The downy mildew fungus on *Chenopodium* was first recorded under *Botrytis effusa* [1], now known as *Peronospora effusa*, a fungus that was recently proved to be restricted to *Spinacia* [2]. Later, Schlechtendal [3] introduced *P. chenopodii*, for the downy mildew pathogen of *C. hybridum*. Gäumann [4] considered that several downy mildew fungi were parasitic on *Chenopodium* and introduced seven additional species. Unlike the previous investigators, Yerkes and Shaw [5] were unable to find morphological differences between these species, and considered that all members of the family Chenopodiaceae are parasitized by a single species, *P. farinosa*. Following cross-inoculation experiments, Byford [6] divided *P. farinosa* into three *formae speciales*, viz. f. sp. *chenopodii* on *Chenopodium* spp., f. sp. *spinaciae* on *Spinacia oleracea*, and f. sp. *betae* on *Beta* spp. As a consequence, the name currently used for the downy mildew pathogen of *Chenopodium* is *P. farinosa* (Fr.:Fr.) Fr. f. sp. *chenopodii* Byford. Nevertheless, in some recent

monographic studies [7–11] Gäumann's [4] multi-species approach is applied.

The sequence analysis of the ITS rDNA has recently been shown to be a very useful tool for the comparison of closely related species, and was recently applied to resolve taxonomic and phylogenetic relationships of various species within *Peronospora* and allied genera [2, 12–14]. The purpose of this study was to analyze the morphological characters and phylogenetic relationships of *Peronospora* species parasitic on five species of *Chenopodium*.

Materials and Methods

Fungal Specimens

Thirty-seven herbarium specimens were examined morphologically in this study. Of these, 17 were sequenced, due to difficulty in the extraction of DNA from some samples. For comparison with previously published sequences, 15 sequences were obtained from GenBank (Table 1). Herbaria abbreviations are those from Holmgren and Holmgren [15].

Morphological Analysis

Herbarium specimens were moistened with 70% alcohol, and fungi were transferred to 60% lactic acid on a glass slide. The microscope preparations were slowly warmed up, covered with a coverslip, and examined by brightfield- and DIC-light microscopy. Measurements were performed at 1,000× for conidia and 100–1,000× for other organs. Approximately 70 conidia were measured from each sample, in accordance with the findings of Hamilton and Cunnington [16] who demonstrated that in order to obtain an accurate estimate of mean spore length in *Peronospora*, 40–70 spores needed to be measured.

DNA Extraction, PCR Amplification, and Sequencing

Genomic DNA was extracted using conidiophores and conidia formed on the lower or upper surface of the infected leaves or using the infected host tissue of herbarium specimens. The extraction of genomic DNA was undertaken according to the method of Lee and Taylor [17]. The DC6 [18] and ITS4 [19] primers were used for the selective amplification of the complete ITS region of the rDNA. The PCR products were visualized on a 1% agarose gels, and purified using a QIAquick gel extraction kit (Qiagene, Hilden, Germany). Purified DNA was directly sequenced on an automatic sequencer (ABI Prism TM 377 DNA Sequencer) with primers ITS1, ITS2, ITS3, and ITS4 [19].

Sequence Alignment and Phylogenetic Analysis

Sequences were edited with the DNASTAR computer package. An alignment of the sequences was performed using the CLUSTAL W [20] program. Bayesian analysis was performed using the MRBAYES version 3.0b4 [21]. The most appropriate evolutionary model was determined for a given dataset using PAUP version 4b10 [22] and Modeltest 3.06 [23]. The general time reversible model (GTR) with a gamma-distributed substitution rate was chosen. A maximum parsimony (MP) heuristic search was performed with ten random sequence additions, branch swapping by tree bisection-reconnection (TBR), and MAXTREES set at 20,000, using PAUP, version 4b10. The relative robustness of the individual branches was estimated by bootstrapping using 10,000 replicates.

Results and Discussion

The PCR products of 1,200–1,300 bp, containing a partial 18S and complete ITS region (ITS1, 5.8S rDNA, and ITS2), were amplified from each specimen. The 32 sequences were adjusted to the length of the complete ITS region. The sequences of *Peronospora* specimens ranged from 794 to 798 bp. All sequences have been deposited in GenBank (Table 1). The phylogenetic relationship was inferred from Bayesian (MCMC) analysis and heuristic MP analysis of the ITS alignment. For the Bayesian inference, all five analyses resulted in the same tree topology and almost identical posterior probability values. After running one million generations, a 50% majority rule consensus tree was obtained (Fig. 1). Out of 823 total characters, 144 were parsimony-informative, and parsimony analysis resulted in

Table 1 Information about the specimens used for phylogenetic analysis

Species	Host	Geographical origin/year of collection (source or herbarium number)	GenBank accession no.
Peronospora astragalina	*Astragalus membranaceus*	Korea, Samchok, 2001 (KUS-F18200)[a]	AY608608
P. campestris	*Arenaria serphyllifolia*	Korea, Kangnung, 2001 (KUS-F18203)[a]	AY608609
P. chrysosplenii	*Chrysosplenium flagelliferum*	Korea, Hongchon, 2002 (KUS-F19232)[a]	DQ643839
P. corydalis	*Corydalis ochotensis*	Korea, Pyeongchang, 2000 (KUS-F17368)[a]	AY211015
P. effusa	*Spinacia oleracea*	Korea, Namyangju, 1998 (BPI877761)[a]	DQ643876
P. boni-henrici	*Chenopodium bonus-henricus*	Austria, Tirol, 2000 (WU22886)[a]	AY198286
	C. bonus-henricus	Bulgaria, Rhodopes, 1982 (SOMF15654)	EF614952
	C. bonus-henricus	Germany, Westfalen, 1920 (BPI787221)	EF614953
	C. bonus-henricus	Switzerland, Granbunden, 1971 (BPI787219)	EF614954
P. chenopodii	*C. hybridum*	Germany, Sachsen-Anhalt, 2001 (HAL) (ex H. Jage 3550/01)	EF614955
P. chenopodii-polyspermi	*C. polyspermum*	Austria, Upper Austria, 2000 (WU22891)[a]	AY198291
	C. polyspermum	Germany, Mittelfranken, 1946 (BPI787538)	EF614956
P. sp.	*C. ambrosioides*	Australia, N.S.W., 1965 (UPS) (ex DAR45530)	EF614957
	C. ambrosioides	Korea, Jeju, 2003 (BPI877765; KUS-F20063)	EF614958
P. variabilis	*C. album*	China, Heilungjiang, 1981 (HMAS57036)	EF614959
	C. album	China, Xingjiang, 1959 (HMAS57057)	EF614960
	C. album	Germany, Oberhessen, 1964 (BPI791617)	EF614961
	C. album	Ireland, Dublin Co., 1935 (UPS)	EF614962
	C. album	Italy, Montesilvano, - (BPI791615)	EF614963
	C. album	Korea, Chunchon, 2000 (KUS-F17266)[a]	AF528556
	C. album	Korea, Chunchon, 2000 (KUS-F17547)[a]	AY211017
	C. album	Korea, Chunchon, 2002 (KUS-F18830)	EF614964
	C. album	Korea, Chunchon, 2003 (KUS-F19787)	EF614965
	C. album	Korea, Pyeongchang, 2000 (KUS-F17768)	EF614966
	C. album	Korea, Samchok, 2000 (KUS-F17289)[a]	AF528557
	C. album	Latvia, Vidzema, 1932 (BPI791620)	EF614967
	C. album	Netherland, Zuid-Holland, 1932 (UPS)	EF614968
	C. album	Romania, 2000 (UPS)[a]	AF465762
P. lamii	*Lamium amplexicaule*	Korea, Kimhae, 2002 (KUS-F19412)[a]	DQ643902
P. manshurica	*Glycine soja*	Korea, Chunchon, 1999 (KUS-F17669)[a]	AY211019
P. rumicis	*Rumex acetosa*	Finland, 1939 (Liro. Myc. fenn. 616, UPS)[a]	AF465758
P. trigonotidis	*Trigonotis peduncularis*	Korea, Wonju, 2002 (KUS-F19287)[a]	AY608611

[a] Sequence obtained from GenBank

the two most parsimonious trees of 434 steps, with a CI of 0.6820 and an RI of 0.8184. As no differences were found between the tree topologies from the MCMC and MP analyses, only the MCMC tree is shown in Fig. 1.

The phylogenetic tree of the ITS rDNA sequences revealed five groups corresponding to the species of *Chenopodium* they originated from. These groups were supported by a strong probability value (1.00 in all groupings) in the MCMC tree (Fig. 1). *Peronospora* specimens from *C. hybridum* appeared as an independent group from those originating from *C. album, C. bonus-henricus, C. polyspermum*, and *C. ambrosioides*. The phylogenetic distances between this species and the listed species were considerably greater as 4.7–5.1, 2.6, 4.9–5.0, and 4.8–5.1%, respectively.

Fig. 1 Phylogenetic tree inferred from Bayesian analysis of the complete ITS region (ITS1, 5.8S rDNA, and ITS2), showing mean branch lengths of a 50% majority rule consensus tree, obtained from an MCMC analysis of one-million generations. Posterior probabilities are indicated above the branches, and MP BS values greater than 50% are indicated below the branches. An asterisk (*) denotes a sequence from GenBank

All sequences from *C. album* formed a monophyletic group regardless of their different geographic origins. *Peronospora boni-henrici* and *P. chenopodii-polyspermi* were identical with GenBank sequences of *C. bonus-henricus* and *C. polyspermum*, respectively. *Peronospora* specimens from *C. ambrosioides* and *C. polyspermum* each formed distinct clades.

All specimens examined could be divided into five groups based on the morphological characteristics of their conidiophores and conidia (Table 2 and Fig. 2). By conidial shape (Fig. 2), downy mildews from five species of *Chenopodium* were divided into two groups. The conidia of *Peronospora* from *C. bonus-henricus* (Fig. 2a, b) and *C. polyspermum* (Fig. 2g, h) were subglobose to broadly ellipsoidal, with a significantly lower l/w ratio, while those from *C. album* (Fig. 2j, k), *C. ambrosioides* (Fig. 2m, n), and *C. hybridum* (Fig. 2 d, e) were broadly ellipsoidal to ellipsoidal and had a higher l/w ratio. The *Peronospora* on *C. polyspermum* was clearly different from other *Chenopodium* mildews by its small conidial size. The *Peronospora* on *C. album* was easily distinguished from that on *C. ambrosioides* and *C. hybridum* by its flexuous to curved ultimate branchlets and the pedicellated conidia. The larger size and higher l/w ratio of conidia allowed us to distinguish the *Peronospora* on *C. hybridum* from the species on *C. ambrosioides*.

Taxonomy

The molecular and morphological results support the existence of five species of *Peronospora* that are represented by their host species. When Gäumann introduced *P. boni-henrici*, *P. chenopodii-polyspermi*, and *P. variabilis*, he did not refer type

Table 2 Comparison of morphological features of *Peronospora* parasitic on five species of *Chenopodium*

Host	*C. album*	*C. ambrosioides*	*C. bonus-henricus*	*C. hybridum*	*C. polyspermum*
Conidiophores					
Length	240–580 μm	220–500 μm	350–600 μm	160–400 μm	200–350 μm
Trunk width (below the first branch)	8–10 μm	5–10 μm	8–12 μm	6–10 μm	6–11 μm
Branch type	Subdichotomous	Subdichotomous or monopodial	Monopodial	Monopodial	Monopodial
No. of branch orders	5–6(–7)	4–6	4–7	4–6	5–7
Callose plugs	Often present	Absent	Very rarely present	Very rarely present	Very rarely present
Ultimate branchlets					
Shape	Flexuous to curved	Straight to slightly curved	Substraight to slightly curved	Straight to slightly curved	Straight to substraight
Length	8–23 (ca. 12.3) μm	5–11 μm	6–15(–22) μm	6–13(–19) μm	6–8 μm
Base width	2–3.5 (ca. 2.6) μm	1.5–2 μm	1.5–2.5 μm	2–3.5 μm	2–3 μm
Tips	Obtuse	Obtuse	Obtuse, rarely subacute	Subacute or obtuse	Obtuse, rarely subtruncate
Conidia					
Shape	Broadly ellipsoidal to ellipsoidal, sometimes obovoidal or napiform	Broadly ellipsoidal to ellipsoidal	Subglobose to broadly ellipsoidal	Ellipsoidal	Subglobose to broadly ellipsoidal
Color	Olivaceous with grayish tint	Olivaceous with grayish tint	Olivaceous or yellowish	Olivaceous to pale brown	Olivaceous
Length	24–35 (ca. 29.5) μm	17.5–27.5 (ca. 23.7) μm	22.7–30 (ca. 25.5) μm	26–33 (ca. 30) μm	15.5–20.8 (ca. 18.4) μm
Width	22–26 (ca. 23) μm	12.5–21.7 (ca. 17.9) μm	20.3–26 (ca. 22.4) μm	17–22.5 (ca. 19.8) μm	13–17 (ca. 15.2) μm
l/w ratio	1.2−1.5 (ca. 1.35)	1.21−1.51 (ca. 1.32)	1.06−1.29 (ca. 1.13)	1.33−1.63 (ca. 1.52)	1.04−1.36 (ca. 1.23)
Pedicel	Present and short-conical	Mostly absent, rarely protruding	Absent, but have scar	Absent, but have scar	Absent, but have scar
Resting organs					
Oogonia	32−55 μm diameter	Not seen	40−55(–68) μm diameter	27−53 μm diameter	Not seen
Oospores	22.5−32.5 (ca. 28) μm diameter	Not seen	25−33 μm diameter	(17-)22−33 μm diameter	Not seen

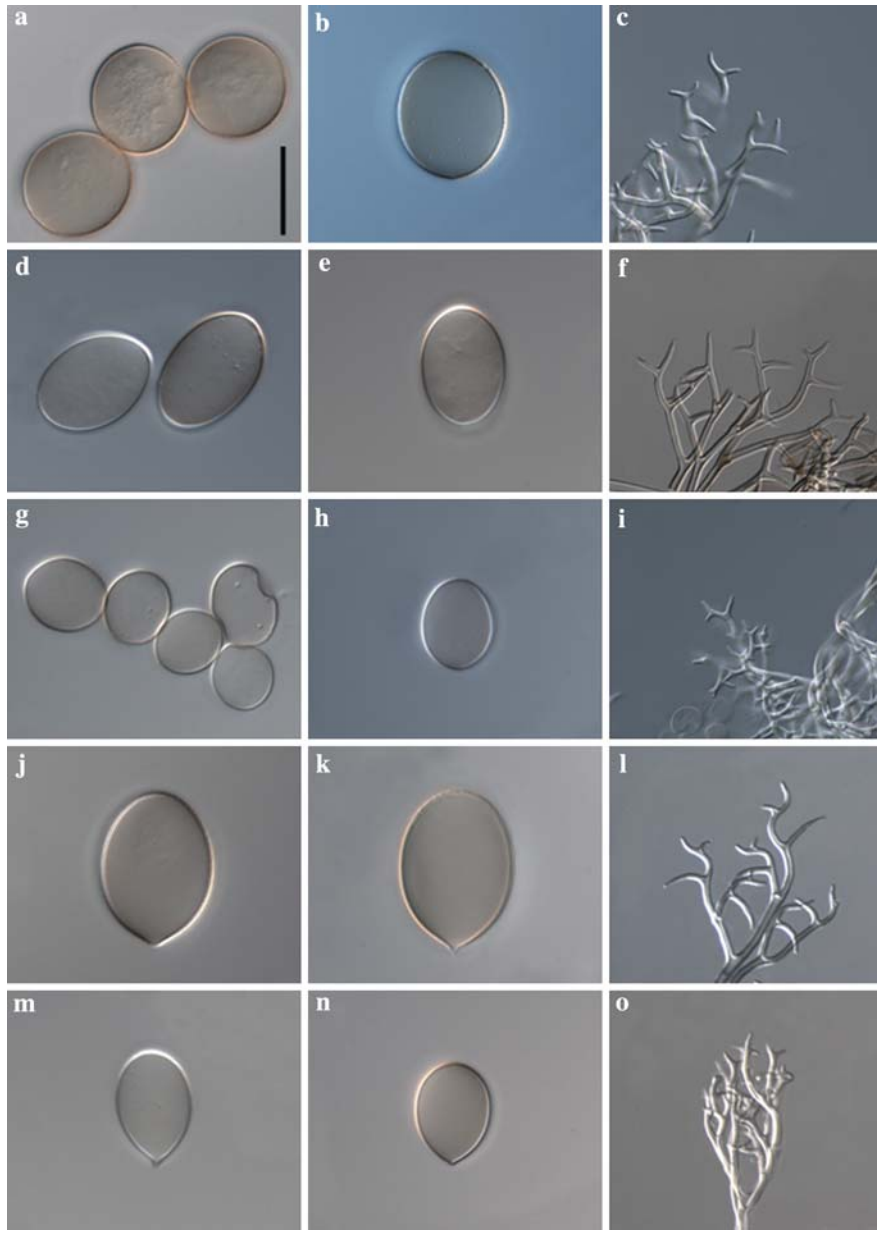

Fig. 2 *Peronospora* species on *Chenopodium*. *P. boni-henrici*: **a**, **b** (conidia), and **c** (ultimate branchlets); *P. chenopodii*: **d**, **e** (conidia), and **f** (ultimate branchlets); *P. chenopodii-polyspermi*: **g**, **h** (conidia), and **i** (ultimate branchlets); *P. variabilis*: **j**, **k** (conidia), and **l** (ultimate branchlets); *P.* sp. (on *C. ambrosioides*): **m**, **n** (conidia), and **o** (ultimate branchlets). Scale bar is 20 μm for conidia and 50 μm for the ultimate branchlets

materials, and therefore in the present study we only added the information about original collection [24] in each description. Morphologically, they can be distinguished by using the key below.

Key for the Identification of *Peronospora* on Five Species of *Chenopodium*

1. Ultimate branchlets flexuous to curved, conidia with obvious pedicel ..... *P. variabilis*
1. Ultimate branchlets straight to slightly curved, conidia without pedicel 2
2. Conidia subglobose to broadly ellipsoidal, l/w ratio mostly 1.1–1.2 3
2. Conidia broadly ellipsoidal to ellipsoidal, l/w ratio mostly 1.3–1.5 4
3. Conidia small (ca. 18.4 × 15.2 μm), ultimate branchlets 6–8 μm long .... *P. chenopodii-polyspermi*

3. Conidia significantly larger (ca. 25.5 × 22.4 µm), ultimate branchlets 6–15(–22) µm long ..... *P. boni-henrici*
4. Conidia large (ca. 30 × 19.8 µm), l/w ratio of ca. 1.52 ..... *P. chenopodii*
4. Conidia somewhat smaller (ca. 23.7 × 17.9 µm), l/w ratio of ca. 1.32 ..... *P.* sp.

Peronospora boni-henrici Gäum. ([4], p. 63) (Figs. 2a–c)

Original collection: on *Chenopodium bonus-henricus* L., Switzerland: Canton Neuchâtel, Rebberge near Cortaillod, June 1915, E. Gäumann.

Conidiophores 350–600 µm long, slender, colorless; trunk substraight to slightly curved, 140–300 µm long, 8–12 µm wide below the first branch, basal end not differentiated, rarely slightly bulbous, callose plugs very rarely present; branches monopodial, occasionally appearing subdichotomous, branched in four to seven orders, substraight to slightly curved; ultimate branchlets mostly in pairs, rarely single, substraight to slightly curved, 6–15(–22) µm long, 1.5–2.5 µm wide at the base, apex obtuse, rarely subacute. *Conidia* olivaceous or yellowish, subglobose to broadly ellipsoidal, greatest width median, 22.7–30 (ca. 25.5) µm long, 20.3–26 (ca. 22.4) µm wide, l/w ratio = 1.08–1.22 (ca. 1.13), pedicel absent in most conidia, but a scar is visible at the place of attachment. *Resting organs* visible as dark brown to black dots on the lower leaf surface; oogonia subglobose to irregular, 40–55(–68) µm diameter, wall yellowish to yellow-brown, uniformly 1–2 µm thick; oospores globose, 25–33 µm diameter, wall smooth, brownish, 2.5–3 µm thick.

Host plant: *Chenopodium bonus-henricus* L.

Specimens examined: Bulgaria, Rhodopes, 19 August 1982, S. Vanev & E. Dimitrova (SOMF 15654); Czech Republic, Olomouc, July 1910, R. Picbauer (BRNM 07834/39); Czech Republic, Hranice, 1923, F. Petrak (BPI 787220); ditto, Jesernik, 20 May 1923, F. Petrak (BPI 787222); Germany, Weidenau, June 1919, J. Hruby (BRNM 07813/39); Germany, Westfalen, Weringhausen, near Finnentrop, Meschede Kreis, 13 May 1920, A. Ludwig (BPI 787221); Germany, Tamsel, 14 August 1923, P. Vogel (BPI 787223); Germany, Tamsel, 14 August 1923, P. Vogel (BPI 787224); Switzerland, Graubünden, Susch, 1 September 1971, R.F. Cain (BPI 787219).

Peronospora chenopodii Schltdl. ([3], p. 619) (Figs. 2d–f)

Original collection: on *Chenopodium hybridum* L., Germany: Sachsen, Halle, coll. Schlechtendal (Rabenh., Klotzsch Herb. viv. mycol. 1776; HAL lectotype, selected by Braun in Feddes repert. 90: 412. 1979).

Conidiophores 160–400 µm long, slender, colorless; trunk straight, 100–260 µm long, uniformly 6–10 µm wide below the first branch, basal end not differentiated to slightly bulbous, up to 13 µm wide, callose plugs very rarely present; branches simple, monopodial, branched in four to six orders, straight to slightly curved; ultimate branchlets in pairs or single, straight to slightly curved, 6–13(–19) µm long, 2–3.5 µm wide at the base, apex subacute or obtuse. *Conidia* olivaceous to pale brown, ellipsoidal, greatest width median, 26–33 (ca. 30) µm long, 17–22.5 (ca. 19.8) µm wide, l/w ratio = 1.33–1.63 (ca. 1.52), pedicel absent in most conidia, but a scar visible at the place of attachment. *Resting organs* clearly visible as dark brown dots on the lower leaf surface; oogonia subglobose to irregular, 27–53 µm diameter, wall smooth, yellowish; oospores plerotic, globose, (17-)22–33 µm diameter, wall 1–3(–4) µm thick.

Host plant: *Chenopodium hybridum* L.

Specimens examined: Czech Republic, unknown, 19 August 1911, E. Baudys (BRNM 334936); Germany, Sachsen-Anhalt, Hügelland, Eisleben, Aseleben, 7 October 2001, H. Jage (HAL ex herb. H. Jage No. 3550/01).

Peronospora chenopodii-polyspermi Gäum. ([4], p. 64) (Figs. 2g–i)

Original collection: on *C. polyspermum* L. Switzerland: Canton Bern, Brüggwald near Biel, July 1916, E. Gäumann.

Conidiophores 200–350 µm long, slender, colorless; trunk substraight to slightly curved, 90–180(–230) µm long, 6–11 µm wide below the first branch, basal end somewhat bulbous, up to 13 µm wide,

callose plugs very rarely present; branches monopodial, branched in five to seven orders, straight to slightly curved; ultimate branchlets mostly in pairs, rarely single, straight to substraight, 6–8 μm long, 2–3 μm wide at the base, apex obtuse, rarely subtruncate. *Conidia* olivaceous, subglobose to broadly ellipsoidal, greatest width median, 15.5–20.8 (ca. 18.4) μm long, 13–17 (ca. 15.2) μm wide, l/w ratio = 1.04–1.36 (ca. 1.23), pedicel absent in most conidia, but a scar visible at the place of attachment. *Resting organs* not seen.

Host plant: *Chenopodium polyspermum* L.

Specimens examined: Czech Republic, Hranice, 28 July 1924, F. Petrak (BPI 787536); Germany, Westfalen, Siegen, 15 July 1929, A. Ludwig (BPI 787537); Germany, Mittelfranken, Hersbruck Kr., 16 September 1946, K. Starcs (BPI 787538); Romania, Transsilvania, Bistriţa-Năsăud District, Beclean, 9 July 1931, T. Săvulescu & T. Rayss (BPI 787539).

Peronospora variabilis Gäum. ([4], p. 62) (Figs. 2j–l)

Original collection: on *Chenopodium album* L., Switzerland: Canton Bern. Wabern near Bern, July 1915, E. Gäumann.

Conidiophores 240–580 μm long, slender; trunk substraight to slightly curved, 120–350 μm long, basal end not differentiated, rarely bulbous, up to 15 μm wide, 10–13 μm wide at the base, 8–10 μm wide below the first branch, callose plugs often present; branches subdichotomously branched in 5–6(–7) orders slightly curved to sigmoid; ultimate branchlets in pairs or single, flexuous to curved, 8–23 (ca. 12.3) μm long, 2–3.5 (ca. 2.6) μm wide at the base, apex obtuse. *Conidia* olivaceous with a grayish tint, broadly ellipsoidal to ellipsoidal, sometimes appearing as obovoid or napiform due to pedicel, greatest width median in most conidia, rarely supra- or sub-median, 24–35 (ca. 29.5) μm long, 22–26 (ca. 23) μm wide, l/w ratio = 1.2–1.5 (ca. 1.35), pedicel present and then short-conical, slightly eccentrically positioned at the base of conidia. *Resting organs* often present and clearly visible as dark brown to yellowish dots on the lower leaf surface; oogonia subglobose to irregular, 32–55 μm diameter, wall smooth, 1–3 μm thick; oospores globose, 22.5–32.5 (ca. 28) μm diameter, golden brown to brown, wall 1.5–3.5 μm thick.

Host plant: *Chenopodium album* L.

Specimens examined: China, Heilungjiang, Haerbin, 3 August 1981, Y. Gong-yi (HMAS 57036); China, Xingjiang, Zhaosu, 1 June 1959, coll.: L. Heng-ying and L. Rong, det.: Y. Gong-yi (HMAS 57057); Korea, Kangnung, 31 October 1994, H.D. Shin (KUS-F 13292); Chunchon, 2 May 1999, H.D. Shin (KUS-F 15722); *ditto*, 1 May 2002 (KUS-F 18830); *ditto*, 7 October 2003 (KUS-F 19787); *ditto*, 4 November 2004 (KUS-F 20950); *ditto*, 27 May 2005 (KUS-F 21135), (KUS-F 21147); *ditto*, 11 November 2005 (KUS-F 21685); Samchok, 5 May 1999, H.D. Shin (KUS-F 15765); Pyeongchang, 3 October 2000 (KUS-F 17768); *ditto*, 11 June 2003 (KUS-F 19561); Yangpyeong, 26 May 2004 (KUS-F 20243); Hongchon, 3 October 2004 (KUS-F 20762); Germany, Oberhessen, Alsfeld Kreis, Romrod, Ruderalstellen, 22 June 1964, H. Hupke (BPI 791617); Ireland, Dublin Co., Tinknoch, 6 July 1935, - (UPS); Netherland, Zuid-Holland, Katwijk-aan-Zee, June 1932 (UPS); Italy, Montesilvano, - (BPI 791615); Latvia, Vidzeme Province, Vestiena, 28 July 1932, K. Starcs (BPI 791620).

Peronospora sp. (Figs. 2m–o)

Conidiophores 220–500 μm long, slender, colorless; trunk straight, 150–310 μm long, 5–10 μm wide below the first branch, basal end slightly bulbous, up to 13 μm wide, callose plugs absent; branches subdichotomous or monopodial, branched in four to six orders, substraight to slightly curved; ultimate branchlets in pairs or single, straight to slightly curved, 5–11 μm long, 1.5–2 μm wide at the base, apex obtuse. *Conidia* olivaceous with gray tint, broadly ellipsoidal to ellipsoidal, greatest width supra-median or median, 17.5–27.5 (ca. 23.7) μm long, 12.5–21.7 (ca. 17.9) μm wide, l/w ratio = 1.21–1.51 (ca. 1.32), pedicel absent in most conidia, sometimes protruding at the place of attachment. *Resting organs* not seen.

Host plant: *Chenopodium ambrosioides* L.

Specimens examined: Australia, N.S.W., Parramatta Park, near Weir, 14 November 1965, J. Walker

(UPS) (ex DAR 45530); Korea, Jeju, Cheju National University, 12 November 2003, H.D. Shin & Y.J. Choi (KUS-F 20063).

Note: For *Peronospora* on *C. ambrosioides*, Golenia [25] introduced *P. chenopodii-ambrosioides*, an invalid name [24]. Kochman and Majewski [8] treated *P. chenopodii-ambrosioides* as a synonym of *P. chenopodii-polyspermi*. The present study, however, shows that the fungus on *C. ambrosioides* is genetically and morphologically distinct from the fungus on *C. polyspermum*.

Conclusion

In this study, *Peronospora* specimens from five species of *Chenopodium* were divided into five different groups by morphological and molecular analyses. These groups correlated with the host plant species. Therefore, the name *P. farinosa* should not be used for all *Peronospora* species on the *Chenopodium*. This work has clarified the identity of several *Peronospora* species infecting *Chenopodium*, but further studies are still needed in order to resolve the taxonomic problems of the remaining species of *Peronospora* found on this genus.

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