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ORIGINAL ARTICLE

Perofascia is not monotypic: the description of the second taxon affecting the South American crop maca (*Lepidium meyenii*)

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Abstract Maca (*Lepidium meyenii*) is an Andean crop of narrow distribution, but because of the nutritional and health value, its cultivation area is rapidly expanding. By a broad-spectrum resistance mechanism against various pathogens, just a few diseases have been reported on maca, among which downy mildew is a potential threat to its cultivation. The occurrence of this disease was, so far, restricted to the native area of maca. However, here we report that it was recently introduced into South Korea. As the causal pathogen has initially been attributed to *Hyaloperonospora parasitica* (syn. *Peronospora parasitica*), which was thought to affect various Brassicaceae, but is, in fact, restricted to *Capsella bursa-pastoris*, the identity of this pathogen remains uncertain. In this study, morphological and phylogenetic data revealed that maca downy mildew is unrelated to any species of *Hyaloperonospora* and instead belongs to the previously monotypic genus *Perofascia*. It differs markedly from the type species, *Perofascia lepidii*, and consequently *Perofascia macaicola* sp. nov. is described and illustrated here. Considering the quick expansion of cultivated land with maca, quarantine measures for this pathogen might be appropriate for hindering the spread of the disease through the international trade of maca.

Keywords Brassicaceae · *Lepidium* · Seed transmission · Species concepts

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Introduction

Downy mildews (Peronosporaceae; Oomycota) are a group of obligate biotrophic fungal-like microorganisms infecting a wide range of hosts from mono- to dicotyledonous plants, and contain many economically relevant species (Thines and Choi 2016), e.g., *Bremia lactucae* on lettuce, *Plasmopara viticola* on grape, *Pseudoperonospora cubensis* on cucurbitaceous crops. In the family Brassicaceae, downy mildew pathogens can cause severe damage to a variety of crops, e.g., horseradish (*Armoracia rusticana*), mustard greens (*Brassica juncea*), rapeseed (*B. napus*), cabbage (*B. oleracea*), Chinese cabbage (*B. rapa*), arugula (*Eruca vesicaria*), wasabi (*Eutrema japonicum*), watercress (*Nasturtium officinale*), and radish (*Raphanus sativus*). Also, several ornamental plant genera, such as *Aubrieta*, *Cheiranthus*, *Iberis*, and *Matthiola*, are affected by downy mildew, as is *Arabidopsis thaliana*, a key model organism in the study of the flowering plants. Despite substantial efforts to resolve host ranges and the phylogeny of Brassicaceae-infecting downy mildew species (Constantinescu and Fatehi 2002; Choi et al. 2003; Göker et al. 2004, 2009), only a fraction of the known hosts could be included in these

investigations so far, leaving the majority of brassicolous downy mildew (BDM) diversity unexplored.

As a consequence of the huge species diversity in downy mildews, many studies have focused on economically important downy mildew groups (Choi et al. 2009, 2011b, 2015b; Thines et al. 2009; Constantinescu and Thines 2010; Runge et al. 2011; Thines 2011; Telle and Thines 2012; Denton et al. 2015), as resolving their species boundaries has immediate importance for phytosanitary measures and quarantine (Thines and Choi 2016). The importance of understanding species boundaries and the biology of downy mildew pathogens is emphasized by the fact that several downy mildew species, such as *Peronospora belbahrii* (Thines et al. 2009), *Pe. tabacina* (Schiltz 1981), *Plasmopara halstedii* (Leppik 1966), and *Pl. viticola* (Viennot-Bourgin 1981), have been causing high economic losses outside their native range after their introduction. Due to increased global trade, the introduction of new downy mildew diseases has accelerated over the past two decades, with several new reports of downy mildew disease on a variety of crops and ornamentals, e.g., *Pe. belbahrii* on basil (Thines et al. 2009), *Pe. salviae-officinalis* on culinary sage (Choi et al. 2009), *Peronospora* sp. on *Aquilegia* (Denton et al. 2015), *Pl. wilsonii* on *Geranium phaeum* (Kruse et al. 2016), and *Pl. destructor* on busy Lizzie (*Impatiens walleriana*) (Görg et al. 2017).

Maca (or Peruvian Ginseng; *Lepidium meyenii*) is grown for the nutritional and health value of its root. For the last few decades, an increase in demand has occurred, promoting a quick expansion of cultivated land in both the restricted native range in the Peruvian Andes and also in other countries, especially in South-East Asia. The occurrence of downy mildew disease on maca has been reported in the native area of maca (Icochea et al. 1994), but, to our knowledge, there are no reports from outside South America.

Since November 2014, maca plants showing typical symptoms of downy mildew (Fig. 1a, b) have been found in experimental plots in the Gochang and Pyeongchang counties of South Korea, where several field trials have recently been conducted to determine the ability of the crop to grow in Korean climate and soil conditions. To our knowledge, this constitutes the first report of downy mildew on this plant in Asia. Downy mildew has to be considered as a potential threat to the cultivation of maca, but the taxonomic identity of the causal agent is uncertain. Initially, the downy mildew disease of maca has been attributed to *Peronospora parasitica*, which was thought to be a generalist species occurring on a broad range of the Brassicaceae and allied families. However, BDM have been generally recognized to be members of the genera *Hyaloperonospora* or *Perofascia* (Constantinescu and Fatehi 2002) and to be fairly host-specific (Göker et al. 2003, 2004, 2009; Choi et al. 2011a; Voglmayr et al. 2014). Importantly, as both *Hyaloperonospora* and *Perofascia* affect various species of *Lepidium*, the identification of the downy mildew pathogen of maca is uncertain even at the genus level, although from the first description of this pathogen (Icochea et al. 1994), it seems to be more likely that the species belongs to *Perofascia*. Given the mostly very narrow host range of BDM, e.g., with the type species *H. parasitica* s. str. having been confirmed only on *Capsella bursa-pastoris* (Gäumann 1918; Wang 1944; Chang et al. 1964; Dickinson and Greenhalgh 1977), it seemed possible that the downy mildew of maca is caused by an undescribed species of BDM. Thus, it was the aim of the present study to clarify if maca downy mildew is caused by *Perofascia* or *Hyaloperonospora* and if it can be attributed to a previously known species or represents a new species overlooked so far.

Materials and methods

Oomycete samples

Six herbarium specimens of downy mildew pathogens originating from five species of *Lepidium* (*L. coronopus*, *L. latifolium*, *L. meyenii*, *L. ruderale*, and *L. virginicum*) were analyzed in this study, along with sequences of nine additional specimens from additional species (*L. densiflorum*, *L. draba*, *L. ruderale*, and *L. virginicum*) for which sequence data were previously published (Choi et al. 2003; Göker et al. 2004, 2009) and available from GenBank. Information on all specimens is shown in Table 1.

Morphological analysis

Herbarium specimens were moistened with 70% ethanol and then transferred to 60% lactic acid on a slide. The microscope preparations were warmed up, covered with coverslips, and examined using a Zeiss Imager M2 AX10 microscope (Carl Zeiss, Göttingen, Germany). Differential interference contrast (DIC) micrographs were captured with a Zeiss Axiocam MRc5 digital camera (Carl Zeiss, Göttingen, Germany) and processed using the AxioVision software (Carl Zeiss, Göttingen, Germany). Measurements were performed at 100–200× for conidiophores and at 400× for conidia and ultimate branchlets. Measurements are reported as follows; (minimum) – standard deviation towards the minimum – mean – standard deviation towards the maximum – (maximum).

DNA extraction, PCR, sequencing, and phylogenetic analysis

In total, 5–20 mg of infected plant tissue from herbarium specimens were disrupted in a mixer mill (MM2, Retsch, Haan, Germany), using three iron beads of 3 mm and 1 mm diameter per sample and shaking at 30 Hz for 3 min. Genomic DNA was extracted using the BioSprint 96 DNA Plant Kit (Qiagen, Hilden, Germany) on a KingFisher Flex (Thermo

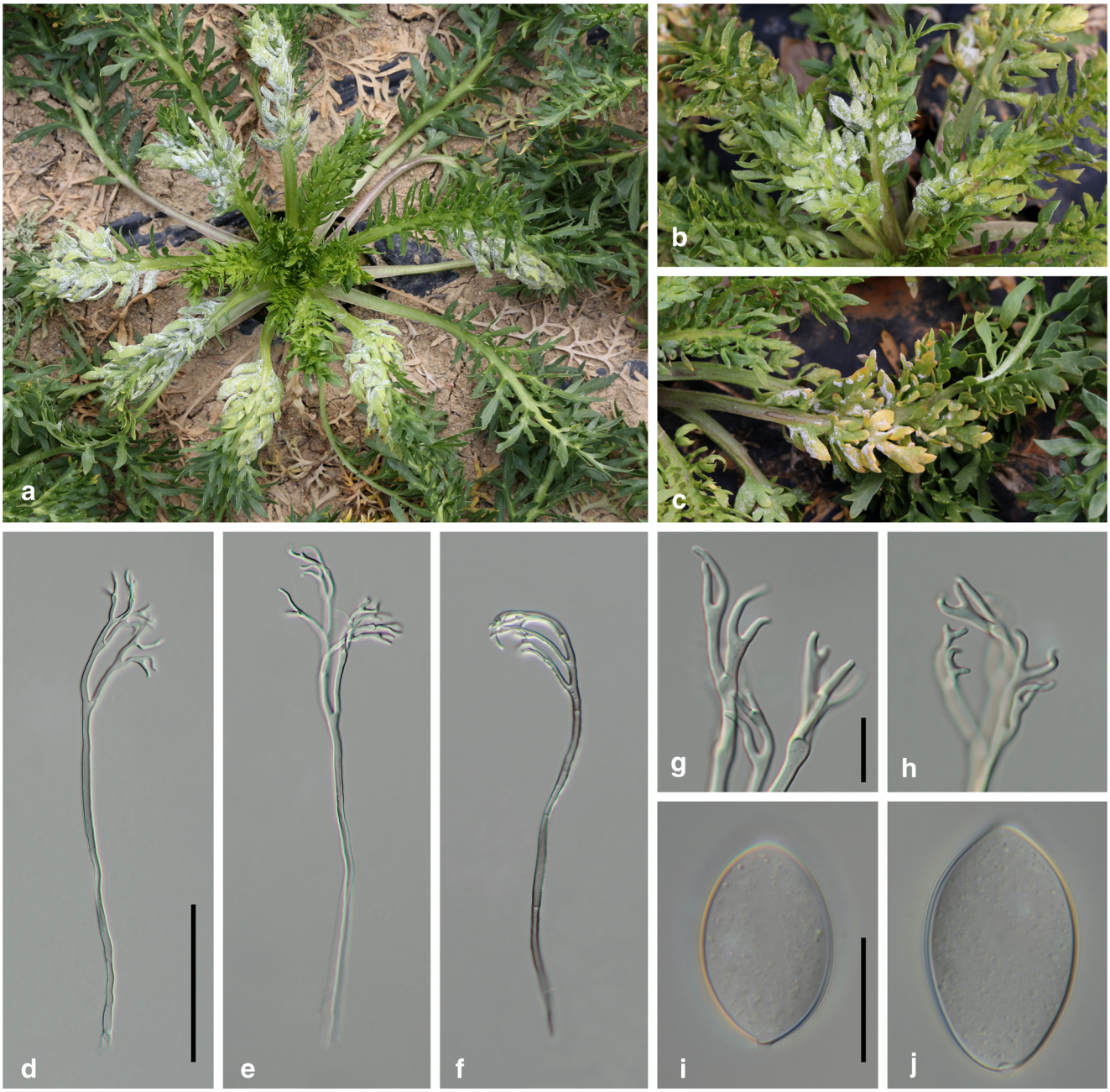

Fig. 1 Downy mildews associated with *Perofascia macaicola* sp. nov. on maca (*Lepidium meyenii*) (KUS-F28527 – holotypus). **a** A typical symptom of downy mildew disease on leaves and stems; **b**, **c** close-up of dense felt-like sporulation on the upper surface; **d**–**f** conidiophores; **g**, **h** ultimate branchlets; **i**, **j** conidia. Scale bars: **d**–**f** = 100 μm; **g**–**j** = 20 μm

Scientific, Dreieich, Germany) robot. Polymerase chain reaction (PCR) amplification was performed with the primers ITS1-O (Bachofer 2004) and LR-0 (reverse complementary to LR-0R; Moncalvo et al. 1995) for ITS rDNA and cox2-F (Hudspeth et al. 2000) and cox2-RC4 (Choi et al. 2015a) for *cox*2 mtDNA. The PCR conditions for ITS and *cox*2 amplifications were as reported by Choi et al. (2015b). Amplicons were sequenced at the Biodiversity and Climate Research Centre (BiK-F) laboratory using primers identical to those used for amplifications.

Sequences were edited using the DNAStar software package (DNAStar Inc., Madison, WI, USA), version 5.05. An alignment of each locus was performed using MAFFT 7 (Katoh and Standley 2013) employing the Q-INS-i algorithm (Katoh and Toh 2008). We used three different tree construction methods: minimum evolution (ME), maximum likelihood (ML), and Bayesian inference (BI). ME analysis was done using MEGA 6.0 (Tamura et al. 2013), with the default settings of the program, except for using the Tamura–Nei model. For ML analyses,

Table 1 Summary of oomycete herbarium specimens investigated in this study

Taxon	Host plant	Geographic origin	Herbarium voucher	GenBank accession no.	
				ITS	*cox*2
Hyaloperonospora sp. [*P. lepidii-sativi*]	*Lepidium draba*	Austria, Burgenland	HV115–116 (WU)	AY531462	–
Hyaloperonospora sp. [*P. lepidii-sativi*]	*Lepidium draba*	Austria, Niederösterreich	HV246 (WU)	AY531463	–
Hyaloperonospora sp. [*P. lepidii-sativi*]	*Lepidium draba*	Germany, Sachsen-Anhalt	J729/01 (TUB 12463)	EU049260	–
Hyaloperonospora sp. [*P. lepidii-sativi*]	*Lepidium draba*	Germany, Sachsen-Anhalt	J484/01 (TUB 12461)	EU049261	–
Hyaloperonospora sp.	*Lepidium ruderale*	Germany, Sachsen-Anhalt	J3189/01 (TUB 1241)	AY531446	–
Perofascia lepidii	*Lepidium coronopus*	Germany, Sachsen-Anhalt	GLM75965	KY986669	KY986663
Perofascia lepidii	*Lepidium latifolium*	Germany, Sachsen-Anhalt	GLM74415	KY986670	KY986664
Perofascia lepidii	*Lepidium densiflorum*	Romania	Myc. Rom. 2659, UPS	AF465760	–
Perofascia lepidii	*Lepidium ruderale*	Germany, Sachsen-Anhalt	J2068/01 (TUB 12409)	AY531467	–
Perofascia lepidii	*Lepidium ruderale*	Germany, Sachsen-Anhalt	GLM74332	KY986671	KY986665
Perofascia lepidii	*Lepidium virginicum*	Korea, Seoul	KUS-F17250	AY211013	–
Perofascia lepidii	*Lepidium virginicum*	Korea, Seoul	KUS-F17311	AY211014	KY986666
Perofascia sp.	*Lepidium meyenii*	Korea, Gochang	KUS-F28527	KY986672	KY986667
Perofascia sp.	*Lepidium meyenii*	Korea, Gochang	KUS-F28528	KY986673	KY986668

1000 rounds of random addition of sequences as well as 1000 fast bootstrap replicates were performed using RAxML 7.0.3 (Stamatakis 2006), as implemented in raxmlGUI 1.3 (Silvestro and Michalak 2012) using the GTRCAT variant. BI analysis was done using siMBa (Mishra and Thines 2014), with the following parameters: GTR (substitution type), 100,000 (number of generations), 25% (fraction of samples to be discarded).

Results

Morphology

The downy mildew pathogen of maca had hyphal haustoria and appressed (parallel) branches, typical features of the monotypic genus *Perofascia*. However, the downy mildew pathogen of maca differs markedly from *Perofascia lepidii* in terms of several morphological characteristics (Table 2). The length of the conidiophores was longer in the maca downy mildew pathogen (290–350 μm) than in *Pf. lepidii* (180–250 μm). This difference is mostly due to the different length of trunks; 160–240 μm in maca downy mildew pathogen vs. 100–135 μm in *Pf. lepidii*. Branches and ultimate branchlets were mostly curved in the former species, but mostly sub-straight in the latter species. Conidia from infected maca specimens were ellipsoidal and measured, on average, 35.1 × 22.8 μm, with a length to width ratio of 1.55, but in *Pf. lepidii*, they were broadly ellipsoidal to ellipsoidal and measured, on average, 28.5 × 20.7 μm, with a length to width ratio of 1.44.

Molecular phylogenetic inference

In the ITS rDNA and *cox*2 mtDNA regions, barcoding loci for oomycetes, the maca downy mildew pathogen and *Pf. lepidii* exhibited significant sequence divergences of 5.8% (46 of 790 characters are different) and 7.8% (44 of 566 characters), respectively. These divergences are higher than for closely related species of *Hyaloperonospora*, e.g., four species parasitic to *Cardamine* s.l. (in Fig. 2a), *H. nasturtii-aquatici*, *H. cardamines-laciniatae*, *H. cardamines-enneaphyllos*, and *H. dentariae-macrophyllae*, which have significantly lower mean pairwise distances of a maximum of 1.5% in ITS and 4.2% in *cox*2.

In the phylogenetic reconstructions based on ITS rDNA (Fig. 2a) and *cox*2 mtDNA (Fig. 2b), the maca downy mildew pathogen was placed as a sister group to *Pf. lepidii* with maximum support in ME, ML, and BI analyses. All specimens of *Pf. lepidii* originating from *L. coronopus*, *L. densiflorum*, *L. latifolium*, *L. ruderale*, and *L. virginicum* grouped together to form a monophyletic group with maximum support in all analyses in the ITS-based tree and high to maximum support in the *cox*2-based tree. Downy mildew pathogens from *Lepidium ruderale* belonged to either *Perofascia lepidii* or a presumably undescribed species of *Hyaloperonospora*. Downy mildew pathogens from *Lepidium draba* grouped together in the *Hyaloperonospora* clade with maximum support.

Discussion

In the first half of the 20th century, a huge amount of *Peronospora* species has been described (e.g., Gäumann

Table 2 Distinctive morphological characters of *Perofascia macaicola* and *Perofascia lepidii*

	Perofascia macaicola (KUS-F28527)	*Perofascia lepidii* (GLM74332)
Host plant	*Lepidium meyenii*	*Lepidium ruderale*
Length of conidiophores	(250–)290–350(−420) (av. 320 μm)	(140–)180–250(−300) (av. 215 μm)
Length of trunk	(150–)160–240(−270) (av. 200 μm)	(85–)100–135(−145) (av.120 μm)
Shape of branches	Mostly curved	Sub-straight to slightly curved
Shape of conidia	Ellipsoidal to broadly ellipsoidal	Broadly ellipsoidal to ellipsoidal
Length of conidia	(25–)30–39(−45) (av. 35.1 μm)	(24–)26–33(−35) (av. 28.5 μm)
Width of conidia	(18–)21–24(−26) (av. 22.8 μm)	(18–)19–22.5(−24) (av. 20.7 μm)
Length/width ratio	(1.3–)1.41–1.66(−1.8) (av. 1.55)	(1.2–)1.38–1.48(−1.55) (av. 1.43)

1918, 1923), based on morphological differences and host ranges. This view, however, had been challenged later, mainly by applied plant pathologists (e.g., Yerkes and Shaw 1959) and has been followed by most plant pathologists until the advent of molecular phylogenetics. Thus, for almost half a century, only the name "*Peronospora parasitica*" has been

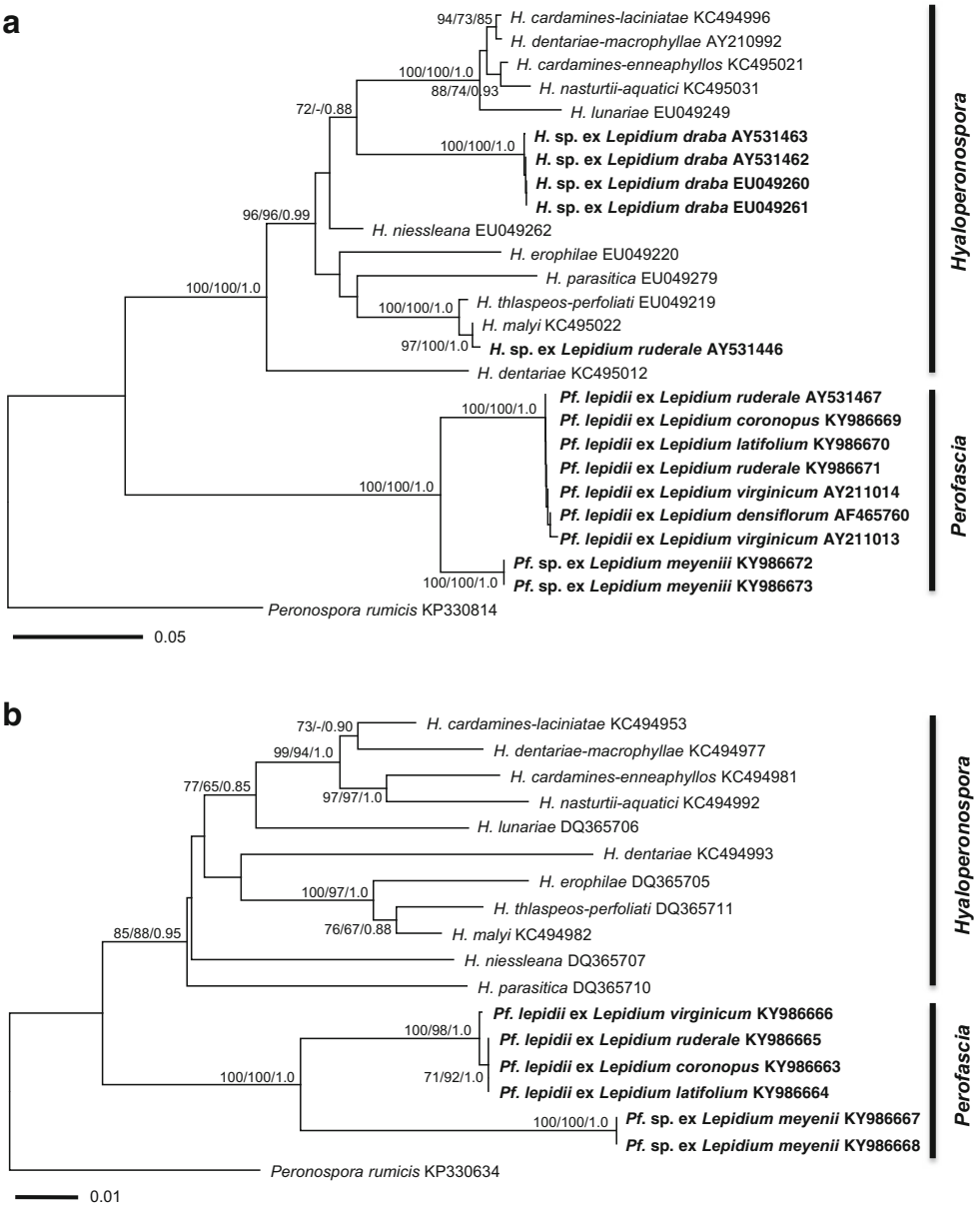

Fig. 2 Minimum evolution trees based on the ITS rDNA (**a**) and *cox*2 mtDNA (**b**) sequences, with support values in maximum likelihood and Bayesian ineference. Support values (ME BS/ML BS/BI PP) higher than 60% are given above or below the branches. Specimens which originated from *Lepidium* spp. are in **bold**. The scale bars equal the number of nucleotide substitutions per site

widely used for any downy mildew pathogen on Brassicaceae, instead of the about 140 other names of downy mildews occurring on Brassicaceae. Presumably, the broad species concept of Yerkes and Shaw (1959) has also influenced the initial identification of maca downy mildew as "*P. parasitica*" (Icochea et al. 1994), instead of considering one of the eight names available for *Peronospora* which have previously been described on members of the genus *Lepidium* (also including *Cardaria* and *Coronopus*); *P. cardariae-repentis*, *P. coronopi*, *P. coronopi-procumbentis*, *P. lepidii*, *P. lepidii-perfoliati*, *P. lepidii-sativi*, *P. lepidii-virginici*, and *P. maublancii* (Constantinescu 1991). However, molecular phylogenetic studies since the turn of the century clearly favored the narrow species circumscription of Gäumann (1918, 1923) that a species of downy mildew is usually restricted to a particular host genus or species (Riethmüller et al. 2002; Choi et al. 2003, 2011a; Göker et al. 2003, 2004, 2009; Voglmayr 2003, 2014; ; Voglmayr and Göker 2011). Based on apparent morphological differences and phylogenetic divergence, the maca downy mildew pathogen is not only distinct from *H. parasitica* but also from *Perofascia lepidii*, the sole other species of *Perofascia*. This is again in line with the narrow species delimitation of downy mildews. Importantly, the presence of the second species in the genus *Perofascia* provides a hint that several genera of downy mildews with only one species might probably contain additional species on other hosts. This is also supported by the recent finding that the genus *Basidiophora* with the only previously accepted species, *B. entospora*, does, indeed, consists of several phylogenetic lineages in need of description as a new species (Sökücü and Thines 2014).

When Constantinescu and Fatehi (2002) established the genus *Perofasica*, they synonymized four out of the eight species described on members of the genus *Lepidium* (*P. coronopi*, *P. coronopi-procumbentis*, *P. lepidii-virginici*, and *P. maublancii*) with *P. lepidii*. This was supported for several of these species by subsequent phylogenetic studies (Choi et al. 2003; Göker et al. 2004, 2009). Similarly, the present phylogenetic study revealed that specimens from *Lepidium coronopus* (type host of *P. coronopi-procumbentis*) and *L. virginicum* (type host of *P. lepidii-virginici*) form a well-supported group with *Pf. lepidii*. Although specimens from the type plants of *P. coronopi* and *P. maublancii* could not be examined in this study, the description and illustration of the two species (Gäumann 1918; Săvulescu and Rayss 1934) are reminiscent of *Pf. lepidii*, as previously suggested by Constantinescu and Fatehi (2002). However, these species are morphologically different from *Perofascia* from maca, supporting the introduction of a second species in this genus. It is notable that all previously described species and specimens investigated so far were derived from the holarctis and that the maca downy mildew pathogen is the first one investigated that originates from the southern hemisphere.

Apart from the five names of *Peronospora* on *Lepidium* which have been transferred to *Perofascia*, three names of *Peronospora* on *Lepidium* have either been combined into *Hyaloperonospora* (*P. lepidii-perfoliati*) or synonymized with *H. parasitica* (*P. cardariae-repentis* and *P. lepidii-sativi*). In the present study, two phylogenetically distant lineages of *Hyaloperonospora* on *Lepidium* were observed, specific to *L. draba* or *L. ruderale*. Including these two hosts, five species of *Lepidium* have been listed as hosts of *P. lepidii-sativi* in the original description by Gäumann (1918). Thus, a specimen from the type host plant, *Lepidium sativum*, should be examined in future studies to determine if either of the two lineages observed in the current study corresponds to this name. On *Lepidium ruderale*, both *Hyaloperonospora* and *Perofascia* were found in this study, in line with the previous morphological investigations, which reported the co-existence of the two different genera on this plant species (Constantinescu and Fatehi 2002).

Little is known about the biology and epidemiology of BDM, and this is the first report of downy mildew disease on maca outside of the high Andes of Peru. The disease has recently been introduced to South Korea, probably along with maca seeds, which would be in line with the systemic nature of the infections observed in the field and also with the fact that oospores of this pathogen have been found inside seeds (Pérez 1999). This also suggests that the pathogen is capable of spreading rapidly into other areas by seed trade. Since the cultivation of maca has recently started on a commercial scale in various countries, the downy mildew has the potential to become a serious threat to the production of this root crop. Thus, maca downy mildew should be closely monitored, and adequate quarantine and phytosanitary measures for hindering further spread should be considered.

Taxonomy

Based on differences in morphology and molecular phylogeny as well as host range, *Perofascia macaicola* is described here as a new species.

Perofascia macaicola Y.J. Choi, Thines, I.Y. Choi & H.D. Shin, sp. nov. Fig 1.

MycoBank no.: MB821212.

Etym.: '*macaicola*' refers to the common name of the host plant, *Lepidium meyenii*.

Down hypophyllous, whitish to yellowish, dense, felt-like. Haustoria filling the host cell partly to almost completely, hyphal, branched. Conidiophores emerging through stomata, up to 20 in a fascicle, hyaline, slender, straight to slightly curved, (250–)290–350(−420) μm; trunk slightly curved,

(150–)160–240(−270) μm long, (3.5–)4.0–5.5(−6.5) μm wide, more or less uniform; basal end not or somewhat swollen, callose plugs absent; branches appressed, slightly parallel, of uniform width, from simple to elaborate structured, branching mostly monopodially but rarely trichotomously (4–)5–6 times, first branching at (1/2–)2/3–3/4 of conidiophore. Ultimate branchlets in pairs or single, curved to reflexuous, (5–)12–20(−25) μm long, 1.5–2(−2.5) μm wide at the base; tip variable, from round to truncate. Conidia hyaline, ellipsoidal to broadly ellipsoidal, (25–)30–37(−40) μm long, (18–)21–24(−26) μm wide, l/w ratio (1.3–)1.41–1.62(−1.7), greatest width median, rarely sub- or supra-median, tip often apiculate in mature conidia but round in young ones, base gradually narrowing; pedicel slightly protruding, 2.5 μm wide and 2 μm long. Resting organs not seen.

Typus: South Korea: Jeollabuk-do; Gochang-gun; Gogneum-myeon, in an experimental plot, on living leaves of *Lepidium meyenii* affected by downy mildew disease, November 13 2014, Shin, Hyeon-Dong & Choi, In-Young (KUS-F28527 – holotypus). Ex-type sequences: KY986667 (ITS nrDNA) and KY986672 (*cox*2 mtDNA).

Habitat: On living leaves of *Lepidium meyenii* Walp. (Brassicaceae).

Distribution: Peru and South Korea.

Additional specimens examined: ditto, November 17 2014 (KUS-F28528); ditto, February 26 2015 (KUS-F28594).

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