The Korean Journal of Mycology www.kjmycology.or.kr

RESEARCH NOTE

Pseudoperonospora urticae Occurring on *Urtica angustifolia* in Korea

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Abstract

The genus *Pseudoperonospora* (Peronosporales, Oomycota) comprises six accepted species, including *Ps. cubensis*, which causes downy mildew on many economically important cucurbitaceous crops, and *Ps. humuli*, which occurs on hops. During a survey of downy mildew flora in Korea, a previously unreported species of *Pseudoperonospora* was found on *Urtica angustifolia*. Based on molecular phylogenetic and morphological analyses, the causal agent was identified as *Pseudoperonospora urticae*. This is the first report of *Pseudoperonospora urticae* occurring on *Urtica angustifolia* in Korea.

Keywords: *cox*2 mtDNA, Internal transcribed spacer rDNA, Oomycetes, *Pseudoperonospora cubensis*, *Pseudoperonospora humuli*

Kor. J. Mycol. 2017 June, 45(2): 160-166
https://doi.org/10.4489/KJM.20170020

pISSN : 0253-651X
eISSN : 2383-5249

Received: 13 May, 2017
Accepted: 22 May, 2017

Downy mildews (Peronosporaceae; Oomycota) are an obligate biotrophic group that infects a wide range of mono-and dicotyledonous plants, including many economically relevant crops [1]. *Pseudoperonospora cubensis* is a notorious species infesting many cucurbitaceous crops, such as cucumber, gourd, pumpkin, and watermelon [2, 3], and *Ps. humuli* is one of the most important threats to the cultivation of hops (Cannabaceae) [4, 5]. Given their association with high economic losses, many recent studies have focused on the biology, host specificity, population structure, detection, and control of *Pseudoperonospora* species [3, 6-11], as well as their taxonomy and phylogeny [12, 13].

To date, four species of *Pseudoperonospora* have been reported in Korea [14, 15], *Ps. cannabina*, *Ps. celtidis*, *Ps. cubensis*, and *Ps. humuli*. In September 2009, symptoms typical of downy mildew were found on the leaves of *Urtica angustifolia* Fisch. ex Hornem. (Urticaceae) growing near Jangjeon valley in Pyeongchang, Korea (N37°29'36"; E128°32'33"). *Urtica angustifolia* is distributed in the wastelands, grasslands, valleys, wet places, and ridges of East Asian countries, including China, Korea, and Russia [16], and it is used as a traditional medicinal plant due to its high hypoglycemic activity [17]. The downy mildew

infection resulted in slight discoloration of the leaf tissues, with yellow or pale green spots on the upper leaf surfaces that developed dark grey fungal growth on the lower surfaces. The lesions were poly-angular, and were delimited by the leaf veins (Fig. 1A, 1B). As the disease progressed, the spots turned blackish and often merged to cover larger areas. A representative sample was deposited in the National Institute of Biological Resources (KZITFG0000000017) and the Korea University Herbarium (KUS-F24488).

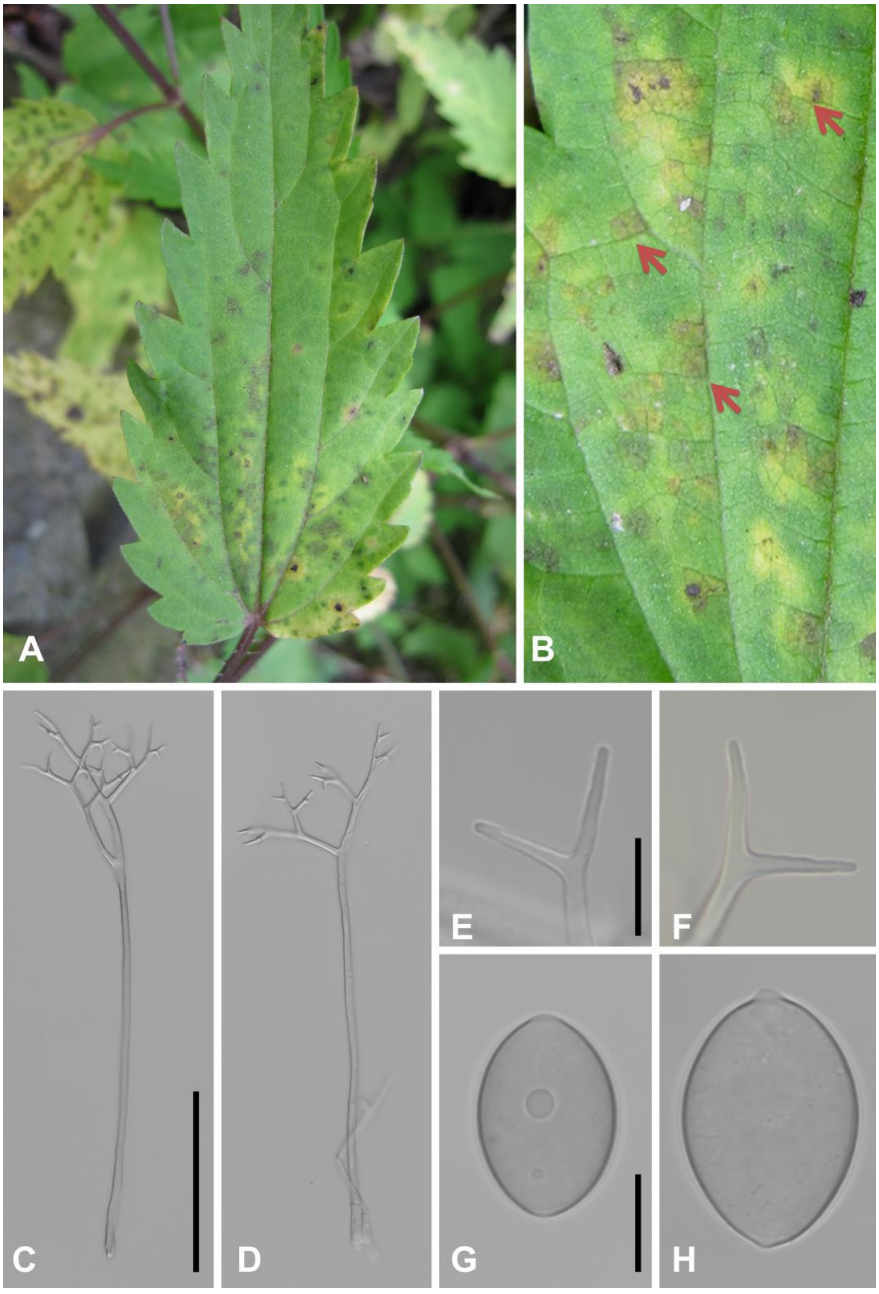

Fig. 1. *Pseudoperonospora urticae* occurring on *Urtica angustifolia*. A, symptoms on the upper surface of *Urtica angustifolia* leaves; B, focus on the vein-limed spots; C, D, sporangiophores; E, F, ultimate branchlets; G, H, sporangia (scale bars: C, D = 100 μm, E~H = 20 μm).

A detailed microscopic examination was performed using an Olympus BX53 microscope (Olympus, Tokyo, Japan), and DIC micrographs were captured with a DigiRetina 16M camera (Tucsen, Fuzhou, China). The following morphological characteristics were observed at 100~200 × for sporangiophores and at 400 × for sporangia and ultimate branchlets. The sporangiophores emerging through the stomata were tree-like, hyaline, straight, slender, and 120~400 μm in height (Fig. 1C, 1D). Trunks were straight, 4~6 μm wide below the first branch, of nearly uniform width, with no callose plug, and slightly swollen at the bases. Branching was monopodial, ramified 3~5 orders, but sometimes appeared subdichotomous. Ultimate branchlets were mostly in pairs but rarely single, straight to substraight, 7~12 μm long, and 1.5~2.5 μm wide at the base, with obtuse or subtruncate tips (Fig. 1E, 1F). Sporangia were pale brown to violet, ellipsoidal, 22~32(~42) μm long, and 14~20 μm wide, with a round or gradually narrowing tip and base and a somewhat protruding pedicel. The length to width ratio of the sporangia was 1.4~1.8 (n = 69), with the greatest width mostly at the median, but rarely supra-median. In the dehiscence apparatus, the inner layer of the wall was discontinuous, with a pore of 3~5 μm diameter and a papilla of 1.5~2.3 μm thick (Fig. 1G, 1H). Resting organs were not seen. The morphological observations revealed that this fungus unequivocally belongs to the genus *Pseudoperonospora*, and were well consistent with the known characteristics of *Ps. urticae* (Lib.) E.S. Salmon and Ware [18-20], except for the slight differences in sporangial size (Table 1). Waterhouse and Brothers [19] noted that the sporangia of *Ps. urticae* (maximum 40 μm and average 30 μm) were larger than those of other *Pseudoperonospora* species; however, others reported smaller sporangia: 19~32 × 13~21 (ave. 25.5 × 16.6) μm by Ito [21], 18~25(~30) × 12~16(~20) μm by Kochman and Majewski [22]; 19~33.5 × 12.5~23 μm by Vanev et al. [23]; 16~28 × 14~21 (mostly 23~25 × 18~20) μm by Ul'yanishchev et al. [24]; 22~32 × 14~22 μm by Mazelaitis and

Table 1. Morphological comparison of the Korean specimen and previously reported *Pseudoperonospora urticae*

	The present study	Salmon and Ware [18]	Constantinescu [20]
Sporangiophores			
shape	simple, straight	simple, straight	almost straight
branching	branched acute angle	branched acute angle	-
Ultimate branchlet's tip	obtuse or subtruncate	-	round to subacute
Sporangia			-
shape	ellipsoidal	ovate	-
tip	round or gradually narrowing	apiculate	-
operculum	clearly present	-	present
base	papillate	papillate	papillate
length	22~32 (~42) μm long,	22~40 (average 27) μm	25~30 μm
width	14~20 μm wide	14~22 (average 18) μm	17~19 μm
Host plant	*U. angustifolia*	*U. dioica, U. urens**	*U. dioica, U. kioviensis*

*Constantinescu [20] stated that *U. urens* is not a host plant of *Ps. urticae*.

Stanevicienė [25]; and 25~30 × 17~19 μm by Constantinescu [20]. In the Korean sample, such large sporangia were quite rare, but unambiguously present among mature sporangia with darker color, sympathizing with the opinion of Waterhouse and Brothers [19], along with a study of Yu [26] (maximum 40 μm).

Genomic DNA was extracted from the infected plant tissue of the herbarium specimen using the DNeasy Plant Mini Kit (Qiagen, Germantown, MD, USA). PCR amplification was performed with the primers ITS1-O & LR-0 for internal transcribed spacer (ITS) rDNA [27] and cox2-F & cox2-RC4 for *cox*2 mtDNA [28]. Amplicons were sequenced by a DNA sequencing service (Macrogen, Seoul, Korea) with the primers used for amplification. The resulting sequences ware deposited in GenBank (accession numbers: KY986684 for ITS rDNA and *cox*2 mtDNA for KY986683). The sequences were edited using the DNASTAR Lasergene software package (DNASTAR, Madison, WI, USA), version 5.05. Alignments of each locus were generated using MAFFT 7 [29] with the Q-INS-1 algorithm. Minimum evolution (ME) and maximum likelihood (ML) methods were used to construct two different trees. ME analysis was done using MEGA 7.0 [30], with the default settings of the program, except for replacement with the Tamura-Nei model. For ML analysis, 1,000 rounds of random addition of sequences as well as 500 fast bootstrap replicates were performed with RAxML 7.0.3 [31] using the GTRCAT model. In the ITS rDNA and *cox*2 mtDNA regions, the barcoding loci of oomycetes [28], the Korean isolate from *Urtica angustifolia* exhibited a high similarity of 99.5% (4 out of 750 characters are different) with *Ps. urticae* sensu stricto from *Urtica dioica* (AY198307, HM636048, HM636049) for the ITS sequencse, but 98% (12 out of 550) with three sequences (DG3657644, HM635952, HM635953) for the *cox*2 sequences. The phylogenetic trees for a combined alignment of ITS rDNA and *cox*2 mtDNA were inferred using the ME and ML methods. As the two trees were congruent, only a ME tree is shown in Fig. 2. The Korean sample was a close sister-lineage to *Ps. urticae* s. s., with a maximum support in both ME and ML trees. The phylogenetic divergence between the Korean sample and *Ps. urticae* s. s. may be due to either the different host species (*Urtica angustifolia* vs. *Urtica dioica*) or the distant geographic origins (Korea vs Europe), as in this study, no morphological differences were found between them (data not shown). Further study with additional collections is needed to investigate the precise relationship of the two lineages.

Based on the morphological and phylogenetic analyses, the pathogen was identified as *Pseudoperonospora urticae*. This fungus has now been reported on six species of *Urtica*; *U. angustifolia*, *U. dioica*, *U. fissa*, *U. gracilis* (often regarded as a subspecies of *U. dioica*), *U. kioviensis*, and *U. urens* [32]. After a detailed review of downy mildews parasitic to *Urtica* spp., Constantinescu [20] suggested that both *U. gracilis* and *U. urens* are not the host plant of *Ps. urticae*, but instead they are infected by *Peronospora debaryi*, another downy mildew species occurring on *Urtica* spp. The present study confirmed that *U. angustifolia* is a rare host plant of *Ps. urticae*, and infection by *Pseudoperonospora* has

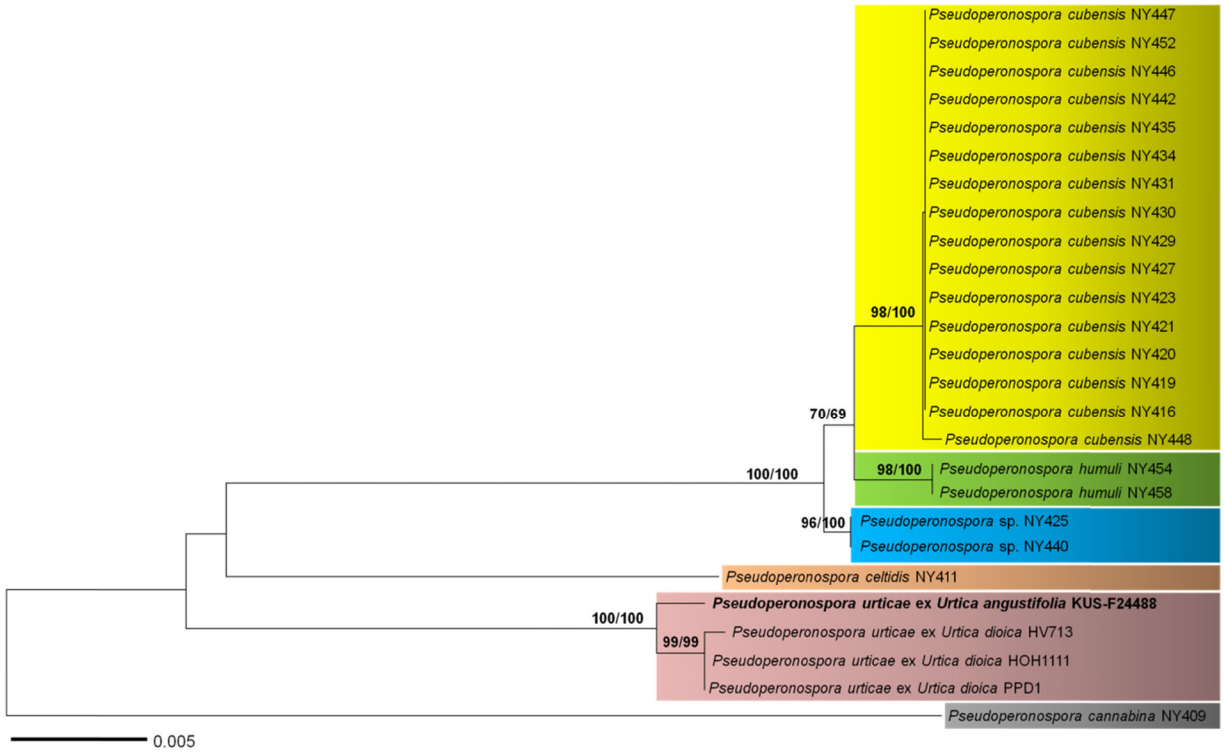

Fig. 2. Minimum evolution tree of *Pseudoperonospora* species using a combined alignment of internal transcribed spacer (ITS) rDNA and *cox2* mtDNA sequences. Bootstrapping values (minimum evolution BP/ maximum likelihood BP) higher than 60% are shown above the branches (1,000 replicates). The scale bar equals the number of nucleotide substitutions per site.

been reported only once in Far Eastern Russia [33]. To our knowledge, this is the first report of *Ps. urticae* occurring on *U. angustifolia* in Korea.

Acknowledgements

This study was supported by a grant from the National Institute of Biological Resources (NIBR), funded by of the Ministry of Environment (MOE) and the National Research Foundation of Korea (NRF), funded by the Ministry of Science, ICT & Future Planning (2016R1C1B2008013), Republic of Korea.

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