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FULL PAPER

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Erysiphe wadae: a new species of *Erysiphe* sect. *Uncinula* on Japanese beech

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Abstract A new species of *Erysiphe* sect. *Uncinula* is described and illustrated from Japan. *Erysiphe wadae* sp. nov., found on Japanese beech (*Fagus crenata*, Fagaceae), is characterized by having two types of appendages, i.e., a long (true) appendage arising from the equatorial zone of the ascomata, and a short appendage arising from the upper part of the ascomata. This characteristic is shared by *E. simulans*, *E. australiana*, *E. flexuosa*, *E. liquidambaris*, *E. prunastri*, and *E. togashiana*. *Erysiphe wadae* differs from the latter five species in its brown-colored appendage. *Erysiphe simulans* is most similar to *E. wadae*, but differs in its loosely uncinate appendage and smaller number of ascospores. Identity of the nucleotide sequences of the rDNA ITS region is 92.3% between the two species. The significance of the two types of appendage in taxonomy and phylogeny of powdery mildews is discussed based on molecular phylogenetic analysis.

Key words Erysiphaceae · Fagaceae · *Fagus crenata* · Powdery mildew · Secondary appendage

Introduction

Only one species of *Erysiphe* sect. *Uncinula* (formerly the genus *Uncinula*), *Erysiphe curvispora* (Hara) U. Braun & S. Takamatsu [≡*Uncinula curvispora* (Hara) Hara], has been reported on *Fagus crenata* Blume (Hara 1915). This fungus is unique among the Erysiphales in its septate and numerous appendages arising from the upper half of the ascomata and its curved ascospores. Although a specimen of this species is not available now, molecular phylogenetic analysis revealed that the allied species, *E. septata* (E.S. Salmon) U. Braun and S. Takam. (≡*Uncinula septata* E.S. Salmon), is placed at the primitive base of the Erysiphales (Mori et al. 2000). We found occurrence of a powdery mildew belonging to *Erysiphe* sect. *Uncinula* on *F. crenata* at Aomori, Akita, and Toyama in 2001 and at Fukushima and Shiga in 2002. The present fungus distinctly differs from *E. curvispora* in its smaller numbers of appendages arising from the equatorial zone of ascomata and smaller ascomata. The most conspicuous characteristic of this fungus is having two types of appendages, i.e., a long (true) appendage arising from the equatorial zone of the ascomata, and a short appendage arising from the upper part of the ascomata. Here, we describe and illustrate the newly found fungus as a new species, *Erysiphe wadae*. We also discuss the significance of two types of appendages in taxonomy and phylogeny of the Erysiphales based on the nucleotide sequences of the internal transcribed spacer (ITS) regions of nuclear ribosomal DNA (rDNA).

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Materials and methods

Morphological observation

Anamorph

Fresh hyphae, conidiophores, and conidia were stripped off from the leaf surfaces with clear adhesive tape, mounted on a microscope slide with the fungal materials uppermost, and examined in water under a light microscope. The following information was noted during the examination: size and shape of conidia; presence or absence of fibrosin bodies; nature of conidiogenesis; characteristics of the conidiophore, e.g., size and shape of foot cell, position of the basal septum; shape and position of hyphal appressoria; position of germ tubes of conidia; and shape of appressoria on germ tubes of conidia.

Teleomorph

Ascomata were transferred onto a microscope slide with a needle under a dissecting microscope and observed in 3% NaOH under a light microscope. The following information was noted during the examination: size and shape of ascomata, asci, and ascospores; characteristics of appendages, e.g. number, length, color, and shape of the apex; and number of asci and ascospores.

DNA extraction, PCR, and sequencing

Whole-cell DNA was isolated from mycelia by the chelex method (Walsh et al. 1991; Hirata and Takamatsu 1996). The nuclear rDNA region, including the ITS regions (ITS 1 and ITS 2), and the 5.8S rRNA gene were amplified by the polymerase chain reaction (PCR) using the primers ITS 5 (White et al. 1990) and P3 (Kusaba and Tsuge 1995). PCR reactions were carried out in 50-μl volumes as previously described (Hirata and Takamatsu 1996). A negative control lacking template DNA was included for each set of reactions. One microliter of the first reaction mixture was used for the second amplification with the partial nested primer set ITS 1 (White et al. 1990) and P3. The PCR product was subjected to preparative electrophoresis in 1.5% agarose gel in TAE buffer (40 mM Tris-acetate, 1 mM EDTA, pH8.0). The DNA product of each amplification was then excised from the ethidium-stained gel and purified using the JETSORB kit (Genomed, Oeynhausen, Germany) following the manufacturer's protocol. Nucleotide sequences of the PCR products were obtained for both strands using direct sequencing in an Applied Biosystems 373A sequencer (Applied Biosystems, Foster City, CA, USA). The sequence reactions were conducted using the PRISM Dye Terminator Cycle Sequencing kit (Applied Biosystems) following the manufacturer's instructions.

Molecular phylogenetic analysis

The sequences were initially aligned using the Clustal V package (Higgins et al. 1992). The alignment was then visually refined with a word processing program, using color-coded nucleotides, and ambiguously aligned sites were removed from the data set in the following analyses. The alignment is available upon request to the corresponding author. Phylogenetic trees were obtained from the data using maximum-likelihood (ML) and parsimony methods. For ML analysis, the most appropriate evolution model was determined for a given data set using PAUP* 4.0b4 (Swofford 1999) and Modeltest 3.06 (Posada and Crandall 1998). A starting tree was obtained with the neighbor-joining method. With this tree, likelihood scores were calculated for 56 alternative models of evolution by PAUP*. The output file was then imported to Modeltest to compare the models by likelihood ratio test. Once a model of evolution was chosen, it was used to construct phylogenetic trees with the ML method by a heuristic search option of PAUP*.

For the parsimony analysis, we used the maximum-parsimony (MP) method with a heuristic search using PAUP*. This search was repeated 100 times with different random starting points, using the stepwise addition option to increase the likelihood of finding the most parsimonious tree. All sites were treated as unordered and unweighted, with gaps treated as missing data. The branch-swapping algorithm was TBR, the MULPARS option was in effect, and zero-length branches were collapsed. The strength of the internal branches from the resulting trees was tested by bootstrap analysis using 100 (ML) or 1000 (MP) replications (Felsenstein 1985).

The Kishino–Hasegawa (KH; Kishino and Hasegawa 1989) test was conducted to evaluate the taxonomic and phylogenetic significance of the two types of appendages. A constrainted tree was constructed using the Tree Window option of MacClade 3.08 (Maddison and Maddison 1992) and was imported into PAUP* to find a ML tree consistent with a specific constraint hypothesis. The log likelihoods of the constrained tree and unconstrained ML tree were calculated using PAUP*. The evolution model appropriate for the data set was determined by PAUP* and Modeltest as already described to calculate log-likelihoods in the KH test.

Results

Taxonomy

Erysiphe wadae S. Takamatsu & Y. Sato, sp. nov.

Figs. 1–8

Mycelium hypophyllum, raro amphigenum in foliis, evanescens, tenue; hyphae hyalinae. Appressoria lobulata. Conidiophora erecta, cellulis basalibus cylindraceis, 2–3 cellulis sequentibus longitudine aequalibus. Conidia solitaria, ellipsoidea, 19.8–23.8 × 7.9–11.9 μm. Ascomata

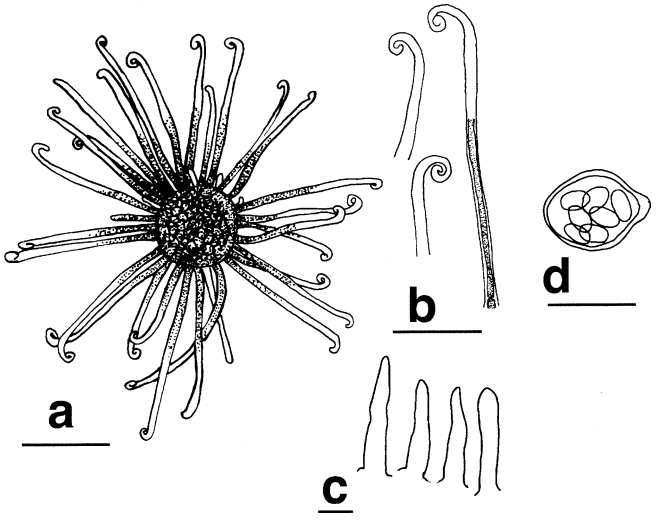
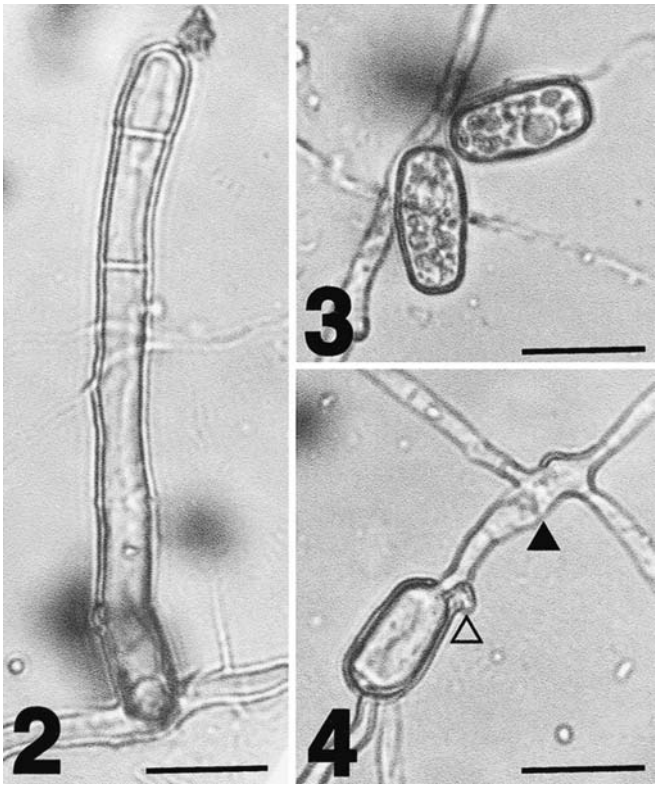


Fig. 1. *Erysiphe wadae*. **a** Ascoma. **b** Long (true) appendages with circinate tip. **c** Short appendages. **d** Ascus and ascospores. *Bars* **a** 100 μm; **b**, **d** 50 μm; **c** 10 μm



Figs. 2–4. Anamorph of *Erysiphe wadae.* **2** Conidiophore. **3** Conidia containing large oil drops. **4** Germ tube having two appressoria. *White arrowhead*, multilobed appressorium at the base; *black arrowhead*, simply lobed appressorium at the end of germ tube. *Bars* 20 μm

sparsa, globosa, (74–)84–105(–125) μm diam, in maturitate fusco-brunea. Appendices biformis. Appendices longiores (12–)18–32, aequatoriales, rectae vel curvatae, in latitudine aequales, uniseptatae vel aseptatae, apice circinatae vel uncinatae, attenuatae, basim brunneae et sursum pallide brunneae, (129–)153–215(–225) μm longae, diametro ascomatis 1.5–2.5 plo longiores). Appendices breviores (3–)4–10(–13), hyalinae, rectae vel facatae, (10–)13–23 (–30) μm longae. Asci (3–)4–6, ovatae, hyalinae, (40–)43–53(–55) × (30–)33–37.5(–43) μm. Ascosporae (4–)6–8, ovatae vel ellipsoideae, hyalinae, (13–)15–18(–20) × (8–)10–13 μm.

Holotypus. In foliis vivis *Fagi crenatae* Blume (buna), Iwakisan, Iwaki-machi, Aomori, Japonica, Sept. 10, 2001, leg. S. Takamatsu and Y. Sato, in Herbario Musei Scientiae nationalis, Tsukuba, Japonia (TNS-F-5685) conservatus.

Etymology. The new species is named in honor of the late Ms. Kumiko Wada who worked on the Erysiphales at Niigata University, Japan.

Colonies. Mycelia on leaves hypophyllous, rare amphigenous, hyaline, very thin, evanescent. Appressoria simply lobed, single or opposite in pairs (see Fig. 8).

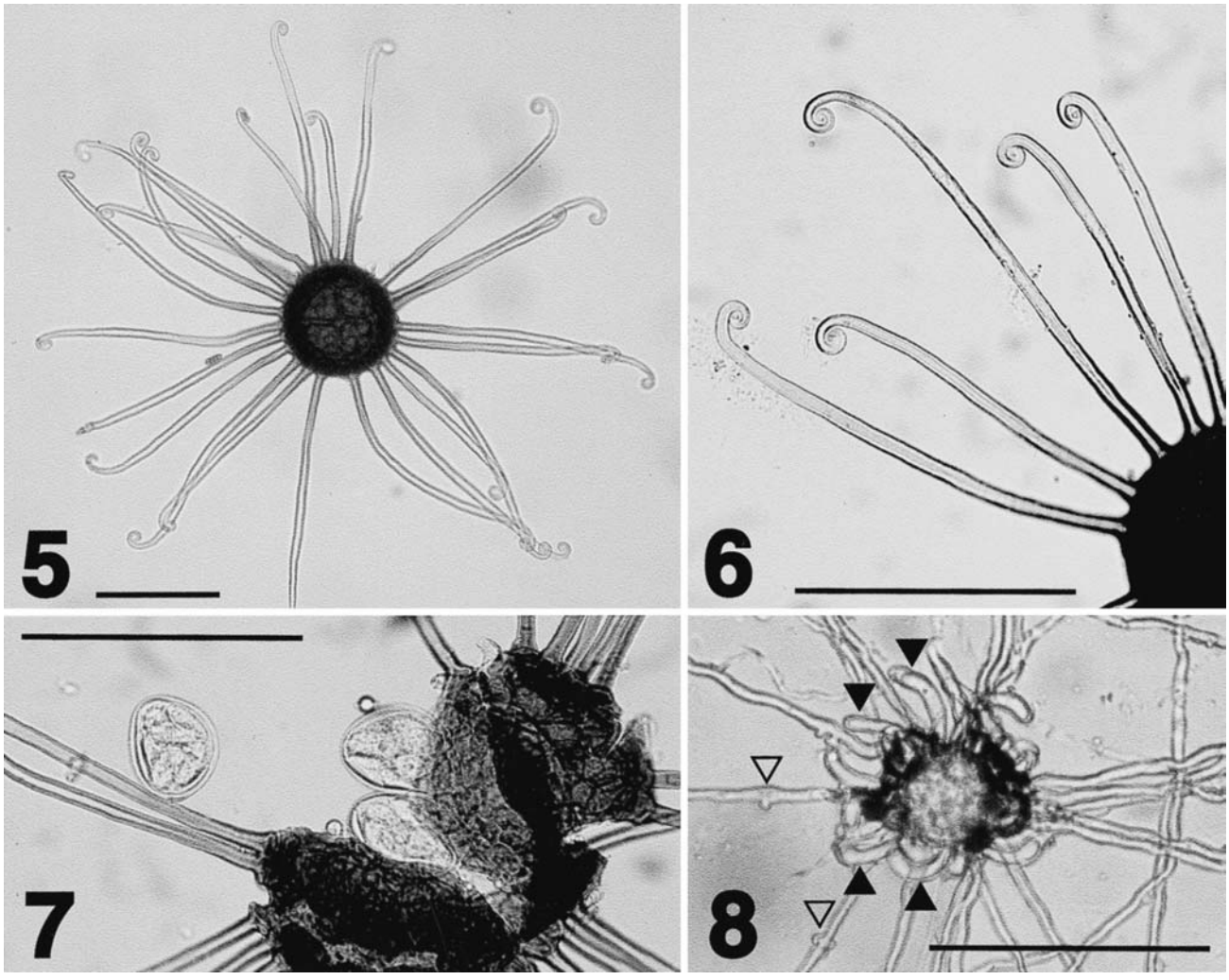
Anamorph. *Oidium* subgenus *Pseudoidium* Jacz. Conidiophores somewhat rare, arising from the upper part or the side of mother cells, 75.2–104.9 × 7.9–11.9 μm (Fig. 2). Foot cells straight or curved at the base, relatively long, up to 89.1 μm, followed by 2–3 cells. Conidia produced solitary, unicellular, hyaline, ellipsoid, 19.8–23.8 × 7.9–11.9 μm, without conspicuous fibrosin bodies, usually containing large oil drops, producing germ tubes on the shoulder (Fig. 3). Germ tubes having two appressoria: multilobed appressorium at the base and simply lobed appressorium at the end of the germ tube (Polygoni type; Braun 1987) (Fig. 4).

Teleomorph. Ascomata scattered, blackish-brown, globose, (74–)84–105(–125) μm in diameter, having two types of appendages (Figs. 1a, 5). Long appendages (12–)18–32 in number, equatorial, simple, straight to mildly curved, width subequal throughout, apex closely circinate, sometimes loosely circinate or subhelicoid, tapering toward the tip, 4–8 μm wide, (129–)153–215(–225) μm in length (1.5–2.5 times as long as the ascomatal diameter), dark brown at the base and gradually paler upward, thick-walled at the base, gradually thinner upward, aseptate or uniseptate at the base (Figs. 1b, 6). Short appendages (3–)4–10(–13) in number, substraight to sickle-shaped, (10–)13–23(–30) μm long, 5–7 μm wide, hyaline (Figs. 1c, 8). Asci (3–)4–6 in an ascoma, (40–)43–53(–55) × (30–)33–37.5(–43) μm, shortly stalked, ovoid, hyaline, thick-walled (Figs. 1d, 7). Ascospores (4–)6–8 in an ascus, unicellular, hyaline, ovoid, ellipsoid to oblong, (13–)15–18(–20) × (8–)10–13 μm.

Materials studied. Sukayu Onsen, Hakkoda Mountain, Aomori-shi, Aomori, Japan, Sept. 8, 2001, leg. S. Takamatsu, MUMH1534; Iwaki Muntain, Iwaki-machi, Aomori, Japan, Sept. 10, 2001, leg. S. Takamatsu and Y. Sato, TNS-F-5685 (holotype), MUMH1660 (isotype); Oirase, Towadako-machi, Aomori, Japan, Sept. 12, 2001, leg. S. Takamatsu and Y. Sato, MUMH1550; Tamagawa Onsen, Tazawako-machi, Akita, Japan, Sept. 14, 2001, leg. S. Takamatsu and Y. Sato, MUMH1632; Shirakimine, Toyama, Japan, Oct. 8, 2001, leg. Y. Sato and G. Mimuro, TPU-3830; Buna-ga-take, Shiga, Japan, Sept. 19, 2002, leg. S. Takamatsu, MUMH1728; Tochiore-toge, Yatsuo-machi, Toyama, Japan, Oct. 14, 2002, leg. Y. Sato, TPU-3963; Hibarako lake, Kita-shiobara village, Fukushima, Japan, Oct. 14, 2002, leg. S. Takamatsu and Y. Nomura, MUMH1729.

Phylogenetic analysis

To evaluate the significance of two types of appendages as a phylogenetic character, we determined the ribosomal DNA ITS sequences for three species of *Erysiphe* sect. *Uncinula* having the two types of appendages. These three sequences were deposited at DDBJ under the accession numbers AB091774 to AB091776 (Table 1). These sequences were aligned with a total of 15 sequences of *Erysiphe* spp., *Typhulochaeta japonica* S. Ito & Hara, and *Brasiliomyces trina* (Harkn.) R.Y. Zheng obtained from DNA databases (Table 1). The alignment data matrix consists of 18 taxa and 636 characters, of which 146 sites were removed because of ambiguous alignment. Of the 496 remaining characters, 208 sites were variable and 123 sites were phylogenetically informative for parsimony analysis. *Erysiphe australiana* (McAlpine) U. Braun & S. Takam. (≡*Uncinula australiana*



Figs. 5–8. Teleomorph (ascoma) of *Erysiphe wadae*. **5** Ascoma. **6** Appendages with circinate tip. **7** Asci and ascospores. **8** Young, immature ascoma. Note secondary appendages (*black arrowheads*) arising at the early stage of ascomatal formation. *White arrowheads*, appressoria on hyphae produced singly or in opposite pairs. *Bars* 100 μm

McAlpine) was used as an outgroup taxon based on Mori et al. (2000). Using Modeltest (Posada and Crandall 1998) under the likelihood ratio test criterion, we concluded that the Tamura–Nei model (Tamura and Nei 1993), with equal base frequencies, a gamma-distributed rate heterogeneity model (Yang 1994; four rate categories, G = 0.3318), and an estimated proportion of invariant sites (0), was the most appropriate model of evolution for this data set. A heuristic search with this model produced a single ML tree with −ln likelihood score of 2950.75606 (Fig. 9, left). MP analysis found six equally parsimonious trees of 494 steps consistency index (CI) = 0.632, retention index (RI) = 0.572, rescaled consistency index (RC) = 0.361) (Fig. 9, right). There is no conflict between the two trees. *Erysiphe wadae* was included in a large clade composed of fungi parasitic to Fagaceae (*Typhulochaeta japonica*, *Brasiliomyces trina*, and *E. gracilis* R.Y. Zheng & G.Q. Chen), Rosaceae [*E. simulans* (E.S. Salmon) U. Braun & S. Takam. (≡*Uncinula simulans* E.S. Salmon) and *E. prunastri* DC. (≡*U. prunastri* (DC.) Sacc.)], Hippocastanaceae [*E. flexuosa* (Peck) U. Braun & S. Takam. (≡*U. flexuosa* Peck)], and Moraceae [*E. mori* (I. Miyake) U. Braun & S. Takam. (≡*U. mori* I. Miyake)] with high bootstrap support (87%–89%). In the clade, *E. wadae* grouped with *E. simulans* with low bootstrap support (51%–53%). Nucleotide sequence identity was 92.3% between *E. wadae* and *E. simulans*.

Of the 18 taxa used in this analysis, 6 taxa [*E. australiana*, *E. flexuosa*, *E. prunastri*, *E. simulans*, *E. togashiana* (U. Braun) U. Braun & S. Takam. (≡*U. togashiana* U. Braun), and *E. wadae*] have two types of appendages. These 6 species did not group into a clade; instead, they mixed with taxa having a single type of appendages in the phylogenetic trees. A constrainted tree assuming these 6 taxa to be monophyletic was significantly worse than the best tree (see Fig. 9). The differences of ln *L* values (−in *L*), the standard deviation of the difference (SD), and the *P* value for significance between the best and constraint tree were as follows: $-\ln L = 44.90252$, SD = 13.30935, $P < 0.001$.

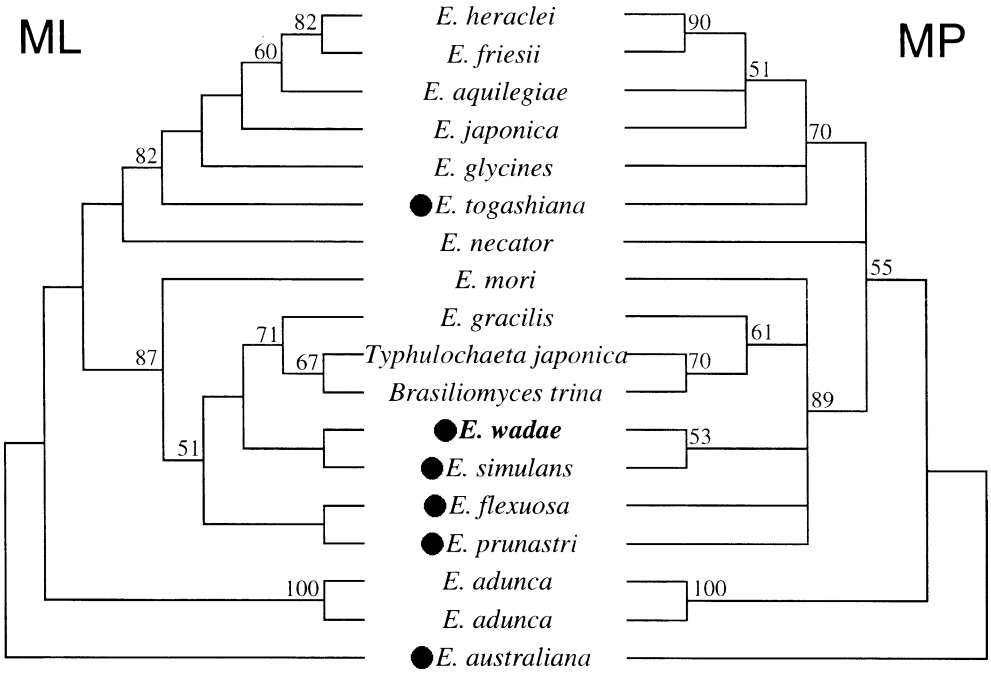


Fig. 9. Phylogeny of *Erysiphe wadae* inferred from internal transcribed spaces (ITS) data for a total of 18 isolates of *Erysiphe* spp., *Brasiliomyces trina*, and *Typhulochaeta japonica*. *Solid circles* indicate species having two types of appendages. *Left:* A maximum-likelihood (*ML*) tree. Model parameters: equal base frequencies with rate heterogeneity; gamma shape parameter = 0.3318; proportion of invariable sites = 0; six rate categories; Tamura–Nei model (Tamura and Nei 1993) with transformation parameters [A–C] = 1.0000, [A–G] = 2.7829, [A–T] = 1.0000, [C–G] = 1.0000, [C–T] = 4.0387, [G–T] = 1.0000. Percent bootstrap support (100 replications) is indicated *above nodes*. *Right:* Strict consensus of six equally parsimonious trees of 484 steps. Percent bootstrap supports (1000 replications) are shown *above nodes*. The consistency index (CI) is 0.632; the retention index (RI) is 0.572; and the rescaled consistency index (RC) is 0.361. *MP*, maximum parsimony

Table 1. Fungal materials and sequence database accession numbers used for phylogenetic analysis

Fungus	Host plant	Isolate[a]	Country of origin	Database accession no.
Erysiphe adunca	*Populus* sp.	UC1512287	USA	AF011324
E. adunca	*Salix vulpina*	MUMH39	Japan	D84383
E. aquilegiae var. *ranunculi*	*Cimicifuga simplex*	TPU-495	Japan	AB000944
E. australiana	*Lagerstroemia indica*	DNA	Japan	AB022408
E. flexuosa	*Aesculus hippocastanum*	MUMH1429	Germany	AB091774[c]
E. friesii var. *dahurica*	*Rhamnus japonica* var. *decipiens*	MUMH6	Japan	AB000939
E. glycines	*Desmodium podocarpum* subsp. *oxyphyllum*	MUMH52	Japan	AB015927
E. gracilis	*Quercus glauca*	MUMH122	Japan	AB022358
E. heraclei	*Daucus carota*	MUMH73	Japan	AB000942
E. japonica	*Swida controversa*	MUMH90	Japan	AB000941
E. mori	*Morus australis*	MUMHs77	Japan	AB000946
E. necator	*Vitis vinifera*	VPRI19719	Australia	AF073346
E. prunastri	*Prunus spinosa*	MUMH652	Switzerland	AB046983
E. simulans	*Rosa miltiflora*	TPU-439	Japan	AB015926
E. togashiana	*Styrax japonica*	MUMH84	Japan	AB091775[c]
E. wadae	*Fagus crenata*	MUMH1534	Japan	AB091776[c]
Brasiliomyces trina	*Quercus agrifolia*	MUMH114	USA	AB022351
Typhulochaeta japonica	*Quercus cuspidata*	MUMHs75	Japan	AB022416

[a] MUMH, Mie University Mycological Herbarium; TPU, Herbarium of Toyama Prefectural University; VPRI, Plant Disease Herbarium, Institute for Horticultural Development, Victoria, Australia; UC, University of California Herbarium; DNA, preserved by DNA, herbarium specimen not available

[b] DDBJ, EMBL, and GenBank database accession number of the nucleotide sequence data

[c] DNA sequence determined in this study

Discussion

According to Viennot-Bourgin (1966), 15 powdery mildew species covering six genera have been recorded on *Quercus* L. (Fagaceae). Because there are no other plant genera recorded with so many kinds of powdery mildew, a close evolutionary relationship between *Quercus* and powdery mildews has been suggested in several reports (Viennot-Bourgin 1966; Hirata 1968; Gardner et al. 1972; Amano 1986; Mori et al. 2000). In contrast, 5 species, *E. erineophila* (Peck) U. Braun & S. Takam. (≡*Microsphaera erineophila* Peck), *E. alphitoides* (Griffon & Maubl.) U. Braun & S. Takam. (≡*M. alphitoides* Griffon and Maubl.), *E. curvispora*, *Phyllactinia guttata* (Wallr.: Fr.) Lév., and *P. angulata* (E.S. Salmon) S. Blumer, have been recorded so far on *Fagus* spp. (Braun 1987). Although the present fungus clearly belongs to *Erysiphe* sect. *Uncinula* based on its appendages with circinate tips, it distinctly differs from *E. curvispora* in its fewer number of appendages arising from the equatorial zone of the ascomata, short secondary appendages arising from the upper part of the ascomata, and smaller ascomata.

The most conspicuous character of the present fungus is having two types of appendages. Among species of *Erysiphe* sect. *Uncinula*, this characteristic is shared by *E. simulans*, *E. australiana*, *E. flexuosa*, *E. liquidambaris* (R.Y. Zheng and G.Q. Chen) U. Braun & S. Takam., *E. prunastri*, and *E. togashiana*. *Erysiphe wadae* differs from the latter five species in its brown-colored appendages. *Erysiphe simulans* is most similar to *E. wadae*, but differs in its loosely uncinate appendage and smaller number of ascospores. The moderately high identity (92.3%) of rDNA ITS sequences between *E. simulans* and *E. wadae* supports their morphological similarity, and also confirms that *E. wadae* is an independent species.

Zheng and Chen (1979) reported the existence of two types of appendages, i.e., "normal" appendages and bristle-like secondary appendages arising from the upper part of the ascomata, in *Uncinula simulans* (≡*E. simulans*) on *Rosa* spp., and transferred the fungus to their newly raised genus *Uncinuliella*. Since then, several *Uncinula* species have been transferrred into the genus *Uncinuliella* based on this characteristic. However, Braun (1995) regarded the bristle-like secondary appendages as immature normal appendages, and reduced the genus *Uncinuliella* into a subgenus of *Uncinula*. Scanning electron microscopic examinations of the ontogeny of these appendages showed that the secondary appendages arise at the early stage of ascomatal formation together with hyphal-like appendages arising from the lower part of the ascomata (Takamatsu et al. 1979). The normal appendages begin elongating at the later stage when the ascomata grow to almost the full size of a matured one. Therefore, the bristle-like secondary appendages have an origin quite different from the normal appendages.

Molecular phylogenetic analysis based on the nucleotide sequences of rDNA indicated that the taxa having two types of appendages do not group into a clade, which supported the taxonomic treatment of Braun (1995; Mori et al. 2000). In the present study, we newly determined the nucleotide sequences of the rDNA ITS region for three species, including *E. wadae*, having two types of appendages and used them for phylogenetic analysis with other taxa of the genus *Erysiphe*. Again, the result indicates that the taxa do not group into a clade. Instead, they are mixed with taxa having a single type of appendages. The Kishino–Hasegawa test significantly rejected the monophyly of the taxa having two types of appendages. Therefore, the existence of bristle-like secondary appendages is a criterion of species level, but not of generic level.

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