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Erysiphe paracarpinicola: A new species of *Erysiphe* sect. *Uncinula* on *Carpinus cordata* (Betulaceae)

*Jamjan Meeboon, Susumu Takamatsu**

Department of Bioresources, Graduate School, Mie University, 1577 Kurima-Machiya, Tsu, Mie Prefecture 514-8507, Japan

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ABSTRACT

A phylogeny of *Erysiphe* sect. *Uncinula* on *Carpinus* spp. was reconstructed using the 28S rDNA sequences and a combined alignment of the 28S, ITS, and IGS rDNA sequences. The analysis was supplemented with morphological data obtained from examination of voucher specimens. A sequence of *Erysiphe* sect. *Uncinula* on *C. cordata* formed a distinct lineage separated from sequences of other *Erysiphe* species on *Carpinus* spp., indicating a cryptic species, which is described as *E. paracarpinicola*. The new species is genetically as well as morphologically most similar to *E. carpinicola* s. str., but differs in having fewer asci per chasmothecium (mainly 3–5 vs 4–10) and shorter chasmothecial appendages. A key to species of *Erysiphe* sect. *Uncinula* on *Carpinus* spp. is provided.

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1. Introduction

Carpinus L. (Betulaceae) is the largest genus in the subfamily Coryloideae with about 35 species distributed in Eastern Asia, Europe and North America (Yoo and Wen 2007). Nine species of powdery mildews have been recorded parasitizing various species of *Carpinus* trees (Braun 1987; Braun et al. 2006; Braun and Cook 2012), namely, *Phyllactinia carpini* (Rabenh.) Fuss, *Ph. carpinicola* U. Braun & S. Takam. *Erysiphe fimbriata* S. Takam. Masuya & Y. Nomura (sect. *Erysiphe*), *E. ellisii* (U. Braun) U. Braun & S. Takam. (sect. *Microsphaera*), and five species belonging to *Erysiphe* sect. *Uncinula*—*E. wuyiensis* (Z.X. Chen & R.X. Gao) U. Braun & S. Takam. *E. carpini-cordatae* (Tanda & Y. Nomura) U. Braun, *E. arcuata* U. Braun, V.P. Heluta & S. Takam. *E. carpinicola* (Hara) U. Braun & S. Takam. *E. carpini-laxiflorae* U. Braun, V.P. Heluta & S. Takam. Among them, species belonging to *E.* sect. *Uncinula* are mainly distributed in East Asia (Japan, China, Korea, and Russian Far East), and only *E. arcuata* is distributed in Europe as well as in Asia (Braun and Cook 2012).

The morphological descriptions and phylogenetic affinities among *E.* sect. *Uncinula* parasitizing *Carpinus* spp. were previously described by Braun et al. (2006). The fungus on *C. cordata* Blume was described as *E. carpini-cordatae*. However, a sequence from a specimen on *C. cordata* (MUMH207) differed from sequences of *E. carpini-cordatae* (similarity = 66.5%). This sequence differed from all other *Uncinula* species on *Carpinus* spp. as well and belonged to a clade comprising *E. carpinicola* and *E. carpini-laxiflorae* (Braun et al. 2006). However, the authors did not discuss the morphological characteristics of this fungus in detail, and maintained it as *Erysiphe* sp. The morphology of the herbarium specimen (MUMH207) collected in October 1996 has recently been re-examined, and nucleotide sequences of the intergenic spacer (IGS) region of rDNA

* *Corresponding author*. Tel.: +81 59 231 9497; fax: +81 59 231 9637.
E-mail address: takamatu@bio.mie-u.ac.jp (S. Takamatsu).
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for *Uncinula* species on *Carpinus* spp. have been determined to confirm the result of Braun et al. (2006). Our morphological examination and molecular phylogenetic analysis showed that the sequence is distinct from those of other closely related species of *E.* sect. *Uncinula* on *Carpinus* spp. Thus this fungus is described as a new species in this paper.

2. Materials and methods

2.1. Morphological examination

A voucher specimen of powdery mildew on *C. cordata* was obtained from Mie University Mycological Herbarium (MUMH 207), collected from Niigata Prefecture (Japan) in 1996, with the GenBank sequence code AB252464 derived from the same collection, which was also used by Braun et al. (2006). Additional specimens with the same location and collection date—MUMH 208, MUMH 297 and MUMH 183 – were also examined. The method of morphological examination refers to Meeboon and Takamatsu (2012). Holo- and isotype material of the new species are deposited at the National Museum of Nature and Science (TNS), Japan and Mie University Mycological Herbarium (MUMH), Japan.

2.2. DNA sequencing and phylogenetic analysis

Whole-cell DNA was extracted from chasmothecia using the chelex method (Walsh et al. 1991) as described in Hirata and Takamatsu (1996). The IGS region was amplified by polymerase chain reaction (PCR) using the primer pairs IGS-12A/NS1R (Carbone and Kohn 1999). KOD FX Neo DNA polymerase (Toyobo, Japan) was used in the PCR reaction according to the manufacturer's protocol. The amplicons of IGS were sent to SolGent Co. Ltd. (Daejeon, South Korea) for sequencing using the primer pairs IGS-12A and NS1R. Representative sequences determined in this study were deposited in the DNA Data Bank of Japan (DDBJ) under the accession numbers of AB731687–AB731694 (Table 1).

Two kinds of phylogenetic analyses, viz. 28S rDNA, and combined analysis of 28S, internal transcribed spacer (ITS), and IGS rDNA sequences, were performed in this study. In the 28S rDNA analysis, 41 sequences of the 28S rDNA sequences including the sequences of *Erysiphe* sect. *Uncinula* on *Carpinus* spp. retrieved from GenBank were aligned using MUSCLE (Edgar 2004) implemented in MEGA 5 (Tamura et al. 2011). This alignment was edited manually by eye. A sequence of *E. australiana* (McAlpine) U. Braun & S. Takam. was used as an outgroup. Maximum likelihood (ML) and neighbor joining (NJ) analyses were performed by MEGA 5 and maximum parsimony (MP) analysis was performed by PAUP* 4.0b10 (Swofford 2002). In the ML and NJ analyses, the best-fit evolution model for the alignment was chosen from the 24 alternative models by the Bayesian information criterion using MEGA 5. The Kimura 2-parameter (Kimura 1980) + G + I model was selected as the best evolution model to construct trees of the 28S rDNA. Partial deletion was set as gap/missing data treatment with site coverage cutoff was set at 95%. Nearest-Neighbor-Interchange (NNI) was selected for ML heuristic method and initial tree for ML was set automatically. MP analysis was done with the heuristic search option using the 'tree-bisection-reconstruction' (TBR) algorithm. All sites were treated as unordered and unweighted, with gaps treated as missing data. The strength of the internal branches of the resulting tree was tested with bootstrap analysis using 1000 replications (Felsenstein 1985) in all ML, NJ and MP analyses.

In the combined analysis, the partition homogeneity test (Farris et al. 1995) was conducted using PAUP* 4.0b10 (Swofford 2002) to determine whether the 28S, ITS, and IGS data sets were in conflict, with 100 replicates. Only ML analysis was performed for this data set by the same conditions described above using MEGA 5. Tamura 3-parameter (Tamura 1992) + G + I model was selected as the best evolution model for this data set and the tree was rooted with mid-point rooting method. The alignments used in this study were deposited in TreeBASE (http://www.treebase.org/) under the accession number S12900.

Shimodaira–Hasegawa test (Shimodaira and Hasegawa 1999) of the 28S rDNA tree was conducted to test the hypothesis that *Erysiphe* sect. *Uncinula* on *Carpinus* spp. are monophyletic. The constraint tree was constructed in MacClade version 4 (Maddison and Maddison 2000) and executed in PAUP* 4.0b10 (Swofford 2002).

3. Results

3.1. Taxonomy

Erysiphe paracarpinicola Meeboon & S. Takam., sp. nov. Figs. 3a–i, 4a–c.
MycoBank no.: MB800802

Table 1 – Sources of fungal material used for molecular analyses and DNA database accession numbers.

Host	Specimen no.	Location and year	Fungal species	Voucher no.	Accession no.	
					ITS+28S	IGS
Carpinus cordata	MUMH207	Niigata, Japan; 1996	*Erysiphe paracarpinicola*	KW30170	AB252464	AB731687
C. betulus	MUMH3197	Halle, Germany; 2004	*E. arcuata*	GLM53866, HAL1012F	AB252460	AB731688
C. betulus	MUMH3237	Saxony, Germany; 2004	*E. arcuata*	GLM53866, HAL1012F	AB252461	AB731689
C. cordata	MUMH3408	Sapporo, Japan; 2004	*E. carpini-cordatae*		AB252466	AB731690
C. japonica	MUMH243	Shiga, Japan; 1996	*E. carpinicola*	HAL1902F	AB252467	AB731691
C. japonica	MUMH3547	Gifu, Japan; 2004	*E. carpinicola*	KW30173	AB252468	AB731692
C. laxiflora	MUMH3503	Gifu, Japan; 2004	*E. carpini-laxiflorae*	KW30176	AB252470	AB731693
C. laxiflora	MUMH3640	Shiga, Japan; 2004	*E. carpini-axiflorae*	KW30179	AB252471	AB731694

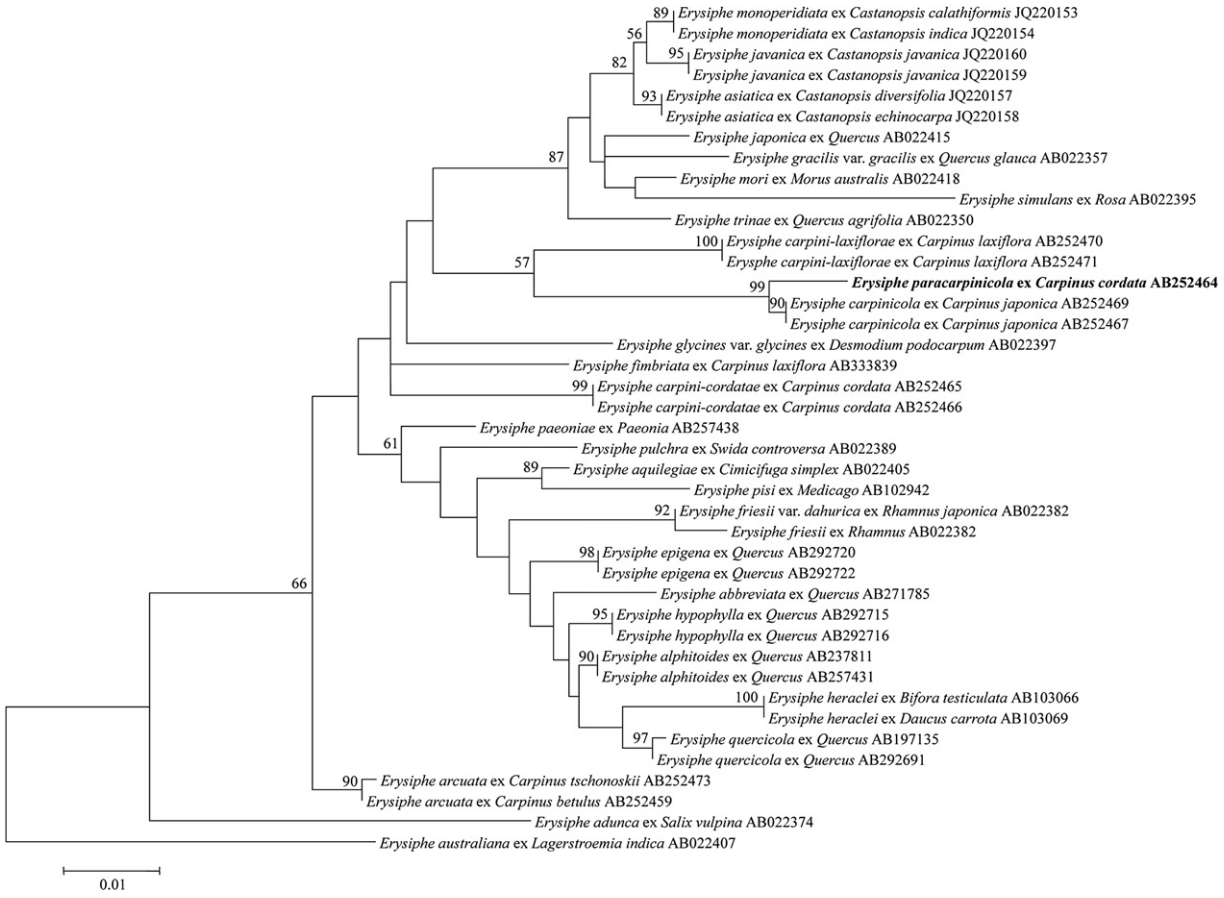

Fig. 1 – Phylogeny of *Erysiphe paracarpinicola* inferred from 28S rDNA sequences using the maximum likelihood method. The percentage bootstrap supports (1000 replications; ≥50%) are shown on the branches.

Similar to *Erysiphe carpinicola* s. str., but differing in having fewer asci (3–5 vs 4–10) per chasmothecium and shorter chasmothecial appendages (only up to 150 μm long vs 90–220 μm).

Type: on *Carpinus cordata* Blume (Betulaceae), Japan, Niigata Prefecture, Mt. Myojo, 19 Oct. 1996, S. Takamatsu (Holotypus, TNS-F-46914; Isotypus, MUMH 207). rDNA sequence ex-holotype: AB252464 (ITS+28S), AB731687 (IGS).

Etymology: The new species is named based on the similarity to *E. carpinicola*.

Mycelium amphigenous, forming patches or effuse, thin, grayish white, often conspicuous on the upper surface, inconspicuous below; hyphae 3–4 μm wide, hyaline, smooth, thin-walled; appressoria lobed; mother cells 13–35 × 3–4 μm; conidiophores (30–)38–76(–86) × (4–)4.5–6.5(–7) μm, erect, arising from the upper surface of the mother cells or usually laterally; foot-cells (7–)16.5–44(–50) × (3.5–)4.5–6(–6.5) μm, cylindrical, curved to sinuous at the base, followed by (1–)2 shorter cells, forming conidia singly; conidia (14–)18–21 × (5.5–)7–11 μm, cylindrical, doliiform, exhibiting microcylic conidiogenesis.

Chasmothecia (80–)82–107(–114) μm diam., amphigenous, mainly hypophyllous, scattered to gregarious, subglobose; peridium 7–10.5 μm thick, dark brown, multilayered,

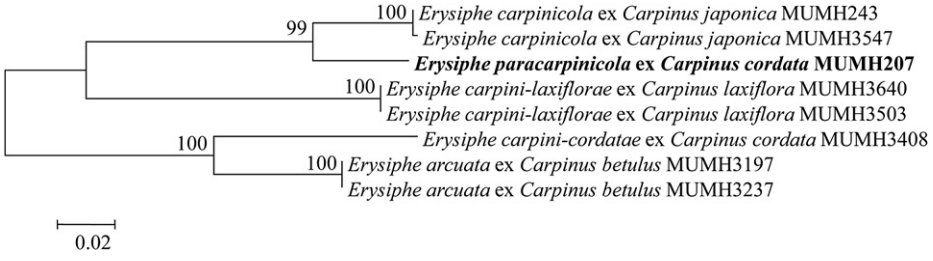

Fig. 2 – Phylogeny of *Erysiphe paracarpinicola* inferred from the 28S, ITS, and IGS ribosomal DNA sequences using the maximum likelihood method. The percentage bootstrap support (1000 replications; ≥50%) is shown on the branches.

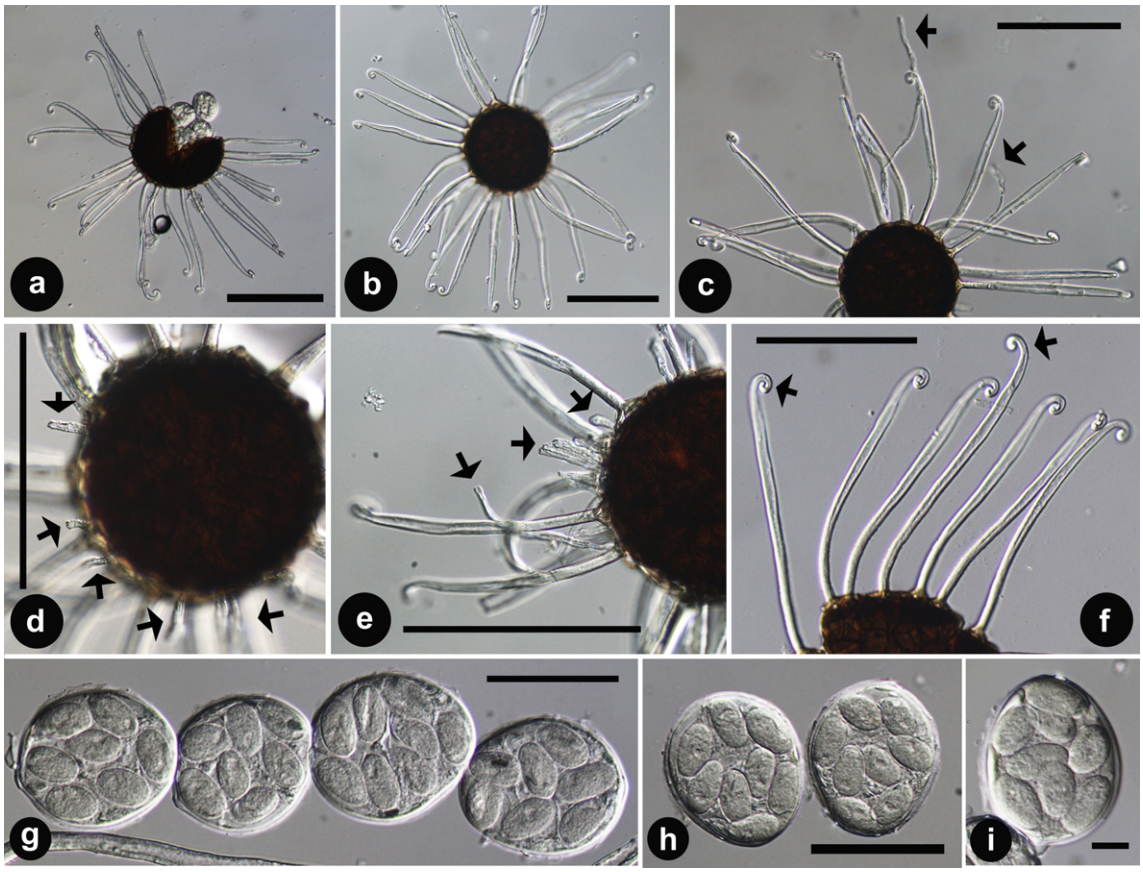

Fig. 3 – *Erysiphe paracarpinicola*. a, b: Chasmothecium. c: Appendages with anchor hyphae (arrows). d, e: Short secondary appendages and broken anchor hyphae (arrows). f: Appendages with uncinate–circinate tips. g, i: Asci and ascospores. *Bars* a–c = 100 μm; d, e = 105 μm; f = 75 μm; g, h = 50 μm; i = 10 μm.

composed of polygonal to rounded or irregularly shaped peridial cells; appendages 13–36, (95–)112–154(–157) × (4–)5.5–6.5(–8) μm, about 1–1.5(–2) times as long as the chasmothecial diam., rough from the base up to the middle, smooth toward the apex, hyaline, wall thick below, straight to curved, sometimes sinuous to slightly geniculate, becoming gradually wider toward the apex, but then narrower toward the very tip with uncinate to circinate, not enlarged apices; anchor hyphae (deviating short appendages in the upper half) present, 11–20 × 3–5 μm; asci usually 3–5 per chasmothecium, rarely 7–13, (40–)42–53(–55.5) × (31–)32.5–41.5(–46.5) μm, broadly ellipsoid to saccate, almost sessile to short-stalked, 7–8–spored; ascospores (15–)15.5–21.5(–24) × (8–)8.5–12(–13) μm, broadly ellipsoid–ovoid, hyaline.

3.2. *Phylogenetic analyses*

The alignment of the 28S rDNA consisted of 41 sequences and 815 total characters. *Erysiphe paracarpinicola* grouped with *E. carpinicola* on *C. japonica* with 99–100% bootstrap (BS) supports in all three constructing methods (Fig. 1; NJ and MP trees not shown). Then, the clade grouped with *E. carpini-laxiflorae* on *C. laxiflora* with 57–70% BS supports, suggesting that these three *Erysiphe* species on *Carpinus* spp. were derived from a common ancestor. However, the phylogenetic placement of this clade was not consistent among the three tree constructing methods. All ML, NJ and MP trees suggested that the five *Erysiphe* species on *Carpinus* spp. are not monophyletic. Especially, *E. arcuata* formed a lineage separated from other species on *Carpinus*. In order to clarify whether the *Erysiphe* species on *Carpinus* spp. are really polyphyletic, we conducted a Shimodaira–Hasegawa test with a null hypothesis that the *Carpinus* mildew species form a monophyletic group. This hypothesis was not rejected by the Shimodaira–Hasegawa test with *p*-value 0.200. Thus, the monophyly of the *Erysiphe* species on *Carpinus* should be re-evaluated using other DNA sequences.

Since the result of the partition homogeneity test showed no direct conflict among the 28S, ITS, and IGS ribosomal DNA sequences, we combined these data into a single dataset. From this analysis, eight sequences of *E.* sect. *Uncinula* on *Carpinus* spp. and 1817 total characters were used. We did not use an outgroup sequence in this analysis because we could not find an appropriate outgroup especially in IGS sequences due to too many substitutions in this DNA region. Alternatively, we used mid-point rooting option of MEGA 5 (Fig. 2). *Erysiphe paracarpinicola* was the sister to *E. carpinicola* on *C. japonica* and formed a monophyletic clade with 99% BS support, indicating a close phylogenetic relationship

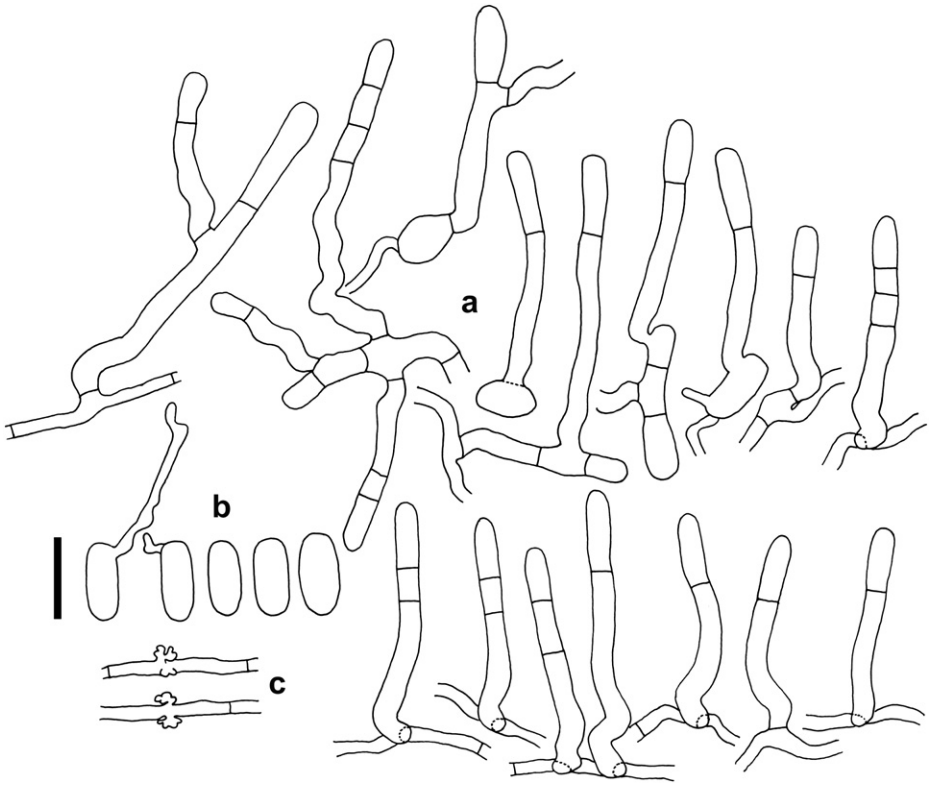

Fig. 4 – Anamorphic state of *Erysiphe paracarpinicola*. a: Conidiophores with microcyclic conidiogenesis. b: Conidia. c: Lobed appressoria. *Bar* = 20 μm.

between *E. paracarpinicola* and *E. carpinicola*. The ITS sequence pair-wise similarity between *E. paracarpinicola* to *E. carpinicola* and *E. carpini-laxiflorae* are 86.9% and 75.8%, respectively.

4. Discussion

The present phylogenetic analysis based on the 28S rDNA sequences and the combined alignment of 28S, ITS, and IGS rDNA sequences confirmed the report of Braun et al. (2006) and Heluta et al. (2009) that "*Erysiphe* sp. MUMH207" on *C. cordata* belongs to a species different from *E. carpini-cordatae* on the same host species. The presence of anchor hyphae on the chasmothecia showed that *E. paracarpinicola* belongs to subsect. *Uncinuliella* (R.Y. Zheng & G.Q. Chen) U. Braun of *E.* sect. *Uncinula*. Three other *Carpinus* powdery mildews, viz. *E. arcuata*, *E. carpinicola*, and *E. carpini-laxiflorae*, belong to this morphological group as well. *Erysiphe paracarpinicola* is phylogenetically closer to *E. carpinicola* s. str. rather than to other species of *E.* sect. *Uncinula* on *Carpinus* spp. (Figs. 1 and 2). *Erysiphe paracarpinicola* is also morphologically similar to *E. carpinicola* in many structures. The number of asci is usually 3–5 per chasmothecium in *E. paracarpinicola*, very rarely exceeding five asci, which differs from 4 to 10 asci in *E. carpinicola* (Braun et al. 2006). The chasmothecial appendages of *E. paracarpinicola* [(95–)112–154(–157) μm] are slightly shorter than those of *E. carpinicola* (90–220 μm). *Erysiphe carpini-laxiflorae* differs from *E. paracarpinicola* in having longer appendages (up to 300 μm) and the coiled apex with more or less constant to somewhat increasing width (Braun et al. 2006). The conidiophores in *E. paracarpinicola* are characteristically curved to sinuous at the base as in *E. carpini-laxiflorae*. Details of the structure of the conidiophores base in *E. carpinicola* and *E. carpini-cordatae* are not yet known. *Erysiphe arcuata* is characterized by having larger conidia (25–45 × 10–19 μm), usually straight conidiophores and longer chasmothecial appendages (up to 360 μm) that are mostly curved throughout (arched). This species is reported parasitizing *C. betulus* and *C. tschonoskii*, and being distributed in Asia and Europe (Braun et al. 2006). *Erysiphe carpini-cordatae* was reported to be found on the same host species and having a similar number of asci per chasmothecium compared with *E. paracarpinicola*. However, this species is distinct from *E. paracarpinicola* by lacking anchor hyphae. Both species are also phylogenetically distant from each other (Figs. 1 and 2). Based on the phylogenetic and morphological differences described above, the specimen MUMH207 on *C. cordata* is regarded as a new species.

During the examination of the anamorph, we found microcyclic conidiogenesis (MC) in *E. paracarpinicola* (Fig. 4). MC was defined by Hanlin (1994) as the germination of spores by the direct formation of conidia without the intervention of mycelial growth (secondary conidia formed directly from spores). This phenomenon was not reported for *E. carpinicola*, *E. carpini-laxiflorae*, *E. carpini-cordatae*, and *E. arcuata* (Braun and Cook 2012). However, the formation of MC is not new for powdery mildews. MC was found in several species of powdery mildews such as *E. necator* ex grapevine, *Podosphaera leucotricha* ex apple, *Golovinomyces orontii* ex tobacco, and *Neoërysiphe galeopsidis* ex *Lamium purpureum* (Pintye et al.

2011). Although the importance of MC in powdery mildews is still not clear, Kiss et al. (2010) noted that MC should be taken into consideration during species identification, because MC occurs in several phylogenetically different powdery mildews.

Key to the species of *Erysiphe* sect. *Uncinula* on *Carpinus* (modified from Braun et al. 2006; Braun and Cook 2012)

1) Appendages 6–12 times as long as the chasmothecial diam., apex uncinate, ultimate tips very short bi- to trifid, with recurved or uncinate branchlets; on *C. londoniana*, China..... ***E. wuyiensis***
1) Appendages shorter, 1–2.5 times the chasmothecial diam., apex simply circinate, bi- or trifid, very short, recurved or uncinate branchlets lacking (*E. carpinicola s. lat.*)..... **2**
2) Anchor hyphae (short, bristle-like "appendages") lacking, chasmothecia with few appendages (8–15); on *C. cordata* .....***E. carpini-cordatae***
2) Anchor hyphae (short, bristle-like "appendages") present, chasmothecia with numerous appendages (about 10–40) **3**
3) Conidia relatively large, 25–45 × 10–19 μm; chasmothecia with (6–)10–20(–25) appendages, up to 360 μm long, mostly curved throughout (arched), with a few anchor hyphae in the upper part, 7–12, asci 2–6-spored, ascospores 15–28 × 10–19 μm; on *C. betulus* and *C. tschonoskii*..... ***E. arcuata***
3) Conidia relatively small, 20–30 × 9–14 μm; chasmothecia with numerous appendages, 15–40, shorter, up to 300 μm, straight to flexuous, somewhat curved-sinuous, but not typically arched, anchor hyphae numerous, usually more than 15 (subsect. *Uncinuliella*), asci (4–)6–8-spored; ascospores smaller, 13–20 × 7–12 μm; on other hosts **4**
4) Chasmothecia with abundant anchor hyphae, appendages up to 300 μm long, width within the apical coil ± uniform to slightly increasing; on *C. laxiflora* .....***E. carpini-laxiflorae***
4) Appendages shorter than 300 μm, width within the apical coil slightly decreasing..... **5**
5) Chasmothecial appendages up to 220 μm, asci 4–10 per chasmothecium; on *C. japonica*, Japan .....***E. carpinicola***
5) Chasmothecial appendages shorter (up to 150 μm long), asci usually 3–5 per chasmothecium; on *C. cordata*, Japan .....***E. paracarpinicola***

Disclosure

The authors declare no conflict of interest. All the experiments undertaken in this study comply with the current laws of Japan.

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