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Short communication

Phyllactinia fraxinicola, another Asian fungal pathogen on *Fraxinus excelsior* (common ash) introduced to Europe?

Markus Scholler [a], Anke Schmidt [b], Jamjan Meeboon [c], Uwe Braun [d], Susumu Takamatsu [c, *]

[a] *Staatliches Museum T. Naturkunde, Abt. Biowissenschaften, Erbprinzenstr. 13, D-76133 Karlsruhe, Germany*
[b] *Holunderweg 2b, 23568 Lübeck, Germany*
[c] *Graduate School of Bioresources, Mie University, Tsu 514-8507, Japan*
[d] *Martin-Luther-Universität, Institut für Biologie, Bereich Geobotanik, Herbarium, Neuwerk 21, 06099 Halle (Saale), Germany*

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ABSTRACT

Since the mid-nineties *Phyllactinia fraxini* has become frequent in Germany. This species has hitherto been characterized by having straight conidiophore foot cells. However, we found that recent collections from Germany have conidiophores with sinuated and twisted foot cells. So far sinuated foot cells were only known from the related *P. fraxinicola*, another species with Eastern Asian origin. We thus hypothesized that recent collections from Germany belong to *P. fraxinicola* which might have been introduced to Europe. Using morphological and molecular rDNA data we found that no introduction took place and that there is only *P. fraxini* in Germany.

Fraxinus excelsior (common ash; Oleaceae) is a native tree of Europe with an eastern border at the Caucasus and the Alborz mountains (Iran). It is fast growing, widely cultivated, also outside the natural area of distribution such as the USA and New Zealand. It has outstanding wood properties and high economic value (e.g. Dobrowolska et al. 2011). The Asian ascomycete pathogenic to *F. excelsior*, *Hymenoscyphus fraxineus* (T. Kowalski) Baral et al. (= *Hymenoscyphus pseudoalbidus* Queloz et al.), was introduced to Europe before 1998 (Drenkhan, Riit, Adamson & Hanso, 2016). In Europe, it causes ash dieback characterized by leaf loss and crown dieback. It is closely related to the very similar saprotrophic *H. albidus* (Gillet) W. Phillips, a species native to Europe that does not cause economic ash disease losses. Today, *H. fraxineus* is much more common than *H. albidus* in Europe and the invasive species seems to have replaced the native one (Drenkhan et al. 2016). Similar to *Hymenoscyphus*, there are two related powdery mildews of the genus *Phyllactinia* on *Fraxinus*, one with almost holarctic [*P. fraxini* (DC.) Fuss] the other with eastern Asian (*P. fraxinicola* U. Braun & H.D. Shin) origin. There is no overlap of the host range between the two species (Braun and Cook 2012). An introduction of *P. fraxinicola* to Europe/North Africa or North America is not documented so far. Braun and Shin (in Braun and Cook 2012) showed clear morphological differences: *P. fraxini* has straight conidiophore foot cells whereas those of *P. fraxinicola* are sinuous-twisted. In addition, Takamatsu et al. (2008) showed clear differences between the two species in a molecular phylogenetic analysis using rDNA internal transcribed spacer (ITS) region.

In 2016, one of us (A. S.) studied the asexual morph of fresh German material of *Phyllactinia* on *F. excelsior*. The conidiophores clearly showed sinuous-twisted foot cells which characterize *P. fraxinicola*. A possible introduction of *P. fraxinicola* simultaneously to the introduction of *H. fraxineus* was also supported by an increase of the species reflected by a much higher number of specimens deposited in German public herbaria since the 90s. The herbarium of the natural history museum in Görlitz (GLM) contains most powdery mildew collections from Germany with continuous additions since the 70s. There are 70 specimens of *Phyllactinia* on *Fraxinus*, but none from before 1997 (U. Damm, curator of GLM, pers. comm.). Herbarium KR houses 23 specimens collected 1994 and after. Scholler (1996) noted *P. fraxini* for the state of Mecklenburg-Vorpommern (NE Germany) in 1991 and later on

* Corresponding author.
E-mail address: takamatu@bio.mie-u.ac.jp (S. Takamatsu).

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after decades of missing. An increasing incidence of infections of common ashes by *P. fraxini* has also been observed around Halle (Saale), including the first occurrence of this powdery mildew on *Wisteria sinensis* (Sims) Sweet in the Botanical Garden of Halle University (Takamatsu et al. 2008). For a long time, *P. fraxini* was considered a rare and endangered species in Germany (Foitzik 1996). We hypothesized that recent collections from Germany belong to *P. fraxinicola* which might have been introduced to Europe. In order to clarify this, we studied asexual morph features and rDNA sequence data of recent findings of *P. fraxini* from Germany and compared them with *P. fraxinicola*.

1. Morphology: description of the asexual morph (Fig. 1)

Specimens of German origin from 2016 were studied. For light microscopy asexual structures of fresh specimens (from KR) were examined in tap water mounts using an Olympus BH 2 (Hamburg) and Zeiss Axioskop 2 Plus (Oberkochen). Pertinent features were measured at magnifications of 400× and 1000× and documented by line drawings of fresh material. From each specimen up to 25 conidia were measured. To induce conidial germination, the method of Schmidt and Scholler (2002) was applied. Fresh conidia were spread on a microscope slide and placed in a Petri dish with moist cellulose tissue. The closed Petri dishes were incubated at room temperature and exposed to daylight through a north-facing window for 24 h.

Phyllactinia fraxini

Mycelium on leaves, hypophyllous, often around leaf veins, effuse, initially very thin, soon afterwards thicker and more conspicuous, whitish; hyphae hyaline, 4–6 μm wide. Hyphal appressoria mostly elongated and curved or branched, sometimes lobed or nipple-shaped. Conidiophores 85–240 μm long and 5–8 μm wide. Foot cells 60–145 μm long, mostly sinuous, twisted or spirally twisted, basal septum of the foot cell mostly raised above the junction with the hyphal mother cell, mostly centrally, rarely somewhat towards one end, followed by 1–3 shorter cells, conidia formed singly. Conidia mostly spatulate, sometimes clavate, apex rounded, base mostly rounded, rarely truncate, swelling in water, particularly in width, 54–91 × 16–25.5 μm, length/width ratio 2.4–4.9 (average 3.5), base of conidia 7–11 μm wide. Germ tubes subapically or subbasally, rarely apically inserted, mostly non-septate, usually shorter than length of conidia, 6–48 (−120) × 3.6–6 μm, terminating simply or in a lobed appressorium.

Specimens studied (fresh material): On *F. excelsior* L., GERMANY, Schleswig–Holstein, Lübeck, Karlshof, Forstmeisterweg/Glas-hüttenweg, 11 Aug 2016, leg. A. Schmidt (KR-M-0048247); Schleswig–Holstein, Ostholstein, Scharbeutz, spa gardens, 13 Aug 2016, leg. A. Schmidt (KR-M-0048244); Bayern, Kreis Weilheim-Schongau, Pähl, Hartschimmel, Moorkoppel, edge of forest, 14 Sep 2016, leg. M. Scholler (KR-M-0048605).

2. Phylogenetic analyses

PCR and DNA sequencing were conducted according to the procedures described in Takamatsu et al. (2016) except for primers used. *Phyllactinia* specific primer sets, viz. ITS5/Ph8 (5′-GCCCCAAGACCAAGCC-3′) and Ph7 (5′-TGTTGCTTTGGYAGGCCG-3′)/NLP2, were used to amplify 5′-half of the internal transcribed spacer (ITS) region (fragment 1), and 3′-half of ITS and 5′-end of the 28 S rRNA gene (including domains D1 and D2) (fragment 2), respectively. Ph8, Ph7, and NLP2 were used as sequence primers for fragments 1 and 2. The newly obtained sequences were deposited in DNA Database of Japan (DDBJ) with the accession numbers of LC307195–LC307202. These sequences were aligned with other sequences of *P. fraxini* and *P. fraxinicola* using MUSCLE (Edgar 2004) implemented in MEGA 6 (Tamura, Stecher, Peterson, Filipski & Kumar, 2013). Alignments were further manually refined using the MEGA6 program and were deposited in TreeBASE (http://www.treebase.org/) under the accession number S21263. Phylogenetic trees were obtained from the data using the maximum parsimony (MP) and maximum likelihood (ML) methods as described in Takamatsu et al. (2016).

Specimens sequenced: *Phyllactinia fraxini* on *F. excelsior*, GERMANY, Baden-Württemberg, Weikersheim, NSG Steinriegellandschaft, 2 Aug 2007, leg. M. Scholler (KR-M-0024918) (DDBJ ID: LC307198); Mecklenburg-Vorpommern, Neustrelitz, 15 Oct 1994, leg. M. Scholler (KR-M-0048243) (DDBJ ID: LC307199); Schleswig–Holstein, Ostholstein, Scharbeutz, spa gardens, 16 Oct 2016, leg. A. Schmidt (KR-M-0048246) (DDBJ ID: LC307200); Schleswig–Holstein, Lübeck, Karlshof, Forstmeisterweg/Glashüttenweg, 11 Aug 2016, leg. A. Schmidt (KR-M-0048247) (DDBJ ID: LC307201); On *F. ornus* L., GERMANY, Sachsen, Görlitz-Rauschwalde, Hilde-Coppi-Straße, near Rosa-Luxemburg-Straße, 15 Sep 2007, leg. S. Höflich (GLM-F080911) (DDBJ ID: LC307196); Sachsen, Görlitz-Innenstadt, Struwestraβe, Heilig-Kreuz-Kirche, opposite Villa Conti, 23 Sep 2008, leg. S. Höflich (GLM-F091208) (DDBJ ID: LC307197); On *F. pennsylvanica* Marshall, GERMANY, Sachsen-Anhalt, Dessau, Beckerbruch, 4 Nov 2001, leg. H. Jage (GLM-F056423) (DDBJ ID: LC307195); *P. fraxinicola* on *F. lanuginosa* Koidz. *F. serrata* (Nakai) Murata, JAPAN, Hokkaido, Chitose-shi, Shikotsu Lake, 4 Oct 2003, leg. S. Takamatsu (TSU-MUMH 2902) (DDBJ ID: LC307202).

The sequences determined in this study were aligned with 16 sequences of *Phyllactinia fraxini* and *P. fraxinicola* reported in Takamatsu et al. (2008). The data set consisted of 24 sequences and 1299 characters, of which 35 (2.7%) characters were variable and 31 (2.4%) characters were informative for parsimony analysis. MP analyses were done by two different gap treatments, i.e. treat gaps as missing data or 5th character. As a result, because both analyses generated similar tree topologies, a tree constructed by "gap = 5th character" setting was shown in Fig. 2. Because *P. fraxini/fraxinicola* group forms an isolated clade distantly related to other *Phyllactinia* species, we did not use outgroup taxa and assumed rooting point based on Takamatsu et al. (2008). ML analysis generated almost identical tree topology and only BS values were shown on MP tree (Fig. 2). All the seven sequences from the specimens collected in Germany belonged to the clade of *P. fraxini* with 100% BS supports in both MP and ML analyses. Six Asian "*P. fraxinicola*" sequences were divided into three distinct clades with strong BS supports, suggesting that "*P. fraxinicola*" is a species complex. The sequence from SMK10643 on *F. mandshurica* is an ex-type sequence. Thus, the group including this sequence could be *P. fraxinicola* s. str. Detailed morphological and molecular re-examinations are necessary for this group.

Our molecular phylogeny shows clear difference with high support for two species, *P. fraxinicola* species complex and *P. fraxini* (Fig. 2). The European specimens including one Iranian specimen all belong to *P. fraxini* whereas those from Japan and Korea belong to *P. fraxinicola* species complex. *Fraxinus excelsior* was proven as host of *P. fraxinicola* (KUS-F17216), showing that the host range is overlapping which is inconsistent with Braun and Cook (2012). In former European descriptions of *P. fraxini* conidiophores where not at all (e.g. Blumer 1967; Sałata 1985) or very briefly (Braun 1987; Piątek 2003) described. Braun and Cook (2012: 249) were the first to provide an illustration of *P. fraxini* showing straight conidiophores. We found that occasionally almost straight conidiophores are formed (Fig. 1A, left and right conidiophore). But in most cases conidiophore foot cells where sinuated or sinuated and twisted (Fig. 1A). The differences between Braun and Cook (2012)

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