

# *Erysiphe platani*: monitoring of an epidemic spread in Germany and molecular characterization based on rDNA sequence data

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## Abstract

This work deals in two sections with the North American plane powdery mildew *Erysiphe platani*, an epidemiological study and a molecular phylogenetic analysis based on rDNA ITS sequence data. Most likely, the species was introduced in South Europe at the beginning of the 1960s. In 2007, it was observed for the first time in Germany near Freiburg (SW Germany) and obviously did not reach other German states until 2009. A detailed monitoring from 2009 to 2011 shows that the fungus continually spread north- and northeastward with a speed of roughly 190 km/year. The northernmost record is from Arendsee in the north of Sachsen-Anhalt from 2011. We assume that the species has come from the Rhone valley and the Burgundian Gate finally entering Germany in the Upper Rhine plain.

The molecular phylogenetic analyses of material of different geographic origins indicate that specimens from Germany and Italy are identical, differ slightly from those from Greece and strongly from extra-European (Australia, USA) material. This might indicate a considerable rate of mutation of this powdery mildew with North American origin in the new European area. In addition, the phylogenetic analyses confirm that *E. platani* is related to other tree-inhabiting powdery mildew species previously accommodated in the genus *Microsphaera*.

## Kurzfassung

### *Erysiphe platani*: Monitoring einer epidemischen Ausbreitung in Deutschland und molekulare Charakterisierung basierend auf rDNS-Sequenzdaten

Die vorliegende Arbeit fasst die Ergebnisse zweier Untersuchungen des aus Nordamerika stammenden Echten Mehltaupilzes der Platane, *Erysiphe platani*, zusammen: Eine epidemiologische Untersuchung und eine Sequenzanalyse von rDNS ITS-Sequenzen. Die vermutlich Anfang der 1960er Jahre nach Südeuropa eingewanderte Art wurde 2007 erstmals in Deutschland bei Freiburg (SW-Deutschland) nachgewiesen. Für den Zeitraum von August 2009 bis 2011 wird die epidemische Ausbreitung der Art innerhalb Deutschlands dokumentiert. Von 2007 bis 2009 breitete sich *Erysiphe platani* nur langsam aus und erreichte vermutlich noch nicht die anderen Bundesländer. Danach beschleunigte sich die Ausbreitung auf rund 190 km/Jahr und die Art breitete sich kontinuierlich nord- und nordostwärts aus. Der nördlichste Nachweis von 2011

ist Arendsee im nördlichen Sachsen-Anhalt. Vermutet wird, dass die Art über das Rhonetal und die Burgundische Pforte in die Oberrheinebene nach Deutschland eingedrungen ist.

Die Sequenzanalyse zeigt, dass Material aus Deutschland und Italien identisch ist und sich von Material aus Griechenland leicht und deutlich von außereuropäischem Material (Australien, USA) unterscheidet. Dies deutet auf eine erhebliche Mutationsrate im neuen europäischen Areal hin. Die phylogenetische Auswertung der Sequenzdaten bestätigt, dass *Erysiphe platani* mit anderen auf Gehölzen vorkommenden *Erysiphe*-Arten verwandt ist, die genau wie *E. platani* früher zur Gattung *Microsphaera* gestellt wurden.

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## 1 Introduction

Powdery mildews (Erysiphales, Ascomycota) are obligate plant-parasitic microfungi predominantly on dicots forming white conspicuous “powdery” conidial layers on plant surfaces, predominantly on leaves, which are often deformed (Fig. 1). Fruitbodies are black, globose, without opening (called cleistothecia or chasmothecia) and with appendages. Conidia, ascospores and fruitbodies may be dispersed by the wind, conidia in highest quantities (e.g. BLUMER 1967). In an unpublished inventory, SCHOLLER et al. (2011) list 141 powdery mildew species for Germany. Fifty species, i.e. more than 1/3 of all species, are introduced (so-called neomyces and ephemeromyces; KREISEL & SCHOLLER 1994) with a culmination of introductions in the past two decades. Interestingly, many species have woody host plants. One of the most recently introduced species in Germany is the plane powdery mildew *Erysiphe pla-*



Figure 1. The anamorph of the Plane Powdery Mildew *Erysiphe platani* on deformed leaves of *Platanus acerifolia* in Karlsruhe, Main Railway Station, August 27, 2009. – Photograph: M. SCHOLLER.

*tani* (HOWE) U. BRAUN & S. TAKAM. (= *Microsphaera platani* HOWE) with North American origin. The species was recorded for the first time in Europe by CIFERRI & CAMERA (1962) on *Platanus orientalis* in Italy. However, the authors did not provide any record data. Later GULLINO & RAPETTI (1978) found the species in Liguria in Italy and documented its spread toward the French Mediterranean coast. In the following years the species spread throughout the warm regions of the Mediterranean countries, southern Switzerland, the mild atlantic France, and southern UK but not to Central or Eastern Europe with increasingly continental

climates (ANSELMINI et al. 1994)<sup>1</sup>. *Erysiphe platani* was reported on the host plants *Platanus occidentalis* L., *P. orientalis* L., and the hybrid *P. acerifolia* (AITON) WILLD. forming the anamorph only. In the beginning of the 1990s the species appeared in south and southwestern Switzerland near lake Geneva (BOLAY 2005) indicating that the species is spreading northward and may be expected in Germany as well. Although there are several “first record” publications from some countries with morphological descriptions in Europe, little is known about the details of the spreading mode, e.g. speed of expansion and if progressing over long or short distances. This lack of information does not concern only *Erysiphe platani*, but almost all other powdery mildew species introduced to Europe. In the following we present the results of a monitoring of the epidemic spread of *E. platani* in Germany. In addition to this, molecular phylogenetic analyses were carried out

<sup>1</sup> In their distribution map ANSELMINI et al. (1994: 165, Fig. 3) report of isolated records of *Erysiphe platani* in Southern Sweden. Since the fungus was not observed in neighboring countries we wanted to get this confirmed and asked the authors directly, but did not get any response. The Swedish Erysiphales expert LENA JONSELL could not confirm the occurrence of *E. platani* in Sweden.

in order to elucidate the phylogenetic placement and compare sequence data from specimens of different geographic origin.

## 2 Materials and methods

### 2.1 Documentation of the epidemic spread in Germany

After finding *Erysiphe platani* at a parking lot in Tul (Lorraine, France) in 2008 the first author looked methodically for this fungus in the Karlsruhe region (Baden-Württemberg, Germany). After finding it in August 2009 a call to mycologists and botanists was started in September 2009 to look for this fungus and help to document its spread in Germany. Collaborators were asked via email to provide both positive and negative record data and voucher specimens. They were regularly informed about the present distribution of the fungus in order to look for it where it was still missing. Voucher specimens were all checked by the first author and finally deposited in the fungus herbarium of the Natural History Museum in Karlsruhe (KR). A few specimens are also deposited in the fungus herbarium in Görlitz (GLM). All specimen data including information on anamorph and teleomorph formation, hyperparasites etc. will be available via *diversity collection* and *IMDAS* online databases. The following is a list of collaborators (authors not mentioned): HERBERT BOYLE, ADRIEN BOLAY, UWE BRAUN, WALTER GAMS, PATRICK DORNES, HORST JAGE, ROLAND KIRSCHNER, FRIEDEMANN KLENKE, HANNS KREISEL, JULIA KRUSE, VOLKER KUMMER, DIRK MATALLA, BERTOLD METZLER, BERND OERTEL, HARALD OSTROW, MARÇIN PIATEK, UDO RICHTER, HARRY REGIN, ANNEMARIE RUBNER, ANKE SCHMIDT, MARTIN SCHNITTLER, DIETRICH SCHOLLER, KATHARINA SCHOLLER, MANFRED SCHUBERT, LEOPOLD SCHRIMPL, HORST STAUB, and HJALMAR THIEL.

### 2.2 Molecular analyses

#### DNA extraction, PCR, and sequencing

The specimens examined in the course of this study are listed in Table 1. The voucher specimens are deposited in KR. Genomic DNA was isolated directly from the herbarium specimens. For methods of isolation and crushing of fungal material, DNA extraction, amplification, purification of PCR products, sequencing, and processing of the raw data see LUTZ et al. (2004). ITS 1 and ITS 2 regions of the rDNA including the 5.8S rDNA (ITS) were amplified using the primer pair

ITS1-F (GARDES & BRUNS 1993) and ITS4 (WHITE et al. 1990). The 5'-end of the nuclear large subunit ribosomal DNA (LSU) was amplified using the primer pair NL1 and NL4 (O'DONNELL 1993). Primers were used for both PCR and cycle sequencing. For amplification the annealing temperature was adjusted to 45° C. DNA sequences determined in this study were deposited in GenBank. GenBank accession numbers are given in Fig. 2 and Table 1.

#### Phylogenetic analyses

The *Erysiphe* specimens examined in this study are listed in Table 1. For molecular phylogenetic analyses the following ITS sequences from GenBank were additionally used (ATTANAYAKE et al. 2010; BRAUN et al. 2006; COOK et al. 2004, 2006; CUNNINGTON et al. 2003; FRANCIS et al. 2007; HELUTA et al. 2009; HIRATA & TAKAMATSU 1996; KHODAPARAST et al. 2003; KIRSCHNER 2010; KOVACS et al. 2011; LEE et al. 2011; LIMKAIKANG et al. 2006; MORI et al. 2000; SAENZ & TAYLOR 1999; SEKO et al. 2011; SHIROYA & TAKAMATSU 2009; STANOSZ et al. 2009; and TAKAMATSU et al. 1998, 1999, 2006, 2007, 2008): *Erysiphe abbreviata* (PECK) U. BRAUN & S. TAKAM. AB271785, *E. adunca* (WALLR.) FR. var. *adunca* D84382, *E. alphitoides* (GRIFFON & MAUBL.) U. BRAUN & S. TAKAM. AB292710, *E. aquilegiae* DC. HQ286643, *E. arcuata* U. BRAUN, V. P. HELUTA & S. TAKAM. AB252462, *E. astragali* DC. AB104515, *E. baeumleri* (MAGNUS) U. BRAUN & S. TAKAM. AB015933, *E. betae* (VAÑHA) WELTZIEN DQ164436, *E. buhrii* U. BRAUN AB128924, *E. carpinicola* (HARA) U. BRAUN & S. TAKAM. AB252469, *E. carpini-laxiflorae* U. BRAUN, V. P. HELUTA & S. TAKAM. AB252470, *E. catalpae* SIMONYAN DQ359695, *E. circaeae* L. JUNELL AB104517, *E. clandestina* BIV. AB475115, *E. convolvuli* DC. AF011298, *E. corylopsidis* SHIROYA & S. TAKAM. AB478990, *E. cruciferarum* OPIZ EX L. JUNELL EU140958, *E. densa* BERK. DQ005439, *E. deutziae* (BUNKINA) U. BRAUN & S. TAKAM. GU196146, *E. elevata* (BURRILL) U. BRAUN & S. TAKAM. AY587013, *E. euonymi-japonici* (VIENN.-BOURG.) U. BRAUN & S. TAKAM. AB250228, *E. fimbriata* S. TAKAM., MASUYA & Y. NOMURA AB333839, *E. friesii* var. *dahurica* (U. BRAUN) U. BRAUN & S. TAKAM. AB000939; *E. glycines* F. L. TAI var. *glycines* AB015934, *E. glycines* var. *lespedezae* (R. Y. ZHENG & U. BRAUN) U. BRAUN & R. Y. ZHENG AB015923, *E. helwingiae* (SAWADA) U. BRAUN & S. TAKAM. AB015916, *E. heraclei* DC. AB104510, *E. howeana* U. BRAUN AF011301, *E. huayinensis* R. Y. ZHENG & G. Q. CHEN AB015914, *E. hypogena* S. TAKAM. &

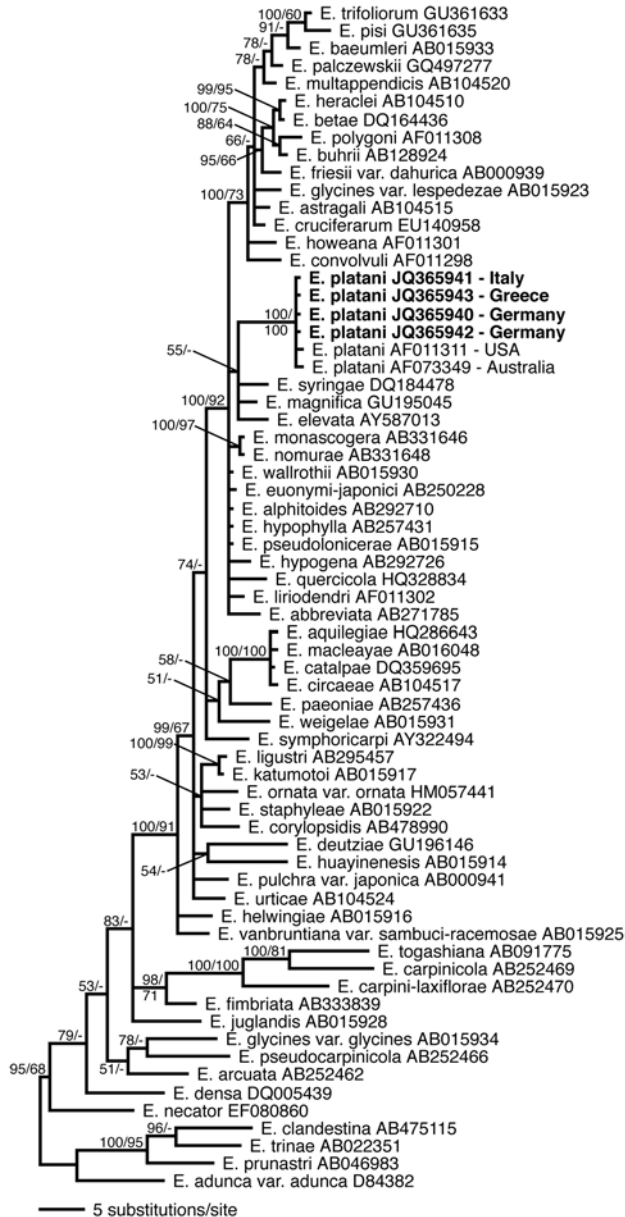


Figure 2. Bayesian inference of phylogenetic relationships within the sampled *Erysiphe* species: Markov chain Monte Carlo analysis of an alignment of ITS base sequences using the GTR+I+G model of DNA substitution with gamma-distributed substitution rates and estimation of invariant sites, random starting trees and default starting parameters of the DNA substitution model. A 50% majority-rule consensus tree is shown computed from 45.000 trees that were sampled after the process had reached stationarity. The topology was rooted with *Erysiphe adunca* var. *adunca*, *E. clandestina*, *E. prunastri*, and *E. trinae*. Numbers on branches before slashes are estimates for a *posteriori* probabilities, numbers after slashes are ML bootstrap support values. Branch lengths were averaged over the sampled trees. They are scaled in terms of expected numbers of nucleotide substitutions per site. Specimens examined in this study are printed in bold. E. = *Erysiphe*.

Table 1. List of *Erysiphe platani* specimens examined in the course of this study with host plants, GenBank accession numbers, and reference specimens.

<i>Platanus</i> Host	GenBank acc. no. (ITS/LSU)	Reference specimens <sup>1</sup>
<i>P. acerifolia</i> (AITON) WILLD.	JQ365940/JQ365936	Germany, Baden-Württemberg, Tübingen, Herrenberger Straße, 21.08.2010, leg. M. LUTZ, KR26134.
<i>P. acerifolia</i>	JQ365941/JQ365937	Italy, Toscana, Livorno, Isola d'Elba, Capoliveri, 30.06.2011, leg. M. LUTZ, KR27975
<i>P. acerifolia</i>	JQ365942/JQ365938	Germany, Baden-Württemberg, Denzlingen, 22.10.2007, leg. B. METZLER, KR26434
<i>P. orientalis</i> L.	JQ365943/JQ365939	Greece, Évia, Karystos, Palaia Styra (Old Styra), 24.08.2011, leg. M. SCHOLLER, KR29265

<sup>1</sup> KR – Fungus collections of the herbarium of Staatliches Museum für Naturkunde, Karlsruhe, Germany.

U. BRAUN AB292726, *E. hypophylla* (NEVOD.) U. BRAUN & CUNNINGT. AB257431, *E. juglandis* (GOLOVIN) U. BRAUN & S. TAKAM. AB015928, *E. katumotoi* (U. BRAUN) U. BRAUN & S. TAKAM. AB015917, *E. ligustri* (HOMMA) U. BRAUN & S. TAKAM. AB295457, *E. liriodendri* SCHWEIN. AF011302, *E. macleayae* R. Y. ZHENG & G. Q. CHEN AB016048, *E. magnifica* (U. BRAUN) U. BRAUN & S. TAKAM. GU195045, *E. monascigera* SHIROYA, C. NAKASH. & S. TAKAM. AB331646, *E. multappendicis* (Z.Y. ZHAO & Y.N. YU) U. BRAUN & S. TAKAM. AB104520, *E. necator* SCHWEIN. EF080860, *E. nomurae* (U. BRAUN) U. BRAUN AB331648, *E. ornata* (U. BRAUN) U. BRAUN & S. TAKAM. var. *ornata* HM057441, *E. paeoniae* R. Y. ZHENG & G. Q. CHEN AB257436, *E. palczewskii* (JACZ.) U. BRAUN & S. TAKAM. GQ497277, *E. pisi* DC. GU361635, *E. platani* (HOWE) U. BRAUN & S. TAKAM. AF011311, AF073349, *E. polygoni* DC. AF011308, *E. prunastri* DC. AB046983, *E. pseudocarpinicola* (Y. NOMURA & TANDA) U. BRAUN & S. TAKAM. AB252466, *E. pseudoloniceriae* (E. S. SALMON) U. BRAUN & S. TAKAM. AB015915, *E. pulchra* var. *japonica* (P. HENN) U. BRAUN AB000941, *E. quercicola* S. TAKAM. & U. BRAUN HQ328834, *E. staphyleae* (SAWADA) U. BRAUN & S. TAKAM. AB015922, *E. symphoricarpi* (HOWE) U. BRAUN & S. TAKAM. AY322494, *E. syringae* SCHWEIN. DQ184478, *E. togashiana* (U. BRAUN) U. BRAUN & S. TAKAM. AB091775, *E. trifoliorum* (WALLR.) U. BRAUN GU361633, *E. triniae* HARKN. AB022351; *E. urticae* (WALLR.) S. BLUMER AB104524, *E. vanbruntiana* var. *sambuci-racemosae* (U. BRAUN) U. BRAUN & S. TAKAM. AB015925, *E. wallrothii* (U. BRAUN) U. BRAUN & S. TAKAM. AB015930, *E. wei-*

*geliae* Z. X. CHEN & S. B. LUO AB015931.

To elucidate the phylogenetic position of *Erysiphe platani* ITS sequences were analysed within a dataset covering all *Erysiphe* species of which sequences were available in GenBank. Sequence alignment was obtained using MAFFT 6.853 (KATO H et al. 2002, 2005, KATO H & TOH 2008) using the L-INS-i option. To obtain reproducible results, manipulation of the alignment by hand as well as manual exclusion of ambiguous sites were avoided as suggested by GIRIBET & WHEELER (1999) and GATESY et al. (1993), respectively. Highly divergent portions of the alignment were omitted using GBlocks 0.91b (CASTRESANA 2000) with the following options: 'Minimum Number of Sequences for a Conserved Position' to 34, 'Minimum Number of Sequences for a Flank Position' to 34, 'Maximum Number of Contiguous Non-conserved Positions' to 8, 'Minimum Length of a Block' to 5 and 'Allowed Gap Positions' to 'With half'.

The resulting alignment [new number of positions: 526 (31% of the original 1645 positions) number of variable sites: 213] was used for phylogenetic analyses using a Bayesian Approach (BA) and Maximum Likelihood (ML). For BA a phylogenetic inference using a Markov chain Monte Carlo technique was used as implemented in the computer program MrBayes 3.1.2 (HUELSENBECK & RONQUIST 2001, RONQUIST & HUELSENBECK 2003). Four incrementally heated simultaneous Markov chains were run over 5 000 000 generations using the general time-reversible model of DNA substitution with gamma-distributed substitution rates and estimation of invariant sites, random

starting trees and default starting parameters of the DNA substitution model as recommended by HUELSENBECK & RANNALA (2004). Trees were sampled every 100<sup>th</sup> generation, resulting in an overall sampling of 50 001 trees. From these, the first 5 001 trees were discarded (burnin = 5 001). The trees sampled after the process had reached stationarity (45 000 trees) were used to compute a 50 % majority rule consensus tree to obtain estimates for the *a posteriori* probabilities of groups of species. This Bayesian approach to phylogenetic analysis was repeated five times to test the independence of the results from topological priors (HUELSENBECK et al. 2002).

Maximum likelihood analysis (FELSENSTEIN 1981) was conducted with the RAxML 7.2.6 software (STAMATAKIS 2006), using raxmlGUI (SILVESTRO & MICHALAK 2010), invoking the GTRCAT and the rapid bootstrap option (STAMATAKIS et al. 2008) with 1000 replicates.

In line with the results of molecular analyses of a sampling that covered both all *Erysiphe* ITS sequences available in GenBank and representatives of all erysiphalean genera of which sequences were available in GenBank, trees were rooted with *E. adunca* var. *adunca*, *E. clandestina*, *E. prunastri*, and *E. trinae*.

### 3 Results

#### Spread of *Erysiphe platani* in Germany

After starting the public monitoring in September 2009 an earlier German record from 2007 was reported by one of the collaborators (Baden-Württemberg, Kr. Emmendingen, Denzlingen, Hauptstr., 22.10.2007, leg. B. METZLER, teleomorph and anamorph with hyperparasite *Ampelomyces quisqualis* CES., voucher specimen KR26434). The methodical documentation of the distribution and spread from September 2009 to December 2011 showed that the fungus was heading continuously north(east)ward. The northernmost record in 2011, Sep. 17 is from Arendsee in northern Sachsen-Anhalt (ca. 52.8° latitude). The fungus progressed northward for approximately 375 km in about two years, i.e. almost 190 km/year (Figs. 3a-d). All records were found on London plane (*Platanus acerifolia*).

#### Phylogenetic analyses

The ITS sequences of the *Erysiphe platani* specimens KR26134, KR26434, and KR27975 were identical, the ITS of KR29265 differed in

one bp from the others. Considering the two *Erysiphe platani* sequences available in GenBank AF011311 differed in five bp in four loci, from the above three specimens and in four bp in three loci from KR29265; AF073349 differed in one bp from KR26134, KR26434, and KR27975. LSU sequences were identical.

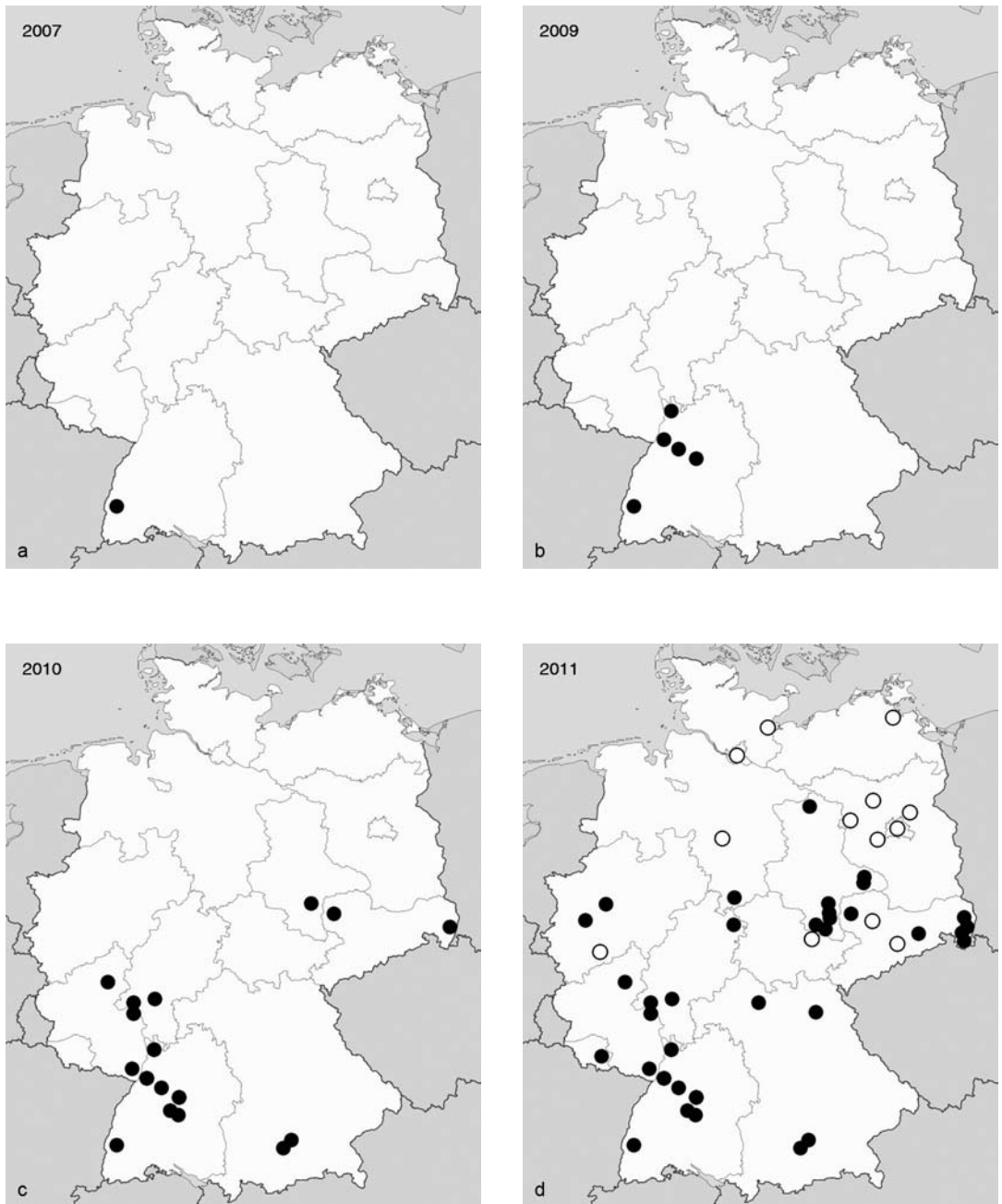
The different runs of BA performed were congruent with the results of the ML analysis in respect to well-supported branchings (ML bootstrap support values greater than 58). To illustrate the results, the consensus tree of one run of the Bayesian phylogenetic analyses is presented (Fig. 2). Estimates for *a posteriori* probabilities are indicated on branches before slashes, numbers on branches after slashes are ML bootstrap support values.

In all analyses the *Erysiphe platani* specimens formed a well-supported clade that clustered within *Erysiphe*. The subgrouping of *Erysiphe platani* with *E. elevata*, *E. magnifica*, and *E. syringae* received only weak support.

### 4 Discussion

#### Epidemic spread

The species was found for the first time in SW Germany in the Upper Rhine Plain near Freiburg. Together with the Upper Rhone valley/Saône valley and the Belfort Gap (Burgundian Gate), the Upper Rhine Plain forms a passage that allows warm Mediterranean flows to advect northeastwards into Germany. Numerous authors think that a great part of southern plant and animal species entered the Rhine plain via this passage (e.g. NÄHRIG & HARMS 2003, LENZIN et al. 2011). This may also count for fungi such as the octopus stinkhorn *Clathrus archeri* (BERK.) DRING (STRICKER 1955) and the Asteraceae rust *Puccinia lagenophorae* COOKE (SCHOLLER 1996), both species native of Australia, or the vine white rot fungus *Fomitiporia mediterranea* M. FISCHER (see the contribution by M. FISCHER in this issue). So did *Erysiphe platani*, most probably. This theory is not only supported because of the first German record in the Upper Rhine Plain. It is also supported by two additional observations: First, the fungus has been widespread in the Rhone valley around the lake Geneva before 2007 (BOLAY 2005, FISCHER HUELIN et al. 2008) and secondly, there were no records from other German states until 2009 such as Bayern with its eastern passage, the Danube valley.



We can hardly reconstruct the spread of the fungus in Germany between 2007 and August 2009. We assume, however, that the species has spread rather slowly and may have reached only a major part of Baden-Württemberg in September 2009. From 2009 onward, however, the spread of *E. platani* could be documented in much detail and probably better than in any other fungal species before in Germany. In 2009 we traced it in northern Baden-Württemberg (Mannheim) and in central Baden-Württemberg (Stuttgart), but not in the south at Lake Constance. Also, the species could not be recorded in the neighboring states of Bayern, Hessen and Rheinland-Pfalz the same year. Negative records were also sent from Nordrhein-Westfalen, Saarland and Sachsen-Anhalt. So most probably, the species had not reached states other than Baden-Württemberg in 2009.

From this point, the monitoring shown in Fig. 3 b-d documents the spread of this fungus in Germany. Considering the northernmost records in 2009 (Mannheim, Baden-Württemberg) and in 2011 (Arendsee, Sachsen-Anhalt) the fungus progressed approx. 190 km/year, i.e. much faster than in the previous years. Given, that the fungus was introduced in SW Germany and wandered also northward to Sachsen-Anhalt, the spread is even 215 km/year. For powdery mildews with woody plant hosts, this fast progressing is not exceptional. We know from powdery mildew epidemic spreads of the 19<sup>th</sup> and the first part of the 20<sup>th</sup> century (see compilation and description in BLUMER 1967) and more recently introduced species such as the elder powdery mildew *Erysiphe vanbruntiana* var. *sambuci-racemosae* (U. BRAUN) U. BRAUN & S. TAKAM. (SCHOLLER 1996 as *Microsphaera sambucicola* Henn.) that other powdery mildews may progress even faster.

KIRSCHNER (2011) provided two possible explanations for the sudden migration northward: Firstly, the global warming as it is assumed for many other migrating southern fungal species (e.g. SCHOLLER & MÜLLER 2008) and secondly, the formation of fruitbodies that are better adapted to overwintering than mycelium or conidia. They were first recorded between 2000 and 2002 in Montenegro (RANKOVIĆ 2003), in 2006 in Hungary (PASTIRČÁKOVÁ & PASTIRČÁK 2008) and finally 2007 in Switzerland (FISCHER et al. 2008) and in Germany. So migrating northward goes along with the formation of the sexual state. We agree with this and considering our results we expect this species to appear in the northernmost European plane plantations within the next years.

### Phylogenetic placement

Molecular phylogenetic analyses of the ITS clearly place *Erysiphe platani* within *Erysiphe*, however, they do neither resolve well the placement of *E. platani* within *Erysiphe* nor reveal any groups of species that can be correlated with morphological traits or relatedness of host plants.

Fig. 2 shows *E. platani* forming a subclade together with three other *Erysiphe* spp. (*E. elevata*, *E. magnifica*, and *E. syringae*) on woody plants of three different families formerly placed in the genus *Microsphaera*. This subclade, however, received only very low support. Many *Erysiphe* species have recurved dichotomous branchings at the end of the fruitbody appendages, so do all species of this subclade. However, species previously accommodated in *Microsphaera* (see BRAUN & TAKAMATSU 2000) and/or having woody plant hosts also occur in other clades. This has already been found by previous authors (e.g. TAKAMATSU et al. 1999).

Analyses of ITS sequence data may not be suitable for resolving the phylogeny of *Erysiphe*. However, the analyses including specimens from distant places in Europe, the USA (SAENZ & TAYLOR 1999), and Australia (CUNNINGTON et al. 2003) show that *Erysiphe platani* can clearly be distinguished by its ITS sequence from other – in several cases – morphologically similar *Erysiphe* species (e.g. *E. alphitoides*, *E. magnifica*, *E. ornata*, *E. syringae*, *E. wallrothii*). That is in line with SCHOCH et al. (2012) who proposed the ITS as universal DNA barcode marker for fungi.

The genetic distance of *Erysiphe platani* to its closest relatives of which ITS sequences are available is considerable. That may be explained by a high phylogenetic age of the species, a high mutation rate or simply by missing data of the closest relatives.

The relatively high variability of the ITS of specimens from different geographic regions (up to five bp in four loci) points at the potential of the ITS as marker in mycogeographical studies of spreading powdery mildews.

### Acknowledgements

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## References

- ANSELMINI, N., CARDIN, L. & NICOLOTTI, G. (1994): Plane decline in European and Mediterranean countries: associated pests and their interactions. – Bulletin OEPP / EPPO Bulletin, **24**: 159-171.
- ATTANAYAKE, R. N., GLAWE, D. A., MCPHEE, K. E., DUGAN, F. M. & CHEN, W. (2010) *Erysiphe trifolii* - a newly recognized powdery mildew pathogen of pea. – Plant Pathol., **59**: 712-720.
- BLUMER, S. (1967): Echte Mehltupilze. Ein Bestimmungsbuch für die in Mitteleuropa vorkommenden Arten. – Jena (G. Fischer).
- BOLAY, A. (2005): Les Oïdiums de Suisse (Erysiphacées). – Cryptogamica Helvetica, **20**: 1-176.
- BRAUN, U. & TAKAMATSU, S. (2000): Phylogeny of *Erysiphe*, *Microsphaera*, *Uncinula* (Erysiphaceae) and *Cystotheca*, *Podosphaera*, *Sphaerotheca* (Cystothecaceae) inferred from rDNA ITS sequences – some taxonomic consequences. – Schlechtendalia, **4**: 1-33.
- BRAUN, U., TAKAMATSU, S., HELUTA, V., LIMKAISANG, S., DIVARANGKON, R., COOK, R. & BOYLE, H. (2006): Phylogeny and taxonomy of powdery mildew fungi of *Erysiphe* sect. *Uncinula* on *Carpinus* species. – Mycol. Prog., **5**: 139-153.
- CASTRESANA, J. (2000): Selection of conserved blocks from multiple alignments for their use in phylogenetic analysis. – Molecular Biology and Evolution, **17**: 540-552.
- CIFERRI R. & CAMERA, C. (1962): Tentativo di elencazione dei funghi italiani. – Istituto Botanico della Università Laboratorio crittogamico, **21**: 1-46.
- COOK, R. T., HENRICOT, B., HENRICI, A. & BEALES, P. (2006): Morphological and phylogenetic comparisons amongst powdery mildews on *Catalpa* in the UK. – Mycol. Res., **110**: 672-685.
- COOK, R. T., HENRICOT, B. & KISS, L. (2004): First record of *Erysiphe elevata* on *Catalpa bignonioides* in the UK. – Plant Pathol., **53**: 807-807.
- CUNNINGTON, J. H., TAKAMATSU, S., LAWRIE, A. C. & PASCOE, I. G. (2003): Molecular identification of anamorphic powdery mildew fungi. – Australas. Plant Pathol., **32**: 421-428.
- FELSENSTEIN, J. (1981): Evolutionary trees from DNA sequences: a maximum likelihood approach. – Journal of Molecular Evolution, **17**: 368-376.
- FISCHER HUELIN, D., JEANMONOD, D. & LOIZEAU, P.-A. (2008): Conservatoire et Jardin Botaniques de la ville de Genève. – Rapport annuel 2007.
- FRANCIS, S. A., RODEN, B. C., ADAMS, M. J., WEILAND, J. & ASHER, M. J. (2007): Comparison of ITS sequences from UK and North American sugar-beet powdery mildews and the designation of *Erysiphe betae*. – Mycol. Res., **111**: 204-212.
- GARDES, M. & BRUNS, T. D. (1993): ITS primers with enhanced specificity for basidiomycetes – application to the identification of mycorrhizae and rusts. – Molecular Ecology, **2**: 113-118.
- GATESY J., DESALLE, R. & WHEELER, W. (1993): Alignment-ambiguous nucleotide sites and the exclusion of systematic data. – Molecular Phylogenetics and Evolution, **2**: 152-157.
- GIRIBET, G. & WHEELER, W. C. (1999): On gaps. – Molecular Phylogenetics and Evolution, **13**: 132-143.
- GULLINO, G. & RAPETTI, S. (1978): Un mal bianco del Platano. – Inf.tore Fitopatol., **11/12**: 65-66.
- HELUTA, V., TAKAMATSU, S., VOYTYUK, S. & SHIROVA, Y. (2009): *Erysiphe kenjiana* (Erysiphales), a new invasive fungus in Europe. – Mycol. Prog., **8**: 367-375.
- HIRATA, T. & TAKAMATSU, S. (1996): Nucleotide sequence diversity of rDNA internal transcribed spacers extracted from conidia and cleistothecia of several powdery mildew fungi. – Mycoscience, **37**: 283-288.
- HUELSENBECK, J. P. & RANNALA, B. (2004): Frequentist properties of Bayesian posterior probabilities of phylogenetic trees under simple and complex substitution models. – Systematic Biology, **53**: 904-913.
- HUELSENBECK, J. P. & RONQUIST, F. (2001): MRBAYES: bayesian inference of phylogenetic trees. – Bioinformatics Applications Note, **17**: 754-755.
- HUELSENBECK, J. P., LARGET, B., MILLER R. E. & RONQUIST, F. (2002): Potential applications and pitfalls of Bayesian inference of phylogeny. – Systematic Biology, **51**: 673-688.
- KATO, K., KUMA, K., TOH, H. & MIYATA, T. (2005): MAFFT version 5: improvement in accuracy of multiple sequence alignment. – Nucleic Acids Research, **33**: 511-518.
- KATO, K., MISAWA, K., KUMA, K. & MIYATA, T. (2002): MAFFT: a novel method for rapid multiple sequence alignment based on fast Fourier transform. – Nucleic Acids Research, **30**: 3059-3066.
- KATO, K. & TOH, H. (2008): Recent developments in the MAFFT multiple sequence alignment program (outlines version 6). – Briefings in Bioinformatics, **9**: 286-298.
- KHODAPARAST, S. A., HEDJAROUDE, G. A. & TAKAMATSU, S. (2003): Phylogenetic relationships between Iranian isolates of *Microsphaera* and *Erysiphe* s. lat. based on rDNA internal transcribed spacers sequences. – Rostaniha, **4**: 79-86.
- KIRSCHNER, R. (2010): First record of *Erysiphe magnifica* on lotus, a host outside the Magnoliales. – Mycol. Prog., **9**: 417-424.
- KIRSCHNER, R. (2011): Observations on *Erysiphe platani* in Germany. – Plant Pathology & Quarantine, **1(2)**: 115-119.
- KOVACS, G. M., JANKOVICS, T. & KISS, L. (2011): Variation in the nrDNA ITS sequences of some powdery mildew species: do routine molecular identification procedures hide valuable information? – Eur. J. Plant Pathol., **131**: 135-141.
- KREISEL, H. & SCHOLLER, M. (1994): Chronology of phytoparasitic fungi introduced to Germany and adjacent countries. – Bot. Acta, **107**: 387-392.
- LEE, H. B., KIM, C. J., MUN, H. Y. & LEE, K.-H. (2011): First report of *Erysiphe quercicola* causing powdery mildew on Ubame oak in Korea. – Plant Dis., **95**: 77.
- LENZIN, H., NAGEL, P. GROSS, A. & HUCK, C. (2011): Nischendifferenzierung zweier nah verwandter Neophyten im urbanen Raum. – Bauhinia, **21**: 17-24.

- LIMKAISANG, S., CUNNINGTON, J. H., WUI, L. K., SALLEH, B., SATO, Y., DIVARANGKON, R., FANGFUK, W., TO-ANUN, C. & TAKAMATSU, S. (2006): Molecular phylogenetic analyses reveal a close relationship between powdery mildew fungi on some tropical trees and *Erysiphe alphitoides*, an oak powdery mildew. – *Mycoscience*, **47**: 327-335.
- LUTZ, M., BAUER, R., BEGEROW, D., OBERWINKLER, F. & TRIEBEL, D. (2004): *Tuberculina*, rust relatives attack rusts. – *Mycologia*, **96**: 614-626.
- NÄHRIG, D. & HARMS, K. H. (2003): Rote Listen und Checklisten der Spinnentiere (Arachnida) Baden-Württembergs. – *Naturschutzpraxis, Artenschutz*, **7**: 1-204.
- MORI, Y., SATO, Y. & TAKAMATSU, S. (2000): Evolutionary analysis of the powdery mildew fungi nucleotide sequences of the nuclear ribosomal DNA. – *Mycologia*, **92**: 74-93.
- O'DONNELL, K. L. (1993): *Fusarium* and its near relatives. In: REYNOLDS, D. R. & TAYLOR, J. W. (eds.): The fungal holomorph: mitotic, meiotic and pleomorphic speciation in fungal systematics: 225-233; Wallingford (CABI).
- PASTIRČÁKOVÁ, K. & PASTIRČÁK, M. (2008): *Erysiphe platanii* causing powdery mildew of London plane in Hungary. – *Acta Phytopathologica et Entomologica Hungarica*, **43**: 31-36.
- RANKOVIĆ, B. (2003): Powdery mildew fungi (order Erysiphales) on plants in Montenegro (Chernogoria). – *Mikol. Fitopatol.*, **37**: 42-52.
- RONQUIST, F. R. & HUELSENBECK, J. P. (2003): MRBAYES 3: Bayesian phylogenetic inference under mixed models. – *Bioinformatics*, **19**: 1572-1574.
- SAENZ, G. S. & TAYLOR, J. W. (1999): Phylogeny of the Erysiphales (powdery mildews) inferred from internal transcribed spacer ribosomal DNA sequences. – *Can. J. Bot.*, **77**: 150-168.
- SCHOCH, C. L., SEIFERT, K. A., HUHDORF, S., ROBERT, V., SPOUGE, J. L., LEVESQUE, C. A., CHEN, W. and Fungal Barcoding Consortium (2012): Nuclear ribosomal internal transcribed spacer (ITS) region as a universal DNA barcode marker for Fungi. – *Proc. Natl. Acad. Sci.*, **109**(16): 6241-6246.
- SCHOLLER, M. (1996): Die Erysiphales, Pucciniales und Ustilaginales der Vorpommerschen Boddenlandschaft. Ökologisch-floristische, florensgeschichtliche und morphologisch-taxonomische Untersuchungen. – *Regensb. Mykol. Schr.*, **6**: 1-325.
- SCHOLLER, M., KLENKE, F., KUMMER, V. & JAGE, H. (2011): Checklist und Rote Liste der obligat-pflanzenparasitischen Kleinpilze (Erysiphales, Microbotryales, Peronosporales, Pucciniales, Ustilaginales) Deutschlands (unpublished manuscript).
- SCHOLLER, M. & MÜLLER, G. (2008). Projekt „Pilzflora von Karlsruhe“ – erste Ergebnisse. – *Carolinea*, **66**: 87-93.
- SEKO, Y., HELUTA, V., GRIGALIUNAITE, B. & TAKAMATSU, S. (2011): Morphological and molecular characterization of two ITS groups of *Erysiphe* (Erysiphales) occurring on *Syringa* and *Ligustrum* (Oleaceae). – *Mycoscience*, **52**: 174-182.
- SHIROYA, Y. & TAKAMATSU, S. (2009): *Erysiphe corylopsidis* sp. nov., a new powdery mildew fungus found on *Corylopsis spicata* and *C. pauciflora*. – *Mycoscience*, **50**: 409-414.
- SILVESTRO, D. & MICHALAK, I. (2010): raxmlGUI: a graphical front-end for RAXML. Available at <http://sourceforge.net/projects/raxmlgui/>.
- STAMATAKIS, A. (2006): RAXML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. – *Bioinformatics*, **22**: 2688-2690.
- STAMATAKIS, A., HOOVER, P. & ROUGEMONT, J. (2008): A rapid bootstrap algorithm for the RAXML web servers. – *Systematic Biology*, **57**: 758-771.
- STANOSZ, G., SMITH, D., & BERNIER, L. (2009): First report of *Erysiphe palczewskii* powdery mildew of Siberian pea tree (*Caragana arborescens*) in Wisconsin and Quebec. – *Plant Dis.*, **93**: 1352.
- STRICKER, P. (1955): Die Ausbreitung des Tintenfischpilzes. – *Beiträge Naturk. Forschung Südwestdeutschland*, **13**: 93-98.
- TAKAMATSU, S., BOLAY, A., LIMKAISANG, S., KOM-UN, S. & TO-ANUN, C. (2006): Identity of a powdery mildew fungus occurring on *Paeonia* and its relationship with *Erysiphe hypophylla* on oak. – *Mycoscience*, **47**: 367-373.
- TAKAMATSU, S., BRAUN, U., LIMKAISANG, S., KOM-UN, S., SATO, Y. & CUNNINGTON, J. H. (2007): Phylogeny and taxonomy of the oak powdery mildew *Erysiphe alphitoides* sensu lato. – *Mycol. Res.*, **111**: 809-826.
- TAKAMATSU, S., HIRATA, T. & SATO, Y. (1998): Phylogenetic analysis and predicted secondary structures of the rDNA internal transcribed spacers of the powdery mildew fungi (Erysiphaceae). – *Mycoscience*, **39**: 441-453.
- TAKAMATSU, S., HIRATA, T., SATO, Y., NOMURA, Y. & SATO, Y. (1999): Phylogenetic relationships of *Microsphaera* and *Erysiphe* section *Erysiphe* (powdery mildews) inferred from the rDNA ITS sequences. – *Mycoscience*, **40**: 259-268.
- TAKAMATSU, S., MASUYA, H., DIVARANGKON, R. & NOMURA, Y. (2008): *Erysiphe fimbriata* sp. nov.: a powdery mildew fungus found on *Carpinus laxiflora*. – *Mycoscience*, **49**: 185-191.
- WHITE, T. J., BRUNS, T., LEE S. & TAYLOR, J. (1990): Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: INNIS, M. A., GELFAND, D. H., SNINSKY, J. J. & WHITE, T. J. (eds.): PCR protocols, a guide to methods and applications. 315-322; San Diego (Academic Press).