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ORIGINAL ARTICLE

The only known white blister rust on a basal angiosperm is a member of the genus *Albugo*

Sebastian Ploch[1] · Young-Joon Choi[2] · Marco Thines[1,3,4]

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Abstract

Rare pathogens on unusual hosts are often providing valuable insight into the evolution of the pathogen group concerned, but it is often challenging to obtain sequence data for these, as because only very few, often decades-old specimens are available. One such example is *Albugo tropica*, the white blister pathogen of a basal angiosperm in the genus *Peperomia* (Piperaceae). For this species, only two, more than 70 and over 120-year-old collections available. Here, sequence data for *A. tropica* are reported and phylogenetic reconstructions reveal it as the sister group to all other white blister rusts of the genus *Albugo*. Its isolated position is also reflected by several morphological differences to the other species of the genus, such as very thin-walled sporangia and almost smooth oospores. The isolated phylogenetic position of the pathogen and its host might indicate that it is a relict species trapped on its host. The sister-group relationship to all members of the genus *Albugo* s.str., which have been investigated using molecular phylogenetics, hints at the possibility, that *Albugo* might have originated in South America or Gondwana and has later radiated in the holarctic on members of the Brassicales.

Keywords *Albugo* · Ancient DNA · *Peperomia* · Plant-pathogen evolution · Relict species · White blister disease

Introduction

Rare pathogens on unusual hosts have always spurred the curiosity of plant pathologists and evolutionary biologists, as their investigation often offers important insights into the evolution of the pathogen groups in question. The oomycetes, fungal-like organisms of the Kingdom Straminipila, related to brown algae and diatoms, are no exception in this respect. For example, the investigation of some rare graminicolous downy mildews (Göker et al. 2003; Thines et al. 2007, 2008) has revealed that downy mildew evolution might have started from hosts in Poaceae (Thines 2009). The investigation of *Albugo mauginii* has unravelled that similar to downy mildews (Voglmayr 2003; Voglmayr et al. 2004; Runge et al. 2011; Choi and Thines 2015) also white blister rusts (Albuginaceae) are able to jump over huge phylogenetic distance to colonise previously unaffected host families (Choi et al. 2009, 2011). The cosmopolitan Albuginaceae are a family of Peronosporomycetes (Oomycota), which, with only two known exceptions, parasitise eudicots (Patouillard and Lagerheim 1892; Wilson 1907; Walker and Priest 2007; Thines and Voglmayr 2009, Thines 2010, 2014). Three genera are currently accepted in the Albuginaceae, *Wilsoniana*, *Pustula* and *Albugo*, primarily infecting members of the Caryophyllidae, Asteridae and Brassicales, respectively. Besides, there are reports of rare white blister rusts infecting other host plant groups, which could hold the key to a better understanding of the evolution of oomycete

✉ Sebastian Ploch
sebastian.ploch@senckenberg.de

✉ Marco Thines
m.thines@thines-lab.eu

[1] Senckenberg Gesellschaft fuer Naturforschung, Biodiversity and Climate Research Centre (SBiK-F), Senckenberganlage 25, D-60325 Frankfurt am Main, Germany

[2] Department of Biology, Kunsan National University, Gunsan 54150, South Korea

[3] Department of Biological Sciences, Institute of Ecology, Evolution and Diversity, Goethe University Frankfurt am Main, Max-von-Laue-Str. 13, D-60438 Frankfurt am Main, Germany

[4] LOEWE Integrative Fungal Research Cluster (IPF), Georg-Voigt-Str. 14-16, D-60325 Frankfurt am Main, Germany

pathogens. *Albugo macalpiniana* is the only species known so far that parasitizes monocots—*Pterostylis* species of the Orchidaceae—and had been described only recently (Walker and Priest 2007) from historic herbarium specimens. The age of the specimens might have been the reason why the authors did not present a molecular phylogenetic analysis, leaving the evolutionary origins of the species unresolved. *Albugo tropica* was described infecting *Peperomia* (Piperaceae) more than 100 years ago by Lagerheim as *Cystopus tropicus* on *Peperomia* sp. (Patouillard and Lagerheim 1892), and soon transferred to the genus *Albugo* by Wilson (1907). The pathogen was also collected by Fosberg in Columbia infecting *Peperomia pellucida* and was determined by Stevenson in April 1943. These two collections seem to be the only ones available in various public herbaria. Even though the original collections by Lagerheim (Patouillard and Lagerheim 1892) were widely recognised (Biga 1955, Choi and Priest 1995, Thines and Voglmayr 2009), no sequence data are available for *Albugo tropica*, despite being the only white blister rust species reported from a basal angiosperm.

Therefore, it was the aim of the current study to provide sequence data for the rare species, *Albugo tropica*, to assess its phylogenetic position and to clarify its genus-level classification.

Material and methods

DNA extraction, PCR and sequencing

A list of vouchers investigated in this study is given in Table 1. Herbarium acronyms are according to Thiers (2017). A small part of symptomatic leaf tissue (~ 5 mg) was transferred to a 2-ml reaction vial containing sterile metal beads. DNA extraction was done as outlined by Telle and Thines (2008).

PCRs were carried out using the Phusion High-Fidelity DNA Polymerase (ThermoFisher Scientific, Waltham, MA, USA) with 1× Phusion HF buffer, 200 µM dNTPs, 0.4 mM of each primer, 0.8 mg ml^{-1} bovine serum albumine (BSA, Carl Roth, Karlsruhe, Germany), 0.25 U Phusion DNA Polymerase, and 1 µl DNA sample in a 25 µl PCR reaction. The ITS (internal transcribed spacer) regions were amplified with the primers ITS1-O (Bachofer 2004) and LR0 (reverse complement to LR0R (Moncalvo et al. 1995)), LSU (large ribosomal subunit) was amplified with the primers LR0R-O and LR6-O (Riethmüller et al. 2002), and *cox*2 (cytochrome C oxidase II) was amplified using the primers cox2-F and cox2-R (Hudspeth et al. 2000). The PCR conditions were set to an initial denaturation at 98 °C for 30 s, 36 cycles at 98 °C for 10 s, 53 °C for 30 s and 72 °C for 60 s, and concluded with a final extension at 72 °C for 600 s for all three genes. If multiple bands occurred after electrophoresis through 1% agarose gels, the bands were cut out and purified using the illustra GFX PCR DNA and Gel Band Purification kit (GE Healthcare, Chicago, USA). Amplicons were sequenced using the primers used for PCR by the laboratory centre of the Senckenberg Biodiversity and Climate Research Centre (SBiK-F, Frankfurt am Main, Germany).

Sequence analyses and phylogenetics

Forward and reverse sequences were merged and edited using Geneious 5.6 (Biomatters, Auckland, New Zealand). Consensus sequences were subjected to BLAST (Altschul et al. 1990) searches on NCBI (https://blast.ncbi.nlm.nih.gov/Blast.cgi), reference sequences were selected from the resulting sequence list (Table 2), aligned with MEGA 7 (Kumar et al. 2016) using the muscle algorithm (clustering option: neighbour joining, gap opening penalty: − 600, gap extension penalty: − 6). After assessing that support for a conflicting topology was not found comparing trees based on ITS to those based on *cox*2, the two loci were concatenated. Phylogenetic trees were inferred using MEGA 7 (Minimum Evolution, Tamura-Nei model (Tamura and Nei 1993), with a gamma shape parameter of 0.3765 (as calculated by MEGA 7), pattern among lineages heterogeneous and partial deletion with a site coverage cut-off of 80%). Robustness of the phylogenies was tested by conducting 1000 bootstrap replicates. In addition, maximum likelihood analysis was done using RAxML (GTRGAMMA model with 1000 bootstrap replicates) and

Table 1 Vouchers investigated in this study

Voucher	Collection details	Collector
PC 0723702	*Cystopus tropicus* on *Peperomia* sp., Ecuador, Puente de Chimbo, Sept. 1891	G. Lagerheim
PC 0723703	*Cystopus tropicus* on *Peperomia* sp., Ecuador, Puente de Chimbo, Sept. 1891	G. Lagerheim
G 00110382	*Albugo tropica* on *Peperomia* sp., Ecuador, Puente de Chimbo, Sept. 1891	G. Lagerheim
US 185812	*Albugo tropica* on *Peperomia pellucida*, Colombia, Samaria, on Rio Timba, 2 km w. of Timba, Dept. E. Valle, April 13, 1943	coll. F. R. Fosberg det. J. A. Stevenson

Table 2 Samples, herbarium numbers and GenBank accession numbers. The specimen in the focus of this study is highlighted in bold

Pathogen	Host species	Herbarium voucher	ITS	*cox*2
Albugo candida s.str.	*Arabis alpina*	BP 54584	GU292130	GU292086
	Calepina irregularis	BP 75212	GU292131	GU292088
	Arabidopsis arenosa	BP 54980	GU292127	FJ468359
	Arabis albida	DAR 30284	GU292129	GU292085
	Biscutella laevigata	BPI 184686	DQ418494	DQ418506
	Brassica juncea	KUS-F 15570	AY929826	AY927046
	Aubrieta deltoidea	BPI 184659	DQ418500	DQ418511
	Diplotaxis muralis	BP 92198	GU292134	GU292093
	Erysimum diffusum	BP 54587	GU292139	GU292098
	Berteroa incana	BPI 184200	DQ418495	DQ418508
	Raphanus sativus	KUS-F 10614	AY929841	AY927059
	Sisymbrium luteum	KUS-F 19086	AY929844	AY913808
	Erophila verna	BP 74487	GU292135	GU292094
	Heliophila meyeri	BPI 184888	DQ418493	DQ418515
	Iberis amara	BPI 184897	DQ418499	DQ418522
	Lunaria sp.	CUP 65639	AY929840	AY913797
	Capsella bursa-pastoris	HOH-HUH 505	GU292132	GU292089
	Erysimum cuspidatum	BPI 199988	DQ418498	DQ418519
Albugo candida s.l.	*Armoracia rusticana*	VPRI 30454	GU292126	GU292084
	Rapistrum rugosum	DAR 53178	GU292141	GU292101
Albugo sp.	*Barbarea vulgaris*	BPI 748543	HQ377368	HQ377360
	Barbarea vulgaris	BPI 184667	HQ377367	HQ377358
Albugo rorippae	*Rorippa palustris*	KRAM F-000130	HQ377371	HQ377364
Albugo leimonia	*Cardamine pratensis*	KR 12323	GU292159	GU292124
	Cardamine pratensis	G 00110294	GU292157	GU292122
Albugo leimonia s.l.	*Cardamine amara*	BP 74489	GU292148	GU292106
Albugo hohenheimia	*Cardamine hirsuta*	G 00110292	GU292154	GU292118
	Cardamine hirsuta	DAR 30279	GU292151	GU292115
Albugo lepidii	*Lepidium apetalum*	KUS-F 13747	AY929835	AY927054
	Lepidium virginicum	KUS-F 17312	AY929839	AY927058
	Lepidium sativum	HOH-HUH 965	GU292145	GU292105
Albugo sp.	*Erysimum cheiranthoides*	BP 74486	GU292137	GU292096
	Diptychocarpus strictus	SOMF 19659	AY929833	AY927052
	Hymenolobus procumbens	BP 74484	GU292140	GU292100
Albugo voglmayrii	*Draba nemorosa*	KUS-F 15732	AY929834	AY927053
Albugo laibachii	*Arabidopsis thaliana*	DAR 73071	GU292128	FJ468368
	Arabidopsis thaliana	SL Nc14	FJ468374	FJ468367
Albugo sp.	*Allysum caliacrae*	BPII 199995	HQ377366	HQ377356
	Descurainia sophia	SOMF 19655	AY929832	AY927051
	Thlaspi arvense	BP 75205	GU292142	GU292102
Albugo koreana	*Capsella bursa-pastoris*	KUS-F 13752	AY929829	AY927048
	Capsella bursa-pastoris	KUS-F 17254	AY929831	AY927050
	Capsella bursa-pastoris	KUS-F 15670	AY929830	AY927049
Albugo tropica	***Peperomia* sp.**	**PC 0723703**	**MF527264**	**MF527263**
Albugo ipomoeae-panduratae	*Ipomoea hederacea*	KUS-F 19628	DQ643920	AY913804

Fig. 1 Phylogenetic tree of concatenated *cox*2 and ITS sequences inferred using the Minimum Evolution criterion. Numbers on the branches indicate bootstrap support in Minimum Evolution and Maximum Likelihood, as well as posterior probabilities from Bayesian Inference greater than 50 % / 50 % / 0.9, in the respective order. The tree is rooted with *Albugo ipomoeae-panduratae* parasitic to Convolvulaceae, which constitutes an own distinct clade of Albuginaceae, as already recognised by Voglmayr and Riethmüller (2006). The bar indicates the amount of substitutions per site

Bayesian inference was done using MrBayes (Huelsenbeck and Ronquist 2001) as implemented in the graphic user interface siMBa (Mishra and Thines 2014) (GTR model, 10 M generations, sampling every 100th tree, and 30% burn-in) using the TrEase webserver (Mishra et al. unpublished). The concatenated alignment and the Minimum Evolution tree were deposited in TreeBase under the accession number S21367.

Light microscopy

Small pieces of the herbarium samples were moistened with 70% alcohol, transferred to 60% lactic acid and heated up. Microscopic pictures were taken with a AxioCam MRc5 mounted on to a Zeiss Axio Imager M1 and recorded with Axiovision Rel. 4.8 (Carl Zeiss GmbH, Göttingen, Germany).

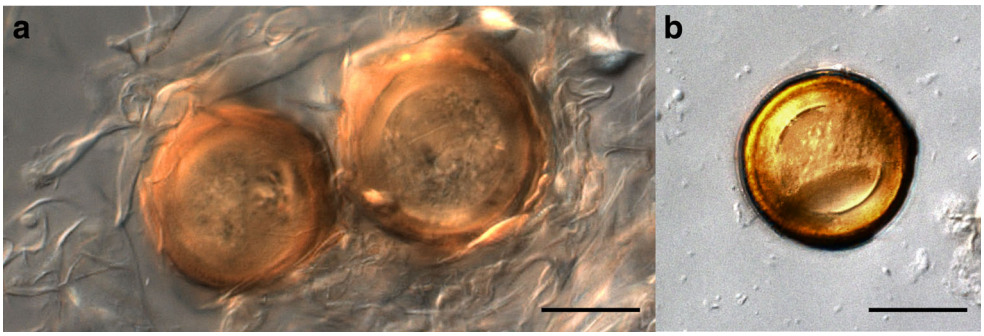

Fig. 2 Oospores of *Albugo tropica* as seen in DIC light microscopy. **a** Within the oogonia. **b** liberated from an oogonium. Scale bars represent 20 μm in all pictures

Scanning electron microscopy

Scanning electron microscopy was performed on small fragments of the herbarium specimens as outlined in Thines (2006). In short, fragments were glued onto aluminium stubs, desiccated over silica gel and sputtered with a gold-palladium alloy. Samples were then analysed in a Zeiss DSM microscope at 5 kV with a target distance of about 1cm.

Results

Sequences from the historic specimen of *A. tropica* were of high quality and the consensus sequence derived from bidirectional sequences did not contain ambiguous bases. The phylogenetic tree of the concatenated *cox*2 and ITS sequences (Fig. 1) revealed three major lineages within *Albugo*—*A. candida* infecting various brassicaceous host genera, a clade containing host-specific *Albugo* species from Brassicaceae and *A. tropica*, placed basal to the former two groups. The branch leading to *A. tropica* is short, as compared to the crown group.

The morphology of the oospores (Fig. 2) overall agreed with the original description of Lagerheim (Patouillard and Lagerheim 1892). However, neither large warts nor other conspicuous protuberances could be seen on oospores. Instead, oospores exhibited an almost smooth surface.

Scanning electron microscopy of the sporangia (Fig. 3) revealed marked differences in the ornamentation of the secondary sporangia. While the surface ornament was made up by rounded warts arranged along spiral lines in *A. candida*, no pronounced surface ornamentation was observed in *A. tropica*. In addition, while primary sporangia revealed a similar degree of resistance to collapsing during desiccation, secondary sporangia in *A. tropica* were collapsing in an almost bowl-shaped manner, suggesting little resistance by the walls, whereas an irregular collapsing pattern was observed in *A. candida*.

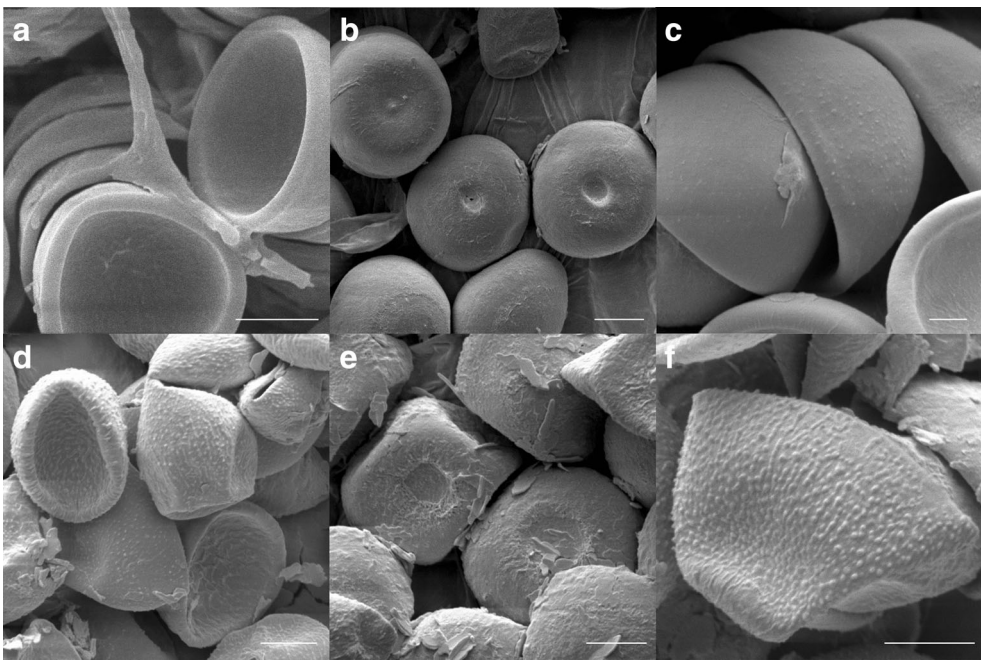

Fig. 3 Scanning electron micrographs of sporangia of *Albugo tropica* (**a**–**c**) and *A. candida* from *Capsella bursa-pastoris* (**d**–**f**). **a**, **d** Overview with secondary sporangia. **b**, **e** Primary sporangia. **c**, **f** Surface ornamentation of secondary sporangia. Scale bars represent 2 μm in (**c**) and 5 μm for the remaining pictures

Discussion

Albugo tropica is an unusual species of obligate biotrophic oomycetes, as it is the only known species of white blister rusts affecting a member of the basal angiosperms. In the original description of *A. tropica*, Lagerheim (Patouillard and Lagerheim 1892) and Wilson (1907) reported large warts on the oospore surface, in contrast to the smooth oospore surface observed in this study. It cannot be excluded that their reports were based on a misinterpretation of the wrinkled oogonium, which sometimes gives the impression of large warts arising from the oospore surface. As due to material restrictions, only small fragments could be investigated, it cannot be ruled out, that oospore ornamentation is variable and some oospores bear warts. However, a smooth oospore surface was also reported for *A. arenosa* infecting *Strigosella* species (Mirzaee et al. 2013).

The lack of a prominent surface ornamentation of the secondary sporangia is unusual for members of the white blister rusts (Thines and Spring 2005; Voglmayr and Riethmüller 2006; Constantinescu and Thines 2006; Thines and Voglmayr 2009). As the function of the surface ornament of spores of white blister pathogens is so far speculative, it is unclear, which consequences the lack of a prominent surface ornamentation in *A. tropica* has for the species. It seems possible that the hydrophobic nature of the sporangia is due to the surface ornamentation and helps the attachment to hydrophobic plant surfaces or delays desiccation, but this has to be tested in future experiments. However, it is noteworthy that the secondary sporangia of *A. tropica* readily collapse into a bowl-shaped pattern, probably suggesting an adaptation to a moist environment in which sporangia face less desiccation stress, or a reduced importance for the spread of the pathogen sporangia, similar to the situation in some graminicolous downy mildews (Bock et al. 1997; Telle and Thines 2012; Thines 2014). This is also in line with the rather thin sporangium wall already noted by Lagerheim in his description of the species (Patouillard and Lagerheim 1892).

The rather low genetic divergence of *A. tropica* to the split of the two main brassicolous lineages of *Albugo* indicates that this white blister rust might have colonised *Peperomia* early in the evolution of *Albugo* and formed an 'odd couple' (Constantinescu 1998) with its host in which situation it stayed genetically relatively unchanged, due to reaching an evolutionary balanced relationship with its host. Thus, it could probably defy the necessity to jump to other hosts after an evolution towards resistance, which otherwise seems to be an important driving force in the diversification of plant pathogens (Thines 2014; Choi and Thines 2015). With achieving a stable balance with its host, *A. tropica* might have lost some of the variability and pre-adaptations needed to jump hosts, leading to it becoming a relict species trapped on its host, similar to the situation in other relict species, such as *Mixia osmundae* (Toome et al. 2014), *Uleiella chilensis* (Riess et al. 2016) and *U. paradoxa*, and *Bartheletia paradoxa* (Scheuer et al. 2008; Mishra et al. 2017).

The phylogenetic placement of *A. tropica* as a sister group to all other species of *Albugo* s.str. investigated to date and some morphological peculiarities might make it seem desirable to separate *A. tropica* from the genus *Albugo*. However, it was felt that because smooth oospores are also known from *A. arenosa* and the phylogenetic distance to *Albugo* s.str. was low, *A. tropica* should be retained in the genus *Albugo*.

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