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Tuberculina-Helicobasidium: Host specificity of the *Tuberculina*-stage reveals unexpected diversity within the group[1]

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Abstract: *Tuberculina* species are mitosporic parasites of rust fungi. It was demonstrated recently that *Tuberculina* represents the asexual life stage of the plant-parasitic genus *Helicobasidium*. Here we reveal the host specificities of *Tuberculina* and *Helicobasidium* species on rust fungal hosts by means of infection experiments and molecular analyses. We inoculated species of the rust genera *Chrysomyxa, Coleosporium, Cronartium, Gymnosporangium, Puccinia, Tranzschelia* and *Uromyces* with conidia and with basidiospores of *Helicobasidium longisporum* and *H. purpureum* and with conidia of *Tuberculina maxima, T. persicina* and *T. sbrozzii*. In addition we analyzed base sequences from the nuclear ITS region of 51 *Tuberculina* and *Helicobasidium* specimens collected in the field together with the sequences from the *Tuberculina* infections obtained by infection experiments. The resulting data show that at least six monophyletic lineages are within the *Tuberculina/Helicobasidium*-group that can be unambiguously distinguished by combining molecular and morphological characters and by specific host spectra of the *Tuberculina*-stage. This diversity opens up new vistas on the evolution of this exceptional mycoparasitic-phytoparasitic fungal group.

Key words: *Helicobasidium,* host specificity, infection experiments, ITS, *Tuberculina,* molecular phylogeny, mycoparasitism, Urediniomycetes

INTRODUCTION

Tuberculina species occur all over the world, living in association with more than 150 rust species from at least 15 genera. Growing between the hyphae of their rust fungal hosts in leaves and stems of plants, *Tuberculina* species become visible during sporulation as hemispherical, lilac to violet mycelia that burst through the plant surfaces generally close to rust sori, releasing a powdery mass of conidia. *Tuberculina* recently was demonstrated phylogenetically to be closely related to its associates, the rusts, some species representing the asexual life stage of species of the plant-parasitic genus *Helicobasidium* (Lutz et al 2004a, b). In contrast to the mycoparasitic genus *Tuberculina, Helicobasidium* species are serious plant pathogens causing the economically important violet root rot. They are unspecific in their choice of hosts (Buddin and Wakefield 1924, Hering 1962) and reported as parasites of more than 120 plant species representing more than 50 families of the spermatophytes and ferns (Itô 1949, Viennot-Bourgin 1949). Extensive research has been conducted on the biology of and the combat against *Helicobasidium,* but the diversity of the genus remains poorly understood (Aimi et al 2003a, b; Uetake et al 2002) and hypotheses on the life cycle conflict (Aimi et al 2003b, Lutz et al 2004a).

Although the strong inhibitory effect of *Tuberculina* on rust spore production has resulted in extensive research dealing with *Tuberculina* as an agent in biological rust control (for review see Wicker 1981), the relationship among *Tuberculina,* rusts and plants was the subject of controversy. *Tuberculina* species have been interpreted as mycoparasites specific to rusts (Kirulis 1940, Tubeuf 1901, Tulasne 1854, Zambettakis et al 1985), as nonspecific parasites on several substrates (Petrak 1956, Schroeter 1889) or even as specialized parasites on rust-infected plant tissues (Biraghi 1940; Hulea 1939; Marchal 1902; Wicker and Woo 1969, 1973). Thus plant parasites and parasites of non-rust fungi were included in the genus adopting a concept based on morphological characters (Ellis 1893, Patouillard and Gaillard 1888). Other authors used a species concept based on host specificities, distinguishing *Tuberculina* species on different rust hosts (Spegazzini 1880, 1884) or even plant hosts (Gobi 1885). However, Barkai-Golan (1959) demonstrated that *Tuberculina* could not infect rust-free plants and we recently substantiated the mycoparasitic nature of *Tuberculina persicina* on rust fungal hosts by ultrastructural observations that reveal a specific and morphologically uncommon cellular in-

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teraction via large fusion pores with a direct cytoplasm-cytoplasm connection and interspecific transfer of *Tuberculina*-nuclei to rust hyphae (Bauer et al 2004, Lutz et al 2004b).

Even though several authors described *Tuberculina* species based on the occurrence on different hosts (e.g., Spegazzini 1880, 1884) and the vast majority of *Tuberculina maxima* specimens are described from *Cronartium*- and *Gymnosporangium*- hosts, no experimental proof for host specificity of any *Tuberculina* species was provided. On the contrary, Barkai-Golan (1959) demonstrated that *Tuberculina persicina* specimens from one rust host are capable of infecting several other rust species.

In this report, we present both results of infection experiments and molecular data that reveal the host specificities of several *Tuberculina* and *Helicobasidium* lineages, respectively, on their rust fungal hosts. These findings yield new insights in the *Tuberculina–Helicobasidium* life cycle and in the diversity of the group.

MATERIALS AND METHODS

Infection experiments.—The aim of our infection experiments was to test whether differences among *Tuberculina maxima, T. persicina, T. sbrozzii, Helicobasidium longisporum* and *H. purpureum* exist in the choice of their rust hosts. In addition we wanted to test whether both basidiospores of *Helicobasidium* and conidia produced in *Tuberculina* and *Helicobasidium* cultures and from *Tuberculina* sporodochia, respectively, are capable of causing *Tuberculina* infections of rust hosts.

We have gathered data on inoculated rust species, the species used as inocula, the results of the different inoculations, the respective kind of inoculum and the respective experimental approach of the different inoculations (TABLE I).

The experimental approach depended on the kind of inoculum applied and on the way the rusts for inoculation were obtained.

We used two kinds of inocula: (i) We collected intensively sporulating basidiocarps of *Helicobasidium longisporum* and *H. purpureum* in the field, tore the fresh basidiocarps into pieces of about 4 square cm and tagged the pieces to pear trees at the same places where we had fixed the rust inocula before (cf. below); (ii) basidiospores from freshly collected basidiocarps (pieces of about 4 cm^2 were used) or conidia were shaken vigorously by hand in tap water (75 mL). The conidia were either from freshly collected *Tuberculina* sporodochia or from sporodochia that were conserved at −18 C in a freezer (cf. Wicker and Wells 1968) (in both cases we used about 20 sporodochia) or from *Tuberculina* or *Helicobasidium* cultures (about 3 g of fungal material were used). The inoculum was spread over the top and the underside of rust-infected leaves with a brush. In every case we inoculated the rusts with their hyperparasites as soon as the rust spermogonia were mature.

The rusts for inoculation were obtained in four different ways. Accordingly, four experimental settings were established. (i) Rust-infected plants growing in nature were used (*Chrysomyxa rhododendri* on *Picea abies, Coleosporium tussilaginis* on *Pinus sylvestris, Cronartium ribicola* on *Pinus aristata, Gymnosporangium cornutum* on *Sorbus aucuparia, Puccinia sessilis* on *Allium ursinum,* and *Tranzschelia prunispinosae* on *Anemone ranunculoides*). We chose places where we were not able to detect any *Tuberculina* infection in the previous growth season. We divided the places in isolated areas where we inoculated the rust infected plants and areas without inoculation for control. (ii) We inoculated pear trees growing in nature with *Gymnosporangium sabinae* to obtain heavy rust infections. To this end we attached freshly collected branches of *Juniperus sp.,* which showed intensive infections with germinating and sporulating teliospores of *Gymnosporangium sabinae* to several distant trees where we were not able to detect any *Tuberculina* infection in the previous vegetation period. One rust-inoculated, isolated tree at each location served as control and was not inoculated with the hyperparasite. (iii) We transferred *Euphorbia cyparissias* plants that systemically were infected by *Uromyces pisi* s.l., young shoots of *Vinca major* infected with *Puccinia vincae* and little *Pinus sylvestris* trees infected with *Coleosporium tussilaginis* from nature to our greenhouse. The plants inoculated with the hyperparasite and control plants were cultivated in different houses. After inoculation the single plants were incubated 3 d under transparent plastic bags to provide high relative humidity. (iv) We cultivated *Taraxacum officinale* agg. in our greenhouse and infected the plants with *Puccinia silvatica* using the wire net method (Kakishima et al 1999). Freshly collected leaves of *Carex brizoides* L. harboring sori of teliospores of the rust, which were incubated at room temperature 3 d in plastic bags at high humidity, served as inoculum. Inoculation with the hyperparasite and control were handled as in (iii).

Inoculated plants and the control then were observed until the respective rusts completed their life cycles on the respective host plant. If sporodochia were detected, the *Tuberculina* infection was verified microscopically and a part of the material was conserved for further examinations.

Field observations.—We checked several rust species growing in an area of 200 square m around several sporulating basidiocarps of *Helicobasidium purpureum* for *Tuberculina* infections during 2 y, and we introduced some additional plant species harboring young spermogonia of different rust species at that place (TABLE II).

Cultures.—We obtained cultures from *Tuberculina* conidia as well as from *Helicobasidium* basidiospores on (i) malt-extract agar (20 g malt extract, 20 g glucose, 15 g agar, 1 g universal peptone/L distilled water); and (ii) malt-yeast-peptone agar (15 g agar, 7 g malt extract, 1 g universal peptone, 0.5 g yeast extract/L distilled water). *Helicobasidium* cultures were started by fixing little pieces of freshly collected sporulating basidiocarps to the underside of the lid of culture dishes. After 1 d of incubation at room temperature the basidiocarps were removed and the germination of the basidiospores was checked microscopically. After

TABLE I. Results of infection experiments. The inoculated rust species, the species used as inocula, the results of the different inoculations, the respective kind of inoculum and the respective experimental approach of the different inoculations.

	Inoculum	
Inoculated rust species	*Helicobasidium purpureum*	*Helicobasidium longisporum*
Chrysomyxa rhododendri	—I1/2A	—I4/2A
Coleosporium tussilaginis	—I1/2A/—I1/2C	—I3/2A/—I3/2C
Cronartium ribicola	—I1/2A	—I3/2A
Gymnosporangium cornutum	—I1/2A	*T. persicina* (AY254194)I4/2A
Gymnosporangium sabinae	—I1/1B	*T. persicina* (AY254195)I3/1B
Puccinia sessilis	*T. persicina* (AY254193)I1/2A	nd
Puccinia silvatica	*T. persicina* (AY254190)I1/2D/*T. p.* (AY60167)I2/2D	—I3/2D/—I4/2D
Puccinia vincae	—I2/2C	—I4/2C
Tranzschelia pruni-spinosae	*T. persicina* (AY254192)I1/2A	—I3/2A
Uromyces pisi s.l.	*T. persicina* (AY254191)I1/2C/*T. p.* (AY460174)I2/2C	—I3/2C/—I4/2C

− = No *Tuberculina* infection observed. nd = The particular experiment was not done. I1–I8 = Indication of the different kinds of inoculum used: I1 are basidiospores from freshly collected basidiocarps (AY254189), I2 are conidia from a culture. Obtained from basidiospores from freshly collected basidiocarps (AY460132), I3 are basidiospores from freshly collected basidiocarps (AY254187), I4 are conidia from a culture obtained from basidiospores from freshly collected basidiocarps (AY254188), I5 are conidia from freshly collected sporodochia on *Gymnosporangium sabinae* (AY460141), I6 are conidia from freshly collected sporodochia on *Puccinia silvatica* (AY460169), I7 are conidia from a culture obtained from conidia from freshly collected sporodochia on *Gymnosporangium sabinae* (AY460155), I8 are conidia from freshly collected sporodochia on *Puccinia vincae* (AY460171). 1B, 2A–2D = For a description of the different experimental approaches see Materials and Methods.

a few days cultural growth could be observed microscopically in some places by a change of hyphal characters and by the change of the color of the medium to violet. For *Tuberculina* a small number of conidia obtained from freshly collected sporodochia were spread over the media with the help of a fine needle. The development of *Tuberculina* conidia essentially was identical compared to that of *Helicobasidium* basidiospores.

Molecular analyses.—We isolated genomic DNA from 41 herbarium specimens and from four cultures on artificial media of *Tuberculina* and *Helicobasidium,* respectively (TABLE III). We followed the methods of isolation and crushing of fungal material, DNA extraction, amplification, purification of PCR products, sequencing and processing of raw data of Lutz et al (2004b). To ensure that the observed *Tuberculina* infections could be ascribed to the respective inocula and to infer the phylogeny of the sampled *Tuberculina* and *Helicobasidium* specimens, we amplified the ITS1/2 region of the rDNA including the 5.8S rDNA (ITS, about 600 bp) using the primer pair ITS1 and ITS4 (White et al 1990). For amplification of the ITS region we adjusted the annealing temperature to 45 C. DNA sequences determined for this study were deposited in GenBank (TABLE III).

To infer the phylogeny of the sampled specimens, we added these sequences from GenBank (accession numbers are in parentheses): *Helicobasidium longisporum* (syn. *H. compactum*) (AY254187, AY254188, AY292426), *Helicobasidium mompa* (AB043964, AY292428, AY292429), *Helicobasidium purpureum* (syn. *H. brebissonii*) (AY254189), *Tuberculina maxima* (AY292435, AY292436, AY292437), *Tuberculina persicina* (AY254190, AY254191, AY254192, AY254193, AY254194, AY254195, AY292440, AY292442, AY292443, AY292444, AY292445, AY292447, AY292449, AY292450, AY292451, AY292454, AY292455) and *Tuberculina sbrozzii* (AY292456, AY292457, AY292458).

DNA sequences were aligned under default settings with Clustal X (Thompson et al 1997). The alignment subsequently was improved with Rascal (Thompson et al 2003). We did not manipulate the alignment by hand or excluded any positions as recommended by Giribet and Wheeler (1999) and Gatesy et al (1993), respectively. The final alignment (75 sequences; length 598 bp, 103 variable sites) and the published tree are deposited in TreeBase (http://treebase.bio.buffalo.edu/treebase/) with the study accession number S1061 and the matrix accession number M1811. Sequence distances were computed with the MEGALIGN module of the Lasergene package (DNASTAR Inc., Madison, Wisconsin).

To estimate phylogenetic relationships we used a Bayesian approach of phylogenetic inference using a Markov Chain Monte Carlo (MCMC) technique as implemented in the computer program MrBayes 3.0b4 (Huelsenbeck and Ronquist 2001). For Bayesian analysis, the data first were analyzed with MrModeltest 1.0b (J.A.A. Nylander, Upsala University, Sweden; Posada and Crandall 1998) to find the most appropriate model of DNA substitution. The hierarchical likelihood ratio test proposed the DNA substitution model of Hasegawa, Kishino and Yano (HKY, Hasegawa et al 1985) with gamma distributed substitution rates (see

TABLE 1. Extended

Inoculum			
	Tuberculina persicina		
Tuberculina maxima	From sporodochia on *Puccinia silvatica*	From sporodochia on *Gymnosporangium sabinae*	*Tuberculina sbrozzii*
—I5/2A	—I6/2A	—I7/2A	nd
—I5/2A/—I5/2C	nd	—I7/2A/—I7/2C	nd
—I5/2A	nd	nd	nd
nd	—I6/2A	*T. persicina* (AY460152)I7/2A	nd
T. maxima (AY460138)I5/2B	—I6/2B	*T. persicina* (AY460153)I7/2B	nd
nd	nd	nd	nd
—I5/2D	*T. persicina* (AY460168)I6/2D	—I7/2D	nd
—I5/2C	—I6/2C	—I7/2C	*T. sbrozzii* (AY460172)I8/2C
—I5/2A	nd	—I7/2A	nd
—I5/2C	*T. persicina* (AY460175)I6/2C	—I7/2C	nd

Swofford et al 1996). Thus, four incrementally heated simultaneous Markov chains were run over 2 000 000 generations using the HKY model of DNA substitution with gamma distributed substitution rates, random starting trees and default starting parameters of the DNA substitution model. Trees were sampled every 100 generations, resulting in an overall sampling of 20 001 trees. From these, the first 1001 trees were discarded (burn in = 1001). The trees (19 000)* remained static and 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 of phylogenetic analysis was repeated 10 times to test the independence of the results from topological priors (cf. Huelsenbeck et al 2002).

RESULTS

Infection experiments.—*Tuberculina* infections of several rusts were obtained with several involved species and in different experimental approaches (TABLE I). Infections of rust aecia were observed for inoculations with both *Tuberculina* and *Helicobasidium*. No differences were found among inoculations with basidiospores, conidia from *Tuberculina* sporodochia or conidia from *Tuberculina* and from *Helicobasidium* cultures. Sporodochia were observed 2–6 wk after inoculation in 20–50% of the rust aecia and rapidly increased to include up to 95% of the aecia by the

TABLE II. Result of the 2 y observation of the rust flora around *Helicobasidium purpureum* basidiocarps (AY254189, Germany, Baden-Württemberg, Tübingen, on *Carpinus betulus* L., 5. 4. 2001. TUB 011542). The observed rust species, their life stages and observed *Tuberculina* infections are given. Experimentally introduced rust species are labeled by asterisks

Rust species (observed life stages)	Infection (specimen)
*Coleosporium tussilaginis** (0–III[1])	—[2]
*Gymnosporangium cornutum** (0, I)	—
*Gymnosporangium sabinae** (0, I)	—
Melampsora sp. (0, I)	*T. persicina* (AY460137)
Ochropsora ariae (0, I)	*T. persicina* (AY292449)
*Puccinia punctiformis** (0, II, III)	—
Puccinia senecionis-acutiformis (I)	*T. persicina* (AY460165)
Puccinia sessilis (0, I)	—
*Puccinia silvatica** (0, I)	*T. persicina* (AY460166)
Puccinia urticata (0, I)	*T. persicina* (AY460170)
Tranzschelia fusca (0, III)	*T. persicina* (AY292450)
Tranzschelia pruni-spinosae (0, I)	*T. persicina* (AY460173)
*Uromyces pisi** s.l. (0, I)	*T. persicina* (AY460176)
*Uromyces poae** (0, I)	*T. persicina* (AY292451)

[1] 0 = spermogonia, I = aecia, II = uredia, III = telia.

[2] — = no *tuberculina* infection observed.

* computed after the process.

TABLE III. List of studied species, GenBank (http://www.ncbi.nlm.nih.gov) accession numbers, and reference materials

Species	GenBank acc. no.[a]	Reference material[b]
Helicobasidium longisporum Wakef. (syn. *H. compactum* Boedijn)	AY254187*	Germany, Baden-Württemberg, Stuttgart, on *Pyrus communis* L., 17. 10. 2000. (TUB 011540)
	AY254188*	Germany, Baden-Württemberg, Nürtingen, Raidwangen, on *Sambucus nigra* L., 25. 4. 2001. (TUB 011541) - culture
	AY292426*	USA, on *Coffea* sp. (CBS 296.50) - culture
Helicobasidium mompa Tanaka	AB043964*	Japan, Kumamoto, on *Ipomoea batatas* L. - culture
	AY292428*	Japan, on *Asparagus officinalis* L. (ATCC56070) - culture
	AY292429*	Japan. (CBS 278.51) - culture
Helicobasidium purpureum Pat. (syn. *H. brebissonii* (Desm.) Donk)	AY254189*	Germany, Baden-Württemberg, Tübingen, on *Carpinus betulus* L., 5. 4. 2001. (TUB 011542)
	AY460132	Germany, Baden-Württemberg, Tübingen, on *Carpinus betulus* L., 5. 4. 2001. (TUB 011542) - culture
Tuberculina maxima Rostr.	AY292435*	USA, Idaho, Kaniksu National Forest, on *Cronartium ribicola* J.C. Fisch. on *Pinus albicaulis* Engelm., 7. 10. 1965. (CBS 135.66) - culture
	AY292436*	Canada, British Columbia, Wap Lake, on *Cronartium ribicola* J. C. Fisch. on *Pinus monticola* Dougl. ex D. Don., 12. 9. 1965. (CBS 136.66) - culture
	AY292437*	USA, Wyoming, Teton, on *Cronartium comandrae* Peck on *Pinus contorta* Dougl., 22.9. 1965. (CBS 137.66) - culture
	AY460135	Netherlands, Baarn, Hooge Vuursche, on *Cronartium ribicola* J.C. Fisch. (CBS 357.33) - culture
	AY460136	USA, Idaho, Bonner Co., Kaniksu National Forest, on *Cronartium ribicola* J.C. Fisch. on *Pinus monticola* Dougl. ex D. Don., 6. 6. 1965. (CBS 445.65) - culture
	AY460138	Grown experimentally from conidia of *Tuberculina maxima* (TUB 011600) on *Gymnosporangium sabinae* (Dicks.) G. Winter on *Pyrus communis* L., 1. 9. 2001. (TUB 011614)
	AY460139	Germany, Baden-Württemberg, Tübingen, on *Gymnosporangium sabinae* (Dicks.) G. Winter on *Pyrus communis* L., 2. 8. 1999. (TUB 011615)
	AY460140	Germany, Baden-Württemberg, Kirchheim/Teck, Ochsenwang, on *Gymnosporangium sabinae* (Dicks.) G. Winter on *Pyrus communis* L., 25. 8. 2001. (TUB 011599)
	AY460141	Germany, Baden-Württemberg, Stuttgart, on *Gymnosporangium sabinae* (Dicks.) G. Winter on *Pyrus communis* L., 17. 4. 2001. (TUB 011600)
	AY460142	Germany, Baden-Württemberg, Crailsheim, on *Gymnosporangium sabinae* (Dicks.) G. Winter on *Pyrus communis* L., 26. 10. 2000. (TUB 011610)
	AY460143	Germany, Thüringen, Weimar, on *Gymnosporangium sabinae* (Dicks.) G. Winter on *Pyrus communis* L., 16. 1. 2003. (TUB 011601)
	AY460144	Austria, Kärnten, Villach, Faak am See, on *Gymnosporangium sabinae* (Dicks.) G. Winter on *Pyrus communis* L., 11. 9. 2002. (TUB 011603)
	AY460145	Germany, Schleswig-Holstein, Steinburg, Glückstadt, on *Gymnosporangium sabinae* (Dicks.) G. Winter on *Pyrus communis* L., 5. 10. 2000. (TUB 011604)
	AY460146	Germany, Baden-Württemberg, Riedlingen, on *Gymnosporangium sabinae* (Dicks.) G. Winter on *Pyrus communis* L., 5. 10. 2002. (TUB 011602)
	AY460147	Germany Baden-Württemberg, Wildgarten bei Gaildorf, on *Gymnosporangium sabinae* (Dicks.) G. Winter on *Pyrus communis* L., 26. 10. 2000. (TUB 011611)
	AY460148	Germany, Baden-Württemberg, Mittelfischbach, on *Gymnosporangium sabinae* (Dicks.) G. Winter on *Pyrus communis* L., 26. 10. 2000. (TUB 011612)
	AY460149	Germany, Baden-Würtemberg, Welzheim, on *Gymnosporangium sabinae* (Dicks.) G. Winter on *Pyrus communis* L., 26. 10. 2000. (TUB 011597)

TABLE III. Continued

Species	GenBank acc. no.[a]	Reference material[b]
Tuberculina maxima (Rostr.)	AY460150	Germany, Baden-Württemberg, Schorndorf, Hohengeren, on *Gymnosporangium sabinae* (Dicks.) G. Winter on *Pyrus communis* L., 26. 10. 2000. (TUB 011598)
	AY460151	Austria, Steiermark, Graz, on *Gymnosporangium sabinae* (Dicks.) G. Winter on *Pyrus communis* L., 16. 2. 1997. (GZU 07-97)
Tuberculina persicina (Ditmar) Sacc.	AY254190*	Grown experimentally from basidiospores of *Helicobasidium purpureum* (TUB 011542) on *Puccinia silvatica* J. Schröt. on *Taraxacum officinale* agg. F. H. Wigg., 25. 4. 2001. (TUB 011532)
	AY254191*	Grown experimentally from basidiospores of *Helicobasidium purpureum* (TUB 011542) on *Uromyces pisi* s.l (DC.) G. H. Otth on *Euphorbia cyparissias* L., 30. 4. 2001. (TUB 011533)
	AY254192*	Grown experimentally from basidiospores of *Helicobasidium purpureum* (TUB 011542) on *Tranzschelia pruni-spinosae* (Pers.) Dietel on *Anemone ranunculoides* L., 6. 5. 2001. (TUB 011536)
	AY254193*	Grown experimentally from basidiospores of *Helicobasidium purpureum* (TUB 011542) on *Puccinia sessilis* W. G. Schneider in J. Schröt. On *Allium ursinum* L., 9. 5. 2001. (TUB 011534)
	AY254194*	Grown experimentally from conidia of *Helicobasidium longisporum* (TUB 011541-culture) on *Gymnosporangium cornutum* (Pers.) Arthur on *Sorbus aucuparia* L., 28. 7. 2001. (TUB 011537)
	AY254195*	Grown experimentally from basidiospores of *Helicobasidium longisporum* (TUB 011540) on *Gymnosporangium sabinae* (Dicks.) G. Winter on *Pyrus communis* L., 10. 10. 2001. (TUB 011538)
	AY292440*	Slovakia, Dolina, on *Puccinia symphyti-bromorum* F. Muell. on *Pulmonaria obscura* Dumort., 14. 7. 2000. (SAV)
	AY292442*	Italy, Lombardia, Padenghe sul Garda, on *Tranzschelia fusca* (Pers.) Dietel on *Anemone nemorosa* L., 18. 4. 1999. (MFE 0650)
	AY292443*	Greece, Thessalia, Kalambaka, on *Gymnosporangium sabinae* (Dicks.) G. Winter on *Pyrus communis* L., 17. 9. 1981. (M 5803)
	AY292444*	Austria, Steiermark, Graz, on *Gymnosporangium sabinae* (Dicks.) G. Winter on *Pyrus communis* L., 29. 8. 1999. (TUB 011539)
	AY292445*	Dominica, on *Puccinia* sp., 21. 12. 1966. (CBS 271.67) - culture
	AY292447*	Costa Rica, Liberia, Rincon de la Vieja, on *Crossopsora notata* (Arthur, & J. R. Johnst.) Arthur on *Byrsonima crassifolia* H. B. & K., 31. 3 1992. (private collection R. Berndt 3159C)
	AY292449*	Germany, Baden-Württemberg, Tübingen, on *Ochropsora ariae* (Fuck.) Ramsb. on *Anemone nemorosa* L., 25. 4. 2002. (TUB 011546)
	AY292450*	Germany, Baden-Württemberg, Tübingen, on *Tranzschelia fusca* (Pers.) Dietel on *Anemone nemorosa* L., 25. 4. 2002. (TUB 011547)
	AY292451*	Germany, Baden-Württemberg, Tübingen, on *Uromyces poae* Rabenh. on *Ranunculus ficaria* L., 25. 4. 2002. (TUB 011548)
	AY292454*	Germany, Berlin, on *Puccinia coronata* Corda on *Rhamnus cathartica* L., 20. 5. 2002. (TUB 011549)
	AY292455*	Italy, Sardinia, Olbia, on aecia of *Ranunculus* sp., 8. 1. 2003. (TUB 011550
	AY460133	Italy, Liguria, Laigueglia, on *Endophyllum centranthi-rubri* Poir. on *Centranthus ruber* DC., 14. 5. 1978. (GZU 48-97)
	AY460134	Germany, Baden-Württemberg, Tübingen, on *Kuehneola uredinis* (Link) Arthur on *Rubus fruticosus* agg. L., 7. 11. 1999. (TUB 011622)
	AY460137	Germany, Baden-Württemberg, Tübingen, on *Melampsora* sp. on *Allium ursinum* L., 9. 5. 2001. (TUB 011593)
	AY460152	Grown experimentally from conidia of *Tuberculina persicina* (TUB 011539 - culture) on *Gymnosporangium cornutum* (Pers.) Arthur on *Sorbus aucuparia* L., 12. 8. 2001. (TUB 011607)
	AY460153	Grown experimentally from conidia of *Tuberculina persicina* (TUB 011539 - culture) on *Gymnosporangium sabinae* (Dicks.) G. Winter on *Pyrus communis* L., 12. 8. 2001. (TUB 011535)

TABLE III. Continued

Species	GenBank acc. no.[a]	Reference material[b]
Tuberculina persicina (Ditmar) Sacc.	AY460154	Germany, Baden-Württemberg, Stuttgart, Fellbach, on *Gymnosporangium sabinae* (Dicks.) G. Winter on *Pyrus communis* L., 27. 9. 2001. (TUB 011617)
	AY460155	Austria, Steiermark, Graz, on *Gymnosporangium sabinae* (Dicks.) G. Winter on *Pyrus communis* L., 29. 8. 1999. (TUB 011539) - culture
	AY460156	Germany, Baden-Württemberg, Tübingen, on *Phragmidium tuberculatum* Jul. Müll. on *Rosa canina* L., 25. 5. 2003. (TUB 011625)
	AY460157	Germany, Baden-Württemberg, Tübingen, on *Puccinia circaeae-caricis* Hasler on *Circaea lutetiana* L., 24. 6. 2001. (TUB 011621)
	AY460158	Germany, Baden-Württemberg, Tübingen, on *Puccinia convolvuli* (Pers.) Cast. on *Calystegia sepium* (L.) R. Br., 6. 8. 1999. (TUB 011623)
	AY460160	Germany, Brandenburg, Großer Klobisch See, on *Puccinia graminis* Pers. on *Berberis vulgaris* L., 21. 5. 2002. (TUB 011628)
	AY460161	Germany, Baden-Württemberg, Münsingen, on *Puccinia sessilis* W. G. Schneider in J. Schröt. on *Arum maculatum* L., 28. 5. 2001. (TUB 011616)
	AY460162	Germany, Baden-Württemberg, Tübingen, on *Puccinia poarum* Nielsen on *Tussilago farfara* L., 9. 1999. (TUB 011624)
	AY460163	Austria, Kärnten, Mallnitz on *Puccinia symphyti-bromorum* F. Muell. on *Symphytum* × *uplandicum* Nym., 28. 5. 2002. (TUB 011619)
	AY460164	Germany, Baden-Württemberg, Blumberg, Achdorf, on *Puccinia symphyti-bromorum* F. Muell. on *Symphytum officinale* L., 1. 7. 1999. (TUB 011620)
	AY460165	Germany, Baden-Württemberg, Tübingen, on *Puccinia senecionis-acutiformis* Hasler, Mayor & Cruchet on *Senecio ovatus* Willd., 5. 6. 2001. (TUB 011594)
	AY460166	Germany, Baden-Württemberg, Tübingen, on *Puccinia silvatica* J. Schröt. on *Taraxacum officinale* agg. F. H. Wigg., 5. 6. 2001. (TUB 011591)
	AY460167	Grown experimentally from conidia of *Helicobasidium purpureum* (TUB 011542 - culture) on *Puccinia silvatica* J. Schröt. on *Taraxacum officinale* agg. F. H. Wigg., 20. 6. 2001. (TUB 011627)
	AY460168	Grown experimentally from conidia of *Tuberculina persicina* (TUB 011618) on *Puccinia silvatica* J. Schröt. on *Taraxacum officinale* agg. F. H. Wigg., 20. 7. 2002. (TUB 011605)
	AY460169	Germany, Baden-Württemberg, Tübingen, on *Puccinia silvatica* J. Schröt. on *Taraxacum officinale* agg. F. H. Wigg., 4. 6. 2002. (TUB 011618)
	AY461070	Germany, Baden-Württemberg, Tübingen, on *Puccinia urticata* F. Kern on *Urtica dioica* L., 5. 6. 2001. (TUB 011592)
	AY460173	Germany, Baden-Württemberg, Tübingen, on *Tranzschelia pruni-spinosae* (Pers.) Dietel on *Anemone ramunculoides* L., 24. 4. 2001. (TUB 011608)
	AY460174	Grown experimentally from conidia of *Helicobasidium purpureum* (TUB 011542 - culture) on *Uromyces pisi* s.l. (DC.) G. H. Otth on *Euphorbia cyparissias* L., 1. 6. 2001. (TUB 011606)
	AY460175	Grown experimentally from conidia of *Tuberculina persicina* (TUB 011618) on *Uromyces pisi* s.l. (DC.) G. H. Otth on *Euphorbia cyparissias* L., 22. 7. 2002. (TUB 011626)
	AY460176	Germany, Baden-Württemberg, Tübingen, on *Uromyces pisi* s.l. (DC.) G. H. Otth on *Euphorbia cyparissias* L., 20. 5. 2001. (TUB 011590)
Tuberculina sbrozzii Cavara & Sacc.	AY292456*	France, Pouzols Minervois, Le Soleil d'Oc, on *Puccinia vincae* (DC.) Berk. on *Vinca major* L., 21. 5. 1996. (CBS 182.97) - culture
	AY292457*	United Kingdom, England, Berkshire, East Burnham, on *Puccinia vincae* (DC.) Berk. on *Vinca major* L., 31. 3. 2000. (K (M): 76122)
	AY292458*	Portugal, Madeira, Camacha, Levada da Serra, on *Puccinia vincae* (DC.) Berk. on *Vinca major* L., 18. 3. 1995. (K (M). 28801)

TABLE III. Continued

Species	GenBank acc. no.[a]	Reference material[b]
Tuberculina sbrozzii Cavara & Sacc.	AY460159	Italy, Lombardia, Brescia, Soiano del Lago, on *Puccinia cribrata* Arthur & Cummins on *Vinca minor* L., 18. 4. 1999. (M 0067220)
	AY460171	Germany, Baden-Württemberg, Tübingen, on *Puccinia vincae* (DC.) Berk. on *Vinca major* L., 10. 8. 2002. (TUB 011609)
	AY460172	Grown experimentally from conidia of *Tuberculina sbrozzii* (TUB 011609) on *Puccinia vincae* (DC.) Berk. on *Vinca major* L., 27. 5. 2003. (TUB 011613)

[a] Sequences taken from GenBank are denoted by *.

[b] Source acronyms: ATCC (American Type Culture Collection, Manassas, Virginia, USA); CBS (Centraalbureau voor Schimmelcultures, AG Baarn, The Netherlands); GZU (Herbarium of the Institut für Botanik, Karl-Franzens-Universität Graz, Austria); K (Herbarium of the Royal Botanic Gardens, Kew, England); M (Botanische Staatssammlung München, Germany); MFE (Microfungi exsiccati, Botanische Staatssammlung München, Germany); SAV (Institute of Botany, Slovak Academy of Sciences, Bratislava, Slovakia); TUB (Herbarium of the Spezielle Botanik/Mykologie, Eberhard-Karls-Universität Tübingen, Germany).

end of the aecial phase of the rust life cycle. *Tuberculina* infections never were observed in the control experiments. The actual derivation of the infections from the inocula was approved by the distribution of the infections being localized at the places of inoculation and by analyzing ITS base sequences of inoculum and infection with *Tuberculina* infections showing identical base sequences compared to the respective inocula (cf. below). For other inoculations, infections never were observed.

Field observations.—We have provided results of our 2 y observation of the rust flora, including some experimentally introduced rust species, around *Helicobasidium purpureum* basidiocarps (TABLE II).

Infection pattern.—Analyzing the pattern of infection and noninfection of the sampled rust species, the differences in the susceptibility of different rust hosts with respect to *Helicobasidium* and *Tuberculina* species and even to specimens of *Tuberculina persicina* became obvious. *Tuberculina* obtained from *Helicobasidium purpureum* infections were restricted to *Puccinia* spp., *Tranzschelia pruni-spinosae* and *Uromyces pisi* s.l., and in either case the inoculations resulted in *Tuberculina persicina* infections. *Tuberculina persicina* conidia from sporodochia on *Puccinia silvatica* could infect only *Puccinia silvatica* and *Uromyces pisi* s.l. For both inocula, we never obtained infections of *Chrysomyxa, Coleosporium, Cronartium* and *Gymnosporangium* species. The field observation is of the same tenor. *Tuberculina persicina* infections presumably caused by *Helicobasidium purpureum* basidiospores never were observed on *Coleosporium* and *Gymnosporangium* species but on several other rusts, whereat the host spectrum is extended to *Melampsora, Ochropsora* and other *Puccinia, Tranzschelia* and *Uromyces* species (TABLE II). In contrast, *Helicobasidium longisporum* caused only *Tuberculina persicina* infections of *Gymnosporangium* spp. and *Tuberculina persicina* conidia from sporodochia on *Gymnosporangium sabinae* were successful only on *Gymnosporangium* spp. For both inocula, we never obtained infections of *Chrysomyxa, Coleosporium, Cronartium, Puccinia, Tranzschelia* and *Uromyces* species. *Tuberculina maxima* conidia from sporodochia on *Gymnosporangium sabinae* were successful only on the original host *Gymnosporangium sabinae,* never on *Chrysomyxa, Coleosporium, Cronartium, Puccinia, Tranzschelia* and *Uromyces* species. *Puccinia vincae* was susceptible only to *Tuberculina sbrozzii* conidia from sporodochia, which were collected on the same host, and not to *Tuberculina maxima, T. persicina, Helicobasidium longisporum* and *H. purpureum.*

Molecular analyses.—The first aim of molecular analyses was to test whether the *Tuberculina* infections obtained in the infection experiments actually originated from the respective inocula. In fact we observed identical ITS base sequences for *Helicobasidium purpureum* (AY254189, AY460132) and *Tuberculina persicina* inocula (AY460169) and the respective *T. persicina* infections (AY254190, AY254191, AY254192, AY254193, AY460167, AY460168, AY460174, AY460175), *H. longisporum* (AY254187, AY254188) and *T. persicina* inocula (AY460155) and the respective *T. persicina* infections (AY254194, AY254195, AY460152, AY460153), *T. maxima* inoculum (AY460141) and the respective infection (AY460138), and *T. sbrozzii* inoculum (AY460171) and the respective infection (AY460172).

Furthermore, we inferred the phylogenetic relationships of the specimens used in the infection ex-

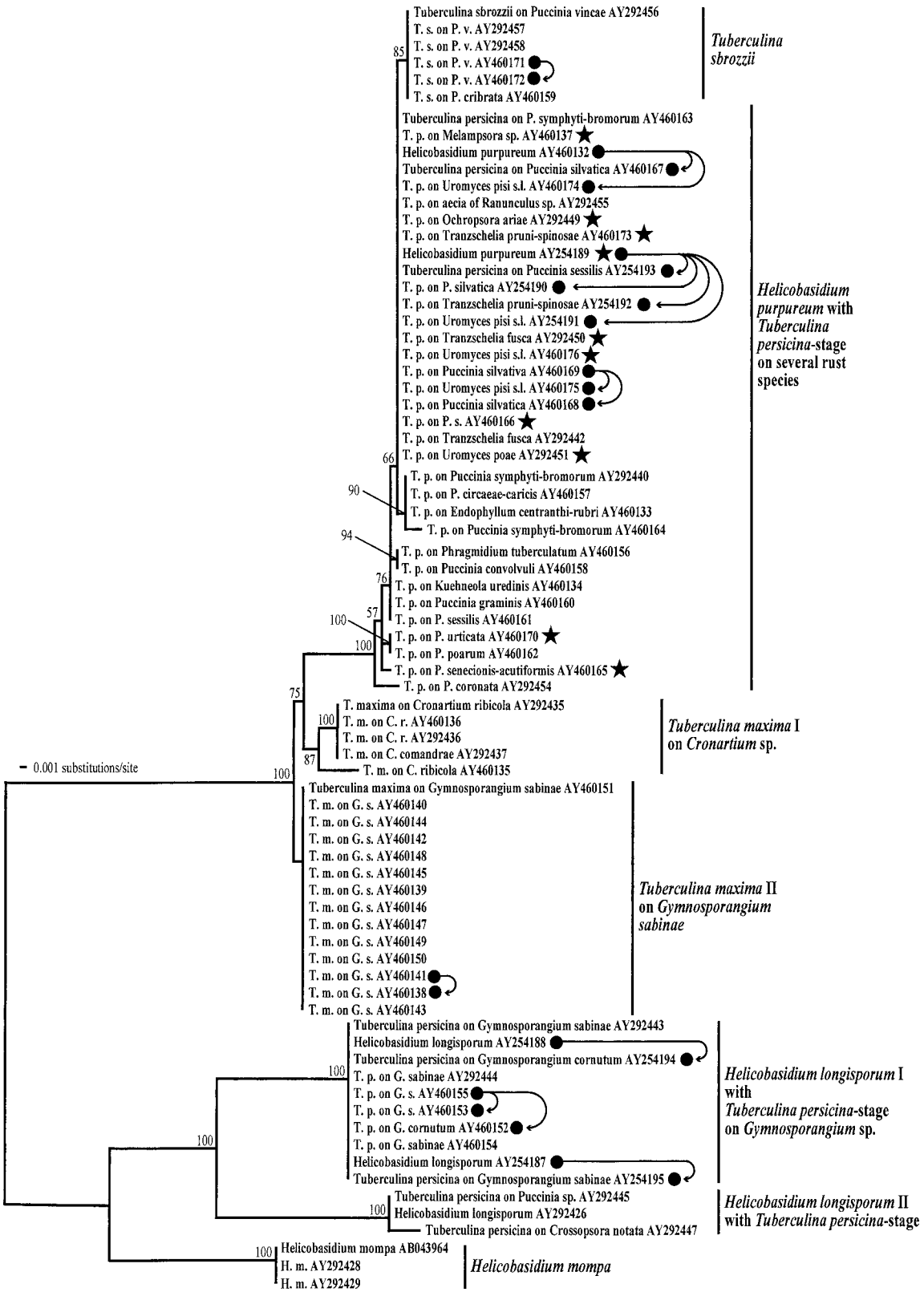

FIG. 1. Bayesian inference of phylogenetic relationships within selected *Tuberculina* and *Helicobasidium* specimens: Markov Chain Monte Carlo analysis of an alignment of base sequences from the ITS1/2 region of the nuc-rDNA including the 5.8S rDNA using the HKY model of DNA substitution with gamma distributed substitution rates, random starting trees and default starting parameters of the DNA substitution model. Majority rule consensus tree from 19 000 trees that were sampled after the process remained static. The topology was rooted with the specimens of the *Helicobasidium longisporum* I-, *H. longisporum* II-,

periments together with specimens collected in the field and sequences from GenBank that cover most of the hitherto known diversity of the *Helicobasidium/Tuberculina*-group. The Bayesian phylogenetic analyses yielded consistent topologies. We present the consensus tree of one run to illustrate the results (FIG. 1). The topology is consistent with recent analyses of ITS (Uetake et al 2002), LSU (Lutz et al 2004b) and combined ITS/LSU (Lutz et al 2004a) sequence datasets, but more taxa are included and it provides higher resolution. The phylogenetic hypothesis reveals two major groups with considerable genetic distance. The first consists of *Helicobasidium mompa* and the sister taxa *H. longisporum* I and *H. longisporum* II (each with the mitosporic life stage *Tuberculina persicina*). The second group comprises two clusters of *T. maxima,* one composed of specimens with *Gymnosporangium sabinae*-hosts (designated as *T. maxima* II), the other with *Cronartium*-hosts (designated as *T. maxima* I), and a group of *H. purpureum, T. persicina* and *T. sbrozzii* specimens. While the a posteriori probabilities for the branching in the *H. mompa/H. longisporum*-group are ideal, the *Tuberculina maxima* II-group lacks any support and the sister-group relationship of the highly supported *H. purpureum*-group (a posteriori probability 100%) and of the moderately supported *Tuberculina maxima* I-group (87%) is supported only weakly (75%). Within the *H. purpureum*-cluster, all specimens of *T. sbrozzii* fall into one clade.

Because we observed always identical sequences for inocula and the respective *Tuberculina* infections, the fact that inoculum and respective infection always occur in the same cluster is expected (cf. black spots and arrows in FIG. 1). In addition, including the sequences from GenBank and the specimens collected in the field, the phylogenetic analysis reveals that the groups within *Helicobasidium/Tuberculina,* as far as tested in the infection experiments, refer to the pattern of infection and noninfection of rust hosts. The only exception is the *H. longisporum* II-group with its *T. persicina*-stage on *Puccinia* sp. and *Crossopsora notata*.

All *Tuberculina* specimens collected in the field observation area around *H. purpureum* basidiocarps cluster in the *H. purpureum*-group (cf. black stars in FIG. 1). In contrast to the infection experiments, two sequences of *Tuberculina* specimens collected in the field observation area (AY460165, AY460170) show a divergence of 0.5% compared to that of the presumable inoculum (*Helicobasidium purpureum* AY254189) and compared to those of the other observed infections and a divergence of 0.4% compared to each other.

It is of interest to note that ITS sequences from specimens collected at distant places are identical in the groups *H. mompa* (the sampled sequences just represent many others from GenBank, which are identical), *H. longisporum* I, *T. maxima* II, and *T. sbrozzii*. In contrast, sequence variability ranges from identity to 1.3% divergence in *H. purpureum* and from identity to 0.7% divergence in *H. longisporum* II. In *T. maxima* I the specimens from North America (AY292435, AY292436, AY292437, AY460136) possess identical ITS sequences and show 1.3% divergence compared to the European specimen (AY460135).

DISCUSSION

Relation of Tuberculina *and rust hosts.*—Results of the presented infection experiments in connection with the phylogenetic hypothesis show clearly that lineages within the *Tuberculina/Helicobasidium*-group are characterized by host specificity of the *Tuberculina*-stage. *Tuberculina maxima* has two lineages, one restricted to *Gymnosporangium* (*T. maxima* II) the other to *Cronartium* (*T. maxima* I); *Tuberculina persicina* has at least two lineages, one restricted to *Gymnosporangium* with the sexual life stage *Helicobasidium longisporum* (*Helicobasidium longisporum* I) the other on several rust genera, but not on *Chrysomyxa-, Coleosporium-, Cronartium-* or *Gymnosporangium-* hosts with the sexual life stage *Helicobasidium purpureum* (*Helicobasidium purpureum*) and on *Tuberculina sbrozzii* restricted to *Puccinia vincae* and the closely related *P. cribrata* (Gäumann 1959). That lineages seem to be restricted to host species of one rust genus (*Tuberculina maxima* I, *T. maxima* II, *Helicobasidium longisporum* I) or even to few closely related species (*Tuberculina sbrozzii*) was of interest to us. In contrast the *Tuberculina*-stage of *Helicobasidium purpureum* is capable of parasitizing species of several rust genera (*Endophyllum, Kuehneola, Melampsora, Ochropsora, Phragmidium, Puccinia, Tranzschelia* and *Uromyces*),

←

and *H. mompa*-clade. Numbers on branches are estimates for a posteriori probabilities. Branch lengths were estimated with PAUP* version 4.0b10 (Swofford 2001) using maximum likelihood and the DNA substitution model proposed by MrModeltest 1.0b. They are scaled in terms of expected numbers of nucleotide substitutions per site. Black spots and arrows refer to specimens from the infection experiments. Big stars refer to specimens collected in the field-observation area (see Materials and Methods).

which represent diverse phylogenetic lineages of the rusts (for rust phylogeny see Maier et al 2003). Two members of the rust family Coleosporiaceae (the type genus *Coleosporium* and *Chrysomyxa*) conversely were not susceptible at all to *Tuberculina,* and despite intensive collection no *Tuberculina* infections of those genera have been reported. In contrast, *Gymnosporangium* species are susceptible for both *Tuberculina maxima* II and the *Tuberculina*-stage of *Helicobasidium longisporum* I. The observed host specificities of the *Tuberculina*-stage are in contrast to the unspecific choice of plant hosts of the *Helicobasidium*-stage (Duggar 1915, Hering 1962, Itô 1949, Viennot-Bourgin 1949).

The combination of host specificities, morphological and molecular characters reveals more lineages within the *Tuberculina/Helicobasidium*-group than hitherto distinguished (in the case of *Tuberculina* see Ellis and Ellis 1988; for *Helicobasidium* see Roberts 1999, Uetake et al 2002) and consequently demands a revision of the species concept within the group to cope adequately with the diversity. The genus *Tuberculina* should be restricted to rust parasites, and species delimitation requires both morphological and molecular characters, as well as information about the host spectrum. Taxonomical conclusions are in preparation.

Diversity and evolution of the Tuberculina/Helicobasidium*-group.*—Synopsis of host specificities, ecology, morphological and molecular characters reveals these seven groups (cf. FIG. 1):

(i) *Helicobasidium mompa* causes the economically important violet root rot and seems to be restricted to east Asia. A definite morphological characterization still is lacking, whereas the species can be distinguished clearly by its specific ITS base sequence (Uetake et al 2002). Unfortunately, no *Tuberculina* specimens from Asia were available. *Helicobasidium mompa* was demonstrated to have a uniform population structure, presumably reproducing mainly or exclusively asexually via hyphae or sclerotia (Katsumata et al 1996). The clonal population structure is suggested by the uniformity of ITS sequences of *Helicobasidium mompa* isolates. Uetake et al (2002) showed that among 17 isolates from several host plants in Japan and Korea only two had a single base substitution. Consequently, one could think of a species that is adapted to the life in annually disturbed habitats (cf. Uetake et al 2003) and that possibly is not dependent on the *Tuberculina*-stage for reproduction.

(ii) *Helicobasidium longisporum* II and its small-spored *Tuberculina*-stage clearly are distinguished by molecular characters but cannot be discussed with any satisfaction here for three reasons. Because no sporulating material was available from specimens of that group, we were not able to include the group in our infection experiments. The *H. longisporum* specimen examined in this study is available only as a culture without formation of basidia. For that reason the validation of *H. longisporum* was impossible. The spore morphology of the *Tuberculina* specimen (He R. Berndt 3159C, AY292447) belonging to that group is in contrast to all other *T. persicina* specimens because it exhibits slightly warty spore walls (data not shown). Further investigations are necessary to clarify the morphological characters of specimens of that group.

(iii) *Helicobasidium longisporum* I is characterized and distinguished from *H. purpureum* by size (16–21 × 4,5–6 μm versus 8–13 × 4,5–6 μm) and shape (elongated clavate versus oblong to suballantoid) of basidiospores (Roberts 1999, data corresponding to our observations) and by its small-spored *Tuberculina*-stage, which seems to be restricted to *Gymnosporangium* species. Even though both the *Helicobasidium*- and the *Tuberculina*-stage are present and, as a consequence sexual reproduction could be assumed, the specimens collected in Greece (AY292443), Austria (AY292444) and Germany (AY254187, AY460154) show identical ITS base sequences.

(iv) The clade *Tuberculina maxima* II is not supported by our molecular analyses, but compared to *Tuberculina persicina* all specimens possess larger conidia (10–14 μm diam versus 7–10 μm, our observations) with thicker spore walls (about 1 μm, our observations) and all were obtained from *Gymnosporangium sabinae*- hosts. The specimens, which were collected from northern Germany to southern Austria possess identical ITS base sequences, which are distinguished by five base substitutions (positions 75, 132, 335, 442 and 546 of the alignment) from *T. maxima* I. The species could be assumed to parasitize other *Gymnosporangium* species too (Farr et al 2003).

(v) *Tuberculina maxima* I exhibits resembling spore features as *T. maxima* II (cf. Hubert 1935) but is restricted to *Cronartium*- hosts. We found it interesting to note that the specimens from North America clearly are separated by the molecular data from the European specimen. This supports the indigenous nature of that lineage that was assumed by Mielke (1933), considering the distribution of *T. maxima* on several *Cronartium* species.

(vi) The basidiospores of *Helicobasidium purpureum* are shorter (8–13 × 4,5–6 μm versus 16–21 × 4,5–6 μm) compared to *H. longisporum* and oblong to suballantoid (versus elongated clavate) (Roberts 1999, data corresponding to our observations). The species' *Tuberculina*-stage is small-spored (7–10 μm

diam, our observations) and occurs on several rust genera.

(vii) By means of a light microscope, *Tuberculina sbrozzii* was not distinguishable from *Tuberculina persicina*. However because all *Tuberculina* specimens from *Puccinia vincae,* which were collected at places distant from each other (England, France, Germany, Madeira) clustered together in the molecular analyses and because they all are characterized by a single base substitution (position 450 of the alignment), we decided to designate the specimens *T. sbrozzii*. In addition, the *Tuberculina* specimen parasitizing *Puccinia cribrata,* which is presumably the closest relative of *P. vincae* (Gäumann 1959), clusters in that group, too. Moreover, in the infection experiments, we were not able to obtain *Tuberculina* infections of *P. vincae* from inocula other than *T. sbrozzii*. Consequently, *T. sbrozzii,* which was erected in 1899 for a *Tuberculina* specimen parasitizing *Puccinia vincae* (Cavara and Saccardo 1899), is presumably a monophyletic lineage, even though *Helicobasidium purpureum* appears then as paraphyletic in the molecular analyses. However, this might be due to lacking resolution of the DNA region.

Our results further suggest that lineages might have evolved that have lost the *Helicobasidium*-stage (*Tuberculina maxima* I, *T. maxima* II and *T. sbrozzii*). *T. maxima* II is common in Germany, but we were not able to detect any referring *Helicobasidium* specimen. The same is true for *T. maxima* I in North America. Both species parasitize rusts that are perennial, and both species are proven to be capable of overwintering as perennial mycelia with their rust fungal hosts in plant tissues and as spores (Wicker and Wells 1968, our observations). The capacity to overwinter might be due to the larger spores with thicker spore walls compared to *Tuberculina persicina* or *T. sbrozzii*. Thus, the soil-borne, perennial *Helicobasidium*-stage is not needed. In addition, *Tuberculina* sclerotia, the very structures that presumably mark the changeover from the *Tuberculina*- to the *Helicobasidium*-stage (Lutz et al 2004a), are unknown for *T. maxima*. The loss of the *Tuberculina*-stage involves a loss of sexual reproduction and consequently should result in a clonal population structure, which might be reflected by the uniformity of the ITS base sequences. Without exception the ITS sequences of *T. maxima* II specimens collected from northern Germany to southern Austria are identical, just as the *T. maxima* I sequences from North American specimens are. In that context, *T. maxima* I AY460135 from Europe has to be assigned to a clone of itself. The sequences of the *T. sbrozzii* specimens collected in England, France, Germany, Italy and Madeira are identical too, it should be noted. In addition, their host rusts *Puccinia cribrata* and *P. vincae* are perennial (Gäumann 1959). *T. sbrozzii* possibly has evolved as clonal lineage accompanying *P. vincae* and closest relatives. The *Tuberculina* species that have lost the phytoparasitic *Helicobasidium*-stage still might be considered potential agents in biological rust control.

Our infection experiments finally reveal that not only *Tuberculina* conidia (cf. Barkai-Golan 1959, Vladimirskaya 1939, Wicker and Kimmey 1967) but also both *Helicobasidium* conidia formed in culture and basidiospores serve the same purpose: the infection of rust hosts. This might explain the observation of Ikeda et al (2003) that single basidiospore isolates of *Helicobasidium mompa* are not pathogenic with respect to plants; it also might explain the observation that, despite extensive research, no *Helicobasidium* infections of plants from inoculations with *Helicobasidium* basidiospores were reported.

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