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Tuberculina – Thanatophytum/Rhizoctonia crocorum – Helicobasidium: a unique mycoparasitic–phytoparasitic life strategy†

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Tuberculina species are mitosporic parasites of rust fungi. Phylogenetically they belong to the *Urediniomycetidae*, therefore being closely related to their rust fungal hosts. We reveal by means of molecular analyses, ultrastructural and morphological features, observations in the field, and infection experiments that species of the genus *Tuberculina* and the violet root rot (*Helicobasidium/Rhizoctonia crocorum*) are stages of the life-cycle of one holomorph. This opens up new perspectives on parasitic life strategies as the resulting life-cycle is based on interkingdom host jumping between rusts and spermatophytes. In addition, we point at the consequences for any practical application dealing with *Helicobasidium* as an economically important plant pathogen and *Tuberculina* as a biological agent in rust control.

INTRODUCTION

In parasitic fungi, complex life strategies have evolved in several lineages (e.g. Gäumann 1964). For example, the rust fungi produce up to five different spore forms, normally depending on two unrelated host plants (e.g. Gäumann 1959) or the smut fungi possess a two-phase life-cycle with a saprobic haploid phase and a phytoparasitic dikaryophase (e.g. Bauer *et al*. 2001). In some cases the life stages of one fungal species are so different from each other that they have been regarded as separate species for a long time (e.g. Begerow, Bauer & Boekhout 2000). Here, we present data that show that species of the mycoparasitic fungal genus *Tuberculina* and species from the plant-parasitic genus *Helicobasidium* are stages of the life-cycle of one holomorph. Thereby, we reveal a hitherto unknown life strategy, which is characterised by, in respect to sexual reproduction, obligate host alternation between rust fungi and plants, each serving as hosts for a biologically distinct life stage. The life-cycle presented with its interkingdom host jump opens up new vistas on parasitic life strategies and the evolution of fungal parasitism. Furthermore, that the rust-parasitic *Tuberculina* and the plant-parasitic *Helicobasidium* are different stages in one life-cycle, calls for a re-evaluation of attempts to use *Tuberculina* as a biological agent in rust control and, finally, offers new perspectives to combat the important plant pathogen *Helicobasidium*.

The mycoparasitic stage of the life-cycle is represented by the genus *Tuberculina*. *Tuberculina* species occur all over the world, living in association with over 150 rust species from at least 15 genera. They are restricted to the haploid stages of their rust fungal hosts (Wicker 1980) growing between the hyphae of their hosts in leaves and stems of plants. When sporulating, *Tuberculina* becomes visible as hemispherical, lilac to violet mycelia that burst through the plant surface, generally close to rust sori, releasing a powdery mass of conidia. The strong inhibitory effect of *Tuberculina* on rust spore production has resulted in extensive research dealing with *Tuberculina* as a biological agent in rust control (Wicker 1981). Recently, we demonstrated that *Tuberculina* species are phylogenetically closely related to their associates, the rust fungi. Additionally, we substantiated the mycoparasitic nature of *Tuberculina* species on rusts, showing cellular interaction *via* large fusion pores (Lutz *et al*. 2004).

Helicobasidium species represent the other stage of the life-cycle presented here. Surprisingly, they are serious plant pathogens causing the economically important violet root rot. Worldwide over 120 species representing more than 50 families of spermatophytes are known as hosts (Itô 1949). *Helicobasidium* species

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Table 1. List of material studied, DNA isolation numbers, reference materials, characters studied and GenBank accession numbers.

Species	DNA isolation no.	Reference material[a]	Characters studied[b]	GenBank accession no.
Helicobasidium longisporum (syn. *H. compactum*)	ml33	**Germany**: *Baden-Württemberg*: Stuttgart, on *Pyrus communis*, 17 Oct. 2000, *M. Lutz 742* (TUB 011540)	I, S: ITS, LSU	AY254187, AY292401
	ml103	**USA**: on *Coffea* sp. (CBS 296.50) – culture	S: ITS	AY292426
	ml123	**Austria**: *Steiermark*: Graz, on *Iris germanica* (cult.), 28 Sept. 1999 (GZU 74-99)	S: ITS, LSU	AY292427, AY254177
	ml423	**Germany**: *Baden-Württemberg*: Nürtingen, Raidwangen, on *Sambucus nigra*, 25 April 2001, *M. Lutz 848* (TUB 011541) – culture	C, I, S: ITS, LSU	AY254188, AY292402
	ml560	**Germany**: *Baden-Württemberg*: Tübingen, April 1977, *F. Oberwinkler 24482*	E	
H. mompa	ml127	**Japan**: on *Asparagus officinalis* (ATCC 56070) – culture	S: ITS, LSU	AY292428, AY254178
	ml490	**Japan** (CBS 278.51) – culture	S: ITS, LSU	AY292429, AY254179
H. purpureum (syn. *H. brebissonii*)	ml139	**Germany**: *Baden-Württemberg*: Tübingen, on *Carpinus betulus*, 5 April 2001, *M. Lutz 828* (TUB 011542)	C, I, S: ITS, LSU	AY254189, AY254180
	ml153	**Germany**: *Baden-Württemberg*: Nürtingen, Raidwangen, on *Prunus spinosa*, 25 April 2001, *M. Lutz 847* (TUB 011544)	S: ITS, LSU	AY292430, AY292403
	ml418	**Belgium**: Antwerpen, on hardwood, 15 April 1995, *De Meulder 10644* (BR 37479-37)	S: ITS, LSU	AY292431, AY292404
Thanatophytum crocorum (syn. *Rhizoctonia crocorum*)	ml88	**Germany**: on *Solanum tuberosum* (CBS 162.24) – culture	S: ITS, LSU	AY292432, AY292405
	ml491	**Germany**: on *Beta vulgaris* (CBS 163.24) – culture	S: ITS, LSU	AY292433, AY254181
	ml592	**Italy**: on *Phaseolus* sp. (CBS 324.47) – culture	S: ITS, LSU	AY292434, AY292406
Tuberculina maxima	ml26	**USA**: *Idaho*: Kaniksu National Forest, on *Cronartium ribicola* on *Pinus albicaulis*, 7 Oct. 1965 (CBS 135.66) – culture	S: ITS, LSU	AY292435, AY292407
	ml29	**Canada**: *British Columbia*: Wap Lake, on *Cronartium ribicola* on *Pinus monticola*, 12 Sept. 1965 (CBS 136.66) – culture	S: ITS	AY292436
	ml593	**USA**: *Wyoming*: Teton, on *Cronartium comandrae* on *Pinus contorta*, 22 Sept. 1965 (CBS 137.66) – culture	S: ITS, LSU	AY292437, AY292408
T. persicina	ml1	**Austria**: *Burgenland*: Neusiedler See, on *Uromyces junci* on *Pulicaria dysenterica*, 19 June 1998 (GZU 11-98)	S: ITS, LSU	AY292438, AY292409
	ml22	**Russia**: St Petersburg, on *Peridermium* sp. (CBS 389.39) – culture	S: ITS, LSU	AY292439, AY292410
	ml69	**Slovakia**: Dolina, on *Puccinia symphyti-bromorum* on *Pulmonaria obscura*, 14 July 2000 (SAV)	S: ITS, LSU	AY292440, AY292411
	ml71	**Germany**: *Baden-Württemberg*: Tübingen, on *Puccinia poarum* on *Tussilago farfara*, 17 Aug. 1999, *R. Bauer 3032* (TUB 011531) – culture	S: ITS, LSU	AY292441, AY254184
	ml79	**Italy**: *Lombardia*: Padenghe sul Garda, on *Tranzschelia fusca* on *Anemone nemorosa*, 18 April 1999 (MFE 0650)	S: ITS, LSU	AY292442, AY292412
	ml81	**Greece**: *Thessalia*: Kalambaka, on *Gymnosporangium sabinae* on *Pyrus communis*, 17 Sept. 1981 *P. Döbbeler 4194* (M 5803)	S: ITS, LSU	AY292443, AY292413
	ml100	**Austria**: *Steiermark*: Graz, on *Gymnosporangium sabinae* on *Pyrus communis*, 29 Aug. 1999, *R. Bauer 3035* (TUB 011539)	C, S: ITS, LSU	AY292444, AY292414
	ml125	**Dominica**: on *Puccinia* sp., 21 Dec. 1966 (CBS 271.67) – culture	S: ITS, LSU	AY292445, AY254185
	ml149	Grown experimentally from basidiospores of *Helicobasidium purpureum* (*M. Lutz 828*, TUB 011542) on *Puccinia silvatica* on *Taraxacum officinale* agg., 25 April 2001, *M. Lutz 854* (TUB 011532)	I, S: ITS	AY254190
	ml155	**Germany**: *Baden-Württemberg*: Nürtingen, Raidwangen, on *Tranzschelia pruni-spinosae* on *Anemone ranunculoides*, 25 April 2001, *M. Lutz 851* (TUB 011530)	C, E, S: ITS	AY292446
	ml164	Grown experimentally from basidiospores of *Helicobasidium purpureum* (*M. Lutz 828*, TUB 011542) on *Uromyces pisi* on *Euphorbia cyparissias*, 30 April 2001, *M. Lutz 869* (TUB 011533)	I, S: ITS	AY254191
	ml173	Grown experimentally from basidiospores of *Helicobasidium purpureum* (*M. Lutz 828*, TUB 011542) on *Tranzschelia pruni-spinosae* on *Anemone ranunculoides*, 6 May 2001, *M. Lutz 877* (TUB 011536)	I, S: ITS	AY254192

Table 1. (*Cont.*)

Species	DNA isolation no.	Reference material[a]	Characters studied[b]	GenBank accession no.
	ml191	Grown experimentally from basidiospores of *Helicobasidium purpureum* (*M. Lutz 828*, TUB 011542) on *Puccinia sessilis* on *Allium ursinum*, 9 May 2001, *M. Lutz 892* (TUB 011534)	I, S: ITS	AY254193
	ml199	**Costa Rica**: *Liberia*: Rincon de la Vieja, on *Crossopsora notata* on *Byrsonima crassifolia*, 31 March 1992, *He R. Berndt 3159C*	S: ITS, LSU	AY292447, AY292415
	ml316	**Germany**: Berlin: on *Puccinia symphyti-bromorum* on *Symphytum officinale*, 15 July 2001, *M. Lutz 992* (TUB 011545)	S: ITS, LSU	AY292448, AY292416
	ml340	Grown experimentally from conidia of *Helicobasidium longisporum* (*M. Lutz 848*, TUB 011541) on *Gymnosporangium cornutum* on *Sorbus aucuparia*, 28 July 2001, *M. Lutz 1217* (TUB 011537)	I, S: ITS	AY254194
	ml362	Grown experimentally from basidiospores of *Helicobasidium longisporum* (*M. Lutz 742*, TUB 011540) on *Gymnosporangium sabinae* on *Pyrus communis*, 10 Oct. 2001, *M. Lutz 1184* (TUB 011538)	I, S: ITS	AY254195
	ml445	**Germany**: *Baden-Württemberg*: Tübingen, on *Ochropsora ariae* on *Anemone nemorosa*, 25 April 2002, *M. Lutz 1271* (TUB 011546)	S: ITS, LSU	AY292449, AY292417
	ml446	**Germany**: *Baden-Württemberg*: Tübingen, on *Tranzschelia fusca* on *Anemone nemorosa*, 25 April 2002, *M. Lutz 1270* (TUB 011547)	S: ITS, LSU	AY292450, AY292418
	ml447	**Germany**: *Baden-Württemberg*: Tübingen, on *Uromyces poae* on *Ranunculus ficaria*, 25 April 2002, *M. Lutz 1269* (TUB 011548)	S: ITS, LSU	AY292451, AY292419
	ml451	**Namibia**: Waterberg, on *Aecidium pergulariae* on *Pergularia daemia*, 18 April 2002, *M. Mennicken NA 315* (WIND)	S: ITS, LSU	AY292452, AY292420
	ml452	**Namibia**: Otavi Mountains, on *Puccinia agrophila* on *Solanum* delagoense, 17 April 2002, *M. Mennicken NA 311* (WIND)	S: ITS, LSU	AY292453, AY292421
	ml464	**Germany**: Berlin, on *Puccinia coronata* on *Rhamnus cathartica*, 20 May 2002, *M. Lutz 1337* (TUB 011549)	S: ITS, LSU	AY292454, AY292422
	ml548	**Italy**: *Sardinia*: Olbia, on aecia of *Ranunculus* sp., 8 Jan. 2003, *M. Lutz 1452* (TUB 011550)	S: ITS, LSU	AY292455, AY292423
	ml601	Grown experimentally from conidia of *Tuberculina persicina* (*R. Baner 3035*, TUB 011539 – culture) on *Gymnosporangium sabinae* on *Pyrus communis*, 15 Sept. 2002, *M. Lutz 1423* (TUB 011595)	C	
T. sbrozzii	ml174	**France**: Pouzols Minervois, Le Soleil d'Oc, on *Puccinia vincae* on *Vinca major*, 21 May 1996 (CBS 182.97) – culture	S: ITS, LSU	AY292456, AY292424
	ml394	**UK**: *Berkshire*: East Burnham, on *Puccinia vincae* on *Vinca major*, 31 March 2000 (K (M) 76122)	S: ITS	AY292457
	ml395	**Portugal**: *Madeira*: Camacha, Levada da Serra, on *Puccinia vincae* on *Vinca major*, 18 March 1995 (K (M) 28801)	S: ITS, LSU	AY292458, AY292425

[a] Source acronyms: ATCC, American Type Culture Collection, Manassas, VA; BR, Jardin Botanique National de Belgique, Meise; CBS, Centraalbureau voor Schimmelcultures, Utrecht; GZU, Herbar des Instituts für Botanik, Karl-Franzens-Universität Graz; K, Royal Botanic Gardens, Kew; M, Botanische Staatssammlung München; MFE, Microfungi exsiccati, Botanische Staatssammlung München; SAV, Institute of Botany, Slovak Academy of Sciences, Bratislava; TUB, Herbarium of the Spezielle Botanik/Mykologie, Eberhard-Karls-Universität Tübingen, Tübingen; WIND, National Botanical Research Institute, Windhoek, Namibia.

[b] C, culture; E, electron microscopy; I, infection experiment; S, sequence; ITS, ITS1/2 region of the rDNA including the 5.8S rDNA localized between the nuc-SSU rDNA and nuc-LSU rDNA; LSU, 5′-end of the nuc-LSU rDNA including the domains D1/D2.

occur mainly in the sterile form known as *Rhizoctonia crocorum* or *Thanatophytum* (*cfr* Gams 2001), respectively. They live as soil-borne root pathogens covering the roots of their host plants with a dense hyphal coat. The *Helicobasidium*-stage of sexual reproduction is developed above the ground, forming a fine hymenium arising on perennial mycelial coats that normally cover the base of the infected plants. In the case of *H. mompa*, the infected plants quickly wither and die, whereas other species seem to be less destructive. Being economically important, extensive research has been conducted on the biology of and combat against *Helicobasidium* resulting in detailed knowledge of host range (Itô 1949, Hering 1962a), conditions of growth (Garrett 1949,

Valder 1958, Suzuki 1978, Fukushima 1998, Uetake *et al*. 2003), metabolites (Nishikawa 1962, Takai 1966), and parasite–host interaction (Hering 1962b, Ieki 1975a, b, Matsuzaki, Haneda & Mitsueda 1986). But fundamental questions on its life-cycle remained so far unanswered (Ikeda, Nakamura & Matsumoto 2003).

MATERIALS AND METHODS

Fungal material

For the studied species, DNA isolation numbers, reference materials, characters studied, and GenBank accession numbers, see Table 1.

Molecular analyses

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). We determined base sequences of the 5′-end of the nuc-LSU rDNA including the domains D1/D2 (LSU) and of the ITS1/2 region of the rDNA including the 5.8S rDNA (ITS) of 42 *Helicobasidium*, *Thanatophytum* and *Tuberculina* specimens (Table 1), including all commonly distinguished species. The ITS region (about 600 bp) was amplified 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.

To estimate phylogenetic relationships, we added sequences of the closest relatives (Lutz *et al*. 2004) including two *Tuberculina* hosts from GenBank (accession nos. in parentheses): *Eocronartium muscicola* (L20280), *Gymnosporangium sabinae* (AF426209), *Helicobasidium longisporum* (syn. *H. compactum*) (AY222046), *Herpobasidium filicinum* (AF426193), *Melampsora lini* (L20283), *Pachnocybe ferruginea* (L20284), *Septobasidium carestianum* (L20289), *Tranzschelia pruni-spinosae* (AF426224), *Tuberculina maxima* (AY222044), *Tuberculina persicina* (AY222043), and *Tuberculina sbrozzii* (AY222045). 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 & Ronquist 2001). Four incrementally heated simultaneous Markov chains were run over 2 000 000 generations using the general time reversible model of DNA substitution with gamma distributed substitution rates (Rodriguez *et al*. 1990, Gu, Fu & Li 1995) and estimation of invariant sites, random starting trees and default starting parameters of the DNA substitution model (Huelsenbeck & Ronquist 2001). Trees were sampled every 100 generations resulting in an overall sampling of 20 001 trees. From these, the first 1000 trees were discarded (burnin = 1000). The trees computed after the process reached stationarity (19 001 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 of phylogenetic analysis was repeated ten times to test the independence of the results from topological priors (*cfr* Huelsenbeck *et al*. 2002). DNA sequences determined for this study were deposited in GenBank, accession numbers are given in Table 1. The alignment (43 sequences; length: 1147 bp; after exclusion of the sites 579–580: 257 variable sites) is available from Tree BASE (matrix accession no. M1726).

Electron microscopy

For transmission electron microscopy (TEM), samples were prepared in two ways: (1) Fixed with 2% glutaraldehyde in 0.1 M sodium cacodylate buffer (pH 7.2) at *ca* 20 ° overnight. Following six transfers in 0.1 M sodium cacodylate buffer, samples were postfixed in 1% osmium tetroxide in the same buffer for 1 h in the dark, washed in distilled water, and stained in 1% aqueous uranyl acetate for 1 h in the dark. After five washes in distilled water, samples were dehydrated in acetone, using 10 min changes at 25, 50, 70, 95, and three times in 100% acetone. Samples were embedded in Spurr's plastic and sectioned with a diamond knife. Ultrathin serial sections were mounted on formvar-coated, single-slot copper grids, stained with lead citrate at room temperature for 5 min, and washed with distilled water. They were examined using a Zeiss EM 109 transmission electron microscope operating at 80 kV. (2) Samples were prepared by high pressure freezing and freeze substitution. Infected areas of leaves were removed with a 2 mm cork borer. To remove air from intercellular spaces, samples were infiltrated with distilled water containing 6% (v/v) (2.5 M) methanol for approx. 5 min at *ca* 20 °. Single samples were placed in an aluminium holder and frozen immediately in the high pressure freezer HPM 010 (Balzers Union, Liechtenstein). Substitution medium (1.5 ml per specimen) consisted of 2% osmium tetroxide in acetone, which was dried over calcium chloride. Freeze substitution was performed at −90 °, −60 °, and −30 °, 8 h for each step, using a Balzers freeze substitution apparatus FSU 010. The temperature was then raised to approximately 0 ° over a 30 min period and samples were washed in dry acetone for another 30 min. Infiltration with an Epon/Araldite mixture was performed stepwise: 30% resin in acetone at 4 ° for 7 h, 70% and 100% resin at 8 ° for 20 h each and 100% resin at 18 ° for approximately 12 h. Samples were then transferred to fresh medium and polymerised at 60 ° for 10 h. Finally, samples were processed as described above for chemically fixed samples, except that the ultrathin sections were additionally stained with 1% aqueous uranyl acetate for 1 h.

Cultures

We obtained cultures from *Tuberculina* conidia as well as from *Helicobasidium* basidiospores on malt-extract

agar (20 g malt extract, 20 g glucose, 15 g agar, 1 g universal peptone per litre distilled water), and on malt–yeast–peptone agar (15 g agar, 7 g malt extract, 1 g universal peptone, 0.5 g yeast extract l^{-1} distilled water). The *Helicobasidium* cultures were started by fixing small pieces of freshly collected sporulating basidiomata at the underside of the lid of culture dishes. After 1 d of incubation at *ca* 20 °, the basidiomes were removed and the germination of the basidiospores was checked microscopically. After a few days, cultural growth could be observed microscopically in some places by a change of hyphal characters and by the change of the colour of the medium to violet. For *Tuberculina*, a small number of conidia obtained from freshly collected sporodochia was spread over the media with the help of a very fine needle.

Infection experiments

The rusts for inoculation were obtained in three different ways: (1) *Puccinia sessilis* on *Allium ursinum*, *Tranzschelia pruni-spinosae* on *Anemone ranunculoides*, *Gymnosporangium sabinae* on *Pyrus communis*, and *G. cornutum* on *Sorbus aucuparia* collected growing in nature in places where we were not able to detect any *Tuberculina* infection in the previous growth season. We divided the places into isolated areas where we inoculated the rust infected plants and areas without inoculation for control. (2) *Euphorbia cyparissias* plants that were systemically infected by *Uromyces pisi s. lat.* in nature were transferred to our greenhouse. The plants inoculated with the hyperparasite and control plants were cultivated in different houses. After inoculation, the single plants were incubated for 3 d under transparent plastic bags to provide high humidity. And (3) cultivated *Taraxacum officinale* aggr. in our greenhouse was infected with *Puccinia silvatica* using the wire net method (Kakishima, Yokoi & Harada 1999). As inoculum we used freshly collected leaves of *Carex brizoides* harbouring sori of teliospores of the rust. Inoculation with the hyperparasite and control were handled as in (2).

For the inoculation of rusts, we vigorously shook basidiospores from freshly collected basidiomes or conidia from *Helicobasidium* cultures in tap water and spread the inoculum with a small brush over the upside and the underside of rust infected leaves, or, for the inoculation of *Gymnosporangium sabinae*, we collected intensively sporulating basidiomes of *Helicobasidium longisporum* and *H. purpureum* in the field, tore the fresh basidiomata into pieces *ca* 4 cm^2 and tagged the pieces to pear trees, which we had already inoculated with *G. sabinae* to obtain heavy rust infections. In every case, we inoculated the rusts with their mycoparasites as soon as the rust pycnia were mature. After inoculation, the inoculated plants and the control were monitored for the time the respective rusts needed to complete their life-phase on the respective host plant.

The plants for inoculation with *Tuberculina* conidia and for control were grown from seed or young seedlings in our greenhouse. For the inoculation of plants, we vigorously shook freshly collected *Tuberculina* conidia in tap water, spread the inoculum over the roots, and re-planted the young seedlings. After inoculation, the inoculated plants and the control were grown in the greenhouse and observed as long as they lived.

RESULTS AND DISCUSSION

Molecular analyses

Molecular analyses gave rise to the idea of *Tuberculina* and *Helicobasidium* being stages of the life-cycle of one holomorph. Spacer regions of the rDNA (ITS, IGS) in particular change rapidly and therefore show a relatively high amount of dissimilarities between closely related taxa (Diaz & Fell 2000, Scorzetti *et al.* 2002) or even within species (O'Donnell 1992). In contrast, identical ITS or IGS base sequences have never been observed for morphologically or physiologically distinct species (Gardes & Bruns 1993, Horton 2002). Consequently, sequence analyses using ITS data is recommended for species identification in, for example, basidiomycetous yeasts (Scorzetti *et al.* 2002) and ectomycorrhiza forming fungi (Horton 2002).

All LSU- and ITS-sequences obtained from *Tuberculina* specimens showed the highest similarities to *Helicobasidium* and '*Rhizoctonia crocorum*' (*Thanatophytum*) sequences, respectively, compared to the sequence data available in GenBank. Including the data from GenBank, identical ITS- and LSU + ITS-sequences of *Tuberculina* and *Helicobasidium* or *Thanatophytum* specimens, respectively, occurred in four groups labelled in Fig. 1 as *H. purpureum* I and II, and *H. longisporum* I and II (see Table 2). Specimens from distant collection sites shared identical ITS sequences (see Table 2). Interestingly, we never found a *Tuberculina* specimen belonging to the serious plant pathogen *Helicobasidium mompa*, and we only observed *Helicobasidium* specimens belonging to *T. persicina*, and never to *T. maxima* or *T. sbrozzii* (Fig. 1).

Morphology

Morphological studies support the hypothesis of a shared identity of *Tuberculina* and *Helicobasidium*. Buddin & Wakefield (1927) already demonstrated that cultures of *Helicobasidium* on artificial media produce '… small raised tubercles, which eventually become pustules of conidia of the type which is characteristic of the genus *Tuberculina* …'. That observation was repeated several times (Valder 1958, Sayama, Kobayashi & Ogoshi 1994, Fukushima 1998) but without explicit conclusions or further investigations. We observed morphological conformity not only in the type of conidiogenesis and morphologies of conidiophores and conidia of *Tuberculina* and *Helicobasidium*, but also in

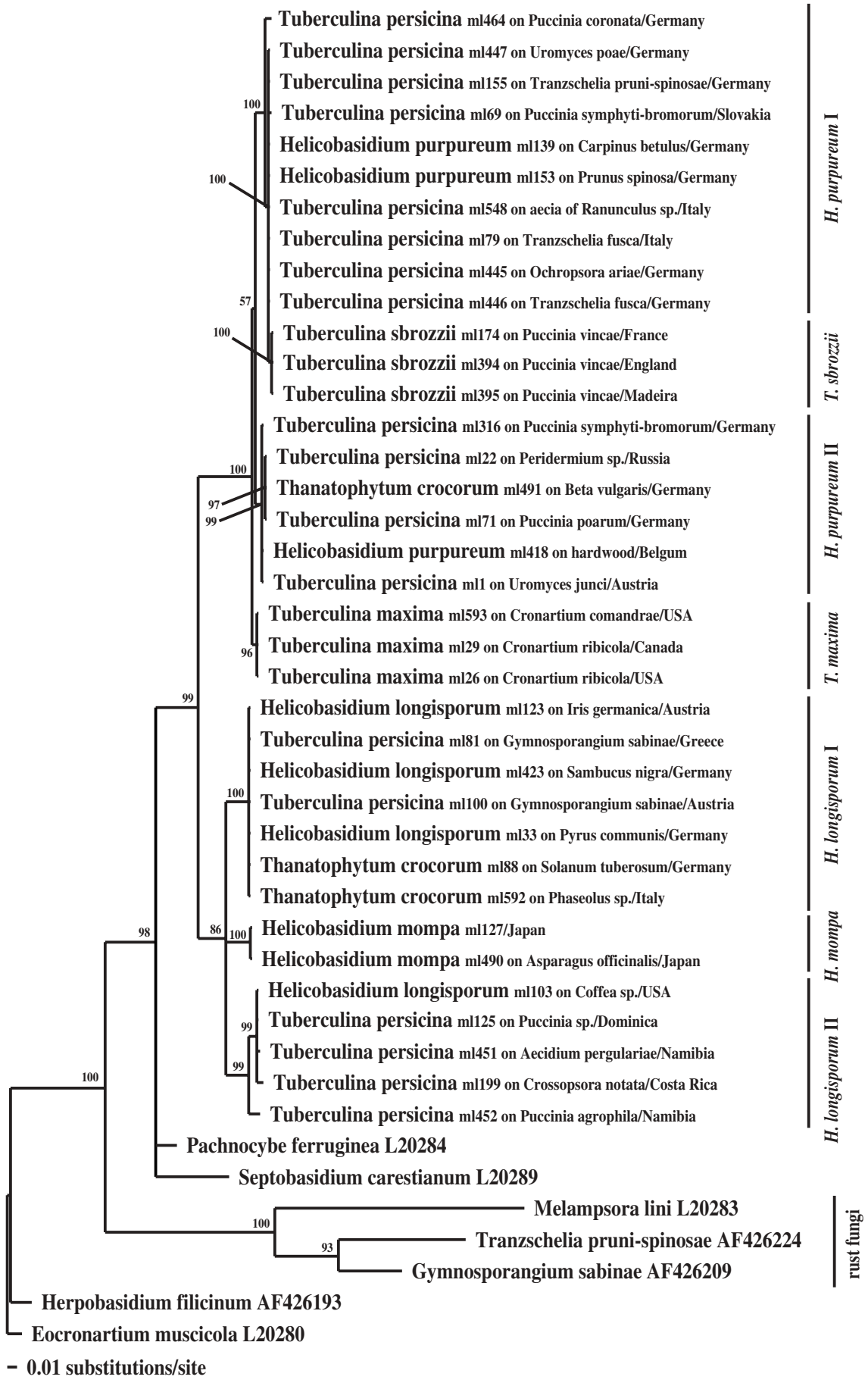


Fig. 1. For caption see opposite page.

Table 2. List of specimens sharing identical base sequences with DNA isolation number or GenBank accession number and their collection site.

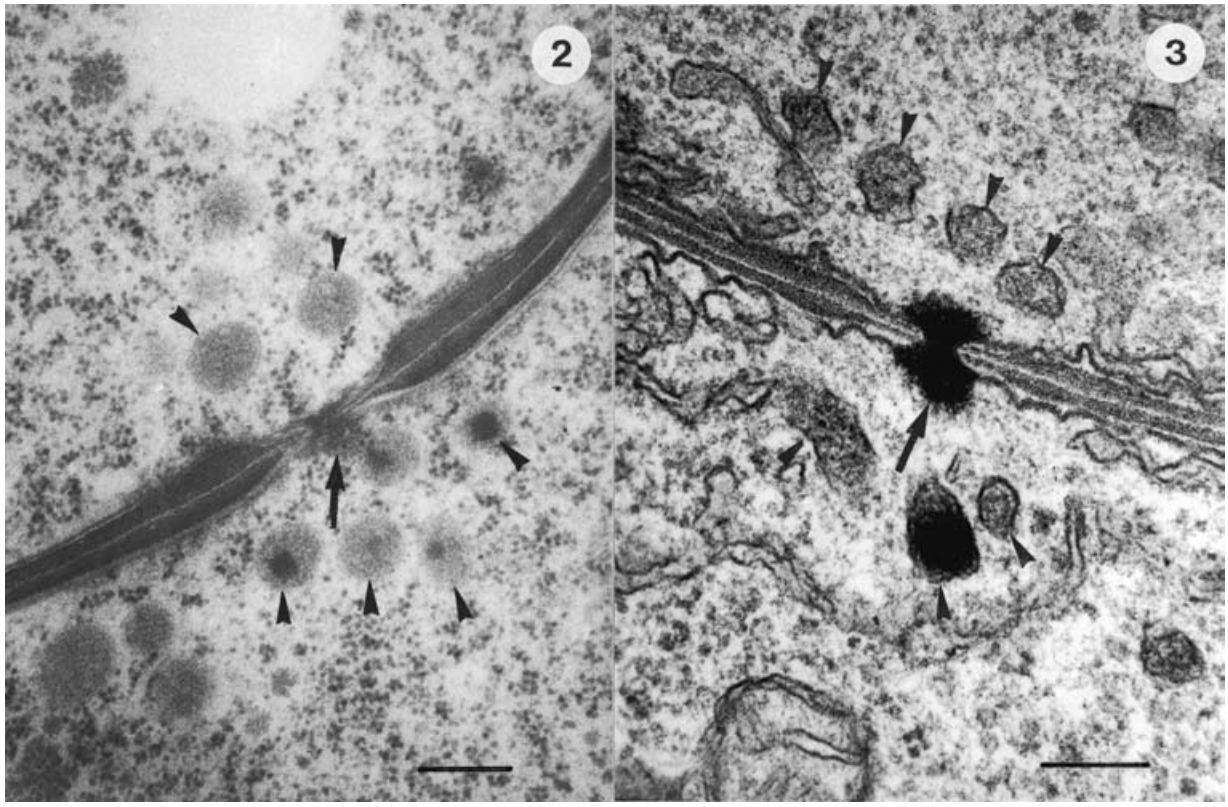
Identical ITS and LSU base sequences		Identical ITS base sequences
***Helicobasidium purpureum* I**		
Helicobasidium purpureum	ml139 – Germany ml153 – Germany	ml139 – Germany ml153 – Germany
Tuberculina persicina	ml79 – Italy ml155 – Germany ml445 – Germany ml446 – Germany ml447 – Germany ml548 – Italy	ml79 – Italy ml155 – Germany ml445 – Germany ml446 – Germany ml447 – Germany ml548 – Italy
***Helicobasidium purpureum* II**		
Helicobasidium purpureum	ml418 – Belgium	ml418 – Belgium
Thanatophytum crocorum		ml491 – Germany AB043969 – New Zealand AB044288 – New Zealand AB044289 – New Zealand AB044290 – New Zealand AB044353 – ?
Tuberculina persicina	ml1 – Austria ml316 – Germany	ml1 – Austria ml22 – Russia ml316 – Germany
***Helicobasidium longisporum* I**		
Helicobasidium longisporum	ml33 – Germany ml123 – Austria	ml33 – Germany ml123 – Austria ml423 – Germany AB044141 – Belgium
Thanatophytum crocorum		ml88 – Germany ml592 – Italy
Tuberculina persicina	ml81 – Greece ml100 – Austria	ml81 – Greece ml100 – Austria
***Helicobasidium longisporum* II**		
Helicobasidium longisporum	ml103 – USA	ml103 – USA
Tuberculina persicina	ml125 – Dominica	ml125 – Dominica

ultrastructural features (Figs 2–3) and for germination and growth in culture of both *Tuberculina*/*Helicobasidium* conidia and *Helicobasidium* basidiospores. Microscopic inspection of conidial formation in *Tuberculina* and *Helicobasidium* cultures and in *Tuberculina* infections revealed identical morphologies, which could be designated, after Kendrick & Carmichael (1973), as amerosporous, ceterisporous, phaeosporous, and phialiform. In culture on malt-extract agar and malt–yeast–peptone agar, *Tuberculina*/*Helicobasidium* conidia and *Helicobasidium* basidiospores grew rapidly at first (*ca* 80 μm h^{-1}), and had rarely branched germ tubes ('infection' hyphae 2–3 μm diam; length to 2 mm, sometimes more), which were regularly septate with cytoplasm concentrated at the tip. Some of these hyphae changed their mode of growth without apparent reason, and started to form thicker, multiple branched, regularly septate hyphae, forming a dense mycelium capable of producing sclerotia and conidia of the *Tuberculina*-type, and an intense golden brown secretion into the media.

Infection experiments

To clarify the life-cycle, we tried to grow one form out of the other. *Tuberculina* infections of several rusts were

Fig. 1. Phylogenetic relationships of selected *Tuberculina*, *Thanatophytum*, and *Helicobasidium* species. Bayesian Markov chain Monte Carlo analysis of an alignment of nuclear rDNA sequences from the 5′-end of the LSU and from the ITS region 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 substitution model, and four incrementally heated simultaneous Markov chains. 50% majority rule consensus tree from 19 001 trees that were sampled after the Markov chains reached stationarity (trees were sampled every 100 generations). The topology was rooted with the sampled urediniomycetidaceous species not belonging to the *Tuberculina* – *Thanatophytum* – *Helicobasidium* cluster. Numbers on branches are estimates for *a posteriori* probabilities, i.e. probabilities that the respective groups are monophyletic given the alignment. Branch lengths were averaged over the sampled trees. They are scaled in terms of expected numbers of nucleotide substitutions per site.



Figs 2–3. Septal pore apparatus of *Tuberculina persicina* ml155 (Fig. 2) and *Helicobasidium longisporum* ml560 (Fig. 3) seen by transmission electron microscopy, prepared by high pressure freezing and freeze substitution for *Tuberculina persicina* and prepared by chemical fixation for *Helicobasidium longisporum*. In each case the section shows a simple pore (arrows) surrounded by microbodies (arrowheads) in a more or less circular arrangement. Note that the pore channel is plugged by amorphous, electron-opaque material. Bar = 0.2 μm.

obtained in infection experiments with several experimental approaches from inoculations with *Helicobasidium* basidiospores collected in the field and from inoculations with conidia from cultures of *Helicobasidium* (Table 3). Infections were detected from the inoculation of the *Puccinia* species, *Tranzschelia*, and *Uromyces* with *Helicobasidium purpureum*, and for both inoculations of the *Gymnosporangium* species with *H. longisporum*. The actual derivation of the infections from the inocula was checked by the control experiments where no infections occurred, the infections being localized at the points of inoculation, and by analysing ITS base sequences of inoculum and infection thereby *Tuberculina* infections showing identical sequences compared to the respective *Helicobasidium* inocula. In addition, *Tuberculina* conidia were demonstrated to be capable of re-infecting rust fungi (Vladimirskaya 1939, Barkai-Golan 1959).

In contrast, we never obtained any *Helicobasidium* infection in any plant inoculated with *Tuberculina* conidia (Table 3) and, despite intensive research, there is no report of *Helicobasidium* or *Thanatophytum* infections, respectively, from inoculations with *Helicobasidium* basidiospores.

Tuberculina sclerotia, which are formed when the *Tuberculina* hosts, the rust fungi, enter dormancy in the autumn, may be the initial structure for the change from the haploid *Tuberculina*-stage to the dikaryotic *Thanatophytum*-stage. At least, our ultrastructural observations revealed cell fusion between *Tuberculina* hyphae and paired nuclei in *Tuberculina* sclerotia (data not shown). Furthermore, in our experiments the *Tuberculina* sclerotia germinated on several agar media with dikaryotic hyphae (Fig. 4), forming typical *Thanatophytum* (*Rhizoctonia crocorum*) cultures. Interestingly, for the very common *Tuberculina maxima* on *Gymnosporangium sabinae* (not represented in Fig. 1) we did not observe either the formation of sclerotia nor the existence of a *Helicobasidium*-stage. In contrast, *T. persicina* on *G. sabinae* produced sclerotia in about 75% of infected rust sori in the autumn, and the related *H. longisporum* (Fig. 1) was found in two of places nearby.

Field observations

Field observations could help in the understanding of the identity and interplay of the two life stages, *Tuberculina* and *Helicobasidium*. In the neighbourhood of Tübingen, we were able to detect *H. longisporum* or *H. purpureum*, respectively, close to *T. persicina* parasitic on at least one rust species in five places during the last two years. In one of those sites, *T. persicina*

Table 3. Experimental approaches and results of infection experiments. The species used as inocula, the respective kind of inoculum, the inoculated rust or plant species, the respective life stages of the inoculated rusts, and the results of the different inoculations are given.

Inoculum: species	Inoculum: used material (specimen)	Inoculated rust/plant (rusts: inoculated life stage)	Infection (specimen)
Helicobasidium purpureum	Basidiospores from freshly collected basidiomes (ml139)	*Gymnosporangium sabinae* (0, I[a])	–[b]
		Gymnosporangium cornutum (0, I)	–
		Puccinia sessilis (0, I)	*T. persicina* (ml191)
		Puccinia silvatica (0, I)	*T. persicina* (ml149)
		Tranzschelia pruni-spinosae (0, I)	*T. persicina* (ml173)
		Uromyces pisi s.l. (0, I)	*T. persicina* (ml164)
H. longisporum	Basidiospores from freshly collected basidiomes (ml33)	*Gymnosporangium sabinae* (0, I)	*T. persicina* (ml362)
		Puccinia silvatica (0, I)	–
		Uromyces pisi s.l. (0, I)	–
	Conidia from a culture obtained from basidiospores from freshly collected basidiomes (ml423)	*Gymnosporangium cornutum* (0, I)	*T. persicina* (ml340)
		Puccinia silvatica (0, I)	–
		Uromyces pisi s.l. (0, I)	–
Tuberculina persicina	Conidia from freshly collected infections of several rusts	*Aster* sp. cult.	–
		Brassica oleracea	–
		Daucus carota	–
		Fraxinus excelsior	–
		Solanum lycopersicum	–
		Solanum tuberosum	–

[a] 0, spermogonia; I, aecia.
[b] –, no infection was observed.

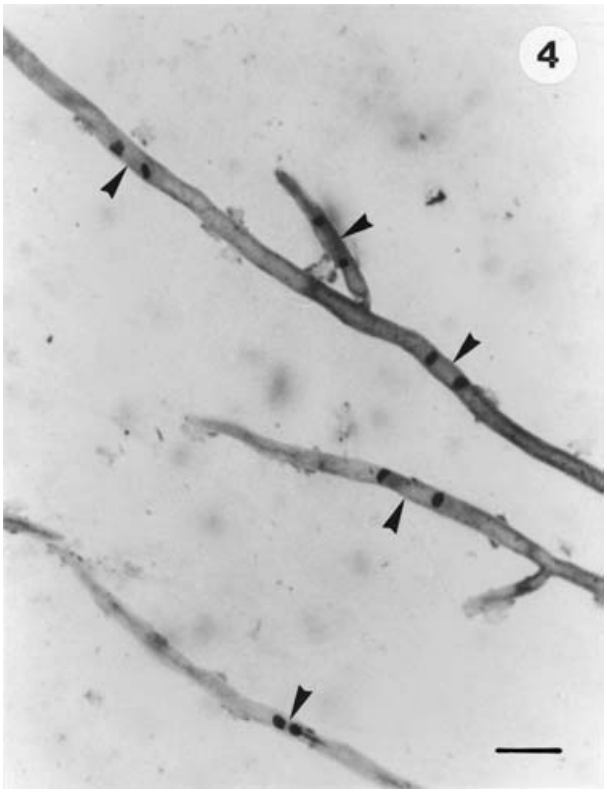


Fig. 4. Dikaryotic hyphae of *Tuberculina persicina* ml601 from germinating sclerotia on malt-extract agar seen by light microscopy after nuclear staining with Giemsa (Bauer 1987). Dikarya are visible at arrowheads. Bar = 20 μm.

parasitised nine different rust species (Table 4), growing in an area of 200 m² around five fructifications of *H. purpureum*. The sporulation time of *Helicobasidium* in a short period of time in spring, harmonises with the occurrence of pycnia and aecia of several rust species in typical *Helicobasidium* habitats. In the autumn when the rust fungi enter dormancy, *Tuberculina* forms sclerotia that fall to the ground together with the leaves, apparently initiating the change to the *Helicobasidium*-stage.

Life-cycle

The recognition of *Helicobasidium* and *Tuberculina* species as stages of the life-cycle of one holomorph reveals a unique life-cycle (Fig. 5) and a fascinating life strategy: sexual reproduction and asexual multiplication are each linked to distinct life stages that are characterised by special morphological and ecological characters. The dependency of the sexual and asexual life stages of one species on hosts from two kingdoms is unique among organisms. The sterile *Thanatophytum*-stage grows as a soil-borne plant parasite covering the roots of spermatophytes with a dense, dikaryotic mycelium causing the disease known as violet root rot (Itô 1949, Valder 1958, Suzuki 1978). The host-parasite-interaction of the *Thanatophytum*-stage is initially realised by complex interaction structures called infection cushions that allow the intrusion of the parasite through the plant cortex (Hering 1962b, Ieki 1975a, Matsuzaki *et al*. 1986). Then, the internal root tissues are decomposed (Itô 1949, Ieki 1975a). *Thanatophytum* survives and is dispersed *via* the formation of soil-borne sclerotia (Suzuki *et al*. 1957, Valder 1958). *Helicobasidium* fructification takes place by forming a fine hymenium arising on perennial mycelial coats covering substrata above the ground (Buddin & Wakefield 1927). As the first step of sexual reproduction, nuclear fusion and subsequent meiosis take place in the

Table 4. List of rust species infected with *Tuberculina persicina* observed around five fructifications of *Helicobasidium purpureum* ml139 (**Germany**: *Baden-Württemberg*: Tübingen, on *Carpinus betulus*, 5 April 2001, *M. Lutz 828*, TUB 011542).

Rust species	Host	Reference material[a]
Melampsora sp.	*Allium ursinum*	**Germany**: *Baden-Württemberg*: Tübingen, 9 May 2001, *M. Lutz 886* (TUB 011593)
Ochropsora ariae	*Anemone nemorosa*	**Germany**: *Baden-Württemberg*: Tübingen, 25 April 2002, *M. Lutz 1271* (TUB 011546)
Puccinia senecionis-acutiformis	*Senecio ovatus*	**Germany**: *Baden-Württemberg*: Tübingen, 5 June 2001, *M. Lutz 948* (TUB 011594)
P. silvatica	*Taraxacum officinale* aggr.	**Germany**: *Baden-Württemberg*: Tübingen, 5 June 2001, *M. Lutz 949* (TUB 011591)
P. urticata	*Urtica dioica*	**Germany**: *Baden-Württemberg*: Tübingen, 5 June 2001, *M. Lutz 947* (TUB 011592)
Tranzschelia fusca	*Anemone nemorosa*	**Germany**: *Baden-Württemberg*: Tübingen, 25 April 2002, *M. Lutz 1270* (TUB 011547)
T. pruni-spinosae	*Anemone ranunculoides*	**Germany**: *Baden-Württemberg*: Tübingen, 24 April 2001, *M. Lutz 852* (TUB 011608)
Uromyces pisi s. lat.	*Euphorbia cyparissias*	**Germany**: *Baden-Württemberg*: Tübingen, 9 May 2001, *M. Lutz 885* (TUB 011590)
U. poae	*Ranunculus ficaria*	**Germany**: *Baden-Württemberg*: Tübingen, 25 April 2002, *M. Lutz 1269* (TUB 011548)

[a] Source acronym: TUB, Herbarium of the Spezielle Botanik/Mykologie; Eberhard-Karls-Universität Tübingen, Tübingen.

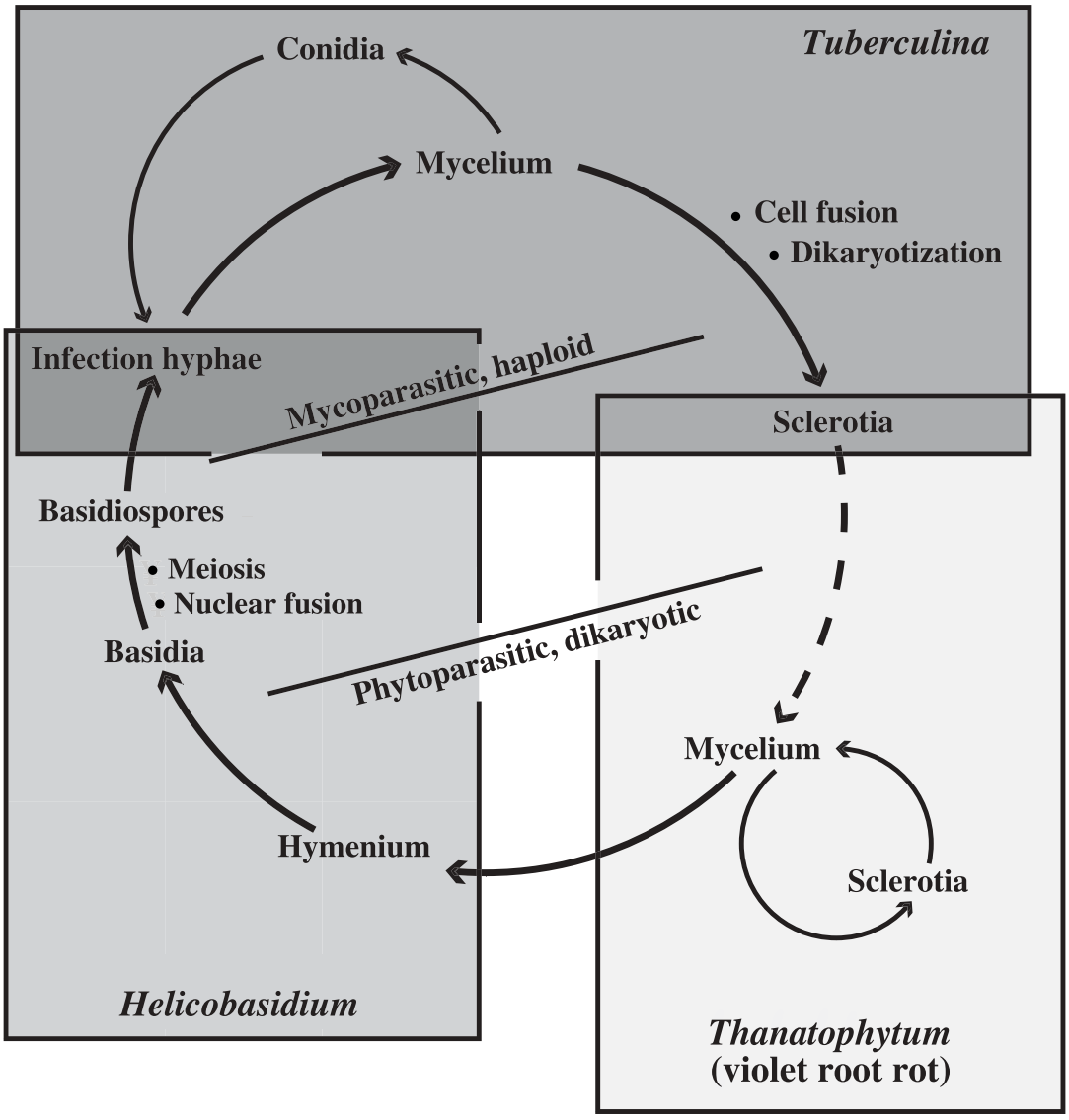


Fig. 5. Life cycle of *Tuberculina* – *Thanatophytum* –*Helicobasidium*. Explanation in text. Continuous lines indicate that the progression is experimentally proven. The dashed line indicates that the progression from *Tuberculina* sclerotia to *Thanatophytum* is suggested by circumstantial evidence given in the text, but not yet experimentally proven.

basidia resulting in haploid, ballistic basidiospores (data not shown). The basidiospores germinate to produce infection hyphae capable of infecting pycnia (data not shown) and presumably the aecia of rust fungi, resulting in a rust-parasitic mycelium. The parasitic interaction between the *Tuberculina*-stage and the rust fungal hosts is realised by large intercellular fusion pores (Lutz *et al*. 2004) and is thus quite different to the parasitism of the *Thanatophytum*-stage. Shortly after infection (Wicker & Wells 1970), anemochorous *Tuberculina* conidia may be formed as units of asexual reproduction and dispersion. They germinate like the basidiospores, and are capable of re-infecting potential rust hosts (Vladimirskaya 1939, Barkai-Golan 1959). The change from the *Tuberculina*- to the *Thanatophytum*-stage, including cell fusion and dikaryotisation, which is the second and completing step of sexual reproduction, is apparently realised *via Tuberculina* sclerotia.

Considering the exceptional life-cycle, the question is: how could a fungus evolve to jump between two kingdoms, i.e. to parasitize organisms from such phylogenetically distinct groups as fungi and plants and, above all, with such different modes of parasitic interaction? *Helicobasidium* and its closest relatives are plant parasites (Bauer & Oberwinkler 1994). Therefore, it seems that this group arose as plant parasites. The crucial point is the mycoparasitic *Tuberculina*-stage, and accordingly the question how the drastic host jump from plants to fungi could have been evolved. A promising approach to clarify the evolution of the *Tuberculina*-stage may be analyses of the genetic background of the remarkable cellular interaction between *Tuberculina* and rust hyphae.

However, the recognition of *Helicobasidium* and *Tuberculina* as the same fungi not only opens new perspectives on fungal life strategies and evolutionary pathways, but also has practical consequences. The knowledge of *Tuberculina* as a stage of multiplication and dispersal might help combat the severe plant pathogens *Helicobasidium* or *Thanatophytum*, respectively. In contrast, knowing that *Tuberculina* is followed by the plant-parasitic *Helicobasidium* stage argues against the possible use of *Tuberculina* as a biological agent in rust control. However, we could not find a *Helicobasidium*-stage for the very common *Tuberculina maxima* or for *T. sbrozzii*. Consequently, there may be *Tuberculina* species which have lost the phytoparasitic stage and which could still be considered as potential agents in rust control.

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REFERENCES

Barkai-Golan, R. (1959) *Tuberculina persicina* (Ditmar) Sacc. attacking rust fungi in Israel. *Bulletin of the Research Council of Israel/D* **8**: 41–46.

Bauer, R. (1987) *Uredinales* – germination of basidiospores and pycnospores. *Studies in Mycology* **30**: 111–125.

Bauer, R., Begerow, D., Oberwinkler, F., Piepenbring, M. & Berbee, M. L. (2001) *Ustilaginomycetes*. In *The Mycota*. Vol. 7B. *Systematics and Evolution* (D. J. McLaughlin, E. G. McLaughlin & P. A. Lemke, eds): 57–83. Springer-Verlag, Berlin.

Bauer, R. & Oberwinkler, F. (1994) Meiosis, septal pore architecture, and systematic position of the heterobasidiomycetous fern parasite *Herpobasidium filicinum*. *Canadian Journal of Botany* **72**: 1229–1242.

Begerow, D., Bauer, R. & Boekhout, T. (2000) Phylogenetic placements of ustilaginomycetous anamorphs as deduced from nuclear LSU rDNA sequences. *Mycological Research* **104**: 53–60.

Buddin, W. & Wakefield, E. M. (1927) Studies on *Rhizoctonia crocorum* (Pers.) DC. and *Helicobasidium purpureum* (Tul.) Pat. *Transactions of the British Mycological Society* **12**: 116–140.

Diaz, M. R. & Fell, J. W. (2000) Molecular analyses of the IGS & ITS regions of rDNA of the psychrophilic yeasts in the genus *Mrakia*. *Antonie van Leeuwenhoek* **77**: 7–12.

Fukushima, C. (1998) Environmental factors important for the occurrence of the violet and white root rots (*Helicobasidium mompa* Tanaka and *Rosellinia necatrix* (Hartig) Berlese) in apple orchards and their control methods. *Bulletin of the Aomori Apple Experiment Station* **30**: 98–112.

Gams, W. (2001) Report of the Committee for Fungi: 9. *Taxon* **50**: 269–272.

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.

Garrett, S. D. (1949) A study of violet root rot. *Transactions of the British Mycological Society* **32**: 217–223.

Gäumann, E. (1959) *Die Rostpilze Mitteleuropas*. Buechler, Bern.

Gäumann, E. (1964) *Die Pilze*, 2nd edn. Birkhäuser Verlag, Basel.

Gu, X., Fu, Y. X. & Li, W. H. (1995) Maximum likelihood estimation of the heterogeneity of substitution rate among nucleotide sites. *Molecular Biology and Evolution* **12**: 546–557.

Hering, T. F. (1962a) Host range of the violet root rot fungus, *Helicobasidium purpureum* Pat. *Transactions of the British Mycological Society* **45**: 488–494.

Hering, T. F. (1962b) Infection cushions of *Helicobasidium purpureum* Pat. *Transactions of the British Mycological Society* **45**: 46–54.

Horton, T. R. (2002) Molecular approaches to ectomycorrhizal diversity studies: variation in ITS at a local scale. *Plant and Soil* **244**: 29–39.

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.

Huelsenbeck, J. P. & Ronquist, F. (2001) MRBAYES: Bayesian inference of phylogenetic trees. *Bioinformatics Applications Note* **17**: 754–755.

Ieki, H. (1975a) Ultrastructural change of root of mulberry trees infected with violet root rot fungus, *Helicobasidium mompa* Tanaka. *Annals of the Phytopathological Society of Japan* **41**: 356–363.

Ieki, H. (1975b) Observations on infection process of *Helicobasidium mompa* Tanaka to mulberry radicle. *Annals of the Phytopathological Society of Japan* **41**: 405–407.

Ikeda, K., Nakamura, H. & Matsumoto, N. (2003) Mycelial incompatibility operative in pairings between single basidiospore isolates of *Helicobasidium mompa*. *Mycological Research* **107**: 847–853.

Itô, K. (1949) Studies on "Murasaki-mompa" disease caused by *Helicobasidium mompa* Tanaka. *Bulletin of the Government Forest Experiment Station* **43**: 1–126.

Kakishima, M., Yokoi, M. & Harada, Y. (1999) *Puccinia caricis-adenocauli*, a new rust fungus on *Carex*, and its anamorph, *Aecidium adenocauli*. *Mycoscience* **40**: 503–507.

Kendrick, W. B. & Carmichael, J. W. (1973) Hyphomycetes. In *The Fungi: an advanced treatise*. Vol. 4A. *A Taxonomic Review with Keys: ascomycetes and fungi imperfecti* (G. C. Ainsworth, F. K. Sparrow & A. S. Sussman, eds): 323–509. Academic press, New York.

Lutz, M., Bauer, R., Begerow, D., Oberwinkler, F. & Triebel, D. (2004) *Tuberculina*: rust relatives attack rusts. *Mycologia*: in press.

Matsuzaki, I., Haneda, H. & Mitsueda, T. (1986) Anatomical observations on the entrance of *Helicobasidium mompa* into the root of asparagus. *Annual report of the Society of Plant Protection of North Japan* **37**: 93–95.

Nishikawa, H. (1962) Biochemistry of filamentous fungi. XIII. Metabolites of *Helicobasidium mompa* Tanaka. *Agricultural and Biological Chemistry* **26**: 696–698.

O'Donnell, K. (1992) Ribosomal DNA internal transcribed spacers are highly divergent in the phytopathogenic ascomycete *Fusarium sambucinum* (*Gibberella pulicaris*). *Current Genetics* **22**: 213–220.

Rodriguez, F., Oliver, J. L., Marín, A. & Medina, J. R. (1990) The general stochastic model of nucleotide substitution. *Journal of Theoretical Biology* **142**: 485–502.

Sayama, A., Kobayashi, K. & Ogoshi, A. (1994) Morphological and physiological comparisons of *Helicobasidium mompa* and *H. purpureum*. *Mycoscience* **35**: 15–20.

Scorzetti, G., Fell, J. W., Fonseca, A. & Statzell-Tallman, A. (2002) Systematics of basidiomycetous yeasts: a comparison of large subunit D1/D2 and internal transcribed spacer rDNA regions. *FEMS Yeast Research* **2**: 495–517.

Suzuki, N., Kasai, K., Araki, T. & Takanashi, T. (1957) Studies on the violet root rot of sweet potatoes caused by *Helicobasidium mompa* Tanaka. *Bulletin of the National Institute of Agricultural Science* **8**: 1–27.

Suzuki, T. (1978) Studies on the ecology and the control of asparagus violet root rot caused by *Helicobasidium mompa* Tanaka. *Research Bulletin of the Hokkaido National Agricultural Experiment Station* **122**: 87–165.

Takai, S. (1966) Studies on helicobasidin, a pigment isolated from *Helicobasidium mompa* Tanaka, and its toxic action to some higher plants and microorganisms. *Bulletin of the Government Forest Experiment Station* **195**: 1–55.

Uetake, Y., Nakamura, H., Ikeda, K., Arakawa, M. & Matsumoto, N. (2003) *Helicobasidium mompa* isolates from sweet potato in continuous monoculture fields. *Journal of General Plant Pathology* **69**: 42–44.

Valder, P. G. (1958) The biology of *Helicobasidium purpureum* Pat. *Transactions of the British Mycological Society* **41**: 283–308.

Vladimirskaya, M. E. (1939) A parasite of crop plant rust, *Tuberculina persicina* (Ditmar) Sacc. *Bulletin of Plant Protection* **1**: 103–110.

White, T. J., Bruns, T. D., Lee, S. & Taylor, J. W. (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In *PCR Protocols: a guide to methods and applications* (M. A. Innis, D. H. Gelfand, J. J. Sninsky & T. J. White, eds): 315–322. Academic Press, San Diego.

Wicker, E. F. (1980) Biocontrol of conifer stem rusts the purple mold. *Phytopathologia Mediterranea* **19**: 21–26.

Wicker, E. F. (1981) Natural control of white pine blister rust by *Tuberculina maxima*. *Phytopathology* **71**: 997–1000.

Wicker, E. F. & Wells, J. M. (1970) Incubation period for *Tuberculina maxima* infecting the Western white pine blister rust cankers. *Phytopathology* **60**: 1693.

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