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Repeated formation of correlated species in *Tranzschelia* (Pucciniales)

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Abstract

Heteroecism, or alternation between two unrelated hosts, is a widespread phenomenon among rust fungi (Pucciniales). In addition to heteroecism, rust fungi have evolved elaborate life cycles ranging from the five spore stages of macrocyclic species with many variations down to microcyclic species that may produce just two of these stages to complete their life cycles on a single host species. Considering the large number of nearly 8000 described rust fungi species and the high proportion that are host-alternating, heteroecism apparently is a successful strategy for these fungi, at least in terms of species diversity. However, the cost of maintaining a heteroecious strategy with respect to spore production and two different host plant species must be high. In Pucciniales, sister-species pairs that include one host-alternating (heteroecious) and one non-host-alternating (autoecious) species that share a common host are called correlated species. In this study, we tested *Tranzschelia* species for the existence of correlated species using molecular phylogenetic data. We reveal the presence of three pairs of correlated species within this single genus and suggest that this is a repeating process in the evolution of rust fungi. We show that a heteroecious macrocyclic strategy can be the starting point for deriving microcyclic autoecious species. The high cost of host alternation may be compensated by the fact that it is a facultative process in *Tranzschelia* with numerous strategies for the species to persist in one or the other host or as overwintering spore. Consequently, the advantage of host alternation seems higher than the cost of (facultative) heteroecism.

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Introduction

Rust fungi (Basidiomycota, Pucciniales) are obligate plant pathogens that likely evolved in the mid-Jurassic (Aime et al. 2018). They comprise the largest group of plant pathogenic fungi consisting of nearly 8000 known species (Kirk et al. 2008) which includes the single most speciose fungal family, Pucciniaceae. This dimension of speciation is astonishing considering the complexity and plasticity of the rust life cycle. For instance, a single species may possess up to five different spore states, each with a different function, and up to seven morphologically different spore types (e.g., Bruckart et al. 2010), all obligately dependent on their specific plant hosts. Moreover, most striking and nearly unique within the kingdom Fungi is the fact that the majority of species are host-alternating (heteroecious). They develop spermogonia (abbreviated 0) forming spermatia and aecia (I) forming aeciospores on a host plant herein referred to as the aecial host. Later in the vegetation season, aeciospores serve to infect another host belonging to an unrelated plant family that is referred to as the telial host. Uredinia (II) with

urediniospores followed by telia (III) with teliospores are formed on the telial host. In temperate climates, thick-walled teliospores often serve as overwintering spores germinating in spring to form basidia (IV) that produce haploid basidiospores after meiosis and serve to reinfect the aecial host. Species forming all spore generations except uredinia (II) are called autopsis-forms and those forming all five spore states are called macrocyclic or eu-forms; host-alternating ones are termed opsis- and heterou-forms, respectively. Reduced life cycles include, among others, auteu-forms (forming all spore states but not host-alternating), brachy-forms (I missing), hemi-forms (0, I missing), autopsis-forms (II missing), and micro-forms that are the most reduced life cycle type, forming only 0 (or no 0 at all), III, and IV (nomenclature of spore states after Wilson and Henderson 1966).

More than 50% of the species of Pucciniales are host-alternating (Malloch 1995), especially in temperate regions (e.g., Berndt 2012; Klenke and Scholler 2015). This proportion suggests that heteroecism is a successful strategy in the evolution of rust fungi, despite an extremely high cost according to Savile (1976), who calculated, considering host changes in both directions from aecial host to telial host and vice versa, an "annual population loss between 1/625 and 1/15,625." De Bary (1879), Dietel (1897, 1899), Fischer (1898), and Tranzschel (1904) observed that for some micro-form rust species, the host was congeneric or conspecific with the aecial host of a heterou-form rust species. In contrast to the popular classifications of the time, these workers hypothesized that life cycle types may not necessarily indicate taxonomic relationships. Tranzschel (1904) concluded that when heteroecious species reduce their life cycle to form a micro-form, it will be formed on the aecial host via intermediate auteu-forms. Tranzschel applied his hypothesis in searching for and describing previously unknown "parallel" species (Tranzschel, l. c.). Later refinements to this idea were initiated by Arthur (1921, 1929, 1934) and Jackson (1931), who coined the term "correlated" species for Tranzschel's parallel species and "Tranzschel's Law" to explain how derived auteu- and micro-forms may occur on the aecial host (and not on the telial host) of heterou-form species. Today, Tranzschel's Law and the concept of correlated species is widely accepted among mycologists, and some molecular phylogenetic studies, e.g., by Zambino and Szabo (1993) on grass rusts in the genus *Puccinia*, Roy et al. (1998) within the *Puccinia monoica* complex, and Vogler and Bruns (1998) on the genus *Cronartium* have confirmed this. While many researchers (e.g., Dietel 1897, 1899; Orton 1927; Jackson 1931; Shattock and Preece 2000) have hypothesized that micro-forms are derived from heterou-forms the reverse hypothesis—that complex forms evolved from microcyclic rusts—has also been invoked, most frequently to explain how macrocylic species could have evolved from what were

considered the most "primitive" groups of rust fungi (e.g., Dietel 1899; Hennen and Buriticá 1980).

The genus *Tranzschelia* includes 16 species (Scholler et al. 2014) with mainly micro- and heterou-forms and one auteu-form (*T. cohaesa* (Long) Arthur, the type species), one autopsis-form (*T. tucsonensis* (Arthur) Dietel), and possibly several hemi-forms (*T. arasbaranica* M. Abbasi & M. Scholler, *T. mexicana* M. Scholler & M. Abbasi, *T. viornae* (Arthur) Arthur). It is possible that some hemi-form species may actually be heterou-form species for which the aecial host is not known. In *Tranzschelia* spp., 0 and I of heterou-form species, all spore states of auteu-form species and 0, III and IV of micro-form species are formed on Ranunculaceae. Telial hosts in heterou-form species are stone fruit trees classified in the genus *Prunus* s.l. (plums and peaches, blackthorn, etc.).

Maier et al. (2005) sequenced three European *Tranzschelia* specimens demonstrating that *T. fusca* (Pers.) Dietel and *T. pruni-spinosae* (Pers.) Dietel (heterou-form) on their telial hosts are indeed more closely related to each other than to another heterou-form species, *T. discolor* (Fuckel) Tranzschel & M.A. Litv. In this study, we used this three-species model for molecular phylogenetic analyses including further *Tranzschelia* specimens from different host plant species and continents to show that the formation of correlated species was not a single but a repeated event in *Tranzschelia* evolution. Against this background, the function and potential advantages of this process and of heteroecism in general are discussed.

Materials and methods

Specimen sampling

Specimens of species of *Tranzschelia*, *Leucotelium*, and *Ochropsora*—the latter two chosen because they also utilize Ranunculaceae species as their aecial hosts—were selected from public herbaria for molecular phylogenetic analyses from as broad a geographic and host range as possible including heterou-/hemi- and micro-form species (Table 1). Specimens were assigned to species on the basis of host plant species, morphology, geographic distribution, and the results of the molecular phylogenetic analyses. However, five specimens could not be assigned to a specific taxon (*Tranzschelia* spp. 1–5).

DNA extraction, PCR, and sequencing

Genomic DNA was isolated directly from herbarium specimens. For each specimen, several sori from a single leaf were excised and extracted using the UltraClean plant DNA extraction kit (MoBio Laboratories Inc., Solana Beach, California). DNA extractions were diluted 1:9 in sterile water and

Table 1 List of examined specimens, with host plant species, spore state, life cycle type, GenBank accession numbers, origin, and reference specimens (for abbreviations of life cycle types see Fig. 1)

Species	Host	Spore state	Life cycle type	GenBank acc. no. (ITS/LSU)	Origin	Collector, collection year/public herbarium accession number	Source
Leucotelium cerasi	Eranthis hyemalis	0, I	HeA	-/MG948657	Austria, Steiermark, Graz, Geidorf, Botanical Garden	C. Scheuer 2010/KR-M-0024931	This paper.
Leucotelium cerasi	Prunus domestica	II, III	HeT	KX228771/KX228776	Germany, Baden-Württemberg, Stuttgart, Rohr	M. Lutz 2013/KR-M-0037198	This paper.
Ochropsora ariae	Anemone nemorosa	0, I	HeA	KX228773/KX228778	Germany, Baden-Württemberg, Münsingen, Buttenhausen	M. Lutz 2010/KR-M-0042604	This paper.
Ochropsora ariae	Sorbus aucuparia	II, III	HeT	KX228772/KX228777	Germany, Sachsen-Anhalt Salzmünde	H. Jage 2001/KR-M-0043444	This paper.
Tranzschelia arasbaranica	Prunus mahaleb	II, III	HeT	-/MG948658	Iran, Ooli, Arasbaran forest	G. Tavanai 1998/ KR-M-0012355/ IRAN 12359 F	Aime 2006.
Tranzschelia arthurii	Prunus cf. serotina	II, III	HeT	MG947386/MG948659	USA, Indiana, West Lafayette,	M. Abbasi 2000/KR-M 0012357/ IRAN 12366F	This paper.
Tranzschelia asiatica	Prunus grayana	II, III	HeT	-/MG948660	Japan, Minamiaizu-gun, Showa-mura, Yanohara	Y. Ono 1993/KR-M-0012106/IBA 6988	This paper.
Tranzschelia discolor	Anemone coronaria	0, I	HeA	-/MG948661	Greece, Kríti, Ida (Psiloritis), Armeni	M. Scholler 1999/KR-M-0010961	This paper.
Tranzschelia discolor	Prunus domestica	II, III	HeT	DQ354542/DQ354542	Iran, Golestán, Nassrabad village	R. Zare 2004/KR-M-0010966/ IRAN 12326 F	This paper.
Tranzschelia fusca	Anemone nemorosa	III	Au	-/MG948662	Germany, Baden-Württemberg, Mannheim, NSG Silberpappel	M. Scholler 2005/KR-M-0012289	This paper.
Tranzschelia fusca	Anemone nemorosa	III	Au	KX228770/KX228775	Germany, Baden-Württemberg, Münsingen, Buttenhausen	M. Lutz 2010/KR-M-0042602	This paper.
Tranzschelia hyrcanica	Prunus cerasifera	II, III	HeT	MG947387/MG948663	Iran, Azarbaijan-e Sharqi, Kaleybar	M. R. Asef 2004/KR-M 0010968/ IRAN 12328 F	This paper.
Tranzschelia mexicana	Prunus serotina var. capuli	II	HeT	-/KP308391	USA, California, Santa Barbara	H. Scheck 2014/KR-M-0040855	Blomquist et al. 2015.
Tranzschelia microcerasi	Prunus microcarpa	II, III	HeT	-/MG948664	Iran, Khorasan, Shirvan, Cheshmeh Gabr, Kopet dagh	M. Abbasi, J. Fatehi, O. Foitzik/ KR-M-0012351/IRAN 8646F	This paper.
Tranzschelia pruni-spinosae	Prunus spinosa	II, III	HeT	-/MG948665	Germany, Sachsen-Anhalt, Hoyersbürg, NSG "Bürgerholz"	H. Jage/KR-M-0029489	This paper.
Tranzschelia pruni-spinosae	Anemone ranunculoides	0, I	HeA	KX228769/KX228774	Germany, Baden-Württemberg, Tübingen	M. Lutz 2000/KR-M 0002755	This paper.
Tranzschelia pseudofusca	Anemone quinquefolia	III	Au	-/MG948666	USA, Minnesota, Hubbard County, lake George	MIN 885887	This paper.
Tranzschelia pseudofusca	Anemone quinquefolia	III	Au	-/MG948667	USA, Minnesota, Hubbard County, ½ mile east of Badoura Nursery	MIN 885891	This paper.
Tranzschelia pulsatillae	Anemone halleri	III	Au	-/MG948668	Slovakia, Malá Fatra Mts., Tiešňevy	J. Piątek, M. Piątek, W. Paul/KR-M-0024825/KRAM F-47945	This paper.
Tranzschelia sp. 1	Prunus pensylvanica	II, III	HeT	-/MG948669	USA, Minnesota, Hubbard County	MIN 885890	This paper.
Tranzschelia sp. 2	Anemone quinquefolia	0, I	HeA	-/MG948670	USA, Minnesota, Anoka County	MIN 885889	This paper.
Tranzschelia sp. 3	Thalictrum polygamum	III	Au	-/MG948671	Canada	DAOM 187607	This paper.

Table 1 (continued)

Species	Host	Spore state	Life cycle type	Origin	GenBank acc. no. (ITS/LSU)	Collector, collection year/public herbarium accession number	Source
Tranzschelia sp. 4	*Prunus pensylvanica*	II, III	HeT	USA	DQ425036/DQ425036	JGPI066-B	Ordonez ME, Groth JV, Szabo LJ. Unpublished.
Tranzschelia sp. 4	*Anemone quinquefolia*	0, I	HeA	USA, Minnesota, Pine County	-/MG948672	MIN 885892	This paper.
Tranzschelia sp. 5	*Prunus grayana*	II, III	HeT	Japan, Ibaraki-ken, Kanto, Ogawa, Sadanami	-/MG948673	Y. Ono/KR-M-0012108/IBA 8565	This paper.
Tranzschelia thalictri	*Thalictrum minus*	III	Au	Germany, Thüringen, Bad Frankenhausen, Napptal	-/MG948674	V. Kummer 2001/KR-M-0002927	This paper.

amplified with the rust-specific primer Rust2inv (Aime 2006) and LR6 (Vilgalys and Hester 1990) in 25 μL reaction volumes with 12.5 μL of PCR Master Mix (Promega Corp., Madison, WI), 1.25 μL of each 10 μM primer, and 10 μL of diluted DNA template with PCR parameters described in Aime (2006), amplifying approximately 1400 bp of a region of the ribosomal repeat spanning the 5.8S subunit, the internal transcribed spacer region 2 (ITS2), and the large subunit (28S) (LSU). Amplifications with weak products were cleaned and reamplified with nested primers Rust28SF (Aime et al. 2018) and LR5 (Vilgalys and Hester 1990) as described by Aime et al. (2018). PCR products were sequenced with amplification primers on an ABI3100 Automated Sequencer (Applied Biosystems, Foster City, California) after cleaning by ethanol precipitation or sent to Beckman-Coulter (Danvers, MA) for sequencing on an ABI 3730XL. Contiguous sequences were assembled and edited in Sequencer v.5.2.3 (Gene Codes Corp., Ann Arbor, MI). DNA sequences were deposited in GenBank; accession numbers are provided in Table 1 and Fig. 1.

Phylogenetic analyses

To infer the phylogenetic relationships of the examined specimens, we assembled an ITS + LSU dataset that included sequences from every species/clade for which sequence data were available. For GenBank accession numbers of the sequences used (Aime 2006; Blomquist et al. 2015), see Fig. 1.

ITS and LSU DNA regions were aligned separately. Sequence alignment was obtained using MAFFT 7.273 (Katoh and Standley 2013) using the L-INS-i option. To obtain reproducible results, manipulation of the alignments by hand and manual exclusion of ambiguous sites were avoided as suggested by Gatesy et al. (1993) and Giribet and Wheeler (1999), respectively. Instead, highly divergent portions of the alignments were omitted using GBlocks 0.91b (Castresana 2000) with the following options for the LSU dataset: "Minimum Number of Sequences for a Conserved Position": 14, "Minimum Number of Sequences for a Flank Position": 14, "Maximum Number of Contiguous Non-conserved Positions": 8, "Minimum Length of a Block": 5, and "Allowed Gap Positions" to "With half," and for the ITS dataset: "Minimum Number of Sequences for a Conserved Position": 6, "Minimum Number of Sequences for a Flank Position": 6, "Maximum Number of Contiguous Non-conserved Positions": 8, "Minimum Length of a Block": 5, and "Allowed Gap Positions" to "With half."

The resulting alignments [LSU dataset: new number of positions: 962 (53% of the original 1782 positions), number of variable sites: 145; ITS dataset: new number of positions: 563 (62% of the original 896 positions), number of variable sites: 219] were then concatenated. Phylogenetic analyses were computed for the dataset in maximum likelihood
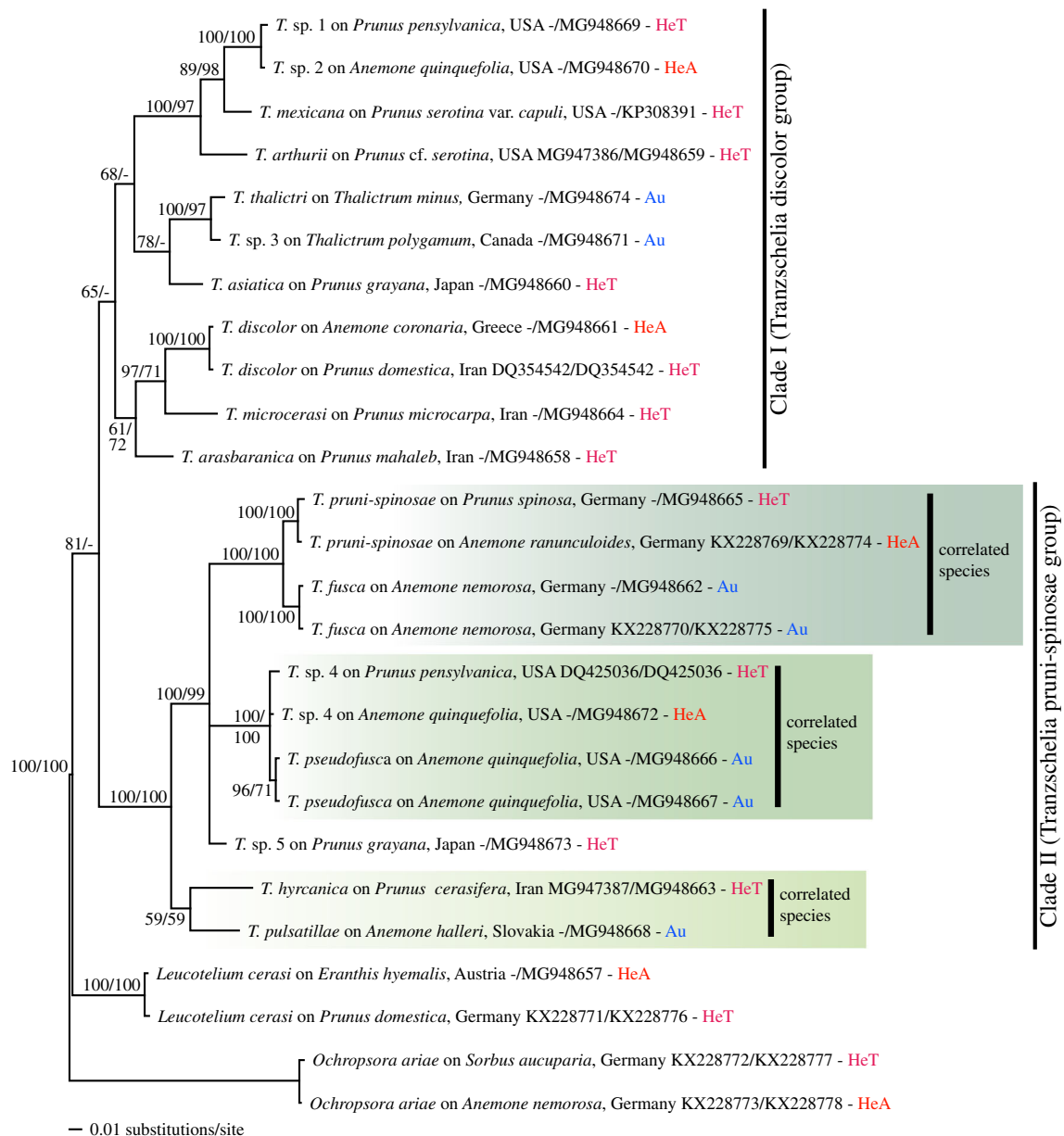
Fig. 1 Bayesian inference of the phylogenetic relationships between the sampled *Leucotelium* and *Tranzschelia* species: Markov chain Monte Carlo analysis of an alignment of concatenated ITS + LSU base sequences using the GTR + I + G model of DNA substitution with gamma distributed substitution rates and estimation of invariant sites, random starting trees, and default starting parameters of the DNA substitution model. A 50% majority-rule consensus tree is shown computed from 75,000 trees that were sampled after the process had reached stationarity. The topology was rooted with *Ochropsora ariae*. Numbers on branches before slashes are estimates for a posteriori probabilities; numbers on branches after slashes are ML bootstrap support values. Branch lengths were averaged over the sampled trees. They are scaled in terms of expected numbers of nucleotide substitutions per site. Au = autoecious; HeA = hetereu-form, aecial host; HeT = hetereu-form, telial host; T. = *Tranzschelia*. HeT include possible hemi-form species (*Tranzschelia arasbaranica*, *T. hyrcanica*, *T. mexicana*)

analyses (ML) with combined rapid bootstrapping under the GTRCAT model from 1000 runs using RAxML 8.0.17 (Stamatakis 2014). Additional posterior probability nodal support values were determined in a Bayesian phylogenetic MCMC search (BA) using MrBayes 3.2.2 (Ronquist et al. 2012) under the general time reversible model with gamma-distributed rate variation (GTR + G). Each search comprised two runs of four chains each for 10×10^6 generations sampled every 100 generations with the first 2.5×10^6 generations discarded as burn-in. This Bayesian approach of phylogenetic analysis was repeated five times to test the independence of the results from topological priors (Huelsenbeck et al. 2002).

Trees were rooted with *Ochropsora ariae*, which also alternates between *Anemone* spp. (and *Aruncus* spp.) and Rosaceae.

Results

Phylogenetic analyses

Bayesian (BA) and maximum likelihood (ML) analyses of ITS and LSU sequence data yielded congruent topologies (see Fig. 1 for BA analyses). These resolved *Tranzschelia* spp. into two major lineages, the *Tranzschelia discolor* clade (herein termed clade I) and the *T. pruni-spinosae* clade (herein termed clade II). A third clade was represented by the hetereu-form species *Leucotelium cerasi*, which was—although weakly supported—resolved as the earliest diverging of the three lineages.

In clade II, three pairs of correlated species were revealed: i. *Tranzschelia pruni-spinosae* (hetereu-form)/*T. fusca* (micro-form) (Eurasia); ii. *T.* sp. 4 (hetereu-form)/*T. pseudofusca* (micro-form) (North America); iii. *T. hyrcanica* (hetereu-form or hemi-form)/*T. pulsatillae* (micro-form) (Eurasia). No correlated species were found in clade I. The sister-species to micro-form species were found to be macrocyclic species except for *Tranzschelia thalictri* and *T.* sp. 3 in clade I, both of which are micro-form species.

Hetereu-form species were present in all clades.

Discussion

Phylogeny and pathways of speciation

Our phylogenetic reconstruction of species of the rust genus *Tranzschelia* (Fig. 2) provides evidence for Arthur's concept of correlated species (Jackson 1931; Arthur 1934) and Tranzschel's Law (Tranzschel 1904; Arthur 1929; Cummins 1959) within clade II for which we uncovered three pairs of
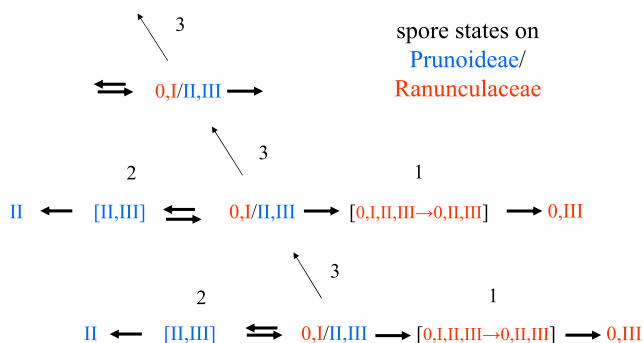

Fig. 2 Major evolutionary and ontogenetic pathways in *Tranzschelia* spp. with host-alternating species as starting point. 1. Reduction via instable autoecious forms to micro-form (correlated species) on Ranunculaceae host (former aecial host) according to Tranzschel's Law. 2. Reduction (but no speciation) on telial host (Prunoideae) via hemi-form (II, III, IV) and finally pure mitotic life cycle with uredinia (II) only as a consequence of missing aecial hosts. 3. Co-evolution with host plants (host shift) and development of new host-alternating species. Roman numerals stand for rust spore states

correlated species, two from Eurasia and one from North America (Fig. 1). In clade I, no correlated species were discovered, maybe due to incomplete sampling. *Tranzschelia* sp. 3 (North America) and *T. thalictri* (Europe) are microcyclic species that parasitize *Thalictrum* spp. Although hardly distinguishable by morphology (Scholler et al. 2014), they are different in ITS 2 sequences (8 bp) and distribution (North America vs. Europe). Therefore, we consider them different species that may have speciated as a consequence of geographic isolation. Following Tranzschel's Law, a correlated macrocyclic species for these could be sought among hetereu-forms with their aecial states on *Thalictrum* sp. *Tranzschelia* spec. has been reported from the USA with 0, I on *Thalictrum purpurascens* DC. (= *Th. dioicum* L.) (Arthur 1934; Farr and Rossman 2017). Both records are named "*T. pruni-spinosae*." However, no relation to a telial host is known so far and no sequence data of the corresponding specimens are available. Sequence data of *Leucotelium cerasi*—a hetereu-form—are located at the base of this lineage as the earliest diverging member, suggesting a macrocyclic and heteroecious ancestry for *Tranzschelia* spp. The overall pattern within the group appears to be a repeated reduction from hetereu-forms to new hetereu-forms and micro-forms.

We suggest two major pathways of speciation within *Tranzschelia*, i. formation of micro-forms (0, III, IV) via auteu-forms (Fig. 2, pathway 1), and ii. formation of new host-alternating species by co-evolution/host shift (Fig. 2, pathway 3). Pathway 2 with hemi-forms (II, III, IV) and asexual uredo-forms (II) does not lead to new species but simply leads to reduced life cycles which finally (as uredo-form) prevent a return to heteroecism. Other speciation pathways such as co-speciation of auteu-forms and micro-forms with their host(s) are possible but cannot be proven by available data. Our data do not provide evidence of jumps of rust species to non-related host species as described in other studies (e.g., McTaggart et al. 2016) or for the hologenetic ladder hypothesis of Leppik (1955) and Savile (1968). It is possible that these events take place on a deeper evolutionary time scale than we have examined. *Tranzschelia* spp. are strictly restricted to Ranunculaceae hosts (autoecious species) and Ranunculaceae/Pomoideae (heteroecious species), respectively.

The function of heteroecism

According to Savile (1976), the cost of heteroecism in rust fungi is extremely high and he calculated a specific "loss of populations" when rust fungi infect aecial hosts with basidiospores or telial hosts with aecial spores. However, considering the high proportion of host-alternating rust species (Mulloch 1994), especially in temperate regions (Berndt 2012; Klenke and Scholler 2015), heteroecism seems a very successful strategy at least in terms of species diversity. Also for

Tranzschelia, host-alternating species make up the majority of extant species (López-Franco and Hennen 1990; Scholler et al. 2014) and our study reveals that hetereu-form species are present in all recovered clades. Thus, the question remains: what may be the function of heteroecism in *Tranzschelia* and other rust fungi? According to Malloch (1995), two benefits for host-alternating species may be additional nutrient sources and that the additional host helps to increase the lifetime reproductive output. However, this does not explain why the many plurivorous rusts such as the grass rusts *Puccinia coronata* Corda and *P. graminis* Pers. that have hundreds of available perennial telial host species (Cummins 1971) or species of woody plants like almost all species of the family Pucciniastraceae are host-alternating.

There are simpler alternatives of disseminating rust spores, namely by transfers from telial host to nearby other telial hosts via mitotic urediniospores—regardless of whether the aecial host is present or absent. This can be shown indirectly by the number of published floristic records and specimen collections. For example, in the inventory of the fungus herbarium of the natural history museum in Karlsruhe, Germany (KR), the majority (80%) of 6078 specimens of host-alternating species are specimens on the telial host (as of 25 May 2016). In *Tranzschelia pruni-spinosae*, there are 30 telial and 19 aecial host specimens, in *T. discolor* the relation is 87:11. *Tranzschelia* spp. and other heteroecious species do not host-alternate in certain regions, where no aecial host population is available. So the fungus' life cycle apparently is reduced to a hemi-form (II, III, IV) only capable of continued clonal reproduction on *Prunus* spp. If karyogamy and subsequent meiosis become useless in host-alternating species because of missing aecial hosts for the basidiospores to infect, teliospore/basidiospore production will finally be stopped and only mitotic urediniospores will be produced ensuring the transfer from the former telial host to telial hosts (*Prunus*

spp.). This corresponds to path 2 in Fig. 2. This reduction to hemi-forms (II, III) and finally mere mitotic production of uredinia (II) are documented for *Tranzschelia pruni-spinosae* and *T. discolor* and may finally lead to allopatric speciation: *T. discolor*, with eastern Mediterranean/Near East origin, has been distributed worldwide with the cultivation of stone fruits like peaches (*Prunus persica* (L.) Batsch) and plums (*P. domestica* L.). In regions where there are no aecial hosts (*Anemone coronaria* L. and related *Anemone* spp.) available, the fungus no longer produces III and IV (e.g., Josifovič 1953; Blumer 1960; Deadman et al. 2007) allowing the species to follow its telial hosts (grown by men) and conquer areas outside their natural area of distribution. In general, fungi that rely on asexual propagation may risk extinction due to the loss of genetic diversity and the inability to adapt to changing ecological conditions (e.g., Fisher et al. 2005). But in the case of *Tranzschelia discolor* and *T. pruni-spinosae*, sexual populations still exist in Europe and Western Asia in their natural area of distribution.

Considering overwinter survival, there are alternative modes for species that rarely or not at all form teliospores, which commonly function as winter or resting spores (e.g., Kirk et al. 2008), especially in areas with temperate climate. *Tranzschelia pruni-spinosae* can overwinter as urediniospores and by systemic infection of the (perennial) host tissue (Blumer 1960). Blumer also found that urediniospores may tolerate low temperatures (2–3 °C) better than teliospores. The micro-form species *Tranzschelia fusca* (Fig. 3a) and probably all other micro-form *Tranzschelia* spp. on Ranunculaceae overwinter as mycelium in the rhizome buds of *Anemone nemorosa* near the vegetation point. Infected rhizomes may produce infected shoots again in the following year (Klebahn 1914). This mode may apply accordingly for rhizomes of *Anemone ranunculoides* (Fig. 3c), the aecial host of the correlated hetereu-form species *Tranzschelia pruni-spinosae* thus

Fig. 3 Correlated species in *Tranzschelia*: **a** Micro-form species *T. fusca* forming spermogonia (0) and telia (III) on etiolated sterile *Anemone nemorosa* host. Background above: uninfected flowering host plants. **b** Rhizome of *A. ranunculoides* with one systemically infected sterile plant with spermogonia (0) and aecia(I) on deformed leaves and one non-infected flowering plant. **c** Uredinia (II) and telia (III) with teliospores on the lower side of a leaf of *Prunus spinosa* (telial host). Photographs taken in SW Germany by M. Scholler; **b** and **c** from Klenke and Scholler 2015
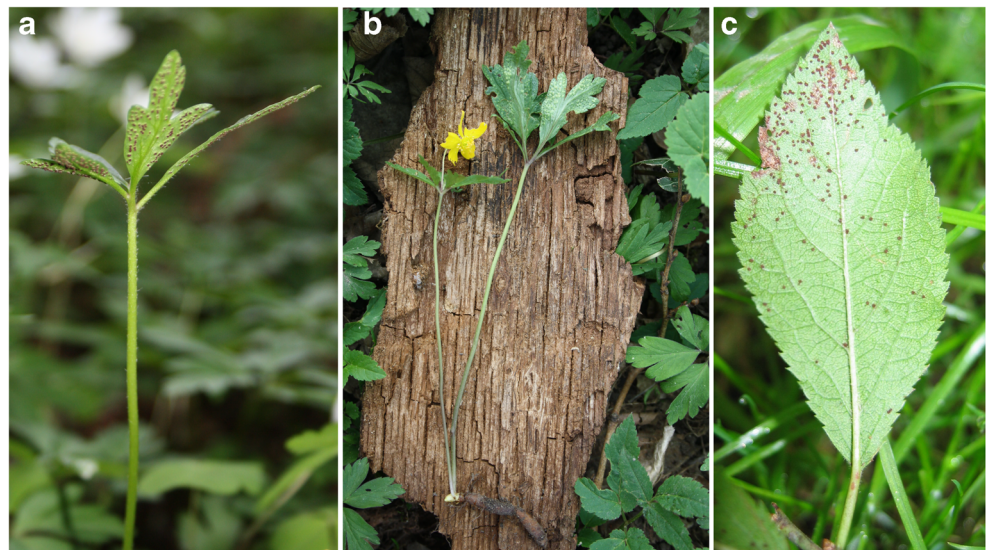
eliminating the need for overwinter survival on the telial host (Fig. 3c) or as urediniospore or teliospore. Since rhizomes are perennial, the fungus may survive in the host tissue for decades. This may be a great advantage for heteroecious species when telial hosts are rare or completely missing over long periods of time. Thus the fungus survives, producing vast quantities of aeciospores. But when telial hosts return to proximity, the fungus may host-alternate again and reproduce sexually. We do not know any "obligate" (e.g., Wilson and Henderson 1966; Zwetko 1990) or "essential" (Feau et al. 2011) heteroecism and we cannot provide any advantage or function connected with "obligate heteroecism" for hetereuforms. Even in heteropsis-form species (0, I/III, IV) of the genus *Gymnosporangium* that lack a mitotic short cycle host-alternating is not obligate, because species of this genus may also survive as mycelium in the woody tissues of both hosts (e.g., Bernaux 1956; EPPO 2006; Dervis et al. 2010). In *Tranzschelia* spp. (and probably in all other host-alternating rust species), we consider the host-alternating process as facultative and talk about "facultative heteroecism," a term that was used and introduced long time ago (Meinecke 1916; Meinecke 1920; group 1 in Peterson 1961).

Taxonomic considerations

Our molecular phylogenetic analyses based on ITS and LSU rDNA (Fig. 1) neither support broad morphology-based species concepts of *Tranzschelia fusca* and *T. pruni-spinosae* (e.g., Hylander et al. 1953; Wilson and Henderson 1966; Urban and Markova 2009), nor do we find support for the hypothesis that life cycle types have taxonomic value in this complex as proclaimed by Schröter (1889) and Arthur (1906, 1921). Specimens of the "morphological species" *Tranzschelia pruni-spinosae* var. *americana* López-Franco & J.F. Hennen belong to three different clades in our phylogeny (*Tranzschelia* sp. 1, 2 and 4). Considering morphology, host plant species, geographic distribution, and our molecular phylogenetic hypothesis, six specimens (*Tranzschelia* sp. 1–5, see Table 1, Fig. 1) cannot be assigned to any known species. This indicates that *Tranzschelia* requires further taxonomic research worldwide—even in North America where *Tranzschelia* spp. are fairly well studied (López-Franco and Hennen 1990; Ono 1994; Scholler et al. 2014; Abbasi and Aime 2015; Blomquist et al. 2015).

Conclusion

Among the oldest extant rust fungi are heteroecious taxa, that likely evolved in conjunction with early Angiosperms during the Jurassic (Aime 2006; Aime et al. 2018). Our results confirm that correlated species evolved several times among species of *Tranzschelia*. In *Tranzschelia*, but probably in all other

genera with host-alternating species, hetereu-form species do both: they most likely represent the starting point for speciation and other life cycle variations and they persist due to the plasticity of their life cycle in terms of reproduction, overwinter survival, and hosts.

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