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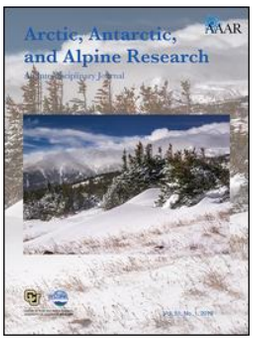
Originally published in:
Arctic, Antarctic, and Alpine Research 51(1), https://doi.org/10.1080/15230430.2019.1676951



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Arctic, Antarctic, and Alpine Research

An Interdisciplinary Journal

ISSN: 1523-0430 (Print) 1938-4246 (Online) Journal homepage: https://www.tandfonline.com/loi/uaar20

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To cite this article: Thomas N. Sieber (2019) The endophyte *Allantophomopsis cytisporea* is associated with snow blight on *Calluna vulgaris* in the Alps—An effect of climate change?, Arctic, Antarctic, and Alpine Research, 51:1, 460-470, DOI: 10.1080/15230430.2019.1676951

To link to this article: https://doi.org/10.1080/15230430.2019.1676951

Published online: 28 Oct 2019.

ARCTIC, ANTARCTIC, AND ALPINE RESEARCH
2019, VOL. 51, NO. 1, 460–470
https://doi.org/10.1080/15230430.2019.1676951

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The endophyte *Allantophomopsis cytisporea* is associated with snow blight on *Calluna vulgaris* in the Alps—An effect of climate change?

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ABSTRACT

Shoots of *Calluna vulgaris, Erica carnea, Juniperus communis* subsp. *nana, Picea abies*, and *Pinus mugo* subsp. *mugo* covered with felty, melanized epiphytic mycelia typical for brown felt blight caused by *Herpotrichia pinetorum* were collected at several locations in the Swiss Alps. Most cultures prepared from the mycelia on *J. communis* subsp. *nana* and *P. abies* were *H. pinetorum*, whereas the majority of cultures from *P. mugo* subsp. *mugo* and *C. vulgaris* were identified by internal transcribed spacer ITS1-5.8S-ITS2 sequencing and morphology as *Allantophomopsis cytisporea*. The fungus tolerates low temperatures, has an optimum between 16°C and 24°C, and ceases to grow at 28°C. 35°C is lethal. *A. cytisporea* is known as the causal agent of cranberry black rot on *Vaccinium macrocarpon* but has never been described as a snow mold. *A. cytisporea* is an endophyte in *C. vulgaris* but seems able to kill leaflets and whole shoots during winter. The epiphytic mycelium can expand from *C. vulgaris* to neighboring shoots of *P. mugo* subsp. *mugo* and *J. communis* subsp. *nana* below the snow where it forms epiphytic mycelial mats reminiscent of *H. pinetorum*. *H. pinetorum* has a strong antibiotic effect against *A. cytisporea* at 4°C and 20°C, whereas *A. cytisporea* grows faster at these temperatures. The effects of climate change on the interaction between the two snow mold fungi and their consequences on regeneration of woody plants at timberline are discussed.

ARTICLE HISTORY
Received 25 April 2019
Revised 28 August 2019
Accepted 24 September 2019

KEYWORDS
Snow mold; climate change; timberline; fungal interaction; forest regeneration

Introduction

Snow blight pathogens such as *Herpotrichia pinetorum* and *Gremmenia* (*Phacidium*) *infestans* affect natural regeneration of conifers in alpine, subalpine, and boreal zones and probably play a decisive role in determining conifer regeneration near timberline (Hartig 1888; Gäumann, Roth, and Anliker 1934; Roll-Hansen 1989). During a study about the population structure of *H. pinetorum* in the region of Arosa, Switzerland, three of more than eighty strains prepared from melanized mycelial mats covering shoots of juniper (*Juniperus communis* subsp. *nana*), dwarf mountain pine (*Pinus mugo* subsp. *mugo*), and Norway spruce (*Picea abies*) in the Swiss Alps were not *H. pinetorum* but a species of *Allantophomopsis*, indicating that *H. pinetorum* is the main but not the only fungus associated with brown felt blight (Schneider et al. 2009). Consequently, not all of the brown to black mycelial mats covering dead shoots of young conifers are due to colonization by *H. pinetorum*. This observation was the starting point for the present study. Melanized, superficial mycelia on *J. communis* subsp. *nana*, *P. mugo* subsp. *mugo*, *P. abies*, *Erica carnea* (winter heath), and *Calluna vulgaris* (common heather) were collected during snowmelt at different locations in the alpine zone in Switzerland over several years. *C. vulgaris* was included because this plant species and its accompanying vegetation close to melting snow patches in the alpine zone were repeatedly observed to be covered by mats of felty gray brown mycelia reminiscent of *H. pinetorum* (Figure 1 and Table 1). Whereas the brown mycelial mats formed on conifers by *H. pinetorum* are visible to the naked eye mostly during the whole vegetation period, the mats on *C. vulgaris* and *E. carnea* are macroscopically visible for a very short period of time only after the snow melts away (Figure 1d). Recognition of the mats is best right at the edge of the retreating snow cover in spring.

The aims of this study were to (1) identify the fungi forming the melanized mycelial mats, (2) describe the fungus most frequently found besides *H. pinetorum*, (3) determine its temperature requirements for growth, and (4) identify the most closely related fungal species.

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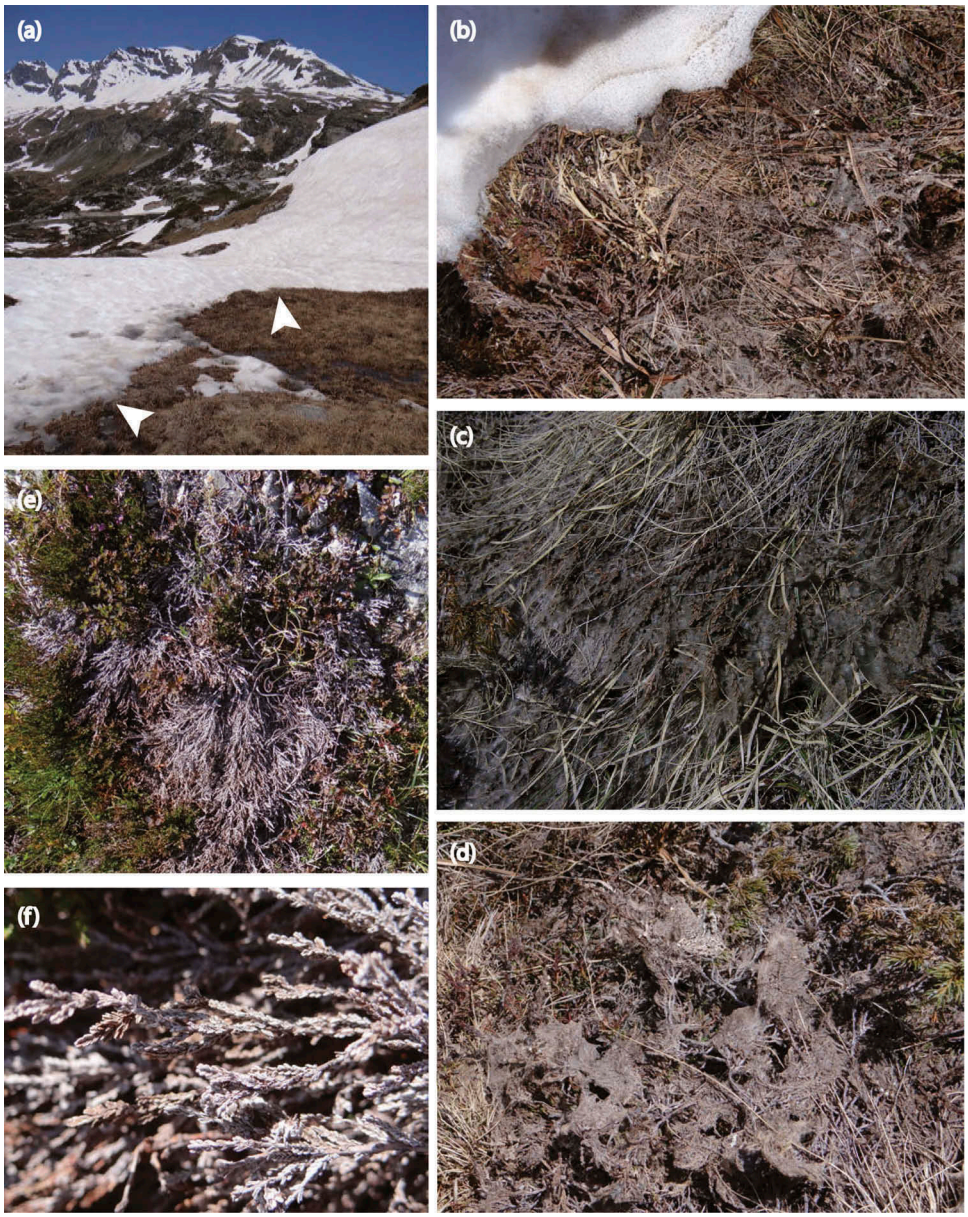

Figure 1. (a) The San Bernardino site (1930 m a.s.l.) during snowmelt on 8 June 2014; arrowheads indicate places where felty brown to black mycelial mats of *Allantophomospsis cytisporea* as shown in (b), (c), and (d) can be observed. (b) Mycelial mat right at the edge of melting snow. (c) Mycelial mat covering horsts of dead leaves of the grass *Nardus stricta*. (d) Mycelial mat covering *Calluna vulgaris*. (e) Shoot blight on *C. vulgaris* observed at the San Bernardino site on 24 August 2014. (f) Close-up of (e).

The ecology of the fungus and its possible significance in the context of climate change are discussed.

Materials and methods

Isolates

Shoots of *C. vulgaris, E. carnea, J. communis* subsp. *nana, P. abies*, and *P. mugo* subsp. *mugo* covered with brown to black superficial mycelia reminiscent of brown felt blight caused by *H. pinetorum* and *H. coulteri* were collected during snowmelt at the edge of retreating snow patches at different locations between 1,800 and 2,400 meters above sea level (m.a.s.l.) in the Alps between 2002 and 2015 (Figure 1 and Table 1). Shoots were stored at 4°C and processed within 72 hours. Small tissue samples covered by epiphytic mycelium were incubated on water agar (15 gl^{-1} agar, 50 mgl^{-1} terramycine [oxytetracycline]) at 4°C for at least 5 weeks. At least three replicates were prepared per collection. Some dead leaves of *C. vulgaris* from shoots covered with melanized mycelium were dipped into 96 percent ethanol for 1 minute prior to incubation to reduce the number of living microbes on the leaf surface. Single hyphal tip cultures

Table 1. Sampling sites, collection dates and plant species collected.

Geographic origin	Coordinates[a]	Altitude (m.a.s.l.)	Hosts examined[b]	Collection date	Collector
Arosa, Sandböden	46°48'06.6" N 9°40'23.8" E	2,050	*Juniperus communis* subsp. *nana*, *Picea abies*, *Pinus mugo* subsp. *mugo*	28 June 2006	M. Schneider
Arosa, Maraner Hauptji	46°47'48.9" N 9°40'15.8" E	2,150	*Calluna vulgaris*	5 June 2015	O. Holdenrieder
Davos, Dischma	46°45'12.4" N 9°54'5.9" E	1,840	*Calluna vulgaris*, *Erica carnea*	3 June 2015	N. Luschka
Davos, Sertig	46°43'28.5" N 9°51'3.4" E	1,870	*Calluna vulgaris*	14 May 2015	N. Luschka
Davos, Kühalptal	46°42'44.3" N 9°51'37.8" E	1,980	*Juniperus communis* subsp. *nana*, *Erica carnea*	14 May 2015	N. Luschka
Davos, Rinerhorn	46°45'5.0" N 9°49'7.5" E	2,080	*Erica carnea*	6 June 2015	N. Luschka
Davos, Geisswaldji	46°49'59.9" N 9°52'42.3" E	1,800	*Calluna vulgaris*	24 May 2015	N. Luschka
Göschenenralp	46°40'9.8" N 8°32'38.4" E	1,820	*Juniperus communis* subsp. *nana*, *Picea abies*	3 August 2012	A. Gross
Lötschental	46°27'14.3" N 7°54'34.7" E	2,328	*Juniperus communis* subsp. *nana*, *Picea abies*, *Calluna vulgaris*	26 July 2012	O. Holdenrieder
San Bernardino	46°28'49.2" N 9°10'31.5" E	1,930	*Calluna vulgaris*	3 April 2002	T. N. Sieber
San Bernardino	46°28'49.2" N 9°10'31.5" E	1,930	*Calluna vulgaris*, *Juniperus communis* subsp. *nana*, *Pinus mugo* subsp. *mugo*	23 June 2012	T. N. Sieber
San Bernardino	46°28'49.2" N 9°10'31.5" E	1,930	*Calluna vulgaris*, *Pinus mugo* subsp. *mugo*	8 June 2014	T. N. Sieber

[a]Collections were made within a radius of 200 m.
[b]Samples only collected from plants with shoots covered by superficial mycelia. *Calluna vulgaris* occurred at all sites but was not colonized by snow molds at all sites and was therefore not collected at all sites.

were prepared from emerging melanized mycelia on malt extract agar (MEA; 20 gl^{-1} malt extract, 15 gl^{-1} agar). The three *Allantophomopsis* strains (A_AR_1_06, A_AR_2_06, A_AR_3_06) isolated by Schneider et al. (2009) were also included (Table 2).

Growth rates and temperature requirements

Growth rates (mmd^{-1}) of 16 *Allantophomopsis cytisporea* strains were determined on MEA at 20°C (Table 2). The growth increment was measured every 2 to 4 days along two perpendicular axes through the inoculum of each of two colonies per isolate to determine the mean daily growth rate. In addition, strains A_AR_1_06 and A_SB_5_02 were used to study the temperature requirements. Growth rates of these strains were examined at 0, 4, 8, 12, 16, 20, 24, 28, 32, and 36°C on MEA.

Morphology

Conidial morphology was studied for eight selected *A. cytisporea* strains. Conidia were examined both in water and in concentrated lactic acid and observed under phase contrast optics using a Zeiss Axiophot microscope. Ordinary fountain pen ink (Pelikan, blue) was used to visualize conidial appendages.

Mycelial interaction between Allantophomopsis cytisporea *and* Herpotrichia pinetorum

Interactions between each of three *A. cytisporea* isolates (A_AR_1_06, A_DA_21_15, A_SB_7_12) and *H. pinetorum* (73-Fo, 18a, 95-Fi; Schneider et al. 2009) strains were tested on MEA at 4°C and 20°C. Colonized 5-mm-diameter plugs from the margin of fresh colonies on MEA were placed at a distance of 30 mm from each other on MEA and incubated. The progress of mycelial growth was monitored every 2 to 5 days.

Sequencing the internal transcribed spacer

For DNA extraction, aerial mycelium was harvested directly from single hyphal tip culture after 12 weeks of incubation at 4°C and lyophilized. DNA was extracted using the NucleoSpin 96 Plant II Kit (MACHEREY-NAGEL, Düren, Germany) according to the manufacturer's protocol. Nucleotide sequences of the internal transcribed spacer ITS1-5.8S-ITS2 region were determined as described previously using primers ITS1 and ITS4 (White et al. 1990; Ibrahim, Sieber, and Schlegel 2017). Amplification of DNA was performed in 20 µl reaction volumes using approximately 2 ng of template

Table 2. *Allantophomopsis* strains isolated from mycelial mats covering shoots of various plant species at various sites in the Swiss Alps during snow melt.

Strain number[a]	Geographic origin	Altitude (m.a.s.l.)	Host	Growth rate on MEA at 20°C (mmd^{-})[b]	GenBank accession number
A_AR_1_06	Arosa, Sandböden	2,050	*Juniperus communis* subsp. *nana*	6.3	FJ904499
A_AR_2_06	Arosa, Sandböden	2,050	*Juniperus communis* subsp. *nana*	6.6	MK790117
A_AR_3_06	Arosa, Sandböden	2,050	*Juniperus communis* subsp. *nana*	6.9	MK790118
A_SB_4_02	San Bernardino	1,930	*Calluna vulgaris*	7.5	MK790119
A_SB_5_02	San Bernardino	1,930	*Calluna vulgaris*	7.2	MK790120
A_SB_6_12	San Bernardino	1,930	*Pinus mugo* subsp. *mugo*	7.2	MK790121
A_SB_7_12	San Bernardino	1,930	*Calluna vulgaris*	7.0	MK790122
A_SB_8_12	San Bernardino	1,930	*Juniperus communis* subsp. *nana*	6.5	MK790123
A_SB_9_12	San Bernardino	1,930	*Juniperus communis* subsp. *nana*	6.8	MK790124
A_LT_11_12	Lötschental	2,328	*Calluna vulgaris*	n.a.	MK790116
A_SB_12_14	San Bernardino	1,930	*Calluna vulgaris*	n.a.	MK790129
A_SB_14_14	San Bernardino	1,930	*Calluna vulgaris*	5.5	MK790125
A_SB_15_14	San Bernardino	1,930	*Calluna vulgaris*	n.a.	MK790130
A_SB_18_14	San Bernardino	1,930	*Pinus mugo* subsp. *mugo*	6.8	MK790126
A_SB_19_14	San Bernardino	1,930	*Pinus mugo* subsp. *mugo*	6.5	MK790127
A_SB_20_14	San Bernardino	1,930	*Pinus mugo* subsp. *mugo*	6.9	MK790128
A_DA_21_15	Davos, Geisswaldji	1,800	*Calluna vulgaris*	6.8	MK790134
A_DA_22_15	Davos, Geisswaldji	1,800	*Calluna vulgaris*	5.5	MK790131
A_DA_23_15	Davos, Geisswaldji	1,800	*Calluna vulgaris*	n.a.	MK790133
A_DA_24_15	Davos, Geisswaldji	1,800	*Calluna vulgaris*	5.6	MK790132

[a]Strains deposited at UAMH Center for Global Microfungal Biodiversity, Toronto, Canada. [b]n.a. = not available.

DNA. After an initial denaturation step for 2 minutes at 94°C, thirty-five cycles were performed each consisting of a denaturation step at 94°C for 30 seconds, an annealing step at 60°C for 1 minute, and an extension step at 72°C for 1 minute, followed by a final extension step for 10 minutes at 72°C. Polymerase chain reaction (PCR) products were directly purified using the Montage PCR Life Science Kit (Millipore Corporation, Bedford, MA) following the manufacturer's instructions. Sequencing of purified PCR products was performed by Microsynth (Balgach, Switzerland). The sequences were BLASTed against sequences deposited in GenBank to find the most closely matching ones. Closely related species were included in phylogenetic analysis to show the relative position of the collected strains among strains found by other authors (Table 3). Maximum parsimony analyses were performed in PAUP 4.0a. Bootstrapping to generate 100 pseudosamples was used for accuracy estimations. *Gremmenia* (*Phacidium*) *infestans* (U92305) served as the outgroup.

Results

Brown to black superficial mycelial mats were found on all the five plant species examined. Whereas the mats remained clearly visible on *J. communis* subsp. *nana*, *P. abies*. and *P. mugo* subsp. *mugo* throughout the vegetation period, discovery of mycelial mats on *E. carnea* and *C. vulgaris* was pure coincidence because, during dry weather, the mats were conspicuous only for a few hours after the snow cover had melted (Figures 1b–1d). The exposed mycelial mats on these ericaceous plants dried and collapsed and the hyphae were torn apart, and the presence of epiphytic hyphae could only be recognized using magnification glasses. Conspicuous gray patches consisting of dead shoots of *C. vulgaris* or living shoots bearing dead leaves were often observed in spring and summer. A closer look at these shoots revealed the presence of mycelial fragments indicative of the former presence of mycelial mats (Figures 1e, 1f). In addition, examination of leaves under the light microscope showed presence of melanized, septate mycelium on and within the leaves (Figure 2a). Incubation of such leaves usually led to the emergence of *A. cytisporea*.

Melanized mycelia that grew comparatively well at 4°C could be isolated from most samples. Most of these isolates grew fast at 20°C on MEA (5.5–7.5 mmd^{-1}), forming olive gray colonies without or with sparse aerial mycelium (Table 2). The ITS sequences of a majority of these isolates were identical, and nonidentical sequences differed by maximally 0.6 percent (Figure 3). The ITS sequences differed by maximally

Table 3. Collection details and GenBank accession numbers of isolates included in the phylogenetic analysis.

Fungal name[a]	ITS sequence GenBank accession number	Strain number	Host	Substrate	Geographic origin	Lifestyle/disease	Reference
Allantophomopsiella pseudotsugae	KJ663826	CBS 321.53	*Picea abies*	Dead bark	Norway	Dieback	Crous et al. (2014)
Allantophomopsiella pseudotsugae	KJ663829	CBS 841.91	*Pinus* sp.	n.a.	Germany	n.a.	Crous et al. (2014)
Allantophomopsiella pseudotsugae	KJ663827	CBS 437.71	*Pinus sylvestris*	n.a.	The Netherlands	n.a.	Crous et al. (2014)
Allantophomopsiella pseudotsugae	JN033384	CBS 322.53	*Picea abies*	Dead bark	Sunnfjord, Norway	Saprophyte	Han et al. (2014)
Allantophomopsis cytisporea	KJ663830	CBS 262.85	Conifer	Root	Germany	n.a.	Crous et al. (2014)
Allantophomopsis cytisporea	KJ663822	CBS 109.22	*Vaccinium macrocarpon*	Leaf	United States	Black rot	Crous et al. (2014)
Allantophomopsis cytisporea	KR873228	CBS 140061 ex-type	*Vaccinium macrocarpon*	Berry	Aluksne, Latvia	Black rot	Crous et al. (2015)
Allantophomopsis lunata	NR132922	CBS 137781 ex-type	*Vaccinium macrocarpon*	Berry	New Jersey	Black rot	Crous et al. (2015)
Allantophomopsis lycopodina	AB041243	CBS 361.68	*Rhododendron* sp.	Litter	The Netherlands	Saprophyte	Okane et al. (2001)
Allantophomopsis sp.	KJ663839	CBS 322.36	*Pinus radiata*	n.a.	New Zealand	n.a.	Crous et al. (2015)
Fungal sp.	FM172911	agrAP150	*Calluna vulgaris*	Root hair	Bavaria, Germany	Endophyte	n.a.
Fungal sp.	FM200723	AP652	*Calluna vulgaris*	Leaf	Bavaria, Germany	Endophyte	n.a.
Fungal sp.	HM123110	AZ0370	*Pinus arizonica* var. *arizonica*	Dead needle	Arizona	Saprophyte	U'ren et al. (2010)
Fungal sp.	HM123479	AZ0780	*Physcia caesia*	Lichen thallus	Arizona	Endolichenophyte	U'ren et al. (2010)
Phacidiopycnis sp.	HM595538	M5, ZLY-2010b	*Abies beshanzuensis*	Needle	Baishanzu National Nature Reserve, China	Endophyte	Yuan et al. (2011)
Phacidiopycnis washingtonensis	AY608644	CLX2152	*Malus domestica*	Fruit	Chelan, Washington	Postharvest disease	Xiao et al. (2005)
Phacidiopycnis washingtonensis	JF732919	OVB10-001	*Malus domestica*	Fruit	Germany	Postharvest disease	Weber (2011)
Phacidiopycnis washingtonensis	JQ013491	JKM54	*Pinus radiata*	n.a.	New Zealand	n.a.	n.a.
Gremmenia infestans	U92305		*Pinus cembra*	Shoot	Obersulzbachtal, Germany	Snow blight	Gernandt et al. (1997)
Phacidium lacerum	FR717225	ZK205/08	*Picea abies*	Litter	Czech Republic	Saprophyte	Zifcakova et al. (2011)
Phacidium lacerum	FR837911	ZK20/08	*Picea abies*	Needle	Czech Republic	Endophyte	Koukol et al. (2012)
Phacidium lacerum	KJ663842	CBS 338.70	*Ilex aquifolium*	Litter	The Netherlands	Saprophyte	Crous et al. (2014)
Phacidium lacerum	KJ663846	CBS 761.73	*Pinus sylvestris*	Dead needle	France	Saprophyte	Crous et al. (2014)
Phacidium lacerum	KJ663843	CBS 400.81	*Juniperus communis*	Living needle	France	Endophyte	Crous et al. (2014)
Phacidium lauri	KJ663850	CBS 308.68	*Prunus laurocerasus*	Litter	The Netherlands	Saprophyte	Crous et al. (2014)
Phacidium pseudophacidioides	KJ663853	CBS 590.69	*Ilex aquifolium*	Litter	The Netherlands	Saprophyte	Crous et al. (2014)
Potebniamyces pyri	AY608641	CBS 339.78	*Pyrus communis*	Fruit	British Columbia, Canada	Postharvest disease	Xiao et al. (2005)
Potebniamyces pyri	KJ663823	CBS 282.55	*Pyrus communis*	Peduncle	The Netherlands	Saprophyte	Crous et al. (2014)
Potebniamyces pyri	KJ663859	CBS 322.63	*Pyrus communis*	Bark	The Netherlands	Saprophyte	Crous et al. (2014)
Pseudophacidium ledi	KJ663860	CBS 377.59	*Picea abies*	n.a.	Switzerland	n.a.	Crous et al. (2014)

n.a. = not available.
[a]Current name according to Index fungorum (http://www.indexfungorum.org/Names/Names.asp), accessed 12 March 2019.

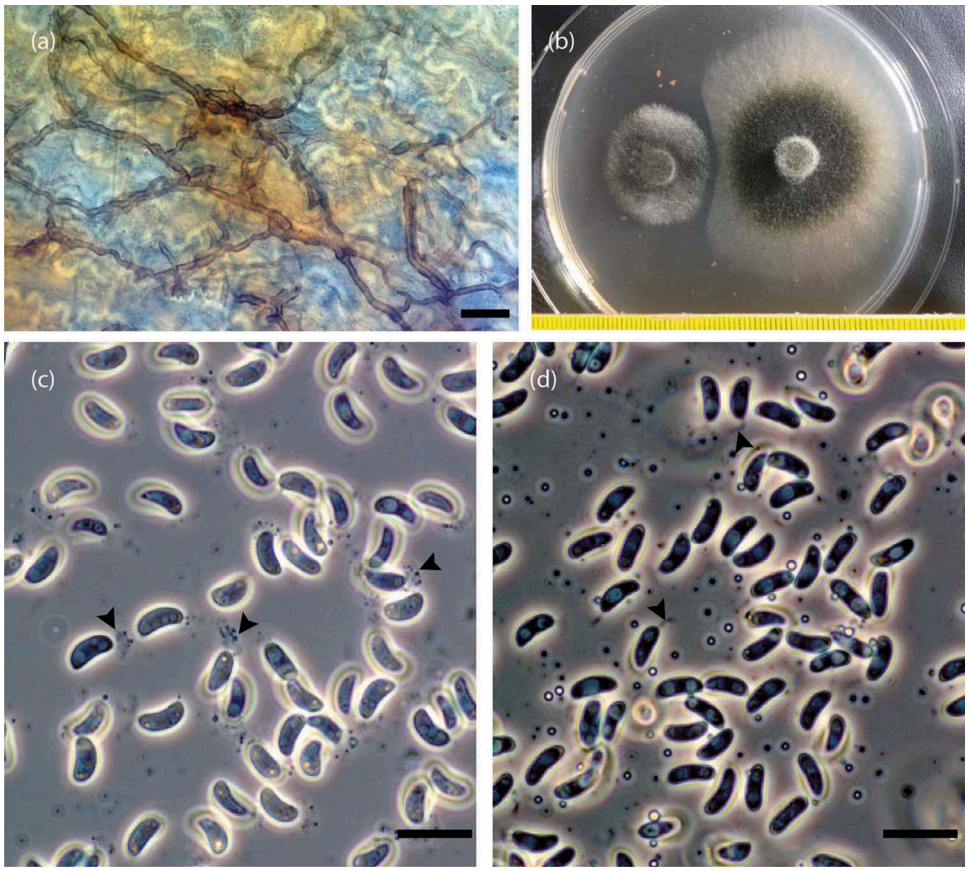

Figure 2. (a) Mycelium of *Allantophomopsis cytisporea* colonizing dead tissue of a *Calluna vulgaris* leaf (scale bar: 20 µm); the bright meandering lines are the cell walls of the puzzle piece–like epidermal cells of *C. vulgaris*. (b) Interaction between *Herpotrichia pinetorum* (strain 95-Fi; Schneider et al. 2009) to the left and *A. cytisporea* (strain A_DA_21_15) to the right after 21 days at 4°C on MEA. Conidia of *A. cytisporea* strains (c) A_SB_8_12 and (d) A_SB_6_12 in H_2O stained with fountain pen ink (Pelikan, blue) and observed using phase contrast optics (scale bar: 10 µm); arrowheads indicate the slimy appendages of the conidia.

0.6 percent from that of an *Allantophomopsis lunata* ex-type isolate (NR132922), by up to 1.7 percent from that of an *A. cytisporea* ex-type isolate (KJ663830), and by up to 1.2 percent from that of an *Allantophomopsis lycopodina* isolate (AB041243). Thus, based on DNA sequences of the ITS regions, at least three different species names are possible for the *Allantophomopsis* species isolated during this study: *A. lycopodina, A. cytisporea*, and *A. lunata*.

Fertile pycnidia developped within 4 to 8 weeks of cultivation. Conidial dimensions did not differ significantly among strains (4.9–7.8 × 1.9–3.0 µm; mean: 6.2 × 2.5 µm) and corresponded well with those of the conidia of *A. cytisporea* (6–8 × 2–2.5 µm; Carris 1990) and *A. lunata* (6–9 × 2–3.5 µm; Nag Raj 1993) (Figures 2c, 2d). The conidia of *A. lycopodina* did not fit because these are distinctly longer and measure (7–)8–15(–17) × 2–3.5 µm (mean: 11.2 × 2.9 µm; Carris 1990). Thus, the snow mold strains isolated during this study are referred to as *A. cytisporea* because *A. cytisporea* and *A. lunata* are considered synonymous, with *A. cytisporea* being the older name (Carris 1990; Nag Raj 1993).

Three snow mold isolates from Davos Geisswaldji (A_DA_22_15, A_DA_23_15, and A_DA_24_15) grew more slowly and produced olive gray colonies with a lot of aerial mycelium, and conidiomata never formed (Table 2). These three strains are closely related but not conspecific with *A. cytisporea* because, in addition to the lack of conidia formation in culture and different colony morphology, their ITS regions differed from those of all other *A. cytisporea* strains at several nucleotide positions. Strain A_DA_24_15 is most closely related to the ex-(neo)type strains of *Phacidium lacerum* and *P. pseudophacidioides* (Crous et al. 2015; Figure 3). The identity of the other two strains is less clear, but their ITS sequences were close to a sequence of *Pseudophacidium ledi*.

The ITS regions of *Phacidiopycnis washingtonensis* and *Potebiamyces pyri*, both known for causing postharvest rots on pome fruits (Xiao et al. 2005), and *Allantophomopsiella pseudotsugae* from dead bark and

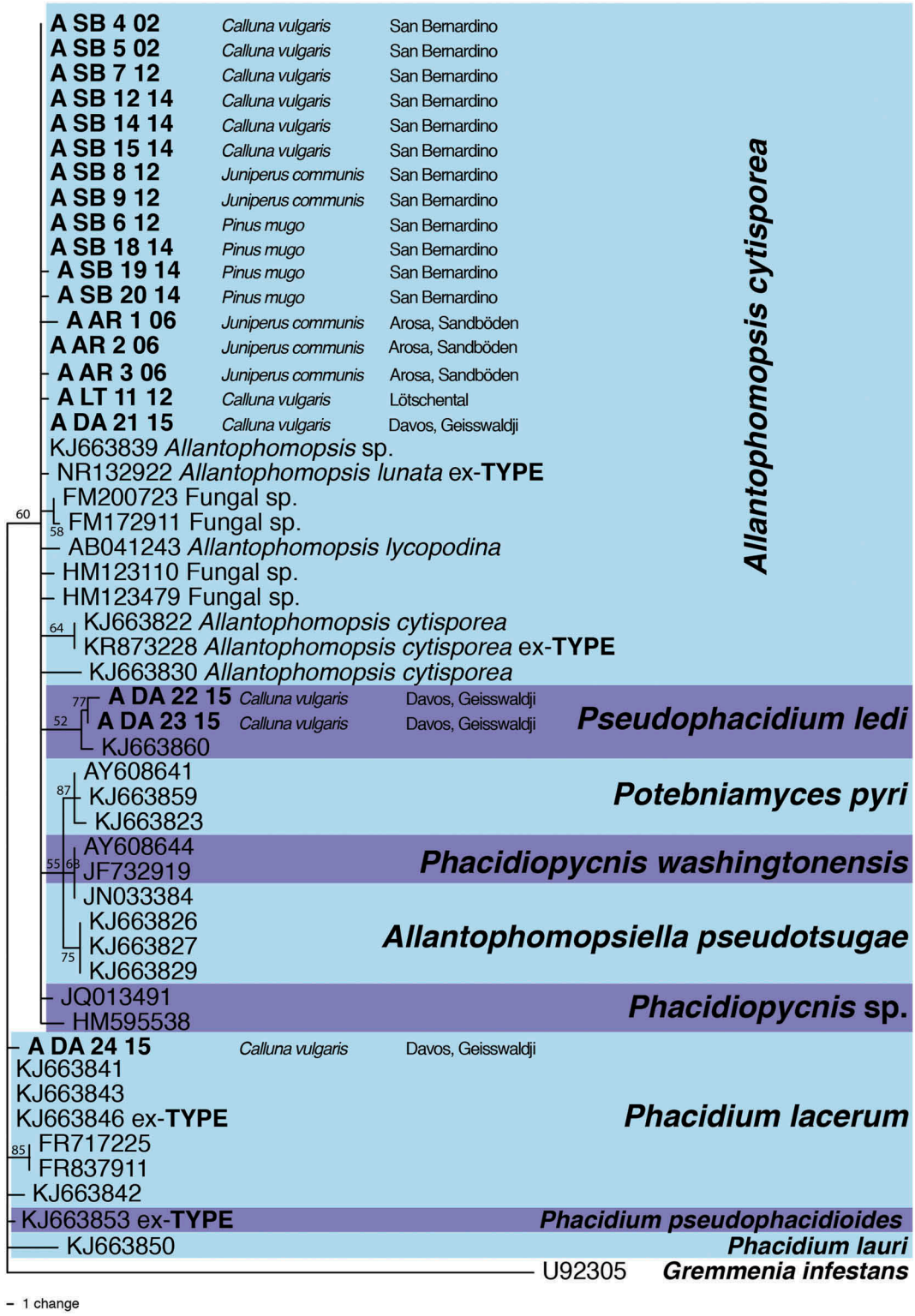

Figure 3. The most parsimonious tree showing the phylogenetic relationships among snow mold strains collected for this study (bold) and related species as inferred from ITS1-5.8s-ITS2 (489 character states including gaps) sequences. The scale bar shows the number of changes, and bootstrap support values of greater than 50 percent from 100 replicates are shown at the nodes. Taxon names are preceeded by GenBank accession numbers. The species boundaries are delimited with colored blocks. The tree was rooted to *Gremmenia* (*Phacidium*) *infestans* (GenBank accession U92305).

needles of conifers (Crous et al. 2014) are also closely related but formed reasonably well-supported clusters separate from the snow mold strains. In addition, the conidia of *P. washingtonensis* and *P. pyri* do not possess an apical appendage, and the conidia of *P. pyri* are distinctly larger (9.0–14.0 × 5.5–9.0 µm; Xiao et al. 2005).

Due to the unpredictability of the discovery of mycelial mats, the number of samples varied considerably among the individual plant species and sites (Table 4). Most strains from *C. vulgaris* and *P. mugo* subsp. *mugo* were *A. cytisporea*, with *Herpotrichia pinetorum* being the second most abundant species. In contrast, *H. pinetorum* dominated on *J. communis* subsp. *nana* and *P. abies*. Neither *A. cytisporea* nor *H. pinetorum* were found on *E. carnea*. Other fungi such as *Cladosporium* spp. and *Mucor hiemalis* were quite often isolated from the two ericaceous hosts. More rarely found fungi were *Truncatella angustata* (GenBank accession number: MK790184), *Phaeosphaeria lycopodina* (MK790186), *Botrytis cinerea*, and a species of *Mycocentrospora* (MK790185).

A. cytisporea was isolated from *J. communis* subsp. *nana* at Arosa Sandböden (Schneider et al. 2009); from *C. vulgaris, J. communis* subsp. *nana*, and *P. mugo* subsp. *mugo* at San Bernardino; from *C. vulgaris* in the Lötschental; and at Davos Geisswaldji (Table 2). *A. cytisporea* was found on *C. vulgaris* at the San Bernardino site in all 3 years of collection (2002, 2012, and 2014). In contrast, *A. cytisporea* was not found at Arosa Maraner Hauptji, at Davos except at the Geisswaldji site, or at Göscheneralp.

Optimum temperature for growth of the collected *A. cytisporea* strains was between 16°C and 24°C. Growth rates were much higher than those of *H. pinetorum* even at 0°C and 4°C (growth rate of approximately 0.7 mmd^{-1} at 0°C; Figure 4). *A. cytisporea* did not grow at 28°C or higher, and

Table 4. Frequency (percentage) of incubated mycelial samples giving rise to *Allantophomopsis cytisporea, Herpotrichia pinetorum*, and other fungi.

Host	Number of samples (*n*)	*Allantophomopsis cytisporea*	*Herpotrichia pinetorum*	Other fungi[a]	No growth
Calluna vulgaris[b]	68	16.2	10.3	51.5	22.1
Erica carnea	9	0	0	88.9	11.1
Juniperus communis subsp. *nana*	99	5.1	84.0[c]	7.1[d]	3.0
Picea abies	6	0	100.0	0	0
Pinus mugo subsp. *mugo*	18	22.2	16.7	11.1	50.0

[a]Including yeasts, *Cladosporium* spp., *Mucor hiemalis, Phaeosphaeria lycopodina* (GenBank accession number: MK790186), *Truncatella angustata* (MK790184), *Botrytis cinerea*, and *Mycocentrospora* sp. (MK790185).
[b]A few samples consisted of a surface-sterilized (1 minute, 96 percent ethanol) leaf.
[c]Two *Herpotrichia* isolates were *Neopeckia coulteri* (FJ904496, FJ904497).
[d]One *Herpotrichia* sp. (FJ904498) isolate, not conspecific with either *N. coulteri* or *H. pinetorum* (see Schneider et al. [2009] for GenBank accessions).

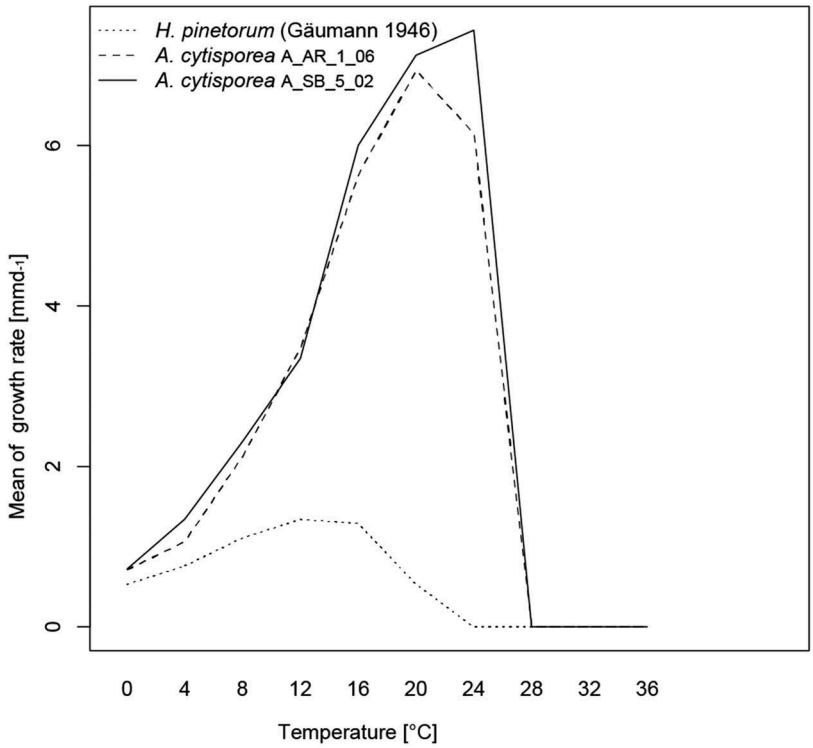

Figure 4. Growth rates of two *Allantophomopsis cytisporea* strains and *Herpotrichia pinetorum* at various temperatures.

temperatures 35°C and higher were lethal. Interaction of the two species on MEA at 4°C showed a strong antibiotic effect of *H. pinetorum* against *A. cytisporea* expressed by a distinct inhibition zone (Figure 2b). A similar effect was observed at 20°C. The type of interaction was always the same independent of the strain combination.

Discussion

Unequivocal species identification of the newly discovered snow molds by ITS sequencing was impossible, but they certainly belong to the *Allantophomopsis cytisporea* sensu lato species complex (Crous et al. 2015). *A. lycopodina* has distinctly longer conidia than those of the strains found in the present study. Thus, the snow mold fungus is either *A. cytisporea, A. lunata*, or a hitherto undescribed species. *A. cytisporea* and *A. lunata* are considered conspecific by some authors (Carris 1990; Nag Raj 1993). In fact, the ITS regions of the ex-type cultures of the two species differ only at a few nucleotide positions (99 percent similarity) and thus probably represent two operational taxonomic units of one and the same species (Schlegel, Queloz, and Sieber 2018). Therefore, the older name *A. cytisporea* is used here. A multigene phylogeny combined with morphological investigations on a larger and geographically more diverse collection of snow molds and closely related taxa, preferentially type specimens, would be required to decide whether the snow mold fungus is a new species. *A. cytisporea* is known from various ericaceous hosts and *Pinus* species and is often associated with cranberry (*Vaccinium macrocarpon*) black rot (Carris 1990; Nag Raj 1993; Olatinwo, Hanson, and Schilder 2003), which is a serious post-harvest disease. *C. vulgaris* and *J. communis* subsp. *nana* can now be added to the list of hosts.

Extented networks of melanized mycelium of *A. cytisporea* can be observed in and on dead leaf tissue (Figure 2a). Interestingly, Pietrowski, Flessa, and Rambold (2012) isolated an *Allantophomopsis* species as an endophyte from *C. vulgaris* leaves that possesses an ITS identical to that of *A. cytisporea* strain A_AR_1_06 isolated by Schneider et al. (2009) from an epiphytic mycelial mat on *J. communis* subsp. *nana* reminiscent of *H. pinetorum* (Table 2, Figure 3). Similarly, Petrini (1985) isolated *A. cytisporea* (as *Apostrasseria lunata*) as the second most frequent endophyte from healthy leaves of *C. vulgaris*. It can be speculated that *A. cytisporea* behaves as a harmless endophyte during the vegetation period but can kill leaves during winter. It probably is a seasonal parasite that profits from the weakness of the dormant host in winter. Under the snow, the mycelium emerges from dead leaves and can form huge mycelial mats, killing leaves and whole shoots of *C. vulgaris*. Adjacent vegetation is also attacked, including *J. communis* subsp. *nana* and *P. mugo* subsp. *mugo*. The fungus has been observed on several ericaceous species and conifers, but it has never been described as a snow mold or as the causal agent of a snow blight. Pathogenicity of *A. cytisporea* is assumed based on the observations made in the field but needs to be tested fulfilling Koch's postulates.

Some *Allantophomopsis* species are asexual states of *Phacidium* species. Consequently, *A. cytisporea* belongs to the family Phacidiaceae. This family contains both pathogenic and saprotrophic species. Well-known pathogenic species are *G.* (*Phacidium*) *infestans* causing snow blight on various conifer species (Björkman 1942; Butin 2011), *P. abietis* causing snow blight on *Abies balsamea* (Smerlis 1962; Dicosmo, Raj, and Kendrick 1983), and *P. coniferarum* causing bark necrosis on Douglas fir, Japanese larch, pines, and firs (Smerlis 1968; Butin 2011). *Allantophomopsis pusilla* was observed on blackened stems of *Rubus fruticosus* in Germany (Nag Raj 1993), and *Phacidiopycnis washingtonensis* is described from diseased twigs and decayed fruits of *Malus* spp. (Xiao et al. 2005).

There are several possible reasons why *A. cytisporea* has so far been overlooked as a snow mold: (1) The mycelial mats on *C. vulgaris* are difficult to observe, because they remain visible only for a very short time after the snow has melted; (2) dark, superficial mycelia were regarded as *H. pinetorum* without further investigation; and (3) the frequency of the snow mold caused by *A. cytisporea* has increased in the course of climate change. Regarding reason 1: The detection of mycelial mats of *A. cytisporea* on *C. vulgaris* requires being at the right place at the right time and searching for snow molds on *C. vulgaris*, which apparently never had been performed previously. Regarding reason 2: In the area of San Bernardino, in summer, long after the snowmelt, branches of *P. mugo* subsp. *mugo* covered with dark mycelium mats were collected several times assuming that the causal agent would be *H. pinetorum*. Surprisingly, only *A. cytisporea* grew from a majority of these samples. Regarding reason 3: An increase in the course of climate change seems plausible, because the snow season now starts 12 days later and ends 26 days earlier on average than in 1970 at elevations between 1,100 and 2,500 m in the Swiss Alps (Klein et al. 2016). *A. cytisporea* and *H. pinetorum* have always been present in the ecosystem, but by shortening the duration of snow cover due to climate change, both fungi have less time to spread under the snow, leading

to a reduction of their biomass. The biomass reduction probably has more serious consequences for *H. pinetorum* than for *A. cytisporea*, because *H. pinetorum* requires 100 percent relative air humidity for mycelium growth, a prerequisite that is only given in the boundary layer between ground and snow cover (Gäumann, Roth, and Anliker 1934). The growth requirements of *A. cytisporea* regarding air humidity are not known, but this fungus seems active as an endophyte in Ericaceae (Petrini 1985) during the vegetation period while *H. pinetorum* is dormant. The absence of a resting period and the use of *C. vulgaris* as a refuge could provide an advantage for *A. cytisporea*. Under a climate change scenario, the way in which *H. pinetorum* and *A. cytisporea* interact is likely to change, as has been shown for other fungi (A'Bear et al. 2013; Hiscox et al. 2016). Interestingly, the frequency of *A. cytisporea* seems to be higher on the southern side of the Alps than on the northern side. This could be due to the warmer climate on the southern side of the Alps. If a more extended monitoring of *A. cytisporea* confirms the preference of *A. cytisporea* for higher temperatures (see Figure 4) under field conditions, it is expected that the frequency of this snow mold will further increase in the course of global warming. However, because global warming will affect all of the interacting organisms, the effects on the ecosystem level are impossible to predict. Climate change will certainly have a strong influence on the natural regeneration of woody plants, possibly with pronounced effects at timberline where inteaction with snow molds is significant. Therefore, the effect of increased temperature on virulence of *A. cytisporea* and the interaction between this fungus and *H. pinetorum* should be studied using inoculation experiments.

Acknowledgments

I thank Andrin Gross, Ottmar Holdenrieder, and Norbert Luschka for collecting snow mold samples and Anja Gall for lab assistance and sequencing of the ITS regions of lots of "snow mold" strains. I also express my gratitude to Ottmar Holdenrieder for reviewing the article and for his helpful comments. The Genetic Diversity Center of ETH Zürich provided excellent laboratory facilities for the molecular part of this work.

Disclosure statement

No potential conflict of interest was reported by the author.

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