



# Reassessment of *Paragnomonium* (*Sydowiellaceae*, *Diaporthales*) and typification of *Paragnomonium fragariae*, the cause of strawberry root rot and petiole blight

Inga Moročko-Bičevska<sup>a, \*</sup>, Jamshid Fatehi<sup>a, b</sup>, Olga Sokolova<sup>a</sup>

<sup>a</sup> Institute of Horticulture, Graudu str. 1, Dobele, LV, 3701, Latvia

<sup>b</sup> Lantmännen BioAgri, Fågelbacksvägen 3, SE-756 51, Uppsala, Sweden

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## ABSTRACT

*Paragnomonium fragariae* is a plant pathogenic ascomycete causing root rot and petiole blight of perennial strawberry in northern Europe. This paper provides a revised description of *Paragnomonium* and *P. fragariae* with lecto- and epitypification based on the species original description, recent collections from four European countries, examination of specimens used in the previous taxonomic studies and phylogenetic analyses of DNA sequences of LSU, ITS/5.8S and *tef1- $\alpha$* . This study presents the first report of *P. fragariae* on cultivated strawberry in Finland and Lithuania. Our study on growth rate showed that *P. fragariae* is a cold-adapted fungus growing almost equally at 5 °C as at 20 °C and attaining maximal growth at 15 °C. New primers were designed for amplification of ca. 0.8 kb fragment of *tef1- $\alpha$*  of *Sydowiella fenestrans*. Additionally, newly generated sequences of *tef1- $\alpha$*  were obtained for the first time from 21 isolates of seven species belonging to five genera of *Sydowiellaceae*, including the type species *S. fenestrans*, therefore considerably contributing to the current knowledge on phylogenetic relationships of this insufficiently studied group of fungi. The phylogenetic analysis has also revealed that the recently described species “*S. centaureii*” is genetically distant from the generic type *S. fenestrans* and other *Sydowiella*.

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## 1. Introduction

*Paragnomonium fragariae*, initially discovered by Klebahn (1918) and described as *Gnomonia fragariae*, is a plant pathogen causing severe root rot and petiole blight of perennial strawberry in northern Europe (Moročko et al., 2006; Moročko-Bičevska and Fatehi, 2011). So far, the fungus has undoubtedly been reported from Germany, Latvia, Sweden, Switzerland, and the United Kingdom (Klebahn, 1918; Bolay, 1972; Moročko, 2006; Moročko et al., 2006). Besides cultivated strawberry, the fungus also has been found on wild species of *Fragaria* and *Potentilla* in Switzerland (Bolay, 1972). Since the first discovery of *P. fragariae* in 1918, the identity of this fungus has been confused in several studies with the another strawberry pathogen *Gnomoniopsis fructicola* (G. Arnaud) Sogonov (syn. *Gnomonia fragariae* f. *fructicola* G. Arnaud; *Gnomonia*

*fructicola* (G. Arnaud) Fall) by misusing the name *Gnomonia fragariae* Kleb. for the specimens of *Gnomonia comari* P. Karst sensu lato (Alexopoulos and Cation, 1952; Parikka, 1981; CMI Distribution maps of plant diseases, No. 438, 1982). This taxonomical confusion has already been notified by several authors (Bolay, 1972; Maas, 1998; Farr et al., 1989; Moročko and Fatehi, 2007; Sogonov et al., 2008; Walker et al., 2010). *G. fructicola* occurs worldwide on strawberry causing leaf blotch, fruit rot, petiole blight, stem end rot and root rot particularly in synergic interaction with nematodes (Alexopoulos and Cation, 1948; Shipton, 1967; Bolay, 1972; Kurppa and Vrain, 1989; Gubler and Feliciano, 1999; Sogonov et al., 2008). The taxonomy of *G. fructicola* was studied and resolved by Sogonov et al. (2008) and Walker et al. (2010).

The genus *Gnomonia* and families in *Diaporthales* have been the subject of detailed morphological and molecular studies over the last decade (Sogonov et al., 2008; Crous et al., 2012; Krusys and Castlebury, 2012; Voglmayr et al., 2012; Walker et al., 2012; Voglmayr and Jaklitsch, 2014; Suetrong et al., 2015; Norphanphoun et al., 2016; Du et al., 2017; Senanayake et al., 2017a; b; Voglmayr et al., 2017). The new concept of *Gnomonia*

\* Corresponding author.

E-mail addresses: [inga.morocko@llu.lv](mailto:inga.morocko@llu.lv) (I. Moročko-Bičevska), [Jamshid.Fatehi@lantmannen.com](mailto:Jamshid.Fatehi@lantmannen.com) (J. Fatehi), [olga.sokolova@llu.lv](mailto:olga.sokolova@llu.lv) (O. Sokolova).

has reduced the number of accepted species from 280 to only a few species sharing common phylogenetic, morphological and host characteristic features (Sogonov et al., 2008). Our previous study on molecular characterisation of *P. fragariae* demonstrated that the genus *Gnomonia* was polyphyletic and *P. fragariae* was genetically distant from the type species of *Gnomonia*, *G. gnomon*, and other members of family *Gnomoniaceae* (Moročko and Fatehi, 2007). Moreover, the phylogeny of *Diaporthales* inferred from rDNA sequences showed that *P. fragariae* belonged to *Sydowiellaceae*, a family harbouring genera with diverse morphology, host range and habitat (Rossman et al., 2007; Kruys and Castlebury, 2012).

In a recent study by Senanayake et al. (2017b), on the basis of previously published rDNA sequences (Moročko and Fatehi, 2007), and apparently some morphological interpretations inferred from the literature related to *P. fragariae* or *G. fruticicola* (Alexopoulos and Citation, 1952; Moročko and Fatehi, 2007), a monotypic genus *Paragnomonium* Senan. & K.D. Hyde in *Sydowiellaceae* and a new combination *P. fragariae* (Kleb.) Senan. & K.D. Hyde were introduced to replace *G. fragariae* Kleb. However, several of the morphological characters presented by the authors in the new description of *P. fragariae* were incorrect and repetitive of those previous taxonomic confusions surrounding *P. fragariae* and *G. fruticicola*. The main misperception is the attribution of an asexual morph for *P. fragariae* that has never been observed or reported by any of the authors who have extensively examined and studied *P. fragariae* specimens and isolates on host plants and living cultures (Klebahn, 1918; Bolay, 1972; Monod, 1983; Moročko and Fatehi, 2007). In addition, characteristics and measurements of asci and ascospores do not correspond to those described by Klebahn (1918) and other authors dealing with *P. fragariae* (Bolay, 1972; Monod, 1983; Moročko and Fatehi, 2007), but they agree more with *G. fruticicola* (Walker et al., 2010). Moreover, *P. fragariae* does not form ascomata on roots in nature, but only on petioles because it requires light for sporulation either on host tissues or agar media (Moročko and Fatehi, 2007).

In a present study, we reassess and provide the revised description of *Paragnomonium* and *P. fragariae* based on the original description of the species by Klebahn (1918), examination of specimens collected previously by other authors, our collection from four European countries, and phylogeny of LSU, ITS/5.8S and *tef1-α* gene sequences. We could not find the type specimens of Klebahn, so we are designating the original drawings by Klebahn (1918) specified in his original publication as a lectotype of *G. fragariae*. In addition, we are designating a freshly collected specimen from Latvia, linked with the fungus morphology on the host and in the culture, ex-type cultures and sequences of three loci, as an epitype in support of the lectotype.

## 2. Materials and methods

### 2.1. Isolates, specimens and morphological examination

The studied fungi were isolated from freshly collected specimens by plating of ascospores on 3 % water agar (WA; Difco), potato dextrose agar (PDA; Difco) or potato carrot agar (PCA; Dhingra and Sinclair, 1995) media amended with rifampicin and streptomycin at final concentrations of 100 and 50 ppm, respectively. Single ascospore isolates were obtained and subcultured on oatmeal agar (OA; Difco). The isolates were maintained in an active state on OA and PDA slants, and in sterile water at 4 °C.

Morphology of *P. fragariae* on host substrate or in pure culture was studied using a stereomicroscope Leica DMLS equipped with phase-contrast objectives C PLAN 4 × /0.10, 10 × /0.22 PH 1 and 100 × /1.25 oil PH 3. Microscopic images were captured with Leica EC3 digital camera and analysed with software Leica LAS EZ v.2.0.

For morphological examination of the fungus in culture, the isolates were grown on OA, PCA, and PDA plates incubated at room temperature on a laboratory bench exposed to natural light or in a growth chamber at 22 °C with 12 h daily illumination of cool-white (Osram L 15W/840) lamps for at least four weeks. Optimal growth temperature of *P. fragariae* was determined for four isolates S1, S4, M1 and UN35 grown in two replicates (plates) on PDA, PCA, corn meal agar (CMA, Difco), and water agar (WA) at temperature regimes from 5 – 30 °C with 5 °C intervals in the dark. The agar plates were inoculated with five mm in diameter plugs of actively growing fungal cultures. The growth was measured daily along two perpendicular lines drawn at the centre of the colonies and continued for two weeks.

The list of fungal isolates studied, the culture and voucher numbers, hosts, collection data and GenBank accession numbers of DNA sequences obtained in this study or extracted from the GenBank are provided in Table 1. Details of specimens used for morphological examinations are listed in the Taxonomy section. The living fungal cultures are maintained at the microbial strain collection at the Institute of Horticulture, Dobeles, Latvia. Representative isolates of the studied fungi were deposited in the Microbial strain collection of Latvia (MSCL), Riga, Latvia and Westerdijk Fungal Biodiversity Centre (CBS-KNAW), Utrecht, The Netherlands. The dry specimens were deposited in the Herbarium of University of Daugavpils (DAU), Daugavpils, Latvia and Swedish Museum of Natural History (S), Stockholm, Sweden.

### 2.2. DNA extraction, PCR amplification and sequencing

Fungal mycelium preparations, DNA extraction, and amplification of partial nuLSU and entire ITS1–5.8S–ITS2 rDNA regions were carried out as described previously (Moročko and Fatehi, 2007), with an exception that REDTaq® ReadyMix™ PCR reaction mix (Sigma–Aldrich) was used in PCR amplification.

Approximately 1.0 kb fragment of translation elongation factor 1 alpha (*tef1-α*) gene was amplified with primers EF1-728F (Carbone and Kohn, 1999) and EF1-1567R (Rehner, 2001). For *Sydowiella fenestrans* ca. 0.8 kb fragment of *tef1-α* gene was amplified using newly designed forward primer EF1-F12 (5' AGCTTGGCAAGGGTTCCTTCA 3') and reverse primer EF1-1567R (Rehner, 2001). The amplification was carried out using DreamTaq Green PCR Master Mix (Thermo Scientific) and a PCR protocol with an initial denaturation step at 95 °C for 3 min, 5 cycles consisting of 45 s at 95 °C, 45 s at 63 °C and 2 min at 72 °C, 5 cycles consisting of 45 s at 95 °C, 45 s at 61 °C and 2 min at 72 °C, 20 cycles consisting of 45 s at 95 °C, 45 s at 59 °C and 2 min at 72 °C followed by a 10 min final extension step at 72 °C. All PCR reactions were carried out in a Mastercycler® thermocycler (Eppendorf AG). The amplified fragments were separated by electrophoresis in 1.5 % agarose gel (Low LE, StarLab), stained with ethidium bromide and visualised under UV light. The size of amplified fragments was estimated with GeneRuler 100 bp DNA Ladder Plus (Thermo Scientific).

Amplicons of partial nuLSU and entire ITS1–5.8S–ITS2 rDNA regions were purified and sequenced as in Moročko and Fatehi (2007). Amplified fragments of *tef1-α* were purified with Qiaquick PCR purification kit (Qiagen AG) and sequenced directly using the same primers as for PCR amplification and EF1-983F (Rehner, 2001) as an internal primer. For isolates of *S. fenestrans* the *tef1-α* gene was sequenced with the same primers as for PCR amplification and with internal primers EF1-983F (Rehner, 2001) and EF-cr (5' TCGAAYTCYCCRGTTACCNGCRGCRAT 3'). The reverse primer EF-cr designed in this work is the reverse complementary of EF-cr primer by Rehner (2001). All sequencing reactions were carried out on a 3130xl Genetic Analyser (Applied Biosystems) automated sequencer as an external service at Latvia State Forest Research

**Table 1**

Hosts, origin, collection data and GenBank accession numbers of the specimens and strains used for phylogenetic analyses. Numbers of herbarium specimens, strains and GenBank sequences indicated in bold have resulted from our collections and the present study.

Species, strain	Voucher, specimen <sup>a</sup>	Host, organ	Origin	Collection date <sup>a,b</sup>	Collected/identified by <sup>a</sup>	GenBank accession No.		
						LSU	ITS	<i>tef1-α</i>
<i>Alborbis galericulata</i> AR4027	Jaklitsch, W. (2288) = BPI872070	<i>Fagus sylvatica</i>	Austria, St. Margareten im Rosental, Kaernten, Stariwald	02 Aug 2003	W.Jaklitsch	–	JF681970	–
AR3811	BPI863767	<i>Fagus grandifolia</i>	USA, Tennessee, Cosby	25 Mar 2002	L.Vasilyeva	–	JF681969	–
AR4004	Jaklitsch, W. (2209) = BPI843559	<i>Fagus grandifolia</i>	USA, Tennessee, Gatlinburg	24 May 2003	W.Jaklitsch	–	JF681968	–
AR3890	BPI871001	<i>Fagus grandifolia</i>	USA, New York, Cranberry Lake	17 Jun 2002	L.Vasilyeva	–	JF681967	–
<i>Cainiella borealis</i> –	Kruys 725 = UPS:BOT:F-567254	<i>Cassiope tetragona</i>	Sweden, Kiruna, Abisko	12 Jul 2008	A.Kruys	–	JF701921	–
<i>Cainiella johansonii</i> –	Kruys 727 = UPS:BOT:F-567261	<i>Dryas octopetala</i>	Sweden, Kiruna, Abisko	15 Jul 2008	A.Kruys	–	JF701922	–
–	Kruys 731 = UPS:BOT:F-567263	<i>Dryas octopetala</i>	Sweden, Kiruna, Abisko	14 Jul 2008	A.Kruys	JF701920	–	–
<i>Calospora innesii</i> <b>APp1.3 = MSCL1594</b>	<b>LVAI60 = DAU100004629 (DAU)</b>	<i>Acer pseudoplatanus</i> ; dead, fallen twig	Latvia, Jelgava	30 May 2011	O.Sokolova/J.Fatehi	<b>MK524455</b>	<b>MK524436</b>	<b>MK524477</b>
<b>APp3.2 = MSCL1595</b> <b>APp3.4</b>	<b>LVAI62 = DAU100004630 (DAU)</b>	<i>Acer pseudoplatanus</i> ; dead twig	Latvia, Liepāja	5 June 2011	I.Moročko-Bičevska/J. Fatehi	<b>MK524456</b> <b>MK524457</b>	<b>MK524437</b> <b>MK524438</b>	<b>MK524478</b> <b>MK524479</b>
AR3925	Jaklitsch W. (2092) = BPI843587	<i>Acer pseudoplatanus</i>	Austria, Kaernten	18 Apr 2003	W.Jaklitsch	–	JF681966	–
AR3639	Jaklitsch W. (1739) = BPI840945	<i>Acer pseudoplatanus</i>	Austria, Triebloch	14 Apr 2001	W.Jaklitsch	EU683071	JF681965	–
AR3831	Jaklitsch W. (1881) = BPI843524	<i>Acer pseudoplatanus</i>	Austria, Kaernten, Oberdoerfl	1 May 2002	W.Jaklitsch	–	JF681964	–
<i>Chapeckia nigrospora</i> AR3809 = CBS125532	BPI863766	<i>Betula</i> sp.; overwintered branches	USA, North Carolina, Balsam Mountain	24 Apr 2002	L.Vasilyeva/A.Rossmann	<b>MK524458</b> EU683068	<b>MK524439</b> JF681957	<b>MK524480</b>
<i>Haplozystis berkeleyi</i> D84 = CBS124568	WU:39959	<i>Platanus x hispanica</i> ; corticated twig	United Kingdom, London, Kew	nd	H.Voglmayr	<b>MK524459</b>	<b>MK524440</b>	<b>MK524481</b>
AR3870	Jaklitsch W. (1909) = BPI843507	<i>Platanus x acerifolia</i>	Austria	01 Jul 2002	W.Jaklitsch	–	JF681958	–
WJ2007	WU24707	<i>Platanus hybrida</i>	Germany	20 Oct 2002	W.Jaklitsch	AY616230	–	–
AR3851	nd	nd	Austria	nd	nd	EU683069	–	–
<i>Haplozystis occidentalis</i> D20-3 = CBS115156	WU24705	<i>Platanus occidentalis</i> ; corticated twig	USA, Tennessee, Great Smokey Mountains National Park	22 May 2003	W.Jaklitsch	<b>MK524460</b>	<b>MK524441</b>	<b>MK524482</b>
<i>Italiomyces centaureii</i> MFLUCC14-0849	IT1976 = MFLU16-2866	<i>Centaurea</i> sp.	Italy, Province of Forli-Cesena, Fiumicello di Premilcuore	02 Apr 2014	E.Camporesi/I.C.Senanayake	KY523494	KY523476	–
MFLU16-2867A	nd	<i>Centaurea</i> sp.	Italy	02 Apr 2014	E.Camporesi/I.C.Senanayake	KY523495	KY523477	–
MFLU16-2867B	nd	<i>Centaurea</i> sp.	Italy	02 Apr 2014	E.Camporesi/I.C.Senanayake	KY523496	KY523478	–
<i>Paragnomonium fragariae</i> IMI100647 = CBS146.64	–	<i>Fragaria x ananassa</i> ; crown	United Kingdom	1964	S.G.Evans	EF212856	EF212844	<b>MK524474</b>
<b>UN22 = MSCL1596</b>	–	<i>Fragaria x ananassa</i> ; crown	Latvia, Tukums, Püre	Oct 2001	I.Moročko-Bičevska	EF212851	EF212833	<b>MK524475</b>
<b>L56</b>	–	<i>Fragaria x ananassa</i> 'Sara'; crown	Latvia, Auce, Vecauce	Oct 2001	I.Moročko-Bičevska	–	EF212838	–
<b>O3</b>	–	<i>Fragaria x ananassa</i> 'Sara'; crown	Latvia, Auce, Vecauce	Oct 2001	I.Moročko-Bičevska	–	EF212837	–

(continued on next page)

Table 1 (continued)

Species, strain	Voucher, specimen <sup>a</sup>	Host, organ	Origin	Collection date <sup>a,b</sup>	Collected/identified by <sup>a</sup>	GenBank accession No.		
						LSU	ITS	<i>tef1-α</i>
<b>M1 = MSCL1597</b>	–	<i>Fragaria x ananassa</i> 'Red Gauntlet'; crown	Latvia, Auce, Vecauce	Oct 2001	I.Moročko-Bičevska	EF212852	EF212834	<b>MK524476</b>
<b>S7 = MSCL1598</b>	–	<i>Fragaria x ananassa</i> ; crown	Latvia, Tukums, Püre	Oct 2001	I.Moročko-Bičevska	–	EF212835	–
<b>S12 = MSCL1599</b>	–	<i>Fragaria x ananassa</i> ; crown	Latvia, Tukums, Püre	Oct 2001	I.Moročko-Bičevska	–	EF212836	–
<b>GF2</b>	–	<i>Fragaria x ananassa</i> ; petiole of overwintered leaf	Latvia, Ventspils	Sept 2004	I.Moročko-Bičevska	EF212850	EF212832	–
<b>F3.1 = MSCL1600</b>	–	<i>Fragaria x ananassa</i> ; root	Sweden, Uppsala, Fredrikslund	May 2004	I.Moročko-Bičevska	<b>MK524453</b>	EF212839	<b>MK524472</b>
<b>F5.6</b>	–	<i>Fragaria x ananassa</i> ; crown	Sweden, Uppsala, Fredrikslund	May 2004	I.Moročko-Bičevska	EF212855	EF212840	–
<b>SER1</b>	–	<i>Fragaria x ananassa</i> 'Honeoye'; root	Sweden, Hunnebustrand	Oct 2004	I.Moročko-Bičevska	EF212854	EF212841	–
<b>SER4 = MSCL1601</b>	–	<i>Fragaria x ananassa</i> 'Honeoye'; root	Sweden, Hunnebustrand	Oct 2004	I.Moročko-Bičevska	–	EF212842	–
<b>SER7 = MSCL1602</b>	–	<i>Fragaria x ananassa</i> ; root	Sweden, Floda	Oct 2004	I.Moročko-Bičevska	<b>MK524454</b>	EF212843	<b>MK524473</b>
<b>F129/P3/1 = MSCL1603</b>	<b>F129 = F367871 (S)</b>	<i>Fragaria x ananassa</i> ; petiole of dead leaf	Latvia, Tukums, Püre	20 Oct 2013	I.Moročko-Bičevska&J.Fatehi/J.Fatehi	<b>MK524447</b>	<b>MK524430</b>	<b>MK524466</b>
<b>F129/P3/2 = MSCL1604</b>	<b>(epitype) = DAU100004631 (DAU)</b> (isoepitype)					<b>MK524448</b>	<b>MK524431</b>	<b>MK524467</b>
<b>F133.1 = MSCL1605</b>	–	<i>Fragaria x ananassa</i> ; crown of diseased plant	Lithuania, Kaunas distr., Babtai	20 May 2014	N.Rasiukevičiūtė/I.Moročko-Bičevska	<b>MK524450</b>	<b>MK524433</b>	<b>MK524469</b>
<b>F137/P1/1 = MSCL1606</b>	<b>F137 = DAU100004632 (DAU)</b>	<i>Fragaria x ananassa</i> 'Dar Select'; dead petiole of diseased plant	Lithuania, Kaunas distr., Babtai	20 May 2014	N.Rasiukevičiūtė/I.Moročko-Bičevska	<b>MK524451</b>	<b>MK524434</b>	<b>MK524470</b>
<b>F168.13 = MSCL1607</b>	<b>F168 = DAU100004633 (DAU)</b>	<i>Fragaria x ananassa</i> ; blighted petiole	Finland, Parainen, Bjursängpolku	16 Oct 2014	I.Moročko-Bičevska	<b>MK524452</b>	<b>MK524435</b>	<b>MK524471</b>
<b>F169.1 = MSCL1608</b>	<b>F169 = DAU100004634 (DAU)</b>	<i>Fragaria x ananassa</i> 'Rumba'; dead petiole of diseased plant	Finland, Parainen, Bjursängpolku	16 Oct 2014	I.Moročko-Bičevska	<b>MK524449</b>	<b>MK524432</b>	<b>MK524468</b>
<i>Ranulospora alnii</i> MFLUCC-13-0793	IT1415 = MFLU16-2868	<i>Alnus incana</i> ; dead branch	Italy, Province of Trento, Mezzana	15 Aug 2013	E.Camporesi/I.C.Senanayake	KY523497	KY523479	–
MFLU16-2869	nd	<i>Alnus incana</i> ; branch	Italy	15 Aug 2013	E.Camporesi	KY523498	KY523480	–
MFLU 16-2870	nd	<i>Alnus incana</i> ; branch	Italy	15 Aug 2013	E.Camporesi	KY523499	KY523481	–
<i>Rossmania ukurunduensis</i> AR3484	BPI747566	<i>Acer ukurunduense</i> ; bark	Russia, Khabarovsk Territory, Siberia	17 Jun 2000	L.Vasilyeva	EU683075	–	–
<i>Sillia ferruginea</i> WJ1455 = AR3440 = CBS126567	BPI843619	<i>Corylus avellana</i> ; overwintered twig	Austria, Zabrde	23 Apr 2000	W.Jaklitsch/A. Rossman	<b>MK524461</b> EU683076	<b>MK524442</b> JF681959	<b>MK524483</b>
<i>Sillia italica</i> MFLU16-0056	nd	<i>Corylus</i> sp.; branch	Italy	11 Jan 2016	E.Camporesi/I.C.Senanayake	KY397950	KY523484	–
MFLU16-0056A	nd	<i>Corylus</i> sp.; branch	Italy	11 Jan 2016	E.Camporesi/I.C.Senanayake	–	KY523485	–
MFLU 16-0056	nd	<i>Corylus</i> sp.	Italy	11 Jan 2016	E.Camporesi	–	KY397949	–
<i>Sillia karstenii</i> MFLU16-2864	IT1611	<i>Corylus avelana</i> ; dead branch	Italy, Province of Forli-Cesena, Bertinoro	30 Dec 2013	E.Camporesi	KY523500	KY523482	–
MFLU16-2865B	nd	–	Italy	30 Dec 2013	E.Camporesi	KY523501	KY523483	–
<i>"Sydowiella" centaureii</i> MFLU16-2858	nd	<i>Centaurea</i> sp.; branch	Italy	02 Apr 2014	E.Camporesi/I.C.Senanayake	KY523502	KY523486	–
MFLU16-2860	nd	<i>Centaurea</i> sp.; branch	Italy, Province of Forli-Cesena, Fiumicello di Prelimicuore	02 Apr 2014	E.Camporesi/I.C.Senanayake	KY523503	KY523487	–

Table 1 (continued)

Species, strain	Voucher, specimen <sup>a</sup>	Host, organ	Origin	Collection date <sup>a,b</sup>	Collected/identified by <sup>a</sup>	GenBank accession No.		
						LSU	ITS	<i>tef1-α</i>
<i>Sydowiella depressula</i> CBS 814.79	M. Monod No. 533	<i>Rubus</i> sp.; stem	Switzerland, Neuchatel, Tourbiere des Ponts de Martel	15 May 1979	M.Monod	<b>MK524445</b>	<b>MK524428</b>	<b>MK524464</b>
<b>Ru13.8 = MSCL1609</b>	<b>LVAI348 = DAU100004635</b> (DAU)	<i>Rubus idaeus</i> (wild); second year stem	Sweden, Uppsala, Skyttorp	13 Aug 2012	J.Fatehi	<b>MK524446</b>	<b>MK524429</b>	<b>MK524465</b>
<i>Sydowiella fenestrans</i> <b>ChA7.1 = MSCL1610</b>	<b>LVAI369 = DAU100004636</b> (DAU)	<i>Chamaenerion angustifolium</i> ; overwintered stem	Latvia, Ape	15 Apr 2014	M.Jundzis/ I.Moročko- Bičevska	<b>MK524443</b>	<b>MK524426</b>	<b>MK524462</b>
AR3777 = CBS125530	BPI843503	<i>Chamaenerion angustifolium</i> ; overwintered stem	Russia, Irkutsk, Verkholsk vic.	16 Aug 2001	T.Morozova/ A.Rossmann	<b>MK524444</b> EU683078	<b>MK524427</b> JF681956	<b>MK524463</b>
<i>Sydowiella urticicola</i> MFLUCC13-0665	MFLU 17-0877	<i>Urtica dioica</i> , branch	Italy	16 May 2013	E.Camporesi/ I.C.Senanayake	KY523504	–	–
<i>Stegosporium pyriforme</i> D2 = CBS117023	WU 28069	<i>Acer pseudoplatanus</i> ; corticated twig	Austria, Wien, Lobau, Ölhafen	29 Oct 2002	W.Jaklitsch	EU039987	EU039971	EU040001
<i>Stilbospora macrosperma</i> D53 = CBS121692	nd	<i>Carpinus betulus</i> ; dead twig	Austria, Niederosterreich	nd	H.Voglmayr	EU039986	JX517285	EU039998
<i>Tenuiappendicula alnicola</i> MFLU16-1265A	MFLU:16-1265A	<i>Alnus cordata</i> ; dead branch	Italy, Province of Forli-Cesena, Fiumicello di Prelimicuore	24 Apr 2013	E.Camporesi/ I.C.Senanayake	KY523505	KY523488	–
MFLU16-2865	nd	<i>Alnus cordata</i>	Italy	24 Apr 2013	E.Camporesi/ I.C.Senanayake	KY523506	KY523490	–
MFLUCC16-1452	nd	<i>Alnus cordata</i>	Italy	24 Apr 2013	E.Camporesi/ I.C.Senanayake	KY523507	KY523489	–
<i>Tortilispora aurantiaca</i> AR4022	Jaklitsch, W. (2299) = BPI872071	<i>Alnus alnobetula</i>	Austria, Steiermark, Kleinsoelk	6 Aug 2003	W.Jaklitsch	–	JF681960	–

<sup>a</sup> nd – data are not available.

<sup>b</sup> If collection date or name of collector was not available, then deposition date and name of depositor in a culture collection is shown.

Institute “Silava”. The obtained sequences were deposited in the GenBank (<https://www.ncbi.nlm.nih.gov>) and are available under accession numbers MK524426–MK524483.

### 2.3. Sequence analyses and phylogeny

The obtained sequences were assembled and manually edited in SeqMan available in the computer program package Lasergene 9.1 (DNASTAR Inc.). The multiple sequence alignments were made using the Clustal W algorithm, and sequence identities were determined by MegAlign (Lasergene 9.1, DNASTAR Inc.). The nucleotide sequences of the isolates studied in this work were aligned with sequences of other *Sydowiellaceae* taxa available in GenBank ([www.ncbi.nlm.nih.gov](http://www.ncbi.nlm.nih.gov)) (Table 1). The ends of the aligned sequences were truncated to ensure the quality and equal length of all sequences. The first alignment was made of entire ITS1–5.8S–ITS2 rDNA region (length 586 nt) sequences of 16 *Sydowiellaceae* taxa sequenced during this study, and 46 sequences from GenBank. The second alignment consisted of concatenated partial nuLSU sequences (length 737 nt) and ITS1–5.8S–ITS2 rDNA region (length 586 nt) of 19 *Sydowiellaceae* taxa sequenced during this study and 26 (including two outgroup taxa) from GenBank. The third sequence alignment (length 747 nt) was made of *tef1-α* sequences of 22 *Sydowiellaceae* taxa sequenced in this work and two outgroup taxa. The fourth sequence alignment included concatenated sequences of all studied genomic regions (total length 2072 nt) of 22 *Sydowiellaceae* taxa that had sequences of

all three genes available and two outgroup taxa. The fifth sequence alignment was a combined sequence data set (total length 2072 nt) consisting of 55 *Sydowiellaceae* and two outgroup taxa. Any missing DNA sequences for the studied taxa were considered missing data in the particular alignment file and the phylogenetic analyses. Sequences of *Stegosporium pyriforme* and *Stilbospora macrosperma* from GenBank were used as an outgroup in all alignments and phylogenetic analyses.

Phylogenetic relationships among *Sydowiellaceae* and *P. fragariae* were determined using maximum parsimony (MP) and Bayesian inference (BI) analyses methods applied to all sequencing data sets. MP analyses were performed using the computer program PAUP v. 4.0b10 (Swofford, 2002). The alignments were subjected to heuristic search with random addition sequence with 1000 replicates, MulTrees option not in effect, Steepest descent option in effect, and tree bisection reconnection (TBR) branch swapping. The same settings as in MP analyses were used in bootstrap (BS) analyses where five random addition sequence in each of 1000 bootstrap replicates were performed.

For all of the data sets, GTR + I + G was estimated as the best-fit model of DNA substitution by Akaike information criterion (AIC) in Mr Modeltest v2 (Nylander, 2004), and was further used in BI analyses. BI analysis was performed with MrBayes (version 3.0b4; Huelsenbeck and Ronquist, 2001). Markov chain Monte Carlo method (Larget and Simon, 1999; Mau et al., 1999) was used in BI, and four incrementally heated simultaneous Markov chains were

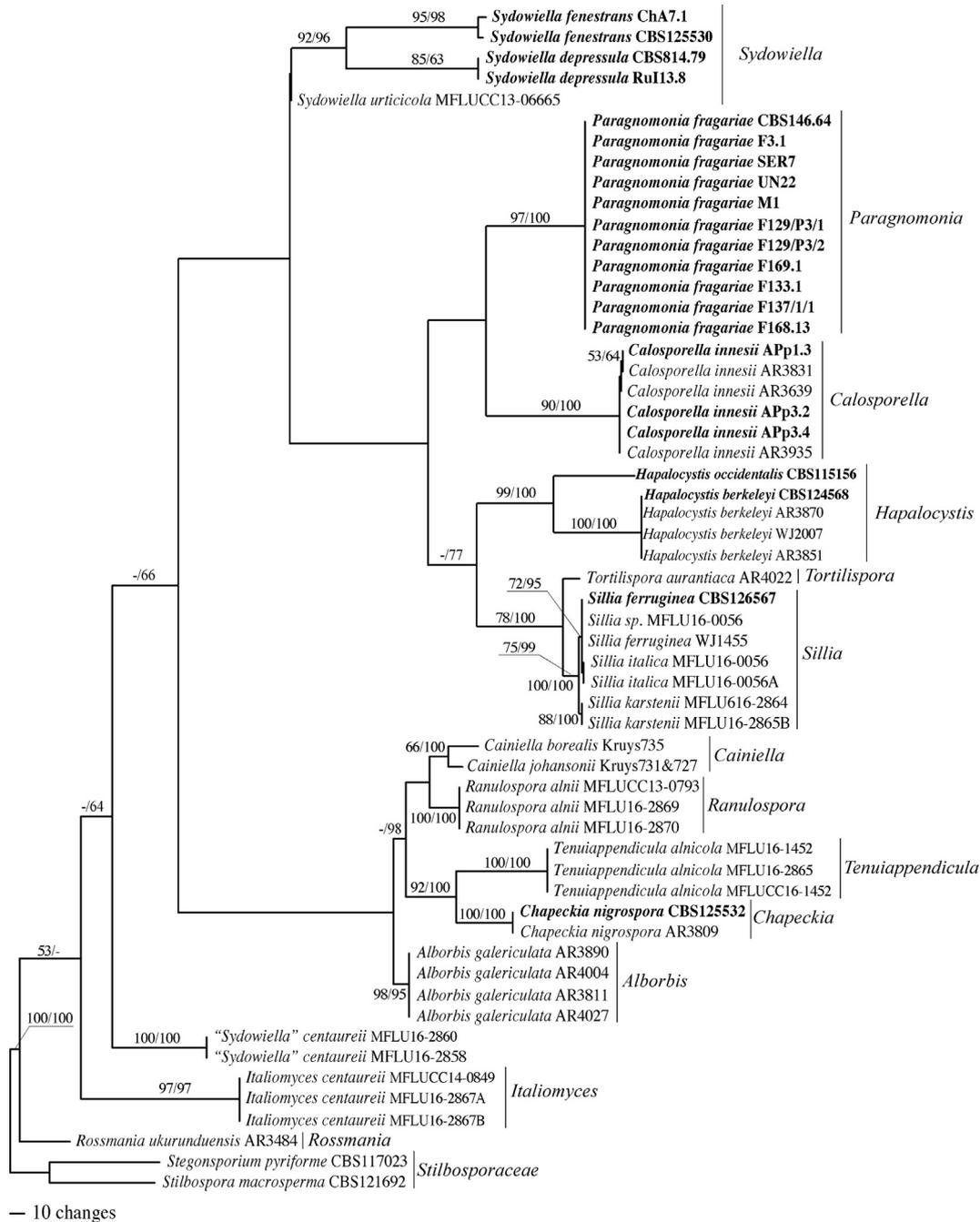
run 1 M generations sampling every 100th tree. The first 1000 trees were discarded, and the remaining trees with stable log likelihood values were included in the analyses for computing of a 50 % majority-rule consensus tree and estimation of posterior probabilities (PPs) of the groups.

### 3. Results

#### 3.1. Molecular phylogeny

The final sequence alignments and obtained trees were deposited in the TreeBase (<http://www.treebase.org>) and are available under a study accession number S24010.

Among 2072 nucleotide positions of three genomic loci included in the phylogenetic analyses 547 were parsimony informative. MP analysis of combined sequence data set (alignment 5) of 55 *Sydowiellaceae* taxa using 547 parsimony-informative characters resulted in 914 most parsimonious trees. One of the trees with topology matching the MP 70 % majority tree is shown in Fig. 1. The backbone topology of the consensus tree obtained from the BI analyses slightly differed from MP consensus tree (data not shown); however, the main phylogenetic groups and all genera were present and supported in both analyses. The posterior probability values of the groups from BI are included in Fig. 1. Most of the nodes on the backbone of the tree had no support in MP analyses but were moderately supported in BI analyses (Fig. 1). The genus of



**Fig. 1.** One of 914 most parsimonious trees (score = 1425) inferred from 2072 nucleotides of the concatenated alignment of LSU, ITS, *tef1- $\alpha$*  sequences of 55 *Sydowiellaceae* and two outgroup taxa *Stegosporium pyriforme* and *Stilbospora macrosperma* using Maximum Parsimony analysis. Numbers above or below branches represent bootstrap supports >50 % in Maximum Parsimony analysis and posterior probabilities in Bayesian analysis, respectively. Taxa in bold represent sequences obtained during the current study.

*Paragnomonina*, comprising isolates of *P. fragariae*, formed a distinct clade within *Sydowiellaceae* with maximum and high supports in all the analyses performed (Fig. 1, Supplementary Figs. S1–S4).

In this study, sequences of three genomic regions (LSU, ITS, *tef1- $\alpha$* ) were analysed. Due to the lack of sequences for several *Sydowiellaceae* taxa, including most of the newly described species (Table 1), separate alignments and analyses were made for each of the studied genomic regions to avoid a large proportion of the missing data in the analyses. The overall topologies of the obtained trees slightly varied among the genomic regions studied and analyses performed, but all genera, including *P. fragariae* clade, were highly supported in all analyses (Fig. 1, Supplementary Figs. S1–S4). Among the genomic regions studied, *tef1- $\alpha$*  had the highest resolution for the phylogenetic species delineation as apparent for the type species of *Sydowiella*, *S. fenestrans*, and *Sydowiella depressula* (Supplementary Fig. S2 – S4).

The phylogenetic analyses separately performed on each of the genomic region alignments revealed a conflict between previously published ITS (JF681956) and LSU (EU683078) sequences of *S. fenestrans* CBS125530 by Kruys and Castlebury (2012). These sequence data have also been used in concatenated alignments in several recent studies on the molecular phylogeny of *Sydowiellaceae* and *Diaporthales* (Senanayake et al., 2017a; b; Voglmayr and Mehrabi, 2018). In our phylogenetic analyses, the ITS sequence JF681956 was grouped in a separate clade together with *S. centaureii*, distant from *S. depressula* and our ITS sequences of *S. fenestrans* from Latvia (Supplementary Fig. S2.), while the LSU sequence EU683078 grouped with LSU sequences of Latvian *S. fenestrans* isolates, *S. depressula*, *S. urticicola* and recently published *S. fenestrans* CBS125530 LSU sequence (MH877859) in GenBank (data not shown). In order to examine the validity of the published sequences assigned to *S. fenestrans* CBS125530, the isolate was obtained from CBS-KNAW fungal collection and sequenced in this study. The phylogenetic analyses of the three genomic regions were concordant placing all isolates of *S. fenestrans* and *S. depressula* together in well-supported clades (Supplementary Fig. S2 – S4). “*Sydowiella*” *centaureii* remained in a separate clade distant from the type species *S. fenestrans* and outside *Sydowiella* with high and maximum bootstrap supports (Fig. 1, Supplementary Figs. S2 and S3).

### 3.2. Growth rate and temperature response

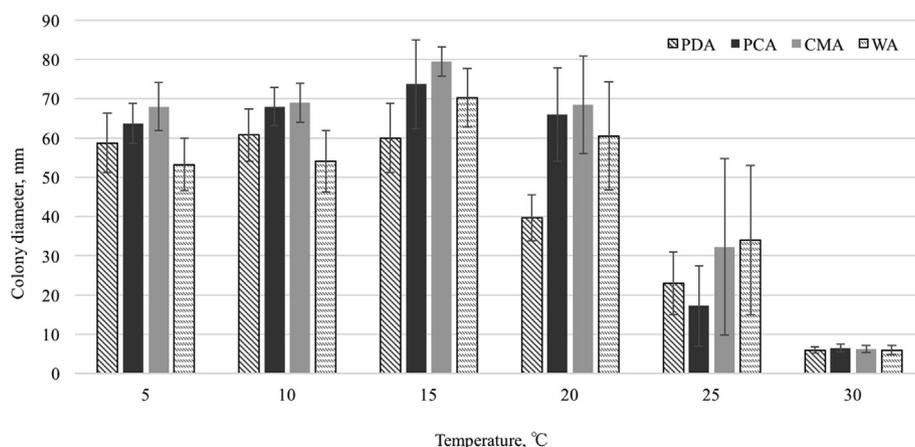
The linear growth of *P. fragariae* isolates S1, S4, M1 and UN35 were measured on agar plates at temperatures ranging from 5 to 30 °C. A maximum growth rate was recorded at 15 °C on most of

the agar media, except PDA. The isolates grew at 5 °C on all of the tested agar media whereas weak or no growth was observed at 25 °C and 30 °C, respectively (Fig. 2). At 30 °C, 1–2 mm radial growth of colonies was detected on PCA and CMA that stopped during the first three d. At 25 °C, the colony development stopped after one week of incubation and no further growth was observed on all agar media tested during the next week of incubation.

## 4. Taxonomy

In the study by Senanayake et al. (2017b) *Gnomonia fragariae* Kleb. was placed in a new genus and combined as *Paragnomonina fragariae* (Kleb.) Senan. & K.D. Hyde. However, typification of the species was not confirmed, and a synonym was erroneously stated as “*Gnomonia fragariae* var. *fragariae* Kleb.” by referring to Klebahn publication in 1918 in which he described *G. fragariae*. The names “*Gnomonia fragariae* var. *fragariae*” and “*Gnomonia fragariae* f. *fragariae*” used in the Senanayake et al. (2017b) have not been used by Klebahn (1918) or other cited authors who later studied *G. fragariae* (Bolay, 1972; Monod, 1983; Moročko and Fatehi, 2007). Moreover, *Gnomoniopsis comari* (P. Karst.) Sogonov (syn. *Gnomonia comari* P. Karst.) and *G. fructicola* (G. Arnaud) Sogonov (syn. *Gnomonia fragariae* f. *fructicola* G. Arnaud), including their various synonyms, have never been listed as the synonyms of *Gnomonia fragariae* Kleb. and vice versa (see Bolay, 1972; Monod, 1983; Sogonov et al., 2008; Walker et al., 2010). The description of *P. fragariae* presented by Senanayake et al. (2017b) corresponds to the characteristics of *G. fructicola* or *G. comari* (see Walker et al., 2010), and it is most likely gathered from the various literature sources related to these two species and not *G. fragariae*. However, Senanayake et al. (2017b) indicated the reference of Klebahn (1918) and included the rDNA sequences of *G. fragariae* in the phylogenetic analysis from an earlier study by Moročko and Fatehi (2017). Thus, here we provide the typification, the corrected basionym and revised descriptions for the species and the genus.

In the original publication of Klebahn (1918) describing *G. fragariae*, there were two illustrations complementing the description of the species and Klebahn had referred to those drawings in his description. However, he did not state any single specimen as a “*typus*” and described the fungus on the host and in the culture, providing a diagnostic description and two illustrations. Attempts to locate any original cultures or specimens collected by Klebahn at several herbaria, including B, BREM, HBG, K, and S, were not successful; therefore, the Klebahn's original illustration



**Fig. 2.** The average colony diameter (mm) of four isolates of *Paragnomonina fragariae* after two weeks of incubation at different temperatures on four agar media in the dark. The error bars represent the standard deviation. PDA – potato dextrose agar, PCA – potato carrot agar, CMA - corn meal agar, WA - water agar.

complementing the diagnostic description of *G. fragariae* is designated as a lectotype here. Since the Klebahn's illustration may not provide sufficient information for the modern identification and taxonomy, a representative freshly collected specimen from Latvia, linked with the fungus morphology, ex-type cultures and sequences of three genomic loci, is designated here as an epitype in support of the lectotype.

***Paragnomonium*** Senan. & K.D. Hyde, *Mycosphere* **8**: 198 (2017). MycoBank No.: MB552723.

A monotypic genus of *Sydowiellaceae*. Sexual morph non-stromatic, perithecia solitary or in groups, immersed or submerged in substrate, black, globose with erupted, long, central,

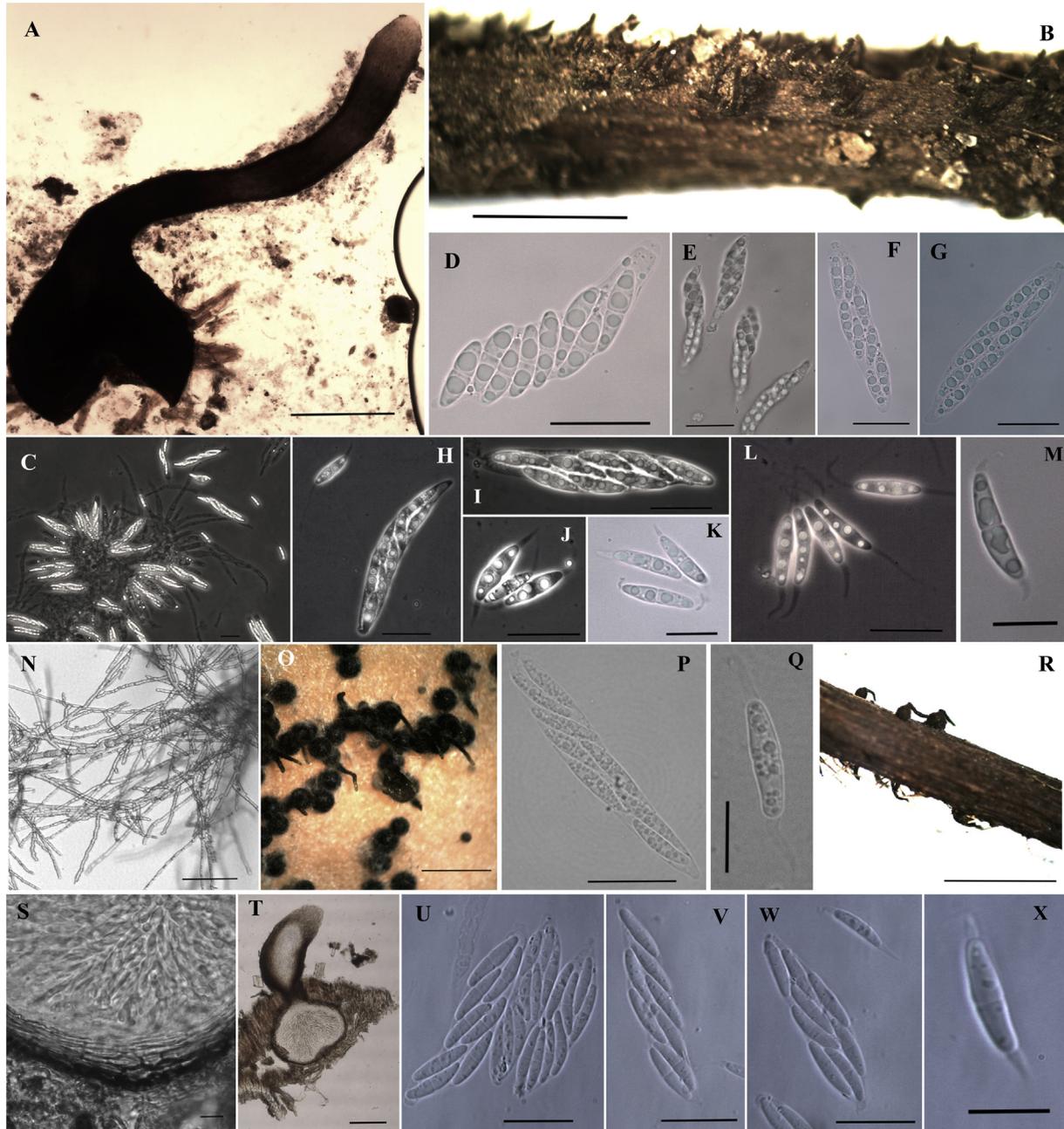
straight or curved, ostiolar necks. Paraphyses elongate, septate, thick, robust, and hyaline. Asci unitunicate, hyaline, fusiform, with eight ascospores, biseriata, obliquely uniseriate, irregularly uniseriate, with short pedicel and distinct apical ring. Ascospores hyaline, fusiform to ellipsoid, straight to slightly curved, two-celled with transverse median septum and with delicate, gelatinous, hyaline, filiform appendages at both ends.

*Asexual morph*: Not known.

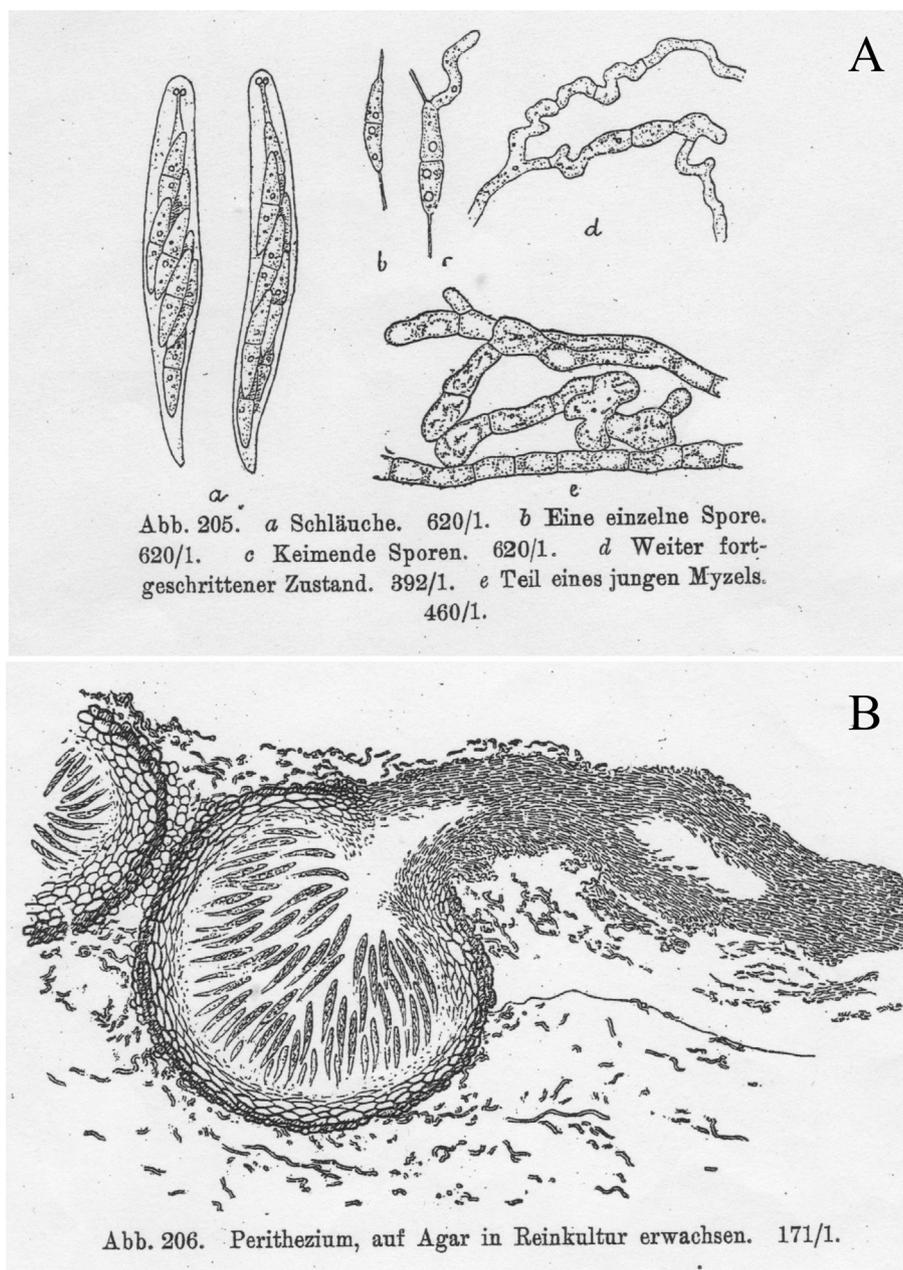
*Type species*: *Paragnomonium fragariae* (Kleb.) Senan. & K.D. Hyde.

***Paragnomonium fragariae*** (Kleb.) Senan. & K.D. Hyde, *Mycosphere* **8**: 199 (2017). Figs. 3–5.

MycoBank No.: MB552724.



**Fig. 3.** Morphology of *Paragnomonium fragariae* on the host and in culture. (A–M) Epitype I. Moročko-Bičevska & J. Fatehi F129 = F367871 (S): (A) Perithecium; (B) Perithecia on dead petiole; (C) Asci and paraphyses; (D–I) Asci; (J–M) Ascospores. (N) Mycelium of ex-epitype culture F129/P3/1 = MSCL1603 on potato dextrose agar. (O–Q) Isolate UN22 on oatmeal agar: (O) Perithecia; (P) Ascus; (Q) Ascospore. (R–T): On the host, I. Moročko-Bičevska F11: (R) Erupted perithecia on petiole; (Q) Cross section of perithecium wall. (T–X) On petiole of strawberry, Switzerland, Tessen, Tenero, A. Bolay, 20 Jun 1958: (T) Cross section of perithecium; (U–W) Asci and ascospores; (X) Ascospore. Bars = K, M, Q, X 10  $\mu$ m; C–J, L, P, S, U–W 20  $\mu$ m; A, N, T 100  $\mu$ m; B, Q, R 2 mm. All mounted in 3% KOH.



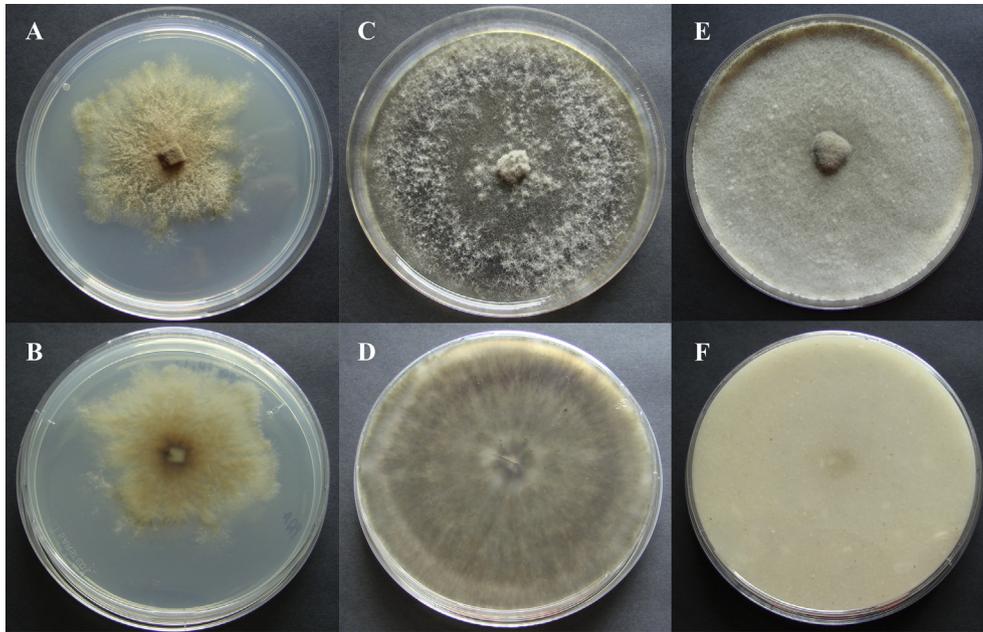
**Fig. 4.** (A) Lectotype of *Gnomonia fragariae* (Illustration Abb. 205, page 286., in H. Klebahn, Haupt- und Nebenfruchtformen der Askomyceten: Eine Darstellung eigener und der in der Literatur niedergelegten Beobachtungen über die Zusammenhänge zwischen Schlauchfrüchten und Konidienfruchtformen, 1918); (B) Perithecium of *Gnomonia fragariae* on agar medium illustrated by Klebahn (1918). The illustrations are reproduced with the permission of Schweizerbart and Borntraeger science publishers ([www.schweizerbart.de](http://www.schweizerbart.de)).

**Basionym:** *Gnomonia fragariae* Kleb., Haupt- und Nebenfruchtformen der Askomyceten: Eine Darstellung eigener und der in der Literatur niedergelegten Beobachtungen über die Zusammenhänge zwischen Schlauchfrüchten und Konidienfruchtformen: 285 (1918).

Mycobank No.: MB101207.

**Sexual morph:** Perithecia solitary or in groups, immersed or submerged in substrate, black, globose, 260–530  $\mu\text{m}$  high (mean = 377, SD 85, n = 13), 320–490  $\mu\text{m}$  wide (mean = 385, SD 62, n = 13), without stroma, convex or irregularly dented when dry, with one or two necks. Erupted, long ostiolar necks central, straight or curved, 101–1190  $\mu\text{m}$  long (mean = 548, SD 241, n = 20), 70–130  $\mu\text{m}$  wide at the base (mean = 94, SD 20, n = 20). Perithecial outer wall pseudo-parenchymatous composed of several outer

layers of dark and thick-walled cells and several layers of colourless inner cells. The perithecial neck composed of the outer thin, brown cell layers and the inner paraphyses lining ostiolar wide canal. Paraphyses elongate, septate, thick, robust, and hyaline. Asci uniseriate, hyaline, fusiform, 42–70  $\mu\text{m}$  long (mean = 56, SD 6, n = 290), 5–12  $\mu\text{m}$  wide (mean = 7, SD 1, n = 290), straight to slightly curved, containing eight ascospores, biseriata, obliquely uniseriate, irregularly uniseriate with short pedicel and distinct apical ring. Ascospores fusiform to ellipsoid, straight to slightly curved, 11–23  $\mu\text{m}$  long (mean = 16, SD 1.7, n = 501), 3–5  $\mu\text{m}$  wide (mean = 3.5, SD 0.5, n = 501), two-celled, hyaline, slightly constricted at transverse median septum, one cell often narrower than other, with usually 2 distinct guttules or up to 6 less distinctive guttules in each cell. The number and size of guttules in each cell



**Fig. 5.** Colony morphology of *Paragonomia fragariae* ex-epitype culture MSCL1603 on three agar media after four weeks of incubation at 22 °C in the dark: (A–B) potato dextrose agar; (C–D) potato carrot agar; (E–F) oatmeal agar.

vary depending on the age of spores. Ascospores at both ends have 2–18 µm long (mean = 7.6, SD 3.2, n = 562), delicate, gelatinous, hyaline, filiform, with pointed ends appendages. Length of appendages variable depending on ascospore maturity, usually 5–7 µm long in mature and longer than 20 µm in immature ascospores.

**Asexual morph:** Not known.

**Culture characteristics:** In culture growth on various media usually attaining full plate within four weeks at room temperature. On PDA colonies usually forming as thick radiating mycelial strands, yellow when young, becoming tawny or grimy brown with age, and having distinct irregular margins. Hyphae mostly submerged, profusely branched, with distinct swellings at most of the septa resulting in dumbbell-shaped or pyriform cells resembling those described and illustrated by Klebahn (1918). Colonies on PCA submerged, grey to olive green, while on OMA whitish, becoming grey with age and with abundant aerial mycelium. Homothallic. Numerous ascomata readily formed on PCA and OMA after at least three to four weeks of incubation at room temperature on a laboratory bench in a natural day–light cycle or in the conditions as described before (Moročko et al., 2006; Moročko and Fatehi, 2007). On PDA, sporulation does not occur.

**Lectotype designated here:** Illustration Abb. 205, page 286., in H. Klebahn, Haupt- und Nebenfruchtformen der Askomyzeten: Eine Darstellung eigener und der in der Literatur niedergelegten Beobachtungen über die Zusammenhänge zwischen Schlauchfrüchten und Konidienfruchtformen, 1918. Here reproduced as Fig. 4A.

Mycobank No.: MBT388393.

**Epitype designated here:** **Latvia:** Tukums, Püre, on dead petioles of *Fragaria x ananassa*, Lat: 57.0323418, Lon: 22.9160658, 20 Oct 2013, I. Moročko-Bičevska & J. Fatehi F129 (epitype F367871(S); isoepitype DAU100004631 (DAU); ex-epitype single ascospore culture F129/P3/1 = MSCL1603 (ex sexual morph); ex-epitype culture sequences MK524447 (LSU), MK524430 (ITS), MK524466 (*tef1-α*) (Fig. 3A–M).

Mycobank No.: MBT388634.

**Habitat:** Primary as a pathogen on cultivated strawberry (*Rosaceae*) causing root and crown rot and petiole blight. Perithecia of

the fungus can be found on the bases of the dead, overwintered petioles in spring to early summer and the dead or blighted petioles in late summer to autumn. The presence of perithecia also confirmed on petioles of naturally infected *Fragaria vesca* and *Potentilla anserina* (*Rosaceae*).

**Distribution:** Confirmed distribution – Germany (Hamburg) (Klebahn, 1918), Switzerland (Vaud, Les Barges, Valais, Tessin) (Bolay, 1972; Monod, 1983), United Kingdom, Latvia (all across the country), Sweden (Uppsala, Västra) (Moročko, 2006; Moročko et al., 2006), Lithuania (Kaunas, Šiauliai) and Finland (Parainen) (current study).

**Additional specimens examined** (all on dead petioles of cultivated *Fragaria x ananassa* and confirmed as *P. fragariae* except were indicated): **Finland:** Parainen, Bjursängpolku, 16 Oct 2014, I. Moročko-Bičevska F168 (DAU100004633; living hyphal tip culture F168.13 = MSCL1607); *ibid.*, 16 Oct 2014, I. Moročko-Bičevska F169 (DAU100004634; living single ascospore culture F169.1 = MSCL1608). –**Latvia:** Tukums: Püre, Sep 2003, I. Moročko-Bičevska Püre-1 (PR1) (DAU100004637); Püre, Sep 2003, I. Moročko-Bičevska Püre-2 (PR2) (DAU100004638); Püre, 2004, I. Moročko-Bičevska Elsanta (EL) (DAU100004639). Saldus: Jaunlutriņi, 6 Jun 2007, I. Moročko-Bičevska F11. –**Lithuania:** Kaunas: Babtai, 20 May 2014, I. Moročko-Bičevska F137 (DAU100004632; living single ascospore culture F137/P1/1 = MSCL1606); Babtai, 20 May 2014, I. Moročko-Bičevska F138. –**Sweden:** Uppsala: Fredrikslund, May 2004, I. Moročko-Bičevska & J. Fatehi F5 (living single hyphal tip culture F5.6). –**Switzerland:** Vaud: Chailly-sur-Clarens, 17 Jun 1958, A. Bolay (LAU); La Conversion sur Lutry, on petioles of cultivated *F. vesca*, 22 May 1958, A. Bolay (LAU); Corbeyrier, 23 Jun 1959, A. Bolay (LAU). Valais: Vouvry, 25 Jun 1958, A. Bolay (LAU); Torgon, on petioles of *P. anserina*, 11 Oct 1961, R. Corbaz (LAU); Miex, 29 Aug 1961, R. Corbaz (LAU). Tessin: Tenero, 20 Jun 1958, A. Bolay (LAU); *ibid.*, on fruit, asexual morph of *G. fructicola*, 20 Jun 1958, A. Bolay (LAU).

**Notes:** *P. fragariae* is a pathogen causing root rot, crown rot and petiole blight on cultivated perennial strawberry (Moročko et al., 2006; Moročko-Bičevska and Fatehi, 2011). During the large scale survey in 2007 in Latvia, it was found in 67 % of the surveyed farms

all across the country in 19 locations (I. Moročko-Bičevska, unpublished). In Lithuania, *P. fragariae* was identified in 40 % of the samples from three locations out of five surveyed in 2014 (data not shown). In Sweden, it was found in 66 % of the samples from three locations out of four surveyed (Moročko, 2006). So far, in Finland, the collection was done only in one location where we found *P. fragariae*. The morphological characters of the examined specimens and more than 200 *P. fragariae* isolates of our collection in culture from four European countries were in a good agreement and concordant with previous descriptions (Klebahn, 1918; Bolay, 1972; Monod, 1983). Among the specimens available from Switzerland (Bolay, 1972; Monod, 1983), besides cultivated *Fragaria x ananassa*, the presence of *P. fragariae* was also confirmed on naturally infected *F. vesca* and *P. anserina*. The specimens on *P. micrantha* and *P. rupestris* from Bolay's work were not available for the examination. Attempts to induce sporulation of isolate CBS146.64 from the United Kingdom were not successful, but the sequences have confirmed it as *P. fragariae* (Moročko and Fatehi, 2007).

In our collections, the other strawberry pathogen *G. fructicola* that causes fruit rot, stem end rot and leaf blotch was seldom found in few samples from Finland, Latvia, and Lithuania (data not shown), and on fruit in one specimen of Bolay's collection from Switzerland, Tessin, Tenero (see material examined). *G. fructicola* can be differentiated from *P. fragariae* based on smaller asci, ascospore arrangement in asci, differences in shape, septation and size of ascospores (see Walker et al., 2010), lack of paraphyses in mature perithecia and the presence of asexual morph. The records in several old plant pathological literatures from mid of last century on distribution of *P. fragariae* in various regions outside Europe are incorrect identifications or misuse of *G. fragariae* for *Gnomonia comari sensu lato* as it is evidenced by disease and pathogen descriptions provided in these publications (summarized by Farr, 1989; Maas, 1998; Moročko, 2006; Moročko et al., 2006).

The number of guttules in each cell of ascospores as a character for the differentiation of species within *Sydowiellaceae* suggested by Senanayake et al. (2017b) is misleading for *P. fragariae*. According to our observations, number, size and arrangement of guttules are variable and change depending on the maturity and age of the ascospores.

## 5. Discussion

### 5.1. Taxonomy

*Paragnomonium fragariae* (Kleb.) Senan. & K.D. Hyde based on *Gnomonia fragariae* Kleb. belongs to the *Sydowiellaceae* and causes strawberry root and crown rot and petiole blight while *Gnomoniopsis fructicola* (G. Arnaud) Sogonov based on *Gnomonia fragariae* f. *fructicola* G. Arnaud belongs to the *Gnomoniaceae* and causes fruit rot, stem end rot and leaf blotch. *G. fructicola* is a worldwide pathogen on strawberry (Bolay, 1972; Punithalingam, 1982; Sogonov et al., 2008), that in the literatures before Sogonov et al. (2008) has been most often referred under names *Gnomonia fructicola* (Arnaud) Fall, *Zythia fragariae* Laibach and *Gnomonia comari* P. Karst. Although confused with *Gnomonia*-like fungi on strawberry, *Gnomoniopsis comari* (P. Karst.) Sogonov based on *Gnomonia comari* P. Karst. also belongs in the *Gnomoniaceae* and occurs only on *Comarum palustre* (Sogonov et al., 2008; Walker et al., 2010).

Despite the obvious morphological differences, *P. fragariae* was confused with *Gnomoniopsis fructicola* by Arnaud and Arnaud (1931) and Alexopoulos and Cation (1948, 1952) leading to the series of misuse of the name *Gnomonia fragariae* Kleb. instead of *Gnomonia comari* P. Karst *sensu lato*, misidentifications and false records on its distribution in various regions of the world during the last century (summarised by Bolay, 1972; Moročko, 2006;

Moročko et al., 2006). Arnaud and Arnaud (1931) in France found a fungus causing strawberry fruit rot and identified it as *Gnomonia fragariae* Kleb.; however, since it formed pycnidia and its asci and ascospores were smaller than those described by Klebahn (1918) for *P. fragariae*, they named it *Gnomonia fragariae* Klebahn f. sp. *fructicola* Arnaud (summarised by Bolay, 1972). Later, in the United States, Alexopoulos and Cation (1948) isolated *Gnomonia*-like species from diseased strawberry that produced pycnidia and identified it as *Z. fragariae* Laibach (in plant pathological literatures before Sogonov et al. (2008) listed as an asexual morph of *Gnomonia comari* P. Karst.; for discussion on synonyms and taxonomy see Bolay, 1972; Monod, 1983; Sogonov et al., 2008; Walker et al., 2010). As summarised by Bolay (1972), the American strains were confirmed by G. Arnaud to be identical with French strains found by Arnaud and Arnaud (1931). Fall (1951) characterised the differences between the fungi described by Klebahn (1918) and Arnaud and Arnaud (1931) and proposed the new combination *Gnomonia fructicola* (Arnaud) Fall for *Gnomonia fragariae* Klebahn f. *fructicola* Arnaud. However, Alexopoulos and Cation (1952) still misused the name *Gnomonia fragariae* Kleb. although it was clear that it is not the same fungus described by Klebahn in 1918. Sogonov et al. (2008) demonstrated that *Gnomonia comari* P. Karst. *sensu lato* comprises two different species, *Gnomoniopsis comari* and *G. fructicola*, now placed in *Gnomoniopsis*, *Gnomoniaceae* family.

When Klebahn (1918) discovered and described *Gnomonia fragariae*, he mentioned the similarity of this fungus to the raspberry pathogen *Gnomonia rubi*, but he also noted that the latter taxon was different by having only four ascospores per ascus. Our previous phylogenetic analyses confirmed that these two species were different (Moročko and Fatehi, 2007).

The misidentification and confusion of *Gnomonia comari* P. Karst. *sensu lato* with *Gnomonia fragariae* Kleb. was discussed and presented in detail by Bolay (1972) and later followed by Monod (1983) in his monograph on *Gnomoniaceae*. Moreover, in the later plant pathological publications by Farr (1989), Maas (1998), and Moročko et al. (2006) the confusion surrounding these two separate strawberry pathogens with different distributions have been discussed and clarified. However, in the recent study by Senanayake et al. (2017b) the confusion surrounding *P. fragariae*, *G. comari* and *G. fructicola* was continued. Hence, in this paper we have provided revised description, lecto- and epitypification of *P. fragariae* based on the species original description, our recent collection, examination of Bolay's material used in the previous taxonomic studies concerning *P. fragariae* by Bolay (1972) and Monod (1983) and phylogenetic analyses of DNA sequences of three genomic loci.

### 5.2. Distribution and hosts

Monod (1983) examined Bolay's collection of *P. fragariae* on strawberry *Fragaria x ananassa* and wild species *F. vesca*, *P. anserina*, *P. micrantha* and *P. rupestris* from Switzerland. In our study, we compared our specimens with Bolay's collection and confirmed *P. fragariae* on these hosts, except on *P. micrantha* and *P. rupestris*, which were not available for examination. Bolay (1972) demonstrated perithecia formation of *P. fragariae* by inoculating autoclaved petioles of various species of *Alchemilla*, *Aruncus*, *Geum*, *Filipendula*, *Potentilla*, *Fragaria*, and *Sanguisorba* in the laboratory. Woodland strawberry *F. vesca*, silverweed or goose grass *P. anserina* and other above listed related plants are common herbaceous plant species in Northern Hemisphere (Davidson, 1995; Liston et al., 2014) and may act as natural inoculum reservoirs for infection of cultivated strawberry. In addition to previous records on the distribution of *P. fragariae* in Europe, to our knowledge, this study presents the first report of *P. fragariae* on cultivated strawberry in

Finland and Lithuania. *Gnomonia fragariae* was reported as an associated fungus with the root rot of strawberry in Finland (Parikka, 1981), but the authenticity of the record remains in question since the references listed for identification of the fungus were related to *G. fructicola*. The presence of *P. fragariae* in other parts of the world cannot be ruled out since the cultivated strawberry, and other hosts are common and widely distributed in various regions of the world. Our study on growth rate in various temperature regimes showed that *P. fragariae* is a cold-adapted fungus growing almost equally at 5 °C as in 20 °C and attaining maximal growth at 15 °C. The cold adaptation, however, might explain its distribution in northern European countries and abundant formation of fruiting bodies on the host in spring and autumn when such temperatures are common.

### 5.3. Molecular phylogeny

*Sydowiellaceae* is a family harbouring genera with diverse morphology, hosts, ecology and habitat (Rossman et al., 2007; Kruys and Castlebury, 2012; Senanayake et al., 2017b). The molecular phylogenetic analyses based on sequences of three genomic loci (LSU, ITS, *tef1- $\alpha$* ) in our study confirmed that *Paragnomonina*, comprising isolates of *P. fragariae* from five European countries, is a distinct, well supported monotypic genus within *Sydowiellaceae*. Phylogenetic studies (Kruys et al., 2012; Senanayake et al., 2017a; b) dealing with family and generic classification of *Diaporthales* and *Sydowiellaceae*, respectively, mainly have been based on LSU-ITS rDNA region shown to be insufficiently informative for a number of taxonomic groups of *Diaporthales* (Voglmayr and Jaklitsch, 2008, 2014; Voglmayr et al., 2017). So far, only LSU and ITS rDNA sequences have been available for the majority of the taxa currently placed in *Sydowiellaceae* or even just a single region, LSU or ITS, has been sequenced for several genera and species (e.g. *Alborbis*, *Cainiella*, *Rossmania*, *S. urticicola*) (Table 1). In the present study, the sequences of *tef1- $\alpha$*  were obtained from 22 isolates of seven species belonging to five genera of *Sydowiellaceae*, including the type species *S. fenestrans*. Additionally, the new primers were designed for amplification of ca. 0.8 kb fragment of *tef1- $\alpha$*  of *S. fenestrans* that failed to amplify with the other tested primers from Carbone and Kohn (1999) and Rehner (2001) (data not showed). Our phylogenetic analysis in the present study has also revealed that the recently described species "*S.*" *centaureii* (Senanayake et al., 2017b) is distant from the generic type *S. fenestrans* and other *Sydowiella*.

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### Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.funbio.2019.08.002>.

### References

- Alexopoulos, C.J., Cation, D., 1948. Stem-end rot of strawberries. *Phytopathology* 38, 698–706.
- Alexopoulos, C.J., Cation, D., 1952. *Gnomonia fragariae* in Michigan. *Mycologia* 44, 221–223.
- Arnaud, G., Arnaud, M., 1931. *Traité de Pathologie Végétale, tome 1 Encyclopédie Mycologique* 4: 1558 – 1562. P. Lechevalier, Paris, France.
- Bolay, A., 1972. Contribution a la connaissance de *Gnomonia comari* Karsten (syn. *G. fructicola* [Arnaud] Fall). Etude taxonomique, phytopathologique et recherches sur sa croissance in vitro. *Berichte der Schweizerischen Botanischen Gesellschaft: Bull. Soc. Bot. Suisse* 81, 398–482.
- Carbone, I., Kohn, L.M., 1999. A method for designing primer sets for speciation studies in filamentous ascomycetes. *Mycologia* 91, 553–556.
- Crous, P.W., Summerell, B.A., Shivas, R.G., Carnegie, A.J., Groenewald, J.Z., 2012. A reappraisal of *Harknessia* (*Diaporthales*), and the introduction of *Harknessiaceae* fam. nov. *Persoonia* 28, 49–65.
- Davidson, C.G., 1995. *Potentilla fruticosa*, taxonomy and international registration. *Acta Hort.* 413, 157–160.
- Dhingra, O.D., Sinclair, J.B., 1995. *Basic Plant Pathological Methods*. CRC Press, US.
- Du, Z., Hyde, K.D., Yang, Q., Liang, Y.M., Tian, C.M., 2017. *Melansporellaceae*: a novel family of *Diaporthales* (*Ascomycota*). *Phytotaxa* 305, 191–200.
- Fall, J., 1951. Studies on fungus parasites of strawberry leaves in Ontario. *Can. J. Bot.* 29, 299–315.
- Farr, D.F., Bills, G.F., Chamuris, G.P., Rossman, A.Y., 1989. *Fungi on Plants and Plant Products in the United States*. APS Press, St. Paul, Minnesota.
- Gubler, W.D., Feliciano, A., 1999. Occurrence of leaf blotch and stem-end rot of strawberry in the Central Coast California. *Plant Dis.* 83, 199.
- Huelsenbeck, J.P., Ronquist, F., 2001. MRBAYES: Bayesian inference of phylogenetic trees. *Bioinformatics* 17, 754–755.
- Klebahn, H., 1918. Haupt- und Nebenfruchtformen der Askomycyeten: Eine Darstellung eigener und der in der Literatur niedergelegten Beobachtungen über die Zusammenhänge zwischen Schlauchfrüchten und Konidienfruchtformen. Verlag von Gebrüder Borntraeger, Leipzig.
- Kruys, A., Castlebury, L.A., 2012. Molecular phylogeny of *Sydowiellaceae* – resolving the position of *Cainiella*. *Mycologia* 104, 419–426.
- Kurppa, S., Vrain, T.C., 1989. Effect of *Pratylenchus penetrans* on the infection of strawberry roots by *Gnomonia comari*. *J. Nematol.* 21, 511–516.
- Larget, B., Simon, D.L., 1999. Markov chain Monte Carlo algorithms for the Bayesian analysis of phylogenetic trees. *Mol. Biol. Evol.* 16, 750–759.
- Liston, A., Cronn, R., Ashman, T.L., 2014. *Fragaria*: a genus with deep historical roots and ripe for evolutionary and ecological insights. *Am. J. Bot.* 101, 1686–1699.
- Maas, J.L., 1998. *Compendium of Strawberry Diseases*, second ed. APS Press, St. Paul, Minnesota, pp. 38–39.
- Mau, B., Newton, M.A., Larget, B., 1999. Bayesian phylogenetic inference via Markov chain Monte Carlo methods. *Biometrics* 55, 1–12.
- Monod, M., 1983. *Monographie taxonomique des Gnomoniaceae* (*Ascomycètes de l'ordre des Diaporthales* I). Beihefte zur Sydowia 9, 1–315.
- Moročko, I., 2006. Characterization of the strawberry pathogen *Gnomonia fragariae*, and biocontrol possibilities. *Acta Universitatis Agriculturae Sueciae, Uppsala*, p. 71. SLU Service/Repro.
- Moročko, I., Fatehi, J., 2007. Molecular characterization of strawberry pathogen *Gnomonia fragariae* and its genetic relatedness to other *Gnomonia* species and members of *Diaporthales*. *Mycol. Res.* 111, 603–614.
- Moročko, I., Fatehi, J., Gerhardson, B., 2006. *Gnomonia fragariae*, a cause of strawberry root rot and petiole blight. *Eur. J. Plant Pathol.* 114, 235–244.
- Moročko-Bičevska, I., Fatehi, J., 2011. Infection and colonization of strawberry by *Gnomonia fragariae* strain expressing green fluorescent protein. *Eur. J. Plant Pathol.* 129, 567–577.
- Norphanphoun, C., Hongsanan, S., Doilom, M., Bhat, D.J., Wen, T.C., Senanayake, I.C., Bulgakov, T., Hyde, K., 2016. *Lamproconiaceae* fam. nov. to accommodate *Lamproconium desmazieri*. *Phytotaxa* 270, 89–102.
- Nylander, J.A.A., 2004. MrModeltest v2. Computer program distributed by the author. Evolutionary Biology Centre, Uppsala University.
- Parikka, P., 1981. Strawberry root rot in Finland. *Annales Agriculturae Fenniae/Seria Phytopathologia* 85, 192–197.
- Punithalingam, E., 1982. *CMI Descriptions of Pathogenic Fungi and Bacteria* No. 737. The Cambrian News, Aberystwyth, UK.
- Rehner, S., 2001. Primers for Elongation Factor 1- $\alpha$  (EF1- $\alpha$ ). Available from: <http://www.aftol.org/pdfs/EF1primer.pdf>.
- Rossman, A.Y., Farr, D.F., Castlebury, L.A., 2007. A review of the phylogeny and biology of the *Diaporthales*. *Mycoscience* 48, 135–144.
- Senanayake, I.C., Crous, P.W., Groenewald, J.Z., Maharachchikumbura, S.S.N., Jeewon, R., Phillips, A.J.L., Bhat, J.D., Perera, R.H., Li, Q.R., Li, W.J., Tangthirasunon, N., Norphanphoun, C., Karunanatha, S.C., Camporesi, E., Manawasinghe, I.S., Al-Sadi, A.M., Hyde, K.D., 2017a. Families of *Diaporthales* based on morphological and phylogenetic evidence. *Stud. Mycol.* 86, 217–296.
- Senanayake, I.C., Maharachchikumbura, S.S.N., Jeewon, R., Proputtha, I., Al-Sadi, A.M., Camporesi, E., Hyde, K.D., 2017b. Morphophylogenetic study of *Sydowiellaceae* reveals several new genera. *Mycosphere* 8, 172–217.
- Shipton, P.J., 1967. A fruit rot of strawberries caused by *Zythia fragariae*. *Plant Pathol.* 16, 123–125.
- Sogonov, M.V., Castlebury, L.A., Rossman, A.Y., Mejia, L.C., White, J.F., 2008. Leaf-inhabiting genera of the *Gnomoniaceae*, *Diaporthales*. *Stud. Mycol.* 62, 1–79.

- Suetrong, S., Klaysuban, A., Sakayaroj, J., Preedanon, S., Ruang-Areerate, P., Phongpaichit, S., Pang, K.L., Jones, E.B.G., 2015. *Tirisporellaceae*, a new family in the order *Diaporthales* (*Sordariomycetes*, *Ascomycota*). *Cryptog. Mycol.* 36, 319–330.
- Swofford, D.L., 2002. PAUP: Phylogenetic Analysis Using Parsimony (And Other Methods). Version 4.0b10. Sinauer Associates, Sunderland, Massachusetts.
- Voglmayr, H., Castlebury, L.A., Jaklitsch, W.M., 2017. *Juglanconis* gen. nov. on *Juglandaceae*, and the new family *Juglanconidaceae* (*Diaporthales*). *Persoonia* 38, 136–155.
- Voglmayr, H., Jaklitsch, W.M., 2008. *Prosthecium* species with *Stegosporium* anamorphs on acer. *Mycol. Res.* 112, 885–905.
- Voglmayr, H., Jaklitsch, W.M., 2014. *Stilbosporaceae* resurrected: generic reclassification and speciation. *Persoonia* 33, 61–82.
- Voglmayr, H., Mehrabi, M., 2018. Molecular phylogeny and a new Iranian species of *Caudospora* (*Sydowiellaceae*, *Diaporthales*). *Sydowia* 70, 67–80.
- Voglmayr, H., Rossman, A.Y., Castlebury, L.A., Jaklitsch, W.M., 2012. Multigene phylogeny and taxonomy of the genus *Melanconiella* (*Diaporthales*). *Fungal Divers.* 57, 1–44.
- Walker, D.M., Castlebury, L.A., Rossman, A.Y., Mejía, L.C., White, J.F., 2012. Phylogeny and taxonomy of *Ophiognomonina* (*Gnomoniaceae*, *Diaporthales*), including twenty-five new species in this highly diverse genus. *Fungal Divers.* 57, 85–147.
- Walker, D.M., Castlebury, L.A., Rossman, A.Y., Sogonov, M.V., White, J.F., 2010. Systematics of genus *Gnomoniopsis* (*Gnomoniaceae*, *Diaporthales*) based on a three gene phylogeny, host associations and morphology. *Mycologia* 102, 1479–1496.