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Sirococcus smithogilvyi: haplotype diversity in Basilicata Region (Southern Italy)

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Abstract Chestnut phytosanitary emergencies are rising in recent years also due to climate changes, which allow new treats to find favourable environmental conditions in previously unsuitable places and the rapidity of movements which aids their survival. Among the emerging fungal pathogens, *Sirococcus smithogilvyi* the causal agent of chestnut brown rot (CBR), a very damaging chestnut disease, stands out. Between 2023-2024, *S. smithogilvyi* was frequently isolated from 1200 nuts collected randomly in three chestnut locations of Basilicata region (Southern Italy) and initially identified based on morphology features. Other fungal taxa were less frequently isolated: *Alternaria alternata*, *Botrytis cinerea*, *Penicillium* sp., *Neofusicoccum parvum*, *Mucor* sp., *Cladosporium* sp. and *Trichoderma* sp. Sequencing of two common fungal barcodes, the Internal Transcribed Spacer (ITS) region of ribosomal DNA and the *β-tubulin* (*tub2*) gene confirmed the morphological identification. Furthermore, phylogenetic analyses of *S. smithogilvyi* isolates, based on *tub2* nucleotide sequences, showed the occurrence of two separate evolutionary lineages in Basilicata, previously recognized as haplotypes A and B. The haplotype A, was predominant registering 86% frequency while the haplotype B registered only a 14% frequency. The prevalent presence of *S. smithogilvyi* haplotype A (reported to be more aggressive) along with other additional factors could explain the serious damage and the chestnut production losses recorded for the past years in Basilicata, which urges to find and implement suitable measures to control this emerging and very damaging chestnut pathogen.

Keywords: chestnut nut rot disease, emerging fungal pathogen, haplotypes diversity, molecular identification.

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Introduction

Sweet chestnut (*Castanea sativa* Mill.), although known as a long living-tree species, throughout the past several years, exhibits pronounced vulnerability to multiple biotic threats, among which fungal pathogens are of relevant importance (Vettraino et al. 2005, 2021, Gothier & Robin 2020, Fernandes et al. 2022, Lema et al. 2023).

One of the emerging and highly destructive fungal pathogens attacking sweet chestnut worldwide is *Sirococcus smithogilvyi* (L.A. Shuttlew., E.C.Y. Liew & D.I. Guest) Ning Jiang (synonyms: *Gnomoniopsis smithogilvyi* L.A. Shuttlew., E.C.Y. Liew & D.I. Guest and *Gnomoniopsis castaneae* Tamietti [as '*castanea*']), causing an economically relevant disease named "chestnut brown rot" (CBR). CBR disease is currently considered a serious threat to chestnut cultivations in many countries (Visentin et al. 2012, Dennert et al. 2015, Shuttleworth et al. 2015, Lione et al. 2019, Sakalidis et al. 2019, Cisterna-Oyarce et al. 2022, Topalidou et al. 2024). Chestnut nuts infected by *S. smithogilvyi* may appear externally healthy but typically exhibit internal symptoms, including a chalky and spongy endosperm associated with dark brown lesions (hence the name of the disease) (Maresi et al. 2013, Shuttleworth & Guest 2017). In addition, their flavour is significantly altered, rendering them unsuitable for consumption.

The presence of *S. smithogilvyi*, previously named *G. castaneae* or *G. smithogilvyi* (Jiang et al. 2026), which is a pathogen of foreign origin, has been reported for the first time in Italy, in Piedmont, and it has now spread to many of the most important chestnut-growing areas of the country (Visentin et al. 2012, Maresi et al. 2013, Bastianelli et al. 2022 Seddaiu et al. 2023, Mang et al. 2024, Carloni et al. 2026). It can infect multiple plant hosts, such as, hazel, holm oak and ash on which it is also capable of causing stem and branch cankers and bud death (Visentin et al. 2012). Although the CBR disease is worldwide recognized by chestnut growers, the biology of causing agent so far remains unclear. The climatic conditions seem to play a very important role in the spread of the disease. In particular, very hot summers with little rainfall (conditions which unfortunately due to global climatic changes occur more and more frequently in many countries) appear to favour pathogen development. The damage caused by *S. smithogilvyi* can be extensive for example, infections of up to 91% and 72% in mature and rooted nuts had been reported in Switzerland and Australia (Dennert et al. 2015, Shuttleworth et al. 2013). Due to the importance of this pathogen posing a threat to both chestnut productions and the native chestnut species in America, Dobry and Campbell (2023) pointed out the essential need to re-evaluate the *S. smithogilvyi* epidemiology, associations and implications.

Understanding genetic variation and host–pathogen interactions is crucial for developing effective and lasting disease management strategies and requires a deep knowledge of the phenotypic and genetic structure of pathogen populations. In the past, Dennert et al. (2015) reported *G. castanea* as the key agent of chestnut nut rot in Switzerland as it was the only pathogen isolated from chestnuts exhibiting CBR symptoms. Same authors, based on combined data of *calmodulin* and *β-tubulin* sequences showed that several haplotypes (named H1-H5 and distinguished based on Single Nucleotide Polymophisms) were present in different localities in Switzerland however, two of them (namely H1 and H2) were most frequent comprising 33 and 9 isolates of the 45 isolates investigated (Dennert et al. 2015). Furthermore, outcomes from *calmodulin* gene alone showed the occurrence of three haplotypes while, results from *β-tubulin* gene alone disclosed two haplotypes matching the two clusters obtained by phylogenetic analysis (Dennert et al. 2015). Studies about the genetic variability of *S. smithogilvyi* populations in north-western Italy and some isolates from southern European countries performed by Simple Sequence Repeats (SSR) markers allowed the identification of at least two putative separate fungal subpopulations (Sillo et al. 2017).

Recently, Seddaiu et al. (2023) and Carloni et al. (2026) investigated the spatial distribution, genetic structure and virulence of *S. smithogilvyi* in Italy and based on the *β-tubulin* gene revealed the occurrence of two separate evolutionary

lineages (named haplotypes A and B) with various frequencies in different Italian geographic regions. Same authors also reported that haplotype A (dominant in central Italy) and haplotype B (dominant in northern Italy) of *S. smithogilvyi* (*Ss*), at the time known as *G. castaneae,* had different aggressiveness (Seddaiu et al. 2023) and this also varied depending on isolate (Carloni et al. 2026). In particular, Seddaiu et al. (2023) demonstrated that *Ss* (previously named *Gc* using the abbreviation of *Gnomoniopsis castaneae)*-haplotype A (4 isolates) appear to be more aggressive than *Ss*-haplotype B (3 isolates), all originating from Sardinia. Nevertheless, very recently Carloni et al. (2026) performing pathogenicity studies involving both A (5 isolates) and B (11 isolates) *Ss*-haplotypes, from different Italian regions, plus 2 reference isolates from a fungal collection from Padova University, found that from the statistical point of view *Ss* isolates of haplotype A were not pathogenically different from the B haplotypes of the same fungus. However, the same authors noticed differences in aggressiveness within each *Ss*-haplotype group and also reported that prolonged water stress promoted aggressiveness only in case of *Ss*-haplotype A isolates (Carloni et al. 2026).

Although both earlier described studies provided significant knowledge about *S. smithogilvyi* haplotypes presence, distribution and virulence in Italy complete data about the occurrence, distribution and, genetic diversity of this fungal pathogen in a southern Italian region like Basilicata, is still missing. In order to fill this gap of knowledge the present study was undertaken aiming to: (a) estimate incidence of brown rot in Basilicata region (Southern Italy), (b) isolate and identify the causal agent of the CBR disease and (c) investigate occurrence, types and frequency of *S. smithogilvyi* haplotypes in Basilicata region.

Materials and Methods

Plant material

Between 2023 and 2024, a total of 1,200 chestnut fruits were randomly collected from three major chestnut-growing areas (Melfi, Rionero in Vulture and Lagonegro) of the Basilicata region (Southern Italy). In particular, chestnuts of "Marroncino di Melfi" variety were collected from Melfi and Rionero in Vulture, while chestnuts from a local ecotype were gathered from Lagonegro. They were brought to laboratory, kept at 4°C and shortly processed. Site management differed among locations, with sites S1 and S2 classified as semi-natural and site S3 as natural (Table 1). Samples were taken equally from fallen chestnuts and from those present on the branches.

Fungi isolation

In order to isolate the fungal taxa, nuts were rinsed under tap water to eliminate any dirt presence, then surface disinfected by immersion in 70% ethanol for 1 min and left to dry at room temperature under laminar flow cabinet. Subsequently, they were bisected and visually inspected for the presence of any endosperm symptoms (necrotic lesion/brown rot). Small pieces (~5 mm^2) of symptomatic tissues were placed on Potato Dextrose Agar (PDA) media amended with antibiotics as fully described by Mang & Figliuolo (2010) and Mang et al. (2024). All pure fungal cultures obtained as fully described in earlier studies (Mang et al. 2015, D'Ippolito et al. 2022) were initially identified morphologically, at least at genus level, based on colony and conidia features using appropriate literature resources (Sogonov et al. 2008, Walker et al. 2010, Visentin et al. 2012).

Table 1 Sampling sites and plant material details.

Sampling site code	Locality	Location Coordinates		Climatic Zone	Number of samples collected
S1	Melfi	40°59'51"00 N	15°9'7"92 E	D	400
S2	Rionero in Vulture	40°55'36"12 N	15°40'14"88 E	E	400
S3	Lagonegro	40°7'45"48 N	15°45'44"28 E	E	400
Total samples					**1200**

DNA Isolation, PCR and sequencing

Identity of the *S. smithogilvyi* along with other fungal isolated taxa was further confirmed by molecular analysis. The genomic DNA was extracted from 7 days old representative pure fungal cultures grown on PDA + antibiotics (Mang & Figliuolo 2010, D'Ippolito et al. 2022) at 24°C in the dark, using the NucleoSpin Plant II™ (Macherey-Nagel, Germany) kit as previously described (Frisullo et al. 2015, Camele & Mang 2019). Its integrity was verified by agarose gel electrophoresis and concentration and purity by readings obtained with a spectrophotometer model NanoDrop ND-1000 (NanoDrop technologies Inc., Wilmington, USA) following the protocols described in detail by Camele & Mang (2019). Two molecular fungal barcodes were used to precisely identify the isolates obtained in this study. In particular, the ITS region of the ribosomal DNA was amplified with the universal primers ITS1/ITS4 (White et al. 1990) and a fragment of the *β-tubulin* gene (*tub2*) was amplified with the Bt2a/Bt2b primers (Glass & Donaldson 1995) following the protocols previously described by Mang & Figliuolo 2010 and by Mang et al. 2024 for all fungal isolates. Outcomes of the PCR reactions were verified by electrophoresis on 1.5% agarose gel run at 70V for 35 minutes in 1X Tris-Acetate-EDTA (TAE) buffer. Amplicons were purified by QIAquick PCR purification Kit (Qiagen, Hilden, Germany) following manufacturer's instructions and subsequently, directly sequenced, in both directions, by BMR Genomics (Padua, Italy) using same PCR primers (Frisullo et al. 2015, Cardellicchio et al. 2023).

Sequence analysis

Nucleotide sequences were initially visualized with freely available software Chromas v. 2.6.6 (Technelysium Pty Ltd., South Brisbane, Australia), a chromatogram editor, downloaded from www.technelysium.com.au/wp/chromas and afterwards analyzed by nucleotide Basic Local Alignment Search Tool (BLAST, Altschul et al. 1990) (https://blast.ncbi.nlm.nih.gov) option BLASTn in the National Centre for Biotechnology Information (NCBI) database and compared with reference sequences already available in GenBank. Species assignment for each fungal isolate was performed for the two barcodes considering >99.7% identity with ex-type cultures or isolates.

Phylogenetic investigation

The obtained nucleotide sequences (of the ITS region and of *β-tubulin* gene) along with those belonging to reference species (downloaded from the GenBank) were aligned with the MAFFT7 software (Kuraku et al. 2013, Katoh et al. 2019) online (https://mafft.cbrc.jp/alignment/server/index.html), which provides a rapid and accurate Multiple Sequences Alignment (MSA), and then manually trimmed to remove poor aligned regions.

Phylogenetic analysis involving 56 *β-tubulin* nucleotide sequences (Table 2) was conducted in MEGA12 software (Kumar et al. 2024) and inferred using the Maximum Likelihood (ML) and Kimura 2-parameter model (Saitou & Nei 1987). Before phylogenetic analysis, to choose the best-fit nucleotide substitution model under Models tool in MEGA12 the option find best DNA models (ML) was selected (Kumar et al. 2024). The Bayesian Information Criterion (BIC) scores were used to select the optimal model and after analysis the K2+I was chosen. Evolutionary rate variation among sites was modeled using a discrete Gamma distribution (+G) with 5 rate categories (Yang 1994) and a fraction of sites were allowed to be evolutionarily invariant (+I).

To test the statistical reliability of the tree branches the percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) was determined and shown above the branches (Felsenstein 1985). Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Joining and BioNJ algorithms (Saitou & Nei 1987) to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with superior log likelihood value. The tree was drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. Codon positions included were 1st+2nd+3rd+Noncoding. The partial deletion option was applied to eliminate all positions with less than 60% site coverage. The evolutionary distances were presented in the units of the number of base substitutions per site.

Table 2 List of *Sirococcus smithogilvyi* isolates used in this study for phylogenetic analysis.

Isolate name	Strain	Haplotype	Host plant	Geographical Origin	GenBank Acc. Nos.[1] ITS	*tub2*	GenBank Reference[2]
G. smithogilvyi	MUT401	A	*C. sativa*	Italy	-	KR072532	Unpublished, 2015
G. smithogilvyi	MUT411	A	*Castanea* sp.	New Zealand	-	KR072533	Unpublished, 2015
S. smithogilvyi	**3-2**	**A**	***C. sativa***	**Italy**	**PV715875**	**PX653209**	**This study**
S. smithogilvyi	**4**	**A**	***C. sativa***	**Italy**	**PV715872**	**PX649999**	**This study**
S. smithogilvyi	**5**	**A**	***C. sativa***	**Italy**	**PV715873**	**PX650000**	**This study**
S. smithogilvyi	**A**	**A**	***C. sativa***	**Italy**	**PV715874**	**PX653210**	**This study**
S. smithogilvyi	**B2**	**A**	***C. sativa***	**Italy**	**PV715876**	**PX653211**	**This study**
S. smithogilvyi	**B3**	**A**	***C. sativa***	**Italy**	**PV715877**	**PX653212**	**This study**
S. smithogilvyi	**D4**	**A**	***C. sativa***	**Italy**	**PV715878**	**PX653213**	**This study**
G. castaneae	E4	A	Unspecified	Italy	MN809485	MN817098	Unpublished, 2020, 2021
G. castaneae	E5	A	Unspecified	Italy	MN809486	MN817099	Unpublished, 2020, 2021
S. smithogilvyi	**E5-1**	**A**	***C. sativa***	**Italy**	**PV715879**	**PX653214**	**This study**
G. smithogilvyi	EFA 924A	A	*C. sativa*	Spain	OM319846	OM417078	Unpublished, 2022
G. smithogilvyi	EFA 925A	A	*C. sativa*	Spain	OM319847	OM417079	Unpublished, 2022
S. smithogilvyi	**I9**	**A**	***C. sativa***	**Italy**	**PV715882**	**PX653215**	**This study**
S. smithogilvyi	**O15**	**A**	***C. sativa***	**Italy**	**PV715885**	**PX650003**	**This study**
S. smithogilvyi	**R18**	**A**	***C. sativa***	**Italy**	**PV715887**	**PX650002**	**This study**
S. smithogilvyi	**S19**	**A**	***C. sativa***	**Italy**	**PV715888**	**PX653216**	**This study**
S. smithogilvyi	**T2**	**A**	***C. sativa***	**Italy**	**PV715883**	**PX653217**	**This study**
S. smithogilvyi	**V21**	**A**	***C. sativa***	**Italy**	**PV715889**	**PX650001**	**This study**
S. smithogilvyi	**Q17**	**A**	***C. sativa***	**Italy**	**PV715886**	**PX653218**	**This study**
G. smithogilvyi	Ge1	A	*C. sativa*	Switzerland	KP824754	KP824768	Unpublished, 2015
G. castaneae	BIA_1_1	A	*C. sativa*	Switzerland	-	KM437889	Unpublished, 2014
G. castaneae	CAS_1_1	A	*C. sativa*	Switzerland	-	KM437890	Unpublished, 2014
G. castaneae	PSB3	A	*C. dentata*	USA	MZ682108	OK335787	Unpublished, 2022
G. castaneae	1A	A	*C. sativa*	Italy	-	PX259707	Unpublished, 2026
G. castaneae	12_2	A	*C. sativa*	Italy	PX242886	PX259715	Unpublished, 2026
G. castaneae	3A	A	*C. sativa*	Italy	PX242877	PX259708	Unpublished, 2026
G. castaneae	V	A	*C. sativa*	Italy	PX242891	PX259719	Unpublished, 2026
G. castaneae	11_1	A	*C. sativa*	Italy	PX242885	PX259714	Unpublished, 2026
G. smithogilvyi	EFA 962.4A	B	*C. sativa*	Spain	OM319848	OM417080	Unpublished, 2022
G. castaneae	UP45119	B	*C. dentata*	USA	MZ681935	OK335785	Unpublished, 2022
G. castaneae	E2	B	Unspecified	Italy	-	MN817096	Unpublished, 2021
G. castaneae	E3	B	Unspecified	Italy	-	MN817097	Unpublished, 2021
G. castaneae	6A	B	*C. sativa*	Italy	PX242880	PX259710	Unpublished, 2026
G. castaneae	D2	B	*C. sativa*	Italy	PX242898	PX259725	Unpublished, 2026
G. castaneae	9A	B	*C. sativa*	Italy	PX242883	PX259713	Unpublished, 2026
G. castaneae	F	B	*C. sativa*	Italy	PX242888	PX259717	Unpublished, 2026
G. castaneae	B2BIS	B	*C. sativa*	Italy	PX242896	PX259723	Unpublished, 2026
G. castaneae	7A	B	*C. sativa*	Italy	PX242881	PX259711	Unpublished, 2026
G. castaneae	E	B	*C. sativa*	Italy	PX242892	PX259720	Unpublished, 2026
G. castaneae	DX1	B	*C. sativa*	Italy	PX242899	PX259726	Unpublished, 2026
G. castaneae	GCAS1	B	*C. sativa*	Greece	MH107826	MH213477	Tziros, 2019
G. castaneae	GCAS2	B	*C. sativa*	Greece	MH107827	MH213478	Tziros, 2019
G. castaneae	GCAS3	B	*C. sativa*	Greece	MH107828	MH213479	Tziros, 2019
G. castaneae	GCAS4	B	*C. sativa*	Greece	MH107829	MH213480	Tziros, 2019
G. castaneae	GCAS5	B	*C. sativa*	Greece	MH107830	MH213481	Tziros, 2019
G. castanea	TAV_1_1	B	*C. sativa*	Switzerland	KM437892	KM437888	Unpublished, 2014
G. smithogilvyi	Ti1	B	*C. sativa*	Switzerland	KP824746	KP824764	Unpublished, 2015
G. smithogilvyi	Ti3	B	*C. sativa*	Switzerland	KP824748	KP824765	Unpublished, 2015
G. castaneae	Ti4	B	*C. sativa*	Switzerland	KP824750	KP824766	Unpublished, 2015
G. castaneae	Ti5	B	*C. sativa*	Switzerland	KP824752	KP824767	Unpublished, 2015
S. smithogilvyi	**1**	**B**	***C. sativa***	**Italy**	**PV715880**	**PX660472**	**This study**
S. smithogilvyi	**2**	**B**	***C. sativa***	**Italy**	**PV715881**	**PX660473**	**This study**
S. smithogilvyi	**T20**	**B**	***C. sativa***	**Italy**	**PV715884**	**PX660474**	**This study**
G. sanguisorbae	CBS 858.79	-	*Sanguisorba minor*	Switzerland	GU320818	GU320790	Walker et al. 2010

Note: [1]Nucleotide sequences written in bold are obtained in this study. They were deposited under the name valid at the moment of deposition which was later updated to the current name (https://www.speciesfungorum.org) showed in the table and also used in phylogenetic analysis. [2]Only the reference and the year reported in the GenBank for each accession are shown in the table regardless to any subsequent publication reporting the same accession numbers.

Results

Both common barcodes (ITS and *tub2*) were successfully amplified and sequenced for all fungal isolates obtained in this study and nucleotide sequences of 18 representative isolates of *S. smithogilvyi* were deposited in GenBank (with the scientific name for the species valid at the moment of deposition) under the following accession numbers: PV715872-86 (ITS sequences); PX649999-PX650003, PX653209-18, and PX660472-74 (*tub2* sequences) (Table 2). Fungi isolated in the present study from chestnut nuts endosperm were identified based on morphological features and molecular data as belonging to eight taxa namely *S. smithogilvyi*, *Alternaria alternata*, *Botrytis cinerea*, *Penicillium* sp., *Mucor* sp., *Neofusiccocum parvum*, *Trichoderma* sp. and *Cladosporium* sp. However, the most frequently isolated fungus was *S. smithogilvyi* (>50% isolation frequency) while the other fungi were only occasionally isolated (<12% isolation frequency) with the exception of *Penicillium* sp. (24,5% isolation frequency) as shown in Figure 1.

The alignment of the ITS nucleotide sequences of all *S. smithogilvyi* isolates from this study along with those downloaded from the NCBI database did not show differences among sequences and consequently, this barcode did not allow any distinction between the two haplotypes. Therefore, a more informative barcode like the *tub2* gene (commonly recommended as secondary DNA barcode for fungi) was used to separate the two lineages. Outcomes of *tub2* gene analysis permitted to distinguish between the A and B haplotypes of *Ss* based on the Single Nucleotide Polymorphisms (SNPs) occurring at conserved sites (Seddaiu et al. 2013, Carloni et al. 2026). It was observed that most of the *Ss* taxa (15 isolates) from Basilicata region grouped together with the isolates of the same species belonging to the haplotype A of diverse geographical origins, including those from other Italian regions, and only three *Ss* isolates (namely the isolate 1, the isolate 2 and the isolate T20) grouped together with the haplotype B isolates of the same fungus from other studies and originated from different countries. In detail, *Ss*-haplotype A isolates grouped together with other members of the same haplotype (73% bootstrap support) and *Ss*-haplotype B isolates clustered closely to similar isolates of different origin. In addition, outcomes from this study showed that together the *Ss*-haplotypes (A, B) formed a distinct clade strongly supported by robust bootstrap values (85%) and also well-separated from the *S. sanguisorbae* (previously named *G. sanguisorbae*) strain CBS 858.79 taken as outgroup (Figure 2).

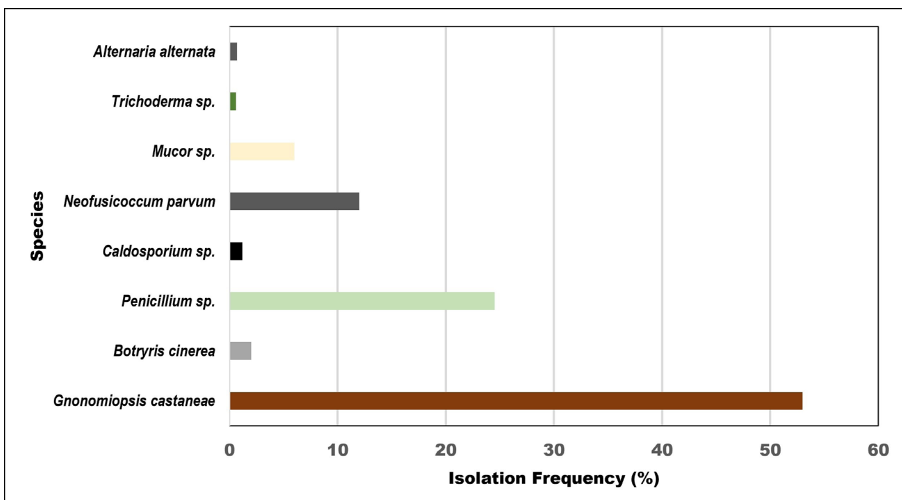

Figure 1 Isolation frequency (IF%) of fungal species from CBR symptomatic chestnut nuts from Basilicata region.

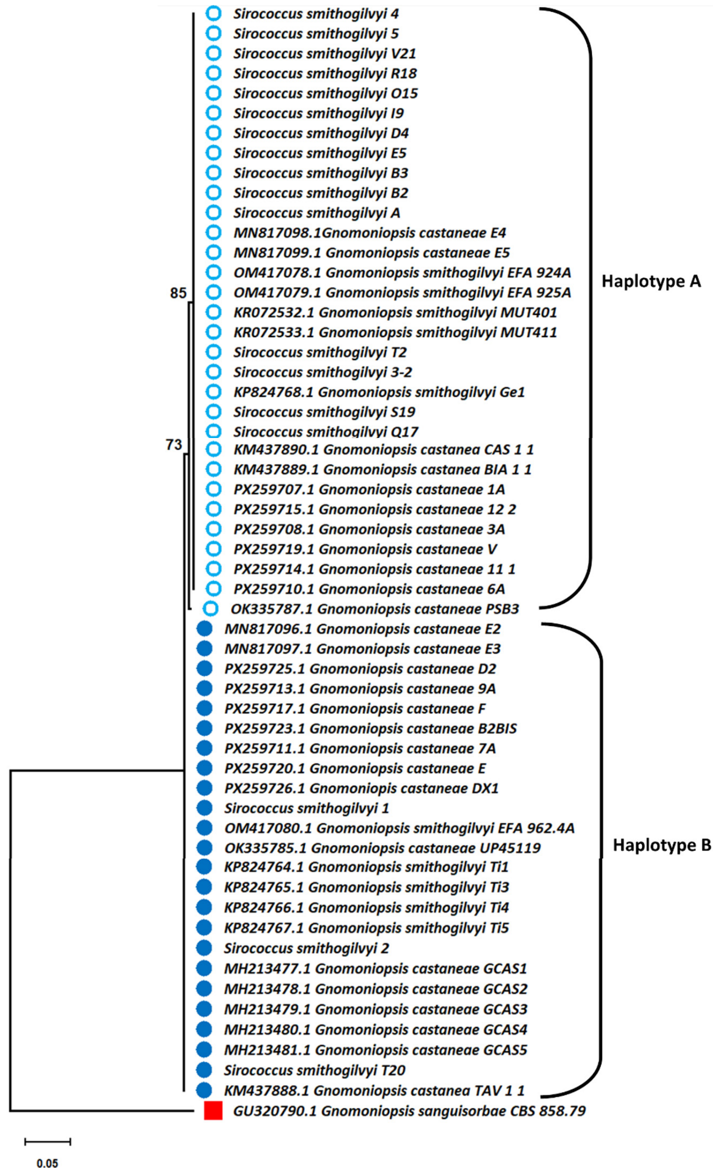

Figure 2 Maximum Likelihood tree based on *S. smithogilvyi β-tubulin* sequences from isolates of diverse geographical origin. The tree is drawn to scale, and the percentages of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) when equal to or greater than 70% (≥70) are shown above the branches. Haplotypes A of *S. smithogilvyi* are indicated by light blue empty circles and the B haplotypes of the same fungus by medium blue circles. The strain CBS 858.79 of *S. sanguisorbae* (syn. *Gnomoniopsis sanguisorbae)* was used as an outgroup (red square). Scale bar represents nucleotide changes/site. Evolutionary analyses were conducted in MEGA12 software.

Looking at the virulence of the haplotypes, majority of *Ss* isolates from Basilicata region belonged to haplotype A which was reported to be more virulent than haplotype B. Interestingly, analysing the distribution of *Ss* haplotypes in Italy, an increasing trend of haplotype A (more virulent) occurrence and a decrease presence of haplotype B (less virulent) from North to South can be observed (Table 3). However, an exception should be pointed out for Sardinia, where a bit higher percentage of haplotype B (23%) and a slightly less of haplotype A (77%) occurrence was reported (Table 3).

Table 3 Percentage of haplotypes A and B of *S. smithogilvyi* found in Basilicata compared with other Italian regions.

Region	No. of isolates	Haplotype A (%) *	Haplotype B (%) *	Reference
Piedmont	5	0	100	Seddaiu et al. 2023
Trentino Alto Adige	6	33	67	Seddaiu et al. 2023
Veneto	32	41	59	Seddaiu et al. 2023
Lazio	45	85	15	Seddaiu et al. 2023
Basilicata	**46**	**86**	**14**	**This study**
Sardinia	19	77	23	Seddaiu et al. 2023

Note: * = Distribution trends of *S. smithogilvyi* haplotypes noticed across Italy are based on data from the present study and previous reference. Data obtained in this study are shown in Bold and highlighted in gray.

Discussion

Outcomes from the present study are in accordance with those recently reported by Seddaiu et al. (2023) which isolated mainly *S. smithogilvyi* (syn. *G. smithogilvyi*) from the chestnut tissues with chestnut brown rot (CBR) symptoms and also few similar fungal species as those found in our study along with others (e.g. *Cryphonectria* sp., *Rutstroemia echinophila*, and *Talaromyces* sp.) which were not detected in the investigated chestnut nuts from Basilicata region. In addition, comparable results were also obtained by Carloni et al. (2026) which reported the isolation of several fungal taxa where *S. smithogilvyi* comprised the majority of the total fungi isolated (68.52%) while few other taxa such as *Fusarium* sp. (9.12%), *Aspergillus* sp. (7.41%), *Alternaria* sp. (6.1%), *Penicillium* sp. (4.85%) and *Thrichoderma* sp. (4,13%) have been less frequently isolated from CBR affected chestnuts being also associated with high disease severity classes (4 or 5).

CBR caused by *S. smithogilvyi*, has recently emerged as an important disease affecting chestnut production in Europe and Oceania (Dobry & Campbell 2022) including several countries such as Italy (Lione et al. 2019, Seddaiu et al. 2023, Mang et al. 2024, Carloni et al. 2026), Ireland (O'Loinsigh et al. 2022), Portugal (Coelho & Gouveia 2021, Lema et al.2023, Possamai et al. 2023), Greece (Tziros, 2019), United Kingdom (Lewis et al. 2017) but also the North and the South of the U.S.A. (Sakalidis et al. 2019, Cisterna-Oyarce et al. 2022).

Different approaches like morphological, molecular and phylogenetic ones based on at least two routine barcodes (ITS and *tub2*) should be used to precisely identify the causal agent of CBR (Silva-Campos et al. 2022, Mang et al. 2024) and they were both utilized in this work.

The presence and distribution of CBR disease is reported in the present study in chestnut nuts of "Marroncino di Melfi" variety and in those of the local ecotype originated from three main chestnut locations Melfi, Rionero in Vulture and Lagonegro from Basilicata region (Southern Italy). These findings confirm that *S. smithogilvyi* is rather widely distributed throughout Italy, from north to south. Outcomes from this study also match those of Dennert et al. (2015) reporting the presence, in Switzerland of two haplotypes of *G. castanaea* based on *β-tubulin* gene alone. Although, both Seddaiu et al. (2023) and Carloni et al. (2026) performed broad studies on the phylogeographic diversity of *Ss* (*Gc*) isolates in Italy still a complete information about haplotypes distribution and diversity in Southern regions of Italy was missing. Therefore, the outcomes of the present study successfully filled the gap of knowledge in this regard furnishing novel and key information about *Ss* haplotypes occurrence, types and frequency in a previously unstudied southern Italian region.

In particular, findings from our study suggest the existence of both *Ss* genetic lineages (previously named *Gc* haplotypes A and B) and also showed that genetic diversity of this fungal pathogen in Basilicata region is quite similar to that previously reported for Lazio region (Central Italy) by Seddaiu et al. (2023). Carloni et al. (2026) reported

that *Ss* (*Gc)* A haplotype was recorded from isolates originated from Campania, Marche and Emilia-Romagna and Sardinia while B haplotype isolates of Ss (*Gc)* were reported from Piedmont, Lombardy and Emilia-Romagna and Sardinia.

Considering that only few studies (Seddaiu et al. 2023, Carloni et al. 2026) investigated the Ss haplotypes variation and distribution in Italy the statement regarding the existence of a latitudinal cline, with *Ss* A haplotypes increasing southward and *Ss* B haplotypes increasing northward, still needs to be further confirmed by additional data. Above all, those originating from Southern Italian chestnut-growing regions like Campania, Basilicata, Calabria and Sicily will certainly expand the current knowledge on the geographic variation and distribution of Ss haplotypes in the country. In addition, previous data on haplotypes occurrence and distribution in other Italian regions suggests that environmental factors or historical host displacement may drive lineage selection (Seddaiu et al. 2023).

Knowledge of *S. smithogilvyi* genetics is relevant since CBR disease caused by this pathogen can even reach an incidence of 80% severely affecting trees health and chestnut production (Bastianelli et al. 2025).

Besides, strategies to control CBR disease are under research including the *in vitro* and in filed efficacity investigation of biologically-based fungicides (Bastianelli et al. 2025, Battaglia et al. 2025). In this perspective, integrated management strategies comprising phytosanitary practices, pruning, removal of infected plant material along with the application of biological and chemical treatments had been assessed under laboratory and field conditions for the containment the *S. smitholgilvyi* pathogen in chestnut orchards in Campania region (Battaglia et al. 2025). The authors, reported a 6% reduction *S. smitholgilvyi* incidence (even under relatively low disease pressure) in chestnut trees treated with copper oxychloride, Eugenol + Geraniol + Thymol, Tetraconazole and Fenhexamid applied in field demonstrating their potential in disease mitigation associated to proper agronomical practices and sound orchard management (Battaglia et al. 2025).

Direct chemical approach to control CBR disease is not advisable due to environmental concerns and significant technical challenges. To maintain a low disease incidence in chestnut groves it is good practice to keep the foliage in good condition, ventilated and it is very important to collect and burn the urchins and chestnuts leftovers (Lizotte et al. 2025; Miller & Lewis Ivey, 2025).

Promising results in CBR disease control have been obtained experimentally with *Trichoderma atroviride* alone or in combination to *B. amyloliquefaciens,* which appear to be potential candidates for the biological control against *S. smithogilvyi* (Pasche et al. 2016, Benigno et al. 2024). Additionally, ozone treatment of chestnuts appears to have a fungistatic effect capable of containing pathogen development (Vettraino et al. 2021). Predictive models had been developed that can estimate the incidence of *S. smithogilvyi* (Lione et al. 2016). However, according to current knowledge different chestnut varieties, breeding methods, and management practices can affect the disease (Dobry & Campbell 2023, Lema et al. 2023).

It is known that *S. smithogilvyi* is able to live as an endophyte without causing any symptoms and the mechanisms that determine the transition from endophyte to pathogen are still unknown (Dobry & Campbell 2023). High temperatures may facilitate the transition of the fungus from an endophyte to a pathogen (Lione et al. 2016, Vannini et al. 2017, Topalidou et al. 2024, Carloni et al. 2026). This transition may be explained by the hypothesis that high temperatures trigger metabolic and functional changes in the fungus, increasing its ability to spread spores and colonize tissues (Possamai et al. 2023). Furthermore, exposure to drought periods can stress plants, making them more receptive to *S. smithogilvyi* and additionally endophyte inoculation can trigger disease-like symptoms (Shultz et al. 1998, Carloni et al. 2026).

Another important aspect concerns the relationship between the Asian chestnut gall wasp ACGW *Dryocosmus kuriphilus,*

Yasumatsu (Hymenoptera: Cynipidae) and CBR epidemiology. It had been already established that ACGW can act as mechanical and ecological CBR vector and ACGW galls are proper micro-environments for the development and spread of *S. smithogilvyi* along with other fungal taxa (Seddaiu et al. 2017, Vannini et al. 2017, Morales-Rodriguez et al. 2019, Dobry & Campbell 2023, Mang et al. 2024). However, further detailed research focusing on vector competence or on modelling temporal correlations between ACGW, necrosis of galls and *S. smithogilvyi* spores release is still required to fully elucidate the potential role played by the ACGW in the transmission of the CBR disease and to find preventive measures for its control.

Conclusions

After the damaging chestnut blight caused by *C. parasitica* and the destructive effects of the ACGW, a new threat strikes chestnut in Basilicata which risks frustrating the efforts made to relaunch chestnut cultivation in the region.

This is the first study which looked into the haplotype diversity of *S. smithogilvyi* (causal agent of the CBR disease) in the region providing new and relevant information about the occurrence of both A and B types of *S. smithogilvyi* evolutionary lineages in Basilicata (Southern Italy).

In addition, further studies should aim to verify the behaviour of different chestnut varieties towards the *S. smithogilvyi* through artificial inoculations using different pathogen strains, exploring different *S. smithogilvyi* strains pathogenicity and virulence and hopping to find at least few tolerant or less susceptible chestnut local varieties or hybrids to be used to restore orchards affected by CBR disease and to be employed in the upcoming breeding programs.

Overall, findings from this study highlight the need for further and more extended research on the ecology (mainly focusing on mechanisms that trigger its shift from innocuous endophyte to destructive pathogen), epidemiology (concentrated also on evaluating climate effects on disease incidence) and population biology of *S. smithogilvyi* to support the development of sustainable disease-management strategies and protect chestnut production in the region.

Conflict of interest

The authors have no financial or personal conflict of interest to declare from the work presented in the present paper.

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Data availability

Data of the outcomes from the present study are included in the manuscript, and may be provided upon reasonable request from the corresponding author. All nucleotide sequences for the two fungal barcodes from in this study were deposited in the NCBI GenBank under the accession numbers reported in Table 2.

Author contribution statement

Conceptualization: S.M.M. and I.C; methodology: S.M.M., G.M., I.C. and C.M.; software: S.M.M.; validation: S.M.M., I.C., A.C., G.M., and C.M.; formal analysis: S.M.M.; investigation: S.M.M.; resources: S.M.M., I.C. and C.M.; data curation: S.M.M., C.M. and I.C.; writing-original draft preparation: S.M.M.; writing-review and editing: S.M.M., I.C., A.C., C.M. and G.M.; visualization: S.M.M.; funding acquisition: I.C and G.M. All authors have read and agreed to the published version of the manuscript.

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