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Fungal diversity in chestnut galls induced by *Dryocosmus kuriphilus* from Basilicata Region (Southern Italy)

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Abstract In recent years, the Asian chestnut gall wasp (ACGW) *Dryocosmus kuriphilus* has been reported to have a high incidence in Italy and other Mediterranean basin countries. In 2021-2022, a study was undertaken in the Basilicata Region (Southern Italy) to investigate the relationship between the galls produced by ACGW on sweet chestnut (*Castanea sativa* Mill.) and fungal pathogens. In particular, the fungal diversity from green and necrotic galls collected from two important sweet chestnut sites (Melfi and Rionero in Vulture) was investigated. Nineteen fungal taxa were identified based on their morphological and molecular traits. In both localities, the most frequent species isolated from green and necrotic galls were *Gnomoniopsis castaneae*, *Colletotrichum acutatum*, and *Pestalotiopsis* sp. It is essential to understand the role played by the galls as an inoculum source for sweet chestnut fungal pathogens, particularly for *G. castaneae*, an emerging pathogen of which biology is still poorly understood. Findings from the present study stressed that the complex relationship between host-insect-microbial community needs to be elucidated to be able to control the pathogenic fungi and consequently maintain sweet chestnut trees' health as they play a key role in the local agriculture (horticulture, forestry) and subsidiary economy.

Keywords: *Dryocosmus kuriphilus*, *Castanea sativa*, forest pathogens, endophyte fungi.

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Introduction

The sweet chestnut (*Castanea sativa* Mill.) trees are attacked by several fungal pathogens and pests, which damage their health and dramatically reduce their production. The most frequent pathogens reported for sweet chestnut are *Cryphonectria parasitica* Murrill. M.E. Barr, the causal invasive agent of chestnut blight (Chira et al. 2017, Rigling & Prospero 2018), *Phytophthora cinnamomi* Rands and *P. cambivora* [(Petri) Buisman], causing root rot (Cristinzio 1986, Camisón et al. 2019, Zhebentyayeva et al. 2019, Marzocchi et al., 2024) and, among the pests, the Asian chestnut gall wasp (ACGW) [*Dryocosmus kuriphilus*

Yasumatsu (Hymenoptera: Cynipidae)] (Brussino et al. 2002, Sartor et al. 2015, Cśoka et al. 2017, Seddaiu et al. 2017).

The ACGW is a quarantine pest, included in the A2 list of the European and Mediterranean Plant Protection Organization (EPPO 2015), and it was reported in Italian chestnut orchards from 2010 (Bosio et al. 2010). In spring, during the bud break period, the larval growth process induces stem, petiole and galls formation, which disturbs both the normal growth of the shoots and also modifies fruit formation (Kato & Hijii 1997, Turchetti et al. 2012, Seddaiu et al. 2017). Furthermore, the ACGW attack weakens the chestnut trees making them more susceptible to the cortical canker attack by facilitating the inoculation and penetration of *C. parasitica* into the dormant buds (Vannini et al. 2017, Morales-Rodriguez et al. 2019). Also, the *D. kuriphilus* outbreak indirectly induces modifications in the chestnut ecosystem by altering the densities and host niches of the natural parasitoids and determining variations in the chestnut tree microbial communities (Panzavolta et al. 2013, Fernandez-Conradi et al. 2019, Vannini et al. 2017, Morales-Rodriguez et al. 2019, Chira et al. 2021).

Attempts to control this damaging pest, including those of the parasitoid *Torymus sinensis* Kamijo use, have been proven to be effective for both the medium and long term (Balsa et al. 2021), but the use of non-native species may pose several negative impacts on parasitoid community (Quacchia et al. 2008, Muñoz-Adalia et al. 2019). In this context, the search for other control tools against the ACGW attack is of critical importance, and alternative perspectives offered by the endophytic fungi seem attractive as they were already had been applied successfully as biocontrol agents (Tosi et al. 2015, Seddaiu et al. 2017, Muñoz-Adalia et al. 2019).

Evidence about the presence and role of fungi in green and necrotic chestnut galls was reported by frequent isolation and molecular detection of *Gnomoniopsis castaneae* Tamietti (syn. *G. smithogilvy*i L. A. Shuttlew., E.C.Y. Liew & D.I. Guest) (Lione et al. 2016, Seddaiu et al. 2017, Fernandez-Conradi et al. 2019, Morales-Rodriguez et al. 2019, Muñoz-Adalia et al. 2019). This fungus, an emerging pathogen causing brown rot in chestnut tissues, was described as a severe threat to the sweet chestnut trees in many countries worldwide, particularly in Switzerland and India (Dar & Rai 2015, Meyer et al. 2017), Italy (Gentile et al. 2010, Visentin et al. 2012, Maresi et al. 2013, Seddaiu et al. 2017), in United Kingdom (Lewis et al. 2017), and in Spain (Trapiello et al. 2018). The disease spread is ongoing and recently was reported in Greece (Tziros 2019), in Portugal (Possamai 2020, Coelho & Gouveia 2021), in Ireland (O'Loinsigh et al. 2022), and in Chile (Cisterna-Oyarce et al. 2022).

Despite its continuous discovery in many countries, the origin of the disease is still unknown (Dobry & Campbell 2023), and the damage produced by this pathogen on chestnuts could reach higher levels, up to 70% (Gehring et al. 2018). The infected chestnuts look healthy, showing no external symptoms. However, they crack after a light pressure and float on the surface if immersed in water. Only after splitting the chestnuts the disease-specific symptoms could be observed by the presence of a more or less dark endosperm (from which the name of the disease derives) based on the infection stage and on chalky texture.

Over the years, the species' name was several times changed, first being called *G. castaneae* Tamietti then, being changed to *G. smithogilvyi* L.A. Shuttleworth, E.C.Y. Liew & D.I. Guest, and finally, according to Index Fungorum and Global Biodiversity Information Facility (GBIF) being accepted, as *G. castaneae* (Tamietti 2016, Dobry & Campbell 2023). The pathogen was reported in Italy in 2013, in Piedmont, Tuscany and Trentino (Maresi et al. 2013), even if earlier studies of Magro et al. (2010) stated the relationship between *Gnomoniopsis* and the necrosis of chestnut leaves and galls induced by *D. kuriphilus* and Visentin et al. (2012) reported and described the *G. castanea* sp. nov. (Gnomoniaceae, Diaporthales) as a causal agent of nut rot in sweet chestnut. At

present, *G. castanea* is spread in many chestnut areas over the Italian peninsula.

Recent studies showed that the fungus is also present in the Basilicata region (unpublished data). *G. castaneae* is a pathogen and an endophyte (Visentin et al. 2012, Maresi et al. 2013, Shuttleworth et al. 2013, Dennert et al. 2015, Pasche et al. 2016, Seddaiu et al. 2017). This fungus is the causal agent of the chestnut nut rot disease and was also reported to be associated with cankers in both chestnut and hazelnut and necrosis on chestnut galls and leaves (Lione et al. 2019). It is possible that cankers, thought to be caused by *C. parasitica* on chestnut, can be caused by *G. castaneae* or by *Sirococcus castaneae* (Prill. & Delacr.) J.B. Meyer Senn-Irlet & T.N. Sieber (Meyer et al. 2017), frequently isolated from them (Pasche et al. 2016).

The incidence of *G. castaneae* attack seems to be influenced by temperature and not by rainfall or chestnut culture density, and it could be estimated locally by mathematical models (Lione et al. 2015). In the European central Mediterranean region, two subpopulations of *G. castaneae* and two haplotypes, A and B, with different virulence and distribution, had been reported (Sillo et al. 2017, Seddaiu et al. 2023).

The present study was carried out to verify the possible role played by ACGW galls in the conservation and dispersion of *G. castaneae* and also to identify eventually other taxa present in galls sampled in two chestnut sites in Basilicata Region (Southern Italy) to find a possible strategy to control *G. castaneae* infections. The general objectives of the present study were i) to identify the taxa associated with green and necrotic chestnut galls produced by *D. kuriphilus* and ii) to characterize the taxa isolated from chestnut galls for their possible uses in biocontrol of *D. kuriphilus*.

Materials and Methods

Investigated sites and sampling

Surveys were performed during 2021-2022 between May and October in two sites, Melfi (S1) and Rionero in Vulture (S2) (Basilicata Region, Southern Italy), aiming to check the phytosanitary status of the chestnut trees. A total of 299 galls were collected: 105 green (75 from Melfi and 30 from Rionero in Vulture) and 194 necrotic (151 from Melfi and 43 from Rionero in Vulture) and considered for further investigations. Climatic data of the investigated sites is shown in Table 1.

Table 1 Climatic and geographical data of sampled sites.

Features	S1 - Melfi		S2 - Rionero in Vulture	
Geographic coordinates	40°59'51"00 N 15°39'7"92 E		40°55'36"12 N 15°40'14"88 E	
Height (m.a.s.l.*)	530		656	
Morphology	Hill		Hill	
Year	2021	2022	2021	2022
Annual rainfall (mm)	1647.8	814.6	1617.3	925.9
Summer rainfall (mm)**	604.2	433.3	646.2	593.6
Mean temperature (°C)	13.81	14.82	13.13	14.45
Maximal temperature (°C)	18.34	19.74	18.37	19.68
Minimal temperature (°C)	9.29	9.88	9.02	9.22
Summer mean temp (°C)	19.92	21.46	19.90	20.33
Summer maximal temp (°C)	25.42	27.37	25.52	26.14
Summer minimal temp (°C)	14.45	15.56	14.29	14.52

Notes: (*) m.a.s.l.= meters above sea level; (**) summer values correspond to May-October. The data presented are the maximum and minimum average temperatures (temp). Source of data: 3Bmeteo (https://3bmeteo.com/meteo/storico).

Fungi isolation and morphological analysis

For fungal isolations, all necrotic and green galls were surface sterilized with ethanol 70% for 5 min followed by immersion for 5 min in sodium hypochlorite 50% and then for 5 min in ethanol 70%, 2-3 times rinsed with distilled water, dried on sterile paper and finally portions of galls tissues were directly placed on Potato Dextrose Agar (PDA) medium amended with ampicillin and streptomycin (40 mg L^{-1}, MerckKGaA, Darmstadt, Germany). Representative samples for each growing fungal colony were subsequently 2-3 sub-cultured on the same medium to obtain pure cultures (PFCs) for morphological and molecular identification, according to the protocol described by Mang et al. (2017, 2020). All PFCs from this study were stored at 4°C in the fungal collection of the Plant Pathology Laboratory (PPL), University of Basilicata, Potenza, Italy. Three replicates of each PFC, grown on PDA and incubated in the dark at 24°C for 7-10 days, were used for morphological identification.

Macroscopic examinations of colony morphology and microscopic observations using an optical microscope model Nikon Eclipse Ei 104c (Nikon, Japan) equipped with Digital Slight DS1000 camera (Nikon, Japan) were performed and appropriate literature sources (Moore & Korf 1963, Ellis & Waller 1975, Jarvis 1977, Jarvis 1980, Domsch et al. 1982, Bell 1983, Burdsall 1985, Melo & Ryvarden 1989, Boerema 1993, Boerema et al. 2004, Jeewon et al. 2004, Leslie et al. 2006, Simmons 2007, Maharachchikumbura et al. 2011, Bensch et al. 2012, Damm et al. 2012, Shuttleworth & Guest 2012, Visentin et al. 2012, Yang et al. 2012, Phillips et al. 2013, Maharachchikumbura et al. 2014, Visagie et al. 2014, Floudas & Hibbett 2015, Shuttleworth et al. 2015, Tamietti 2016, Chen et al. 2017, Meyer et al. 2017, Senanayake et al. 2017, Xu et al. 2020, O'Donnell et al. 2021, Cai et al. 2022, Infantino et al. 2023) and the nomenclature of Index Fungorum and GBIF were considered for fungal identification at least at genus level.

Molecular analysis

Total DNA was extracted from PFCs mycelium using the Macherey-Nagel kit (GmbH & Co KG, Düren, Germany) following manufacturer's instructions with minor modifications (Camele et al. 2005, Mentana et al. 2019). DNA quality and quantity were verified by readings at the Nanodrop ND-1000 spectrophotometer (Wilmington, Delaware, U.S.A.) and the ITS5(5'-GGAAGTAAAAGTCGTAACAAGG-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3') primers (White et al. 1990) were used to amplify by PCR the ITS1, 5.8S and ITS2 regions of the nuclear rDNA as described in a previous study by Mang et al. (2017; 2022). PCR results were checked by gel electrophoresis ran at 80V for 30 min on 1.2% agarose gel stained with 4.5µl of SYBR Safe DNA Gel Stain (Invitrogen Inc., USA). Amplification products were purified by QIAquick PCR purification Kit (Qiagen, Hilden, Germany) and directly sequenced, in both directions, by BMR Genomics (Padua, Italy) using the same primers as for the PCR. Molecular identification of ITS nucleotide sequences obtained in this study was done by querying online the National Centre for Biotechnology Information (NCBI) GenBank database using the nucleotide Basic Local Alignment Search Tool (BLAST, Altschul et al. 1997) (https://blast.ncbi.nlm.nih.gov) search option BLASTn available into the same database. Further sequence similarity analyses, including Pairwise and Multiple Sequence Alignments, were performed in the MEGA11 phylogenetic package (Tamura et al. 2021) using the ClustalW software (Larkin et al. 2007, Kumar et al. 2018). The abundance percentage (Ab%) of the identified taxa was estimated by calculating the rate of a specific taxa from the total percentage of the taxa isolated.

Phylogenetic analysis

Phylogenetic analyses were performed using the MEGA11 software (Tamura et al. 2021). The evolutionary history was inferred using the Neighbor-Joining method (Saitou & Nei 1987). The optimal tree is shown. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown above the branches (Felsenstein 1985). The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Jukes-Cantor method (Juke & Cantor 1969) and are in the units of the number of nucleotide base substitutions per site. Codon positions included were 1st+2nd+3rd+Noncoding. All ambiguous positions were removed for each sequence pair (pairwise deletion option). In addition, ITS nucleotide sequences representative of other similar fungal species (first selection criterion) retrieved from the NCBI database were used to confirm the identity of the investigated fungi (Table 2). Their second selection criterion was to originate from chestnut ACGW galls (this was done when they were available if not other similar sequences despite their origin were considered).

Statistical analysis

Common statistics (analysis of variance; 95% confidence level) were used to analyze the dominant species and their relationship with the type of galls (green vs. necrotic) in the two zones.

Results

Fungi isolation and morphological analysis

Fungal isolations on the PDA medium yielded 299 PFCs. After morphological analyses, these had been identified at least at the genus level (Figure 1).

Molecular characterisation of the fungi

Molecular analyses of the pure fungal cultures DNA based on the ITS region, after direct sequencing and bioinformatic analysis, permitted the identification of most of them at the species level (Figure 2). A total of 226 fungal colonies from the first site and 73 fungal colonies from the second site have been isolated and molecularly identified.

Significant differences were found among the 19 identified taxon inhabiting the galls in both sites. From the 75 green galls originating from Melfi, 12 fungal genera had been identified. In particular, the most frequently identified genera were *Gnomoniopsis* (**dominant), *Fusarium* (*codominant), *Colletotrichum, Trichoderma*, and *Cladosporium,* while the others were isolated with frequencies less than 6%. From the 151 necrotic galls investigated, 11 fungal genera had been identified. The most often isolated were *Gnomoniopsis* (**), *Colletotrichum, Fusarium, Trichoderma*, and *Pyronema* while the other fungal taxa were found

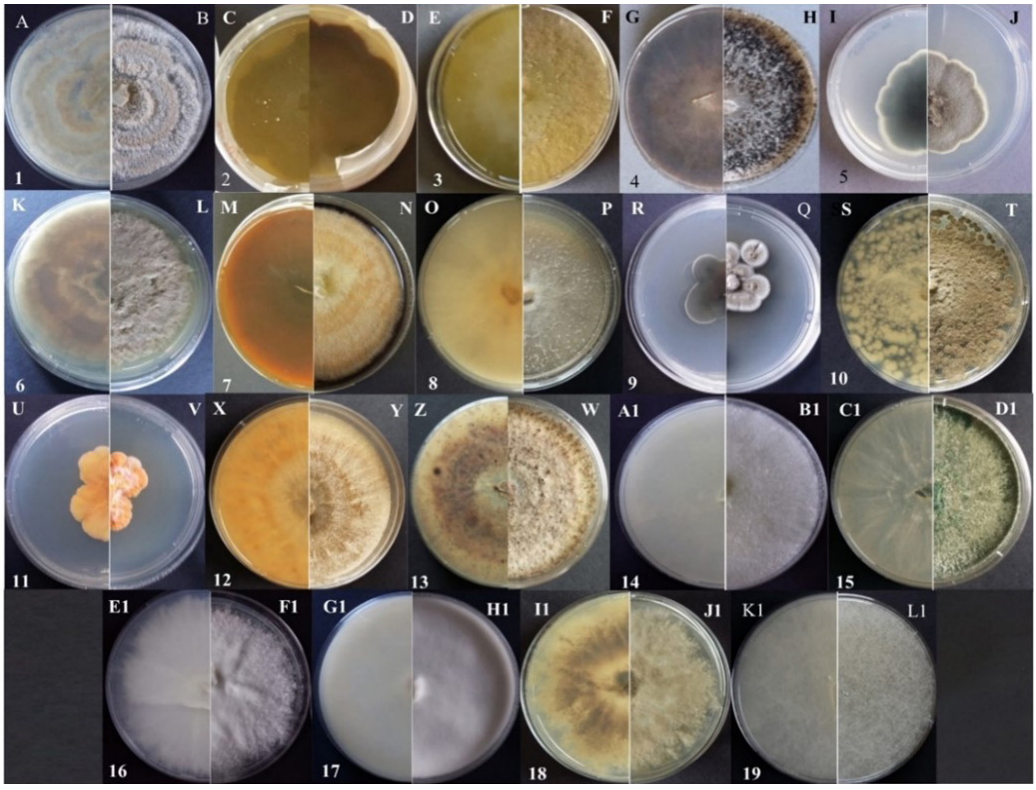

Figure 1 Fungi isolated from sweet chestnut galls. Colonies on PDA - front and reverse: 1 = *Gnomoniopsis castanea*; 2 = *Alternaria* sp.; 3 = *Botryosphaeria dothidea*; 4 = *Sordaria fimicola*; 5 = *Cladosporium cladosporioides*; 6 = *Colletotrichum acutatum*; 7 = *Epicoccum nigrum*; 8 = *Fusarium* sp.; 9 = *Phoma* sp.; 10 = *Penicillium* sp.; 11 = *Fusarium lateritium*; 12 = *Pestalotiopsis* sp.; 13 = *Sirococcus castaneae*; 14 = *Phanerochaete livescens*; 15 = *Trichoderma* sp.; 16 = *Pyronema domesticum*; 17 = *Pilatoporus ibericus*; 18 = *Botrytris cinerea*; 19 = *Mucor* sp.

with frequencies less than 5%.

A total of 10 fungal genera were detected from the 30 green galls, and 8 taxa from 43 necrotic galls originated from Rionero in Vulture. The most frequent genera were *Gnomoniopsis* (dominant in S1 and S2), *Colletotrichum* (codominant), *Sirococcus* (dominant in S2, codominant in S1), and *Pestalotiopsis*, while the others were found with less than 6% frequency (Figures 2 and 3).

Regarding the distribution of the taxa isolated from S1 and S2, it can be observed that some fungal taxa like *G. castaneae*, *C. acutatum*, *Pestalotiopsis* sp. and *Phanerocheate livescens* were detected in both sites, both green and necrotic galls. Other fungal taxa were site-specific: *Fusarium* sp., *Penicillium*

Table 2 Nucleotide sequences of the ITS barcode taken from the NCBI GenBank and used in this study for the phylogenetic analysis.

Taxon	Strain / Isolate / Clone	GenBank Acc. No.
Gnomoniopsis castaneae	TT2016	MF540695
Gnomoniopsis castaneae	360T	MH257580
Alternaria sp.	JP31	OQ297081
Alternaria sp.	Pw-10.1A(3156)JM	OM363497
Sordaria fimicola	Gall 32	KT823797
Sordaria fimicola	CBS 398.63	MH858315
Cladosporium cladosporioides	V-19-5-3	MG888624
Cladosporium cladosporioides	I5 4	LR778221
Colletotrichum acutatum	Gall 7a	KT823766
Colletotrichum acutatum	Gall 7b	KT823767
Colletotrichum acutatum	FEM Ac1	KP064131
Colletotrichum acutatum	V24-2	MG888626
Epicoccum nigrum	Gall 9	KT823769
Epicoccum nigrum	Cas 5 Agalla 2	KU095870
Epicoccum nigrum	Cas 5 Agalla 9	KU095873
Fusarium sp.	Cas 5 Agalla 11	KU095878
Fusarium sp.	PG-145.1-G(44)JM	OM345232
Phoma aliena	Gall 28	KT823793
Phoma herbarum	V8-1	MG888615
Penicillium sp.	Gall 25	KT823790
Penicillium sp.	mcp2526	JN983438
Penicillium sp.	mcp2528	JN983439
Penicillium sp.	1-15ITS1F	KF530744
Fusarium lateritium	V2-1	MG888631
Fusarium lateritium	CBS:165.57	MH857682
Pestalotiospsis sp.	AG108	MG888616
Pestalotiospsis sp.	Cas 5 Agalla 2	KU095868
Sirococcus castaneae	Dca40	KX929736
Sirococcus castaneae	Dca60	KX929737
Sirococcus castaneae	Dca105	KX929758
Sirococcus castaneae	V11-1	MG888629
Phanerochaete livescens	LE RUS:286904	KP994361
Phanerochaete livescens	459E	MZ078483
Trichoderma harzianum	Gall 37	KT823802
Trichoderma harzianum	SF 128	MT529404
Trichoderma atroviridae	C405II	MG888611
Trichoderma atroviridae	AG101	MG888612
Pyronema domesticum	NWFVA4757	MT790324
Pyronema domesticum	NW-FVA2396	MG098284
Pilatoporus ibericus	X1367	KC5959530
Pilatoporus ibercus	LE-BIN 3922	MG722742
Mucor hiemalis	Gall 21	KT823784
Mucor hiemalis	B4	LN714573

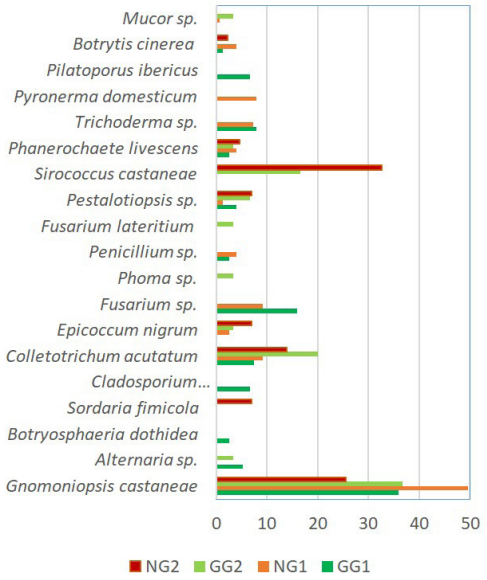

Figure 2 Fungal taxa identified with molecular analyses in chestnut galls from the Basilicata region. GG1/2 = green galls from site 1/2; NG1/2 = necrotic galls from site 1/2.

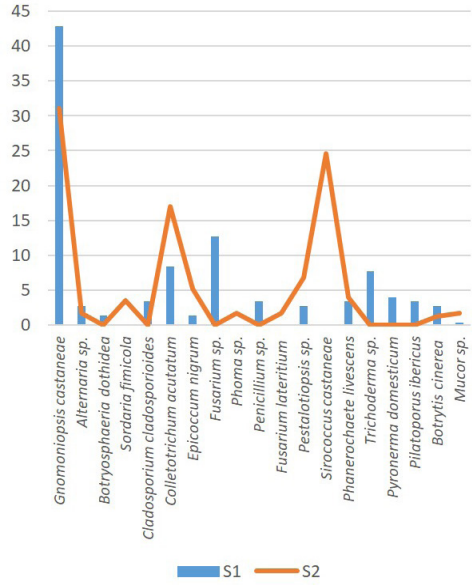

Figure 3 Distribution of fungal taxa isolated from chestnut galls between the two investigated sites S1: Melfi and S2: Rionero in Vulture.

sp., *Trichoderma* sp. and *Pilatoporus ibericus* were specific to site 1, being found in both types of galls (Figure 3). *Sirococcus castaneae* was isolated only from green and necrotic galls originated from site 2 (Figure 3). Also, *S. fimicola* was found only in necrotic galls, and *F. lateritium* and *Phoma* sp. were isolated from green galls only from site 2 (Figure 3).

Furthermore, different types of fungi were found in green and necrotic chestnut galls, and details of their life strategies are presented in Table 3. It was interesting to observe that, only a few of them have been reported to be efficient in controlling *D. kuriphilus* or to have some influence on this gall-wasp, while others proved to affect different gall-wasp insects or to have a relationship with ACGW (Table 3).

Based on morphological features, all fungal species were preliminarily identified, at least at the genus level, and the fungal species were confirmed after direct sequencing of the ITS region. The genetic variability of the fungal isolates was verified through multiple sequence alignments with reference species from the NCBI GenBank database. Sequence alignment of the ITS region had 525 bp length. Out of them, 63 sites were constant, 462 were variable, 461 were parsimony-informative, and one singleton site was observed. All PFCs showed 99% to 100% similarity across all the isolates within the same species, suggesting that they belong to

Table 3 Type of fungi found in chestnut galls and their potential to control *Dryocosmus kuriphilus*.

Nr	Fungal taxa	Lifestyle							Ref.*	Dk Control Potential	
		A	En	L	Ep	P	Y	Ss	W			
1	*Gnomoniopsis castaneae*									Yes	xx	Magro et al. 2010, Vannini et al. 2017
2	*Alternaria* sp.	x	x					x		Yes	x	Addario & Turchetti 2011
3	*Botryosphaeria dothidea*		x							Yes	?	Zimowska et al. 2020
4	*Sordaria fimicola*									Yes		
5	*Cladosporium cladosporioides*	x						x		Yes		Gaffuri 2018, Muñoz-Adalia et al. 2019, Yang et al. 2021
6	*Colletotrichum acutatum*	x								Yes	x	Graziosi & Rieske 2015
7	*Epicoccum nigrum*									Yes	?	Nicoletti et al. 2021, Morales-Rodrigues et al. 2019
8	*Fusarium* sp.									Yes	x(x)	Addario & Turchetti 2011, Tosi et al. 2015
9	*Phoma* sp.									Yes		
10	*Penicillium* sp.	x	x					x		Yes	?	Nicoletti et al. 2021
11	*Fusarium lateritium*							x		Yes	?	Nicoletti et al. 2021
12	*Pestalotiopsis* sp.	x	x					x		Yes	?	Lv et al. 2011
13	*Sirococcus castaneae*		x							Yes		
14	***Phanerochaete livescens***							x		**No**		
15	*Trichoderma* sp.							x		Yes	?	Benigno et al. 2024
16	***Pyronerma domesticum***									**No**		
17	***Pilatoporus ibericus***									**No**		
18	*Botrytis cinerea*		x					x		Yes	x	Addario & Turchetti 2011
19	*Mucor* sp.		x							Yes		

Notes: **Lifestyle** (fungi life strategy): A = animal pathogen; En = endophyte; L = parasite of lichens; Ep = epiphyte; P = plant pathogen; Y = yeast; Ss = saprotroph; W = wood decomposer. **Ref*** (References): fungus presence (Yes: previously reported; No: unreported on chestnut) on chestnut was reported by the following references: *G. castaneae* = Vannini et al. 2017, Seddaiu et al. 2017, Morales-Rodriguez et al. 2019, Fernandez-Conradi et al. 2019, and Muñoz-Adalia et al. 2019. *Alternaria* sp. = Meyer et al. 2015 and Fernandez-Conradi et al. 2019. *Botryosphaeria dothidea* and *Sordaria* sp. = Fernandez-Conradi et al. 2019. *Sordaria fimicola* = Meyer et al. 2015. *Cladosporium cladosporioides* = Muñoz-Adalia et al. 2019. *Colletotrichum acutatum* = Gaffuri et al., 2015; Gaffuri et al., 2018; Morales-Rodriguez et al. 2019 and Muñoz-Adalia et al. 2019. *Epicoccum nigrum* = Morales-Rodriguez et al. 2019 and Muñoz-Adalia et al. 2019. *Fusarium* sp. = Muñoz-Adalia et al. 2019. *Phoma herbarum* = Muñoz-Adalia et al. 2019 and *Phoma aliena* = Meyer et al. 2015. *Penicillium* (different species) = Morales-Rodriguez et al. 2019 and Muñoz-Adalia et al. 2019. *Fusarium lateritium* = Muñoz-Adalia et al. 2019 and Fernandez-Conradi et al. 2019. *Pestalotiopsis* sp. = Muñoz-Adalia et al. 2019. *Sirococcus castaneae* and *Trichoderma* sp. = Muñoz-Adalia et al. 2019. *Botrytis cinerea* = Morales-Rodriguez et al. 2019. *Mucor* sp. = Muñoz-Adalia et al. 2019. **DkControl potential** (potential for *D. kuriphilus* control): xx=proved to be efficient on *D. kuriphilus*, x=proved to have some influence on *D. kuriphilus*;?=proved to other gall-wasps or insects and/or supposed to have a relationship with ACGW.

the same fungal species. In particular, outcomes of BLAST analysis of the 71 representative ITS sequences showed both very high similarities (from 99 to 100%, which was above the threshold level for species determination) and a very high query coverage (>99%) with similar fungal species (Table 2). All ITS sequences originated from ACGW green and necrotic galls of chestnut and identified in this study were deposited in the NCBI GenBank under the accession numbers presented in Table 4.

Phylogenetic analysis

Fungal species isolated from both necrotic and green sweet chestnut galls clustered together with similar species downloaded from the NCBI GenBank, and their ITS sequences showed high identity compared to those already found in the database. Phylogenetic analysis results confirmed the previous morphological and molecular investigation outcomes based on ITS region variation (Figure 4). In the NJ tree based on the ITS region, two groups were distinguished and split into subclades that were highly supported by high bootstrap values (> 90%) (Figure 4). Furthermore, each fungal species was placed in the same cluster with similar species previously isolated from ACGW galls from chestnut. Some new species, previously not reported from sweet chestnut galls or even from *C. sativa*, were isolated for the first time during this study. In particular, *P. livescens* was isolated from both green and necrotic ACGW chestnut galls, while *P. domesticum* and *P. ibericus* were isolated only from necrotic galls. In addition, above mentioned species precise identification was confirmed by phylogenetic analysis as they clustered (bootstrap support 95-100%) nearby the same species downloaded from the GenBank (Figure 4).

Table 4 Fungal taxa isolated from necrotic and green sweet chestnut galls and ITS sequences accession number after deposition into the NCBI GenBank database.

No.	Fungal taxa	Isolate/s name/s*	Accession numbers (NCBI) ITS
1	*Gnomoniopsis castaneae*	GNC1; GNC2; GNC3; GNC4	OR231929; OR231930; OR231931; OR231932
2	*Alternaria* sp.	ALT5; ALT6; ALT7	OR232202; OR232203; OR232204
3	*Botryosphaeria dothidea*	BOTDOT8; BOTDOT9; BOTDOT10	PP399140; PP399141; PP399142
4	*Sordaria fimicola*	SORDFM11; SORDFM12; SORDFM13; SORDFM14	PP391735; PP391736; PP391737; PP391738
5	*Cladosporium cladosporioides*	CLADCD15; CLADCD16; CLADCD17; CADCD18; CLADCD19	PP397139; PP397140; PP397141; PP397142; PP397143
6	*Colletotrichum acutatum*	COLLETAC20; COLLETAC21; COLLETAC22; COLLETAC23; COLLETAC24	PP391744; PP391745; PP391746; PP391747; PP391748
7	*Epicoccum nigrum*	EPICNGR25; EPICNGR26; EPICNGR27	PP391749; PP391750; PP391751
8	*Fusarium* sp.	FUS28; FUS29; FUS30; FUS31; FUS32	PP391752; PP391753; PP391754; PP391755; PP391756
9	*Phoma* sp.	PHOM33; PHOM34; PHOM35	PP474739; PP474740; PP474741
10	*Penicillium* sp.	PENIC36; PENIC37; PENIC38	PP474742; PP474743; PP474744
11	*Fusarium lateritium*	FUSLAT39; FUSLAT40; FUSLAT41	PP474745; PP474746; PP474747
12	*Pestalotiopsis* sp.	PESTALOT42; PESTALOT43; PESTALOT44	PP474748; PP474749; PP474750
13	*Sirococcus castaneae*	SIROCAST45; SIROCAST46; SIROCAST47; SIROCAST48; SIROCAST49	PP474751; PP474752; PP474753; PP474754; PP474755
14	*Phanerochaete livescens*	PHANERLIV50; PHANERLIV51; PHANERLIV52	PP474756; PP474757; PP474758
15	*Trichoderma* sp.	TRICH53; TRICH54; TRICH55; TRICH56; TRICH57	PP474759; PP474760; PP474761; PP474762; PP474763
16	*Pyronerma domesticum*	PYRONDOM58; PYRONDOM59; PYRONDOM60; PYRONDOM61	PP474764; PP474765; PP474766; PP474767
17	*Pilatoporus ibericus*	PILATIB62; PILATIB63; PILATIB64; PILATIB65	PP474768; PP474769; PP474770; PP474771
18	*Botrytis cinerea*	BOTRYC IN66; BOTRYCIN67; BOTRYCIN68; BOTRYCIN69	PP474772; PP474773; PP474774; PP474775
19	*Mucor* sp.	MUCOR70; MUCOR71	PP474776; PP474777

Note: *= Acronyms used for fungal cultures.

Figure 4 Phylogenetic relationships of 71 representative fungal taxa from chestnut galls and their closest relatives based on the internal transcribed spacer (ITS) region. The evolutionary history was inferred using the Neighbor-Joining method. The optimal tree is shown. The percentage of replicates (if >90%) in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown above the branches. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Jukes-Cantor method and are in the units of the number of base substitutions per site. Fungal taxa obtained in this study are marked with red circles.

Discussion

Experimental data from this study showed a moderate fungal diversity in ACGW galls matching the results reported by other studies (Yang et al. 2012, Fernandez-Conradi et al. 2019, Morales-Rodriguez et al. 2019, Muñoz-Adalia et al. 2019). Generally, myco-endophyte diversity is lower in galls compared to unaffected leaf tissues (Fernandez-Conradi et al. 2019).

In the present study, the most abundant taxa isolated from both green and necrotic galls was *G. castaneae*. In almost all European studies it was observed that *G. castaneae* dominated the mycobiota associated with ACGW (Fernandez-Conradi et al. 2019, Muñoz-Adalia et al. 2019), with the exception of fungal communities carried by *D. kuriphilus* adults (Morales-Rodriguez et al. 2019).

Fernandez-Conradi et al. (2019), studying the

fungal endophyte communities in chestnut galls and surrounding foliar tissues, reported that the composition of endophyte communities varies between galls produced by ACGW and surrounding foliar tissue, and a high fungal diversity was found in both. In particular, observing the taxonomical composition of chestnut leaves and galls, the authors found 97 operational taxonomic units (OUTs) in chestnut leaves and 84 OTUs in galls produced by ACGW. They also showed that Ascomycota dominated the studied communities, while low levels represented the other microorganisms. Furthermore, the most abundant species was *G. castaneae* (~56%), followed by other species which were isolated less frequently (6-2%) *Trichothecium roseum*, *Alternaria* sp., *Botryopshaeria dothidea* and *Sordaria* sp. These data are relatively similar to those obtained in the present study. Although the fungal endophyte richness was lower in galls than in surrounded tissue, insect-induced galls still provide a specific habitat for endophytic fungi. It is worth stressing that the higher number of OTUs obtained from ACWG galls compared to our study could be explained using the Illumina sequencing technology versus the culture-based methodology followed by Sanger sequencing, which can limit the discovery of some less abundant species. However, the methodology adopted by the present study allowed us to reveal most of the fungal endophytes found before in both green and necrotic ACGW galls from chestnut. Despite the technology utilized, both the present and Fernandez-Conradi et al. (2019) studies demonstrated that the taxa that dominated in ACGW chestnut galls belonged to *Sordariomycetes* and *Dothiodeomycetes* classes.

The predominance of *G. castaneae* in *D. kuriphilus* galls was previously reported by Seddaiu et al. (2017) also being isolated with high frequency from green and necrotic galls in Basilicata region worries very much as this pathogen is responsible of nut rot in chestnut worldwide. In addition, it was stated that *G. castaneae* may behave both as an endophyte and pathogen, is a causal canker agent in *C. sativa* (Pasche et al. 2016) and is also closely linked with the *D. kuriphilus* spread (Seddaiu et al. 2017, Munõz-Adalia et al. 2019). Furthermore, other fungi isolated in the present study like *Fusarium* sp., *Alternaria* sp., *C. acutatum, B. dothidea*, *B. cinerea, Pestalotiopsis* sp., and *S. casteaneae* are known pathogens attacking many host plants, some of them being destructive canker agents all over the world (Phillips et al. 2013, Zimowska et al. 2020, Yang et al. 2021, Mohali 2023). Munõz-Adalia et al. (2019) also reported isolating these fungal species from necrotic and healthy galls in chestnut trees affected by *D. kuriphilus* from two sites in northern Spain. Similar to our study, the authors from both necrotic and healthy ACGW chestnut gall predominantly *G. castanea* (~34-44%), but also *Fusarium* sp. (~1.5-13%) were isolated. In the necrotic and healthy chestnut ACGW galls from the second site, other less abundant fungal species were found, like *Sydowia polivora* (~6%), *Nigrospora oryzae* (~3%), *Rhizomucor variabilis* and *Diplodia concentrica* (1.5%), along with some other taxa which were never found in our investigated sites. In the first site, they found fewer fungal species from necrotic and green leaves, but still, the same fungi mentioned before were predominant as in our study, and others, such as *Arthrinium arundis*, had not been found in our samples. On the contrary, in our study, the sites harboured quite similar numbers (green galls 12 vs. 10 and necrotic galls 11 vs. 8) of fungal species, registering a slightly small number of taxa isolated from site 2, despite the gall type.

The fact that many of the fungal species isolated from ACGW chestnut galls in the present study are pathogens poses a serious threat to the health of the chestnut trees in the Basilicata region.

Some fungal taxa such as *Fusarium* sp., *C. cladospoiroides*, *S. fimicola*, *E. nigrum*, *C. acutatum*, and *Phoma* sp. can have different behaviour, acting as endophytes, saprophytes,

or pathogens depending on the host as already reported by several authors (Meyer et al. 2017, Munõz-Adalia et al. 2019). Antagonistic fungi like *Trichoderma* sp. presence in ACGW chestnut galls was observed in one site from the Basilicata region, and this is similar to what was found by Munõz-Adalia et al. (2019). The associations between ACGW or other cynipid gall wasps (*Dryocosmus dubiosus*) are well recognized (Raman et al. 2012, Morales-Rodriguez et al. 2019), and the endophytic communities found in galls are established by Ascomycota (type II endophyte taxa) also transmitted by biotic vectors such as insects. Nevertheless, information about the role of Cynipids in the dispersal of Type II endophytes is still unavailable (Morales-Rodriguez et al. 2019).

The presence of entomopathogenic fungi like *C. acutatum*, *Fusarium* sp., *Pestalotiopsis* sp., and *C. cladospoiroides,* which were also found in this study had been linked to *D. kuriphilus*, *Hemiberlesia pityophila*, and *Teteranycus urticae* infections causing their death (Lv et al. 2011, Graziosi & Rieske 2015, Munõz-Adalia et al. 2019).

The fungal community associated with ACGW after the emergence of galls in chestnuts from Central Italy was discovered with the help of novel technologies such as metabarcoding with High Throughput Sequencing (HTS) (Morales-Rodriguez et al. 2019). The authors tried to find *C. parasitica* and understand its possible role in the epidemiology of flagging symptoms (Morales-Rodriguez et al., 2019) as it is known that insects-inducing galls are associated with various species of fungi (Vanini et al. 2017), but they did not find *C. parasitica* in *D. kuriphilus* adults and neither did us in galls in the present study. Instead, some taxa, such as *E. nigrum*, *G. castanea*, *C. acutatum,* and *B. cinerea* (also found in our research), along with others like *Ramularia endophylla* (syn. *Mycosphaerella maculiformis*), *Stromatoseptoria castaneicola*, and *Beauveria bassiana* were the most abundant within the core microbiome. In addition, as in our study, most of the taxa found in ACGW adults were plant pathogens followed by endophytes and wood saprotrophs based on their functional guilds assessment (Morales-Rodriguez et al. 2019). Opposite to what we found in green and necrotic galls caused by ACGW in chestnut, saprotrophs constituted an essential part of the fungal communities identified and some genera such as *Penicillium* and *Cladosporium* were highly abundant (Morales-Rodriguez et al. 2019).

Recent studies stated that *G. castaneae* colonized healthy and necrotic galls with ubiquitous components of fungal communities like *Alternaria* sp., *Aspergillus* sp., *Fusarium* sp. and *Colletotrichum fioriniae*. The authors also highlighted that *T. sinensis* could control the ACGW, while native parasitoids had only a secondary role. Additionally, it was shown that gall necroses slightly impacted *T. sinensis* control, as their efficacy was limited to the early stages of gall development (Pennacchio et al. 2023).

Furthermore, the fungal community associated with ACGW is very different in Asia compared to Europe (Yang et al. 2021). The absence of the competitive alien fungi *G. castaneae* allowed the local Asian taxa to develop. The host specificity (*C. mollissima, C. crenata*, and other Asian species) influenced the fungal spectrum. *Didymella rosea* and *C. delicatulum* dominated the leaf, twig and gall fungal community. Even the sub-dominating species – *Acremonium* sp., *Cryptococcus aureus*, *Cercospora* sp. are not frequent in Europe related to galls. The fungi associated with *D. kuriphilus* adults are different from the gall community, being largely dominated by *Botryosphaeria* sp., other relative frequently taxa being *Aspergillus* sp. and *Diaporthe amygdali* (Yang et al. 2021).

Overall, the diversity of microbiota associated with chestnuts infected by ACGW in Basilicata was similar to that reported in Europe (Muñoz-Adalia et al. 2018, Fernandez-Conradi et al. 2019, Morales-Rodriguez et al. 2019), respectively very different compared to Asia (Yang et al. 2021). Fungal richness was lower than that reported for other host plants

(Koukol et al. 2012, Sanz-Ros et al. 2015, Muñoz-Adalia et al. 2019). Although this is the situation, the presence of endophytes in ACGW galls from chestnuts is important, and knowledge about their biology is critical to use as biocontrol agents against galling insects. The interactions between endophytic fungal taxa and gall-forming insects are very complex and certainly future data delivered by new technologies will help us understand how they influence the chestnut health and productivity.

Conclusion

The present study revealed the dominant presence of *G. castaneae* along with 18 other fungal species, some of which have already been reported in previous studies in Sardinia (southern Italy) and other countries.

The taxonomic composition of the fungal communities found in the two investigated sites in the Basilicata region varied more with the type of gall (necrotic vs green type) and less with the site.

Most of the taxa identified were either plant pathogens or fungal endophytes and some of them deserve supplementary studies as they can affect the chestnut health.

Further investigations are required to establish the role played by these fungal species, especially *G. castaneae,* in the health of the chestnut trees in the Basilicata region and to better understand their interactions with the invasive pest *D. kuriphilus*.

Compliance with ethical standards

Conflict of interest

Authors declare there is no conflict of interest.

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References

Addario E., Turchetti T. 2011. Parasitic fungi on *Dryocosmus kuriphilus* in *Castanea sativa* necrotic galls. Bull. Insectology 64(2): 269-273.

Altschul S.F., Madden T.L., Schäffer A.A., Zhang J., Zhang Z.Z., Miller V., Lipman D.J. 1997. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Res. 25 (17): 3389–402. https//doi: 10.1093/nar/25.17.3389

Balsa C., Bento A., Paparella F. 2021. Biological control of the Asian chestnut gall wasp in Portugal: Insights from a mathematical model. *PLoS ONE* 16(9): e0257990. https://doi.org/10.1371/journal.pone.0257990

Bell A.E. 1983. Dung Fungi. Victoria University Press, New Zealand, 88p.

Benigno A., Aglietti C., Cacciola S.O., & Moricca S. 2024. Trunk injection delivery of biocontrol strains of *Trichoderma* spp. effectively suppresses nut rot by *Gnomoniopsis castaneae* in chestnut (*Castanea sativa* Mill.). Biology, 13(3): 143. https://doi.org/10.3390/biology13030143

Bensch K., Braun U., Groenewald J.Z., Crous P.W. 2012. The genus *Cladosporium*. Stud. Mycol. 71: 1–401. https://doi.org/10.3114/sim0003

Boerema G.H. 1993. Contributions towards a monograph of *Phoma* (Coelomycetes) – II. Section *Peyronellaea*. Persoonia 15: 197–221.

Boerema G.H., de Gruyer J., Noordeloos M.E., et al. 2004. *Phoma* identification manual. Differentiation of specific and infra-specific taxa in culture. CABI Publishing, Wallingford, UK., 425 p.

Bosio G, Gerbaudo C, Piazza E. 2010. *Dryocosmus kuriphilus* Yasumatsu: an outline seven years after the first report in Piedmont (Italy). Acta Hortic. 866: 341–348. https://doi.org/10.17660/ActaHortic.2010.866.43

Brussino G., Bosio G., Baudino M., Giordano R., Ramello F., Melika G. 2002. Pericoloso insetto esotico per il castagno europeo. Inf. Agrar. 58(37): 59–62.

Burdsall H.H. Jr. 1985. A contribution to the taxonomy of the genus *Phanerochaete*. Mycologia Memoirs 10: 1–165.

Cai F., Dou K., Wang P., Chenthamara K., Chen J., Druzhinina I.S. 2022. The current state of *Trichoderma* taxonomy and species identification. in: Amaresan, N., Sankaranarayanan, A., Dwivedi, M.K., Druzhinina,

I.S. (eds). Advances in *Trichoderma* Biology for Agricultural Applications. Fungal Biology. Springer, Cham., Birkhäuser Verlag AG, Switzerland 650p. https://doi.org/10.1007/978-3-030-91650-3_1

Camele I., Marcone C., Cristinzio, G. 2005. Detection and identification of *Phytophthora* species in Southern Italy by RFLP and sequence analysis of PCR-amplified nuclear ribosomal DNA. Eur. J. Plant Pathol. 113: 1–14. https://doi.org/10.1007/s10658-005-8915-1

Camisón Á., Martín M.Á., Sánchez-Bel P., Flors V., Alcaide F., Morcuende D., Pinto G., Solla A. 2019. Hormone and secondary metabolite profiling in chestnut during susceptible and resistant interactions with *Phytophthora cinnamomi*. J. Plant Physiol. 241: 153030. https//doi.org/10.1016/j.jplph.2019.153030

Chen Q., Hou L.W., Duan W.J., Crous P.W, Cai L. 2017. *Didymellaceae* revisited. Stud. Mycol. 87(1): 105–159. http://dx.doi.org/10.1016/j.simyco.2017.06.002

Chira D., Bolea V., Chira F., Mantale C., Tăut I., Șimonca V., Diamandis, S., 2017. Biological control of *Cryphonectria parasitica* in Romanian protected sweet chestnut forests. Notulae Botan. Horti Agrobot. Cluj-Napoca 45(2): 632–638. https://doi.org/10.15835/nbha45210895

Chira F., Ciocîrlan M., Mantale C. 2021. Monitorizarea insectei invasive *Dryocosmus kuriphilus* în România (Monitorizing of the invasive insect *Dryocosmus kuriphilus* in Romania) Rev. Silv. Cineget. 49: 50–55.

Cisterna-Oyarce V., Carrasco-Fernandez J., Castro J.F., Santelices C., Munoz-Reyes V., Millas P., Alan G., Buddie A.G., France A. 2022. *Gnomoniopsis smithogilvyi*: Identification, characterization and incidence of the main pathogen causing brown rot in postharvest sweet chestnut fruits (*Castanea sativa*) in Chile. Australas. Plant Dis. Notes 17(2). https://doi.org/10.1007/s13314-022-00450-6

Coelho V., Gouveia E. 2021. *Gnomoniopsis smithogilvyi*, the causal agent of chestnut brown rot reported from Portugal. New Disease Rep. 43: e12007. https://doi.org/10.1002/ndr2.12007

Cristinzio G. 1986. A new danger for the Italian chestnut cultivation. Atti Giornate Fitopatol. 2: 223–228. https://doi.org/10.1007/s13314-022-00450-6228. [In Italian]

Csóka G., Stone G.N., Melika G. 2017. Non-native gall-inducing insects on forest trees: a global review. Biol. Invasions 19(11): 3161–3181. https://doi.org/10.1007/s10530-017-1466-5

Dar M.A., Rai M. 2015. *Gnomoniopsis smithogilvyi*, a canker causing pathogen on *Castanea sativa*: First report. Mycosphere 6 (3): 327–336. Doi 10.5943/mycosphere/6/3/8

Damm U., Cannon P.F., Woudenberg J.H.C., Crous P.W. 2012. The *Colletotrichum acutatum* species complex. Stud. Mycol.73: 37–113. https://doi.org/10/10.3114/sim0010

Dennert F., Broggini G., Gessler C., Storari M. 2015. *Gnomoniopsis castanea* is the main chestnut nut rot agent in Switzerland. Phytopathologia Mediterrranea 54(2): 199–211. https://doi.org/10.14601/Phytopathol_Mediterr-14712

Domsch K. H., Gams W., Anderson T. H. 1982. Compendium of soil fungi. Volume 1 Academic Press Ltd., London, U.K, 860 p.

Dobry E., Campbell, M. 2023. *Gnomoniopsis castanea*: An emerging plant pathogen and global threat to chestnut systems. Plant Pathol. 72: 218–231. https://doi.org/10.1111/ppa.13670

Ellis M.B., Waller J.M 1975. *Sclerotinia fuckeliana* (conidial state: *Botrytis cinerea*). C.M.I. Descriptions of pathogenic fungi and bacteria 431 ref.4.Commonwealth Mycological Institute, Ferry Lane, Kew, Surrey, UK.

Fernandez-Conradi P., Fort T., Castagneyrol B., Hervé J., Robin C. 2019. Fungal endophyte communities differ between chestnut galls and surrounding foliar tissues. Fungal Ecol. 42: 100876. https://doi.org/10.1016/j.funeco.2019.100876

Floudas D., Hibbett D.S. 2015. Revisiting the taxonomy of *Phanerochaete* (Polyporales, Basidiomycota) using a four gene dataset and extensive ITS sampling. Fungal Biol. 119, 679–719. https://doi.org/10.1016/j.funbio.2015.04.003

Felsenstein J., 1985. Confidence limits on phylogenies; An approach using bootstrap. Evolution 39(4): 783-791. https://doi.org/10.2307/2408678

Gaffuri F., Maresi G., Pedrazzoli F., Longa C., Boriani M., Molinari M., Tantardini A. 2015. *Colletotrichum acutatum* associated with *Dryocosmus kuriphilus* galls on *Castanea sativa*. Forest Pathol. 45 (2): 169–171. https://doi.org/10.1111/efp.12178

Gaffuri F. 2018. Incidence and effects of *Colletotrichum acutatum* JH Simmonds 1968, in *Dryocosmus kuriphilus* Yatsumatsu (Hymenoptera Cynipidae) galls on *Castanea sativa* Mill. PhD thesis, Università degli Studi di Pavia. https://iris.unipv.it/handle/11571/1214833

Gehring E., Bellosi B., Quacchia A., Conedera M. 2018. Evaluating *Dryocosmus Kuriphilus*-induced Damage on *Castanea Sativa.* J. Vis. Exp. 30(138):57564. https://doi.org/10.3791/57564. PMID: 30222155; PMCID: PMC6235062

Gentile S., Valentino D., Visentin I., Tamietti G. 2010. An epidemic of *Gnomonia pascoe* on nuts of *Castanea sativa* in the Cuneo area. Acta Hortic. 866: 363–368. https://doi.org/10.17660/ActaHortic.2010.866.46

Graziosi I., Rieske L.K. 2015. A plant pathogen causes extensive mortality in an invasive insect herbivore. Agric. Forest Entomol. 17 (4): 366–374. https://doi.org/10.1111/afe.12116

Infantino A., Grottoli A., Bergamaschi V., Oufensou S., Burgess L.W., Balmas V. 2023. FusaHelp. A web site program for the morphological identification of *Fusarium* species. Journal of Plant Pathol. 105(2): 429–436. https://doi.org/10.1007/s42161-023-01349-6

Jarvis W.R. 1977. *Botryotinia* and *Botrytis* species: taxonomy, physiology, and pathogenicity. A guide to the literature. Research Branch Canada Department of Agriculture, Monograph No. 15, Hignell Printing

Limited, OTTAWA K1A 0C7, Canada, 208 p.
Jarvis W.R. 1980. Taxonomy. pages 1-18 in: The Biology of *Botrytis.* Koley-Smith J.R., Verhoeff K., Jarvis, W.R. (Eds.). Academic Press, San Diego, California, USA.
Jeewon R., Liew E.C.Y., Hyde K.D. 2004. Phylogenetic evaluation of species nomenclature of *Pestalotiopsis* in relation to host association. Fungal Div. 17: 39–55.
Jukes T.H., Cantor C.R. 1969. Evolution of protein molecules. In Munro HN, editor, Mammalian Protein Metabolism, pp. 21–132, Academic Press, New York
Kato, K., Hijii, N., 1997. Effects of gall formation by *Dryocosmus kuriphilus* Yasumatsu (Hym., Cynipidae) on the growth of chestnut trees. J. Appl. Entomol. 121 (1–5), 9–15. https://doi.org/10.1111/j.1439-0418.1997.tb01363.x
Koukol O., Kolarík M., Kolárová Z., Baldrian P., 2012. Diversity of foliar endophytes in windfallen *Picea abies* trees. Fungal Divers. 54: 69-77 doi: 10.1007/s13225-011-0112-2
Kumar S., Stecher G., Li M., Knyaz C., Tamura K. 2018. MEGA X: Molecular Evolutionary Genetics Analysis across computing platforms. Molec. Biol. Evol. 35: 1547–1549. https://doi.org/10.1093/molbev/msy096
Larkin M., Blackshields G., Brown N.P., Chenna R., Mcgettigan P., McWilliam H., Valentin F., Wallace I., Wilm A., López R., Thomson J.D., Gibson T.J., Higgins D. C. 2007. Clustal W and Clustal X version 2.0. Bioinformatics 23 (21): 2947–2948. https://doi.org/10.1093/bioinformatics/btm404
Leslie J.F., Summerell B.A. 2006. The *Fusarium* laboratory manual. Blackwell Publishing, Ames, Iowa, 388 p.
Lewis A., Gorton C., Rees H., Webber J., Pérez-Sierra A., 2017. First report of *Gnomoniopsis smithogilvyi* causing lesions and cankers of sweet chestnut in the United Kingdom. New Dis. Rep. 35: 20. http://dx.doi.org/10.5197/j.2044-0588.2017.035.020
Lione G., Giordano L., Sillo F., Gonthier P. 2015. Testing and modelling the effects of climate on the incidence of the emergent nut rot agent of chestnut *Gnomoniopsis castanea*. Plant Pathol. 64: 852–863. https://doi.org/10.1111/ppa.12319
Lione G. 2016. Ecology and epidemiology of the emerging plant pathogen *Gnomoniopsis castaneae*. J. Plant Pathol. 98(4) S5. https://doi.org/10.4454/jpp.v98i4sup.3773
Lione G., Danti R., Fernandez-Conradi P., Ferreira-Cardoso J.V., Lefort F., Marques G., Meyer J.B., Prospero S., Radócz L., Robin C., et al. 2019. The emerging pathogen of chestnut *Gnomoniopsis castaneae*: The challenge posed by a versatile fungus. European J. Plant Pathol. 153: 671–685.https://doi.org/10.1007/s10658-018-1597-2
Lv C., Huang B., Qiao M., Wei J., Ding B. 2011. Entomopathogenic Fungi on *Hemiberlesia pitysophila*. PLoS ONE 6 (8): e23649. DOI: 10.1371/journal.pone.0023649
Magro P., Speranza S., Stacchiotti M., Martignoni D., Paparatti B. 2010. *Gnomoniopsis* associated with necrosis of leaves and chestnut galls induced by *Dryocosmus kuriphilus.* Plant Pathol. 59: 1171. https://doi.org/10.1111/j.1365-3059.2010.02336.x
Maharachchikumbura S.S.N, Guo L.D., Chukeatirote E., et al, 2011. *Pestalotiopsis*: morphology, phylogeny, biochemistry and diversity. Fungal Div. 50(1): 167–187. doi:10.1007/s13225-011-0125-x
Maharachchikumbura S.S.N., Hyde K.D., Groenewald J.Z., et al, 2014. *Pestalotiopsis* revisited. Stud. Mycol.79(1): 121–186. doi: 10.1016/j.simyco.2014.09.005
Mang S.M., Zotta T., Camele I., Racioppi R., D'Auria M., & Rana G.L. 2017. Morphological, physico-chemical and molecular investigations on *Tuber bellonae* from Basilicata - Italy. J. Anim. & Plant Sci. 27(2): 528–541.
Mang S.M., Scrano L., & Camele, I. 2020. Preliminary studies on fungal contamination of two rupestrian churches from Matera (Southern Italy). Sustainability 12(7): 6988. https://doi.org/10.3390/su12176988
Mang S.M., Marcone C., Maxim A. & Camele I., 2022. Investigations on fungi isolated from apple trees with diebacksymptoms in Basilicata region (Southern Italy). Plants 11(10): 1374. https://doi.org/10.3390/plants/11101374
Maresi G., Longa O., Turchetti T. 2013. Brown rot on nuts of *Castanea sativa* Mill: an emerging disease and its causal agent. IForest – Biogeosci. Forest. 6(5): 294–301. https://doi.org/10.3832/ifor0952-006
Marzocchi G., Maresi G., Luchi N., Pecori F., Gionni A., Longa C.M.O., Pezzi G., and Ferretti F., 2024. 85 years counteracting an invasion: chestnut ecosystems and landscapes survival against ink disease. Biol. Invasions: 1–14. https://doi.org/10.1007/s10530-024-03292-8
Melo I., Ryvarden L. 1989. *Fomitopsis iberica* Melo & Ryvarden sp. nov. Bol. Soc. Brot. 62: 227–230.
Mentana A., Camele I., Mang S.M., De Benedetto G.E., Salvatore F., Centonze D. 2019. Volatolomics approach by HS-SPME-GC-MS and multivariate analysis to discriminate olive tree varieties infected by *Xylella fastidiosa*. Phytochem. Anal. 30(6): 623–634. https://doi.org/10.1002/pca.2835
Meyer J.B., Gallien L., Prospero S. 2015. Interaction between two invasive organisms on the European chestnut: does the chestnut blight fungus benefit from the presence of the gall wasp? FEMS Microbiol. Ecol. 91 (11): fiv 122. https://doi.org/10.1093/femsec/fiv122
Meyer J.B., Trapiello E., Senn-Irlet B., Sieber T.N., Cornejo C., Aghayeva D., Gonzales A.J., Prospero S. 2017. Phylogenetic and phenotypic characterization of *Sirococcus castaneae* comb. nov. (*Diplodina castaneae*), a fungal endophyte of European chestnut. Fungal Biol. 121 (8): 625–637. https://doi.org/10.1016/j.funbio.2017.04.001
Mohali S.R., 2023. A review of *Botryosphaeriales* in Venezuela with special reference to woody plants. Ann. For. Res. 66(1): 35–62. https://doi.org/10.15287/afr.2023.2492
Moore E.J., Korf R.P. 1963. The genus *Pyronema*. Bull. Torrey Bot. Club 90 (1): 33–42. https://doi.

org/10.2307/2482857

Morales-Rodriguez C., Sferrazza I., Aleandri M.P., Dalla Valle M., Mazzetto T., Speranza S., Contarini M., Vannini A. 2019. Fungal Community associated with adults of the chestnut gall wasp *Dryocosmus kuriphilus* after emergence from galls: Taxonomy and functional ecology. Fungal Biol. 123: 905–912. https://doi.org/10.1016/jfunbio.2019.09.009

Muñoz-Adalia E.J., Rodriguez D., Casado M., Diez J., Fernández M. 2019. Fungal community of necrotic and healthy galls in chestnut trees colonies by *Drycosmus kuriphilus* (Hymenoptera: Cynipidae). iForest 12: 411–417. https://doi.org/10.3832/ifor3014-012

Nicoletti R., Beccaro G.L., Sekara A., Cirillo C., & Di Vaio C. 2021. Endophytic fungi and ecological fitness of chestnuts. Plants 10(3): 542. https://doi.org/10.3390/plants10030542

O'Donnell K., Whitaker B.K., Laraba I., Proctor R.H., Brown D.W., Broders K., Kim H.S., McCormick S.P., Busman M., Aoki T., Torres-Cruz T.J., Geiser D.M. 2021. DNA sequence-based identification of *Fusarium*: a work in progress. Plant Dis. 106(6): 1597–1609. https://doi.org/10.1094/PDIS-09-21-2035-SR

O´Loinsigh B., McAuley D., Bréchon A.L., Lopez Vernaza M., Ryan C., Destefanis M.L., O'Hanlon R. 2022. First report of the fungus *Gnomoniopsis smithogilvyi* causing cankers on sweet chestnut (*Castanea sativa*) in Ireland. New Dis. Rep. 45: e12072. https://doi.org/10.1002/ndr2.12072

Panzavolta T., Bernardo U., Bracalini M., Cascone P., Croci F., Gebiola M., Iodice L., Tiberi R., Guerrieri E. 2013. Native parasitoids associated with *Dryocosmus kuriphilus* in Tuscany, Italy. Bull. Insectology 66: 195–201.

Pasche S., Calmin G., Auderset G., Crovadore J., Pelleteret P., Mauch-Mani B., Barja F., Paul B., Jermini M., Lefort F. 2016. *Gnomoniopsis smithogilvyi* causes chestnut canker symptoms in *Castanea sativa* shoots in Switzerland. Fungal Gen. Biol. 87: 9–21. https://doi.org/10.1016/j.fgb.2016.01.002

Pennacchio F., Binazzi F., Strangi A., Roversi P.F., Gargani E., Turchetti T., Toccafondi P., and Danti R. 2023. Some biotic limiting factors of *Dryocosmus kuriphilus* (Hymenoptera Cynipidae): a survey in chestnut orchards near Florence (Italy). Redia: J. Zoology / Giornale di Zoologia 106: 79. https://doi.org/10.19263/REDIA-106.23.10

Phillips A.J.L., Alves A., Abdollahzadeh J., Slippers B., Wingfield M.J., Groenewald J.Z., Crous P.W. 2013. The *Botryosphaeriaceae*: genera and species known from culture. Stud. Mycol. 76: 51–167. https://doi.org/10.3114/sim0021

Possamai G., Podridão da Castanha em Trás-os-Montes 2020. Caracterização morfológica, ecofisiológica e molecular do agente causal *Gnomoniopsis smithogilvyi*. Master's thesis, Biotechnological Engineering, Instituto Politécnico de Bragança, Lisbon, Portugal p. 85. Available online https//bibliotecadigitalipb.pt/bitstream/10198/23171/4/Possamai_Guilherme Podrida%cc%o_da_Castanha_em_Tra%cc%81s_os_Montes.pdf (accessed on 2024-01-14)

Quacchia A., Moryia S., Bosio G., Scapin I., Alma A. 2008. Rearing, release and settlement prospect in Italy of *Torymus sinensis* the biological control agent of the chestnut gall wasp *Dryocosmus kuriphilus*. *BioControl* 53 (6): 829–839. https://doi.org/10.1007/s10526-007-9139-4

Raman A., Wheatley W., Popay A. 2012. Endophytic fungus-vascular plant-insect interactions. Environ. Entomol. 41(3): 433–447. https://doi.org/10.1603/EN11317

Rigling D., Prospero S. 2018. *Cryphonectria parasitica*, the causal agent of chestnut blight: invasion history, population biology and disease control. Molec. Plant Pathol. 19(1): 7–20. https://doi.org/10.1111/mpp.12542

Saitou N., Nei M. 1987. The neighbor-joining method: A new method for reconstructing phylogenetic trees. Molec. Biol. Evol. 4: 406–425.

Sanz-Ros A.V., Müller M.M., San Martín R., Diez J.J., 2015

Fungal endophytic communities on twigs of fast and slow growing Scots pine (*Pinus sylvestris* L.) in northern Spain. Fungal Biol. 119 (10): 870-883. - https://doi.org/10.1016/j.funbio.2015.06.008

Sartor C., Dini F., Torello-Marinoni D., Mellano M.G., Beccaro G.L., Alm, A., Quacchia A., Botta R. 2015. Impact of the Asian wasp *Dryocosmus kuriphilus* (Yasumatsu) on cultivated chestnut: yield loss and cultivar susceptibility. Sci. Hortic. 197: 454–460. https://doi.org/10.1016/j.scienta.2015.10.004

Seddaiu S., Cerboneschi A., Sechi C., Mello A. 2017. *Gnomoniopsis castaneae* associated with *Dyocosmus kuriphilus* galls in chestnut stands in Sardinia (Italy). *iForest* 10: 440–445. https://doi.org./10.3832/ifor2064-009

Seddaiu S., Mello A., Sarais L., Mulas A., Sechi C., Ruiu P.A., Vettraino A.M., Petruccioli M., Gonthier P., Sillo F., and Bregant C., 2023. Haplotypes distribution and virulence of *Gnomoniopsis castaneae* in Italy. J. Plant Pathol. 105(3): 1135–1140. https://doi.org/10.1007/s42161-023-01459-1

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., Tangthirasunun N., Norphanphoun C., Karunarathna S.C., Camporesi E., Manawasighe I.S., Al-Sadi A.M., Hyde K.D. 2017. Families of *Diaporthales* based on morphological and phylogenetic evidence. Stud Mycol. 86: 217-296. https://doi.org/10.1016/j.simyco.2017.07.003

Shuttleworth L.A., Guest D.I. 2012. *Gnomoniopsis smithogilvyi*. In: Crous P.W. et al. (eds). Fungal Planet description sheets. Sheet no. 108: 142–143. http://edepot.wur.nl/240835

Shuttleworth L.A., Liew E.C.Y., Guest D.I. 2013. Survey of the incidence of chestnut rot in south-eastern

Australia. Australas. Plant Pathol. 42: 63–72. https://doi.org/10.1007/s13313-0170-2

Shuttleworth L.A., Walker D.M. & Guest D.I. 2015. The chestnut pathogen *Gnomoniopsis smithogilvyi* (*Gnomoniaceae*, *Diaporthales*) and its synonyms. Mycotaxon 130: 929–940. https://doi.org/10.5248/130.929

Sillo P., Giordano L., Zampieri E., Lione G., De Cesare S., Gonthier P. 2017. HRM analysis provides insights on the reproduction mode and the population structure of *Gnomoniopsis castaneae* in Europe. Plant Pathol. 66: 293–303. https://doi.org/10.1111/ppa.12571

Simmons E.G. 2007. *Alternaria.* An Identification Manual. CBS Biodiversity Series, vol. 6, Centraalbureau voor Schimmelcultures Publisher, Netherlands, 780 p.

Tamietti G. 2016. On the fungal species *Gnomoniopsis castaneae* ("*castanea*") and its synonym *G. smithogilvyi*. J. Plant Pathol. 98: 189–190. https://doi.org/10.3390/insects12050426

Tamura K., Stecher G., Kumar S. 2021. MEGA 11: Molecular Evolutionary Genetics Analysis Version 11. Molec. Biol. Evol. https://doi.org/10.1093/molbev/msab120

Trapiello E., Feito I., González A. J., 2018. First Report of *Gnomoniopsis castaneae* Causing Canker on Hybrid Plants of *Castanea sativa* × *C. crenata* in Spain. Plant Disease 102(5). https://doi.org/10.1094/PDIS-12-17-1874-PDN

Tosi L., Beccari G., Rondoni G., Covarelli L., Ricci C. 2015. Natural occurrence of *Fusarium proliferatum* on chestnut in Italy and its potential entomopathogenicity against the Asian chestnut gall wasp *Dryocosmus kuriphilus*. J. Pest Sci. 88 (2): 369-381. https: //doi.org/10.1007/s10340-014-0624-0

Turchetti T., Pennacchio F., D'Acqui L., Maresi G., Pedrazzoli F. 2012. Interventi per la gestione dei castagneti invasi dall cinipide (Practices to manage chestnut orchards infested by the Chinese gall wasp). Forest@. 9: 227–235. https://doi.org/10.3832/efor0701-009

Tziros G.T. 2019. First report of nut rot caused by *Gnomoniopsis castaneae* on *Castanea sativa* in Greece. J. Plant Pathol.101: 211. https://doi.org/10.1007/s42161-018-0161-4

Vannini A., Vettraino A.M., Maritgnoni D., Morales-Rodriguez C., Contarini M., Cacci R., Paparatti B., Speranza S. 2017. Does *Gnomoniopsis castanea* contribute to the natural biological control of chestnut gall wasp? Fungal Biol. 121: 44–52. https://doi.org/10.1016/j.funbio.2016.08.013

Visagie C.M., Houbraken J., Frisvad J.C., Hong S.-B., Klaassen C.H.W., Perrone G., Seifert K.A., Varga J., Yaguchi T., Samson R.A. 2014. Identification and nomenclature of the genus *Penicillium*. Stud. Mycol. 78: 343–371. http://dx.doi.org/10.1016/j.simyco.2014.09.001

Visentin I., Gentile S., Valentino D., Gonthier P., Tamietti G., Cardinale F. 2012. *Gnomoniopsis castanea* sp. nov. (*Gnomoniaceae*, *Diapothales*) as the causal agent of nut rot in sweet chestnut. J. Plant Pathol. 94: 411–419.

White T.J., Bruns T., Lee S., Taylor J. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis, M.A., Gelfand D.H., Sninsky J.J. and White T.J., editors.) PCR protocols. A Guide to Methods and Applications. New York: Academic Press. pp 315–322

Xu Y.L., Cao Y.F.., Nakasone K.K., Chen C.C., He S.H. 2020.Taxonomy and phylogeny of *Phanerochaete* sensu stricto with emphasis on Chinese collections and descriptions of nine new species. Mycosphere 11(1): 152.7–1552, https://doi.org/10.5943/mycosphere/11/1/12

Yang X.-L., Zhangc J.-Z., Luo D-Q. 2012. The taxonomy, biology and chemistry of the fungal *Pestalotiopsis* genus. Nat. Prod. Rep. 29:622. https://doi.org/10.1039/c2np00073c

Yang X.H., Li X.M., Zhu D.H., Zeng Y., & Zhao L.Q. 2021. The diversity and dynamics of fungi in *Dryocosmus kuriphilus* community. Insects 12(5): 426

Zhebentyayeva T.N., Sisco P.H., Georgi L.L., Jeffers S.N., Perkins M.T., James J.B., Hebard F.V., Saski C., Nelson C.D., Abbott A.G. 2019. Dissecting resistance to *Phytophthora cinnamomi* in interspecific hybrid chestnut crosses using sequence-based genotyping and QTL mapping. Phytopathology 109: 1594–1604. https://doi.org/10.1094/PHYTO-11-18-0425-R

Zimowska B., Okoń S., Becchimanzi A., Krol E. D., & Nicoletti R. (2020). Phylogenetic characterization of *Botryosphaeria* strains associated with *Asphondylia* galls on species of Lamiaceae. Diversity 12(2): 41. https://doi.org/10.3390/d12020041