Over the footprints of Italian mycology with emphasis on plant-associated *Ascomycota*

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Abstract

Italy is a Mediterranean country in south-central Europe that has a long tradition in mycology. The mycological journey from ancient Italy to modern advanced studies is remarkable. In this study, we outline historical studies on different fungal groups, mycologists' contributions, fungal collections, illustration techniques, and their development, including traditional and modern taxonomic approaches. In addition, the current progress of mycology is discussed, with an emphasis on plant-associated *Ascomycota*. Furthermore, a case study was carried out to better understand the occurrence of saprobic *Ascomycota* on different host plants in terrestrial habitats. We describe a novel genus, *Pigmentatineomassaria* (*Neomassariaceae*, *Pleosporales*), with the type species, *P. italica*, and another novel species, *Alborbis italica* (*Sydowiellaceae*, *Diaporthales*) as well as 16 novel host records, five new geographical records, two new regional records, and two host recurrent species, from *Botryosphaeriaceae*, *Melanopsaceae* (*Botryosphaeriales*), *Hysteriaceae* (*Hysteriales*), *Cucurbitariaceae*, *Didymellaceae*, *Leptosphaeriaceae*,

Melanommataceae, *Nigrogranaceae* (*Pleosporales*) in *Dothideomycetes* and *Cytosporaceae* (*Diaporthales*), *Nectriaceae* (*Hypocreales*), and *Graphostromataceae*, *Diatrypaceae* (*Xylariales*) in *Sordariomycetes*. Identifications are based on detailed morphologies, descriptions, and updated multi-gene phylogenetic analyses, including ecological and mycogeographical data. To evaluate the mycological progress of plant-associated *Ascomycota* during the past decade, study-based data analyses and mapping were performed by focusing on the database of Italian microfungi (https://italianmicrofungi.org/) webpage. The present study provides an overview of Italian mycology and inspires mycologists to explore the hidden fungal diversity in poorly observed natural Italian forest ecosystems. Also, this work paves the way for advanced fungal taxonomy based on a polyphasic approach and promotes ecological and mycogeographical data documentation in Italy as well as in other Mediterranean countries.

Introduction

In south-central Europe, Italy comprises a continental landmass in the north, a peninsular landmass in the central-southern part, two larger islands (Sardinia and Sicily), and minor islands (Wijesinghe et al. 2022). Italian physiography shows two major mountain ranges; the Alps in the north, separating Italy from the rest of Europe, and the Apennines, extending along the length of the Italian peninsula (Abbate et al. 2015, Domina & Zapparoli 2018). The biogeographic subdivisions in the country are categorized into the Alps, the Alpine and subalpine-oro-boreal biomes, the high Mediterranean mountains, the montane beech forests, the sub-Mediterranean deciduous forests, and the Mediterranean biome (Nimis 2016, Wijesinghe et al. 2022). Due to the latitudinal extension and rugged morphology from the Alps to the Mediterranean basin, Italy has a high number of species and endemism rate (Cristofolini 1998, Abbate et al. 2015, Granito et al. 2015, Nimis 2016, Wijesinghe et al. 2022). In such diverse terrestrial biomes, fungal communities are associated with plants and soil habitats and contribute to ecosystem stability and functioning.

In Italy, the historical studies on fungi were based on morphological identification, with line drawings, color paintings, descriptions (sometimes incomplete), and morphological keys, while the majority were not documented in English (Wijesinghe et al. 2022). With time, morphology-based taxonomic revisions, regional checklists, and monographs for different fungal groups were developed with the significant contribution of mycologists. Most of the studies were scattered among different sources and consisted of poorly reported data on fungal hosts, substrates, ecology, and geography. Morphology-based molecular phylogenetic analyses are being used in current studies. As a result, incorrect identifications are avoided, and accurate taxonomic placements, including ecological and mycogeographical data, are updated (Wijesinghe et al. 2022). Investigating Italian mycology throughout history and advances in taxonomy, and data documentation (ecology and geography) are important for the understanding of mycological development.

In this study, we revisit the history of Italian mycology based on macrofungal and microfungal studies, fossil fungal data, contributions of mycologists, and fungal herbaria, illustration techniques, and their development in brief. Additionally, traditional and modern fungal taxonomies are discussed based on both Italian and global perspectives. Furthermore, we discuss modern-day mycology with an emphasis on plant-associated *Ascomycota* in terrestrial habitats in Italy, including their hosts and distribution. A case study was carried out to determine the occurrence of *Ascomycota* on randomly selected plant hosts from the sites of the Emilia-Romagna and Trentino-Alto Adige regions leading toward data on fungal numbers.

History of fungal studies

The documentation of historical mycology in Italy dates back several centuries. Early observations and studies of Italian mycobiota were primarily based on macrofungal investigations

and were geographically limited (Venturella 1991, Saitta et al. 2011, Venturella et al. 2011, Wijesinghe et al. 2021). In Roman times (77 A.D.), Gaius Plinius Secundus (Plinius the Elder) provided detailed information on noxious and edible mushrooms and truffles (*Ascomycoata*), based on Latin and Greek sources (Graniti et al. 1999). Later, in the 16th century, Pier Andrea Mattioli (1501–1577) and Andrea Cesalpino (1524–1603) performed several fungal studies, while Giovan Battista della Porta (1535–1615) investigated fungal spores and their roles in reproduction (Graniti et al. 1999). Alfonso Ciccarelli (1532–1585) produced the first Italian fungal monograph for macrofungi, based on his work on the morphological and organoleptic characteristics of truffles (Ciccarelli 1564). Fabio Colonna (1567–1650) and Fortunio Liceto (1577–1657) provided several books about fungi (Siniscalco et al. 2013).

Many mycologists made significant contributions in the 17th and 18th centuries with regard to macrofungi. Marcello Malpighi (1628–1694) and Paolo Silvio Boccone (1633–1704) observed the microscopic characteristics of fungi. Later, Luigi Ferdinando Marsili (1658–1730) examined the underground part of the fungal fruiting bodies and provided drawings, descriptions, and details for reproductions and the hyphae with microscopic magnification. Paolo Boccone and Giovanni Antonio Battarra (1714–1789) provided several copper plate drawings and engravings of fungi (Siniscalco et al. 2013). However, since the compilation of the *Flora Italica Cryptogama* by Saccardo & Dalla Costa (1915–1916), based on F. Cavara's initiative studies, there was a century-long gap for a comprehensive mycological data listing. Giacomo Lazzari (1907–1993) provided information on the history of Italian mycology in "*Storia della Micologia Italiana*" (Lazzari 1973, Siniscalco et al. 2013).

At the beginning of the 20th century, mycological studies were encouraged by the "Working Group for Mycology of the Italian Botanical Society" to record, map, and evaluate the actual fungal diversity of macrofungi (Lo Bue 1996, Venturella et al. 2011). In the 21st century, Onofri et al. (2005a) updated a national checklist of Italian fungi, including 4,296 records from *Agaricomycetes*, excluding parasitic rusts and smuts (Onofri et al. 2005b, Venturella et al. 2011, Perini & Salerni 2014). Following this checklist, regional fungal listings of Italian basidiomycetes for Campania, Liguria, Sicily, and Tuscany were published. Later, Boccardo et al. (2008) provided a descriptive and iconographic review with 1,616 taxa for *Agaricomycotina* records in Italy. Further, national and regional preliminary red lists for macrofungi were also established (Venturella et al. 1997, 2003, 2011, de Iongh et al. 2003, Antonini et al. 2006). A newly updated checklist of Sicilian macrofungi has been recently published, including 1,919 infraspecific taxa (Ferraro et al. 2022). Compared to the above-reported macrofungal studies, microfungi have received less attention (Wijesinghe et al. 2021).

In considering microfungi, several studies have been reported since the late 17th century. P. A. Micheli (1679–1753) discussed the microscopic structures of a number of micromycetes (molds), while Giovanni Biroli (1772–1824) and Ciro Pollini (1782–1833) provided two hundred species in "*Flora Aconiensis*" and four hundred species in "*Flora Veronensis*", including both macro- and micromycetes (Siniscalco et al. 2013). Since the 19th century, a considerable number of micromycetes have been studied in parallel to macrofungi. Many botanists, mycologists, naturalists, plant pathologists, university professors, and their collaborators, including Antonio Mori, Augusto Napoleone Berlese, Carlo Bagnis, Carlo Vittadini, Ciro Pollini, E. Barsali, Flaminio Tassi, Giuseppe De Notaris, Giovanni Passerini, Pasquale Beccarini, Pier Andrea Saccardo, Pietro Voglino, Rodolfo Farneti, and Vincenzo Cesati have made immense contributions (Siniscalco et al. 2013). In the 20th century, as the first effort of data compilation, Venturella (1991) updated a checklist for Sicilian fungi (1,532 species) based on the literature data extracted from 1814 to 1990, including *Ascomycotina*. In addition, Nimis (1993) published the first national checklist for Italian lichens, which included 2,145 lichenized taxa. From the 21st century onward, there have been many studies on different fungal groups in different habitats, and in different Italian regions that were popularized, such as taxonomy, taxonomic revisions, monographs, and regional and national checklists. The atlas of Italian *Ascomycota* with 400 illustrated taxa was published by Medardi

(2006) and a national checklist for *Pezizomycotina* on decaying wood in Italy was processed by Saitta et al. (2011) as the informative sources.

Fossil fungi

Italian fossil fungal records have a long history, with evidence reported in the Miocene epoch (Cenozoic era), Jurassic, and Triassic periods of the Mesozoic era (Pampaloni 1902, Schmidt et al. 2006, 2012, Neri et al. 2017, Kettunen 2018, Saxena et al. 2021). The oldest Italian fungal fossils were discovered in Triassic amber with filamentous, conidial microfungi that resemble species of the extant *Ramularia* (Schmidt et al. 2006, Kettunen 2018). In 1896, fossil fungi were illustrated by L. Meschinelli in the *Fungorum Fossilium Omnium Hucusque Cognitorum Iconographia XXXI Tabulis Exornata* (Ubrizsy 1999). Pampaloni (1902) reported the fungal fossils on organic remains in the dissodilian beds of Melilli (Sicily). Fossil fungal taxa found in Italy are listed in Table 1, and the classification is based on the *Saccardoan system* and outline provided by Wijayawardene et al. (2022). The unclassified taxa are included under "taxonomically doubtful" or "fungi imperfecti" (Table 1). Among these, a few re-drawn line illustrations from Pampaloni (1902) are shown in Fig. 1, representing different fossil fungal groups (*Ascomycota*, *Chytridiomycota*, and *Phycomycota*).

Table 1 Fossil fungal records in Italy.

Taxonomic classification	Fossil fungal name(s)	Reference(s)
Ascomycota	*Chaethomites intricatus, Hysterites protographis, Melanosporites stefanii, Microthyrites disodilis, Perisporites hirsutus, P. setosus, Phacidium coronatum (= Phacidites coronatus), Phacidites populi, Sphaerites annulus, S. atomarius, S. braunii, S. excipuloides, S. italicus, S. verrucarioides*	Meschinelli (1898), Berbee et al. (2015), Saxena et al. (2021)
Basidiomycota	*Agaricites wardianus, Daedalea quercina (= Daedaleites quercinus), Leptoporus mollis (= Polyporites mollis), Phellinus igniarius (= Polyporites igniarius), P. igniarius (= Polyporites nigricans)*	Meschinelli (1898)
Oomycota	*Peronosporites miocenicus, P. sicculus*	Pampaloni (1902), Berbee et al. (2015)
Fossil fungi *incertae sedis*	*Polystigmites priscus*	Meschinelli (1898)
Taxonomically doubtful or fungi imperfecti	*Erysiphales melilli, Depazites maculans, D. stilbosporoides, Dothidites cypericola, Lenzitites gastaldii, Palaeomycites disodylis, Pythites disodilis, Rhytismites maculifer, Uncinulites baccarinii, Monilites albidus, Xylomites daphnogenes, X. deformis, Xylomites* sp.	Pampaloni (1902), Meschinelli (1898), Berbee et al. (2015), Saxena et al. (2021)
Mycelia sterilia	*Sclerotites pustulifer, S. salisburiae*	Meschinelli (1898)

Historical pioneers in Italian mycology and herbaria

During the 16th and 17th centuries, a significant mycological contribution was made by university professors as botanists and mycologists. In the 19th century, they specialized in studies of algae, fungi, lichens, mosses, and liverworts under the heading of cryptogamology (Siniscalco et al. 2013). At the same time, mycology was well-known and separated from botany, and mycological schools were established in Italy (Siniscalco et al. 2013). In the early 19th century, studies on *Ascomycota* commenced, and mycologists made significant contributions to advancing the knowledge of fungi and preserving mycological data for future. The earliest contributions to Italian and world mycology by Giuseppe de Notaris (1805–1877), Vincenzo de Cesati (1806–1883), and Pier Andrea Saccardo (1845–1920) (Fig. 2) were highly significant. These best-known pioneers

studied fungal specimens and described new fungal taxa from Italy and other countries, either collected by them or sent to them by both Italian and foreign colleagues (Wijesinghe et al. 2021, 2022). Their collections are preserved worldwide in historical herbaria and are considered a great source of fungal genetic materials (Forin et al. 2018).
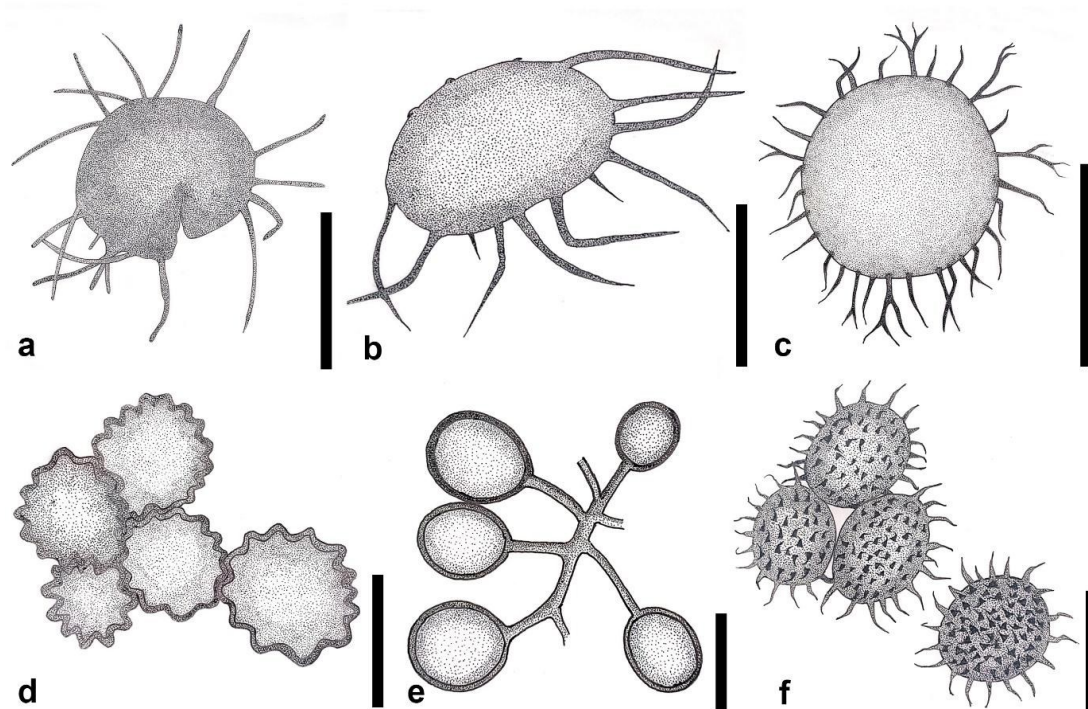
Fig. 1 – The re-drawn fossil records on "Dissodile" beds in Italy by Pampaloni (1902). a *Perisporites setosus* (*Erysiphales*). b *Perisporites hirsutus* (*Erysiphales*). c *Erisiphites melilli*. d *Peronosporites miocenicus* (*Phycomycota*). e *Palaeomycites disodylis* (suggested as *Pythium Disodylis* in *Chytridiomycota*). f *Uncinulites baccarinii*. Scale bars: d = 300, a–b = 150, c, e–f = 100. (*doubtful or incompletely identified, classification updated by Berbee et al. 2015, https://advance.science.sfu.ca/Kalgutkar_and_Jansonius/).

Fig. 2 – Early mycologists in Italy. a Giuseppe de Notaris. b Vincenzo Cesati. c Pier Andrea Saccardo (https://phaidra.cab.unipd.it/o:4479). Photos by L. Zucconi and taken from the PHAIDRA website under the CC BY-NC-SA 4.0 License.

De Notaris was the earliest Italian specialist in cryptogamic botany. He began his studies in Milan and continued them in Turin and Genoa, with a deep interest in the taxonomy of mosses, hepatice, lichens, fungi, and algae (Zucconi & Graniti 1995). In Genoa, he focused entirely on mycological studies and created the first Italian mycological school (Zucconi 1988). De Notaris provided his fundamental contributions to microfungi in the middle of the century, mainly through *Micromycetes Italici* in 1841–1857 and *Sferiacel Italici Centuria* I in 1863 (Zucconi 1988). In 1872, he moved to Rome from Genoa. The General Herbarium of Rome (RO) is a rich source of Italian fungal specimens, where the majority of De Notaris and Cesati's samples are preserved (Zucconi 1988). A total of 17,498 mycological samples (51 bundles of fungi) were deposited in this herbarium by many Italian mycologists (De Notaris, N.A. Pedicino, P.A. Saccardo, G. Bizzozzero, C. Spegazzini, G. Passerini, G. Briosi and, F. Cavara; see Zucconi 1988) and foreign collaborators (G.L. Rabenhorst, F. de Thuemen, M.C. Cooke, C.B. Plowright, J. Desmazieres, J.F. Klotzsch, and M.A. Libert; see Zucconi 1988) who exchanged samples. The greatest activities of mycology were recorded from the late 19th to the early 20th centuries.

The mycological section of the personal herbarium of Vincenzo Cesati is preserved in the Herbarium of Rome. It consists of 18,320 specimens, arranged according to *Sylloge Fungorum* by Saccardo. A number of specimens from other Italian and foreign mycologists are also deposited in this section, including those from P.A. Saccardo (1,339 samples), G. Passerini (517 samples), C. Spegazzini (371 samples), O. Beccari (268 samples), J.F. Klotzsch (37 samples), F. de Thümen (1,916 samples), G. Westendorp (753 samples), C. Roumeguère (1,176 samples), and M.A. Libert (386 samples) (Zucconi 1988).

Francesco Baglietto was a lichen expert (1826–1916) who worked with De Notaris and Cesati. Baglietto gathered fungal specimens from different collectors from different herbaria and preserved them at the Museum of Natural History in Genoa, providing a rich fungal collection (Genta 1999). A total of 5,451 macro- and microfungal specimens, including those from the Baglietto Herbarium, the Durazzo Herbarium, the Balletto Herbarium, and other collections made by different authors (collected under the name of Mycological Herbaria), are deposited at the Museum of Natural History (Genta 1999).

Saccardo's contribution was mainly focused on the basic systematics of ascomycetes and asexual fungi (deuteromycetes) (Montemartini 1999). Saccardo started his studies in the Veneto region and later expanded his studies throughout Italy and around the world. At the same time, the taxonomic value of microscopic fungal characters was highlighted by J.C. Montagne, M.J. Berkeley, and G. De. Notaris (Montemartini & Graniti 1991, Montemartini 1999). Other contributors were the Tulasne brothers (L.R. Tulasne and C. Tulasne), A. de Bary, and O. Brefeld (Montemartini 1999). During Saccardo's study period, he increased the known number of species in the Veneto region from 245 to 1,500. Also, his *Sylloge Fungorum* (1822–1931) with its 25 volumes is still an important reference in bibliographic mycological research (Zucconi 1988, Montemartini 1999). His major mycological collection is preserved in the herbarium of the Botanical Garden of the University of Padua (Forin et al. 2018). This historical collection was started around 1874 and currently contains nearly 70,000 fungal specimens covering over 18,500 different species, most of which have not been subjected to molecular studies (Forin et al. 2018). Also, it contains almost 4,500 type specimens (both by Italian and foreign collectors), which were mostly assigned to *Ascomycota* (Forin et al. 2018, Phukhamsakda et al. 2020). Mycologists from all over the world have used these specimens to conduct taxonomic studies and provided the first morphological descriptions of newly discovered fungal species (Forin et al. 2018, Phukhamsakda et al. 2020).

Finally, the Venice Museum of Natural History hosts a fungal collection of more than 25,000 samples, representing approximately 6,000 species of fungi, including many rare species and for each of them, the DNA sequences are available (https://msn.visitmuve.it/en/research/fields-of-study/micology/projects/barcoding-the-venice-fungal-collection/).

Thus, the contributions of these expert mycologists paved the way for historical mycological collections and preserved a rich source of specimens in different herbaria. Through these extensive

historical studies, Italian mycology has made a significant contribution to global mycology.

Historical illustration techniques and advancement

Fungal morphology is an important tool in both traditional and modern fungal taxonomy. Mycologists used to sketch fungal morphological characters using different approaches. Accurate illustrations are required to preserve characters for identifying fleshy and perishable fungal species (Krieger 1922). The accuracy of available mycological illustrations was initially low because of the illustrator's freedom to follow his imagination as well as technological difficulties (Krieger 1922). Therefore, some of the early pictorial fungal illustrations were not highly informative. However, during the 15th and 16th centuries, water-colored illustrations reached an extremely high artistic standard in scientific textbooks due to the close collaboration between scholars and artists (Ubrizsy 1999). The majority of the illustrations were for macrofungi, particularly *Basidiomycota* species.

The different illustration techniques based on different fungal groups were popularized from time to time in ancient Italy (Table 2). With time, these evolved from primary to advanced techniques such as woodcuts, copper engraving, and lithography to photography (Ubrizsy 1999). Historical illustrations have been discovered in 512 A.D. with illustrations of *Elaphomyces granulatus* (truffle) in the *Dioskorides Vienna Codex* (Ubrizsy 1999). In the 14th century, many illustrations were found in the *Medieval Codex of the Schola Salernitana* (Ubrizsy 1999). During the 15th–16th centuries, several unpublished codexes by U. Aldrovandi, C. Clusius, and F. Cesi were available, and woodcuts and copper engraving were the main mycological illustration techniques in the world (Krieger 1922, Ubrizsy 1999, Siniscalco et al. 2013). In the 16th century, many macrofungal studies were carried out in Italy, and these fungi tended to decay rapidly and could hardly be dried and pressed in the herbarium. Therefore, the drawings of macrofungi became significant to preserve their characters for future requirements (Ubrizsy 1999). At the end of the century, woodcut printing techniques were abandoned and replaced by lithography on zinc or aluminum metal plates as a refined solution (Ubrizsy 1999).

Fungal iconography evolved with the invention of the microscope in the 18th century (Ubrizsy 1999). From the last half of the 18th century onward, high-quality illustrations of higher fungal morphologies were produced by both Italian and foreign mycologists (Ubrizsy 1999). Also, Giuseppe De Notaris and Pier Andrea Saccardo began their fundamental contributions to microfungi, with iconographical activities and morphological descriptions (Figs 3, 4) in the 19th century. Saccardo focused his iconographic activities on higher fungi from Venetia, Italy (Saccardo 1873). He prepared 1,500 plates of microfungi in the *Fungi Italici Autographice Delineati* (Saccardo 1877–1886), and some original plates in the *Sylloge Fungorum Omnium Hucusque Cognitorum* (Ubrizsy 1999, Siniscalco et al. 2013). At that time, black-and-white lithographs and hand-colored plates by typographers or illustrators were popular with both published and unpublished illustrations in monographs and atlases (Saccardo et al. 1882, Saccardo 1901, Graniti 1983, Quadraccia & Ubrizsy 1987, Ubaldi 1990, Ubrizsy 1999). However, some illustrations were extremely pleasing but lacked scientific validity due to misunderstandings between the scientist and the illustrator. Also, in the color plates, sometimes the used colors did not correspond to reality.

After the woodcuts and lithographs of the 17th and 19th centuries, chromolithography was introduced to provide multi-color prints (Ubrizsy 1999). Later, mycological iconography consisted of photographic reproduction in books (Bauer 1976, Ubrizsy 1999). However, some authors preferred to use handmade watercolor drawings that had been photolithographically reprinted rather than photographic reproduction. With the development of computer-assisted photography and printing, high-quality, low-cost reproductions such as mycological iconographic atlases (Ubrizsy 1999) became more common.

Later, plasticized cards with quality photographs, including microscopic details, became popular for Italian fungi (Ubrizsy 1999). Drawings are affected by a series of abstractions, whereas fungal photographs are more descriptive and serve to typify the most common characteristics of the entire species. Therefore, photographic reproduction techniques are more suitable for larger fungi (Ubrizsy 1999). Particularly, electron microscopic pictures are suggested as a preferable solution in

microfungal iconography (Ubrizsy 1999). Well-illustrated monographs of different fungal groups were provided with black-and-white scanning photographs of microscopic characteristics, e.g., A. Bernicchia's *Polyporaceae* s.l. *in Italia* in 1990 and R. Galli's *Le Russule* in 1996 (Ubrizsy 1999). During the 20th century, some historical iconographic manuscripts and other bibliographical rarities have been reprinted thanks to facsimile printing techniques. Despite the destruction or loss of some fungal specimens, the morphological characters of the species are still preserved in their historical illustrations.
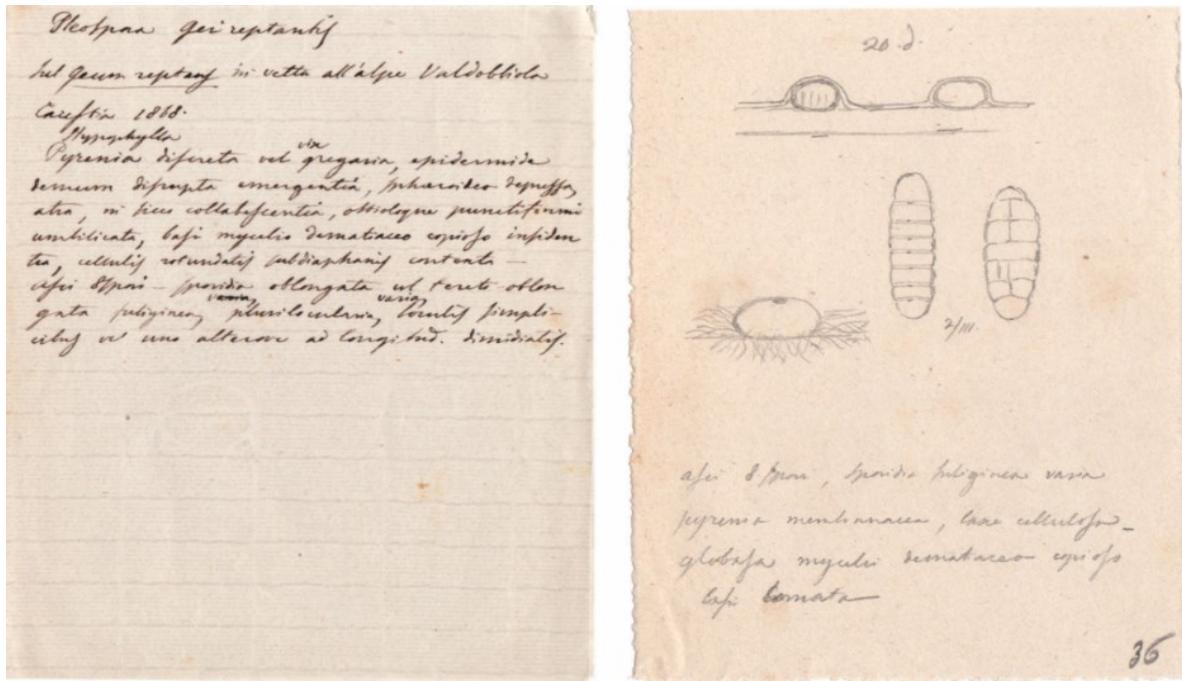
Fig. 3 – Morphological description and line drawings for *Pleospora geireptantis* (*Pleosporaceae*), based on a specimen collected by A. Carestia and studied by G. De Notaris. Photos by L. Zucconi (Graniti 1995).

Fig. 4 – P.A. Saccardo's illustrations: a Pencil and color drawings of *Basidiomycota* species (https://phaidra.cab.unipd.it/o:415462). b Black and white drawings of *Ascomycota* species (https://phaidra.cab.unipd.it/o:415449). c Hand and color drawings of *Ascomycota* (https://phaidra.cab.unipd.it/o:415439). Photos were taken from the PHAIDRA website under the Creative Commons CC BY–NC–SA 4.0 License on September 5, 2022.

Table 2 Temporal changes of different mycological iconographic techniques and examples.

Iconographic technique	Reported main task(s)	Reference(s)
15th century Woodcuts (printed block)	Incunabulum published in Apuleius Platonius' herbarium, Rome in 1481	Ubrizsy (1999)
16th century Copper engraving	P. Nobili's (first user of the technique) *Erbario* in 1580	Ubrizsy (1999)
17th century Lithography	P. Boccone's *Icones et Descriptions Rariorum Plantarum Siciliae, Melitae, Galliae et Italiae* in 1674 and *Museo di Piante Rare* in 1697 including 17 illustrated species	Ubrizsy (1999)
18th century Copper engravings (hand-colored fungal plates)	P.A. Micheli's 108 illustrations in *Nova Plantarum Genera* in 1729	Ubrizsy (1999)
Copper engravings (black and white)	G.A. Battara's *Fungorum Agri Ariminesis Historia* including 40 high-quality illustrations in 1775	Ubrizsy (1999), Siniscalco et al. (2013)
19th century Copper engravings	C. Vittadini's unpublished monograph on Italian *Hymenomycetes*, (destroyed and discarded as waste copper after his death) in 1835	Graniti (1983), Ubrizsy (1999)
Lithography (black-and-white)	D. Viviani's *Funghi d'Italia* in 1834 lithographed by Armanino and partly hand-colored by typographer A. Venturi's color illustrations, lithographs and hand color plates by illustrator F. Joli published in 1860 * The techniques were developed in collaboration with mycologists and typographers or illustrators (lack scientific value)	Krieger (1922), Lazzari (1973), Ubrizsy (1999)
Chromolithography (partial)	*Fungi Siciliani* (Centuria I: 1869, Centuria II: 1879) by G. Inzenga	Ubrizsy (1999)
20th century Photographic reproduction	Reproduced illustrations in printed books	Ubrizsy (1999)
Computer based photo printing methods	*I Funghi Dal Vero* by B. Cetto in 1970–1979 *Funghi E Ambiente* by G. Goidanich and G. Govi in 1982	Ubrizsy (1999)

Progress of fungal taxonomy

Traditional taxonomic approach

Taxonomy is the science of organism classification. Early mycological studies in Italy, as well as worldwide, were based on morphological observations (Wijesinghe et al. 2021). P.A. Micheli (1679–1753), the founder of scientific mycology and a noted Italian botanist, published his studies in "*Nova Plantarum Genera*" in 1729, providing the fundamentals of fungal classification (Siniscalco et al. 2013, Lücking et al. 2021). With his careful microscopic examinations, the reproductive structures of ascomycetes and basidiomycetes were identified as "spores" and "basidia", respectively (Krieger 1922, Barbini & Adversi 1999, Siniscalco et al. 2013). Micheli observed and described basidia and cystidia on the lamellae of *Agaricus* for the first time and provided detailed illustrations (Siniscalco et al. 2013). Micheli's classification of fungal classes was based on the position of the hymenium, the color of different parts (not accurate), and spores on the outer and inner surfaces of fruiting bodies (Siniscalco et al. 2013). In his studies, spore characteristics played a major part in defining genera and higher-order taxa (Barbini & Adversi 1999). The 8-spored asci and the spores of ascomycetes were clearly sketched in an unpublished manuscript deposited in the Botanical Museum at the University of Florence (Barbini & Adversi

1999). After these studies, Christian Hendrik Persoon (1755–1836) made Micheli's work successful (Siniscalco et al. 2013).

At the beginning of the 19th century, Italian mycological studies rapidly increased. However, due to the lack of a mycologist's coordination, incomplete investigations, and poorly developed systematics, a new fungal classification was needed (Siniscalco et al. 2013, Lücking et al. 2021). By this time, a Swedish botanist, E. M. Fries (1794–1878) provided his classification system based on the morphological characteristics (spore color, structures of spore-bearing surfaces) of fungal fruiting bodies. In the contemporary period, De Notaris became interested in fungal microscopy and was the first to use it with lichens. He established new criteria for the classification of lichens based on microscopic characteristics of fungal symbionts and began to revise specimens of Italian microfungal species (De Notaris 1841–1857, Graniti 1999). Then he realized that the existing classification of ascomycetes proposed by E.M. Fries in his *Systema Mycologicum* (1821–1832) needed to be revised because ascomycetes and their asexual morphs were classified without using a microscope. Also, a new revolutionary classification of ascomycetes was proposed based on the microscopic characteristics of ascocarps, asci, and spores (Graniti 1999). This was despite the fact that notable mycologists such as M.J. Berkeley, A.K. Corda, J.H. Léveillé, and J.P. Montagne, had made several important contributions to the micromorphological structures of ascomycetes. However, they believed that these characteristics were transient, unstable, and difficult to verify, making them of little or no taxonomic value (Graniti 1999).

De Notaris, however, stated that these microscopic characters strongly assisted in the development of a better natural classification of so-called "cryptogams" (Graniti 1999), and microscopic fungi were referred to as "Micromycetes". When determining generic and species-level identifications, De Notaris challenged contemporary mycological opinion and used his own methods (De Notaris 1844a, b, 1846), focusing on the texture and organization of the ascocarps or the microscopic characteristics of their asexual morphs (Graniti 1999).

Saccardo's mycological studies in the 1870s, such as *Mycologiae Venetae Specimen* (Saccardo 1873), and *Fungi Veneti Novi Vel Critici* (1873–1877), led to the realization of the taxonomic placements of many new and previously unclassified fungal taxa (Montemartini 1999). His *Fungi Italici Autographice Delineati*, which includes many figures of microscopic species, is graphically simple but extremely useful for identification purposes. Also, it is far more comprehensive than any previous publication from this period (Montemartini 1999). Thus, Saccardo gathered strong knowledge of the systematics of different fungal groups, especially ascomycetes, which had confused many mycologists (Montemartini 1999). Due to his greater interest in ascomycetes, many specimens with accompanying drawings and measurements were available in his herbarium, together with a few other fungal classes (Montemartini 1999). In the first two volumes of *Sylloge Fungorum* (Saccardo 1882a, b), *Pyrenomycetes* were treated, while the third and fourth volumes documented the *Sphaeropsideae Melanconieae* (Saccardo 1884) and hyphomycetes (Saccardo 1886), respectively. Saccardo expanded De Notaris's basic notion of conidial morphology in these volumes. He developed a classifying system for asexual fungi based on conidial morphology (Saccardo 1896). This sporological classification system (Saccardoan system) persisted until the 1950s, and S.J. Hughes conceived the foundation of the "ontogenetic classification" for fungal taxonomy (Hughes 1953) based on the integrated observations of Vuillemin (1910, 1911, 1912) and Mason (1940) (Montemartini 1999). Recent identifications of asexual morphs of *Ascomycota* are distinguished as coelomycete morphs that produce pycnidia and acervuli and hyphomycete morphs that lack pycnidia or acervulus fruiting bodies while developing conidia outside (Seifert & Gams 2011).

Modern taxonomic approaches

The greatest advancement of traditional fungal taxonomy in the world, as well as in Italy, arose in the early 1990s with the advent of DNA-based sequence data. Advanced molecular methods and phylogeny have been linked with morphology (Senanayake et al. 2017a, Wanasinghe et al. 2018, Tennakoon et al. 2020, Wijesinghe et al. 2021). In morphological advancement, the

historical iconographic techniques in Italy have been developed with modern technologies. Mycologists used a combined approach of advanced photographic and microscopic techniques and photographs that can be saved as digital files. The actual color of the outer and inner structures of the fungi can be seen and preserved in the photograph. High-resolution light microscopes equipped with digital cameras (photographic microscopes) are a popular technique for basic fungal morphological studies (Guarro et al. 1999). Some popular techniques used in recent Italian studies are light microscopes and compound stereo microscopes equipped with digital cameras (Forin et al. 2018, Wijesinghe et al. 2020, 2021, Abeywickrama et al. 2022, Knudsen et al. 2022). Also, transmission electron microscopic techniques (TEM) are used for observing the ultrastructure of filamentous fungi and their cells, and scanning electron microscopy (SEM) techniques are used to observe significant surface detail of ascospores and conidia in Italy as well as worldwide (Zucconi & Lunghini 1997, Guarro et al. 1999, Casoli & Fornaciari 2014, Lücking et al. 2021, Faoro et al. 2022). Up to now, a number of taxonomical studies of Italian *Ascomycota* have been conducted by expert mycologists, mainly based on morpho-molecular and phylogenetic analyses (Isola et al. 2016, Wanasinghe et al. 2018, Liu et al. 2019, Marin-Felix et al. 2019, La Rosa et al. 2022).

Ascomycota

Ascomycota in Italian terrestrial habitats

Ecologically, fungi colonize diverse ecosystems including terrestrial, aquatic (freshwater and marine), and harsh environments by adopting the most fitted lifestyle through parasitism, mutualism, and saprotrophy for their macroevolutionary success (Onofri et al. 2011, Lutzoni 2018, Wijesinghe et al. 2022). In terrestrial ecosystems, fungal species are associated with living or dead plants by fulfilling their carbon source, and plants may be responsible for the origin and diversification of major fungal lineages (Lutzoni 2018). The great floral diversity in Italy provides more suitable habitats for different fungal groups, so discussing plant-associated microfungi is topical. In considering plant-associated fungi, *Ascomycota* can be found as pathogens, endophytes, epiphytes, and saprobes on different hosts/substrates in different Italian terrestrial habitats (Ragazzi et al. 2003, Angelini et al. 2012, Harrington & McNew 2018, Li et al. 2020, Tennakoon et al. 2020, Belfiori et al. 2021, Wijesinghe et al. 2022). Saprobic taxa play a key role in decomposition and nutrient cycling and are often specialized in decomposing litter or humus produced by dominant and subdominant trees (Persiani et al. 2011, Saitta et al. 2018). Most mycorrhizal and pathogenic taxa are biotrophs, and their niche may be dependent on specific host species or genera (Saitta et al. 2018). Biotrophic pathogens show host-specificity other than necrotrophic fungal species, and they can form a symbiosis with algae (lichens), plant roots (mycorrhizae), or the leaves and stems of plants as endophytes (Saitta et al. 2018).

The reported plant diversity and composition across different Italian biomes and habitats affect the richness and composition of pathogenic, saprotrophic, and mycorrhizal fungi (Gao et al. 2016). Changes in plant and animal richness are known to occur in the mountains along different altitudinal belts, but data on fungal diversity are still limited (Geml 2017). A study by Saitta et al. (2018) revealed that tree species richness has a positive effect on fungal richness while elevation, climatic, and edaphic parameters have negligible impacts. Still, biodiversity, taxonomy, and ecology of fungi in Mediterranean ecosystems are understudied, even though these ecosystems are considered "hotspots" of biodiversity (Brooks et al. 2002, Saitta et al. 2018). Therefore, a growing awareness of the ecological roles, biology, and diseases caused by fungi is important to maintain conservation and the assessment of biodiversity (Varese et al. 2011).

Distribution and diversity of Italian flora

Italy played a key role in European biogeography by providing refuge areas for the flora during the glacial periods (Svenning et al. 2008, Abbate et al. 2015). The Italian Forest Service (CFS 2004), reported that 35% of the total land cover in Italy is woodlands, shrublands, and Mediterranean maquis. In addition, small reforestation areas and hedges rich in native woody flora

are scattered on agricultural sites (Abbate et al. 2015). Across this high plant cover on the mainland, Abbate et al. (2015) reported 469 species belonging to 133 genera and 61 families, as well as 509 units (species and subspecies). These woody species are varied, including 346 shrubs (74%), 106 trees (23%), and 16 lianas (3%) (Abbate et al. 2015). The familial richness of these records ranged in descending numbers from *Rosaceae*, *Fabaceae*, *Salicaceae*, *Pinaceae*, and *Fagaceae* to *Rhamnaceae*. Recent inventories of native and alien vascular flora in Italy have been provided by Bartolucci et al. (2018) and Galasso et al. (2018), respectively. In native vascular flora 8,195 taxa (6,417 species and 1,778 subspecies), including 23 lycophyte taxa, 108 ferns, and fern allies, 30 gymnosperms, and 8,034 angiosperms, were reported (Bartolucci et al. 2018). As alien vascular flora, 1,597 species, subspecies, and hybrids were listed under 2 lycophytes, 11 ferns, fern allies, 33 gymnosperms, and 1,551 angiosperms (Galasso et al. 2018).

The updated land use/land cover map in Italy (Fig. 5) is generated to understand plant distribution based on the data extracted from the ArcGIS Living Atlas (https://livingatlas.arcgis.com/en/home/). This vegetative land cover is characterized by forests that concentrate in the mountain range, while crops and urban areas concentrate in the plains and along the coastal regions (De Fioravante et al. 2021). Plants are categorized under the terms "trees" (dense woody vegetation, savannas, plantations, swamps, or mangroves), "flooded vegetation" (any vegetation in seasonally flooded areas, including grasses, shrubs, trees, and bare grounds), and "crops" (planted cereals, grasses, and crops by humans).
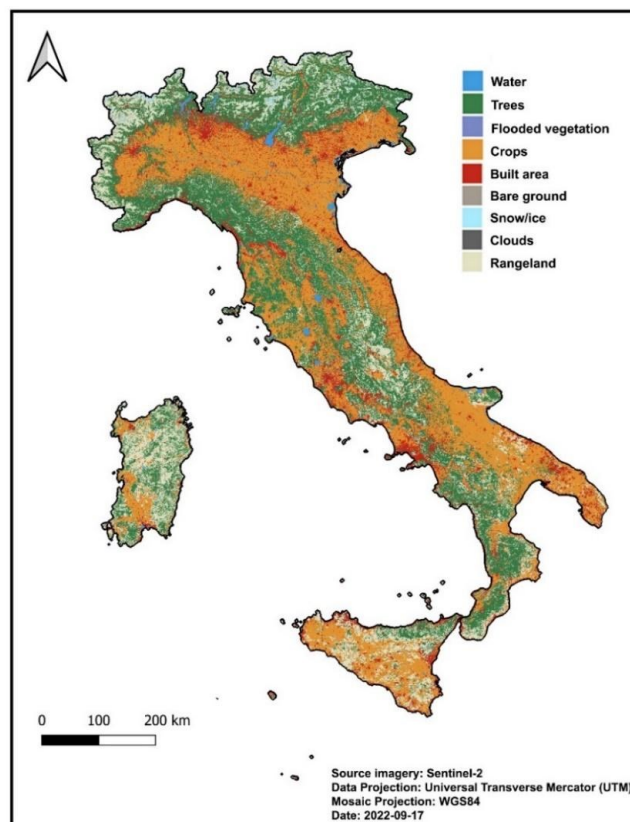

Fig. 5 – Updated Italian land use/land cover map, 2021. Layers of Sentinel–2 10–meter Land Use/Land Cover data extracted from the ArcGIS Living Atlas of the World (https://livingatlas.arcgis.com/en/home/; Karra et al. 2021), produced by Impact Observatory, Microsoft, and Esri, under a Creative Commons by Attribution (CC BY 4.0) license.

Plant-associated *Ascomycota*

While most *Ascomycota* are primarily microscopic, some larger species are included, such as cup-fungi, morels, and truffles (Senn-Irlet et al. 2007, Saitta et al. 2011, Wijesinghe et al. 2022).

They are cosmopolitan, and the majority are associated with numerous host plants in different geographic regions of Italy (Saitta et al. 2011, Wijesinghe et al. 2021, 2022). Studies on the diversity of plant-associated Italian fungi are still understudied (Medardi 2006, Saitta et al. 2011, Granito et al. 2015, Wijesinghe et al. 2021, 2022). In a five-year study, Saitta et al. (2011) recorded 1,582 fungal taxa, including 341 *Ascomycota* from decaying wood (terrestrial) in Italy. Venturella et al. (2011) updated the regional fungal counts in the country following those past records.

The extracted data from Venturella et al. (2011) are arranged in Fig. 6, in order to show the regional *Ascomycota* count in Italy up to 2010. The Sardinia region (1,500 taxa) appears to have the highest number of records, followed by Piedmont (421), Emilia Romagna (322), and Sicily (317), while Abruzzo, Friuli-Venezia Giulia, Lazio, Marche, and Molise have zero records. A few regional checklists have contributed to the Italian list of *Ascomycota*: Venturella (1991) for Sicily; Angelini et al. (2016, 2017), Wagensommer et al. (2018) and Venanzoni et al. (2019) for Umbria; and Medardi (2006) for Emilia-Romagna. Saitta et al. (2011) provided a national checklist of woody fungi. Later, Wijesinghe et al. (2022) provided an updated national checklist for *Fagales* inhabiting *Ascomycota* in the country up to 2021.
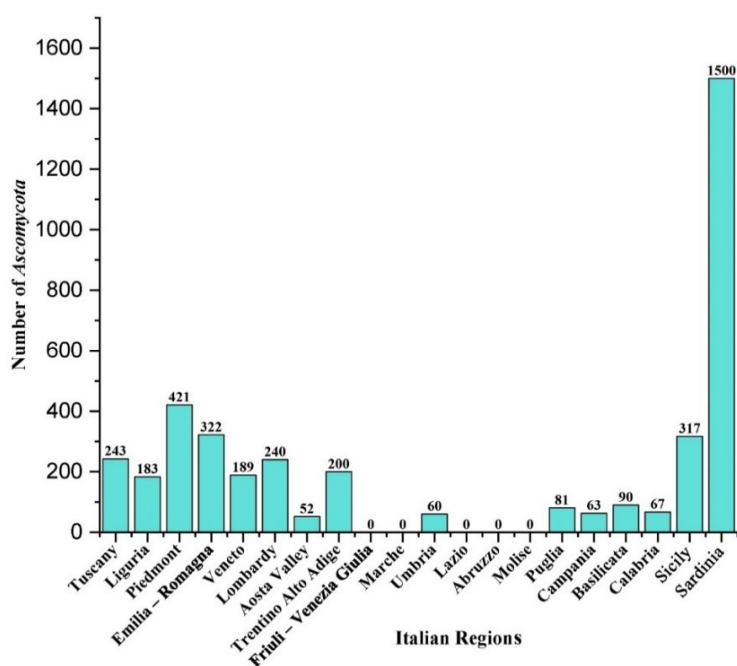
Fig. 6 – Number of *Ascomycota* in Italian regions (up to 2010), data reported by Venturella et al. (2011).

Over the last two decades, several Italian studies were carried out and new records of *Ascomycota* reported from various natural forests and agricultural lands, especially from diseased plants, dead wood, leaf litter, and root substrates (Ragazzi et al. 2003, Zucconi & Pasqualetti 2007, D'Amico et al. 2008, Jensen et al. 2010, Saitta et al. 2011, Angelini et al. 2012, Nigro et al. 2013, Ariyawansa et al. 2015a, Granito et al. 2015, Linaldeddu et al. 2015, 2016, Dissanayake et al. 2016a, 2017a, Tibpromma et al. 2017, Wanasinghe et al. 2018, Hyde et al. 2019, 2020a, b, Liu et al. 2019, Urbez-Torres et al. 2020, Belfiori et al. 2021, Abeywickrama et al. 2022, Manawasinghe et al. 2022, Mang et al. 2022). Also, plant-associated lichens and lichenicolous fungal studies significantly contribute to Italian mycology (Nimis 1993, 2016, von Brackel 2012, 2015, 2021, Gheza et al. 2022).

The majority of the recent taxonomic studies on plants inhabiting microfungi were based on biphasic approaches of morphology and molecular phylogeny (Hyde et al. 2020a, b, Li et al. 2020, Boonmee et al. 2021, Chethana et al. 2021a, Abeywickrama et al. 2022, La Rosa et al. 2022). These studies discovered novel microfungal taxa, genera, families, and orders in different fungal classes

in *Ascomycota*, including novel hosts and geographical records with taxonomic updates. Re-collecting fresh fungal specimens for re-describing species provides novel hosts and regional records of Italy, as well as epitypes and authentic herbarium materials for extant species with sequence data (Ariyawansa et al. 2014, Thambugala et al. 2014, Wijesinghe et al. 2021, 2022). As an example, Thambugala et al. (2014) designated the epitype for *Phaeosphaeriopsis glaucopunctata* (Fig. 7) based on a fresh collection (in 2012) of dead leaves of *Ruscus aculeatus*, from Italy. They provided sexual and asexual morphologies of the fungus with detailed descriptions and sequence data.

Fig. 7 – *Phaeosphaeriopsis glaucopunctata* (MFLU 14-0029, epitype). a–b Ascomata on the host substrate. c Longitudinal section of an ascoma. d Peridium. e Pseudoparaphyses. f–g Asci. h–j Ascospores. k Pycnidia on PDA. l Pycnidial wall. m Conidiogenous cells. n Conidia. Scale bars: k = 500 µm, b = 100 µm, c, f–g = 20 µm. d, l–n = 10 µm, h–j = 5 µm. See Thambugala et al. (2014) for the descriptions.

Data documentation and mapping

In Italy, ecological and mycogeographical data for larger fungi (*Ascomycota* and *Basidiomycota*) are scattered in many publications by a few university-employed mycologists and many amateur groups (Venturella 2009, Venturella et al. 2011). Due to the difficulties in collaboration between academics and amateurs and the diversity of the geography, climate, and geology of Italy, determining the actual fungal count is challenging (Venturella et al. 2011). However, inventorying of previously published mycological data in journal articles, books, book

chapters, theses, and websites provides strong evidence of great fungal diversity in Italy. Saitta et al. (2011) suggested that the published data still needs to be well organized to outline the geographic patterns that demonstrate the ecological diversity within the group for an accurate estimation of species richness.

In terms of gathering and reorganizing scattered mycological data, an online documentation (https://italianmicrofungi.org/) for plant-associated microfungi in Italy was launched by the Centre of Excellence in Fungal Research (CEFR) team (Wijesinghe et al. 2021). Species identification is processed through morpho-molecular and phylogenetic analyses, including ecological data. Also, *Ascomycota* records are provided with their recent taxonomic updates and detailed morphological descriptions and distribution data. A checklist is currently being updated with *Ascomycota* records (https://italianmicrofungi.org/news.php) that are confirmed by biphasic approaches on this website. The updated plant-associated *Ascomycota* count on the web page is 628 (874 records), and the host: fungal ratio is 239:628 over 10 years (Wijesinghe et al. 2021).

For Italian lichens, Nimis (1993) provided a checklist, and later, it was updated by Nimis (2016), which included 2,704 accepted infrageneric taxa, of which 2,565 are lichenized. Currently, lichenized and lichen-associated taxa are being compiled in an online database by Nimis & Martellos (2022) as ITALIC 6.0 (http://italic.units.it/) including data on their taxonomy and geography (maps).

In considering the mapping of Italian mycological data, national or regional programs have provided useful information about fungal diversity, species distribution, rarity, and decline, as well as data for fungal red lists (Courtecuisse 2001, Lo Bue 1996, Venturella 2009, Saitta et al. 2011, Wagensommer et al. 2018). Most of the mapping concerned Italian macrofungal taxa (truffles) in different regions; i.e., Tuscany by Baglioni & Gardin (1998), Emilia-Romagna by Tibiletti & Zambonelli (1999) and Biagioni et al. (2005), Abruzzo by De Laurentis & Spinelli (2006), and Liguria by Pavarino et al. (2011). As a baseline study by mycologists, eight important Italian locations were demarcated in the project, mapping for "Important Plant Areas" under "Important Fungus Areas" based on macromycetes (Venturella et al. 2011). However, no intensive effort has been made to compile scattered mycogeographical data and map plant-associated *Ascomycota* except Italian lichens and lichen-associated taxa.

Rationale

According to the history and current information on Italian mycology, the study of plant-associated *Ascomycota* in terrestrial habitats is still important. Many recent studies have expanded the knowledge of fungal taxonomy by increasing the number of taxa on different plant hosts. However, the hidden fungal diversity in vegetative ecosystems in Italy has not yet been fully investigated. The following research gaps have been identified: 1) fewer taxonomic studies have been conducted on *Ascomycota* compared to *Basidiomycota*; 2) studies have been restricted to certain Italian regions; 3) in some cases, polyphasic taxonomic approaches were poorly applied for species identification; 4) published data were scattered and frequently deficient in ecological and distributional data; 5) there are no maps for the fungal distribution.

Objectives

This study aims to recall the history of Italian mycology and to understand the current study gaps in fungal taxonomy, regional fungal counts, data listing, and mapping based on experiences with *Ascomycota*. Some insights into the occurrence of *Ascomycota* on decaying plant hosts from vegetatively rich habitats in Italy are provided to expand the knowledge of fungal diversity. Based on this, twenty-four microfungal taxa in *Dothideomycetes* and *Sordariomycetes* including a novel genus, two new species, several novel host records, and regional records are described. To bridge the knowledge gaps of modern taxonomy, studies based on biphasic approaches are conducted with complete illustrations, comprehensive descriptions, and updated phylogenetic trees for each taxon. To narrow the ecological and geographical knowledge gaps, host-fungal relationships with mycogeography are addressed. Updated fungal-host distribution maps are provided for our fungal

collection, as well as for the checklist (2013–2022), available on the Italian microfungi website (https://italianmicrofungi.org/news.php). All results will be linked to an online database of Italian microfungi (https://italianmicrofungi.org/) to provide well-documented mycological data. This study will encourage taxonomic studies on plant-associated fungi in order to understand the hidden diversity and host-fungal relationships, including distributions in different Italian regions, especially where such data is lacking.

Materials & Methods

Taxonomy and phylogenetic analyses

Sample collection, morphological studies, and isolation

Decaying branches and stems of dead plant species were randomly collected from different Italian sites in the provinces of Forlì-Cesena, Ravenna (Emilia-Romagna region), and Trento (Trentino-Alto Adige region), in the years 2017 to 2021. The host plants of the collected fungi belong to dead Dicotyledons (*Cornales, Fabales, Fagales, Gentianales, Laurales*), Monocotyledons (*Poales*), and Gymnosperms (*Pinales*). In the laboratory, samples were stored in sterile Ziploc bags at 16–18 °C. A Motic SMZ 168 compound stereo microscope was used to examine the macro-morphological characters of the samples. Hand-sections of structures were examined for micro-morphology using a Nikon ECLIPSE 80i compound stereo microscope equipped with a Canon 600D digital camera. The measurements were obtained using Tarosoft (R) Image FrameWork version 0.9.7. Images were edited with Adobe Photoshop CS6 Extended version 13.0.1 software (Adobe Systems, San Jose, California).

Single-spore isolation was carried out based on the methodologies described by Senanayake et al. (2020). For taxa that lacked cultures, fruiting bodies were used for DNA extraction (Wanasinghe et al. 2018). The observed fungal specimens were deposited at the Mae Fah Luang University Herbarium (MFLU) (Chiang Rai, Thailand). The living cultures were deposited at the Mae Fah Luang Culture Collection (MFLUCC). Both Facesoffungi and Index Fungorum numbers were obtained for taxonomic novelties (Jayasiri et al. 2015, Index Fungorum 2022).

DNA extraction, PCR amplification, and sequencing

The methodologies for DNA extraction, PCR, gel electrophoresis, and sequencing were followed, as detailed in Dissanayake et al. (2020). Genomic DNA was isolated from fruiting bodies or from scraped fresh fungal mycelium grown on PDA media for six weeks at 18 °C, using the Ezgene Fungal Gdna Extraction Kit GD2416 (Biomiga, Shanghai, China), following the manufacturer's instructions. Sequences were generated for different gene regions, namely the internal transcribed spacer region (ITS), partial large subunit (LSU), partial small subunit (SSU), partial RNA polymerase II second-largest subunit (*rpb*2), partial translation elongation factor 1-α (*tef*1-α), and beta-tubulin (*tub*2). The primers used for PCR amplifications and the thermal cycling programs used are reported in Table 3.

Molecular data analyses

Sequences with high similarity indices for all analyses in this study were determined separately in BLASTn searches of the NCBI. Contig sequences were analyzed with other sequences downloaded from GenBank. Each individual gene matrix was aligned with MAFFT version 7 (Katoh & Standley 2013, Katoh et al. 2019) with default parameters. The trimAl v1.4 software was used for the automated removal of spurious sequences or poorly aligned regions in each single gene alignment, and the gappyout automated trimming method was used (Capella-Gutiérrez et al. 2009).

Two separate phylogenetic analyses were conducted: maximum likelihood (ML) and Bayesian inference (BI) using the CIPRES Science Gateway portal (Miller & Pfeiffer 2012) for each data set. The ML trees were generated for the final concatenated alignment using RAxMLHPC2 on the XSEDE (v. 8.2.10) tool (Stamatakis 2014) with 1,000 replicates of

bootstrapping. The BI analyses were computed with MrBayes on the XSEDE 3.2.7a tool in the CIPRES, and six simultaneous Markov chains were run for different generations. Trees were sampled every 1000 generations, with the run ending automatically when the standard deviation of split frequencies dropped below 0.01. For both ML and BI, MrModeltest version 2.3 (Nylander 2004) was run under the Akaike Information Criterion implemented in PAUP version 4.0 b10 (Swofford 2003) to estimate the best evolutionary model. Phylogenetic trees were visualized with FigTree version 1.4.0 (Rambaut 2012) and edited in Adobe Illustrator (Adobe Inc.).

Table 3 Gene regions, related primers, and PCR thermal cycle programs used in this study, with reference(s).

Genes/ loci	PCR primers (forward/ reverse)	PCR conditions	Reference(s)
ITS and LSU	ITS5/ITS4 and LR0R/LR5	94°C; 2 min (95°C; 30 s, 55°C; 50 s, 72°C; 90 s) × 35 thermal cycles, 72°C; 10 min.	White et al. (1990), Vilgalys & Hester (1990)
SSU	NS1/NS4	95°C; 3 min (95°C; 30 s, 55°C; 50 s, 72°C; 30 s) × 35 thermal cycles, 72°C; 10 min.	White et al. (1990)
*tub*2	Bt2a/Bt2b	94°C; 3 min (94°C; 30 s, 55°C; 50 s, 72°C; 1 min) × 35 thermal cycles, 72°C; 10 min.	Glass & Donaldson (1995)
*tef*1-α	EF1-983F/ EF1-2218R	94°C; 2 min (95°C; 30 s, 58°C; 50 s, 72°C; 1 min) × 35 thermal cycles, 72°C; 10 min.	Rehner (2001), Carbone & Kohn (1999)
	EF1-728F/ EF1-986F	95 °C: 5 min, (94 °C: 30 s, 55 °C:45 s, 72 °C: 90 s) × 30 cycles, 72 °C: 10 min	
*rpb*2	RPB2-5f RPB2-7cR	94 °C: 2 min, (95 °C: 45 s, 57 °C:50 s, 72 °C: 90 s) × 35 cycles, 72 °C: 10 min	Liu et al. (1999)
act	ACT-512F/ACT-783R	95°C; 5 min (95°C; 30 s, 55°C; 50 s, 72°C; 1 min) × 39 thermal cycles, 72°C; 10 min.	Carbone & Kohn (1999)

Mapping: Spatial distribution of plant-associated *Ascomycota*

The spatial distribution of *Ascomycota* reported in this study was mapped. Software from QGIS 3.20.3 (Quantum Geographic Information System) was used for mapping and analyses.

Results

Taxonomy & phylogeny

Dothideomycetes incertae sedis

Botryosphaeriales C.L. Schoch, Crous & Shoemaker 2007
Index Fungorum number: IF 501513; Facesoffungi number: FoF 07659
Notes – Schoch et al. (2006) introduced *Botryosphaeriales* (*Dothideomycetes*) to accommodate a single family, *Botryosphaeriaceae*. Sexual morph species are having uni or multilocular ascostromata, multi-layered walls embedded in stromatic tissue, bitunicate, clavate asci with a thick endotunica intermixed with pseudoparaphyses, ellipsoid to ovoid, aseptate or septate, hyaline or pigmented ascospores that lack appendages or a mucilaginous sheath (Phillips et al. 2019, Hongsanan et al. 2020a). Asexual morph species are characterized by uni to multilocular, pycnidial conidiomata, phialidic or annelidic conidiogenous cells, and aseptate or septate, hyaline or pigmented conidia with or without mucoid appendages or sheaths (Phillips et al. 2019, Hongsanan et al. 2020a). Members of *Botryosphaeriales* show worldwide distribution on diverse host plants as saprobes, endophytes, or plant pathogens, and even opportunistic human pathogens (Yang et al. 2017, Phillips et al. 2019, Tennakoon et al. 2021). The estimated crown age of

Botryosphaeriales is to be 110 Mya (Phillips et al. 2019). Currently, six families: *Aplosporellaceae*, *Botryosphaeriaceae*, *Melanopsaceae*, *Phyllostictaceae*, *Planistromellaceae*, and *Saccharataceae* are accepted in the order (Phillips et al. 2019, Wijayawardene et al. 2022, Wu et al. 2021). We follow the updated accounts of *Botryosphaeriaceae* by Zhang et al. (2021), and the latest classification by Wijayawardene et al. (2022) including our justifications.

Botryosphaeriaceae Theiss. & Syd. 1918

Index Fungorum number: IF 80530; Facesoffungi number: FoF 00116

Notes – *Botryosphaeriaceae* was introduced by Theissen & Sydow (1918) to accommodate *Botryosphaeria*, *Dibotryon*, and *Phaeobotryon*. Over the years, many taxonomic revisions and updates for the family were performed based on morphology and molecular data (Dissanayake et al. 2016b, 2021a, Hongsanan et al. 2020a, Rathnayaka et al. 2021, 2022a, b, 2023, Wu et al. 2021). Key sexual morphological characters of *Botryosphaeriaceae* are uni- or rarely multiloculate ascostromata, multi-layered walls, 8-spored, bitunicate, clavate asci with thick endotunica, septate, branched, or unbranched pseudoparaphyses, and 0–2-septate, hyaline to brown ascospores (Phillips et al. 2019, Hongsanan et al. 2020a). Asexual morph species produce uni to multiloculate pycnidial conidiomata, phialidic, hyaline, or pigmented conidiogenous cells with septate or aseptate conidia, spermatogonia that morphologically similar to conidiomata, ampulliform to lageniform or subcylindrical, phialidic spermatogenous cells, subcylindrical or dumbbell-shaped, hyaline, spermatia with rounded ends developing in conidiomata or spermatogonia (Slippers et al. 2013, Phillips et al. 2013, 2019, Hongsanan et al. 2020a, Tennakoon et al. 2021). *Botryosphaeriaceae* diverged approximately 94 Mya in the Cretaceous period, with a crown age of 61 Mya in the Paleogene period (Phillips et al. 2019, Tennakoon et al. 2021). Current taxonomic updates accepted 22 genera in the family, and they were distributed as endophytes, saprobes, and plant pathogens on a wide range of hosts with no host specificity (Rathnayaka et al. 2021, Wijayawardene et al. 2022). In this study, we discuss saprobic botryosphaeriaceous taxa belonging to *Botryosphaeria, Diplodia*, *Dothiorella,* and *Mucoharknessia* collected at different Italian sites.

Botryosphaeria Ces. & De Not. 1863

Index Fungorum number: IF 635; Facesoffungi number: FoF 00141

Notes – *Botryosphaeria* was described by Cesati & De Notaris (1863), including 12 species lacking detailed morphological descriptions. Also, they did not select a type species for the genus. Later, Barr (1972) designated *B. dothidea* (= *Sphaeria dothidea*) as the lectotype species and validated the genus. Sexual morphs are characterized by having aseptate and hyaline ascospores that sometimes become septate and are pale brown at maturity (Phillips et al. 2005, Jayawardena et al. 2018a, Hongsanan et al. 2020a). Asexual morphs have aseptate, hyaline conidia that become pigmented, and 1–2-septate before germination or at maturity (Phillips et al. 2013, Rathnayaka et al. 2022a). *Botryosphaeria* species are pathogens, endophytes, and saprobes and mostly cause die-back and canker diseases (Desprez-Loustau et al. 2006, Marsberg et al. 2017, Dong & Guo 2020, Hattori et al. 2021, Rathnayaka et al. 2022a). They can be found on diverse hosts such as monocotyledonous, dicotyledonous, and gymnosperms worldwide (Darge & Woldemariam 2021, Rathnayaka et al. 2022a). Recently, Zhang et al. (2021) referred previously misidentified specimens to other species, such as *Botryosphaeria auasmontanum*, *B. minutispermatia*, *B. qinlingensis*, *B. quercus*, *B. sinensis*, and *B. wangensis*, which were accepted as synonyms of *B. dothidea*.

Botryosphaeria dothidea (Moug.) Ces. & De Not. 1863   Fig. 9

≡ *Sphaeria dothidea* Moug., in Fries, Syst. mycol. (Lundae) 2(2): 423 (1823)

Index Fungorum number: IF 183247; Facesoffungi number: FoF 03512

Saprobic on a dead and fallen branch of *Robinia pseudoacacia* L. Sexual morph: see Phillips et al. (2013). Asexual morph: Coelomycetous. *Conidiomata* 250–290 × 270–350 μm (x̄ = 280 × 320 μm, n = 5), pycnidial, stromatic, globose to subglobose, semi-immersed, erumpent, aggregate 20–100, uni-loculate, dark brown. *Conidiomata wall* 40–50 μm wide at the sides, 60–70 μm wide at

the base, pseudoparenchymatous, composed 8–10 layered, outermost layers thick-walled brown cells, innermost layers composed of thin-walled pale brown to hyaline cells, arranged in *textura angularis*. *Conidiophores* 6–7 × 4–5 μm, cylindrical, thin-walled, sometimes branched at the base, hyaline, or reduced to conidiogenous cells. *Conidiogenous cells* 7–12 × 2–3 μm (x̄ = 9.5 × 2.5 μm, n = 10), holoblastic, phialidic, subcylindrical, discrete, smooth, and hyaline. *Conidia* 20–30 × 4–7 μm (x̄ = 26 × 6.3 μm n = 20; l/w = 4.1), unicellular, narrowly fusiform, with a sub-truncate to bluntly rounded base, smooth-walled, with a granular content, hyaline. *Spermatophores* 5–12 × 1–2 μm, occasionally branched, cylindrical, hyaline. *Spermatogenous cells* cylindrical to subcylindrical, holoblastic, and hyaline. *Spermatia* 4−6.5 × 1.5−2 μm, allantoid to rod-shaped, unicellular, hyaline.

Material examined – Italy, Province of Forlì-Cesena [FC]), Fratta Terme, on a dead and fallen branch of *Robinia pseudacacia* L. (*Fabaceae*), 28 November 2018, Erio Camporesi, IT 4129 (MFLU 19-0289).

GenBank Accession Numbers – ITS: OQ401062; *tef*1-α: OQ437911; *tub*2: OQ437901.

Notes – In the phylogenetic analysis of *Botryosphaeria*, our strain MFLU 19-0289 grouped with *Botryosphaeria dothidea* (CBS 110302, CMW8000, and PPO 46523) with 94% MLBS and 1.00 BYPP support (Fig. 8, Clade A). The comparison of ITS and *tef*1-α sequence data reveals there is no difference between our strain MFLU 19-0289 and *B. dothidea* strains, while the *tub*2 comparison showed only a single base pair (bp) difference out of a total of 434 bp. Our collection MFLU 19-0289 shows the key characteristics of *B. dothidea*; pycnidial conidiomata, narrowly fusiform, unicellular conidia with a subtruncate to bluntly rounded base. Also, the morphologies of holoblastic and subcylindrical conidiogenous cells, spermatia, and spermatophores resemble those of *B. dothidea* (Slippers et al. 2004, Phillips et al. 2013, Jayawardena et al. 2018a). Based on the above morphology-based phylogenetic analyses, we conclude that our collection is *B. dothidea*, and this is the first report on *Robinia pseudoacacia* in Italy. The known distribution of the fungus on *R. pseudacacia* is in Georgia and the Netherlands (Hanlin 1963, Farr & Rossman 2022).

Diplodia Fr. 1834

Index Fungorum number: IF 8047; Facesoffungi number: FoF 00147

Notes – *Diplodia* was introduced by Montagne (1834) based on the type species *D. mutila*. There are two distinct conidial morphologies reported in *Diplodia* species based on pigmentation and septation (Phillips et al. 2013, Hongsanan et al. 2020a, Tennakoon et al. 2021). Some conidia are initially hyaline and aseptate and later become pale to dark brown and 1-septate. This change occurs after spores have been released from the conidiomata, and in such cases, many brown, 1-septate conidia can be found on the host surface surrounding the pycnidia. The other type of conidia becomes pigmented early in development, even inside enclosed pycnidia, and only rarely becomes septate (Phillips et al. 2013, Hongsanan et al. 2020a). *Diplodia* species have wide distributions on diverse woody hosts as pathogens, saprobes, and endophytes (Phillips et al. 2012, 2013).

Diplodia sapinea (Fr.) Fuckel 1870   Fig. 11

≡ *Sphaeria sapinea* Fr., Syst. mycol. (Lundae) 2(2): 491. 1823
= *Diplodia rosacearum* S. Giambra et al., Mycosphere 7: 983. 2016
= *Diplodia italica* Wijayaw. et al., Fungal Diversity 77: 110. 2016
= *Diplodia intermedia* A.J.L. Phillips et al., Persoonia 29:33. 2012
Index Fungorum number: IF 146913; Facesoffungi number: FoF 05292

Saprobic or *pathogenic* on a dead aerial branch of *Prunus* sp. Sexual morph: Undetermined. Asexual morph: *Conidiomata* 250–300 μm diam., 240–270 μm high (x̄ = 270 × 250 μm, n = 5), pycnidial, stromatic, immersed, solitary, scattered, becoming erumpent at maturity, globose to subglobose, unilocular, ostiolate, dark brown to black. *Ostioles* papillate, central, circular, single. *Conidiomata wall* 30–40 μm wide at the sides, 40–45 μm wide at the base, composed of several layers, outermost layers with thick-walled brown cells, innermost layers with thin-walled, pale brown to hyaline cells, arranged in *textura angularis*. *Conidiophores* reduced to conidiogenous cells. *Conidiogenous cells* 3–10 × 2–3 μm, cylindrical, long, sometimes slightly swollen at the base,

enteroblastic, phialidic, periclinal thickening, and producing single conidia at the tip, determinate, and hyaline. *Paraphyses* lay between and intermingled with conidiogenous cells, wide at the base (1.8–2.2 μm) and gradually becoming thinner towards the apex (0.6–0.7 μm). *Conidia* 12–14 × 5–8 μm ($\bar{x}$ = 12.5 × 7.5 μm, n = 20; l/w = 1.6), oblong to ovoid, widest in the middle, obtuse apex or slightly rounded, truncate at base, aseptate, thick or smooth-walled, guttulate, initially hyaline, brown to dark brown at maturity before releasing from the pycnidia.
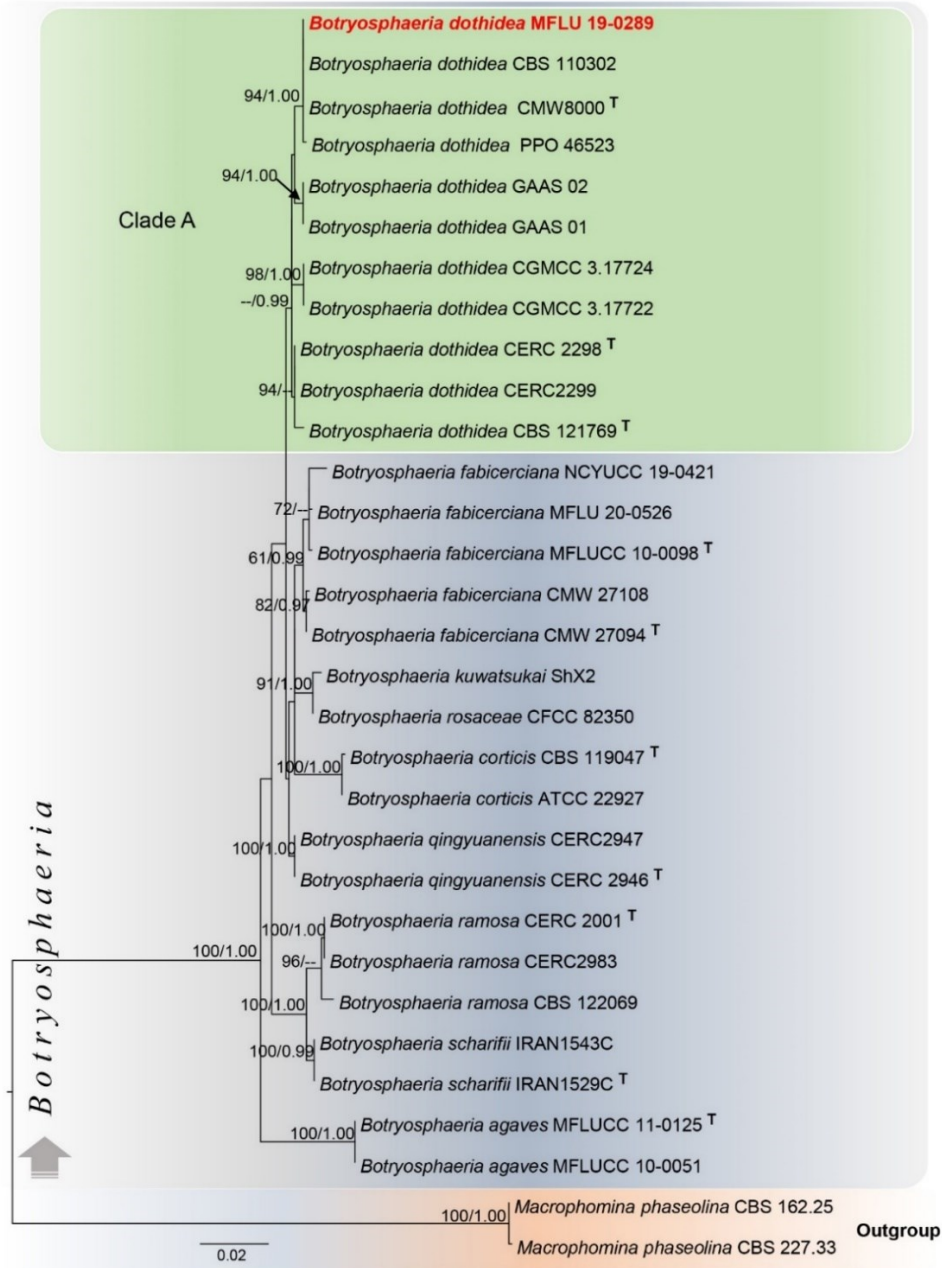
Fig. 8 – Phylogram generated from maximum likelihood analysis based on combined ITS, *tef*1-α and *tub*2 sequenced data. Thirty-one strains were included in the combined sequence analyses, which comprised 1209 characters with gaps (ITS = 492, *tef*1-α = 276, *tub*2 = 434). Single gene analyses were also performed, and topology and clade stability were compared from combined gene analyses. *Macrophomina phaseolina* (CBS 162.25 and CBS 227.33) was used as the outgroup taxon. The final ML optimization likelihood is -2899.991693. The matrix included 187 distinct alignment patterns, with 9.79% undetermined characters or gaps. Estimated base frequencies were obtained as follows: A = 0.208643, C = 0.308619, G = 0.255199, T = 0.227540; substitution rates AC = 0.756466, AG = 3.051284, AT = 1.026958, CG = 0.871125, CT = 5.835318, GT = 1.0;

gamma distribution. Bootstrap support values for ML (first set) equal to or greater than 70%, BYPP equal to or greater than 0.95 are given above or below the nodes. The strain from the current study is in red bold, and the type strains are indicated with [T].
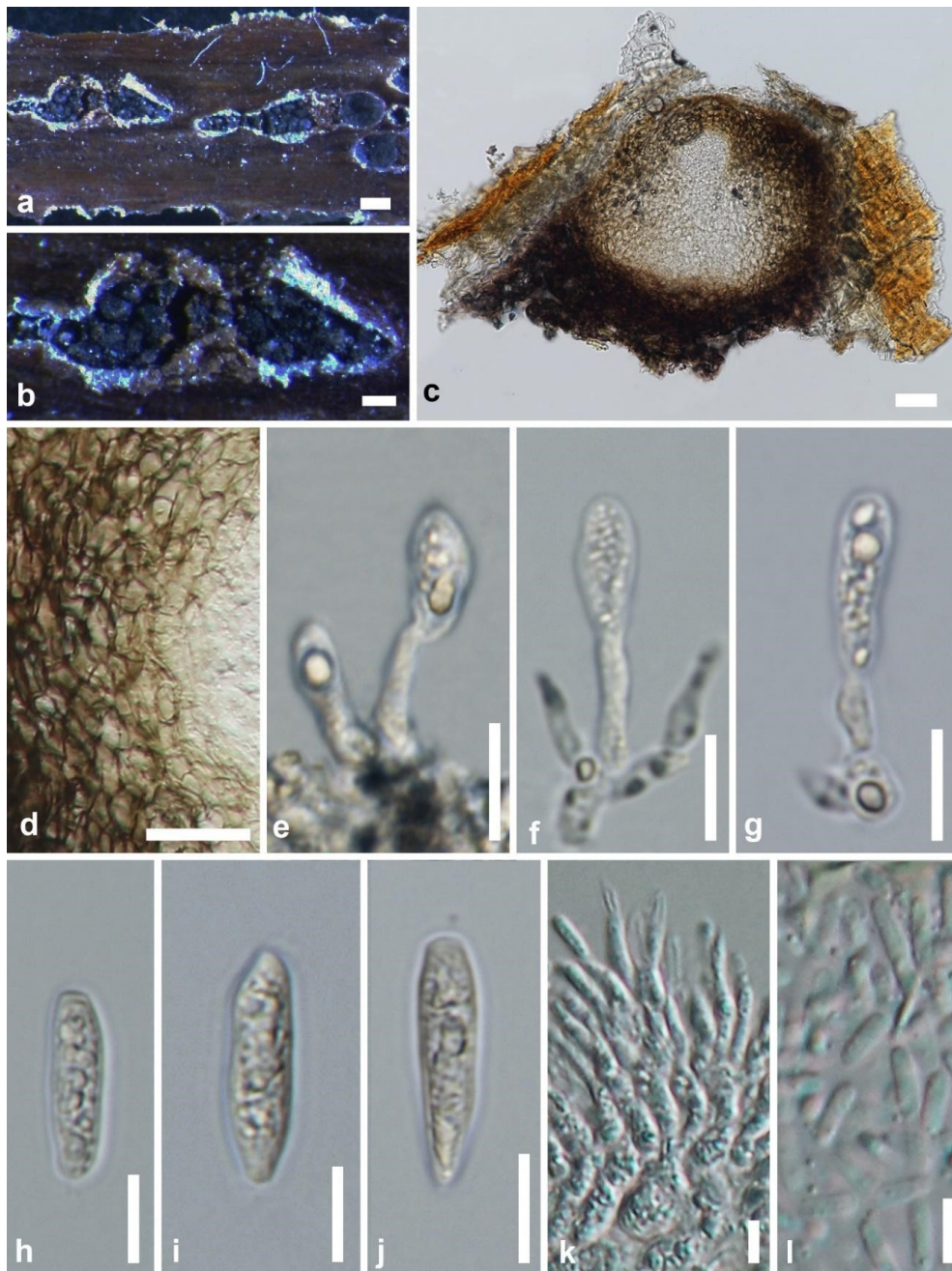
Fig. 9 – *Botryosphaeria dothidea* (MFLU 19-0289, new host record in Italy). a–b Conidiomata on the dead host surface of *Robinia pseudoacacia* L. (*Fabaceae*, *Fabales*). c Longitudinal section of a conidioma. d Conidiomata wall. e–g Conidiophores and stages of conidiogenesis. h–j Conidiospores. k Spermatophores and spermatogenous cells. l Spermatia. Scale bars: a = 100 µm, b, c = 50 µm, d = 20 µm, e–j = 10 µm, k–l = 5 µm.

GenBank Accession Numbers – ITS: OQ401051; *tef*1-α: OQ437907.

Material examined – Italy, Province of Forlì-Cesena [FC]), near Meldola, on a dead aerial branch of *Prunus* sp. (*Rosaceae*), 23 May 2016, Erio Camporesi, IT 978 (MFLU 16-1443).

Notes – The current name of *Diplodia sapinea* name is mentioned as *Sphaeropsis sapinea* in the Species Fungorum (2022). However, in a recent analysis, Zhang et al. (2021) accepted *Diplodia intermedia*, *D. italica* and *D. rosacearum* as synonyms for *D. sapinea* (Fig. 10, Clade A). In our

ITS and *tef*1-α phylogeny, MFLU 16-1443 grouped with the *D. sapinea* group (Fig. 10, Clade A) with 93% MLBS support. Among these species, our strain (MFLU 16-1443) is phylogenetically more closely related to *D. sapinea* (CAP330, CAA802, and MAEC 17) (Fig. 10). These strains share similar morphologies with our specimen (MFLU 16-1443) in having pycnidial, immersed to erumpent, unilocular, conidiomata with cells arranged in *textura angularis,* cylindrical, conidiogenous cells with periclinal thickening and swollen bases, as well as aseptate, ovoid conidia that become pigmented inside the pycnidia (Fig. 11) (Phillips et al. 2013, Giambra et al. 2016). However, we found pycnidial paraphyses between conidiogenous cells of our collection, which *Diplodia* species lack (Fig. 11) (Phillips et al. 2013). Based on the similar key morphologies and phylogenetic relationships, we conclude that our collection is *D. sapinea*. In the past, *D. sapinea* was found on *Crataegus* sp. by Wijayawardene et al. (2016) (as *D. italica*), on *Eriobotrya japonica* by Giambra et al. (2016) (as *Diplodia rosacearum*), and as *D. sapinea* on *Pinus nigra*, *P. sylvestris*, *P. halepensis*, *P. pinaster* and *P. resinosa* by Luchi et al. (2014) in Italy. Additional known distribution of the fungus on *P. persica* is in Western Cape and South Africa (Jami et al. 2017). Our collection is the first report of this fungus on *Prunus* sp. in Italy.

Dothiorella Sacc. 1880

Index Fungorum number: IF 8098; Facesoffungi number: FoF 00148

Notes – *Dothiorella* was introduced by Saccardo (1880), with *D. pyrenophora* as the type species. *Dothiorella* was resurrected by Phillips et al. (2005), to accommodate species characterized by the asexual morph of brown, 1-septate conidia that become brown while attached to the conidiogenous cells and the sexual morph of pigmented, 1-septate ascospores (Phillips et al. 2005, Dissanayake et al. 2016b, You et al. 2017, Rathnayaka et al. 2022a, b). *Dothiorella* taxa have been mistaken for *Diplodia* taxa because they have similar morphologies of 1-septate, pigmented conidia (Denman et al. 2000, Ivanová 2018, Hongsanan et al. 2020a); however, these conidial characters can be seen inside the pycnidium. Previously, *Spencermartinsia* was considered a separate genus based on its apiculi on ascospores (Phillips et al. 2008, 2013), and Yang et al. (2017) synonymized it under *Dothiorella* based on the SSU, ITS, LSU, *tef*1-α, and *tub*2 phylogeny. The sexual morph of *Dothiorella* is rarely found on natural substrates and has never been reported in culture (Hongsanan et al. 2020a). *Dothiorella* species are widely distributed as pathogens, endophytes, and saprobes on diverse woody hosts (Phillips et al. 2013, Dissanayake et al. 2016b, Hongsanan et al. 2020a, Rathnayaka et al. 2022a, b). In this study, we found the sexual morph of two *Dothiorella* species as saprobes on woody substrates from Italy.

Dothiorella iberica A.J.L. Phillips, J. Luque & A. Alves 2005   Fig. 13

Index Fungorum number: IF 344530; Facesoffungi number: FoF 03513

Saprobic on a dead aerial branch of *Quercus ilex* L. Sexual morph: *Ascomata* 250–350 µm diam., 275–400 µm high (x̄ = 275 × 300 µm, n = 5), pseudothecial, globose, scattered on the substrate, semi-immersed, erumpent at maturity, ostiolate, black. *Ostioles* 60–70 µm high, circular, papillate, central, single. *Peridium* 40–55 µm thick, several layers, from outer to inside dark brown to hyaline cells of *textura angularis*. *Hamathecium* comprises 2–3 µm wide, thin-walled, septate, hyaline numerous pseudoparaphyses. *Asci* 60–140 × 12–22 µm (x̄ = 95 × 15 µm, n = 10), stipitate, arising from the base of the ascoma, cylindric-clavate, bitunicate, with a well-developed apical chamber, 8-spored, uniseriate, biseriate or multiseriate. *Ascospores* 19–25 × 7–12 µm (x̄ = 22 × 9 µm, n = 10; l/w = 2.4), elliptical, widest in the middle part, aseptate to 1-septate, slightly constricted at the septum, tapering towards the base, moderately thick-walled, finely verruculose on the inner surface, initially hyaline, becoming dark brown at maturity. Asexual morph: see Phillips et al. (2013).

Material examined – Italy, Province of Forlì-Cesena [FC]), near Colmano, on a dead aerial branch of *Quercus ilex* L. (*Fagaceae*), 27 November 2018, Erio Camporesi, IT 4127b (MFLU 19-0302).
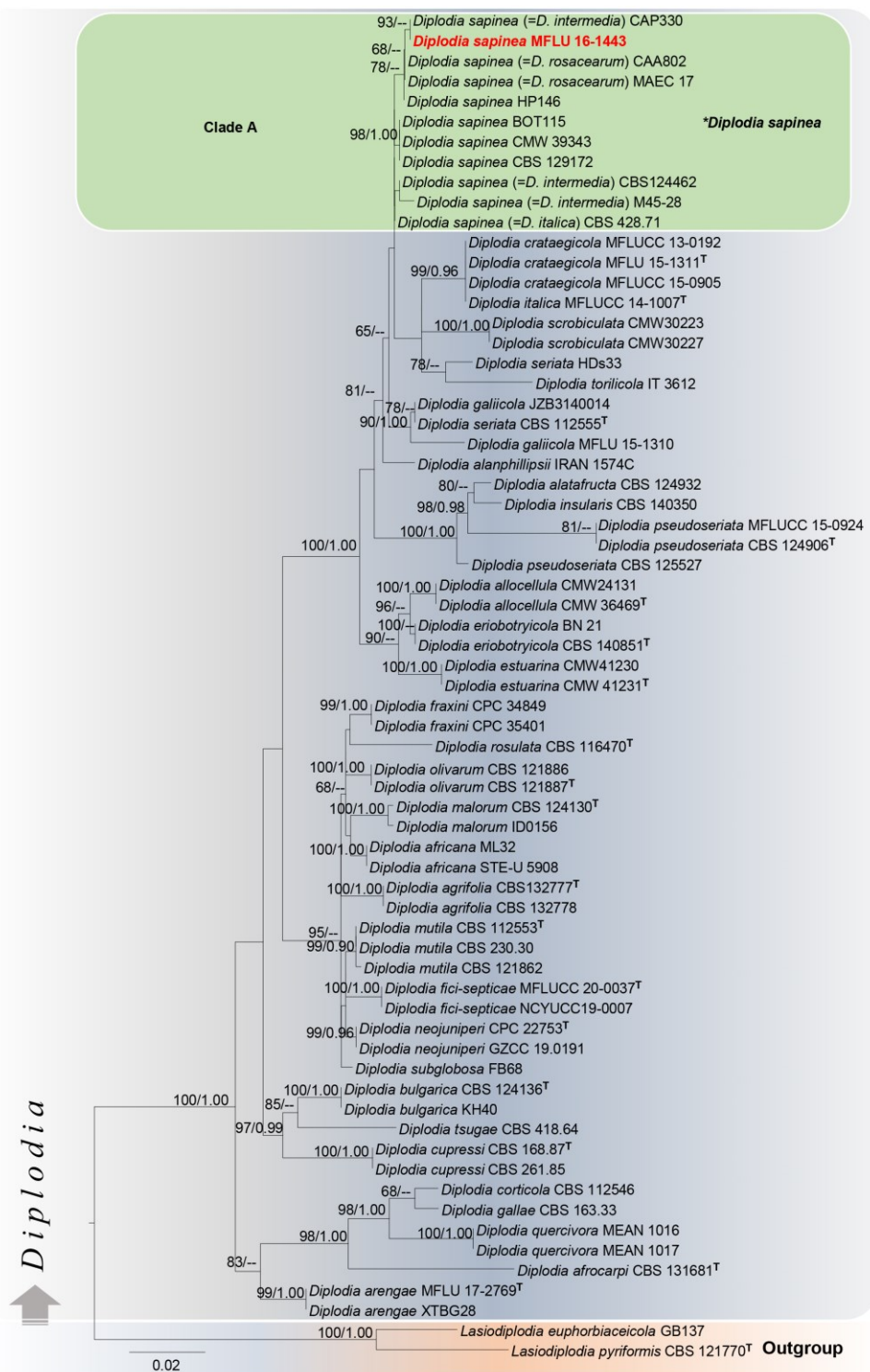
Fig. 10 – Phylogram generated from maximum likelihood analysis based on combined ITS and *tef*1-α sequenced data. Sixty-seven strains were included in the combined sequence analyses, which comprised 797 characters with gaps (ITS = 496, *tef*1-α = 301). Single gene analyses were also performed, and topology and clade stability were compared from the combined gene analyses. *Lasiodiplodia pyriformis* (CBS 121770) and *L. euphorbiaceicola* (GB137) strains were used as the outgroup taxa. The final ML optimization likelihood is -3658.835932. The matrix included 305 distinct alignment patterns, with 9.04% undetermined characters or gaps. Estimated base frequencies were obtained as follows: A = 0.207362, C = 0.299864, G = 0.261385, T = 0.231388; substitution rates AC = 0.795654, AG = 3.073151, AT = 0.830751, CG = 1.336600, CT = 4.187973, GT = 1.0; gamma distribution. Bootstrap support values for ML (first set) equal to or

greater than 65%, BYPP equal to or greater than 0.95 are given above or below the nodes. The strain from the current study is in red bold, and the type strains are indicated with [T].
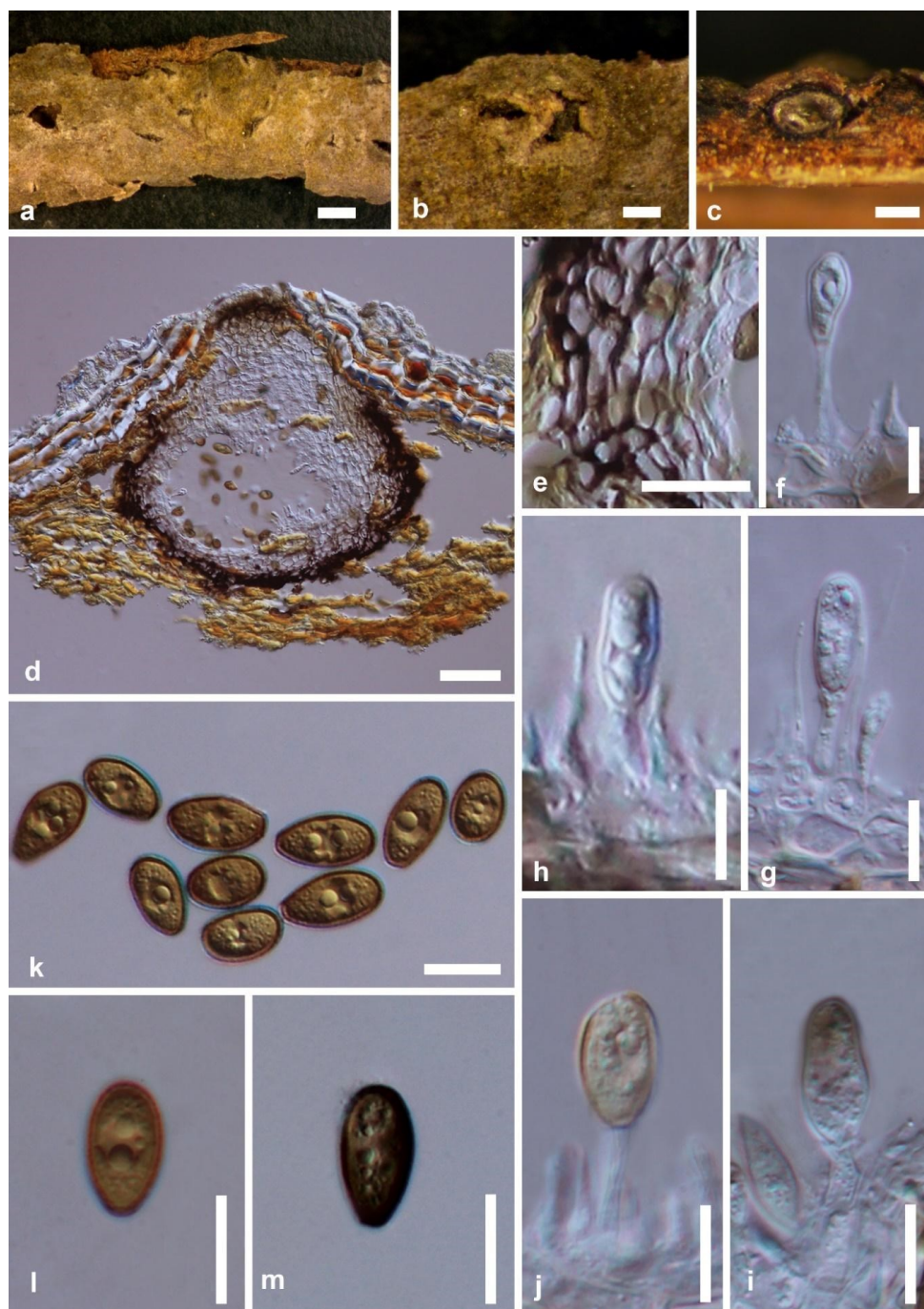

Fig. 11 – *Diplodia sapinea* (MFLU 16-1443, new host record in Italy). a–b Conidiomata on the dead host surface of *Prunus* sp. (*Rosaceae, Rosales*). c–d Longitudinal sections of a conidioma. e Conidiomatal wall. f–j Developmental stages of conidiogenesis. k–m Conidia. Scale bars: a = 1 mm, b, c = 200 µm, d = 50 µm, e = 20 µm, f–m = 10 µm.

GenBank Accession Numbers – ITS: OQ401061; LSU: OQ411146; *tub*2: OQ437900.

Notes – In the multi-gene phylogeny, our strain (MFLU 19-0302) grouped with the type strain of *Dothiorella iberica* (CBS 115041) with 99% MLBS and 0.99 BYPP support (Fig. 12, Clade A). ITS, LSU, and *tub*2 sequences of MFLU 19-0302 are identical to those of CBS 115041. We compared bp differences between *D. italica* (MFLUCC 17-0951) that forms close phylogenetic affinity (96% MLBS and 1.00 BYPP support) to CBS 115041 and MFLU 19-0302 revealing that

ITS sequences are identical while *tub*2 differs from 2 bp (2/342; without gaps). However, based on the ITS, *tef*1-α, and *tub*2 phylogeny, Zhang et al. (2021) suggested that *D. iberica* and *D. italica* are possible synonyms of *D. sarmentorum* (Fig. 12, Clade A). Based on the characters of ascomata, peridium, pseudoparaphyses, asci, and ascospores, our specimen is similar to the holotype of *D. iberica* (ex-type CBS 115041) as described in Phillips et al. (2013). However, in the dimensions of asci (100–125 × 18–25 μm) and ascospores (17.5–29 × 8.5–12.5 μm), they are comparatively different.
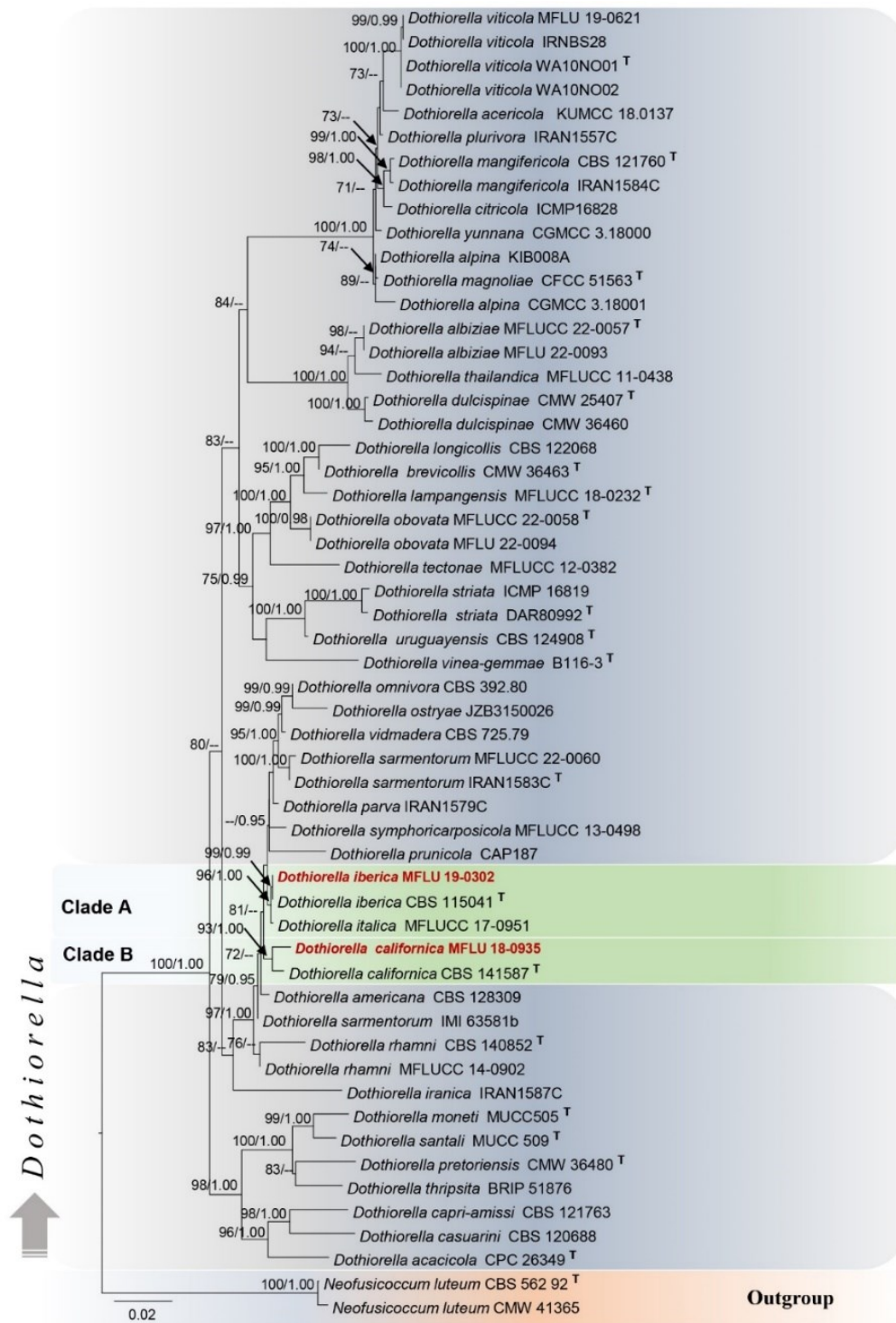
Fig. 12 – Phylogram generated from maximum likelihood analysis based on combined ITS, LSU, *tef*1-α, and *tub*2 sequenced data. Fifty-five strains were included in the combined sequence analyses, which comprised 2042 characters with gaps (ITS = 526, LSU = 794, *tef*1-α = 294, *tub*2 =

428). Single gene analyses were also performed, and topology and clade stability were compared from the combined gene analyses. *Neofusicoccum luteum* (CMW 41365, CBS 562.92) strains were used as the outgroup taxa. The final ML optimization likelihood is -7920.955742. The matrix included 561 distinct alignment patterns, with 33.01% undetermined characters or gaps. Estimated base frequencies were obtained as follows: A = 0.218853, C = 0.280809, G = 0.268756, T = 0.231582; substitution rates AC = 1.494130, AG = 2.646691, AT = 1.239626, CG = 1.250611, CT = 6.621567, GT = 1.0; gamma distribution. Bootstrap support values for ML (first set) equal to or greater than 60%, BYPP equal to or greater than 0.95 are given above or below the nodes. The strains from the current study are in red bold, and the type strains are indicated with ^{T}.
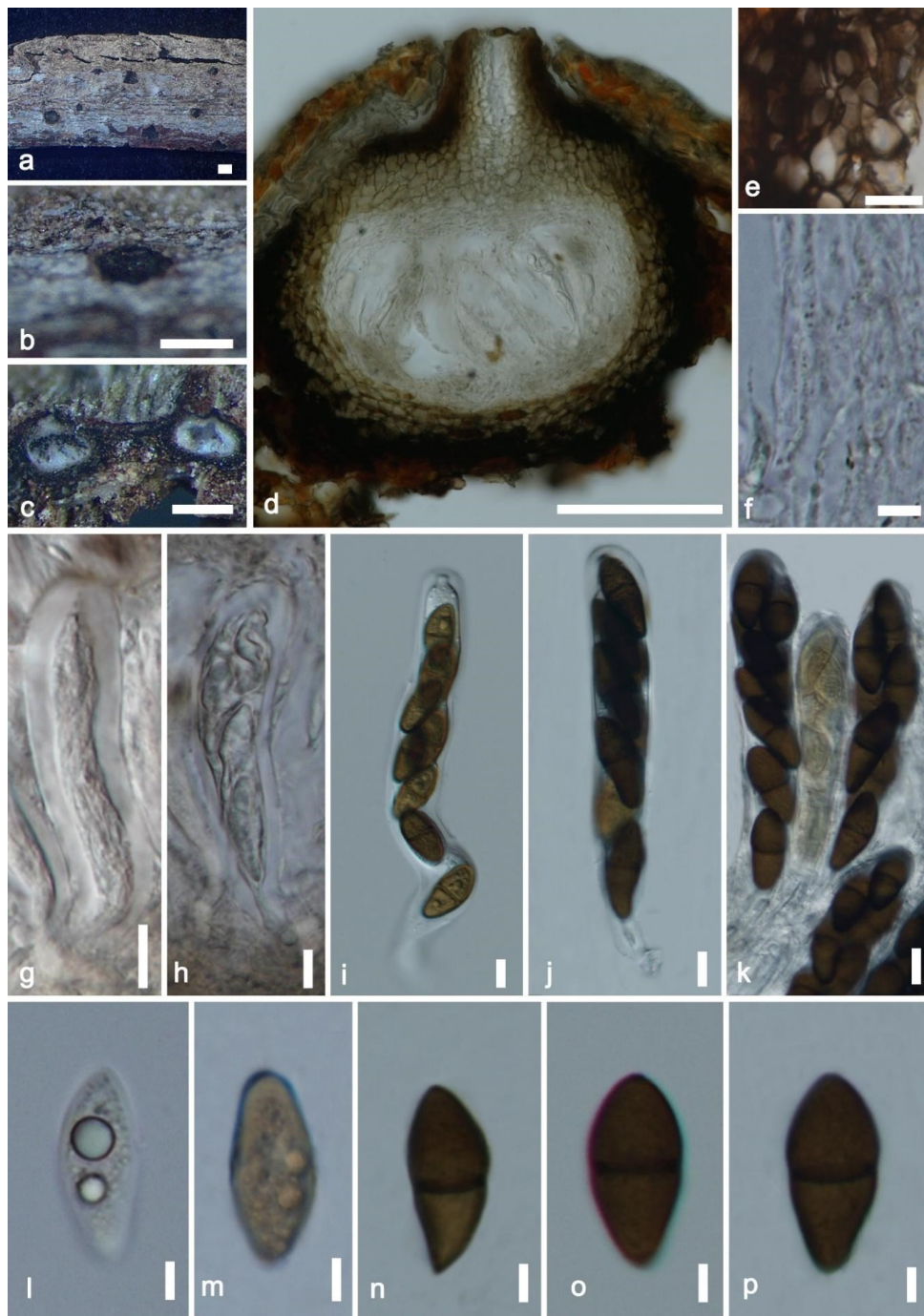
Fig. 13 – *Dothiorella iberica* (MFLU 19-0302, new host record in Italy). a–b Ascomata on the dead host surface of *Quercus ilex* L. (*Fagaceae*, *Fagales*). c–d Sections of ascomata. e Peridium. f Pseudoparaphyses. g–k Asci. l–p Ascospores. Scale bars: a, b = 500 μm, c = 200 μm, d = 100 μm, e–k = 10 μm, l–p = 5 μm.

Dothiorella sarmentorum was introduced by Phillips et al. (2005) based on the asexual morph of *Botryosphaeria sarmentorum*. The sexual morph of *D. sarmentorum* is characterized by partially erumpent ascomata with papillate ostioles, 4–6(–8)-spored asci, and oblong to ovate (0–)1-septate, finely verruculose ascospores, widest in the middle part (Phillips et al. 2013). Based on Phillips et al. (2013) *D. iberica* is similar to *D. sarmentorum* by brown, 1-septate ascospores, but can be distinguished by shorter, more clavate asci, and ascospores that tapering towards the base (Phillips et al. 2013). For *D. italica*, only the asexual morph was identified by Dissanayake et al. (2017b). As only the sexual morph was present in our collection, we were unable to compare morphologies. Therefore, we keep *D. iberica* and *D. italica* without synonymizing and further identify our collection as *D. iberica.* The holotype material was reported from *Quercus ilex* in Spain, and other known distribution is in Algeria, Italy, and Portugal (Farr & Rossman 2022). Italian records of *D. iberica* (asexual morph) were found on *Acer opalus* (*Sapindaceae*), *Rosa canina* (*Rosaceae*), and *Vitis vinifera* (*Vitaceae*) (Dissanayake et al. 2016a, Jawawardena et al. 2018c, Wanasinghe et al. 2018). Our collection was found in the same locality but on a different host species, *Quercus ilex* (*Fagaceae*). Therefore, this is the first record of *D. iberica* on Italian *Q. ilex* trees, and the first sexual morph of *D. iberica* reported from Italian collections.

Dothiorella californica D.P. Lawr. & Trouillas 2017   Fig. 14
     Index Fungorum number: IF 817293; Facesoffungi number: FoF 14005
     Saprobic on a dead aerial branch of *Laurus nobilis* L. Sexual morph: *Ascomata* 300–350 μm diam., 300–370 μm high (x̄ = 320 × 340 μm, n = 5), pseudothecial, globose to subglobose, semi-immersed, scattered or aggregated, erumpent at maturity, ostiolate, black. *Ostioles* 80–90 μm high, circular, papillate, central, single. *Peridium* 40–55 μm thick, composed multi-layered, from outer to inside walls composed of dark brown to pale brown cells of *textura angularis* to *textura globulosa.* *Hamathecium* comprises 2–3 μm wide, thin-walled, hyaline, septate, numerous *pseudoparaphyses*. *Asci* 70–135 × 17–22 μm (x̄ = 95 × 18 μm, n = 10), stipitate, arising from the base of the ascoma, cylindric-clavate, bitunicate, with a well-developed apical chamber, 8-spored, uniseriate to biseriate. *Ascospores* 15–30 × 7–15 μm (x̄ = 21 × 10 μm, n = 20; l/w = 2.1), elliptical to ovate, widest in the middle part, initially aseptate, 1-septate at maturity, slightly constricted at the septum, moderately thick-walled, finely verruculose on the inner surface, initially hyaline, becoming dark brown at maturity. Asexual morph: see Lawrence et al. (2017).
   Material examined – Italy, Province of Forlì-Cesena [FC]), Rocca delle Caminate-Predappio, on a dead aerial branch of *Laurus nobilis* L. (*Lauraceae*), 28 March 2018, Erio Camporesi, IT 3789 (MFLU 18-0935).
     GenBank Accession Numbers – ITS: OQ401057; LSU: OQ411142; *tef*1-α: OQ437910; *tub*2: OQ437897.
     Notes – In the phylogenetic analyses, our strain (MFLU 18-0935) grouped with *D. californica* with 93% MLBS and 1.00 BYPP support (Fig. 12, Clade B). In a bp comparison of the ITS sequences of our strain (MFLU 18-0935) and *D. californica* (CBS 141587), they are identical, while the *tef*1-α and *tub*2 sequences show 7/240 bp (2.91%) and 5/354 bp (1.41%) differences, respectively. *Dothiorella californica* was introduced by Lawrence et al. (2017) with its asexual morph on *Umbellularia californica* dead branches from California, USA. Zhang et al. (2021) suggested that *D. californica* should possibly be synonymized with *D. sarmentorum* based on their ITS, *tef*1-α, and *tub*2 phylogeny. We were unable to undertake a morphological comparison as our collection is the sexual morph of the fungus.
     Based on the suggestion of synonymizing by Zhang et al. (2021), we compared the sexual morph of our collection with the holotype of *D. sarmentorum* (IMI 63581b). Our collection differs from *D. sarmentorum* (IMI 63581b) by having smaller ascomata (300–350 μm diam.) *vs*. IMI 63581b strain (350–400 mm diam.). Also, the peridium wall of our collection consists of *textura angularis* to *textura globulosa*, while the holotype only consists of *textura angularis* (Phillips et al. 2013). When considering the l/w ratio and shape of the ascospores, our collection has l/w = 2.1 and elliptical to ovate shaped ascospores, while the holotype has l/w = 2.2 and oblong to ovate shape

ascospores (Phillips et al. 2013). This could be because of the water content in the surrounding environment. Also, the bp differences of our strain (MFLU 18-0935) and *D. sarmentorum* (IMI 63581b) revealed that identical LSU, 2/447 bp (0.44%) in ITS, 6/240 bp (2.5%) in *tef*1-α and 4/346 bp (1.15%) *tub*2. Based on these morpho-molecular comparisons, we keep our collection as *D. californica* without synonymizing it as *D. sarmentorum*. Hence, we report our new collection as the first sexual morph of *D. californica* and it is the first Italian report as well as on *Laurus nobilis* hosts.
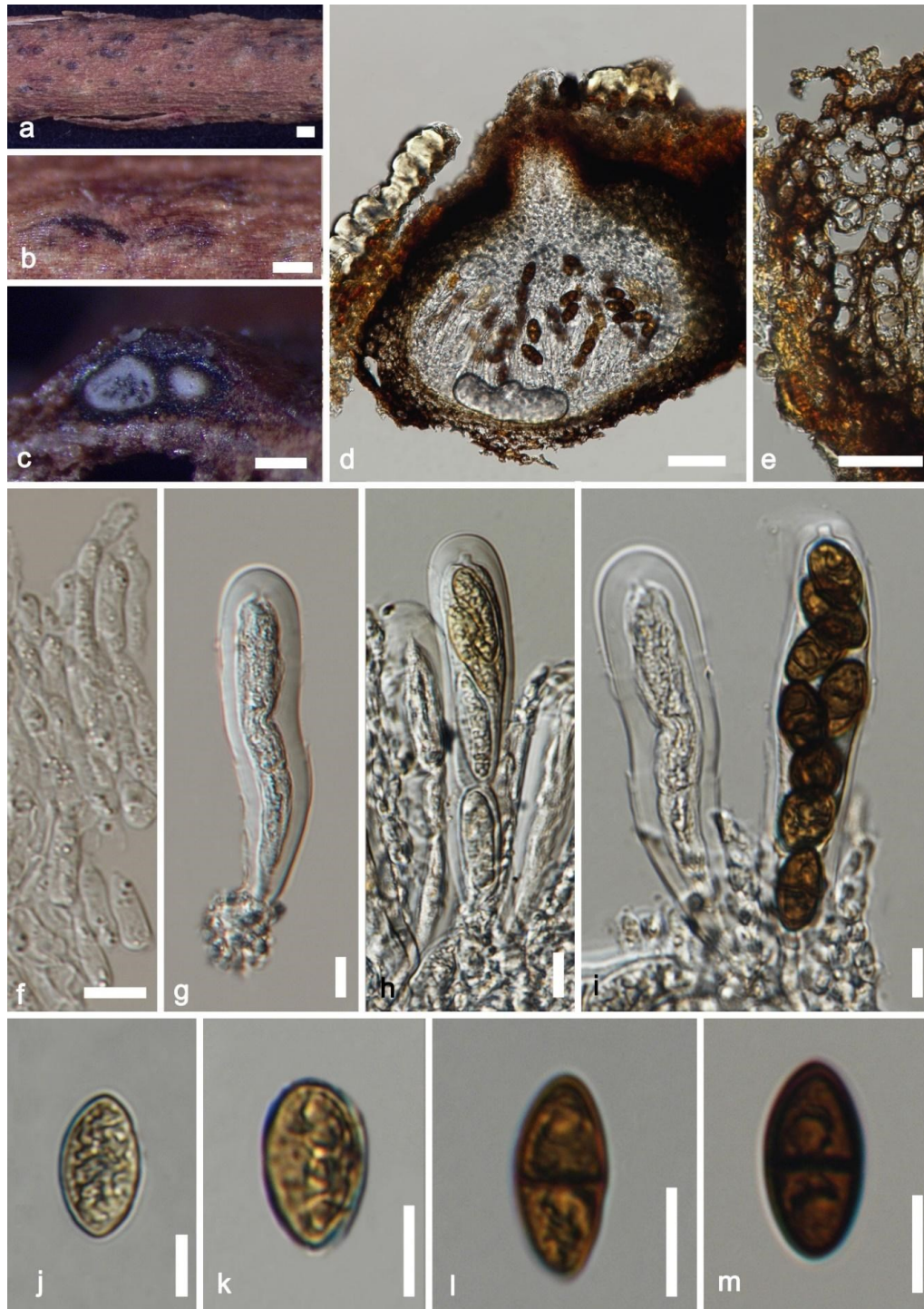

Fig. 14 – *Dothiorella californica* (MFLU 18-0935, new host, and geographical record). a–b Ascomata on the dead host surface of *Laurus nobilis* L. (*Lauraceae*, *Laurales*). c–d Sections of ascomata. e Peridium. f Pseudoparaphyses. g–i Asci. j–m Ascospores. Scale bars: a = 500 μm, b, c = 200 μm, d, e = 20 μm, f–m = 10 μm.

Mucoharknessia Crous 2015

Index Fungorum number: IF 811254; Facesoffungi number: FoF 01651

Notes – *Mucoharknessia* was introduced by Crous et al. (2015) for a species resembling *Harknessia* (*Harknessiaceae*, *Diaporthales*) with the type species *M. cortaderiae*. This asexual genus is characterized by having unilocular, pycnidial conidiomata subepidermal ostioles, peridium with *textura angularis* cells, lageniform to subcylindrical conidiogenous cells, proliferated, percurrently at the apex, a flared collarette, and oval to ellipsoidal, hyaline conidia bearing mucoid appendages (Crous et al. 2015, Hongsanan et al. 2020a). However, the genus is distinguished from *Harknessia* by its pycnidia that lack furfuraceous tissue surrounding the ostiole and conidia that possess a mucoid apical appendage (Crous et al. 2015, Li et al. 2016, Hongsanan et al. 2020a). In the phylogenetic analysis, Crous et al. (2015) understood the genus is allied to genera in the *Tiarosporella* complex in *Botryosphaeriaceae*. There are two species epithets listed under *Mucoharknessia* in the Index Fungorum (2022), including *M. anthoxanthi* and they were found on woody stems and leaves in terrestrial habitats and reported as saprobes.
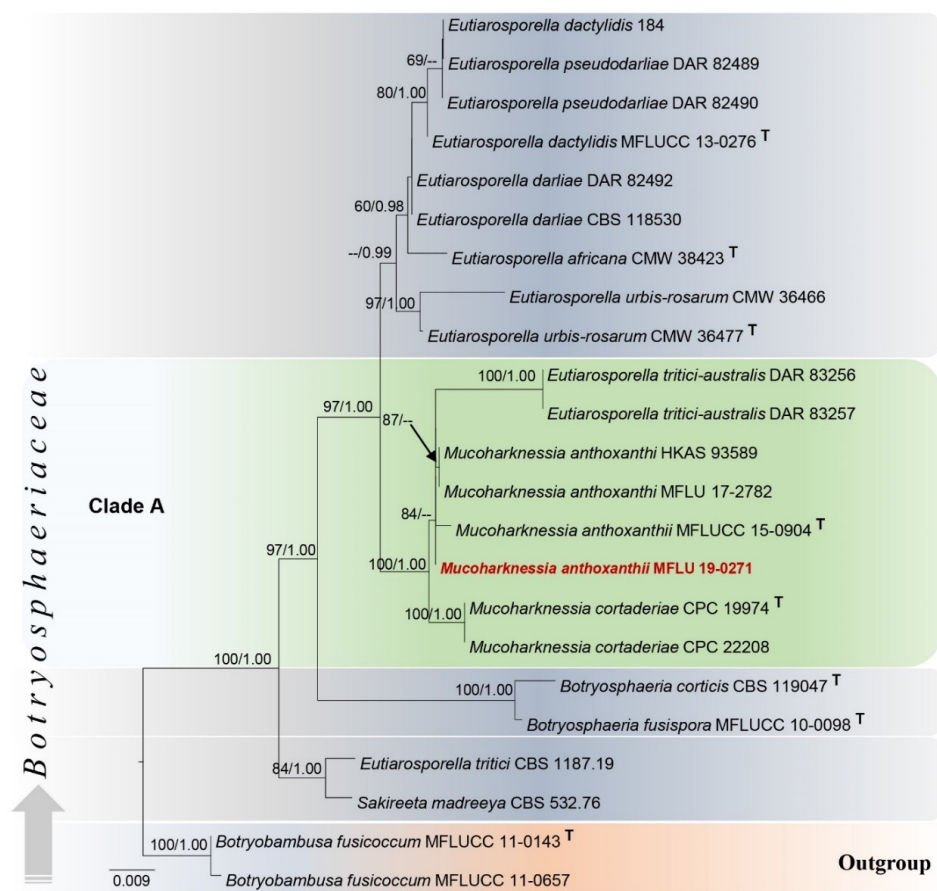
Fig. 15 – Phylogram generated from maximum likelihood analysis based on combined ITS and LSU sequenced data. Twenty-three strains were included in the combined sequence analyses, which comprised 1374 characters with gaps (ITS = 543, LSU = 831). Single gene analyses were also performed, and topology and clade stability were compared with the combined gene analyses. *Botryobambusa fusicoccum* (MFLUCC 11-0143, MFLUCC 11-0657) strains were used as the outgroup taxa. The final ML optimization likelihood is -3186.285068. The matrix included 230 distinct alignment patterns, with 21.53% undetermined characters or gaps. Estimated base frequencies were obtained as follows: A = 0.238961, C = 0.250655, G = 0.287310, T = 0.223074; substitution rates AC = 1.770026, AG = 2.888324, AT = 2.119315, CG = 1.911903, CT = 7.777470, GT = 1.0; gamma distribution. Bootstrap support values for ML (first set) equal to or greater than 60%, BYPP equal to or greater than 0.95 are given above or below the nodes. The strain from the current study is in red bold, and the type strains are indicated with [T].

Mucoharknessia anthoxanthi Dissan., Camporesi & K.D. Hyde 2016   Fig. 16
        Index Fungorum number: IF 551752; Facesoffungi number: FoF 07456
        Saprobic on a dead stem of *Anthoxanthum odoratum* L. Sexual morph: Undetermined. Asexual morph: Coelomycetes. *Conidiomata* 150–230 μm diam., 140–200 μm high (x̄ = 163 × 155 μm, n = 10) pycnidial, solitary, forming in a linear series on the host surface, globose, semi-immersed, erumpent, uniloculate, black. *Conidiomata wall* 20–30 μm diam. composed of the thin-walled, outermost layer, dark brown cells of *textura globulosa*, inner-layers pale brown to hyaline cells of *textura angularis*. *Conidiophores* reduced to conidiogenous cells. *Conidiogenous cells* 5–10 × 3–5 μm, lining the inner cavity, enteroblastic, phialidic, ampulliform to subcylindrical, percurrently proliferating at the apex, sometimes determinate, hyaline. *Conidia* 18–28 × 6–9 μm (x̄ = 25 × 7 μm, n = 30; l/w = 3.5), solitary, smooth-walled, elliptical to fusoid, straight, sometimes with apiculate apex and truncated base, apex with a flared mucoid appendage, basal appendages usually absent, unicellular, hyaline.
        Material examined – Italy, Province of Forlì-Cesena [FC], Montebello – Modigliana, on a dead aerial stem of *Anthoxanthum odoratum* L. (*Poaceae*), 18 November 2018, Erio Camporesi IT 4117 (MFLU 19-0271), *ibid*., 03 December 2018, Erio Camporesi, IT 4117A, (MFLU 19-0271).
   GenBank Accession Numbers – ITS: OQ401060; LSU: OQ411145.
        Notes – In the multi-gene phylogeny, our strain (MFLU 19-0271) grouped together with *Mucoharknessia anthoxanthi* (HKAS 93589, MFLU 17-2782 and MFLUCC 15-0904) with 84% MLBS support (Fig. 15, Clade A). This new collection was from the same host (*Anthoxanthum odoratum*) and locality as the type specimen. In the bp comparison of the ITS and LSU regions between our specimen and the type (MFLU 17-2782), a 1/508 (0.19%) bp and a zero difference were revealed, respectively. When compared with MFLUCC 15-0904, ITS sequences revealed 3/508 (0.59%) bp differences, while LSU sequences were identical. Also, our collection has similar morphologies to the holotype and paratype strains (MFLU 15-3477 and HKAS 93589) with globose conidiomata arranged in a linear series on the host surface, phialidic, ampulliform to subcylindrical conidiogenous cells, and elliptical to fusoid conidia (Li et al. 2016, 2020). Based on the cells of the conidiomata wall, our collection differs from the holotype and paratype by *textura globulosa* to *textura angularis* cells while MFLU 15-3477 has only *textura angularis* cells and HKAS 93589 has *textura angularis* and *textura prismatica* cells. Further, MFLU 19-0271 has smaller conidiomata (140–150 high × 150–160 diam.) compared to MFLU 15-3477 (170–250 high × 600 diam.) and HKAS 93589 (240–320 high × 215–280 diam.) (Li et al. 2016, 2020). Based on morphology and phylogenetic analyses, we conclude our collection is *Mucoharknessia anthoxanthi*, and it is the second collection from *Anthoxanthum odoratum* in Italy. The holotype of *M. anthoxanthi* was collected in Passo delle Forche – Galeata in Forlì-Cesena province (Li et al. 2016) while our collection is from Montebello – Modigliana, in the same province. Another collection of *M. anthoxanthi* was recorded on *Dactylis glomerata* (*Poaceae*) in the same region (Li et al. 2020). There is no record of this fungus in other regions of the world. We suggest that *M. anthoxanthi* can be a host recurrent species on *Poaceae* hosts.

Melanopsaceae A.J.L. Phillips, Slippers, Boissin & Crous 2013
        Index Fungorum number: IF 805796; Facesoffungi number: FoF 07630
        Notes – *Melanopsaceae* was established by Slippers et al. (2013) in *Botryosphaeriales* to accommodate *Melanops*. The key characters of sexual morph taxa in *Melanopsaceae* are multiloculate ascostromata, thick peridium wall with *textura angularis* cells, septate, cellular pseudoparaphyses, bitunicate, 8-spored, clavate asci and ellipsoid to rhomboid, aseptate, hyaline ascospores with mucus sheath (Slippers et al. 2013, Hongsanan et al. 2020a). Asexual morphs are coelomycetous and characterized by conidiomata similar to ascostromata, filiform paraphyses, 1–2-septate conidiophores, subcylindrical, conidiogenous cells with periclinal thickening or percurrently proliferating at the apex, and fusoid, aseptate, hyaline conidia with mucus sheath (Slippers et al. 2013, Phillips et al. 2019, Hongsanan et al. 2020a). Many members of *Botryosphaeriales* are endophytes and latent pathogens, causing plant tissue infection and sporulation on dead tissues, but

the pathogenic or endophytic status of *Melanopsaceae* is not clear (Slippers et al. 2013, Jiang et al. 2018, Hongsanan et al. 2020a). In this study, we collected a *Melanops* species from dead *Quercus* sp. from Italy.
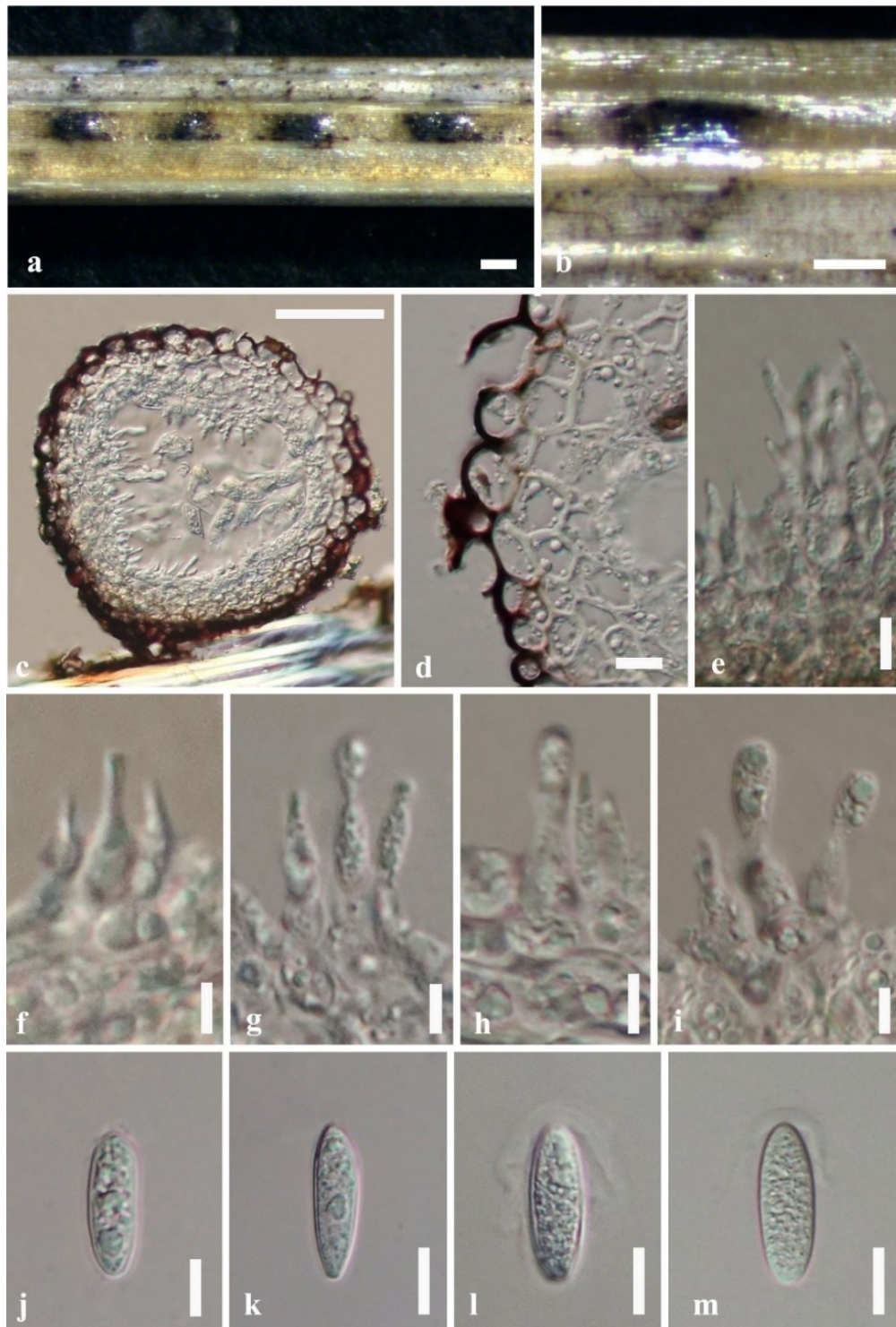

Fig. 16 – *Mucoharknessia anthoxanthi* (MFLU 19-0271, host recurrent species on *Poaceae*). a–b Conidiomata on the dead host surface of *Anthoxanthum odoratum* L. (*Poaceae, Poales*). c Section of a conidioma. d Conidiomata wall. e–i Development stages of conidiogenesis. j–m Conidiospores. Scale bars: a, b = 200 μm, c = 50 μm, d, j–m = 10 μm, e–i = 5 μm.

Melanops Nitschke ex Fuckel 1870
  Index Fungorum number: IF 3078; Facesoffungi number: FoF 07442

Notes – *Melanops* was established by Fuckel (1870) to accommodate *Melanops tulasnei* which was previously described as *Dothidia melanops* by Tulasne (1856) and *M. mirabilis* species. Winter (1887) considered *D. melanops* to be accommodated in *Botryosphaeria* and a new combination was formed as *B. melanops*. Then, von Arx & Müller (1954) included *B. melanops* under their broad concept of *B. quercuum*. Later, Phillips & Pennycook (2004) accepted the fungus as a member of *Botryosphaeria* and corrected the name to *B. melanops* with the designation of a neotype. Based on morphology and phylogeny, Phillips & Alves (2009) epitypified the type species *M. tulasnei* and retained *Melanops* as a separate genus in *Botryosphaeriaceae*. However, the phylogenetic position of this species could not be established due to the absence of culture. *Melanops* is characterized by having both ascomata and conidiomata occurring in the same stroma with ellipsoid to rhomboid ascospores and fusiform conidia surrounded by a mucus sheath (Li et al. 2020). This is the only genus in *Melanopsaceae* and 260 species epithets are listed in the Index Fungorum (2022). However, some have been transferred to different families, and others are still unresolved (Jiang et al. 2018). *Melanops* species were found on woody hosts, and their life modes are unclear as pathogenic or endophytic (Slippers et al. 2013, Hongsanan et al. 2020a). In this study, we describe *M. tulasnei* collected from Italian dead wood.
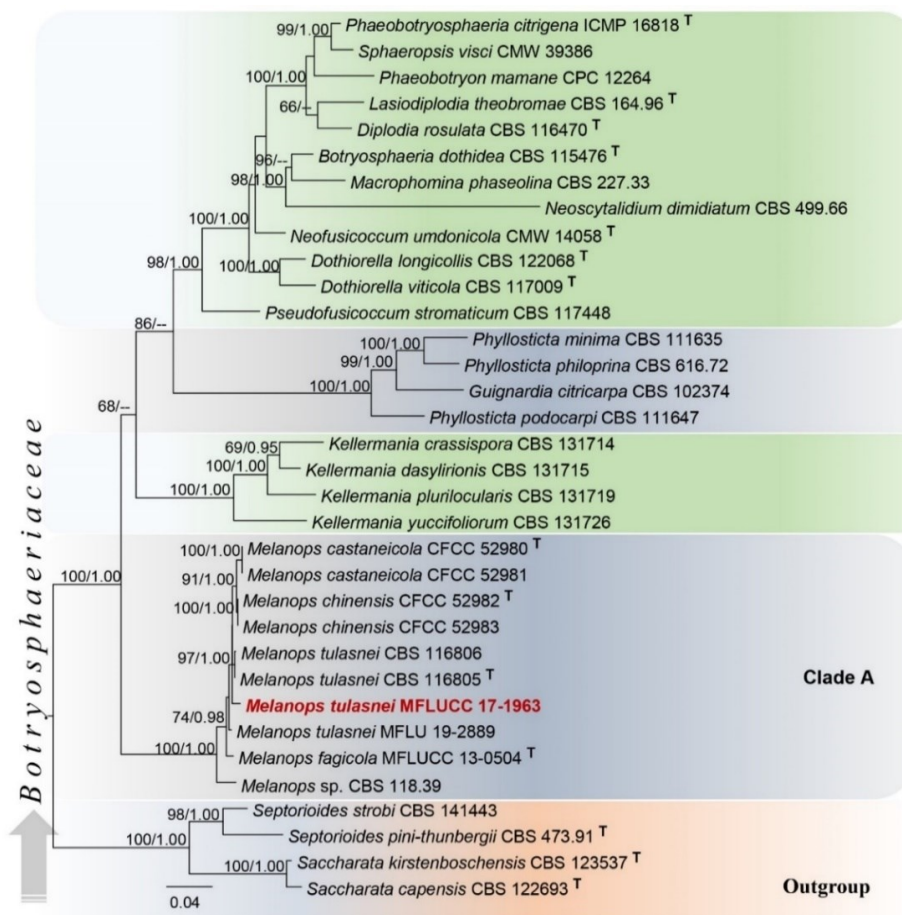
Fig. 17 – Phylogram generated from maximum likelihood analysis based on combined LSU, ITS, *tef*1-α, and *tub*2 sequenced data. Thirty-four strains were included in the combined sequence analyses, which comprised 2003 characters with gaps (LSU = 839, ITS = 511, *tef*1-α = 261, *tub*2 = 320). Single gene analyses were also performed, and topology and clade stability were compared from combined gene analyses. *Saccharata kirstenboschensis* (CBS 123537), *S. capensis* (CBS 122693), *Septorioides pini-thunbergii* (CBS 473.92) and *Se. strobi* (CBS 141442) strains were used as the outgroup taxa. Final ML optimization likelihood is -14562.933145. The matrix included 839 distinct alignment patterns, with 10.12% undetermined characters or gaps. Estimated base frequencies were obtained as follows: A = 0.222159, C = 0.267002, G = 0.286442, T = 0.224397;

substitution rates AC = 1.097206, AG = 2.318042, AT = 1.606116, CG = 1.143176, CT = 4.832204, GT = 1.0; gamma distribution. Bootstrap support values for ML (first set) equal to or greater than 65%, BYPP equal to or greater than 0.95 are given above or below the nodes. The strain from the current study is in red bold, and the type strains are indicated with [T].

Melanops tulasnei Nitschke ex Fuckel 1870   Fig. 18
Index Fungorum number: IF 150956; Facesoffungi number: FoF 07444
Saprobic on a dead and fallen branch of *Quercus* sp. Sexual morph: see Phillips & Alves (2009). Asexual morph: *Conidiomata* 2–3 mm wide, 1–2 mm high, multiloculate, immersed to semi-immersed, partly erumpent at maturity, globose to subglobose, black. *Locules* 260–310 mm diam., 330–450 mm high (x̄ = 279 × 385 μm, n = 5). *Peridium* 40–70 μm composed of thick-walled cells of *textura angularis* to *textura globulosa Ostioles* circular and central on each locule, non-papillate. *Conidiogenous cells* 12–18.5 × 1.9–3.2 μm (x̄ = 13 × 2.8 μm, n = 5), subcylindrical, hyaline, unbranched or branched at the base, formed from the inner wall of the conidioma, proliferating per currently at apex, or with periclinal thickening. *Paraphyses* 1.5–1.8 μm wide, filiform, hyphae-like, branched, arising between the conidiogenous cells. *Conidia* 35–60 × 7–11.8 μm (x̄ = 53 × 10 μm, n = 10; l/w = 5.3), fusiform, wide in the middle, apex acute, base truncate, aseptate, contents granular, surrounded by a narrow, persistent mucus sheath, hyaline.

Culture characteristics – Conidia germinating on MEA within 24 h at 18 °C, colonies on MEA, at first white, becoming gray to black after 7 days, flat, with irregular margins, texture firstly uniform, later forming concentric circles.

Material examined – Italy, Province of Forlì-Cesena [FC]), Rocca delle Caminate–Predappio, on a dead and fallen branch of *Quercus* sp. (*Fagaceae*), 2 March 2017, Erio Camporesi, IT 3260 (MFLU 17-0726), living culture (MFLUCC 17-1963).

GenBank Accession Numbers – ITS: OQ401055; LSU: OQ411139.

Notes – In the multi-gene phylogeny, our strain (MFLUCC 17-1963) formed a well-supported monophyletic clade (Fig. 17, Clade A) with *M. castaneicola*, *M. chinensis*, *M. fagicola*, and *M. tulasnei*. Among these, our strain formed a separate lineage basal to the strains of CFCC 52980, CFCC 52981, CFCC 52982, CFCC 52983 and CBS 116805, CBS 116806 with 97% MLBS and 1.00 BYPP support (Fig. 17, Clade A). However, the strain of *M. tulasnei* (MFLU 19-2889) formed a distinct lineage in Clade A. This collection was made from dead twigs of *Quercus robur* from Russia (Li et al. 2020). Based on the BLAST results of ITS and LSU sequence data of MFLUCC 17-1963, the closest species was *M. tulasnei* CBS 116805 (99.10% high similarity) and (99.54% high similarity), respectively. The bp comparisons for the LSU and ITS regions of our strain with other closely related strains are in Table 4.

Table 4 Base pair comparisons between our strain and closely related taxa in Clade A, Fig. 17.

Fungal species (strains)	LSU (bp)	ITS (bp)
Melanops castaneicola CFCC 52980, CFCC 52981	6/839 (0.71%)	6/500 (1.2%)
M. chinensis CFCC 52982, CFCC 52983	4/839 (0.47%)	5/511 (0.97%)
M. tulasnei CBS 116805, CBS 116806	4/585 (0.68%)	5/510 (0.98%)

Also, we compared the morphologies (Table 5) of our species with those of phylogenetically more closely related species in Clade A. Based on asexual morph, our species shares morphologies with the type collection of *M. tulasnei* but comparatively differs from MFLU 19-2889 (Table 5). The original morphology and descriptions for *M. tulasnei* were provided by Tulasne (1856). Later, Phillips & Pennycook (2004) designated a neotype for the species, using a specimen that had been misapplied as *Botryosphaeria advena*, deposited in the Herbarium Mycologicum of Padua (Italy). The specimen was collected on *Quercus* species from Padua (Veneto region) Italy. Then, Phillips &

Alves (2009) designated an epitype for *M. tulasnei* from samples collected on dead twigs of *Quercus robur* from Germany. Our new isolate was collected from *Quercus species* (Emilia–Romagna region, Italy), as for the neotype and epitype materials, which were collected from Italy and Germany, respectively (Phillips & Alves 2009).
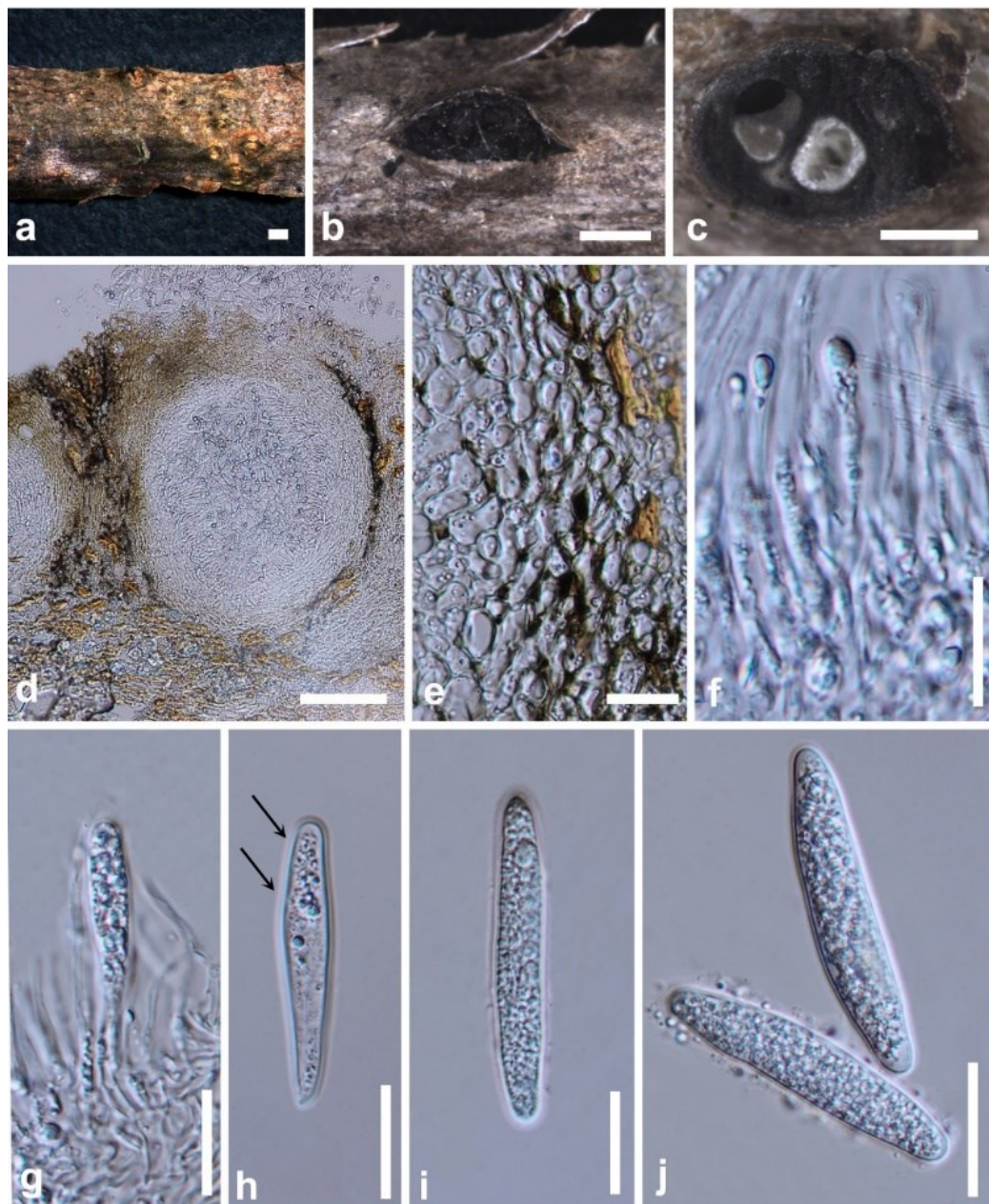
Fig. 18 – *Melanops tulasnei* (MFLU 17-0726, new regional record in Italy). a–b Conidiomata on the dead host surface of *Quercus* sp. (*Fagaceae, Fagales*). c A transverse section through a conidioma. d Longitudinal section of a conidioma. e Conidiomata wall. f–g Development of conidiogenous cells and paraphyses. h–j Conidia with mucus sheath (arrowed). Scale bars: a = 2 mm, b = 1 mm, c = 500 μm, d = 100 μm, e–j = 20 μm.

Also, some similar morphologies in the same range were found in our collection with *M. castaneicola*, *M. chinensis*, and *M. fagicola* (Table 5). However, based on the morphological evidence, we conclude our new collection is *M. tulasnei*, found on the same host genus as the designated types. Our collection is the first record of *M. tulasnei* from the Emilia–Romagna region in Italy. Further collections from different host species and additional coding genes for phylogeny are suggested.

Table 5 Comparison of conidiomata and conidial dimensions of the species grouped in Clade A, Fig. 17.

Species Name	Conidiomata (diam. × high)	Conidiogenous cells (µm)	Conidia (µm)	Host	Reference(s)
MFLUCC 17-1963	2–3 × 1–2 mm	12–18.5 × 1.9–3.2	35–60 × 7–11.8	*Quercus* sp.	This study
Melanops tulasnei	2 mm (based on ascomata)	12–18 × 2–3	(37.2–)45–46.8(–53) × (7.2–)9.1–9.7 (–12.1)	*Quercus* spp.	Phillips & Alves (2009)
M. tulasnei (MFLU 19-2889)	1–1.5 × 0.5–0.75	20–40 × 1.5–3.5	53–85 × 8–11	*Quercus robur*	Li et al. (2020)
M. chinensis	2–4 × 1–2.5 mm	5–20 × 1.5–3.5	(64.4–)68.1–73.7(–75.1) × (11.7–)12.4–14.5 (–15.6)	*Quercus* sp.	Jiang et al. (2018)
M. castaneicola	1–2 × 0.2–0.5 mm	5–15 × 2–4	(50.2–)56.3–66.7(–72.1) × (12.4–)12.9–14.6 (–15.6)	*Castanea mollissima*	Jiang et al. (2018)
M. fagicola	845–1270 × 370–470 µm	13–20 × 3–6	37–52.5 × 9.5–15.5	*Fagus sylvatica*	Li et al. (2020)

Dothideomycetes O.E. Erikss. & Winka 1997

Hysteriales Lindau 1897

Index Fungorum number: IF 90549; Facesoffungi number: FoF 07681

Notes – *Hysteriales* contains hysterothecioid taxa and was introduced by Lindau (1897a) to accommodate *Hysteriaceae* (Hongsanan et al. 2020b, Wijayawardene et al. 2022). In the past, the order has been placed among pyrenomycetes and discomycetes at different times (Rehm 1986, Jayasiri et al. 2018, Dayarathne et al. 2020a). However, molecular data confirmed the placement of *Hysteriales* within *Pleosporomycetidae* in *Dothideomycetes* (Boehm et al. 2009a, b, Shearer et al. 2009, Suetrong et al. 2009, Jayasiri et al. 2018, Dayarathne et al. 2020a, Hongsanan et al. 2020b, Wijayawardene et al. 2022). The key morphologies of sexual *Hysteriales* taxa are thick-walled, navicular ascomata that typically dehisce through an invaginated slit or sulcus (Zogg 1962, Hongsanan et al. 2020b). *Hysteriales* members are saprobes, endophytes or ectomycorrhizal species mainly found on woody and herbaceous plant hosts from terrestrial and aquatic habitats (Jayasiri et al. 2018). The divergence time was estimated as 109 MYA (stem age) for *Hysteriales* by Hongsanan et al. (2020b).

Hysteriaceae Chevall. 1826

Index Fungorum number: IF 80901; Facesoffungi number: FoF 01838

Notes – *Hysteriaceae* was introduced by Chevallier (1826) with *Hysterium* as the type genus. Through taxonomic studies, the term hysterothecium was defined for the unique, darkly pigmented, and carbonaceous ascocarps for the *Hysteriaceae* (Clements 1909) and Zogg (1962) later monographed the family. The key characters of sexual morph taxa in *Hysteriaceae* are hysterothecial thick-walled, carbonaceous ascomata that are navicular and characteristically dehiscing by an invaginated slit or sulcus, small and thick pseudoparenchymatous cells, hamathecium with cellular or trabeculate, septate pseudoparaphyses; 8-spored, bitunicate asci with a distinct ocular chamber, and 1–2-seriate, 1-multi-septate, or muriform ascospores that are hyaline to light or dark-brown (Zogg 1962, Boehm et al. 2009a, Dayarathne et al. 2020a, Hongsanan et al. 2020b). Currently, 13 genera are accepted in *Hysteriaceae* while some genera (*Actidiographium*,

Gloniella, *Hysterocarina*, and *Hysteroglonium*) still lack molecular data (Hongsanan et al. 2020b, Wijayawardene et al. 2022). They can be found as lignicolous or corticolous, mainly on well-decorticated hardwoods and rarely on conifers (Hongsanan et al. 2020b). Hysteriaceous species are not harmful to plants or animals (Hongsanan et al. 2020b). In this study, we collected a *Hysterium* species on *Crataegus* sp. from Italy.

Hysterium Pers. 1797

Index Fungorum number: IF 2464; Facesoffungi number: FoF 0004

Notes – *Hysterium* was lectotypified by *H. pulicare* (Bisby 1923). The members of the genus are characterized by carbonaceous, hysterothecial ascomata, bitunicate, cylindrical, clavate asci and fusiform, 3 or more transversely septate, pigmented ascospores (Boehm et al. 2009a, b, Schoch et al. 2009, Hyde et al. 2017a, Dayarathne et al. 2020a). *Hysterium* is one of the largest genera in *Hysteriales* and they were reported as saprophytic on dead wood in terrestrial habitats (Hyde et al. 2020a). We describe *Hysterium angustatum* as a novel host record with morphology and phylogenetic analyses.

Hysterium angustatum Pers. 1801   Fig. 20

Index Fungorum number: IF 221405; Facesoffungi number: FoF 04579

Saprobic on a dead and fallen branch of *Crataegus* sp. Sexual morph: *Hysterothecia* 250–320 μm diam. × 200–250 μm high × 600–725 μm long (x̄ = 290 × 230 × 660 μm, n = 10), superficial, immersed at the base, elongate and depressed, conchate, solitary to aggregated or scattered, ellipsoid or elongate, longitudinally straight, apex compressed, opening by a longitudinal slit, vertical section globose, black. *Peridium* 45–55 μm (x̄ = 58 μm, n = 10), carbonaceous, brittle, small prosenchymatous cells, heavily pigmented, black. *Hamathecium* comprises 0.5–1.5 μm wide, trabeculate, aseptate, branched, hyaline, and numerous pseudoparaphyses embedded in a glutinous matrix. *Asci* 50–110 × 6–11 μm (x̄ = 83 × 9.3 μm, n = 15), 8–spored, bitunicate, oblong to clavate, with a short pedicel, with a distinct ocular chamber, apically thickened. *Ascospores* 15–21 × 4–8 μm (x̄ = 18.7 × 6.1 μm, n = 20; l/w = 3.0), crowded to biseriate, fusiform, 3–septate, slightly constricted at the septum or not constricted, smooth-walled, ornamented, guttulate, hyaline when young, becoming brown at maturity, mucilaginous sheath absent. Asexual morph: Undetermined.

Material examined – Italy, Province of Forlì–Cesena [FC]), Ladino–Forlì, on a dead and fallen branch of *Crataegus* sp. (*Rosaceae*), 6 March 2017, Erio Camporesi, IT 3271, (MFLU 17-0822).

GenBank Accession Numbers – LSU: OQ411140; SSU: OQ411133; *tef*1–α: OQ437909.

Notes – In the multigene phylogeny, our strain (MFLU 17-0822) grouped with *H. angustatum* (SMH 5216) with 97% MLBS and 1.00 BYPP support (Fig. 19, Clade A) within Group 1. Morphologies were not provided for *H. angustatum* (SMH 5216) and only LUS and *tef*1-α phylogenetic analyses were provided by Mugambi & Huhndorf (2009). *Hysterium angustatum* was described by Persoon & Besemann (1801), on dead wood. Recently, Jayasiri et al. (2018) re-described the taxon based on morphology and molecular data and this was the first report of *H. angustatum* on *Rubus* sp. from Italy. Our collection shares similar morphologies with *H. angustatum* (MFLUCC 16-0623) provided by Jayasiri et al. (2018), however, our collection differs from it in having comparatively larger ascomata, asci, and ascospores (Table 6). Hyde et al. (2017a) opined that the ascomata, asci and ascospore sizes of *H. angustatum* may vary. This may be due to the environmental condition of the substrates collected. Our collection is from a fallen branch and the collection by Jayasiri et al. (2018) was from a hanging branch. Accordingly, larger asci were reported by Lohman (1933) and Zogg (1962) measuring 105–120 × 10–16 μm and 100–120 × 11–14 μm, respectively. Ellis & Everhart (1892) also reported wider asci with similar lengths (75–80 × 12–15 μm). Then, larger ascospores were reported by Ellis & Everhart (1892) and Lohman (1933), as 15–22 × 6–7 μm and 22–26 × 6.5–8(9) μm, respectively. We compared the sizes of ascomata, asci, and ascospores in our collection with MFLUCC 16-0623 (Table 6).
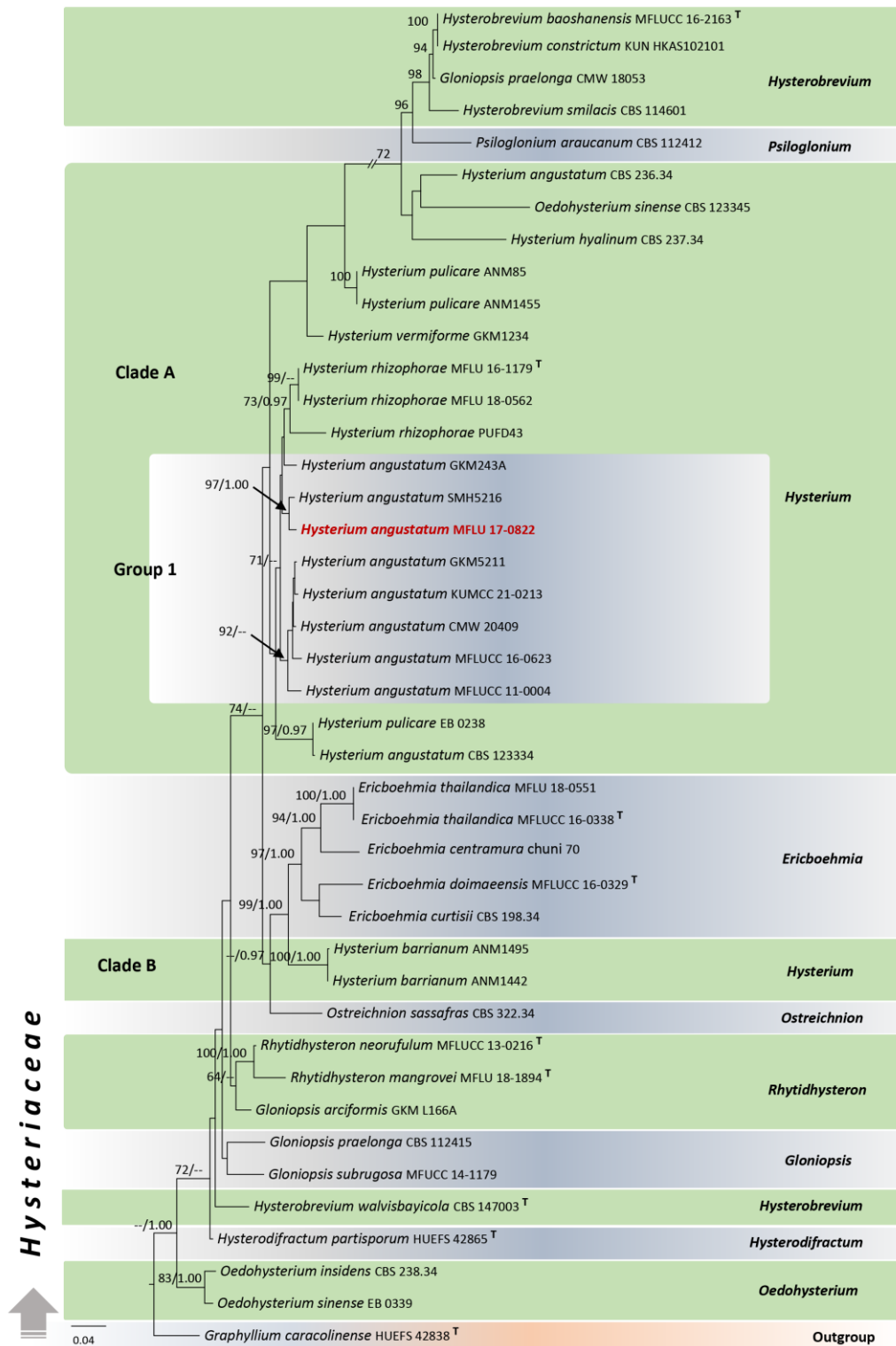
Fig. 19 – Phylogram generated from maximum likelihood analysis based on combined LSU, SSU, *tef*1-α, and *rpb*2 sequenced data. Forty-two strains were included in the combined sequence analyses, which comprised 3856 characters with gaps (LSU = 889, SSU = 1356, *tef*1-α = 899, *rpb*2 = 712). Single gene analyses were also performed, and topology and clade stability were compared from combined gene analyses. *Graphyllium caracolinense* (HUEFS 42838) strain was used as the outgroup taxon. Final ML optimization likelihood is -18707.117719. The matrix included 1300 distinct alignment patterns, with 44.80% undetermined characters or gaps. Estimated base frequencies were obtained as follows: A = 0.248687, C = 0.241529, G = 0.280807, T = 0.228977; substitution rates AC = 1.349687, AG = 2.972923, AT = 1.029279, CG = 0.896521, CT = 8.188144, GT = 1.0; gamma distribution. Bootstrap support values for ML (first set) equal to or

greater than 60%, BYPP equal to or greater than 0.95 are given above or below the nodes. The strain from the current study is in red bold, and the type strains are indicated with [T].

Table 6 Comparison of the sizes of ascomata, asci, and ascospores of *H. angustatum*.

Ascomata (high × wide) μm	Asci (μm)	Ascospores (μm)	Reference(s)
200–250 × 250–320	50(–75)–110 × 6(–9)–11	15–21 × 4–8	This study
208–232 × 256–284	59–66 × 7.6–9.2	14–17 × 4–5.3	Jayasiri et al. (2018)
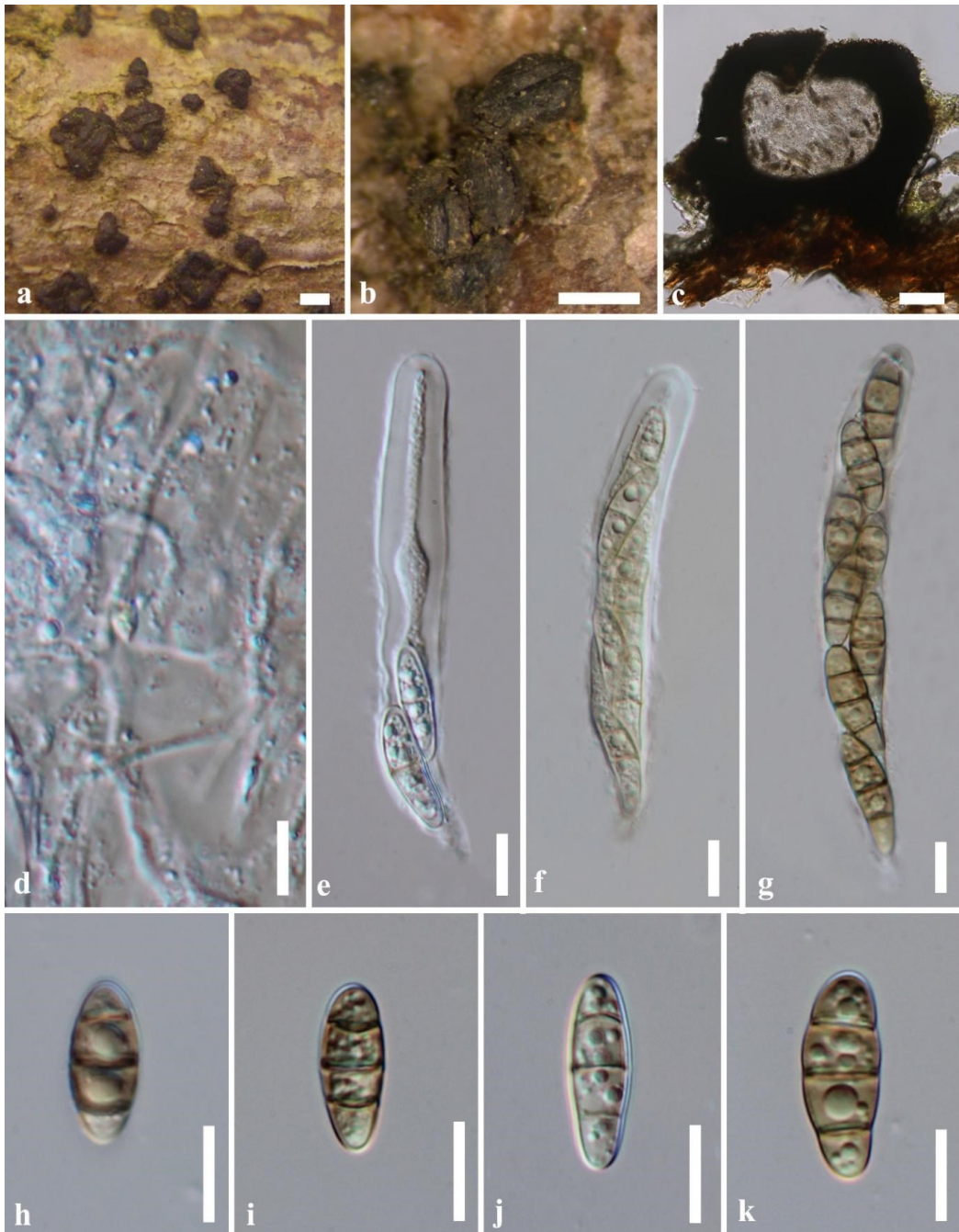
Fig. 20 – *Hysterium angustatum* (MFLU 17-0822, new host record). a–b Ascomata on a dead branch of *Crataegus* sp. (*Rosaceae*, *Rosales*). c Longitudinal section of an ascoma. d Pseudoparaphyses. e–g Asci. h–k Ascospores. Scale bars: a–b = 500 μm, c = 50 μm, d, e–k = 10 μm.

The previous records of *H. angustatum* were found in Kenya, New Zealand, Tennessee, the United States (Mugambi & Huhndorf 2009, Boehm et al. 2009b) and Italy (Jayasiri et al. 2018). The strains of *H. rhizophorae* species are grouped inside Clade A, as closely related to our strain (Fig. 19). The holotype of *H. rhizophorae* (MFLU 18-0562) differs from our specimen in having larger hysterothecia (1.5–2.0 long × 0.15–0.25 wide × 0.2–0.25 high mm), wider peridium (38.5–52 μm), septate pseudoparaphyses, larger asci (40.8–48.6 × 3.5–5 μm) and ellipsoidal, slightly curved ascospores (14.5–16 × 4–5.9) (Dayarathne et al. 2020a). Based on morphology and phylogenetic analyses we conclude our collection (MFLU 17-0822) is *H. angustatum* and it is the first record on *Crataegus* sp. and the second record for Italy.

Pleosporales Luttr. ex M.E. Barr 1987

Index Fungorum number: IF 90563; Facesoffungi number: FoF 08715

Notes – *Pleosporales* is the most speciose order in *Dothideomycetes* with a diverse number of species. The order was introduced by Luttrell (1955) invalidly and later validly established by Barr (1987a) based on *Pleosporaceae* with the type species *Pleospora herbarum* (= *Stemphylium vesicarium*) (Barr 1987b). In Pleosporalean species, sexual morphs are characterized by perithecioid ascomata usually papillate, with bitunicate, fissitunicate asci with different colored and shaped ascospores that are mostly septate and with or without a gelatinous sheath (Hyde et al. 2013, Hongsanan et al. 2020b). Asexual morphs are coelomycetous or hyphomycetous (Hongsanan et al. 2020b). Currently, this order comprises 91 families and 566 accepted genera based on both morphology and phylogenetic evidence (Hongsanan et al. 2020b, Wijayawardene et al. 2022). They have a worldwide distribution as epiphytes, endophytes or parasites, saprobes on dead wood, hyperparasites on fungi or insects, and lichenized taxa (Kirk et al. 2008, Zhang et al. 2012, Hongsanan et al. 2020b, Mortimer et al. 2021). The divergence time of the order was estimated at 205 MYA (stem age) (Hongsanan et al. 2020b). In this study, we report some plant-associated pleosporalean taxa belonging to *Cucurbitariaceae, Didymellaceae, Leptosphaeriaceae*, and *Neomassariaceae* collected from Italy.

Cucurbitariaceae Luerss. 1877

Index Fungorum number: IF 80667; Facesoffungi number: FoF 08179

Notes – *Cucurbitariaceae* was originally described by Luerssen (1877) and later it was typified with the generic type *Cucurbitaria berberidis* Winter (1885). Following recent phylogenetic studies, *Cucurbitariaceae* is a well-supported monophyletic family in *Pleosporales* (Li et al. 2016, Wanasinghe et al. 2017, Jaklitsch et al. 2018). *Cucurbitariaceae* are characterized by solitary to aggregated, perithecioid ascomata with ostiole and seated on a basal stromatic structure, cylindrical to oblong, fissitunicate asci and pigmented, phragmosporous or muriform ascospores in the sexual morphs (Hyde et al. 2013, Wanasinghe et al. 2017), while asexual morphs are coelomycetous, phoma- or pyrenochaeta-like (Jaklitsch et al. 2018). This family comprises 13 genera and they can be necrotrophic or saprophytic on woody plant hosts or parasitic on other fungi (Hongsanan et al. 2020b, Wijayawardene et al. 2022). In this study, we discuss two saprobic species from Italy, referred to as *Neocucurbitaria* and *Fenestella*.

Fenestella Tul. & C. Tul. 1863

Index Fungorum number: IF 1983; Facesoffungi number: FoF 00576

Notes – *Fenestella* was introduced by Tulasne & Tulasne (1863). The genus was accepted into *Dothideomycetes* as it forms valsoid ascostromata, having bitunicate asci and brown to reddish-brown, muriform ascospores (Barr 1979, Huhndorf & Glawe 1990, Hyde et al. 2013, Phookamsak & Hyde 2015, Wanasinghe et al. 2017). *Fenestella* was relatively poorly studied, and the type species of the genus could not be located (Wanasinghe et al. 2017, Hongsanan et al. 2020b). Therefore, Phookamsak & Hyde (2015) revisited the family *Fenestellaceae* and maintained the monotypic genus *Fenestella* in the family due to the lack of a modern taxonomic description of the genus and limited molecular data. Later, an updated phylogeny was conducted by Wanasinghe

et al. (2017) to propose that *Fenestella* be transferred to *Cucurbitariaceae* and *Fenestellaceae* be synonymized with *Cucurbitariaceae*. Jaklitsch et al. (2018) re-described the generic type of *Fenestella* as *F. fenestrate* and provided lecto- and epitypification with DNA sequence data. The recently updated phylogeny was provided by Jaklitsch & Voglmayr (2020). *Pleurostromella* is treated as an asexual morph of *Fenestella*, thus reduced to a synonym with *Fenestella* (Hongsanan et al. 2020b). Currently, 75 species epithets are listed under *Fenestella* in Index Fungorum (2022), with eight species supported by molecular phylogeny.

Fenestella media Tul. & C. Tul. 1863   Fig. 22

    = *Fenestella macrospora* Fuckel, Jahrb. Nassauischen Vereins Naturk. 25–26: 313. 1871

    Index Fungorum number: IF 146499; Facesoffungi number: FoF 14006

    Saprobic on a dead aerial branch of *Corylus avellana* L. Sexual morph: *Pseudostromatic pustules* 0.8–1.5 mm diam. scattered on the host substrate, visible as black ruptures. *Ascomata* 450–600 μm diam., 300–500 μm high (x̄ = 560 × 365 μm, n = 5), subglobose to pyriform, arranged in valsoid configuration, 8–9 per individual ascostroma, semi-immersed to erumpent, on *Cytospora* sp. (sometimes effete conidiomata), connected by dark brown walled subicular hyphae or form pseudostromatic structures. *Ostioles* 100–150 μm diam, indistinct at the surface. *Peridium* 20–80 μm thick, pseudoparenchymatous, composed of dark brown narrow outermost layers and pale brown to hyaline inner layers, comprising cells of *textura angularis*. *Hamathecium* comprises 2–4.5 μm wide, filamentous, branched, septate pseudoparaphyses. *Asci* 180–280 × 20–25 μm (x̄ = 223 × 23 μm, n = 10), 8-spored, cylindrical to oblong, bitunicate, fissitunicate, with an ocular chamber, usually a short stipe and a knob-like base, usually uniseriate, sometimes partly biseriate. *Ascospores* 27–41 × 9–15 μm, (x̄ = 36 × 12 μm, n = 20; l/w = 3.0), thick-walled, ellipsoid to broadly fusoid, hyaline to pale yellow at the beginning, 1–6 transversely septate, yellowish brown to golden brown, with often indistinct septa at maturity, 11–18 transverse and 3–6 longitudinal septa, asymmetric with submedian primary septum, upper part higher than lower, with verruculose surface, concolorous or hyaline terminal cells, projecting as apiculi. Asexual morph: see Jaklitsch & Voglmayr (2020).

    Culture characteristics – Ascospores germinating on PDA within 24 hours at 18 °C, germ tubes produced by all sides of the ascospore, colonies growing on PDA and reaching 20–25 mm diam. after 7 days, circular, with the entire edge, white, dense, and convex surface on the upper side, reverse greenish brown at the center, and pale-yellowish white surrounding.

    Material examined – Italy, Province of Trento [TN], Bagni di Rabbi, on a dead aerial branch of *Corylus avellana* L. (*Betulaceae*), 15 September 2020, Erio Camporesi, IT 4651, (MFLU 20-0622, MFLUCC 23-0018).

    GenBank Accession Numbers – ITS: OQ430683, OQ401064; LSU: OQ428180, OQ411148; *tub*2: OQ437902.

    Notes – In the phylogeny, our strains MFLU 20-0622 and MFLUCC 23-0018, grouped with *Fenestella media* strains (CBS 144860-FP, FP1, and FP3) with 100% MLBS and 1.00 BYPP support (Fig. 21). Based on morphology, our collection shares a lower range of the ascomatal dimensions compared to the type and other representative specimens (Jaklitsch & Voglmayr 2020, this study). Pseudostromatic pustules are also smaller in size (0.8–1.5 mm *vs.* 0.6–3.6 mm), even if the size and development of pseudostromata vary considerably among specimens (Jaklitsch & Voglmayr 2020). Additionally, the width of pseudoparaphyses of our collection is larger (2–4.5 μm) than that of the others, which ranges from 1.5–2.5(–3) μm (Jaklitsch & Voglmayr 2020). *Fenestella media*, *F. subsymmetrica* and *F. viburni* are closely related cryptic species that are difficult to identify by morphology, and all species reported on *Cytospora* spp. associated with a plant host (Jaklitsch & Voglmayr 2020). However, in our phylogenetic analysis, these species are distinct, within Clade A.

    The holotype of *F. media* was collected in France on *Cytospora fagaci* living on *Salix alba*. A lectotype and an epitype of *F. media* (as *F. macrospora*) were designated by Jaklitsch & Voglmayr (2020) on *Cytospora* sp. associated with *Corylus avellana* hosts, collected in Germany and Austria,

respectively. Our two strains of *F. media* were reported on a *Cytospora* sp. (asexual morph) associated with *C. avellana* trees as well, and the majority of the previous records were from *Fagales* species. This is a new geographical record in Italy.
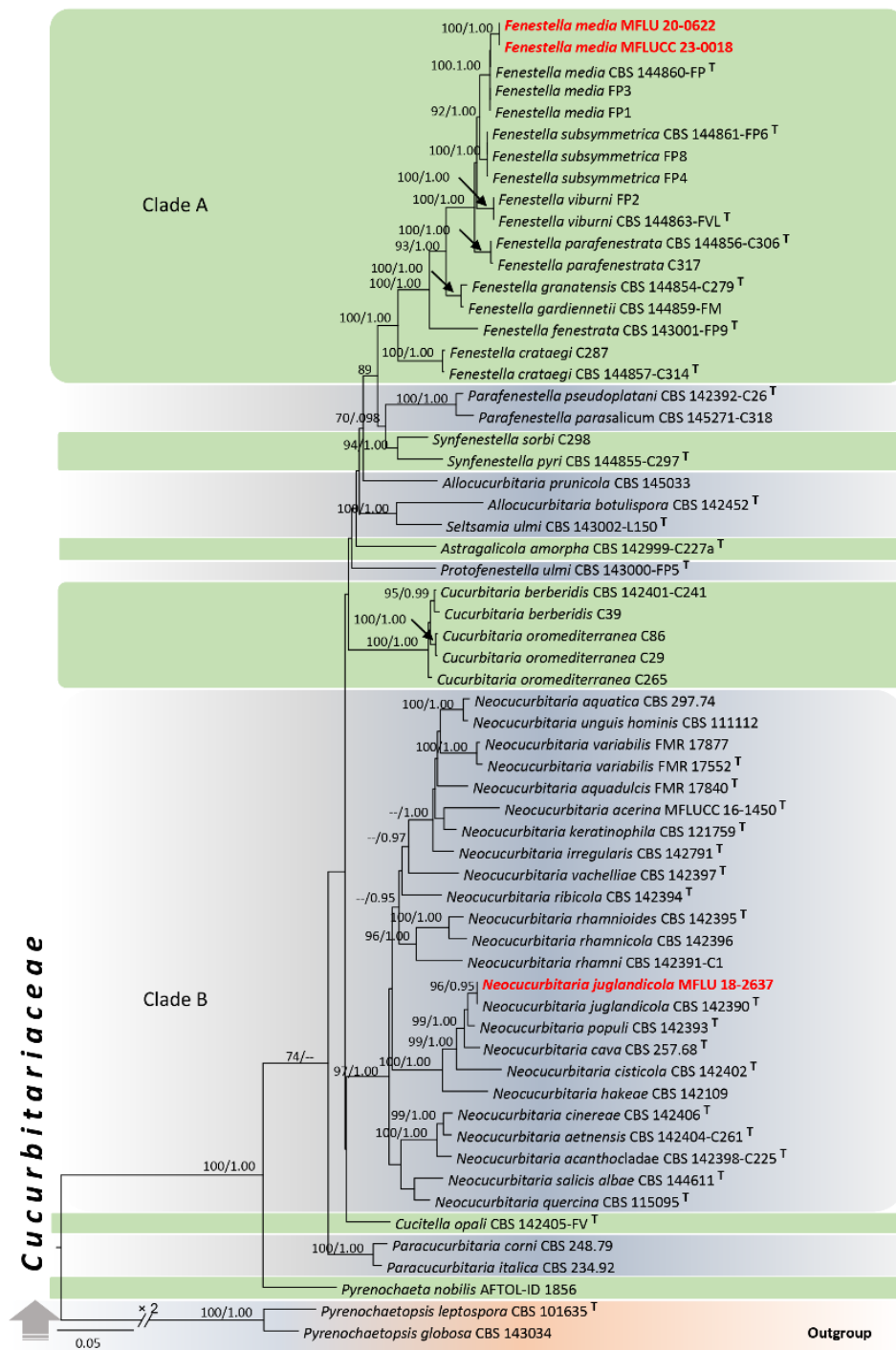
Fig. 21 – Phylogram generated from maximum likelihood analysis based on combined ITS, LSU, *tub*2, and *rpb*2 sequenced data. Sixty-one strains were included in the combined sequence analyses, which comprised 2585 characters with gaps (ITS = 471, LSU = 824, *tub*2 = 379, *rpb*2 = 902). Single gene analyses were also performed, and topology and clade stability were compared from combined gene analyses. *Pyrenochaetopsis leptospora* (CBS 101635) and *Pyrenochaetopsis globosa* (CBS 143034) strains were used as the outgroup taxa. Final ML optimization likelihood is -19137.693857. The matrix included 848 distinct alignment patterns, with 6.07% undetermined characters or gaps. Estimated base frequencies were obtained as follows: A = 0.240618, C = 0.249855, G = 0.273676, T = 0.235851; substitution rates AC = 1.688460, AG = 6.037478, AT =

1.442639, CG = 1.024102, CT = 9.017933, GT = 1.0; gamma distribution. Bootstrap support values for ML (first set) equal to or greater than 70%, BYPP equal to or greater than 0.95 are given above or below the nodes. The strains from the current study are in red bold, and the type strains are indicated with [T].
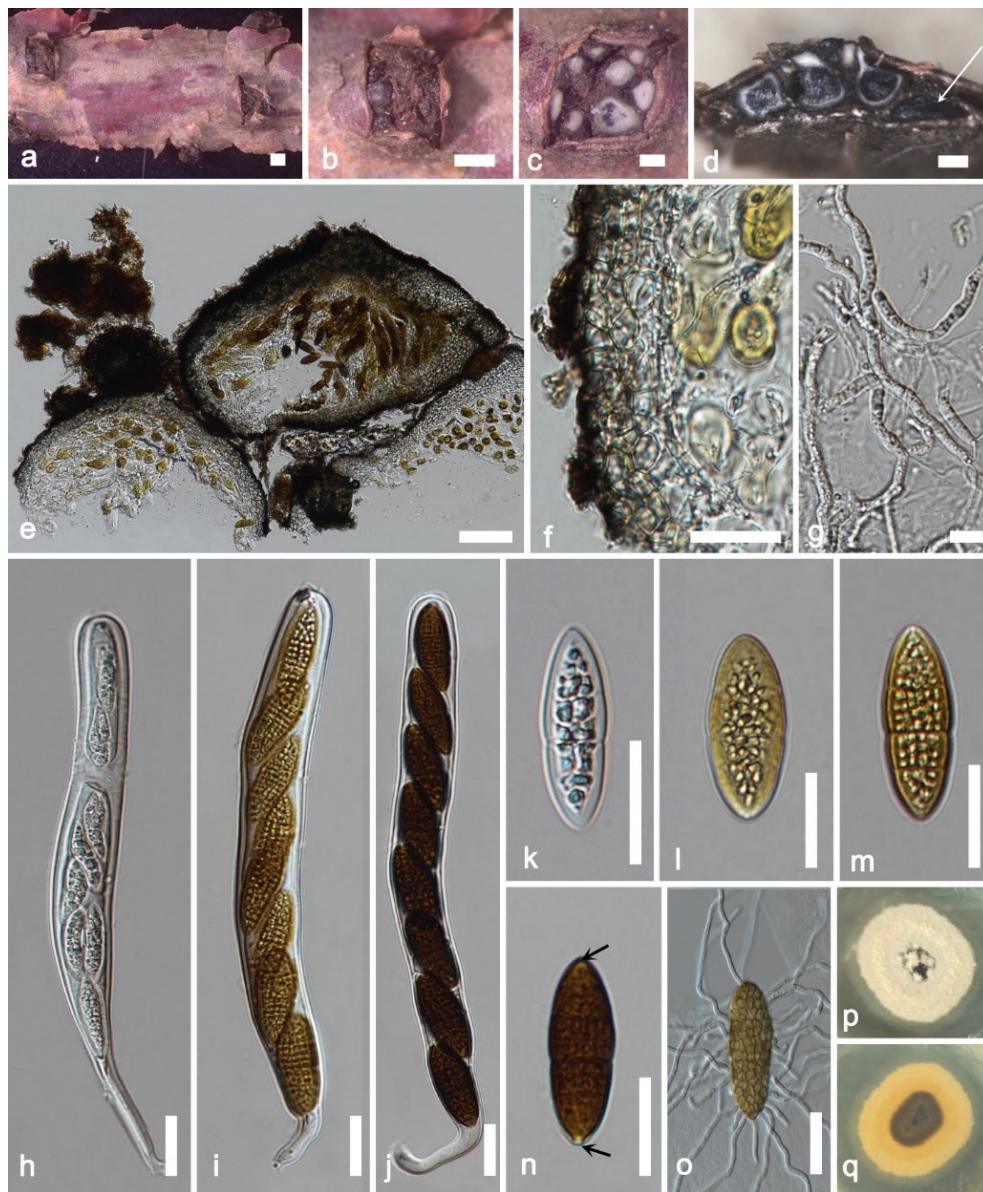
Fig. 22 – *Fenestella media* (MFLU 20-0622, new geographical record). a–c Pseudostromatic pustules erumpent on dead host surface of *Corylus avellana* L. (*Betulaceae*, *Fagales*). d–e Longitudinal section of ascomata. f Peridium. g Pseudoparaphyses. h–j Asci. k–n Ascospores (m: arrowed the projections as apiculi). o A germinated ascospore. p–q Colonies on PDA (p-upper, q-lower). Scale bars: a–d = 200 μm, e = 100 μm, f, h–o = 20 μm, g = 10 μm.

Neocucurbitaria Wanas., E.B.G. Jones & K.D. Hyde 2017

  Index Fungorum number: IF 552832; Facesoffungi number: FoF 02902

  Notes – *Neocucurbitaria* was introduced by Wanasinghe et al. (2017) to accommodate *N. acerina* as the type species of the genus together with *N. unguis-hominis*. The sexual morph of *Neocucurbitaria* is characterized by scattered or aggregated, erumpent, brown to black ascomata, that are globose to pyriform or turbinate, bitunicate, fissitunicate, cylindrical asci containing 4–8 muriform ascospores. Asexual morphs are pycnidial, and have ostiolate conidiomata, abundant with setae, and produce hyaline and aseptate conidia (Sutton 1980, Wanasinghe et al. 2017, Valenzuela-

Lopez et al. 2018). These species were reported as saprobes and pathogens on plants hosts, opportunistic pathogens in human keratitis, and in plant-associated soil and air in terrestrial habitats as well as in marine environments (Jaklitsch et al. 2018, Garcia-Hermoso et al. 2019, Valenzuela-Lopez et al. 2019, Hu et al. 2022). There are 24 species epithets listed in the Index Fungorum under *Neocucurbitaria* (Index Fungorum 2022).

Neocucurbitaria juglandicola Jaklitsch & Voglmayr 2017   Fig. 23
   Index Fungorum number: IF 823007; Facesoffungi number: FoF 14007
   Saprobic on a dead and fallen branch of *Quercus pubescens* Willd. Sexual morph: *Ascomata* 140–270 μm diam., 120–180 μm high, (x̄ = 190 × 150 μm, n = 10), semi-immersed to erumpent, scattered or aggregated, globose to pyriform, sometimes with apical papilla, dark brown to black. *Peridium* 50–60 μm wide, thick-walled, from the outermost layer towards inner layers consisting of dark brown to pale brown cells of *textura angularis*. *Hamathecium* comprises 1–2 μm wide (x̄ = 1.8 μm, n = 10), branched, hyaline, numerous pseudoparaphyses. *Asci* 120–170 × 13–16 μm (x̄ =148 × 14 μm, n = 20), 8-spored, bitunicate, fissitunicate, oblong to cylindrical from immature to mature, short distinct pedicel with furcate or knob-like ends, apically rounded, well-developed ocular chamber. *Ascospores* 12–25 × 6–8.5 μm (x̄ = 15 × 7 μm, n = 30; l/w = 2.1), overlapping, uniseriate, ellipsoid, straight or slightly curved, 3–6 transversely septate, vertically aseptate at the beginning, and 1–2 vertically septate at maturity, constricted at the middle septum, sometimes slightly constricted at other septa, upper half slightly larger at maturity, usually obtuse at apex, smooth-walled, pale brown to dark brown. Asexual morph: see Jaklitsch et al. (2018).
   Material examined – Italy, Province of Forlì–Cesena [FC], Spinello di Santa Sofia, on a dead and fallen branch of *Quercus pubescens* Willd. (*Fagaceae*), 6 November 2018, Erio Camporesi, IT 4104, (MFLU 18-2637).
   GenBank Accession Numbers – ITS: OQ401058; LSU: OQ411143.
   Notes – In the phylogeny, our strain MFLU 18-2637 grouped with *Neocucurbitaria juglandicola* strains (CBS 142390) with 96% MLBS and 0.95 BYPP support (Fig. 21). *Neocucurbitaria juglandicola* was introduced by Jaklitsch et al. (2018) on the bark of *Juglans regia* from Vienna, Austria. The morphology of our collection MFLU 18-2637 shares similar morphology to the ex-holotype culture (CBS 142390) reported by Jaklitsch et al. (2018). Other collections of the fungus were reported on *Quercus rubra* hosts in Austria and *Magnolia grandiflora* in China (Jaklitsch and Voglmayr 2020, Zhang et al. 2022). Therefore, we identified our new strain as *N. juglandicola*, which is the first record on *Quercus pubescens* in Italy and worldwide.

Didymellaceae Gruyter, Aveskamp & Verkley 2009
   Index Fungorum number: IF 508292; Facesoffungi number: FoF 08216
   Microsphaeropsidaceae Q. Chen et al.
   Notes – *Didymellaceae* was established by de Gruyter et al. (2009) to include species traditionally classified as *Phoma* and phoma-like. Species delineation of *Didymellaceae* is mainly based on morphology and plant host association (Aveskamp et al. 2010, Chen et al. 2015, 2017, Hyde et al. 2020b). Initially, the family consisted of three genera: *Ascochyta*, *Didymella*, and *Phoma* (Aveskamp et al. 2010, Hongsanan et al. 2020b). After several studies, Chen et al. (2015) accepted 17 well-supported generic clades. Later, thirty-five genera were listed under *Didymellaceae* by Hongsanan et al. (2020b) and updated to forty-five genera by Wijayawardene et al. (2022). Sexual morphs of *Didymellaceae* are characterized by having immersed or superficial, globose to flattened, ostiolate pseudothecia, 8-spored, bitunicate, fissitunicate, cylindrical to clavate or saccate asci arising from a broad hymenium among pseudoparaphyses, and hyaline to pigmented, 1-septate to multi-septate ascospores (Hyde et al. 2020b). Coelomycetous or hyphomycetous asexual morphs are found on natural substrates or in culture and are characterized by semi-immersed to erumpent, ostiolate, pycnidial conidiomata, enteroblastic, hyaline, thin-walled conidiogenous cells, hyaline or pigmented and septate or aseptate, guttulate conidia (Chen et al.

2015, Hongsanan et al. 2020b, Hyde et al. 2020b). Members of this family are cosmopolitan and show a wide range of hosts with different life modes such as pathogenic, endophytic, and saprobic, as well as fungicolous and lichenicolous (Hongsanan et al. 2020b, Hyde et al. 2020a, b). In this study, we report two new records from Italy, belonging to *Ascochyta* and *Boeremia*.
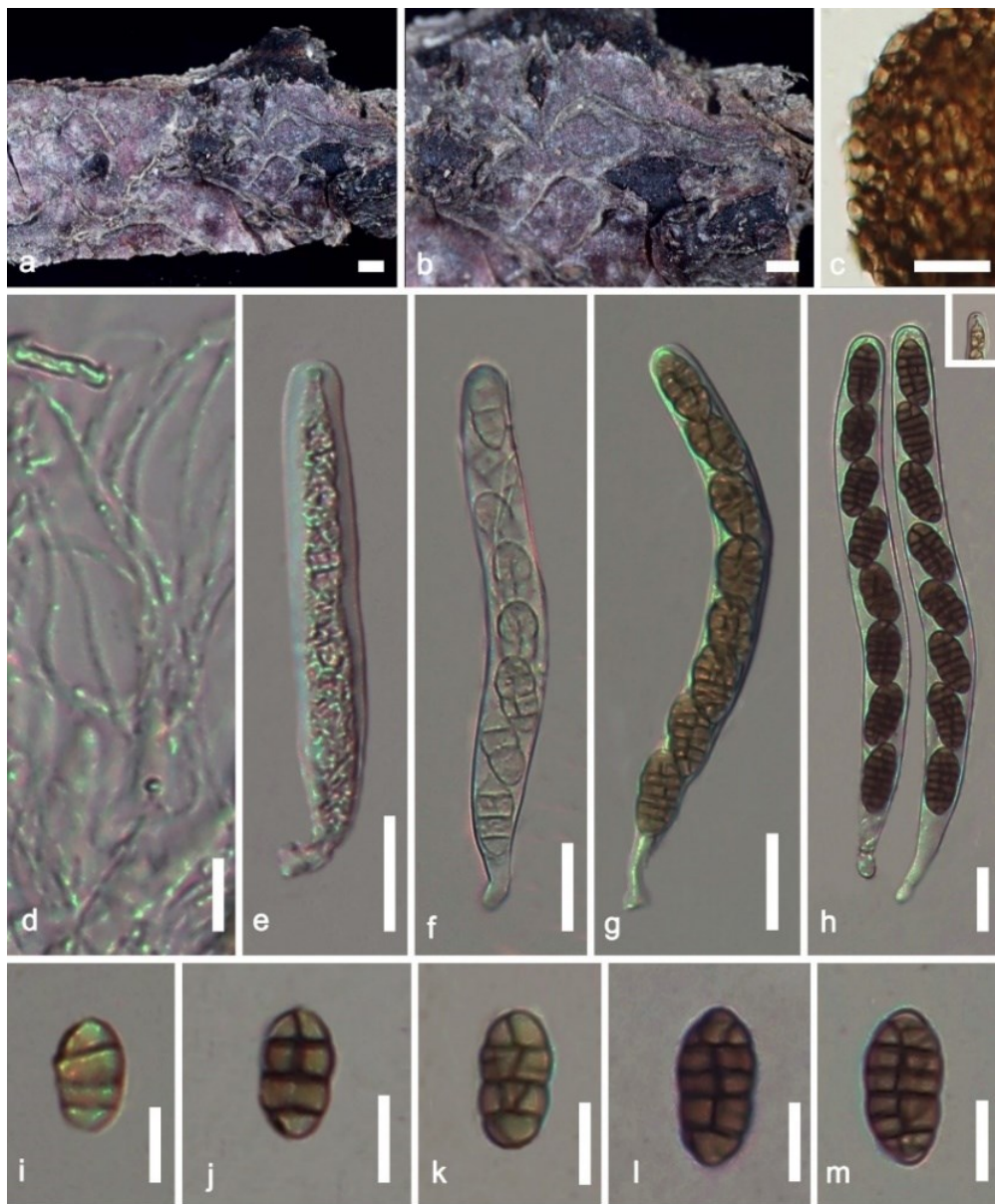
Fig. 23 – *Neocucurbitaria juglandicola* (MFLU 18–2637, new host record). a–b Ascomata on the dead host surface of *Quercus pubescens* Willd. (*Fagaceae*, *Fagales*). c Peridium. d Pseudoparaphyses. e–i Asci. j–n Ascospores. Scale bars: a, b = 500 μm, c, e–h = 20 μm, d, i–m = 10 μm.

Ascochyta Lib. 1830
    Index Fungorum number: IF 7239; Facesoffungi number: FoF 07121
    Notes – *Ascochyta* was introduced by Libert (1830) with *A. pisi* as the type species (Hongsanan et al. 2020b, Hyde et al. 2020b). *Ascochyta* was previously described based on its sexual morphs, and Chen et al. (2015) linked the asexual morph with a coelomycetous state. Key morphological characters of the genus are globose locules with perithecial protuberances immersed in the stroma and oblong to ellipsoidal aseptate conidia (Hongsanan et al. 2020b, Hyde et al. 2020b). Members of this genus include endophytic, pathogenic, and saprobic species found on

different hosts worldwide (Wijayawardene et al. 2017, Hyde et al. 2020a) despite the fact that they mostly occur on *Campanulaceae*, *Chenopodiaceae*, *Leguminosae*, *Poaceae*, *Solanaceae*, and *Umbelliferae* (Valenzuela-Lopez et al. 2018, Hongsanan et al. 2020b). There are 1,429 records of *Ascochya* listed in the Index Fungorum (2022). In this study, we report *Ascochyta medicaginicola* found on *Medicago* sp. from Italy.

Ascochyta medicaginicola Qian Chen & L. Cai 2015   Fig. 25
    = *Phoma medicaginis* Malbr. & Roum., in Roumeguère 1886.
    Index Fungorum number: IF 814129; Facesoffungi number: FoF 00423
    Saprobic on a dead aerial stem of *Medicago* sp. Sexual morph: see Tibpromma et al. (2017). Asexual morph: Coelomycetous. *Conidiomata* 150–190 μm diam., 100–165 μm high, (x̄ = 177 × 150 μm, n = 5), pycnidial, solitary, scattered or gregarious, visible as black dots on the host surface, globose to subglobose, semi-immersed to immersed, unilocular, thin-walled. *Ostioles* circular, single papillate, central. *Pycnidial walls* 15–25 μm wide, 3–4 layers, similarly dense at each side, outer layers dark brown, inner layers hyaline, with cells of *textura angularis*. *Conidiophores* reduced to conidiogenous cells. *Conidiogenous cells* 3–5 μm high, 2–4 μm wide, enteroblastic, phialidic, doliiform, hyaline. *Conidia* 5–10 × 2–4 μm (x̄ = 7.5 × 3.1 μm, n = 20), ellipsoidal to cylindrical, rounded at apex, straight or slightly curved at the middle, aseptate, smooth-walled, hyaline, guttulate.
    Material examined – Italy, Province of Forlì–Cesena [FC]), Passo del Carnaio, on a dead aerial stem of *Medicago* sp. (*Fabaceae*), 20 November 2018, Erio Camporesi, IT 4116, (MFLU 19-0269).
    GenBank Accession Numbers – ITS: OQ401059; LSU: OQ411144.
    Notes – In multi-gene phylogeny, our strain (MFLU 19-0269) grouped with the reference strains of *A. medicaginicola* (CBS 112.53, CBS 316.90, BRIP 450.51, CBS 404.65, and MFLUCC 18-0095) with 95% MLBS and 1.00 BYPP support (Fig. 24, Clade A). Our strain (MFLU 19-0269) is more closely related to *A. medicaginicola* (MFLUCC 18-0095) and no bp differences were revealed in ITS and LSU regions. Base pair differences between the closely related MFLUCC 18-0095 and MFLU 19-0269 and reference strains of *A. medicaginicola* (BRIP 450.51, CBS 404.65, CBS 112.53, CBS 316.90), revealed 2 bp differences (0.4%) out of 422 bp in ITS region while LSU, *rpb*2 and *tub*2 are identical. A strain of *A. medicaginicola* var. *medicaginicola* (MFLUCC 16-0599) grouped basal to other strains. However, we noted 12 bp differences (2.8%) out of 422 bp of ITS region between *A. medicaginicola* strains (MFLUCC 18-0095 and MFLU 19-0269) and the *A. medicaginicola* var. *medicaginicola* (MFLUCC 16-0599) strain, while LSU base pairs are identical. Therefore, we keep MFLUCC 16-0599 strain as *A. medicaginicola* var. *medicaginicola* and further studies are suggested.
   The two varieties of *A. medicaginicola* such as *A. medicaginicola* var. *macrospora* and *A. medicaginicola* var. *medicaginicola* were proposed by Chen et al. (2015) and are currently accepted as *A. medicaginicola* (Jayasiri et al. 2017, Hongsanan et al. 2020b, Hyde et al. 2020b, Li et al. 2020). Our collection (MFLU 19-0269) shares similar morphologies with other *A. medicaginicola* collections (Hyde et al. 2022b). Based on morphology and muli-gene phylogeny, we conclude our collection is *A. medicaginicola* and the second record on *Medicago* sp. in Italy, while Hyde et al. (2020b) reported the fungus on the same host (*Medicago* sp.) and in the Forlì-Cesena province. The known distribution of the fungus on *M. sativa* is in Canada, the Czech Republic, France, and Minnesota (Farr & Rossman 2022). We suggest that *A. medicaginicola* can be a host recurrent species on *Medicago* hosts.

Boeremia Aveskamp, Gruyter & Verkley 2010
    Index Fungorum number: IF 515621; Facesoffungi number: FoF 07128
   Notes – *Boeremia* was established by Aveskamp et al. (2010) to accommodate phoma-like species. Members of this genus are morphologically similar to *Phoma exigua*, but molecular data separates them into two distinct groups (Aveskamp et al. 2010, Jayasiri et al. 2017, Dayarathne et

al. 2020a). *Boeremia* species and varieties are identified primarily through host association (Berner et al. 2015, Dayarathne et al. 2020a). *Boeremia* sexual morph is characterized by ellipsoidal, 1-septate ascospores, while the asexual morph has 0–2-septate conidia in different shapes. Chen et al. (2015, 2017) and Marin-Felix et al. (2017) suggested the importance of using combined DNA sequence data with morphological observations when identifying species of this genus. Jayawardena et al. (2019) synonymized most of the *Boeremia exigua* varieties up to the species level. Species have been reported from both terrestrial and marine habitats (Aveskamp et al. 2010, Jayasiri et al. 2017, Dayarathne et al. 2020a). There are 35 species epithets listed under *Boeremia* in the Index Fungorum (2022).
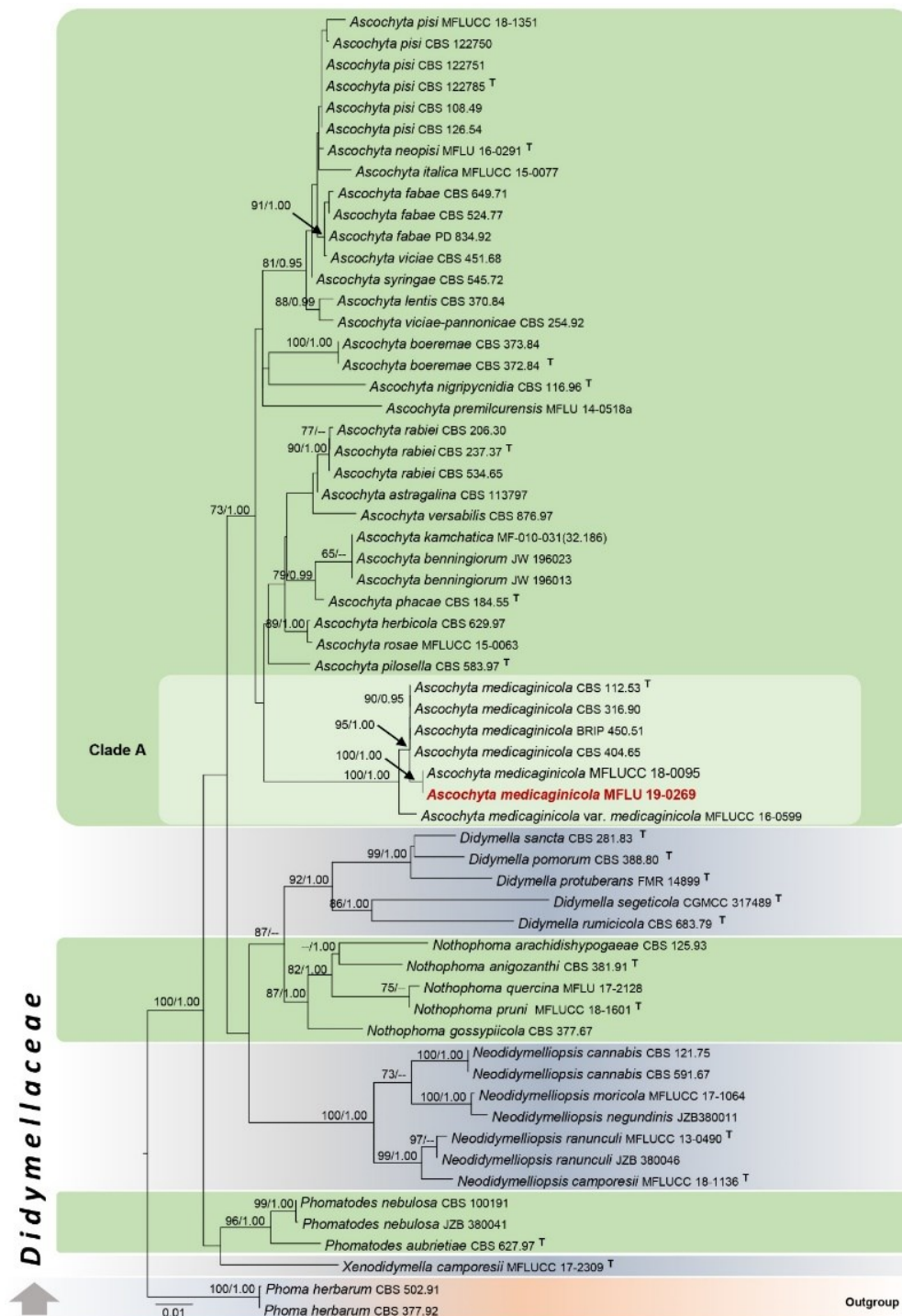
Fig. 24 – Phylogram generated from maximum likelihood analysis based on combined LSU, ITS, *rpb*2 and *tub*2 sequenced data. Sixty-one strains were included in the combined sequence analyses,

which comprised 2131 characters with gaps (LSU = 840, ITS = 435, *rpb*2 = 594, *tub*2 = 262). Single gene analyses were also performed, and topology and clade stability were compared from combined gene analyses. *Phoma herbarum* (CBS 509.91, CBS 377.92) strains were used as the outgroup taxa. Final ML optimization likelihood is -8760.096205. The matrix included 430 distinct alignment patterns, with 14.52% undetermined characters or gaps. Estimated base frequencies were obtained as follows: A = 0.239641, C = 0.238201, G = 0.278009, T = 0.244150; substitution rates AC = 1.505606, AG = 7.652346, AT = 2.930419, CG = 0.943442, CT = 17.514654, GT = 1.0; gamma distribution. Bootstrap support values for ML (first set) equal to or greater than 60%, BYPP equal to or greater than 0.95 are given above or below the nodes. The strain from the current study is in red bold, and the type strains are indicated with [T].
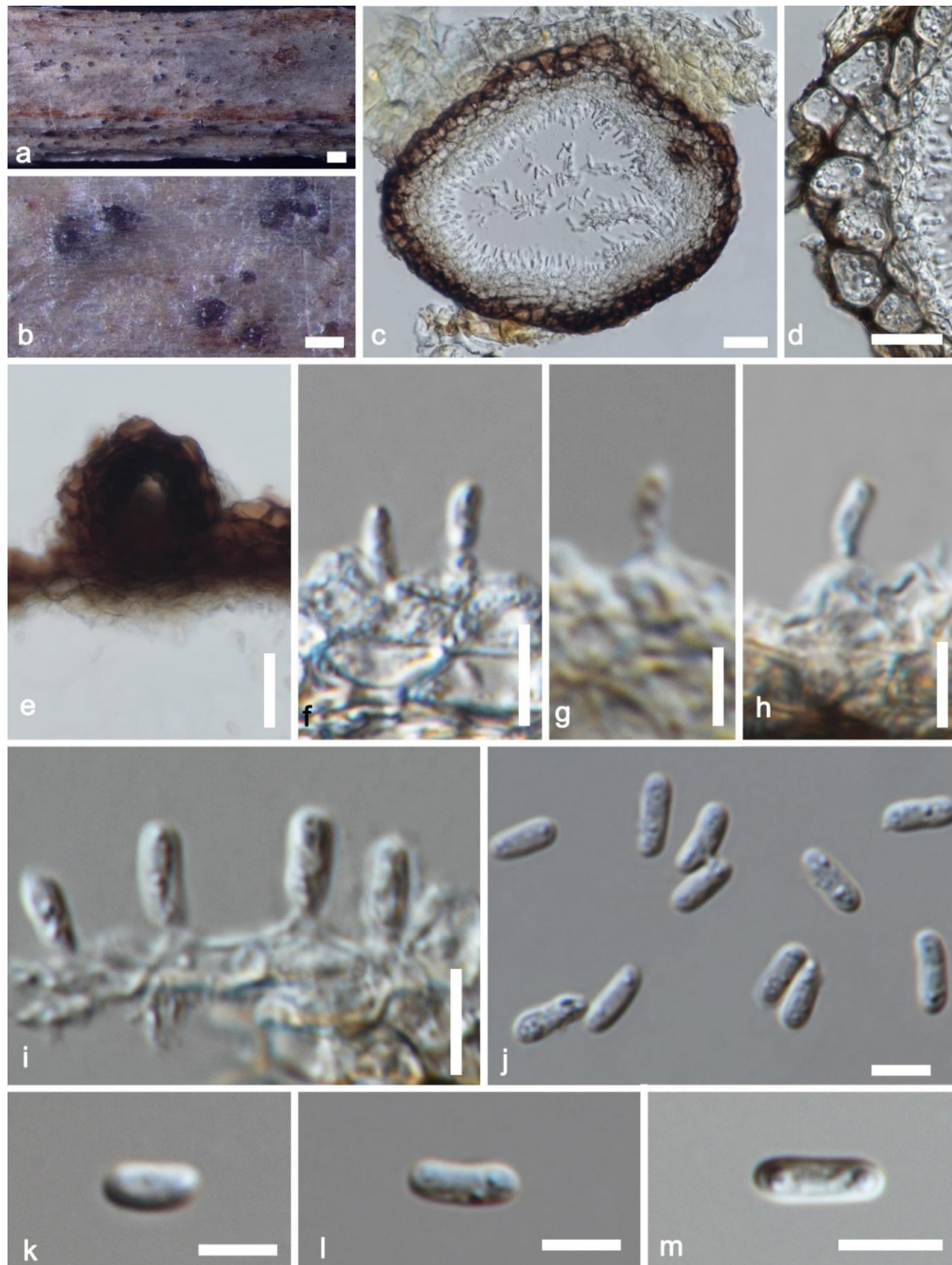
Fig. 25 – *Ascochyta medicaginicola* (MFLU 19-0269, host recurrent species on *Medicago*). a–b Conidiomata on the dead host surface of *Medicago* sp. (*Fabaceae*, *Fabales*). c Longitudinal section of a conidioma. d Conidioma wall. e Longitudinal section of ostiole, f–i Development

stages of conidiogenesis. j–m Conidia. Scale bars: a = 500 μm, b = 100 μm, c, e = 20 μm, d = 10 μm, f–m = 5 μm.

Boeremia galiicola Jayasiri, Camporesi & K.D. Hyde 2017   Fig. 27

Index Fungorum number: IF 552360; Facesoffungi number: FoF 02501

Saprobic on dead aerial stems of *Galium* sp. Sexual morph: *Ascomata* 180–230 μm diam., 150–210 μm high (x̄ = 200 × 180 μm, n = 10), immersed, visible black circular spots on the host surface, without subiculum covering the host, solitary, scattered, globose to subglobose, brown to black. *Ostioles* central, without periphyses. *Peridium* 20–50 μm, 3–4 layers, from outermost towards innermost layers composed of dark brown to hyaline cells of *textura angularis*. *Hamathecium* comprises 1.5–2 μm wide, filiform to cylindrical, hyaline, numerous pseudoparaphyses. *Asci* 70–90 × 8–11 μm (x̄ = 75 × 9 μm, n = 20), 8-spored, cylindric-clavate, bitunicate, fissitunicate, slightly curved, smooth-walled, with an ocular chamber, apically rounded, short-pedicellate with a knob-like base. *Ascospores* 17–22 × 3–5 μm (x̄ = 20 × 5 μm, n = 20), ellipsoid to fusiform, mostly 1-septate, sometimes 2–3 septate, constricted at the middle septum, overlapping, 1–3-seriate, widest at the middle and tapering toward narrow ends, straight or slightly curved, thick and smooth-walled, hyaline, guttulate. Asexual morph: Coelomycetous. see Jayasiri et al. (2017).

Culture characteristics – Ascospores germinated on PDA within 24 hours and germ tubes produced from one end. Colonies on PDA reaching 30 mm diam. after 5 days at 18 °C, flat, irregular margin, forward white to olivaceous gray, brownish gray.

Material examined – Italy, Province of Forlì-Cesena [FC]), near Lago Pontini – Bagno di Romagna, on dead aerial stems of *Galium* sp. (*Rubiaceae*), 20 May 2015, Erio Camporesi, IT 2491, (MFLU 19-2554, HKAS 92494), living culture = KUMCC 15-0522.

GenBank Accession Numbers – ITS: OQ401053; LSU: OQ411136.

Note – In multigene phylogeny, our strain (MFLU 19-2554) clusters with the ex-type strain of *B. galiicola* (MFLUCC 15-0771) with 88% MLBS and 0.95 BYPP support (Fig. 26). In base pair comparison, both strains are identical in ITS and LSU sequences. *Boeremia galiicola* was introduced by Jayasiri et al. (2017) on *Galium* sp. from Italy, based on morphology and phylogeny. This new collection shares similar morphologies with the type in having ascomata, peridium, pseudoparaphyses, asci and ascospores. Thus, we conclude that our new collection is *B. galiicola*. Contrary to the holotype, which was from Arezzo province (Tuscany region) and our collection was found on the same host species, but in a different region, Forlì–Cesena (Emilia–Romagna region). Therefore, our collection is the first record of *B. galiicola* in the Emilia–Romagna region, Italy.

Neomassariaceae H.A. Ariyaw., Jaklitsch & Voglmayr 2018

Index Fungorum number: IF 827113; Facesoffungi number: FoF 08315

Notes – *Neomassariaceae* was proposed for *Neomassaria* species, including two species *N. fabacearum* and *N. formosana*, based on morphology and multi-gene phylogeny (LSU, *rpb*2, SSU, and *tef*1-α) by Ariyawansa et al. (2018). The sexual morph of the family is characterized by having globose to subglobose, immersed ascomata with central ostioles, *textura angularis* peridium cells, cylindrical to filiform, cellular or trabeculate pseudoparaphyses, oblong to cylindrical, 8-spored, bitunicate asci and ellipsoid to fusiform, 1-septate, hyaline ascospores with or without a gelatinous sheath (Hongsanan et al. 2020b). Initially, *Neomassaria* was introduced under *Massariaceae* by Hyde et al. (2016) to accommodate *N. fabacearum*. *Massariaceae* contains species with large subglobose to broadly pyriform ascomata, large oblong, fusoid, or clavate asci with a refractive ring, comprising 2–3-seriate oblong, narrowly ellipsoidal, or fusoid, dark umber to blackish brown, 3-septate ascospores (Voglmayr & Jaklitsch 2011, Hyde et al. 2020b). *Neomassariaceae* characters differ from *Massariaceae* in having comparatively smaller globose to subglobose ascomata, asci lacking a refractive ring, and hyaline, 1-septate ascospores (Ariyawansa et al. 2018, Hyde et al. 2020b). Five species are accepted into *Neomassaria* and the reported species are saprobes (de Silva et al. 2022, Yang et al. 2022a). In this study, we introduce a new genus,

Pigmentatineomassaria to the family based on a polyphasic approach such as morphology, molecular phylogeny and ecological data.
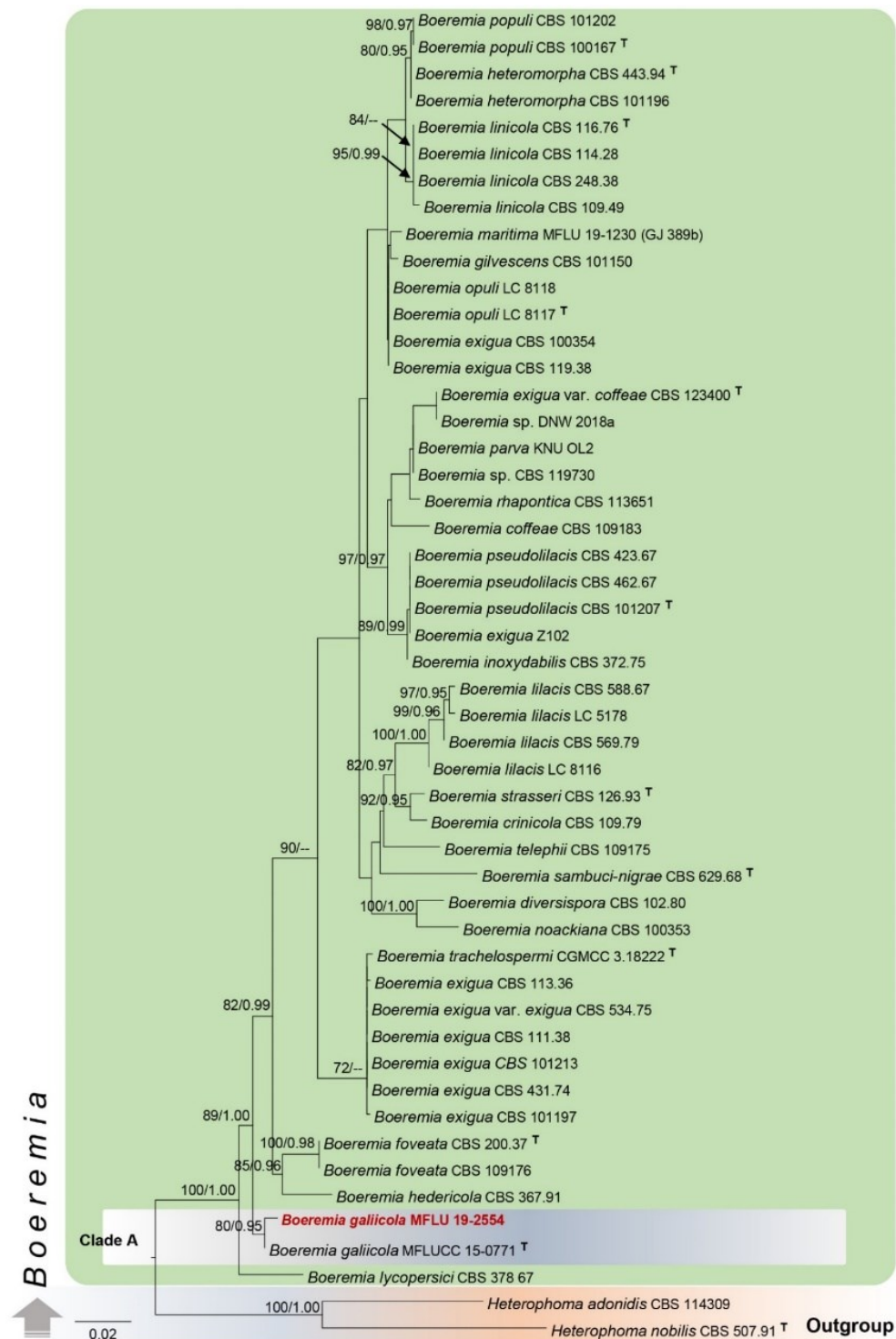
Fig. 26 – Phylogram generated from maximum likelihood analysis based on combined LSU, ITS, *tef*1-α, and *tub*2 sequenced data. Fifty strains were included in the combined sequence analyses, which comprised 2131 characters with gaps (LSU = 899, ITS = 491, *tef*1-α = 284, *tub*2 = 321). Single gene analyses were also performed, and topology and clade stability were compared by combined gene analyses. *Heterophoma nobilis* (CBS 507.91) and *H. adonidis* (CBS 114309) strains were used as the outgroup taxa. Final ML optimization likelihood is -5264.574154. The matrix included 310 distinct alignment patterns, with 11.07% undetermined characters or gaps. Estimated base frequencies were obtained as follows: A = 0.232575, C = 0.244023, G = 0.266456,

T = 0.256946; substitution rates AC = 2.505808, AG = 3.613819, AT = 2.809768, CG = 1.335895, CT = 12.761363, GT = 1.0; gamma distribution. Bootstrap support values for ML (first set) equal to or greater than 70%, BYPP equal to or greater than 0.95 are given above or below the nodes. The strain from the current study is in red bold, and the type strains are indicated with [T].
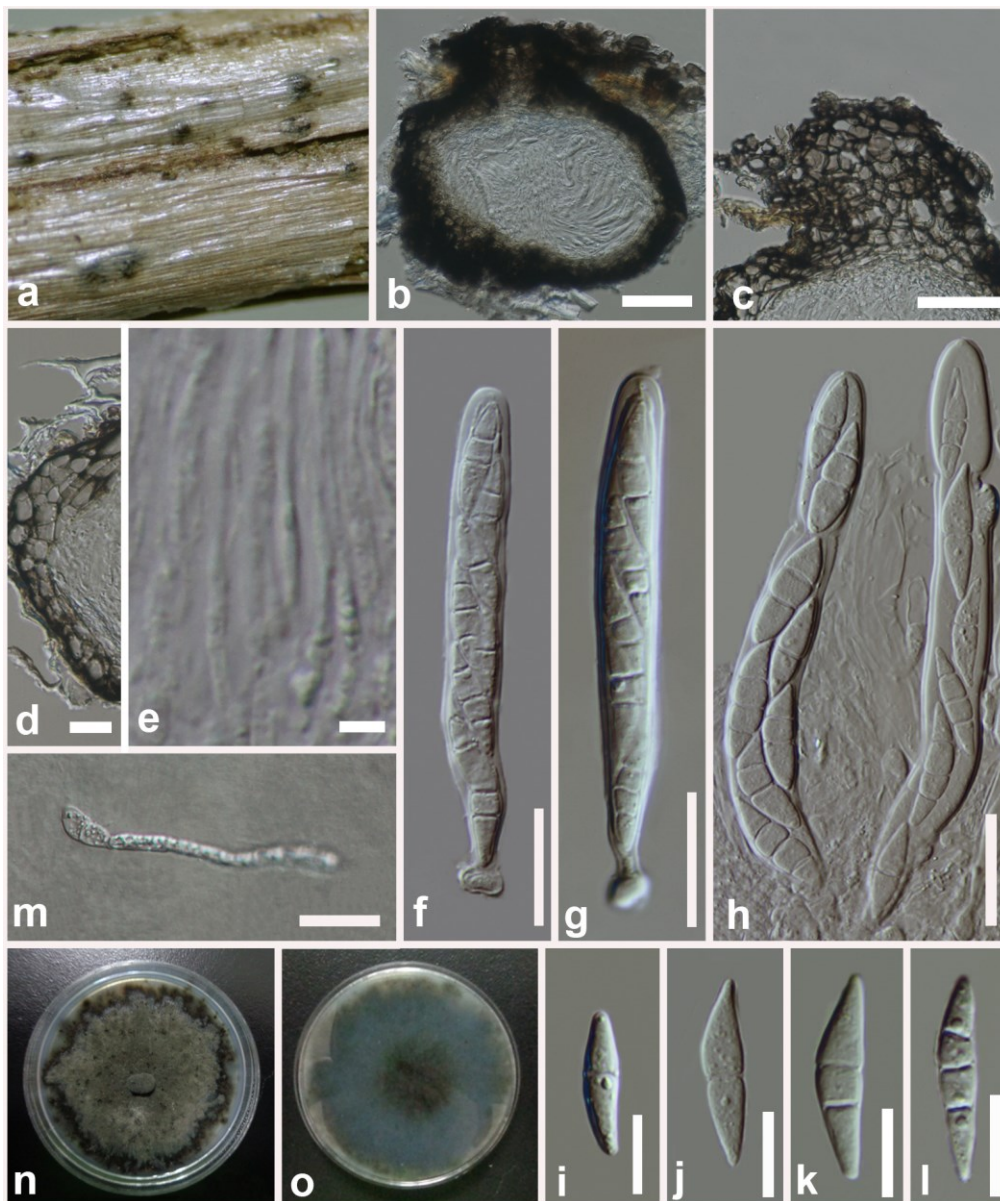
Fig. 27 – *Boeremia galiicola* (MFLU 19–2554, new regional record in Italy). a Ascomata on the dead host surface of *Galium* sp. (*Rubiaceae*, *Gentianales*). b Longitudinal section of an ascoma. c Longitudinal section of ostiole. d Peridium. e Pseudoparaphyses. f–h Asci. i–l Ascospores. m A germinated ascospore. n–o Colonies on PDA (n-upper side, o-lower side). Scale bars: b–c = 50 μm, d, f–h, m = 20 μm, i–l = 10 μm, e = 5 μm.

Pigmentatineomassaria Wijes., Camporesi & K.D. Hyde, gen. nov.

     Index Fungorum number: IF 900090; Facesoffungi number: FoF 14008

     Etymology – Referring to its pigmented ascospores

    Saprobic on the dead branch of *Cornus mas* L. Sexual morph: *Ascomata* pseudothecial, solitary, scattered, immersed to erumpent, globose to subglobose or broadly pyriform, uniloculate, ostiolate, coriaceous, surrounded by a black stromatic zone extending down and thick at sides, with distinct dark clypeus. *Ostioles* central, cylindrical, narrowing toward the apex, lined with hyaline periphyses. *Peridium* pseudoparenchymatous, thick, from outermost to innermost layers comprising

blackish, dark brown to hyaline, thick-walled cells of *textura angularis*. *Hamathecium* comprises dense, filamentous, branched, cellular, occasionally septate, numerous pseudoparaphyses, embedded in a glutinous matrix. *Asci* 8-spored, bitunicate, fissitunicate, cylindrical-clavate, short pedicel with a rounded end, apically rounded, with a distinct ocular chamber. *Ascospores* overlapping, 1–2-seriate, lenticular, fusoid to ellipsoid, initially hyaline, 1-septate, becoming yellowish to brown, and 3-septate at maturity, constricted at the septa, upper and lower cells from central septum wider and dark pigmented, both polar cells pale yellowish brown, acute at the apices, echinulate, without a gelatinous sheath. Asexual morph: Undetermined.

Type species – *Pigmentatineomassaria italica* Wijes., Camporesi & K.D. Hyde

Notes – *Pigmentatineomassaria* is a monotypic genus associated with *Cornus mas* in Italy. The main characteristics of the genus that distinguish it from *Neomassaria* (*Neomassariaceae*), a black stromatic zone surrounding the ascomata, with a distinct dark clypeus, and 1-septate and hyaline ascospores when young, becoming 3-septate, echinulate, and yellowish brown at maturity. However, *Pigmentatineomassaria* resemblances *Neomassaria,* by the presence of coriaceous ascomata with central ostioles, a peridium with cells of *textura angularis*, branched and cellular pseudoparaphyses, 8-spored, bitunicate, and short pedicellate asci with a distinct ocular chamber, and finally fusoid to ellipsoid ascospores (Ariyawansa et al. 2018, Hyde et al. 2020b, Yang et al. 2022a). *Pigmentatineomassaria* also shares some morphologies with *Massaria* species (*Massariaceae*) as having immersed, coriaceous, globose, subglobose or pyriform ascomata with a black stromatic zone, a dark clypeus, a pseudoparenchymatous peridium and hyaline to brown, septate ascospores (Voglmayr & Jaklitsch 2011). Phylogenetically, *Pigmentatineomassaria* forms a basal lineage in *Neomassariaceae* (Fig. 28, Clade A). Based on these morphological characters and phylogenetic analyses, we introduce *Pigmentatineomassaria* as the second genus in *Neomassariaceae*.

Pigmentatineomassaria italica Wijes., Camporesi & K.D. Hyde, sp. nov.   Fig. 29

Index Fungorum number: IF 900091; Facesoffungi number: FoF 14009

Etymology – The epithet refers to Italy, where the holotype was collected

Holotype – MFLU 19-0948

Saprobic on a dead branch of *Cornus mas* L. Sexual morph: *Ascomata* 380–420 μm diam., 320–500 μm high (excluding ostiolar neck) (x̄ = 400 × 450 μm, n = 5), pseudothecial, solitary, scattered, immersed in the substrate, slightly erumpent at maturity, globose to subglobose or broadly pyriform, uni-loculate, ostiolate, coriaceous, surrounded by a black stromatic zone extending down and thick at sides, with distinct dark clypeus. *Ostioles* central, cylindrical, lined with hyaline periphyses, ostiolar neck 160–165 × 18–40 μm, narrowing towards the apex. *Peridium* 25–48 μm wide, pseudoparenchymatous, fusing with host tissues, thick, from the outermost to innermost layers comprising blackish, dark brown to hyaline, thick-walled cells of *textura angularis*. *Hamathecium* comprises 1–2 μm wide (x̄ = 1.5 μm), dense, filamentous, branched, cellular, septate, thin-walled, hyaline, numerous pseudoparaphyses embedded in a glutinous matrix. *Asci* 90–150 × 10–15.5 μm (x̄ = 145 × 13 μm, n = 20), 8-spored, bitunicate, fissitunicate, cylindrical-clavate, short pedicel with a rounded end, apically rounded with an ocular chamber. *Ascospores* 23–35 × 4.8–7 μm (x̄ = 30 × 6 μm, n = 20), overlapping, 1–2-seriate, fusoid to ellipsoid, hyaline, 1-septate when young, becoming yellowish to brown, 3-septate at maturity, constricted at the septa, upper, and lower cells from central septum wide and dark pigmented, two end cells pale yellowish brown, pointed at apices, echinulate, rough-walled, without a gelatinous sheath. Asexual morph: Undetermined.

Material examined – Italy, Province of Forlì–Cesena [FC]), near Strada San Zeno – Galeata, on a dead branch of *Cornus mas* L. (*Cornaceae*), 15 February 2016, Erio Camporesi, IT 2824, (MFLU 19-0948, holotype).

GenBank Accession Numbers – LSU: OQ411138; SSU: OQ411132; *tef*1-α: OQ420449; *rpb*2: OQ420450.

Notes – In our multi-locus phylogeny (Fig. 28, Clade A), we represent the recognized species in *Neomassariaceae*. The new genus *Pigmentatineomassaria*, with a single species *P. italica*, is phylogenetically distinct from *Neomassaria*. In the multi-gene phylogenetic analyses, *Pigmentatineomassaria italica* grouped as a monophyletic affiliate in *Neomassariaceae* (95% ML and 1.00 BI, Fig. 28). The nucleotide differences of the holotype strain of *P. italica* (MFLU 19-0948) compared against the type species of *Neomassaria* are as follows: for *N. fabacearum* (MFLU 16-1875) LSU 49/855 bp difference (5.73%); SSU 18/757 bp difference (2.37%) and *tef*1-α 76/796 bp difference (9.54%).
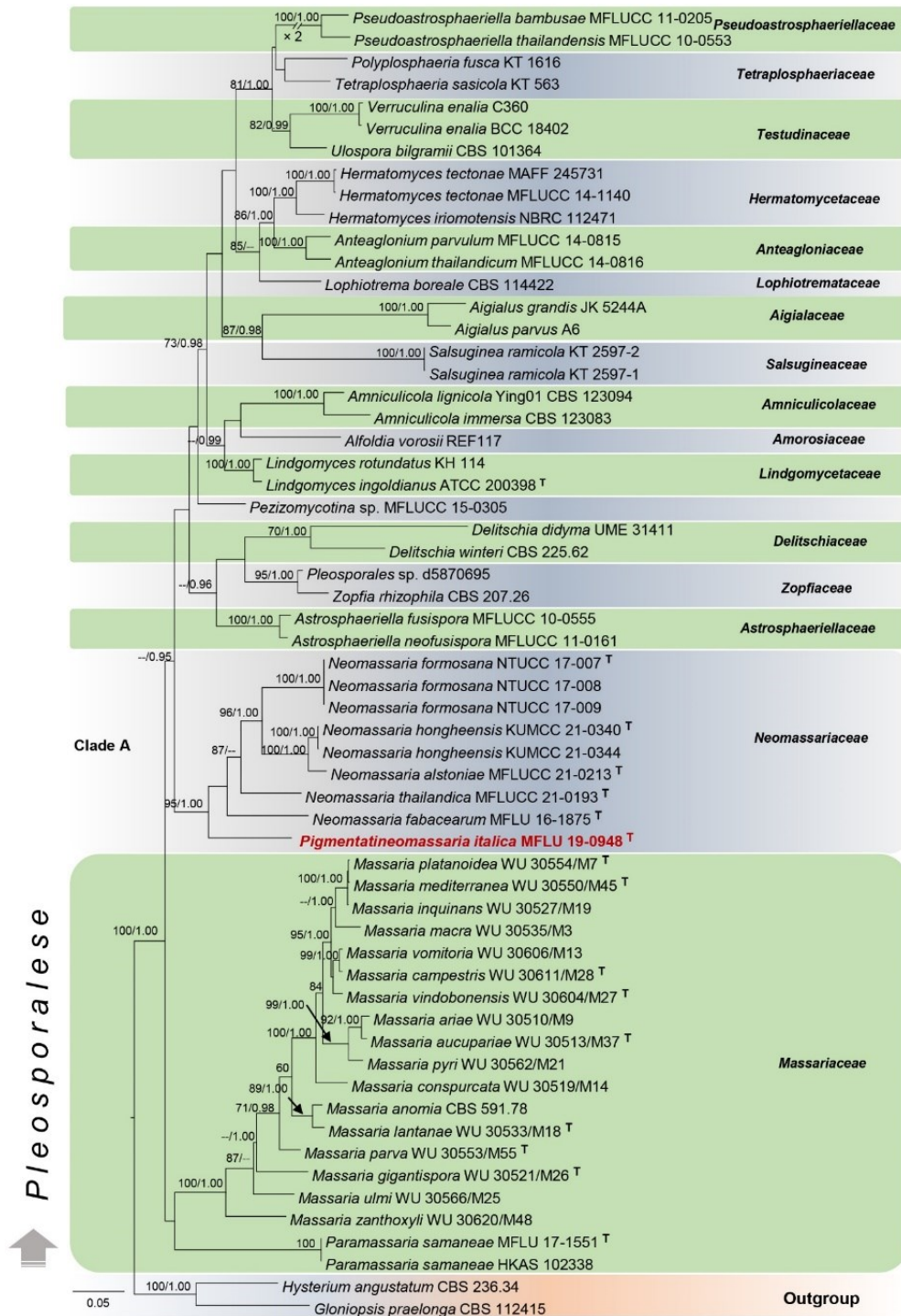
Fig. 28 – Phylogram generated from maximum likelihood analysis based on combined LSU, SSU, *tef*1-α, and *rpb*2 sequenced data. Fifty-nine strains were included in the combined sequence analyses, which comprised 4021 characters with gaps (LSU = 875, SSU = 1276, *tef*1-α = 886, *rpb*2

= 984). Single gene analyses were also performed, and topology and clade stability were compared from combined gene analyses. *Hysterium angustatum* (CBS 236.34) and *Gloniopsis praelonga* (CBS 112415) strains were used as the outgroup taxa. Final ML optimization likelihood is -28746.269781. The matrix included 430 distinct alignment patterns, with 31.57% undetermined characters or gaps. Estimated base frequencies were obtained as follows: A = 0.250196, C = 0.239447, G = 0.276221, T = 0.234136; substitution rates AC = 1.402848, AG = 4.606705, AT = 1.381371, CG = 1.406991, CT = 10.143410, GT = 1.0; gamma distribution. Bootstrap support values for ML (first set) equal to or greater than 70%, BYPP equal to or greater than 0.95 are given above or below the nodes. The strain from the current study is in red bold, and the type strains are indicated with [T].
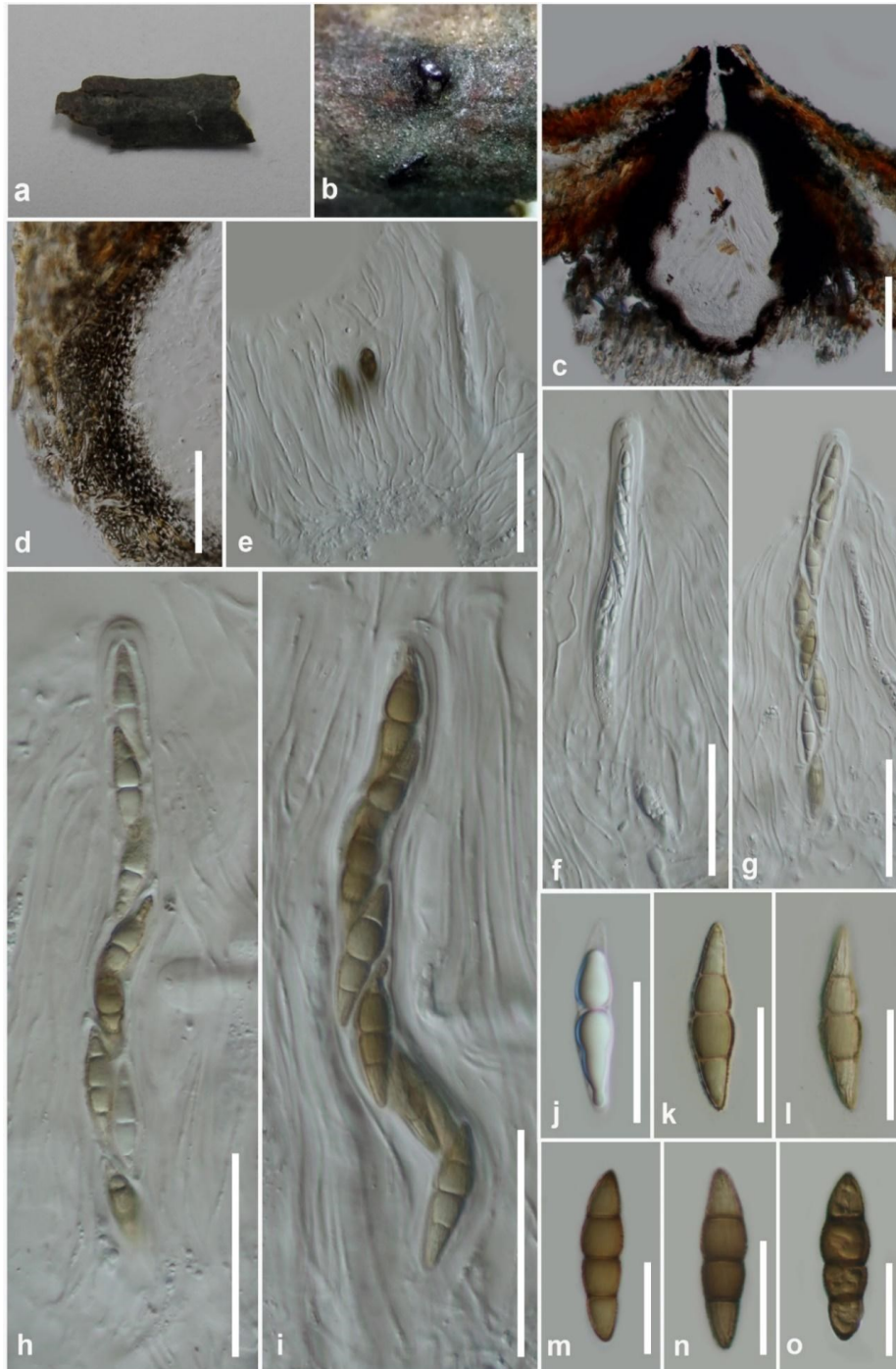
Fig. 29 – *Pigmentatineomassaria italica* (MFLU 19-0948, holotype). a Herbarium material. b Appearance of an ascoma on a dead branch of *Cornus mas* L. (*Cornaceae, Cornales*). c Vertical

section of an ascoma. d Peridium. e Pseudoparaphyses. f–i Asci. j–o Ascospore. Scale bars: c = 200 μm, d–i = 50 μm, j–o = 20 μm.

Pigmentatineomassaria italica shares morphological characters with *Neomassaria alstoniae* (MFLUCC 21-0213), *N. fabacearum* (MFLU 16-1875), *N. formosana* (NTUCC 17-007), *N. hongheensis* (KUMCC 21-0340), and *N. thailandica* (MFLUCC 21-0193) in having coriaceous ascomata with a central ostiole, a peridium with the cells of *textura angularis*, filamentous, branched, septate, hyaline pseudoparaphyses, 8-spored, bitunicate, short pedicellate asci with distinct ocular chamber and 1–2-seriate, fusoid to ellipsoid ascospores (Jayasiri et al. 2015, Hyde et al. 2016, Ariyawansa et al. 2018, Yang et al. 2022a). *Pigmentatineomassaria italica* differs from the above species in having broadly pyriform ascomata with a black stromatic margin, a distinct dark clypeus, a long ostiolar neck, cylindrical-clavate asci, hyaline, 1-septate immature ascospores and 3-septate, yellowish to brown mature ascospores with echinulate walls. These morphological differences of the sexual morphs of *P. italica* from *Neomassaria* species and the multi-locus phylogenetic analysis allow us to establish *Pigmentatineomassaria* as a new genus in *Neomassariaceae* with *P. italica* as its type species.

Leptosphaeriaceae M.E. Barr 1987
Index Fungorum number: IF 81843; Facesoffungi number: FoF 01151
Notes – *Leptosphaeriaceae* was established by Barr (1987a) as a specious family, and it was typified by *Leptosphaeria* (de Gruyter et al. 2013, Hyde et al. 2013, Phookamsak et al. 2019, Lestari et al. 2021, Xu et al. 2022). Based on the familial revision by Ariyawansa et al. (2015b), ten genera were accepted into *Leptosphaeriaceae* including *Leptosphaeria* as the generic type. Leptosphaeriaceous sexual morphs have single, papillate, immersed or erumpent, perithecial ascomata, scleroplectenchymatous or plectenchymatous peridium, cylindrical to clavate asci, and reddish brown or yellowish brown, septate ascospores (Hyde et al. 2013, Ariyawansa et al. 2015b, Hongsanan et al. 2020b, Xu et al. 2022). The asexual morphs are coelomycetous or hyphomycetous (de Gruyter et al. 2013, Hyde et al. 2013, Crous & Groenewald 2017, Aiello et al. 2020, Hongsanan et al. 2020b, Xu et al. 2022). Recently, fifteen genera have been accepted into the family (Wijayawardene et al. 2022). Members of this family have diverse lifestyles that are fungicolous, or epiphytic, parasitic, saprobic, and hemibiotrophic on stems and leaves of herbaceous and woody plant substrates in terrestrial and aquatic habitats (Ariyawansa et al. 2015b, Tennakoon et al. 2017, Sun et al. 2019, Lestari et al. 2021). In this study, we report two species belonging to *Leptosphaeria* and *Plenodomus* associated with *Fabaceae* and *Urticaceae* hosts in Italy.

Leptosphaeria Ces. & De Not. 1863
Index Fungorum number: IF 2800; Facesoffungi number: FoF 02297
Notes – *Leptosphaeria* was established by Cesati & De Notaris (1863) with 26 species and the genus was lectotypified with *Leptosphaeria doliolum* (Shearer et al. 1990). Initially, *Leptosphaeria* was described based on the characteristics of ellipsoid or fusoid, one or multi-septate, and hyaline to dark brown ascospores (Crane & Shearer 1991, Hyde et al. 2013). The species identification was also based on the nature of the pseudothecium, ascospore septation, host, and habitat (Saccardo 1891, Crane & Shearer 1991, Zhang et al. 2012, Hyde et al. 2013). The sexual morph of the genus is characterized by having semi-immersed to erumpent, coriaceous ascomata with a scleroplectenchymatous peridium, cylindrical to cylindric-clavate asci, ellipsoidal to fusiform, reddish to yellowish brown, septate ascospores. Asexual morphs have coelomycetous, coniothyrium-like, and phoma-like characters (Hyde et al. 2013, Phookamsak et al. 2019). Molecular phylogenetic analyses were provided for the type species by Ariyawansa et al. (2015b) and Dayarathne et al. (2015). Members of *Leptosphaeria* have different life modes, as saprobes, hemibiotrophs or plant pathogens on cultivated, wild herbaceous and woody plants (Hyde et al. 2000). More than a thousand records are listed under *Leptosphaeria* in the Index Fungorum (2022),

but only a few species have been confirmed by phylogenetic analysis (Phookamsak et al. 2019). In this study, we report *L. urticae* found in Italy based on both morphology and molecular analyses.

Leptosphaeria urticae D. Pem, E.B.G. Jones & K.D. Hyde 2019 Fig. 31
Index Fungorum number: IF 555597; Facesoffungi number: FoF 04370

Saprobic on a dead aerial stem of *Urtica dioica* L. Sexual morph: *Ascomata* 300–450 μm diam., 500–620 μm high (including ostiolar neck), solitary, scattered or sometimes aggregated, centrally papillate, with a long neck, uniloculate, semi-immersed to broadly superficial, conical to mammiform, dark brown to black, visible as raised, black bumps on the host surface, coriaceous, smooth, easily removed from the host substrate by forming a circular depression on the surface, ostiolate. *Ostioles* central, with periphyses oriented towards the apex, ostiolar neck 190–220 × 100–130 μm, brownish black, shiny, smooth. *Peridium* scleroplectenchymatous, 40–50 μm wide at sides, 60–70 μm at the base, 8–9-layered, outermost layers composed of small, thick-walled, dark brown to brown cells of *textura globulosa* to *textura angulari*, inner layers composed of light brown to subhyaline cells of *textura angularis*, cells near the base densely packed. *Hamathecium* comprises 1.5–2.5 μm wide, dense, filamentous, septate, cellular, anastomosing, branched, hyaline, pseudoparaphyses embedded in a gelatinous matrix. *Asci* 130–165 × 10–12 μm (x̄ = 150 × 11.5 μm, n = 15), 8-spored, bitunicate, fissitunicate, long fusiform to cylindrical, rounded at the apex, with short furcate pedicel, with an ocular chamber. *Ascospores* 30–42 × 4–7 μm (x̄ = 37 × 5 μm, n = 20), overlapping 1–2-seriate, long fusiform, initially hyaline, becoming yellowish brown at maturity, 8(–10)-septate, slightly constricted at the septa, narrowly rounded at both ends, straight or slightly curved, smooth-walled, guttulate, lacking a mucilaginous sheath. Asexual morph: Undetermined.

Material examined – Italy, Province of Forlì-Cesena [FC]), near Dovadola, on a dead aerial stem of *Urtica dioica* L. (*Urticaceae*), 24 February 2016, Erio Camporesi, IT 1289, (MFLU 16-0908).

GenBank Accession Numbers – ITS: OQ401052; LSU: OQ411135; SSU: OQ411131; *tef*1-α: OQ437908.

Notes – In the phylogeny, our new strain MFLU 16-0908 grouped with *Leptosphaeria urticae* (holotype; MFLU 18-0591) with 99% MLBS and 1.00 BYPP support (Fig. 30, Clade B). *Leptosphaeria urticae* was introduced by Phookamsak et al. (2019) on a dead stem of *Urtica dioica* from the United Kingdom based on morphological and phylogenetic analyses. Also, this new collection shares similar morphologies with the holotype of *L. urticae*. However, ascomata (500–620 μm high, with ostiole neck, 300–450 μm diam.) and asci (130–165 × 10–12 μm) are larger in our collection compared to ascomata (100–130 μm high × 70–110 μm diam.) and asci (60–140 × 9–11 μm) of the holotype (Phookamsak et al. 2019). Based on the above morphology and phylogenetic analyses, we conclude our new collection is *L. urticae,* found on the same host species as the holotype, but collected from Italy. Therefore, our collection is the first record of *L. urticae* from Italy.

Plenodomus Preuss 1851
Index Fungorum number: IF 9445; Facesoffungi number: FoF 06403

Notes – *Plenodomus* was introduced by Preuss (1851) and typified by *P. rabenhorstii* (de Gruyter et al. 2013, Ariyawansa et al. 2015b, Xu et al. 2022). The type material of *P. rabenhorstii* was destroyed during World War II. Therefore, *P. rabenhorstii* was replaced by *P. lingam* (asexual morph) (= *Leptosphaeria maculans*; sexual morph) (Boerema & Van Kesteren 1964, Torres et al. 2005, Ariyawansa et al. 2015a, Phookamsak et al. 2019, Brahmanage et al. 2020, Hyde et al. 2020b). The sexual morph of the genus is characterized by having immersed ascomata with scleroplectenchyma cell types of the peridium, and 3–5-distoseptate, broadly fusiform ascospores (Phookamsak et al. 2019, Hongsanan et al. 2020b), while the asexual morph is coelomycetous. *Plenodomus* species are saprophytic or parasitic on plants in terrestrial habitats (Ariyawansa et al. 2015b, Hyde et al. 2020b). There are 101 species epithets listed in the Index

Fungorum (2022) under *Plenodomus*. In this study, we report a new host record of *P. enteroleucus* on *Melilotus officinalis* (*Fabaceae*) from Italy.
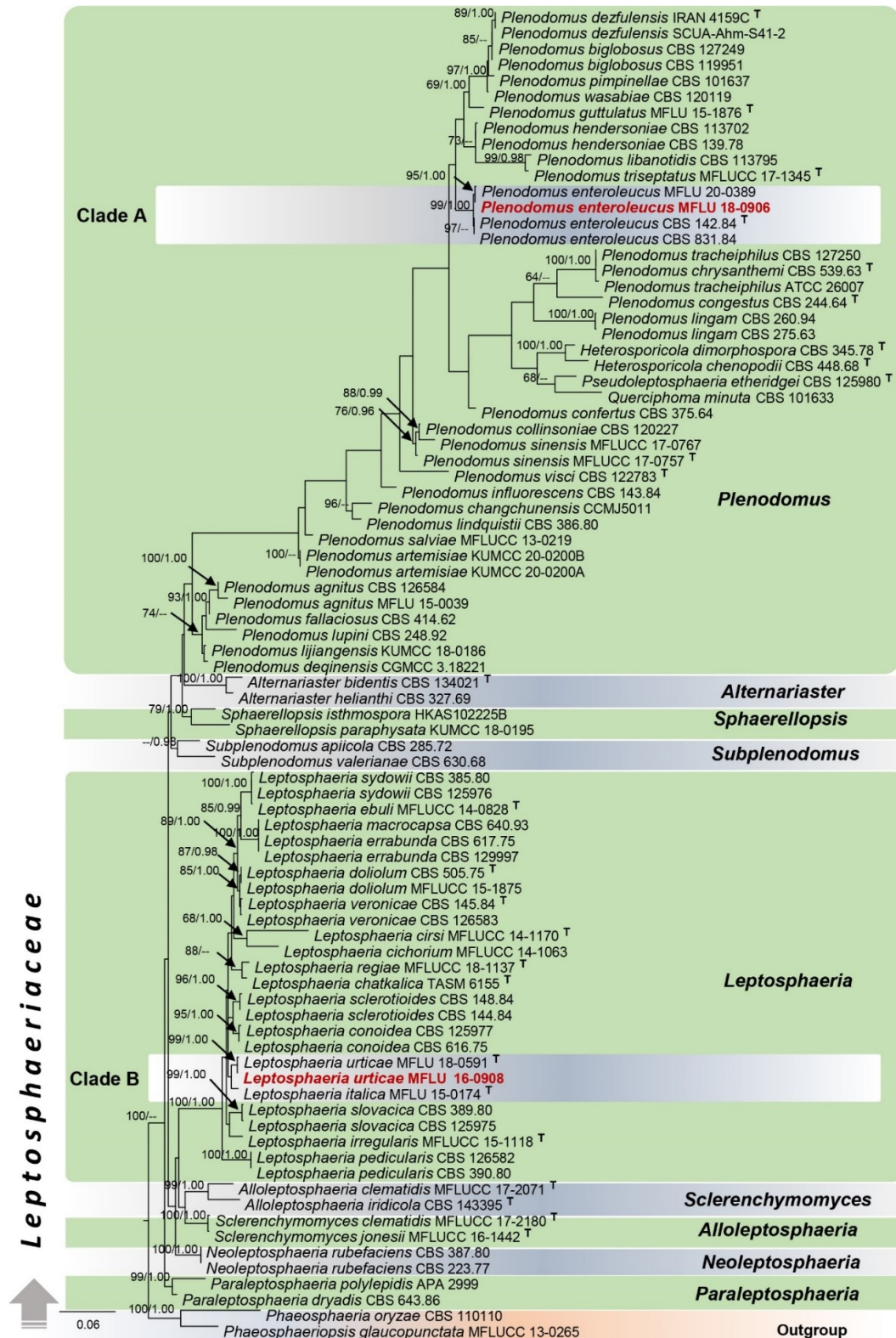
Fig. 30 – Phylogram generated from maximum likelihood analysis based on combined LSU, ITS, SSU, and *tef*1-α sequenced data. Eighty-four strains were included in the combined sequence analyses, which comprised 3750 characters with gaps (LSU = 880, ITS = 585, SSU = 1332, *tef*1-α = 953). Single gene analyses were also performed and topology and clade stability were compared from the combined gene analyses. *Phaeosphaeria oryzae* (CBS 110110) and *Phaeosphaeriopsis*

glaucopunctata (MFLUCC 13-0265) strains were used as the outgroup taxa. Final ML optimization likelihood is -19867.074032. The matrix included 1123 distinct alignment patterns, with 47.68% undetermined characters or gaps. Estimated base frequencies were obtained as follows: A = 0.242113, C = 0.229060, G = 0.270709, T = 0.258117; substitution rates AC = 1.325762, AG = 2.444641, AT = 1.769664, CG = 0.732120, CT = 5.379445, GT = 1.0; gamma distribution. Bootstrap support values for ML (first set) equal to or greater than 60%, BYPP equal to or greater than 0.95 are given above or below the nodes. The strains from the current study are in red bold, and the type strains are indicated with [T].

Fig. 31 – *Leptosphaeria urticae* (MFLU 16-0908, new geographical record). a–b Ascomata on a dead host surface of *Urtica dioica* L. (*Urticaceae*, *Rosales*). c Longitudinal section of an ascoma. d Longitudinal section through ostiole neck. e Peridium. f Pseudoparaphyses. g–j Asci. k–m Ascospore. Scale bars: a–b = 500 μm, c–d = 50 μm, e = 20 μm, f–m = 10 μm.

***Plenodomus enteroleucus*** (Sacc.) Gruyter, Aveskamp & Verkley 2012   Fig. 32

Index Fungorum number: IF 564753; Facesoffungi number: FoF08009

Saprobic on a dead aerial stem of *Melilotus officinalis* (L.) Pall. Sexual morph: Undetermined. Asexual morph: Coelomycetous. *Conidiomata* 100–130 μm diam., 130–160 μm high, (x̄ = 125 × 140 μm, n = 5), pycnidial, globose to subglobose, solitary, scattered, visible as black dots on the host surface, semi-immersed, ostiolate. *Ostioles* slightly papillate. *Pycnidial wall* 10–25 μm wide, composed of several layers with thick-walled, brown to lightly pigmented cells of *textura angularis*, surface heavily pigmented. *Conidiogenous cells* 5–9 μm long, holoblastic, phialidic, globose to oblong, individually hyaline, and pale brown when in a mass, and formed from the inner layer of the pycnidial wall. *Conidia* 5–9 × 3–3.5 μm (x̄ = 6.7 × 3.2 μm, n = 20), hyaline, aseptate, ellipsoidal to oblong, with guttules.

Material examined – Italy, Province of Forlì–Cesena [FC]), Versara – Galeata, on a dead aerial stem of *Melilotus officinalis* (L.) Pall. (*Fabaceae*), 15 March 2018, Erio Camporesi, IT 3778, (MFLU 18-0906).

GenBank Accession Numbers – ITS: OQ401056; LSU: OQ411141.
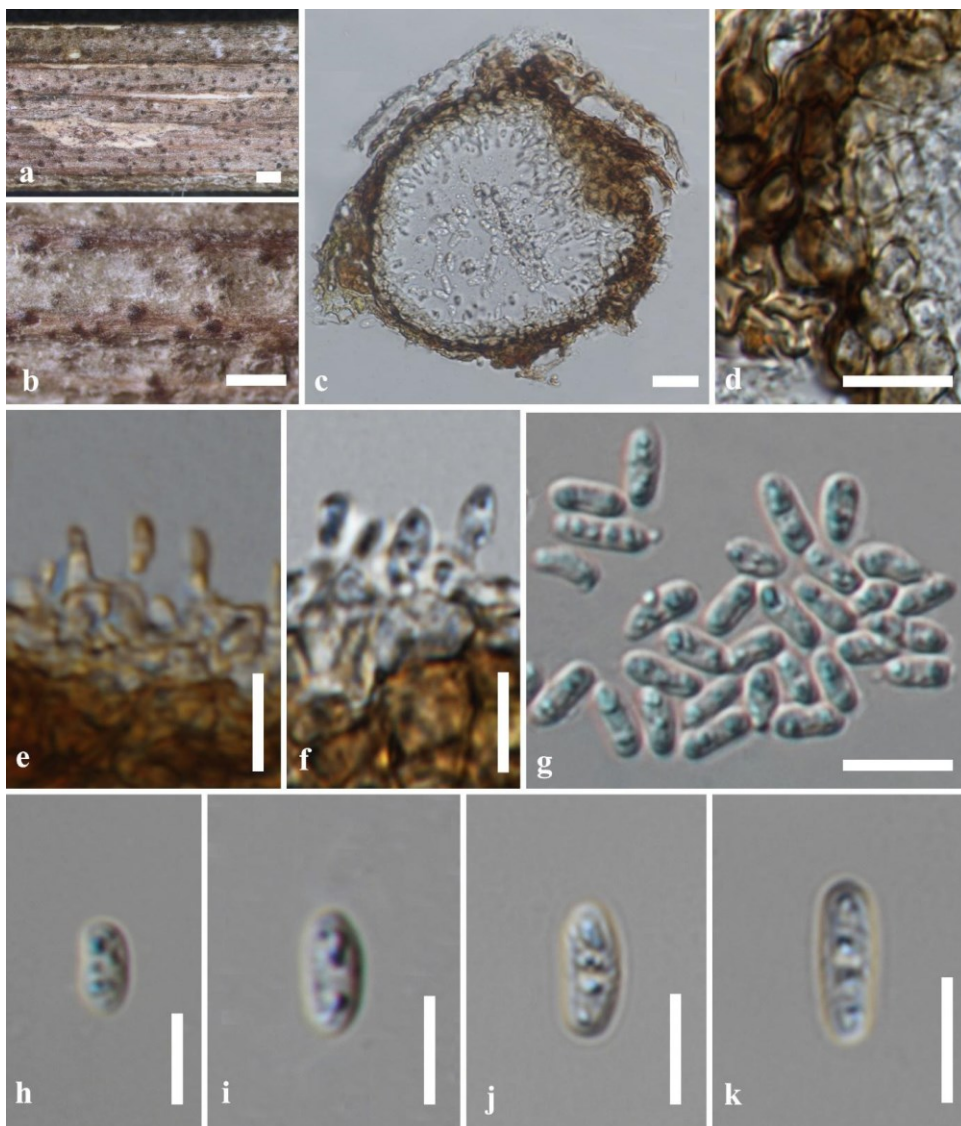
Fig. 32 – *Plenodomus enteroleucus* (MFLU 18-0906, new host record in Italy). a–b Conidiomata on a dead and aerial host surface of *Melilotus officinalis* (L.) Pall. (*Fabaceae*, *Fabales*). c Longitudinal section of a conidioma. d Longitudinal section of a conidioma wall. e–f Development stages of conidiogenesis. g–k Conidia. Scale bars: a–b = 500 μm, c = 20 μm, d–g = 10 μm, h–k = 5 μm.

Notes – In the multi-gene phylogeny, our strain MFLU 16-0906 grouped with *Plenodomus enteroleucus* strains (CBS 142.84, CBS 831.84, and MFLU 20-0389) with 99% MLBS and 1.00 BYPP support (Fig. 30, Clade A). Brahmanage et al. (2020) collected *Pl. enteroleucus* (MFLU 20-0389) on *Picris hieracioides* (*Asteraceae*) in Italy. *Plenodomus enteroleucus* was introduced as *Phoma enteroleuca* by Saccardo (1878) from dead branches of *Pyrus communis* (*Rosaceae*) in France. Later, de Gruyter et al. (2013) synonymized *Ph. enteroleuca* to *Pl. enteroleucus* based on multi-gene phylogenetic analyses. Our new collection shares similar morphologies of conidiomata, peridium, conidiogenous cells and conidia, but different sizes with respect to those provided by Boerema & Loerakker (1985) and Brahmanage et al. (2020). Based on the above morphological and phylogenetic analyses, we conclude our new collection is *Pl. enteroleucus*, and this is the first report of the species on *Melilotus officinalis* (*Fabaceae*) trees in Italy.

Melanommataceae G. Winter 1885

Index Fungorum number: IF 80990; Facesoffungi number: FoF 01023

Notes – *Melanommataceae* was established by Winter (1885) with the generic type *Melanomma* and *M. pulvispyrius* as type species. Tian et al. (2015) provided a detailed monograph for *Melanommataceae*, including morphological and multi-gene phylogenetic data. The sexual morph of the members is characterized by having globose to subglobose, perithecial ascomata that are carbonaceous or coriaceous, trabeculate pseudoparaphyses, bitunicate, and fissitunicate asci, pigmented and phragmosporous ascospores. Asexual morphs are mostly coelomycetous and rarely hyphomycetous (Hyde et al. 2013, Tian et al. 2015, Wanasinghe et al. 2018, Hongsanan et al. 2020b). Currently, 34 genera have been accepted into *Melanommataceae* (Wijayawardene et al. 2022). Members of this family are saprophytic, or parasitic on woody substrates in terrestrial, marine, or freshwater habitats, mainly distributed in temperate and subtropical regions (Zhang et al. 2012, Hyde et al. 2013, Hongsanan et al. 2020b). In this study, we introduce a novel host and geographical record of *Phragmotrichum chailletii* based on morphology and multi-gene phylogeny.

Phragmotrichum Kunze 1823

Index Fungorum number: IF 9376; Facesoffungi number: FoF 08297

Notes – *Phragmotrichum* was introduced by Kunze (1823) with *P. chailletii* as the type species. The genus is characterized by having stromatic to cupulate conidiomata, cylindrical, hyaline conidiophores, cylindrical, thallic, and integrated conidiogenous cells, and muriform, fusoid to ellipsoid, brown conidia in unbranched basipetal chains (Crous et al. 2020, Hongsanan et al. 2020b). *Phragmotrichum* species have been found on the cones of *Pinus* hosts in terrestrial habitats (Crous et al. 2020). There are 18 species epithets listed in the Index Fungorum (2022) under *Phragmotrichum* and one species has molecular data. We introduce the first *P. chailletii* species from Italy on *Picea excelsa* based on morpho-molecular phylogenetic analyses.

Phragmotrichum chailletii Kunze 1823   Fig. 34

≡ *Gymnosporangium chailletii* (Kunze) Spreng., Systema Vegetabilium edit. 16, 4(1): 562. 1827

Index Fungorum number: IF 122161; Facesoffungi number: FoF 11605

Saprobic on a dead and fallen cone of *Picea excelsa* (L.) H. Karst. Sexual morph: Undetermined. Asexual morph: Coelomycetous. *Conidiomata* 300–600 μm diam., 350–450 μm high, ($\bar{x}$ = 450 × 390 μm, n = 5), pycnidial, stromatic to cupulate, semi- immersed to erumpent, sometimes superficial, solitary to aggregate, uni-loculate, visible as black dots on the host surface. *Pycnidial wall* 40–50 μm ($\bar{x}$ = 43 μm), multi-layered, outer layers comprised of thick-walled, dark brown to pale brown cells, inner layers comprised of thin-walled, hyaline, cells, *textura angularis* to *textura globulosa*. *Conidiophores* 15–30 × 3–4.5 μm, branched at the base, cylindrical, originate from the inner layer of conidiomata, 1–3-septate, hyaline. *Conidiogenous cells* 10–17 × 3–4 μm, thallic, integrated, cylindrical, producing unbranched basipetal chains of conidia, smooth, hyaline. *Conidia* 20–45 × 13–20 μm. ($\bar{x}$ = 35.5 × 16 μm, n = 25), fusoid to ellipsoid, initially hyaline and

aseptate or poorly septate, becoming yellowish pale brown at maturity, muriform, with 4–6 transverse, and 2–3 longitudinal septa, truncate at both ends, middle cells darker than end cells, straight to curved, smooth-walled.

Material examined – Italy, Province of Forlì–Cesena [FC]), Campigna – Santa Sofia, on a dead and fallen cone of *Picea excelsa* (L.) H. Karst. (*Pinaceae*), 23 May 2016, Nello Camporesi, IT 2492, (MFLU 16-1342), *ibid.*, 04 April 2016, Erio Camporesi, MFLU 16-1125.

GenBank Accession Numbers – ITS: OQ401054; LSU: OQ411137.
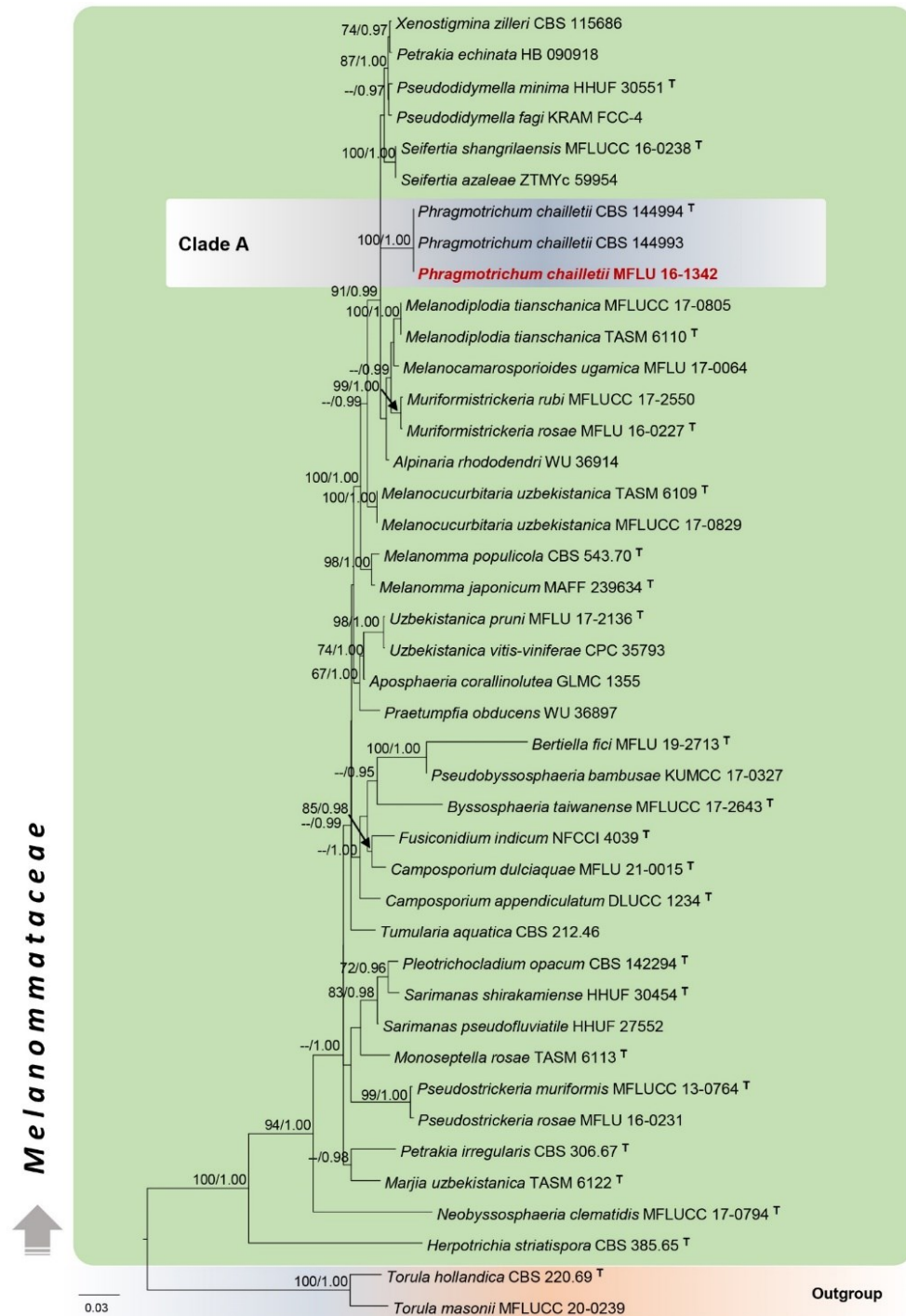
Fig. 33 – Phylogram generated from maximum likelihood analysis based on combined LSU, SSU and ITS sequenced data. Forty-two strains were included in the combined sequence analyses, which comprised 2401 characters with gaps (LSU = 851, SSU = 1025, ITS = 525). Single gene analyses were also performed and topology and clade stability were compared by combined gene analyses.

Torula hollandica (CBS 220.69) and *T. masonii* (MFLUCC 20–0239) strains were used as the outgroup taxa. Final ML optimization likelihood is – 9482.931896. The matrix included 584 distinct alignment patterns, with 26.74% undetermined characters or gaps. Estimated base frequencies were obtained as follows: A = 0.250164, C = 0.220089, G = 0.275658, T = 0.254089; substitution rates AC = 2.582855, AG = 3.063959, AT = 1.962392, CG = 1.463222, CT = 13.214385, GT = 1.0; gamma distribution. Bootstrap support values for ML (first set) equal to or greater than 60%, BYPP equal to or greater than 0.95 are given above or below the nodes. The strain from the current study is in red bold, and the type strains are indicated with [T].

Notes – Our strain (MFLU 16-1342) grouped with the *P. chailletii* strain (ex-neotype: CBS 144994) with 100% MLBS and 1.00 BYPP support (Fig. 33, Clade B). Based on morphology, our collection shares similar morphologies with the neotype (CPC 33263 = CBS 144994) in having stromatic to cupulate conidiomata, cylindrical, hyaline conidiophores that are branched at the base, cylindrical, thallic and integrated, and hyaline conidiogenous cells with fusoid to ellipsoid, muriform conidia with truncated ends (Crous et al. 2020, Hongsanan et al. 2020b). However, the peridium of our collection has mixed cells of *textura angularis* to *textura globulosa*, while the neotype (CBS 144994) shows only *textura angularis* cells in the peridium. The holotype material of *P. chailletii* was collected on fallen cones of *Picea abies* [as *Pinus abies*] (*Pinaceae*) in Switzerland. As Kunze's herbarium was destroyed during World War II and the holotype was lost (Crous et al. 2020, Hongsanan et al. 2020b). Crous et al. (2020) designated a neotype collected from Switzerland on fallen cones of *Picea abies*. Based on morphology and phylogenetic analyses, we conclude that our collection is *P. chailletii* and is the first record on *Picea excelsa* and from Italy.
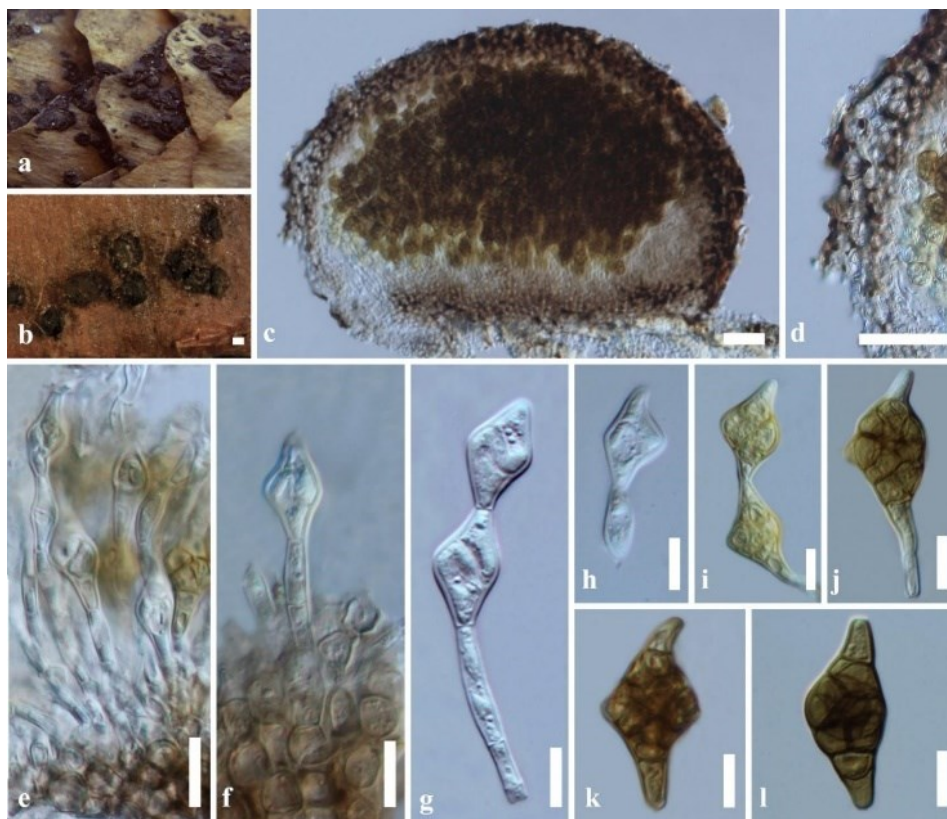
Fig. 34 – *Phragmotrichum chailletii* (MFLU 16-1342, new host and geographical record). a–b Conidiomata on a dead and fallen cones of *Picea excelsa* (L.) H. Karst. (*Pinaceae*, *Pinales*). c Longitudinal section of a conidioma. d Longitudinal section of a conidioma wall. e–g Development stages of conidiogenesis. h–l Conidia. Scale bars: b = 200 μm, c = 50 μm, e–l = 10 μm.

Nigrogranaceae Jaklitsch & Voglmayr 2016

Index Fungorum number: IF 817780; Facesoffungi number: FoF 08317

Notes – Jaklitsch & Voglmayr (2016) introduced the *Nigrogranaceae* to accommodate three *Nigrograna* species based on morphology and phylogeny. *Nigrograna* is the only genus accepted in *Nigrogranaceae* (Hongsanan et al. 2020b, Wijayawardene et al. 2022). The key sexual morph characters of *Nigrogranaceae* species are having globose to subglobose, immersed to erumpent ascomata surrounded by a subiculum, papillate to cylindrical, periphysate ostiole, pseudoparenchymatous peridium, cellular, septate, branched apically free pseudoparaphyses, cylindric clavate to broadly clavate, 8-spored asci and fusoid to narrowly ellipsoid, 1–3-septate, pale to chocolate brown ascospores (Jaklitsch & Voglmayr 2016, Hongsanan et al. 2020b). In *Nigrogranaceae* members, both coelomycetous and hyphomycetous asexual morphs have been reported (Jaklitsch & Voglmayr 2016, Tibpromma et al. 2017, Dong et al. 2020). Pyrenochaeta-like asexual morph taxa are characterized by having pycnidial conidiomata, pseudoparenchymatous peridium, filiform, simple to sparsely branched conidiophores with pegs and terminal phialides, ampulliform, lageniform, or subcylindrical conidiogenous cells with rod-like to ellipsoid, aseptate, hyaline or subhyaline conidia (Jaklitsch & Voglmayr 2016, Hongsanan et al. 2020b). Hyphomycetous asexual morphs are characterized by having black synnemata, immersed mycelium, composed of septate, brown hyphae, macronematous conidiophores, synnematous on substratum, polyblastic, sympodial, ampulliform, denticulate, hyaline conidiogenous cells and ellipsoidal, aseptate, solitary, acrogenous hyaline conidia (Dong et al. 2020). The members of the family are prominent as saprobes on decaying wood in terrestrial, freshwater, and marine environments, as well as endophytes or human pathogens (Dayarathne et al. 2020a, Dong et al. 2020, Hongsanan et al. 2020b). In this study, we discuss a saprobic species of *Nigrograna* collected from Italy.

Nigrograna Gruyter, Verkley & Crous 2012

Index Fungorum number: IF 564794; Facesoffungi number: FoF 08318

Notes – *Nigrograna* was established by de Gruyter et al. (2013) to accommodate *N. mackinnonii* (basionym: *Pyrenochaeta mackinnonii*), a human pathogen, as the type species. Ahmed et al. (2014) transferred *N. mackinnonii* to *Biatriospora* due to its close phylogenetic affinity to the type species of *Biatriospora* (*B. marina*). Also, in the multi-gene phylogenetic analyses by Jaklitsch & Voglmayr (2016), *B. marina* clustered with *Nigrograna*. However, three collections of *Nigrograna* taxa clustered above with two *Biatriospora* species, but they differ morphologically and ecologically from *B. marina*. Therefore, Jaklitsch & Voglmayr (2016) established *Nigrogranaceae* to accommodate *Nigrograna*. *Nigrograna* species are characterized by having black ascomata, clavate, short pedicellate asci consisting of pale to chocolate brown, fusoid to narrowly ellipsoid, asymmetric ascospores with septa (Zhang et al. 2020). They are geographically and ecologically diverse in different ecosystems, including endophytes, saprobes, or pathogens that have been reported worldwide (Kolařík 2018, Zhao et al. 2018, Dayarathne et al. 2020a, Mapook et al. 2020, Wanasinghe et al. 2020, Zhang et al. 2020). Currently, 20 species are listed in the Index Fungorum (2022) under *Nigrograna*. In this study, we provide a collection of *N. thymi* as the first report on *Corylus avellana* from Italy.

Nigrograna thymi Mapook, Camporesi & K.D. Hyde 2017   Fig. 36

Index Fungorum number: IF 552958; Facesoffungi number: FoF 03119

Saprobic on dead aerial branches of *Corylus avellana* L. Sexual morph: *Ascomata* 150–200 μm diam., 300–350 μm high (x̄ = 160 × 320 μm, n = 5), immersed to erumpent through host substrate, scattered or aggregated in small valsoid groups, coriaceous, subglobose to oblate, black. *Ostiole* central. *Peridium* 20–35 μm wide, pseudoparenchymatous, from outer layers towards inner layers comprising dark brown to pale brown cells of *textura angularis*. *Hamathecium* comprises 1–2.1 μm wide, cylindrical to filiform, branched, septate, numerous pseudoparaphyses with free ends, embedded in a mucilaginous matrix. *Asci* 50–75 × 8–10 μm (x̄ = 66 × 9 μm, n = 10), 8-spored,

bitunicate, fissitunicate, cylindrical-clavate, with the small ocular chamber, short pedicellate, rounded to furcate base. *Ascospores* 13–18 × 4–5 μm (x̄ = 15 × 4.4 μm, n = 10), overlapping, 1–2-seriate, broadly fusiform, 1-septate, yellowish brown when young, becoming 3-septate, chocolate-brown at maturity, slightly constricted at the primary median septum, end cells often acute, second upper cell slightly wider than others, straight or curved, guttulate. Asexual morph: Undetermined.
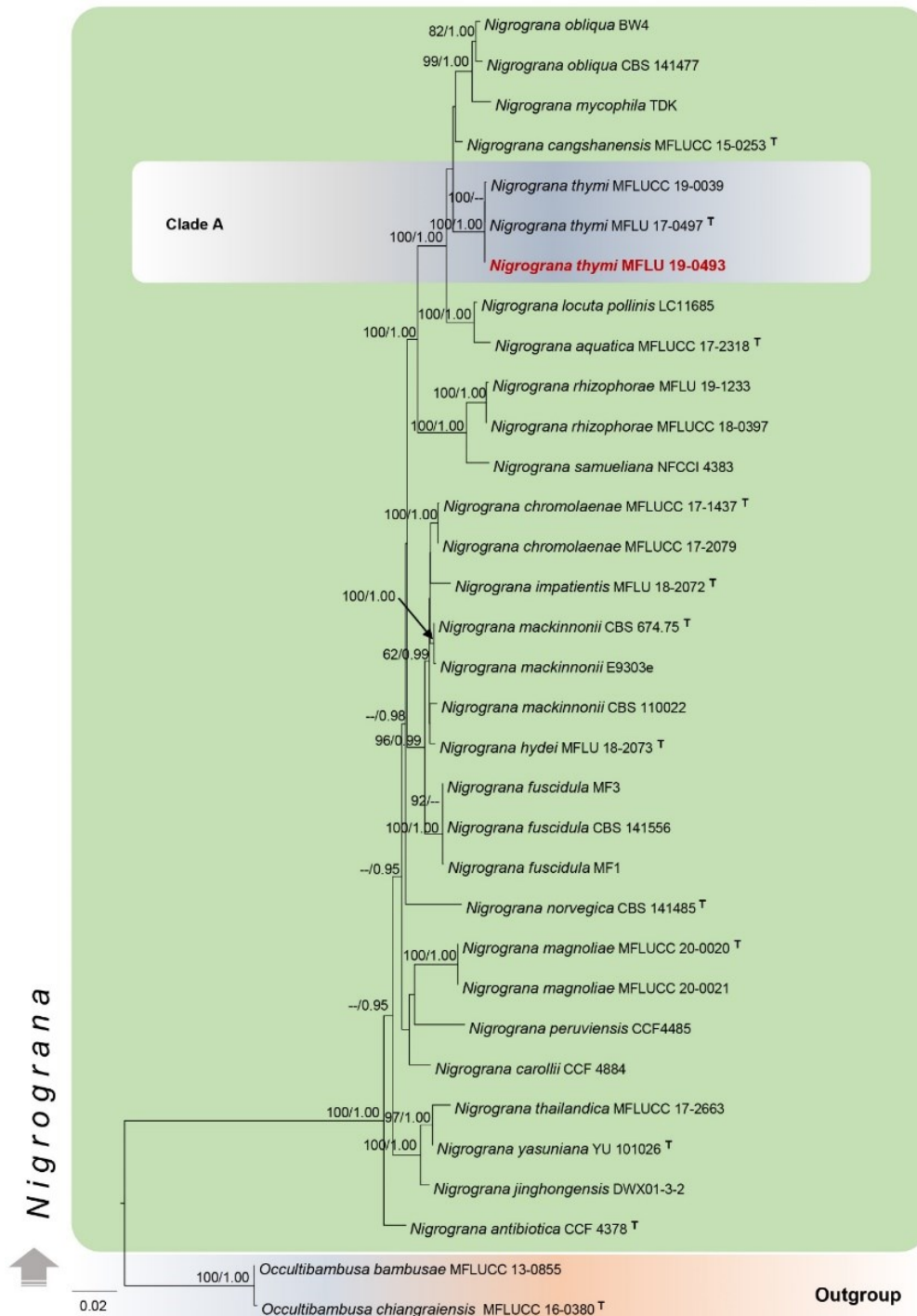
Fig. 35 – Phylogram generated from maximum likelihood analysis based on combined LSU, ITS, SSU and *tef*1-α sequenced data. Thirty-three strains were included in the combined sequence analyses, which comprised 2401 characters with gaps (LSU = 849, ITS = 544, SSU = 1351, *tef*1-α = 903). Single gene analyses were also performed, and topology and clade stability were compared from combined gene analyses. *Occultibambusa bambusae* (MFLUCC 13-0855) and *O. chiangraiensis* (MFLUCC 16-0380) strains were used as the outgroup taxa. Final ML

optimization likelihood is - 9732.444016. The matrix included 609 distinct alignment patterns, with 28.65% undetermined characters or gaps. Estimated base frequencies were obtained as follows: A = 0.245606, C = 0.244207, G = 0.269539, T = 0.240648; substitution rates AC = 1.635575, AG = 3.031390, AT = 1.440914, CG = 0.805051, CT = 10.066249, GT = 1.0; gamma distribution. Bootstrap support values for ML (first set) equal to or greater than 60%, BYPP equal to or greater than 0.95 are given above or below the nodes. The strain from the current study is in red bold, and the type strains are indicated with [T].
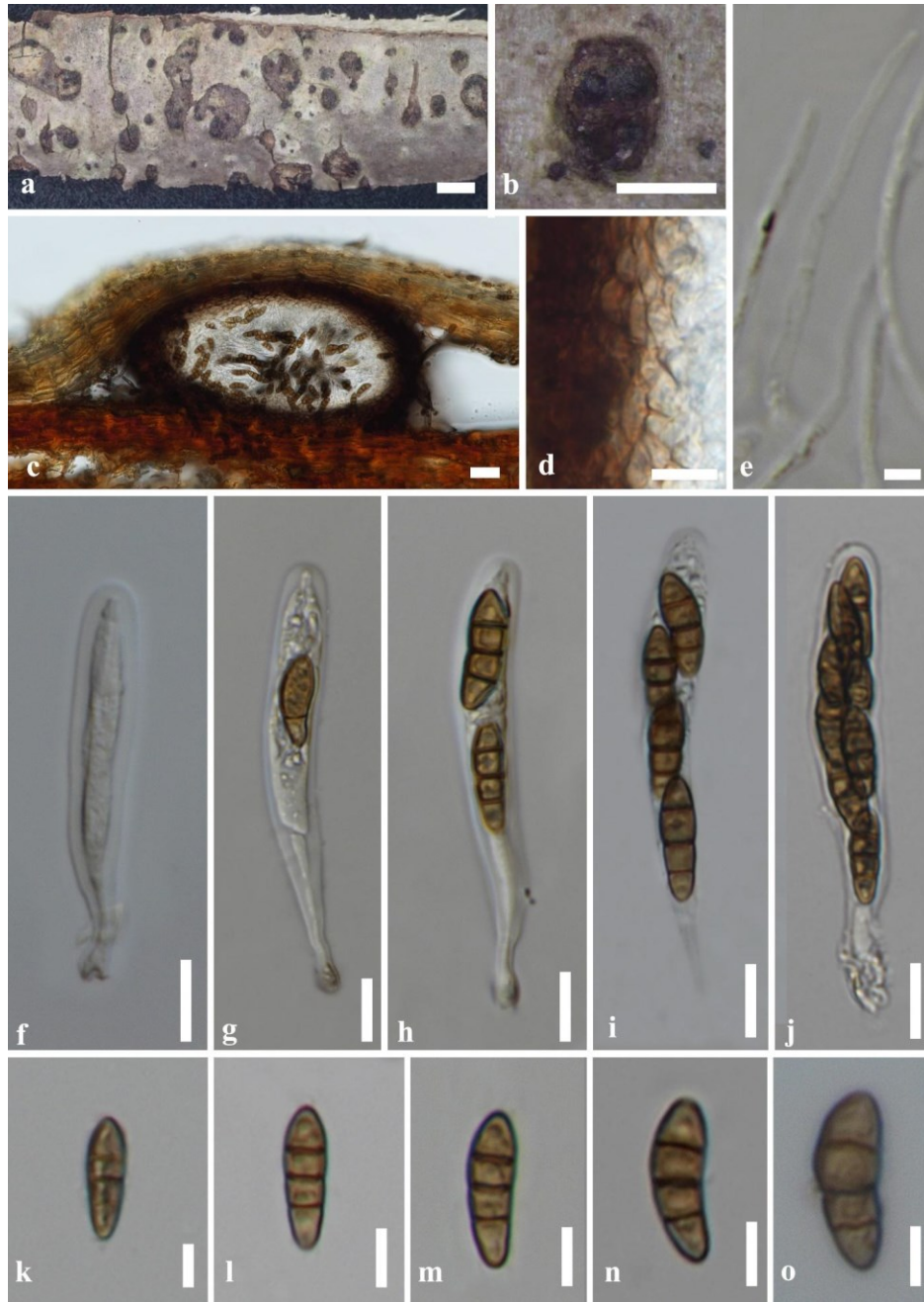
Fig. 36 – *Nigrograna thymi* (MFLU 19-0493, new host record). a–b Ascomata on a dead host surface of *Corylus avellana* L. (*Betulaceae*, *Fagales*). c Longitudinal section of an ascoma. d Peridium. e Pseudoparaphyses. f–j Asci. k–o Ascospores. Scale bars: a = 1 mm, b = 500 μm, c = 20 μm, d, f–j = 10 μm, e, k–o = 5 μm.

Material examined – Italy, Province of Forlì–Cesena [FC]), near Meldola, on dead aerial branches of *Corylus avellana* L. (*Betulaceae*), 19 January 2019, Erio Camporesi, IT 4200, (MFLU

19-0493), *ibid*., 18 February 2019, Erio Camporesi, IT 4200a, (MFLU 19-0632), *ibid.*, 25 February 2019, Erio Camporesi, IT 4200b, (MFLU 19-0653).

GenBank Accession Numbers – ITS: OQ401063; LSU: OQ411147; SSU: OQ411134.

Notes – Phylogenetically, our strain (MFLU 19-0493) grouped with *Nigrograna thymi* strains MFLU 17-0497 (holotype) and MFLUCC 19-0039 with 100% MLBS and 1.00 BYPP support (Fig. 35, Clade A). A base pair comparison between our strain and the MFLU 17-0497 strain revealed only one base pair difference in the ITS and LSU sequences out of 498 and 876, respectively, while SSU sequence data are identical. Based on morphology, our collection shares similar morphologies with type strain (MFLU 17-0497) in having immersed to erumpent, coriaceous ascomata, peridium with the cells of *textura angularis*, cylindrical to filiform, branched, septate, pseudoparaphyses, 8-spored, bitunicate asci with a small ocular chamber and broadly fusiform, hyaline to pale or dark brown ascospores (Hyde et al. 2017a). However, the holotype strain of *N. thymi* has larger dimensions of ascomata (295–390 diam. × 430–460 high) μm, peridium (25–55) μm, asci (90–98 × 8–11 μm) and ascospores (24–26 × 5.5–6.5 μm) compared to our collection (Hyde et al. 2017a). Also, the holotype differs from other *Nigrograna* species in having ascospores with 4–5 septa (Hyde et al. 2017a). Similarly, to other *Nigrograna* species, our collection (MFLU 19-0493) has initially 1-septate, yellowish brown ascospores that become and 3-septate and chocolate-brown when mature. The holotype material of *N. thymi* was collected on a dead stem of *Thymus oenipontanus* (*Lamiaceae*) from, Forlì–Cesena province, while our collection was found on a dead branch of *Corylus avellana* (*Betulaceae*) in the same province of Italy. Based on morphology and phylogenetic analyses, we conclude that our collection is *N. thymi* and the first record on *C. avellana* hosts.

Sordariomycetes O.E. Erikss. & Winka 1997

Diaporthales Nannf. 1932

Index Fungorum number: IF 90468; Facesoffungi number: FoF 00593

Notes – *Diaporthales* was introduced by Nannfeldt (1932) to accommodate the taxa bearing allantoid and non-allantoid ascospores based on the classification of diaporthoid taxa by Von Höhnel (1917). *Diaporthales* taxa are characterized by having solitary to aggregated perithecia with long papilla, 2–32-spored, unitunicate asci with a conspicuous refractive ring, and coelomycetous and rarely hyphomycetous asexual morphs (Rossman et al. 2007, Senanayake et al. 2018, Hyde et al. 2020c). The divergence time for *Diaporthales* was estimated at 180 MYA by Hyde et al. (2020c). The majority of *Diaporthales* taxa can be found in terrestrial and aquatic habitats (Senanayake et al. 2017a, 2018, Hyde et al. 2020c). Their nutritional modes are varied, including pathogens, saprobes, endophytes, and parasites or secondary invaders of injured plant tissues. Coprophilous, hypersaprobes or mycophylic species have not been recorded (Senanayake et al. 2017a, 2018). These taxa inhabit different hosts and substrates, such as trees and crops with high economic value, soil, living animal and human tissues (Barr 1978, Gryzenhout et al. 2006, Senanayake et al. 2017a). Currently, the order consists of 32 families and 36 genera in *Diaporthales* genera *incertae sedis* (Wijayawardene et al. 2022). In this study, we discuss three Italian collections belonging to *Cytosporaceae* and *Sydowiellaceae*.

Cytosporaceae Fr. 1825

Index Fungorum number: IF 82042; Facesoffungi number: FoF 06870

Notes – *Cytosporaceae* was introduced by Fries (1825) to accommodate the type genus *Cytospora*. Later, Tulasne & Tulasne (1861) introduced *Valsaceae* as the sexual morph of *Cytospora* and Senanayake et al. (2017a) accepted *Valsaceae* under *Cytosporaceae*. Sexual morphs of *Cytosporaceae* species are characterized by having immersed to erumpent, globose, perithecia with long neck swollen at the tips, periphysate ostiole, peridium with, hyaline paraphyses, 8-spored, unitunicate, clavate asci with or without apical ring and unicellular or rarely bicellular, allantoid or ellipsoid, and hyaline ascospores. Coelomycetous asexual morph has dark-pigmented conidiomata,

conidiodiophores reduced to hyaline conidiogenous cells, and aseptate, allantoid, hyaline conidia. Senanayake et al. (2017a) delimited *Cytosporaceae* to accommodate *Cytospora sensu-lato* by accepting *Cytospora*, *Pachytrype*, *Paravalsa*, *Waydora*, and *Xenotypa*. *Cytosporaceae* species are pathogens or saprobes in plant tissues, especially *Cytospora* species (Senanayake et al. 2018, Hyde et al. 2020c). Currently, six genera are accepted in the family including the above genera with the addition of *Cryptascoma* (Hyde et al. 2020c, Wijayawardene et al. 2022). In this study, we discuss two saprobic cytosporaceous taxa, belonging to *Cytospora* collected from Italy.

Cytospora Ehrenb. 1818
        Index Fungorum number: IF 7904; Facesoffungi number: FoF 01378
        Notes – *Cytospora* was introduced by Ehrenberg (1818) and typified by *C. chrysosperma* (Hyde et al. 2020c, Shang et al. 2020). The sexual morph of *Cytospora* is characterized by having solitary, immersed to erumpent ascostromata with valsoid or diatrypelloid arrangements, inserted in ectostromatic disc, with or without paraphyses, ellipsoid to clavate asci with refractive, J– apical ring, and ellipsoid to allantoid, aseptate, hyaline ascospores (Maharachchikumbura et al. 2016, Norphanphoun et al. 2018, Fan et al. 2020). The coelomycetous asexual morph is characterized by single or labyrinthine, loculate stromata, filamentous conidiophores, enteroblastic, phialidic conidiogenous cells, and allantoid, aseptate, hyaline conidia (Fan et al. 2020, Zhu et al. 2020, Monkai et al. 2021, Manawasinghe et al. 2022). *Cytospora* species are important pathogens causing cytospora-cankers (valsa-canker) and dieback disease on hardwoods and coniferous trees (Farr et al. 1989, Hyde et al. 2016, 2020c, Norphanphoun et al. 2017, 2018, Fan et al. 2020, Pan et al. 2020). Some species have been described as highly virulent and destructive pathogens on *Prunus* and *Populus* hosts, while some are facultative wound parasites that attack damaged or weakened plants (Biggs 1989, Kepley & Jacobi 2000). More than 650+ *Cytospora* species are listed in the Index Fungorum (2022); however, most are regarded as synonyms (Adams et al. 2005). *Leucostoma*, *Valsa*, *Valsella*, and *Valseutypella* are synonyms of *Cytospora* (Rossman et al. 2015, Pan et al. 2020).

Cytospora cotini Norph., Bulgakov & K.D. Hyde 2016   Fig. 38
   Index Fungorum number: IF 552231; Facesoffungi number: FoF 02365
        Saprobic on a dead and hanging branch of *Corylus avellana* L. Sexual morph: see Shang et al. (2020). Asexual morph: *Conidiomata* 500–1500 μm diam. × 400–600 μm high, (x̄ = 1300 × 500 μm, n = 10) (including ostiole), pycnidial, solitary, scattered, immersed in host tissue, erumpent through the surface when mature, multiloculate, globose to subglobose or discoid to conical, dark brown, ostiolate. *Ostiole* 130–150 μm diam. × 150–250 μm high, conspicuous, flask-shaped to conical, at the same level as the disc surface. *Locules* numerous, irregularly arranged, separated by individual walls. *Peridium* 20–30 μm diam. comprising several layers with cells of *textura angularis*, outer layers dark brown to pale brown, innermost layers thin, hyaline. *Conidiophores* branched at the base, hyaline or reduced to conidiogenous cells. *Conidiogenous cells* 8–11 × 1–2 μm (x̄ = 9.5 × 1.5 μm, n = 10) enteroblastic, phialidic, sub-cylindrical to cylindrical, originating from the inner layers of pycnidial wall, hyaline, smooth. *Conidia* 4.5–7.2 × 1.0–1.5 μm (x̄ = 5.8 × 1.13 μm, n = 30), aseptate, allantoid to subcylindrical, hyaline, smooth-walled.
        Material examined – Italy, Province of Forlì-Cesena [FC]), Campigna – Santa Sofia, on a dead and aerial branch of *Corylus avellana* L. (*Betulaceae*), 15 February 2019, Erio Camporesi, IT 4232 (MFLU 19-0660).
        GenBank Accession Numbers – ITS: OQ411125; LSU: OQ411118: *act*: OQ437904.
        Notes – In the multi-gene phylogeny, our strain (MFLU 19-0660) grouped together with *Cytospora cotini* (MFLUCC 14-1050) with 80% MLBS and 0.95 BYPP support (Fig. 37, Clade A). These two strains (MFLU 19-0660 and MFLUCC 14-1050) have a close phylogenetic affinity to *C. prunicola* (MFLUCC 16-1315), *C. carbonacea* (CBS 219.54), and *Cytospora* sp. (MFLU 19-2857). In bp comparisons of the ITS and LSU region between our strain and the ex-type strain of *C. cotini* (MFLUCC 14-1050), *C. prunicola* (MFLUCC 16-1315), *C. carbonacea* (CBS 219.54),

and *Cytospora* sp. (MFLU 19-2857) in Clade A (Fig. 37) revealed, they are identical. Thus, we speculate that the phylogeny of this clade is complex.
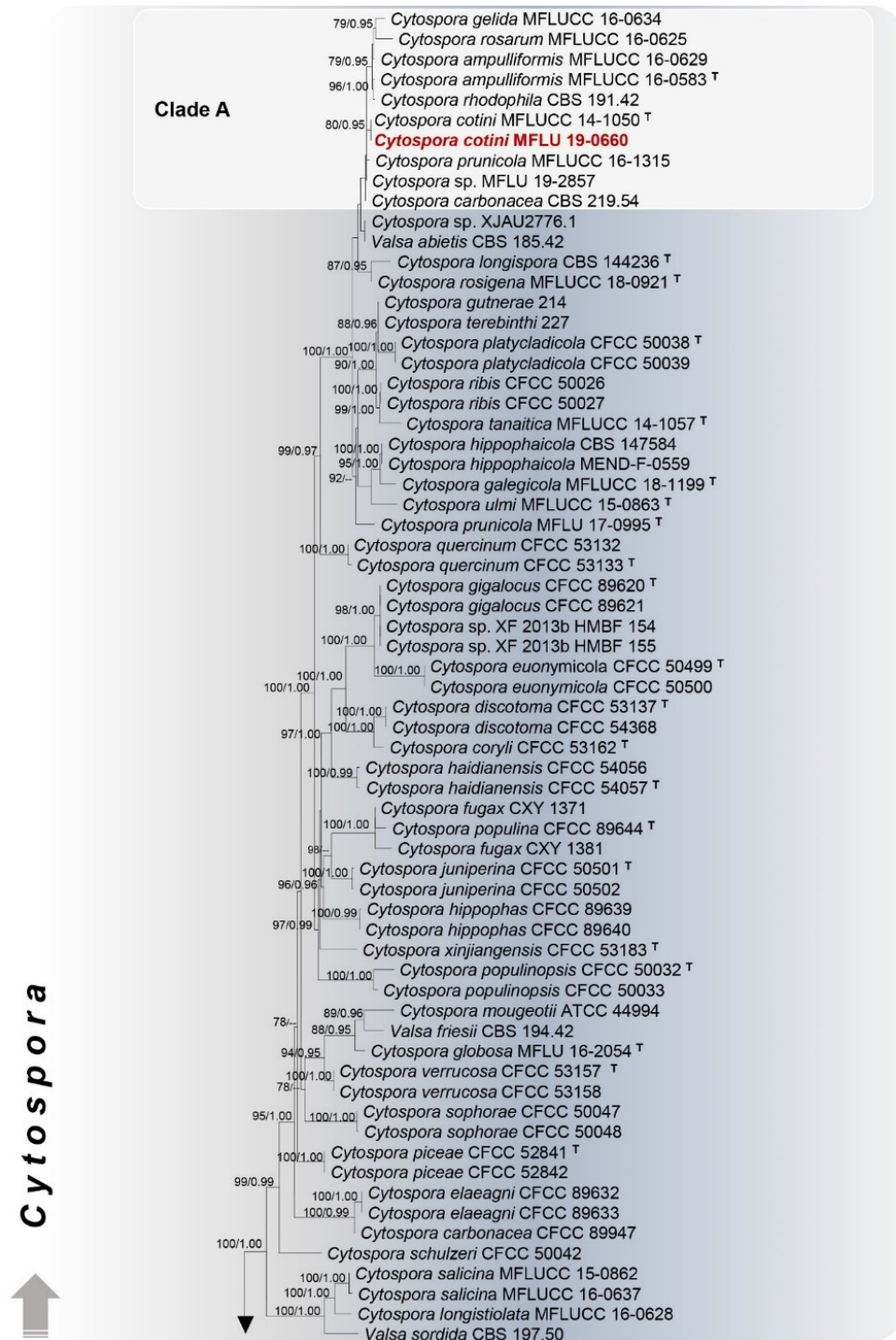
Fig. 37 – Phylogram generated from maximum likelihood analysis based on combined ITS, LSU, *rpb*2, and *act* sequenced data. One-hundred forty-one strains were included in the combined sequence analyses, which comprised 1887 characters with gaps (ITS = 452, LSU = 847, *rpb*2 = 726, *act* = 259). Single gene analyses were also performed, and topology and clade stability were compared from the combined gene analyses. *Cytospora variostrmatica* (CMW1240 and CMW6766) strains were used as outgroup. The final ML optimization likelihood is -17236.415435. The matrix included 811 distinct alignment patterns, with 34.05% undetermined characters or gaps. Estimated base frequencies were obtained as follows: A = 0.245229, C = 0.257402, G = 0.274638, T = 0.222732; substitution rates AC = 1.219437, AG = 4.611597, AT = 1.752016, CG = 1.151254,

CT = 10.092474, GT = 1.0; gamma distribution. Bootstrap support values for ML (first set) equal to or greater than 70%, BYPP equal to or greater than 0.95 are given above or below the nodes. The strains from the current study are in red bold, and the type strains are indicated with [T].
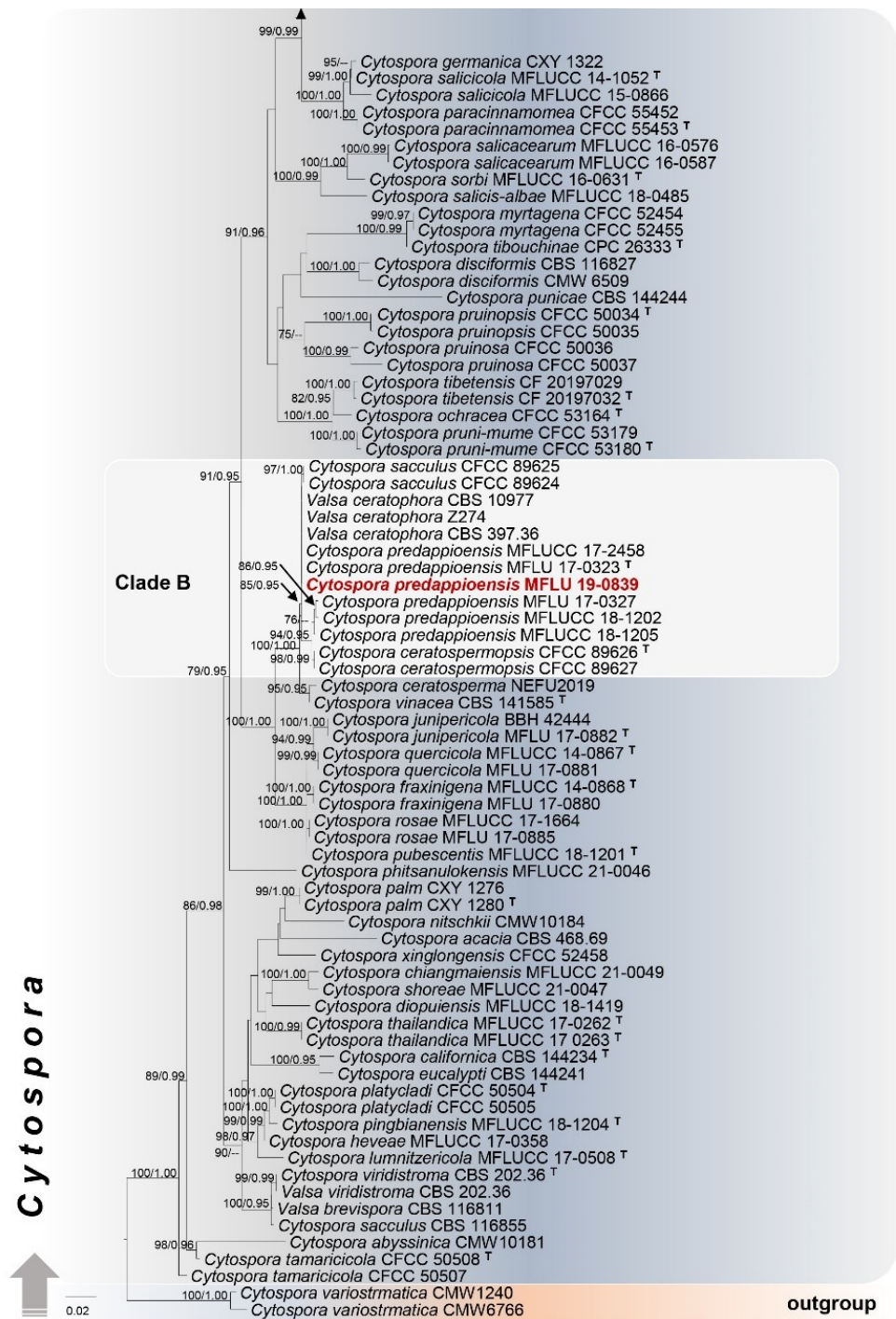

Fig. 37 – Continued.

Morphologies of our collection, except the conidiomata width are identical to *C. cotini*, having pycnidial, immersed, multiloculate conidiomata with an ostiole, peridium with cells of *textura angularis*, enteroblastic, phialidic conidiogenous cells and aseptate, allantoid to subcylindrical hyaline conidia similar sizes (Hyde et al. 2016). Conidiomata in our collection (500–1500 μm) are wider than MFLUCC 14-1050, where they measure 800–1000 μm (Hyde et al. 2016). Based on morphology and phylogenetic analyses, we conservatively identify our collection as *C. cotini.* However, further morpho-molecular analyses with additional coding genes are suggested

for better taxonomic resolution. The known distribution of the species as necrotrophic on dying *Cotinus coggygria* is in Russia and saprobic on *Ostrya carpinifolia* woody substrates is in Italy, respectively (Hyde et al. 2016, Shang et al. 2020). Therefore, our collection is the first record of *C. cotini* on *Corylus avellana* and the second record from Italy.
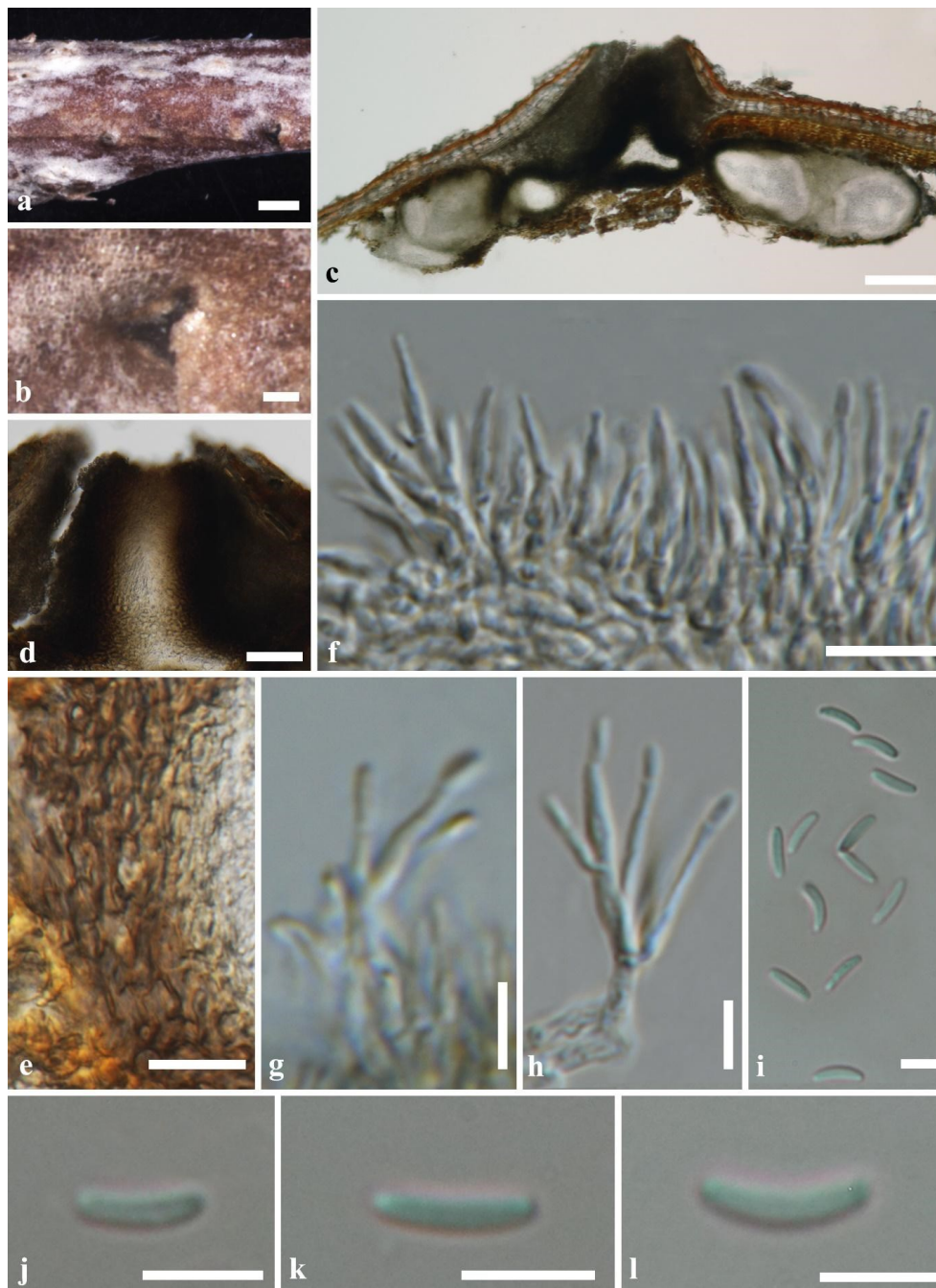
Fig. 38 – *Cytospora cotini* (MFLU 19-0660, new host record in Italy). a–b Conidiomata on the dead host surface of *Corylus avellana* L. (*Betulaceae*, *Fagales*). c Longitudinal section of a conidioma and distribution of locules. d Ostiolar neck. e Conidiomatal wall. f–h Developmental stages of conidiogenesis. i–l Conidia. Scale bars: a = 500 μm, b, c = 200 μm, d = 50 μm, e = 20 μm, f = 10 μm, g–l = 5 μm.

Cytospora predappioensis Q.J. Shang, Norph., Camporesi & K.D. Hyde 2018   Fig. 39
        Index Fungorum number: IF 554083; Facesoffungi number: FoF 3936
        Saprobic on a dead and hanging branch of *Corylus avellana* L. Sexual morph: *Ascostromata* 1200–1600 μm wide, 450–610 μm high, (x̄ = 1400 × 550 μm, n = 5), solitary to gregarious,

immersed, erumpent by ostiole at maturity, multiloculate, ostiolate. *Ascomata* (excluding necks) 250–456 μm high, 200–300 μm diam. ($\bar{x}$ = 350 × 255 μm, n = 10), perithecial, immersed in stroma, globose to subglobose, glabrous, individual ostiolate, brown. *Ostiolar canal* 250–380 μm high, 90–150 μm diam. ($\bar{x}$ = 280 × 110 μm, n = 10), cylindrical, sulcate, periphysate. *Peridium* 14–27 μm wide, composed of several layers, outermost layers comprising pale-brown to brown cells of *textura angularis* to *textura prismatica*, innermost layers thin, hyaline cells of *textura prismatica*. *Hamathecium* comprises 0.5–2.0 μm wide, thin-walled, cylindrical, hyaline, numerous paraphyses mixed with asci. *Asci* 30–40 × 5–7 μm ($\bar{x}$ = 32.7 × 5.8 μm, n = 20), 8-spored, unitunicate, clavate, apically rounded, occasionally truncate, with a J– apical ring, without pedicel. *Ascospores* 5.5–9.2 × 1.5–0.9 μm ($\bar{x}$ = 7.3 × 1.6 μm, n = 30), overlapping 1–2-seriate, narrowly oblong, sometimes allantoid, aseptate, smooth-walled, hyaline. Asexual morph: See Shang et al. (2020).
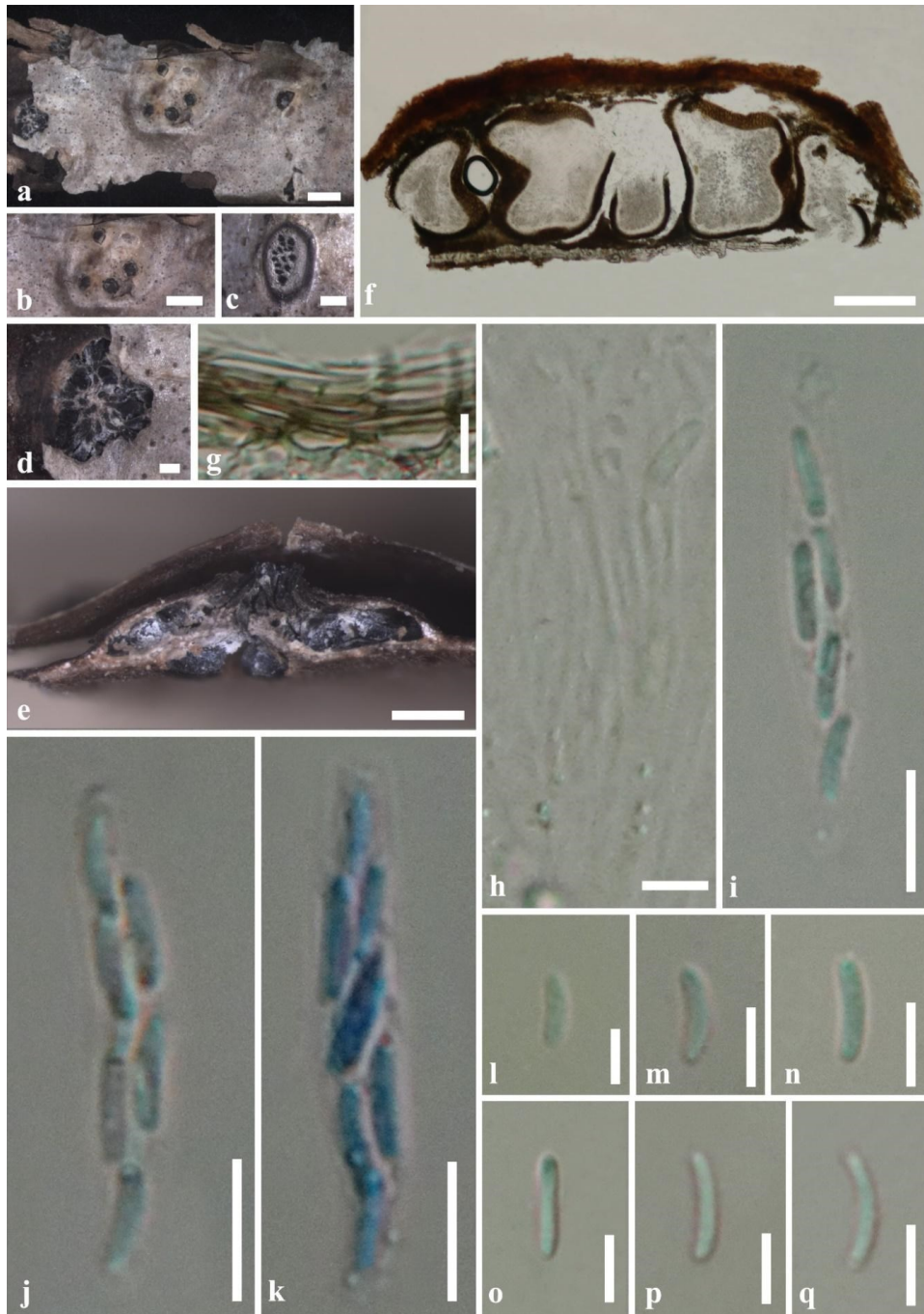
Fig. 39 – *Cytospora predappioensis* (MFLU 19-0839, new host record in Italy). a–b Ascostromata on the dead host surface of *Corylus avellana* (*Betulaceae*, *Fagales*). c–d Upper view of a cross-

section of ascostroma. e–f Longitudinal section of an ascostroma. g Peridium. h Paraphyses. i–k Asci. l–q Ascospores. Scale bars: a, b = 1 mm, c, e = 500 μm, d, f = 200 μm, g = 20 μm, i–k = 10 μm, h, l–q = 5 μm.

Material examined – Italy, Province of Forlì-Cesena [FC]), Corniolo, on a dead and aerial branch of *Corylus avellana* L. (*Betulaceae*), 10 March 2019, Erio Camporesi, IT 4247 (MFLU 19-0839).

GenBank Accession Numbers – ITS: OQ411126; LSU: OQ411119.

Notes – In the multi-gene phylogeny, our strain (MFLU 19-0839) grouped together with *Cytospora predappioensis* (MFLU 17-0323, MFLUCC 17-2458) and *Valsa ceratophora* (CBS 10977, Z274, and CBS 397.36) with 85% MLBS and 0.95 BYPP support (Fig. 37, Clade B). Also, these strains have a close phylogenetic affinity to *C. sacculus* (CFCC 89625, CFCC 89624). In the bp comparisons of ITS and LSU sequence data, between our strain and the type strains of *C. predappioensis* (MFLU 17-0323, MFLUCC 17-2458), *C. sacculus* (CFCC 89625, CFCC 89624), and *V. ceratophora* strains (CBS 10977, Z274, and CBS 397.36) revealed, they are identical and can be identified as *C. predappioensis*. Thus, we speculate that the phylogeny of this clade is complex.

In addition, our collection shares similar ascostromata, asci, and ascospores measurements to *C. predappioensis*, except for ascomata (200–300 *vs*. 450–680 μm diam.) and paraphyses (0.5–2.0 *vs*. 2.5–4 μm) of the holotype (Hyde et al. 2018). Moreover, our collection has *textura prismatica* cells in the innermost peridium, and narrowly oblong to allantoid ascospores, while the holotype (MFLU 17-0323) has *textura angularis* cells in the peridium and fusiform to oblong ascospores (Hyde et al. 2018). However, the illustration of the holotype provided by Hyde et al. (2018), showed peridium cells with both *textura angularis* and *textura prismatica*. Therefore, the morphological differences between our collection and the type material are characterized by comparatively narrower ascomata and paraphyses than the holotype (Hyde et al. 2018).

Based on morphology and phylogenetic analyses, we conservatively identify our collection as *C. predappioensis*. The known distribution of the fungus is in Italy on *Platanus hybrida* and *Ostrya carpinifolia* woody hosts (Hyde et al. 2018, Shang et al. 2020). Therefore, our collection is the first record of *C. predappioensis* on *Corylus avellana* and the second record from Italy.

Sydowiellaceae Lar.N. Vassiljeva 1987

Index Fungorum number: IF 81867; Facesoffungi number: FoF 06882

Notes – Vasilyeva (1987) introduced *Sydowiellaceae* to accommodate *Sydowiella* which was typified by *S. fenestrans* and a collection of morphologically diverse taxa. This is a phylogenetically well-separated monophyletic family in *Diaporthales* and poorly supported with morphology (Kruys & Castlebury 2012, Senanayake et al. 2017b). Generally, *Sydowiellaceae* members are characterized by having solitary or aggregated, coriaceous, ostiolate, papillate ascomata with filamentous, hyaline periphyses, peridium with an outer layer composed of *textura globose* cells and an inner layer of *textura angularis* cells, cellular, septate, branched paraphyses, unitunicate, cylindrical to cylindro-clavate or broadly fusoid asci with J– or bi-lobed apical ring, and 0–11-septate, ellipsoidal or filiform, hyaline or greenish-brown ascospores occasionally baring apical and basal appendages (Maharachchikumburaet al. 2016, Senanayake et al. 2017b, Hyde et al. 2020c). Coelomycetous asexual morph is characterized by having stromatic or pycnidial conidiomata, which are superficial, aggregated, uniloculate, with conidiomatal wall composed of *textura angularis* cells, elongate and branched, conidiophores, cylindrical, ampulliform, phialidic, septate conidiogenous cells, and ovoid to ellipsoid, unicellular, hyaline conidia (Hyde et al. 2020c). Most taxa of *Sydowiellaceae* are saprobes, endophytes or pathogens on dicotyledonous, hardwood trees and herbaceous plants (Senanayake et al. 2017b, Tibpromma et al. 2017). Senanayake et al. (2017b) revised the taxonomy of *Sydowiellaceae* and Voglmayr & Mehrabi (2018) introduced *Caudospora* into the family. Currently, 16 genera are included in *Sydowiellaceae* (Hyde et al.

2020c, Wijayawardene et al. 2022). In this study, we discuss a novel saprobic *Sydowiellaceae* taxon belonging to *Alborbis* collected from Italy.

Alborbis Senan. & K.D. Hyde 2017

 Index Fungorum number: IF 552717; Facesoffungi number: FoF 2835

 Notes – *Alborbis* was introduced by Senanayake et al. (2017b) to accommodate *Alborbis galericulata* which was previously identified as *Cryptodiaporthe galericulata* (= *Valsa galericulata*) by Tulasne & Tulasne (1863). *Cryptodiaporthe* was identified as a genus containing a heterogeneous group of species (Wehmeyer 1933, Senanayake et al. 2017b, Hyde et al. 2020c). Mejía et al. (2011) and Kruys & Castlebury (2012) revealed that *C. galericulata* clustered in *Sydowiellaceae* based on LSU and ITS phylogeny and it differs from the type of *Cryptodiaporthe*. In the LSU, ITS, *rpb*2, and *tef*1-α phylogeny by Senanayake et al. (2017b) the placement of *C. galericulata* was confirmed in *Sydowiellaceae*. The genus is characterized by its sexual morph in having a whitish ectostromatic disc with the appearance of pustulate swellings, containing several immersed, aggregated, coriaceous ascomata, ostiolar canal opening to ectostromatic disc by a wide ostiole covered by hyaline, filamentous periphyses, peridium of *textura angularis* cells, unitunicate, cylindrical to clavate, 8-spored asci with J– apical ring and fusiform, 1-septate ascospores with or without apical and basal appendages (Senanayake et al. 2017b).

Alborbis italica Wijes., E. Camporesi & K.D. Hyde, sp. nov.   Fig. 41

 Index Fungorum number: IF 900089; Facesoffungi number: FoF 14010

 Saprobic on dead aerial branches of *Corylus avellana* L. Sexual morph: *Ascostroma* appears as black dots inside the ruptures on the host surface, with pustulate swellings, containing 1–4 ascomata, solitary, scattered. *Ascomata* 150–290 μm diam. × 430–500 μm high (x̄ = 200 × 450 μm, n = 5), perithecial, globose to subglobose, immersed in stromatic tissues, ostiolate. *Ostiolar neck* 50–75 μm diam. × 200–250 μm long, central, separate, cylindrical, scarcely emergent through host surface, widest at the ostiolar opening (90–120 μm diam.), with hyaline periphyses. *Peridium* 15–30 μm thick, composed of several layers, outermost layers consisting of brown, thick-walled cells of *textura angularis*, innermost layers hyaline, thin-walled cells of *textura prismatica*. *Hamathecium* comprises 3–5 μm wide, thin-walled, cellular, septate, cylindrical, hyaline, numerous paraphyses mixed with asci. *Asci* 60–110 × 17–22 μm (x̄ = 92 × 17 μm, n = 25), 8-spored, unitunicate, cylindrical to clavate, straight or sometimes curved, rounded at apex, with J– apical ring, short pedicellate with a rounded end. *Ascospores* 35–45 × 7–9 μm (x̄ = 39 × 8.1 μm, n = 25), overlapping bi-seriate, fusiform, 1-septate, obtuse at the apexes, mostly 2-guttulate, 1 larger guttule per one cell with miner guttules, constricted at the septum, hyaline, with hyaline apical appendages (7–10 × 2.4–2.7 μm). Asexual morph: Undetermined.

 Culture characteristics – Ascospores germinating on PDA within 24 hours at 18 °C, Colonies on PDA, reaching 6–8 mm diam. after 14 days, irregular, flat, smooth surface with an undulate edge, zonate with different sectors, brown at the margin, yellow at the center, reverse brown at the margin, and yellow at the center.

 Material examined – Italy, Province of Forlì–Cesena [FC]), Monte Mirabello–Predappio, on dead branches of *Corylus avellana* L. (*Betulaceae*), 2 May 2016, Erio Camporesi, IT 356 (MFLU 16-1280, holotype), ex-type living culture, MFLUCC 17-2456, *ibid*., 24 May 2021, Erio Camporesi, (MFLU 23-0019, paratype).

 GenBank Accession Numbers – ITS: OQ411122; LSU: OQ411117.

 Notes – In the multi-locus phylogeny, our strain (*Alborbis italica*; MFLUCC 17-2456) grouped in *Alborbis* sister to *A. galericulata* with 100% MLBS and 1.00 BYPP support (Fig. 40, Clade A). Nucleotide differences (excluding gaps) between our strain (MFLUCC 17-2456) and *A. galericulata* (AR 3811, AR 4027, AR 4004, and AR 3890) revealed that the ITS region has a 17/504 bp difference (3.37%) while LSU is not available for *A. galericulata*. Also, our collection shares similar morphologies to *A. galericulata* in having ruptured ascostromata with pustulate swellings, distinct, periphysate ostiolar neck, cylindrical to clavate asci with J– apical ring, but

smaller sizes (Table 7). The key differences distinguishing our collection from *A. galericulata* are the presence of a peridium with cells of *textura angularis* to *textura prismatica*, and fusiform, 2–guttulate, larger ascospores (35–45 × 7–9 μm) with apical appendages, while *A. galericulata* has only *textura angularis* cells in peridium and smaller ascospores (21–26 × 5–7) lacking appendages. The morphological comparison, in combination with the multi-locus phylogenetic analysis, allows us to establish our isolate as a new species in *Alborbis,* named *A. italica.*
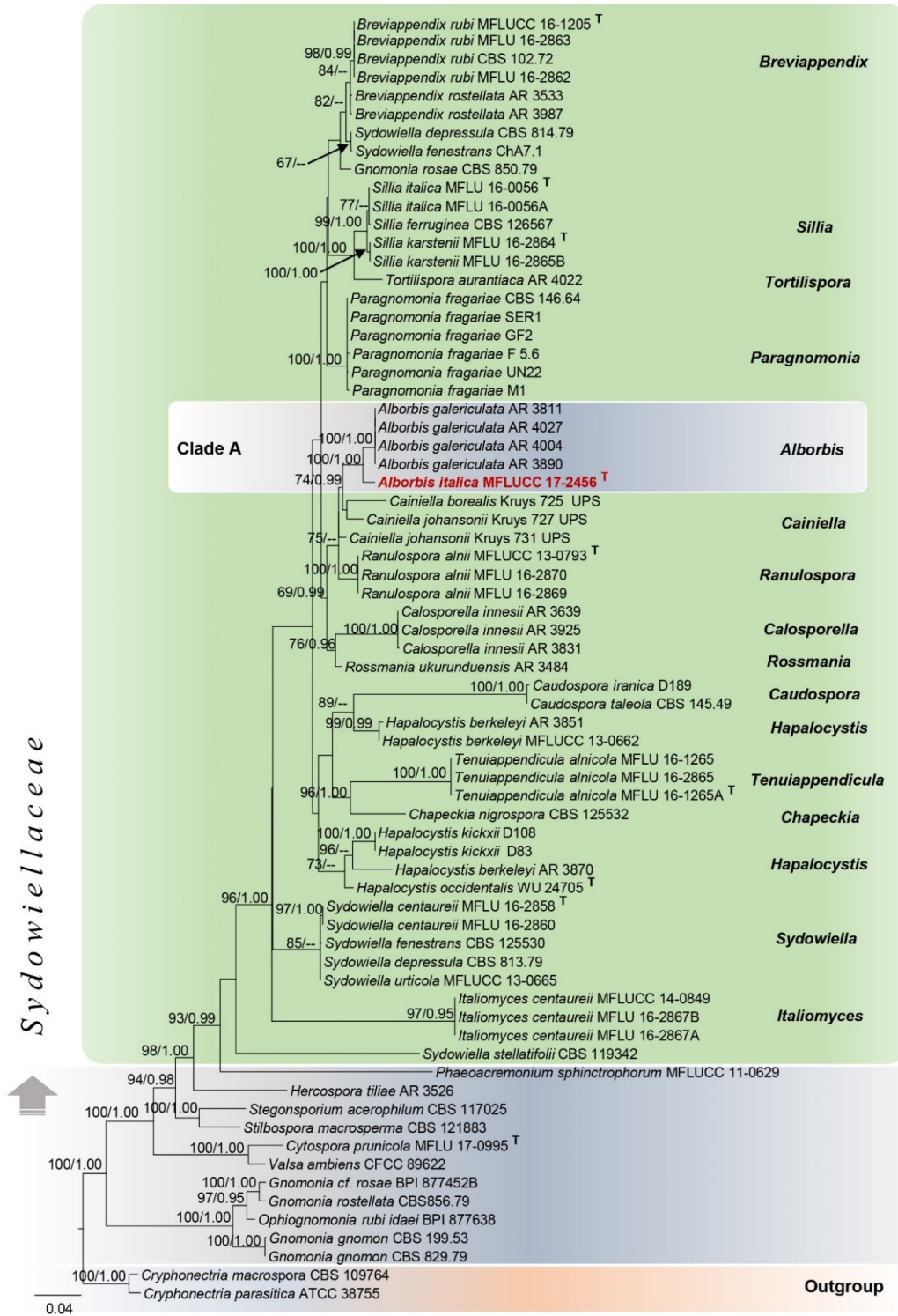
Fig. 40 – Phylogram generated from maximum likelihood analysis based on combined LSU and ITS sequenced data. Seventy strains were included in the combined sequence analyses, which comprised 1344 characters with gaps (LSU = 826, ITS= 518). Single gene analyses were also

performed, and topology and clade stability were compared from the combined gene analyses. *Cryphonectria macrospora* (CBS 109764) and *C. parasitica* (ATCC 38755) strains were used as the outgroup taxa. The final ML optimization likelihood is - 9672.023536. The matrix included 494 distinct alignment patterns, with 19.33% undetermined characters or gaps. Estimated base frequencies were obtained as follows: A = 0.236112, C = 0.263158, G = 0.290834, T = 0.209896; substitution rates AC = 1.425940, AG = 2.017268, AT = 2.019458, CG = 1.384090, CT = 5.431289, GT = 1.0; gamma distribution. Bootstrap support values for ML (first set) equal to or greater than 70%, BYPP equal to or greater than 0.95 are given above or below the nodes. The strain from the current study is in red bold, and the type strains are indicated with [T].

Fig. 41 – *Alborbis italica* (MFLU 16-1280, holotype). a Ascostromata on the dead host surface of *Corylus avellana* L. (*Betulaceae, Fagales*). b Longitudinal section of an ascostroma. c Longitudinal section of ostiolar neck. d. Peridium. e Paraphyses. f–i Asci. j–m Ascospores. n A germinating ascospore. o–p View of culture grown on PDA, upper (o) and lower (p) side. Scale bars: b, c, f–i = 100 μm, d = 20 μm, e, j–m = 10 μm.

Table 7 The key morphological differences in the dimensions of *Alborbis* species.

Species	Ascomata (μm)	Ostiolar neck (μm)	Peridium (μm)	Asci (μm)	Ascospores (μm)	Reference(s)
Alborbis italica	430–500 high, × 150–290 diam.	200–250 high, × 50–75 diam.	15–30	60–110 × 17 – 22	35–45 × 7–9	This study
A. galericulata	600–950 high, 260– diam.	375–400 high, 80–90 wide	30–35	90–110 × 17–20	21–26 × 5–7	Senanayake et al. (2017b)

Hypocreales Lindau 1897

Index Fungorum number: IF 0477; Facesoffungi number: FoF 02091

Notes – *Hypocreales* was established by Lindau (1897b) to accommodate *Hypocreaceae* based on *Hypocrea* which was introduced by Fries (1825). Maharachchikumbura et al. (2016) accepted *Hypocreales* into *Hypocreomycetidae*. Hypocrealean taxa are characterized by white to brightly colored, soft, fleshy, or membranous perithecia, apical paraphyses in the centrum, single layer, thin-walled asci sometimes thickened at the apex, with or without a pore and producing phialides on free conidiophores (Rogerson 1970). Members of the *Hypocreales* are highly diverse in the tropics, subtropics, and temperate regions (Põldmaa 2011). They can be biotrophic, hemibiotrophic, saprobic or hypersaprobic, endophytes, parasitic or pathogenic forming cankers on plant hosts, as well as fungicolous, lichenicolous, or entomogenous myxomyceticolous (Barr 1990, Gams et al. 2004, Perera et al. 2023). Currently, the order consists of 15 families and 30 genera in *Hypocreales* genera *incertae sedis* (Wijayawardene et al. 2022). In this study, we discuss a saprobic species that belongs to *Nectriaceae* from Italy.

Nectriaceae Tul. & C. Tul. 1865

Index Fungorum number: IF 81059; Facesoffungi number: FoF 1396

Notes – *Nectriaceae* was introduced by Tulasne & Tulasne (1865) with *Nectria* as the type genus. The family was accepted into *Hypocreales* based on stromatic and perithecial characteristics (Seaver 1909, Kreisel 1969, Rossman et al. 1999, Lombard et al. 2015, Maharachchikumbura et al. 2016, Hyde et al. 2020c). *Nectriaceae* taxa share similar morphological characteristics to *Bionectriaceae* and therefore, DNA-based sequence data are used for species-level identification (Rossman et al. 1999, Voglmayr & Jaklitsch 2019, Perera et al. 2023). The sexual morphs of *Nectriaceae* are characterized by perithecial, uni-loculate ascomata that are white, yellow, orange-red, brown, purple to black, changing color in KOH, with or without setae, 4–8-spored unitunicate asci with or without an apical ring, and fusiform, aseptate to multi-septate or muriform ascospores (Lombard et al. 2015, Hyde et al. 2020c). Asexual morphs are mostly hyphomycetous and coelomycetous (*Thyronectria*) morphs are rarely available. *Nectriaceae* is characterized by having synnematous, sporodochial or pycnidial conidiomata, penicillate, verticillate conidiophores, monophialidic to polyphialidic conidiogenous cells, aseptate to multi-septate conidia and presence or absence of chlamydospores (Maharachchikumbura et al. 2016, Voglmayr & Jaklitsch 2019, Hyde et al. 2020c, Perera et al. 2023). *Nectriaceae* members are mainly found as soil-borne saprobes, facultative or obligate pathogens and endophytes, foliicolous or saprobes on woody hosts, and entomogenous and human pathogens in terrestrial or aquatic habitats, worldwide (Lombard et al. 2015, Maharachchikumbura et al. 2016, Hyde et al. 2020c). After the studies by Lumbsch & Huhndorf (2010), Lombard et al. (2015), Maharachchikumbura et al. (2016) and Hyde et al. (2020c), currently seventy-seven genera are accepted in the family (Perera et al. 2023). In this study, we discuss a saprobic *Nectria* species collected from Italy.

Nectria (Fr.) Fr. 1849

Index Fungorum number: IF 3431; Facesoffungi number: FoF 02122

Notes – *Nectria* was recognized by Fries (1849) without designating a type species. The first typification of the genus was performed by Clements & Shear (1931) who designated *N. cinnabarina* as the lectotype (Rossman et al. 1999), and *Tubercularia vulgaris* as the asexual morph (Ma et al. 2020). The sexual morph of the genus is characterized by globose, red, fleshy, warted perithecia that become cupulate upon drying and 0–3-septate ascospores (Rossman et al. 1999, Dayarathne et al. 2020a). The key characters of asexual morph are yellow-orange, red-brown or black, synnemata or sessile, stipitate sporodochial to pycnidial conidiomata, branched, septate conidiophores, presence or absence of sterile hyphae, enteroblastic, phialidic, cylindrical to subcylindrical or lageniform conidiogenous cells with or without acropleurogenous branches and aseptate, ellipsoidal, obovate, or cylindrical, allantoid, hyaline conidia (Hirooka et al. 2011, Li et al. 2020). The species congeneric with the type species *N. cinnabarina* were considered as *Nectria sensu stricto* by Rossman et al. (1999). Based on morphology and LSU sequence data *Nectria sensu lato* was divided into different genera (Hirooka et al. 2010, Rehner & Samuels 1995, Rossman et al. 1999). *Nectria* species are saprobic or weakly parasitic on plant hosts in terrestrial and marine habitats (Yang et al. 2019, Dayarathne et al. 2020a, Li et al. 2020).

Nectria dematiosa (Schwein.) Berk. 1873   Fig. 43
        Index Fungorum number: IF 144029; Facesoffungi number: FoF 07461
        Saprobic on dead and fallen branches of *Fagus sylvatica* L. Sexual morph: See Yang et al. (2018). Asexual morph: Sporodochial. *Stromata* solitary to caespitose, erumpent through host substrate, yellowish orange to red. *Conidiomata* 400–1000 μm diam., 300–700 μm high ($\bar{x}$ = 700 × 532 μm, n = 5), sporodochial, astipitate, semi-immersed to superficial, solitary or aggregated, scattered, discoid or cylindrical-capitate, sessile, pustular, yellowish orange. *Hymenium* 2.0–3.5 μm wide, arising directly, at the base consisting of the cells of *textura angularis*, elongating with the cells of *textura prismatica*. *Conidiophores* monoverticillate or biverticillate, developing acropleurogenously, straight, hyaline. *Conidiogenous cells* monophialidic, intercalarate, occurring below each septum, rarely terminal, without collarettes. *Conidia* 5.3–8.2 × 2.4–3.0 μm ($\bar{x}$ = 7.1 × 2.7 μm, n = 30), elongate to sub-cylindrical, straight or slightly curved, aseptate, hyaline.
        Culture characteristics – Conidia germinating on PDA within 24 h at 18 °C, colonies on PDA reaching 10–15 mm diam. after 14 days, with circular, flat, filiform margin, white at the upper surface, reverse pale yellowish white.
        Material examined – Italy, Province of Forlì-Cesena [FC]), Campigna - Santa Sofia, on dead and fallen branches of *Fagus sylvatica* L. (*Fagaceae*), 3 November 2017, Erio Camporesi, IT 3559 (MFLU 17-2739), *ibid*., 10 September 2018, Erio Camporesi, MFLU 18-1937.
        GenBank Accession Numbers – ITS: OQ411123; *act*: OQ437903; *tub2*: OQ437894.
   Notes – In the multi-gene phylogeny, our strain (MFLU 17-2739) grouped together with *Nectria dematiosa* (CBS 126570, CBS 279.48, CFCC 53586, XJAU 2772–2 and CFCC 52137) with 97% MLBS and 0.99 BYPP support (Fig. 42, Clade A). The bp comparison of the ITS region between our strain and the ex-epitype strain (CBS 126570), revealed that they are identical. Also, our collection shares similar morphologies to *N. dematiosa*, provided by Hirooka et al. (2012). Based on morphology and phylogenetic analyses, we conclude our collection is *N. dematiosa.* The known distribution of *N. dematiosa* is in Canada, China, Finland, Japan, Poland, New Zealand, and the USA on different woody hosts (Hirooka et al. 2012, Yang et al. 2018). Therefore, our collection is the first record of *N. dematiosa* from Italy as well as on *Fagus sylvatica*.

Xylariales Nannf. 1932
        Index Fungorum number: IF 90505; Facesoffungi number: FoF 12988
Notes – *Xylariales* was established by Nannfeldt (1932) to accommodate the type family *Xylariaceae* and a few other families, *viz. Diatrypaceae, Hypocreaceae, Hyponectriaceae, Lasiosphaeriaceae*, and *Polystigmataceae*. It is a large order in *Sordariomycetes* of perithecial *Ascomycota* consisting of conspicuous and inconspicuous fruiting bodies (Smith et al. 2003, Sun et al. 2021, Vandegrift 2021, Ma et al. 2022). *Xylariales* was accepted as the only order in

Xylariomycetidae by Maharachchikumbura et al. (2016) and currently, *Amphispheriales* and *Delonicicolales* are accepted (Wijayawardene et al. 2022). The divergence time for *Xylariales* was estimated to be 147 MYA by Hyde et al. (2020c). The majority of xylarialean taxa are endophytes, and also hemibiotrophs, necrotrophs, pathogens, and saprobes can be found on wood, other plant debris, dung, and in some arthropod animals from terrestrial and aquatic habitats (Senanayake et al. 2015, Maharachchikumbura et al. 2016, Sun et al. 2021, Vandegrift 2021, Ma et al. 2022). Currently, the order consists of 22 families and 57 genera in *Xylariales* genera *incertae sedis* (Samarakoon et al. 2022). In this study, we report on several plant-associated xylarialean species belonging to *Graphostromataceae* and *Diatrypaceae* collected from Italy.
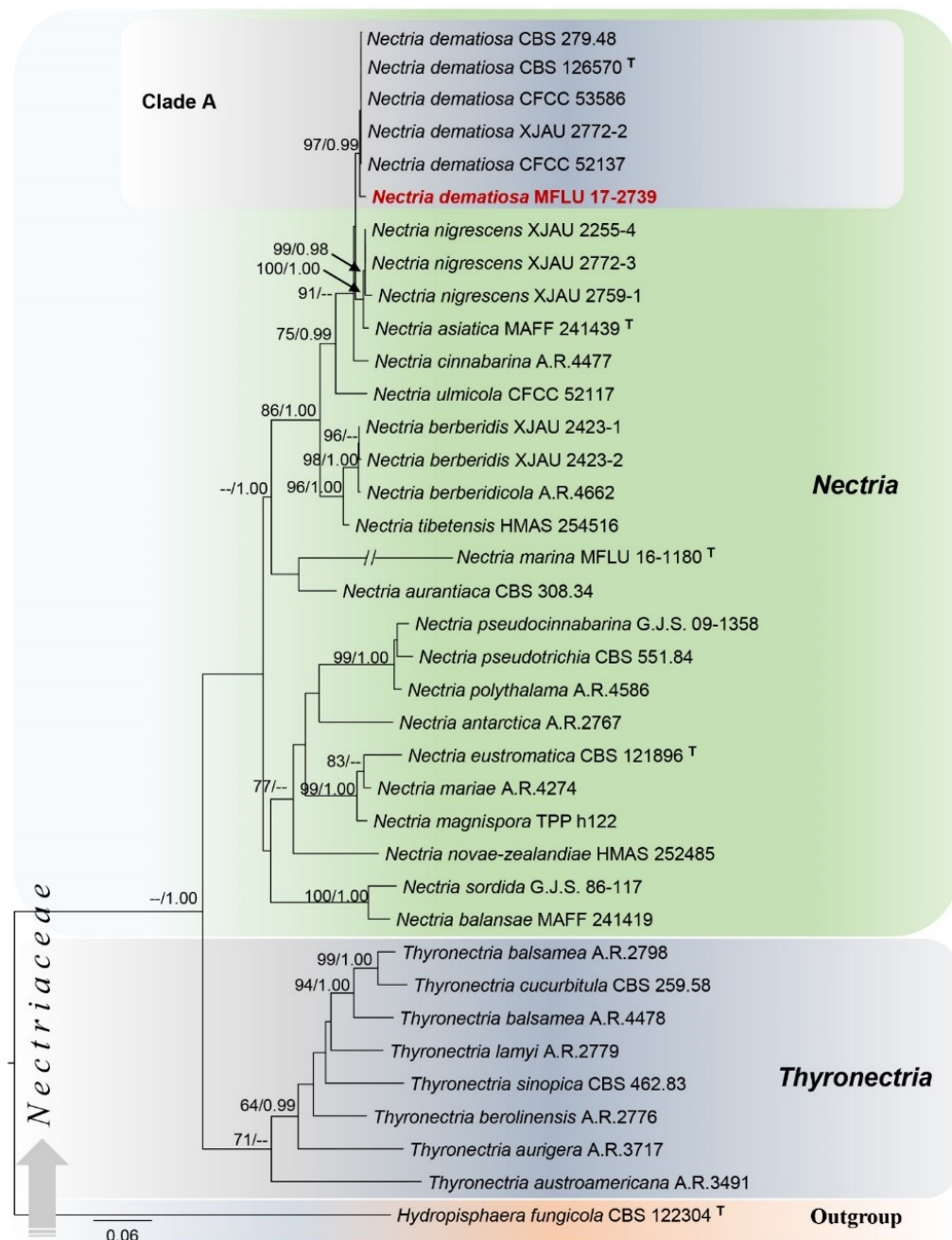
Fig. 42 – Phylogram generated from maximum likelihood analysis based on the combined *act*, ITS, and *tub*2 sequenced data. Thirty-seven strains were included in the combined sequence analyses, which comprised 1450 characters with gaps (*act* = 266, ITS = 591, *tub*2 = 583). Single gene analyses were also performed, and topology and clade stability were compared from combined gene analyses. *Hydropisphaera fungicola* (CBS 122304) strain was used as the outgroup taxon.

The final ML optimization likelihood is -9658.353945. The matrix included 645 distinct alignment patterns, with 18.16% undetermined characters or gaps. Estimated base frequencies were obtained as follows: A = 0.200431, C = 0.310118, G = 0.249117, T = 0.240334; substitution rates AC = 0.547215, AG = 2.169416, AT = 1.292898, CG = 0.773244, CT = 3.618162, GT = 1.0; gamma distribution. Bootstrap support values for ML (first set) equal to or greater than 60%, BYPP equal to or greater than 0.95 are given above or below the nodes. The strain from the current study is in red bold, and the type strains are indicated with ^{T}.
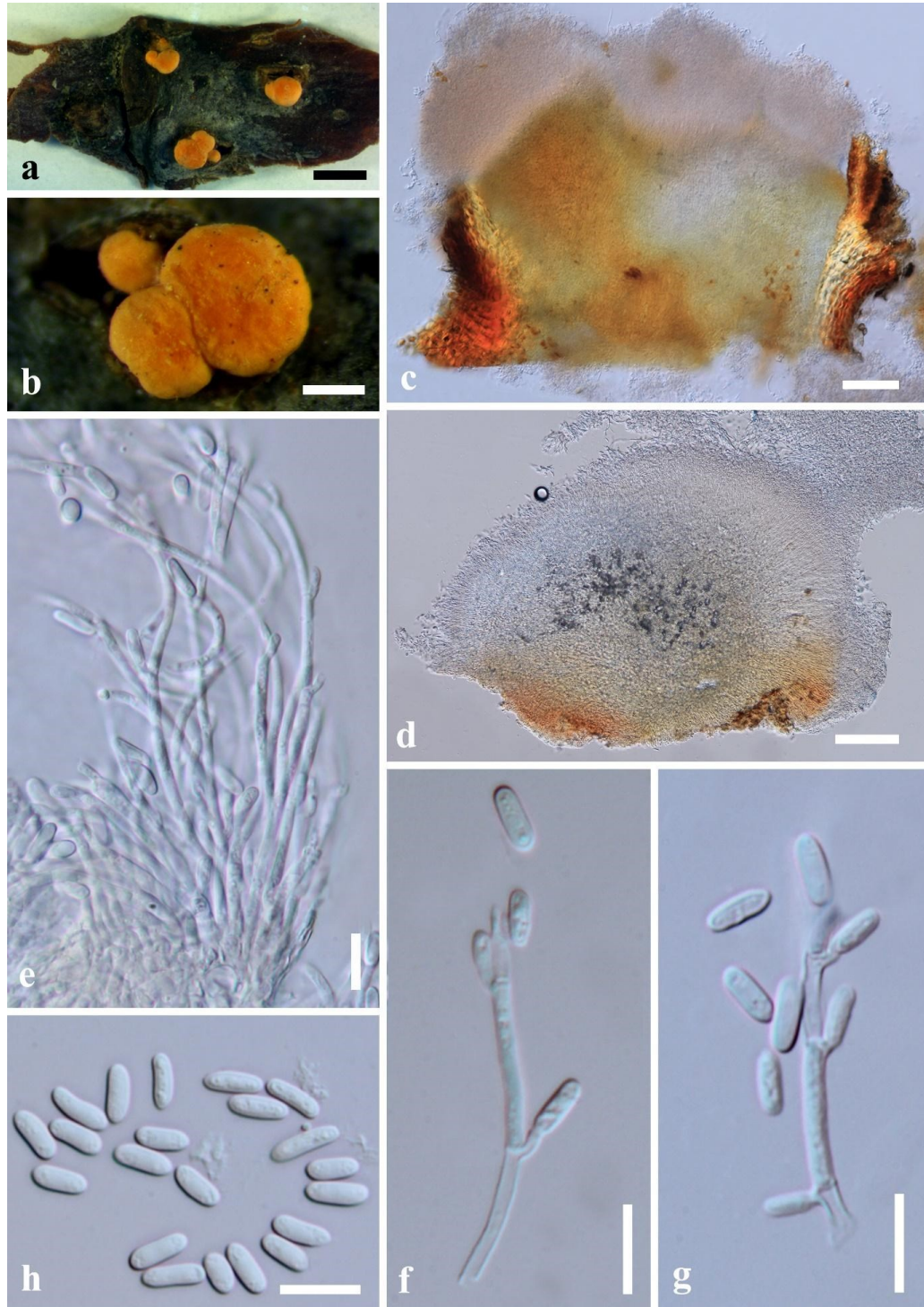
Fig. 43 – *Nectria dematiosa* (MFLU 17-2739, new host and geographical record). a–b Sporodochia on the dead host surface of *Fagus sylvatica* L. (*Fagaceae*, *Fagales*). c Longitudinal section of astipitate sporodochia. d Hymenium. e Conidiophores inside the sporodochium. f–g Conidiophores with attached conidia. h Conidia. Scale bars: a = 1 mm, b = 200 µm, c–d = 100 µm, e–h = 10 µm.

Graphostromataceae M.E. Barr, J.D. Rogers & Y.M. Ju 1993

Index Fungorum number: IF 81957; Facesoffungi number: FoF 00624

Notes – *Graphostromataceae* was introduced by Barr et al. (1993) to accommodate *Graphostroma* by considering a diatrypaceae-like sexual morph (allantoid ascospores) and nodulisporium-like asexual morph. Maharachchikumbura et al. (2016) synonymized *Graphostromataceae* to *Xylariaceae*, and later, based on molecular clock evidence and updated phylogeny, *Graphostromataceae* was accepted as a separate family (Samarakoon et al. 2016, Hyde et al. 2017b, Hongsanan et al. 2017, Wendt et al. 2017, Daranagama et al. 2018, Voglmayr et al. 2018). The key morphologies of sexual morph taxa in the family are irregularly outlined by erumpent stromata, perithecial, bottle-shaped ascomata with diatrypoid configuration, multi-layered peridium, elongate paraphyses, 8-spored, narrowly clavate, short-pedicellate asci with J+ apical ring and ellipsoid, uni to multiseriate, aseptate, allantoid, and hyaline or brown ascospores. Asexual morphs are characterized by having nodulisporium-type, periconiella-like, or xylocladium-like morphologies (Wendt et al. 2017). Most *Graphostromataceae* species are saprobes, endophytes, and pathogens on terrestrial wood (Hyde et al. 2020c). Currently, five genera are accepted in the family *viz. Biscogniauxia, Camillea, Graphostroma, Obolarina* and *Vivantia* (Li et al. 2021, Hyde et al. 2020c, Wijayawardene et al. 2022). This study discusses about two saprobic species under *Graphostromataceae*.

Biscogniauxia Kuntze 1891

Index Fungorum number: IF 582; Facesoffungi number: FoF 02970

Notes – *Biscogniauxia*, previously known as *Nummularia*, was introduced by Tulasne & Tulasne (1863). Pouzar (1979) used the name *Biscogniauxia* for the first time in the sexual morph of *Nummularia*. The key morphologies of the sexual morph of the genus are the presence of widely effuse stromata with ostioles separate from the surface, perithecia in a layer or polystichous, 8-spored, cylindrical asci with or without J+ apical ring, mostly uniseriate, ellipsoid brown ascospores with or without germ slits (Ju et al. 1998, Vasilyeva et al. 2007, Li et al. 2020). The asexual morph is characterized by having nummularia-like morphologies that are similar to *Obolarina* and *Camillea* (Li et al. 2020) and sometimes varying from nodulisporium-like to periconiella-like morphologies (Stadler et al. 2013, Hyde et al. 2020c). The majority of *Biscogniauxia* species are pathogens and both endophytic and pathogenic species are known as causal agents for fungal trunk diseases on different host trees worldwide (Sohrabi et al. 2022). Some species can be found as foliar endophytes and they can turn into pathogens such as *B. mediterranea* in water-stressed conditions on the host plants (Edwards et al. 2003, Raimondo et al. 2016, Hyde et al. 2020c). In this study, we discussed two saprobic *Biscogniauxia* species collected from Italy.

Biscogniauxia anceps (Sacc.) J.D. Rogers, Y.M. Ju & Cand. 1996   Fig. 45

Index Fungorum number: IF 434566; Facesoffungi number: FoF 14011

Saprobic on dead aerial branches of *Corylus avellana* L. Sexual morph: *Ascostromata* 5–8 mm long, applanate, discoid to irregular, erumpent, slightly raising through host surface, wide-spread, carbonaceous, grayish black surface, with distinct margins, scattered sunken gray ostioles on the surface. *Ascomata* 150–350 μm diam., 300–500 high μm ($\bar{x}$ = 300 × 450 μm, n = 5), perithecial, surrounded by carbonaceous stromatal material, pyriform to ovoid, coriaceous, delicate, black, ostiolate. *Ostiole* central, umbilicate, positioned in depressed areas. *Hamathecium* comprises 2–4 μm wide, longer than asci, thin-walled, branched, septate, hyaline, numerous paraphyses with oily contents. *Asci* 80–130 × 8.5–11 μm ($\bar{x}$ = 110 × 9 μm, n = 15), 8-spored, unitunicate, cylindrical, apically rounded, with a J+ apical ring, short pedicellate, obtuse or truncate ends. *Ascospores* 13–17 × 7–8 μm ($\bar{x}$ = 16 × 7.5 μm, n = 25), uniseriate, broadly ellipsoidal, inequilateral ellipsoidal or obovoid, unequally 1-septate, larger upper cell and smaller lower cell, smooth, hyaline, without a gelatinous sheath or appendages. Asexual morph: see Rogers et al. (1996).
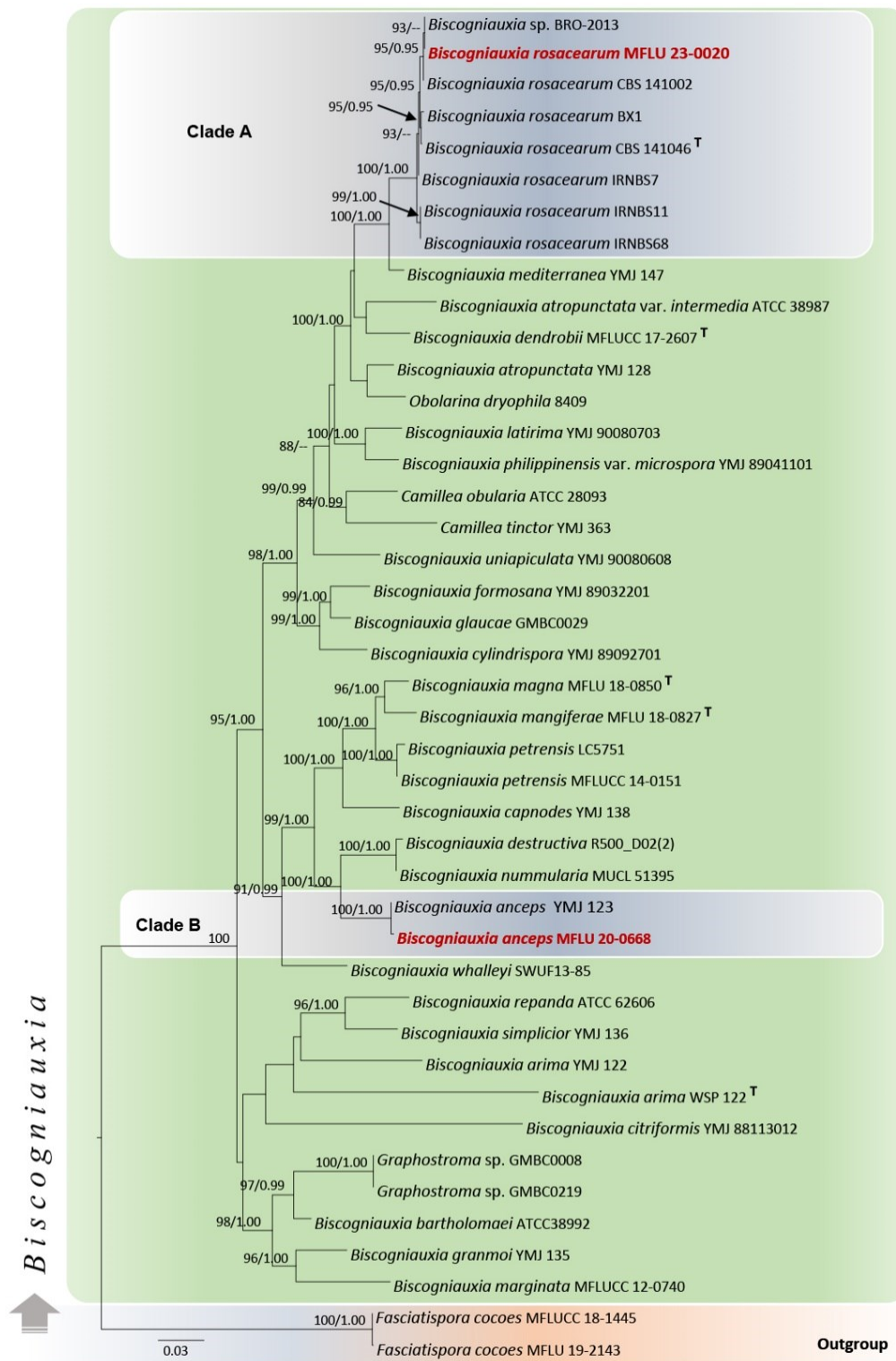
Fig. 44 – Phylogram generated from maximum likelihood analysis based on combined ITS, *tub*2, *act*, and LSU sequenced data. Forty-three strains were included in the combined sequence analyses, which comprised 1993 characters with gaps (ITS = 567, *tub*2 = 448, *act* = 220, LSU = 758). Single gene analyses were also performed, and topology and clade stability were compared from the combined gene analyses. *Fasciatispora cocoes* strains (MFLUCC 18-1445 and MFLU 19-2143) were used as the outgroup taxa. The final ML optimization likelihood is -12900.207868. The matrix included 782 distinct alignment patterns, with 38.55% undetermined characters or gaps. Estimated base frequencies were obtained as follows: A = 0.240708, C = 0.252622, G = 0.252603, T = 0.254067; substitution rates AC = 1.449977, AG = 3.388313, AT = 1.472953, CG = 0.900931, CT

= 6.034996, GT = 1.0; gamma distribution. Bootstrap support values for ML (first set) equal to or greater than 80%, BYPP equal to or greater than 0.95 are given above or below the nodes. The strains from the current study are in red bold, and the type strains are indicated with [T].

Material examined – Italy, Province of Ravenna [RA]), Zattaglia, on dead aerial branches of *Corylus avellana* L. (*Betulaceae*), 9 November 2020, Erio Camporesi, IT 4675 (MFLU 20-0668), *ibid.*, 23 November 2020, Erio Camporesi, IT 4675A (MFLU 20-0669).

GenBank Accession Numbers – ITS: OQ411128; LSU: OQ411120; *act*: OQ437905: *tub*2: OQ437898.

Notes – In the multi-gene phylogeny, our strain (MFLU 20-0668) grouped together with *Biscogniauxia anceps* (YMJ 123) with 100% MLBS and 1.00 BYPP support (Fig. 44, Clade B). The bp comparison between our strain and YMJ 123, revealed that 2/517 bp difference (0.38%) in ITS, 1/442 bp difference (0.22%) in *tub*2, and *act* sequences are identical. Also, these two strains are phylogenetically closer to *B. destructive* and *B. nummularia* by forming separate sister lineages (Fig. 44, Clade B).

In considering the morphology, *Biscogniauxia* species had long been known as *Nummularia* (Raimondo et al. 2016). Based on the discussion about the long-term hyaline nature of *B. anceps* ascospores and their affinity to *Nummularia* by Saccardo (1928), Rogers et al. (1996) observed the holotype of *N. anceps*. They identified the identical fungal morphologies between *N. anceps* and *B. anceps* and, therefore, a new combination was provided. Also, our collection shares similar morphologies to *B. anceps* (stroma, ascomata, asci, paraphyses, and ascospores), provided by Rogers et al. (1996). The ascostromata morphology on the host substrate is always confused with the stromata of *Diatrype stigma* (Rogers et al. 1996). Some ascospores of this fungus were reported with smaller hyaline to subhyaline, larger dark brown to black cell with a full-length germ slit, other than whole hyaline ascospores with smaller cell devoid of a germ slit (Rogers et al. 1996). Sometimes, *B. anceps* shows bicellular ascospores with upper dark and lower hyaline cells, with an appendage, while some have unicellular-hyaline ascospores without an appendage, similar to our collection (Vujanovic et al. 2020). The closely related *Biscogniauxia nummularia* shows unicellular, dark, and hyaline ascospores, without appendages, while *B. destructive* shows a combination of both unicellular-dark ascospores and bicellular-dark or hyaline ascospores with or without germ slits and appendages (Vujanovic et al. 2020). Based on these morphologies and phylogenetic analyses, we conclude that our collection is *B. anceps.* The known distribution of *B. anceps* is in France (*Corylus avellana*, *Fraxinus* sp., *Acer pseudoplatanus*, *Laurus cerasus*, *Crataegus oxyacantha*), Italy (*Quercus pedunculata*), United Kingdom (*Corylus avellana*) Honduras, Mexico and Spain (Rogers et al. 1996, Ju et al. 1998, Hsieh et al. 2005, Vujanovic et al. 2020). Therefore, our collection is the first record of *B. anceps* on *Corylus avellana* in Italy.

Biscogniauxia rosacearum M.L. Raimondo & Carlucci 2016   Fig. 46
Index Fungorum number: IF 816045; Facesoffungi number: FoF 14012
Saprobic on dead and fallen branches of *Quercus cerris* L. Sexual morph: *Ascostromata* 20–40 mm long, applanate, irregularly spread on the substrate, erumpent, slightly raising through host surface, carbonaceous, with distinct margins, grayish black, scattered sunken gray ostioles on the surface. *Ascomata* 200–300 μm diam., 500–800 μm high ($\bar{x}$ = 250 × 600 μm, n = 5), perithecial, surrounded by carbonaceous stromatal material, pyriform to ovoid, coriaceous, delicate, brown to black, ostiolate. *Ostiole* 40–60 μm wide, papillate, with puncate opening, central, umbilicate, positioned in depressed areas. *Hamathecium* comprises 3–5 μm wide, longer than asci, thin-walled, unbranched, hyaline, numerous paraphyses with oily contents. *Asci* 100–150 × 8–13 μm ($\bar{x}$ = 123 × 11.5 μm, n = 15), 8-spored, unitunicate, cylindrical, with oily contents, apically rounded, with a J+ apical ring, short pedicellate, with truncate ends, with oily contents. *Ascospores* 15–19 × 8–9.5 μm ($\bar{x}$ = 16.7 × 8.9 μm, n = 25), uniseriate, broadly ellipsoidal, rounded ends, smooth, aseptate, initially hyaline, becoming pale to dark brown at maturity, guttulate, without a gelatinous sheath. Asexual morph: see Raimondo et al. (2016).
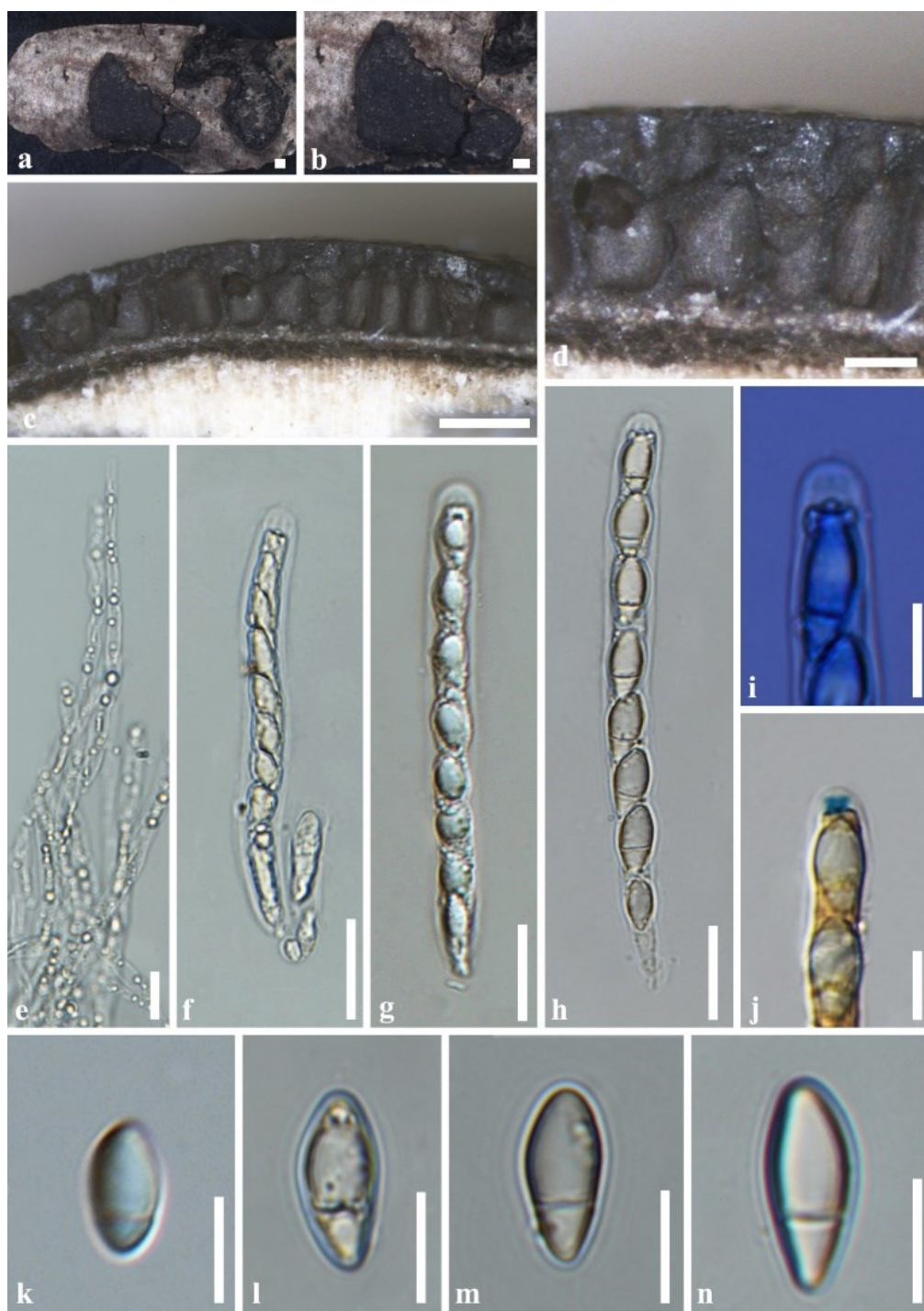
Fig. 45 – *Biscogniauxia anceps* (MFLU 20-0668, new host record in Italy). a–b Ascostromata on the dead host surface of *Corylus avellana* L. (*Betulaceae, Fagales*). c–d Longitudinal section of an ascostroma. e Paraphyses. f–h Asci. i–j Apical ring. k–n Ascospores. Scale bars: a–c = 500 μm, d = 200 μm, f–h = 20 μm, e, i–n = 10 μm.

Material examined – Italy, Province of Forlì-Cesena [FC]), Farazzano-Forlì, on dead and fallen branches of *Quercus cerris* L. (*Fagaceae*), 16 April 2021, Erio Camporesi, IT 4706 (MFLU 23-0020), *ibid.*, 03 May 2021, Erio Camporesi, IT 4706A (MFLU 23-0021).

GenBank Accession Numbers – ITS: OQ411129; LSU: OQ411121; *act*: OQ437906: *tub2*: OQ437899.

Notes – In the multi-gene phylogeny, our strain (MFLU 23-0020) grouped with *Biscogniauxia rosacearum* strains BRO–2013, CBS 141002, BX1, CBS 141046, IRNBS7, IRNBS11, and IRNBS68 with 100% MLBS and 1.00 BYPP support (Fig. 44, Clade A). Also,

B. rosacearum group forms a well-supported sister lineage to *B. mediterranea* (YMJ 147). The bp comparison between our strain and the ex-type strain (CBS 141046), revealed 5/408 bp difference (1.20%) in *tub*2, while ITS and *act* are identical. Also, our collection shares similar morphologies with the holotype by stroma, ascomata, and asci (Raimondo et al. 2016). However, our collection, has wider paraphyses (3–5 μm) and initially hyaline ascospores, while *B. rosacearum* collection described by Raimondo et al. (2016) has narrower paraphyses (1.1–1.5 μm) and brown to dark ascospores. Based on morphology and phylogenetic analyses, we conclude that our collection is *B. rosacearum*. This fungus was described by Raimondo et al. (2016) on *Cydonia oblonga*, *Prunus domestica* and *Pyrus communis* trees and reported as the causal agent of charcoal cankers in southern Italy. Several isolates have been recorded from the Apulia region (Italy) on different hosts, including *Quercus castaneifolia*, *Q. ilex* and *Q. pubescens*, while our collection is from *Q. cerris* in the Emilia-Romagna region. Other known distribution of *B. rosacearum* is in Iran, Portugal and Spain (Raimondo et al. 2016, Bahmani et al. 2021, Sohrabi et al. 2022). Therefore, our collection is the first record of *B. rosacearum* on *Q. cerris* trees in Italy and worldwide.

Diatrypaceae Nitschke 1869

Index Fungorum number: IF 80692; Facesoffungi number: FoF 00679

Notes – *Diatrypaceae* (*Xylariales*) was introduced by Nitschke (1869) and typified by *Diatrype*. The key characters of the sexual and asexual morphs of this family are perithecial ascomata immersed in stroma with ostiolar necks, 8-spored or polysporous, rarely 1–2-spored, long-stalked, unitunicate, asci with J–, or J+, apical ring, allantoid ascospores, and coelomycetes or hyphomycetes asexual morphs (Glawe & Rogers 1984, Rappaz 1987a, Trouillas et al. 2011, Hyde et al.2020c, Konta et al. 2020, Li et al. 2022). Species in this family can be mostly found as saprobes on dead plant substrates, pathogens or endophytes on economic crops and forest trees and on a wide range of hosts in both terrestrial and aquatic habitats worldwide (Senanayake et al. 2015, Dayarathne et al. 2020a, b, Hyde et al. 2020c, Dissanayake et al. 2021b). Currently, 25 genera are accepted in the family (Samarakoon et al. 2022). In this study, we report two saprobic *Diatrypaceous* species belonging to *Cryptovalsa* and *Diatrype* collected in Italy.

Cryptovalsa Ces. & De Not. ex Fuckel 1870

Index Fungorum number: IF 1340; Facesoffungi number: FoF 13137

Notes – *Cryptovalsa* was established by Cesati & De Notaris (1863) to accommodate the type *C. protracta*, along with *C. ampelina*, *C. effuse* and *C. nitschkei* species. The sexual morph characters of *Cryptovalsa* are eutypoid stromata, separately erumpent, diatrypelloid, cylindrical or clavate, polysporous asci with short or long pedicels, consisting of crowded, allantoid, and yellowish ascospores (Spooner 1981, Vasilyeva & Stephenson 2005, Hyde et al. 2020c, Zhu et al. 2021). The majority of *Cryptovalsa* species are reported as pathogens mainly on grapevines and *Prunus* trees from Asia, Europe, North America, Oceania, South Africa, and South America (Mostert et al. 2004, Moyo et al. 2018, Zhu et al. 2021).

***Cryptovalsa ampelina*** (Nitschke) Fuckel 1870   Fig. 48

Index Fungorum number: IF 241474; Facesoffungi number: FoF 01800

Saprobic on a dead and fallen branch of *Quercus ilex* L. Sexual morph: *Ascostromata* 400–700 μm diam., multiloculate, erumpent through the substrate, solitary to aggregated, scattered, appearing as black spots, shiny. *Ascomata* 250–350 μm diam., 450–720 μm high (including ostiolar neck) (x̄ = 304 × 590 μm, n = 5), perithecial, immersed in stroma, globose, semi-immersed, erumpent through the bark, ostiolate. *Ostiole neck* 150–200 μm diam., 230–270 μm high (x̄ = 176 × 258 μm, n = 5), individual, central, cylindrical, outer layers with dark melanised cells, inner layers with pale brown to yellowish cells, with periphyses oriented towards the apical direction. *Peridium* 30–40 μm thick, composed of several layers, outermost layers consisting of thick-walled, brown cells of *textura globulosa,* inner layers with thin-walled, pale brown cells of *textura angularis*, innermost thin layers with the hyaline cells of *textura prismatica*. *Hamathecium* comprises 2.5–4.5

μm wide, thin-walled, unbranched, aseptate, guttulate, hyaline, numerous paraphyses. *Asci* 90–130 × 9–11.5 μm (x̄ = 112 × 10.1 μm, n = 15), polysporous, unitunicate, clavate, with long pedicel, swollen upper portion, with a J– apical ring, apically flat. *Ascopores* 5.5–9.5 × 1.8–2.3 μm (x̄ = 7.9 × 2.0 μm, n = 30), overlapping, bi-seriate to multi-seriate, crowded, initially cylindrical, allantoid to reniform at maturity, aseptate, smooth-walled, yellowish to pale brown. Asexual morph: see Mostert et al. (2004).

Material examined – Italy, Province of Forlì-Cesena [FC]), near Colmano, on a dead and fallen branch of *Quercus ilex* L. (*Fagaceae*), 4 December 2018, Erio Camporesi, IT 4139 (MFLU 19-0307).
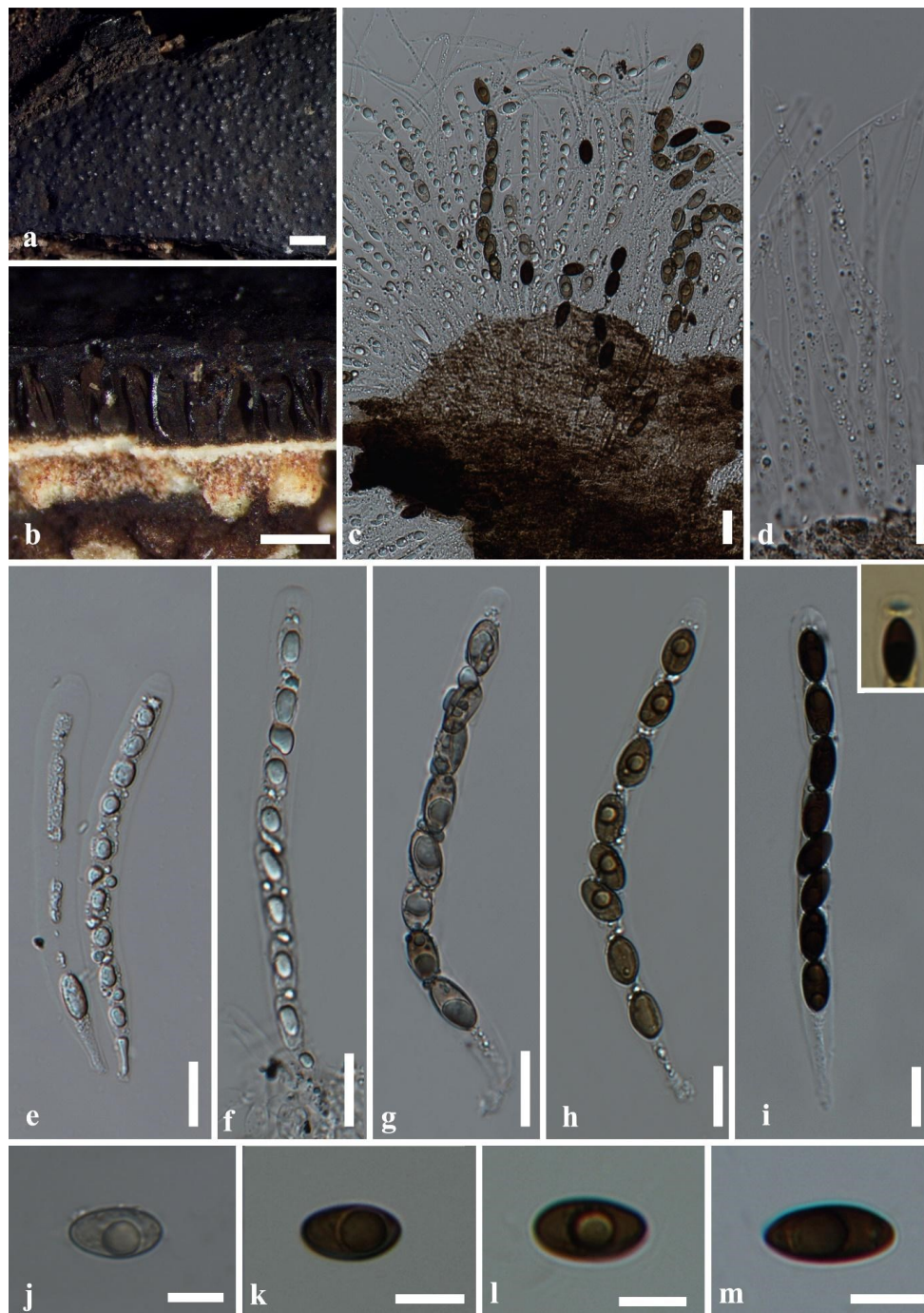
Fig. 46 – *Biscogniauxia rosacearum* (MFLU 23-0020, new host record). a Ascostromata on the dead host surface of *Quercus cerris* L. (*Fagaceae*, *Fagales*). b Longitudinal section of ascostroma. c Structures inside hamathecium d. Paraphyses. e–i Asci. j–m Ascospores. Scale bars: a = 1 mm, b = 500 μm, c–i = 20 μm, j–m = 10 μm.
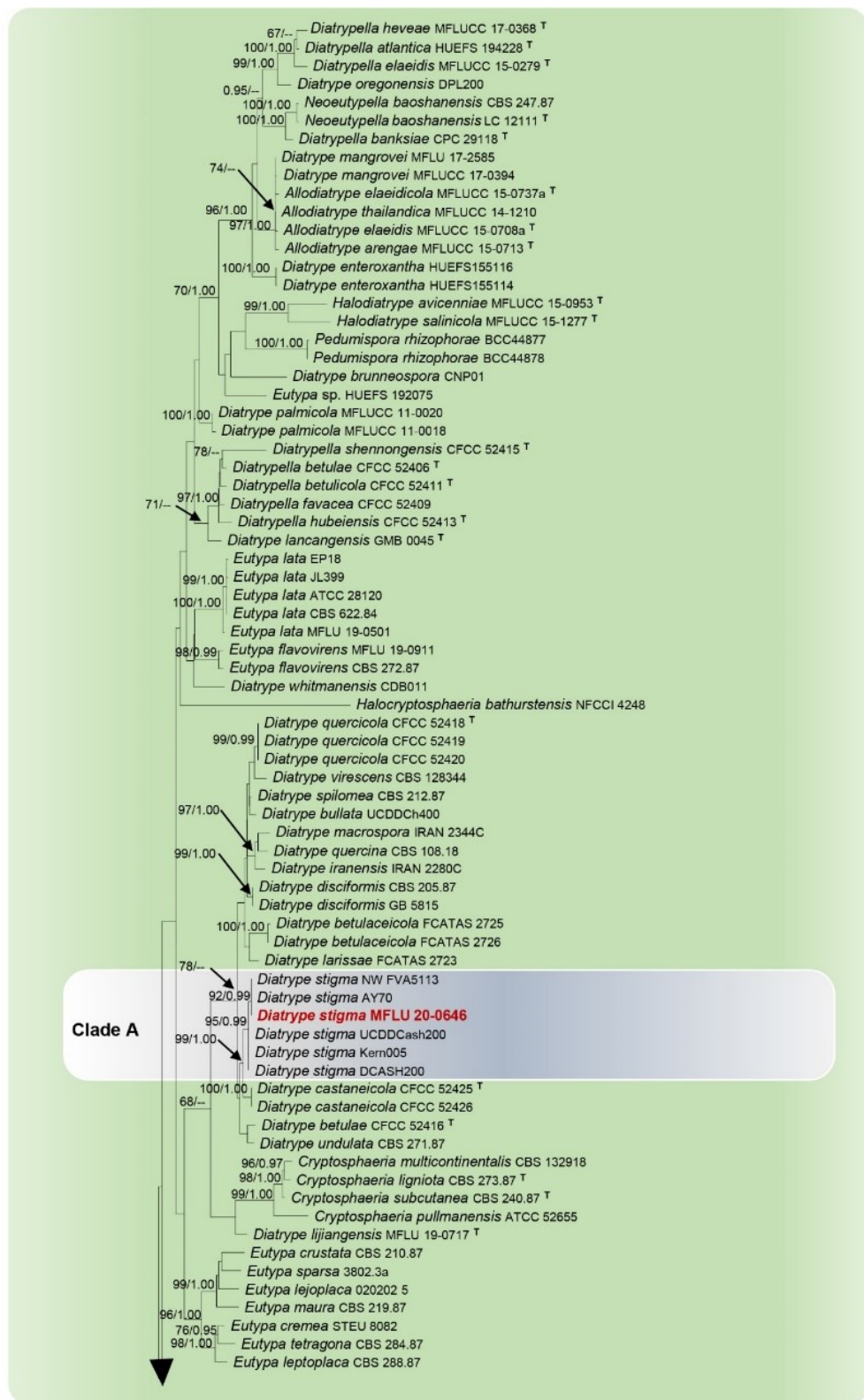
Fig. 47 – Phylogram generated from maximum likelihood analysis based on combined ITS and *tub*2 sequenced data. One hundred-eight strains were included in the combined sequence analyses, which comprised 906 characters with gaps (ITS = 511, *tub*2 = 395). Single gene analyses were also performed, and topology and clade stability were compared from the combined gene analyses. *Xylaria schimicola* (FCATAS 896) strain was used as the outgroup taxon. The final ML optimization likelihood is -14916.754941. The matrix included 615 distinct alignment patterns, with 19.21% undetermined characters or gaps. Estimated base frequencies were obtained as follows: A = 0.227974, C = 0.264089, G = 0.232376, T = 0.275562; substitution rates AC =

1.014067, AG = 3.118118, AT = 1.235617, CG = 0.802325, CT = 4.276671, GT = 1.0; gamma distribution. Bootstrap support values for ML (first set) equal to or greater than 65%, BYPP equal to or greater than 0.95 are given above or below the nodes. The strains from the current study are in red bold, and the type strains are indicated with ᵀ.
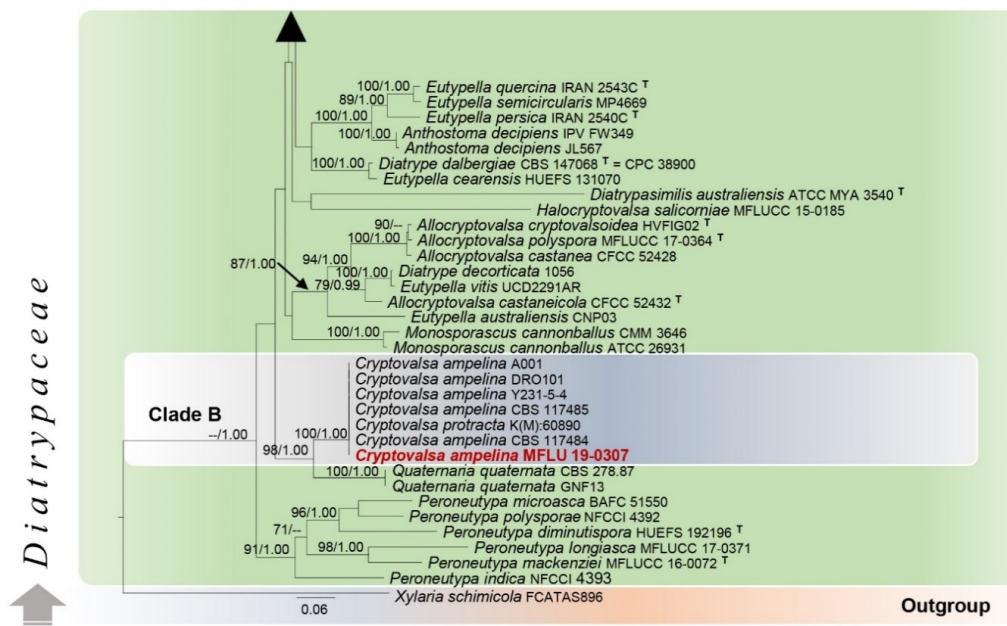

Fig. 47 – Continued.

GenBank Accession Numbers – ITS: OQ411124; *tub*2: OQ437895.

Notes – In the multi-gene phylogeny, our strain (MFLU 19-0307) grouped with *Cryptovalsa ampelina* strains A001, DRO101, Y231-5-4, CBS 117485, and CBS 117484 and *C. protracta* K(M):60890 with 100% MLBS and 1.00 BYPP support (Fig. 47, Clade B). The bp comparison revealed that the ITS and *tub*2 regions of our strain and *C. ampelina* strains are identical. Also, our collection shares similar morphologies to *C. ampelina* in the appearance of stroma, shapes of ascomata, asci, and ascospores (Mostert et al. 2004). However, the dimensions of *C. ampelina* slightly differ from our collection with wider ascomata (300–630 μm high, 300–500 μm diam.), narrower asci (70–125 × 7–9 μm), and longer ascospores (7–11 × 2 μm) (Mostert et al. 2004). Also, in our collection, we found three types of peridial cells from outer *textura globulosa*, inner *textura angularis* and innermost *textura prismatica*, while Mostert et al. (2004) only reported *textura angularis* and *textura globulosa*. Based on its perithecial characters, Nitschke (1867) separated *C. ampelina*, which produces ovoid perithecia, from *C. protracta*, which produces globose perithecia. Based on the holotype of *C. protracta*, no detailed morphological descriptions were provided. However, our phylogenetic analysis of the ITS loci of *C. protracta* strain K(M):60890 (not from the type material) revealed that all strains in Clade B (Fig. 47), are identical to each other. Based on these morphologies and phylogenetic analyses, we conclude that our collection is *C. ampelina*. This fungus was reported on *Vitis vinifera* trees in Australia, Italy, and South Africa (Mostert et al. 2004, Jayawardena et al. 2018a, b, c). Therefore, our collection is the first record of *C. ampelina* on *Quercus ilex* in Italy and the world.

Diatrype Fr. 1849

Index Fungorum number: IF 1504; Facesoffungi number: FoF 01385

Notes – *Diatrype* was established by Fries (1849) to accommodate the type *D. disciformis*, which can often be found as a saprobe on rotting wood (Senanayake et al. 2015, Yang et al. 2022). The key characters of the sexual morph of *Diatrype* species are widely effuse or verrucose, flat or slightly convex stromata with discoid or sulcate ostioles at the surface, 8-spored and long-stalked

asci and hyaline or brownish, allantoid ascospores (Senanayake et al. 2015, Yang et al. 2022b). Species of the genus are commonly reported as saprobes on dead twigs, stems, and barks of various plants, trees, and shrubs worldwide (Chlebicki 2005, Hyde et al. 2020c).
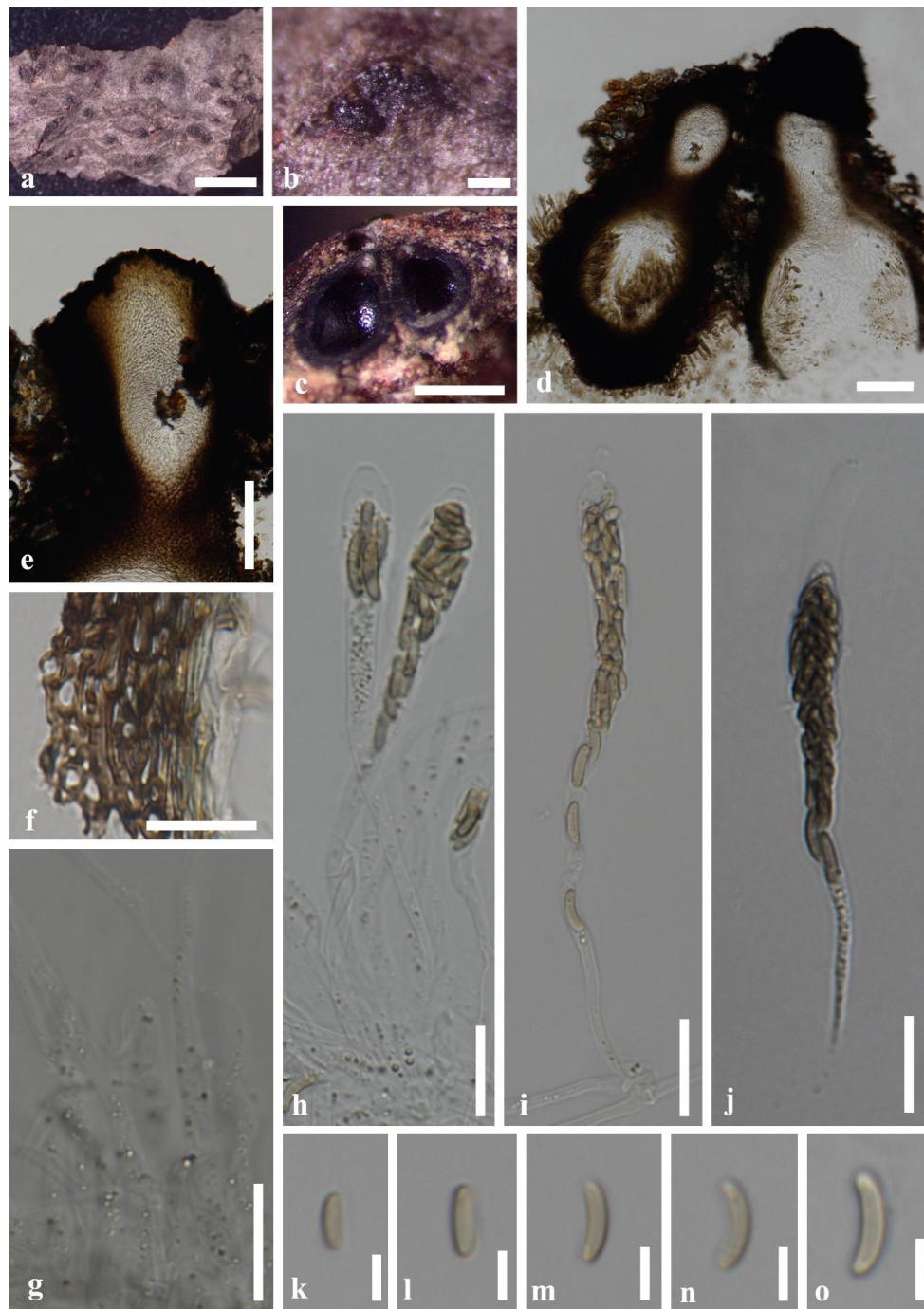
Fig. 48 – *Cryptovalsa ampelina* (MFLU 19-0307, new host record). a–b Ascostromata on the dead host surface of *Quercus ilex* L. (*Fagaceae*, *Fagales*). c–d Longitudinal section of an ascostroma. e Longitudinal section of the ostiolar neck. f Peridium. g Paraphyses. h–j Asci. k–o Ascospores. Scale bars: a = 1 mm, c = 500 μm, b = 200 μm, d, e = 100 μm, f–j = 20 μm, k–o = 5 μm.

Diatrype stigma (Hoffm.) Fr. 1849   Fig. 49
    Index Fungorum number: IF 220872; Facesoffungi number: FoF 14013
    Saprobic on a dead and fallen branch of *Ostrya carpinifolia* Scop. Sexual morph: *Ascostromata* 3.5–6 mm diam., multiloculate, erumpent through the substrate, irregularly outlined, solitary, scattered, widespread on the substrate, appearing as black swellings with punctate ostioles,

coriaceous, shiny. *Ascomycota* 200–300 μm diam., 230–350 μm high (excluding ostiolar neck) (x̄ = 239 × 286 μm, n = 5), perithecial, globose to pyriform, immersed in the stroma, semi-immersed, erumpent through the bark, ostiolate. *Ostiolar neck* 60–70 μm diam., 100–150 μm high (x̄ = 65 × 121 μm, n = 5), central, individual, cylindrical, wider in upper part, periphysate, outer layers composed of dark melanised cells. *Peridium* 17–25 μm thick, several layers, from outer towards inner layers comprising thick-walled, dark brown to pale brown or hyaline cells of *textura angularis*. *Hamathecium* comprises 2.0–3.5 μm wide, thin-walled, unbranched, aseptate, guttulate, longer than asci, hyaline, numerous paraphyses. *Asci* 60–100 × 5–7 μm (x̄ = 85 × 6.5 μm, n = 15), 8-spored, unitunicate, narrowly clavate, with long tapering pedicel, with a J– apical ring, apically flat. *Ascopores* 6.0–8.0 × 1.5–2.0 μm (x̄ = 7.1 × 1.7 μm, n = 30), overlapping, 1–3-seriate, cylindrical to allantoid, aseptate, smooth-walled, without gelatinous sheath and appendages, yellowish to pale brown. Asexual morph: see Rappaz (1987b).
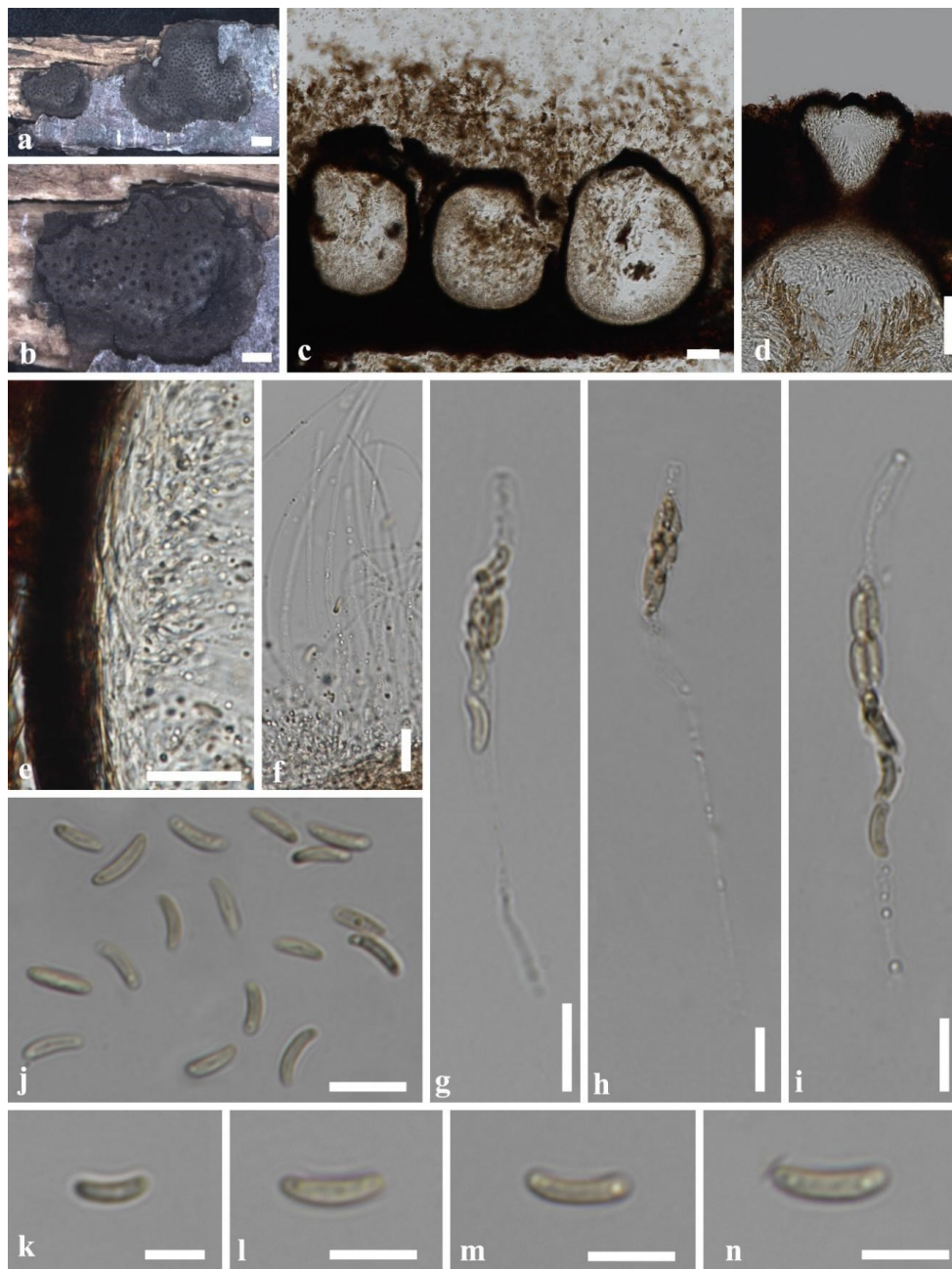
Fig. 49 – *Diatrype stigma* (MFLU 20-0646, new host record). a–b Ascostromata on the dead host surface of *Ostrya carpinifolia* Scop. (*Betulaceae*, *Fagales*). c Longitudinal section of an ascostroma. d. Longitudinal section of the ostiolar neck. e Peridium. f Paraphyses. g–i Asci.

j–n Ascospores. Scale bars: a = 1 mm, b = 500 μm, c, d = 50 μm, e = 20 μm, f–j = 10 μm, k–n = 5 μm.

Material examined – Italy, Province of Forlì-Cesena [FC]), Montecoronaro-Verghereto, on a dead and fallen branch of *Ostrya carpinifolia* Scop. (*Betulaceae*), 8 October 2020, Erio Camporesi, IT 4662 (MFLU 20-0646).

GenBank Accession Numbers – ITS: OQ411127; *tub*2: OQ437896.

Notes – In the multi-gene phylogeny, our strain (MFLU 20-0646) grouped together with *Diatrype stigma* strains NW FVA5113, AY70, UCDDCash200, Kern005, and DCASH200 with 95% MLBS and 0.99 BYPP support (Fig. 47, Clade B). *Diatrype stigma* group forms a sister clade to *D. castaneicola* (CFCC 52425, CFCC 52426). In comparison of base pairs composition between our strain and *D. stigma* strains (NW FVA5113, AY70) revealed that ITS region is identical, and these three strains have 2/507 (0.39%) bp difference to UCDDCash200, Kern005, and DCASH200 strains in the ITS region. Also, our collection shares similar morphologies to *D. stigma* in the characters of stroma, ascomata, asci and ascospores provided by Munk (1957), only differing in having lengthier asci (60–100 *vs* 25–30 μm). This species was reported from *Quercus* sp. in the United States (Vasilyeva & Stephenson 2004, Yang et al. 2022b). Therefore, our collection is the first record of *C. ampelina* on *Ostrya carpinifolia* in Italy as well as in the world.

Spatial distribution of plant-associated *Ascomycota*

In this study, new fungal records from our collections were from different sites in Forlì-Cesena and Ravenna provinces (Emilia-Romagna region, Fig. 50) and one site in Trento province (Trentino–Alto Adige region).

Fig. 50 – Collecting sites of a *Crataegus* species. b A dead *Picea* species. c *Quercus pubescens* species in the Emilia-Romagna region. Photos by Erio Camporesi.

The provincial boundaries of the collecting sites and the distribution of the fungi are demarcated on the maps (Fig. 51). Samples have been randomly collected and contribute to a better understanding of the fungal occurrence on plants in Italy. All the fungi collected from the Emilia-

Romagna region are identified as *Sordariomycetes* (8 species) belonging to *Diaporthales*, *Hypocreales*, and *Xylariales* on *Fagales* hosts. *Dothideomycetes* (16 species) belong to *Botryosphaeriales*, *Hysteriales*, and *Pleosporales* are scattered in both regions on different plant hosts, including *Cornales, Fabales, Fagales, Gentianales, Laurales, Pinales, Poales* and *Rosales*.
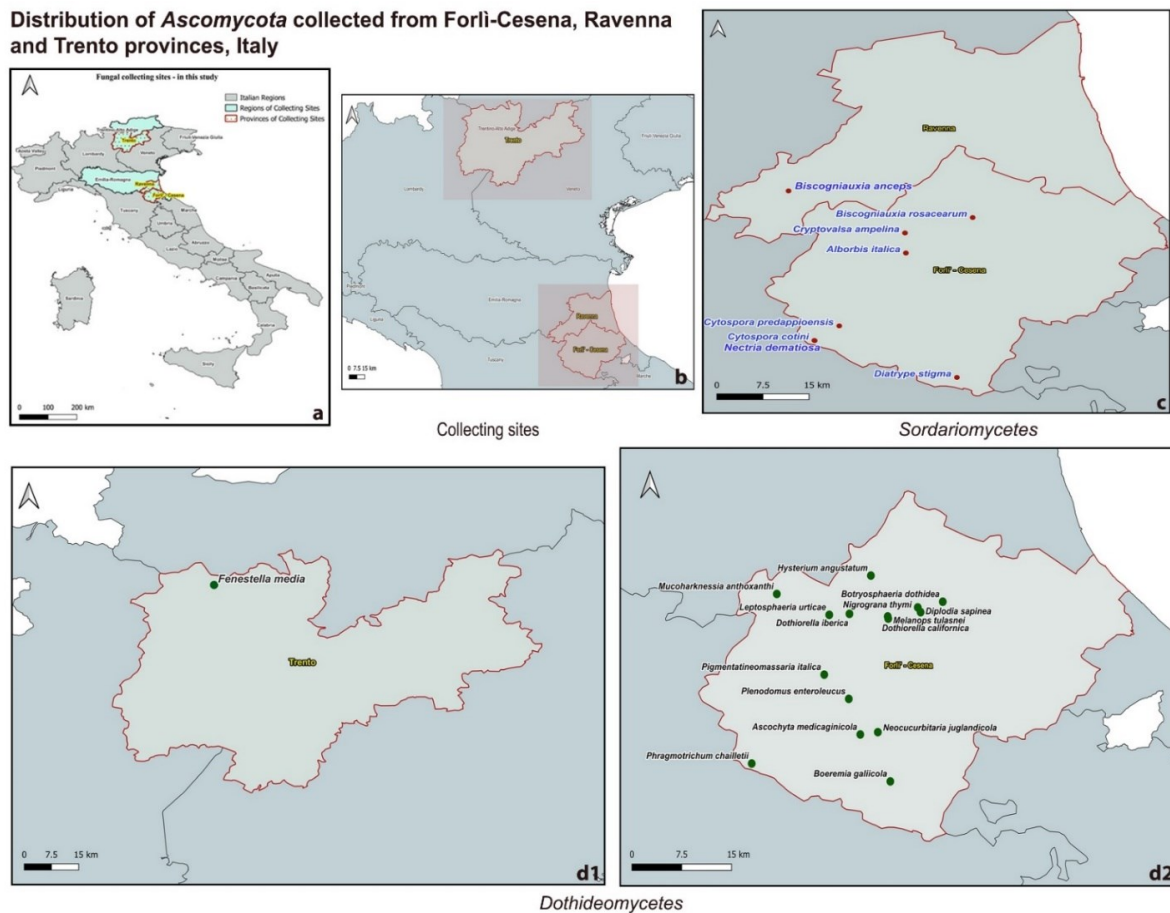
Fig. 51 – The geographical distribution of the discussed *Ascomycota* in this study. a–b Administrative boundaries and provinces of the collecting sites. c *Sordariomycetes* in Ravenna and Forlì-Cesena provinces. d1–d2 *Dothideomycetes* in Trento d1 and Forlì-Cesena d2 provinces.

History of Italian mycology

In this study, we present an overview of Italian mycological studies in terms of macrofungi, microfungi, and fossil fungi. In early studies, most mycological accounts were of the larger fungi including species listing, red data listing, geo-referenced mapping, and conservation strategies. At the same time, a few taxonomic studies of microfungi were confined to limited regions. According to documented data, studies on microfungi progressed slowly from the 17th century but significantly increased by the 19th century (Wijesinghe et al. 2021). Mycologists' contributions, their extensive fungal collections, and different illustration techniques, all directly influenced this golden era of mycology.

As for taxonomy, we outline the path from traditional fungal identification to modern approaches. Historically, fungal identification, classification, and determination of taxonomic relationships have been based on observable morphology. The number of taxonomic studies on fungi has grown over time, and advanced microscopes and cameras have yielded better morphological details of the fungi. In the identification of species complexes, non-sporulating and non-cultivable fungi, cryptic species, phenotypic plasticity, and convergent evolution, morphology became a challenge to worldwide mycologists including those in Italy (Guarro et al. 1999,

Chethana et al. 2021b, Wijesinghe et al. 2021). Also, many confusions have arisen over the asexual and sexual relationships of some fungal species. By overcoming these obstacles in fungal taxonomy, DNA-based molecular data has been linked with morphology as a universally accepted approach since the early 1990s. Additionally, physiological, chemical, ecological, and geographical data have also contributed to a better understanding of fungal taxonomy (Manawasinghe et al. 2019, Wijesinghe et al. 2021, Chethana et al. 2021b).

Plant-associated *Ascomycota*

Considering the high diversity of species and distribution patterns of Italian vegetation, taxonomic studies on plant-associated fungi have become popular in recent years. Ecologically, there are four main climatic regions in Italy, including temperate, Mediterranean, and two transitional regions, with nine bioclimates (Blasi & Michetti 2007, Abbate et al. 2015, Martinelli & Matzarakis 2017), while climate warming is expected to "enhance" plant growth or induce plant expansion in newly favorable areas, in Italy as well as globally. The future climatic scenario, with less precipitation and higher temperatures, is expected to cause latitudinal and altitudinal shifts in the geographical distribution of plant and animal species (Parmesan & Yohe 2003, Parmesan 2006, Saitta et al. 2018), with subsequent changes in the distribution of plant-associated microorganisms. Still, the magnitude of growth stimulation will probably vary among climatic zones, with stronger effects expected for the cold alpine regions (Chelli et al. 2017). Despite the many studies that have been carried out on the effects of the general elevation patterns in terrestrial ecosystems on macro-organisms, especially on changes in the richness and diversity of plants and animals, information on micro-organisms such as fungi, is largely absent (Geml 2017, Saitta et al. 2018). Climate change and biodiversity loss are interdependent, and biodiversity loss may also affect plant-associated fungi. Therefore, monitoring fungal diversity is a challenging goal that can no longer be pushed off in light of climate change.

Species, genera, families, and orders of plant-associated *Ascomycota* in the world have been subjected to rapid taxonomic changes with the biphasic approach to their characterization, including recent Italian contributions (Wijesinghe et al. 2021). In this study, we strongly considered morphological characters together with sequence data and species identification was performed by multi-gene phylogenetic analyses. Thirty fungal specimens were randomly collected from dead, fallen, or aerial branches at different Italian sites. Twenty-four species belonging to *Dothideomycetes* and *Sordariomycetes* were identified, resulting in a new genus, two new species, 16 new host records, five new geographical records, two new regional records, and two host recurrent species. The novel genus *Pigmentatineomassaria* (*Neomassariaceae*, *Pleosporales*) was introduced along with a novel species, *P. italica*. Another novel species, *Alborbis italica* belonging to *Sydowiellaceae* (*Diaporthales*) was also introduced. The orders and families with the described species are displayed in Fig. 52. Fungal orders are listed on the right side of the figure, and families are on the left side, with the described species counts demarcated near the relative colors. The highest fungal count, nine species, belonged to *Pleosporales*, followed by *Botryosphaeriales* (6), *Xylariales* (4), *Diaporthales* (3), *Hypocreales* (1), and *Hysteriales* (1).

Along with fungal taxonomy, investigation of ecology and mycogeography is a prerequisite to understanding fungal biology, diversity, and conservation (Wijesinghe et al. 2022). In terrestrial ecosystems, fungi are assumed to have coevolved with plants through interactions of different life modes, such as parasitism, mutualism, and saprotrophism (Lutzoni 2018). Therefore, growing awareness of plant-associated microfungi in Italy is important with respect to their lower attention in the past (Wijesinghe et al. 2021). In our study, the fungal host species are restricted to a few orders such as *Cornales* (*Cornaceae*), *Fabales* (*Fabaceae*), *Fagales* (*Betulaceae* and *Fagaceae*), *Gentianales* (*Rubiaceae*), *Laurales* (*Lauraceae*), *Pinales* (*Pinaceae*), *Poales* (*Poaceae*) and *Rosales* (*Rosaceae* and *Urticaceae*). The majority of the fungal species in this collection were found on *Fagales* spp. an economically important group of plants in Italian habitats (Wijesinghe et al. 2022). Relationships between fungal species and hosts are demarcated in Fig. 53, and *Betulaceae* and *Fagaceae* are the dominant families. Predominantly, all *Sordariomycetes* (8) reported in the study

were found on *Fagales* hosts, while *Dothideomycetes* occurred on only a few (5). Previous research revealed that *Sordariomycetes* were the dominant species, based on the species listing of *Fagales*-associated *Ascomycota* by Saitta et al. (2011) and Wijesinghe et al. (2022). Therefore, further fungal collections from *Fagales* hosts, in different Italian environments, might reveal hidden fungal diversity.
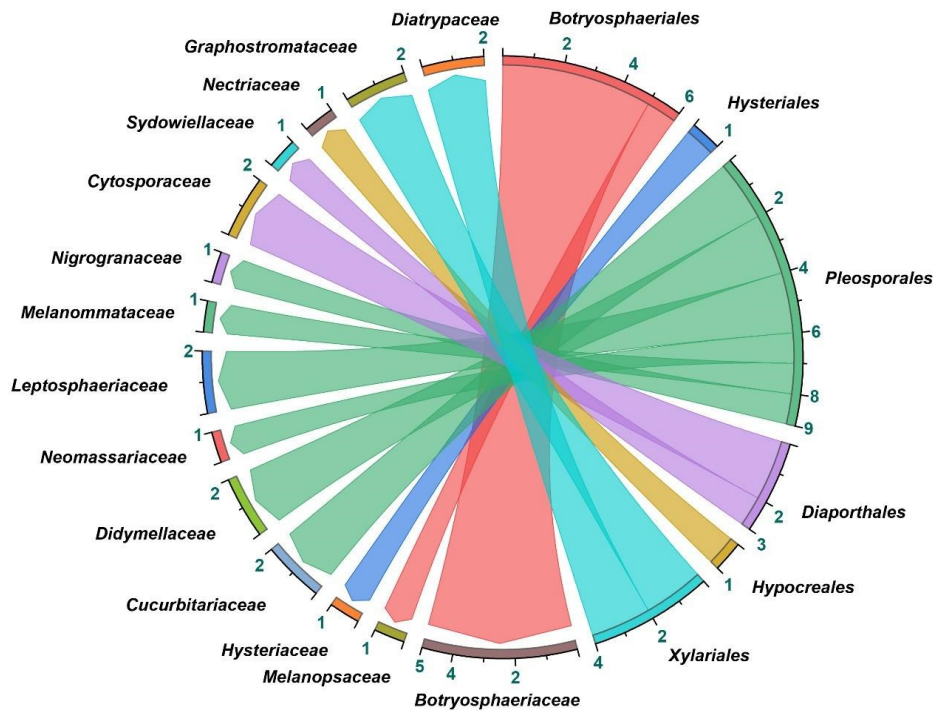

Fig. 52 – Orders and families of *Ascomycota* with the reported number of species in this study.

Studying fungal species and their hosts is important in understanding their host recurrence and host exclusivity. Host recurrent species can be predominantly found on a particular host or a host range and infrequently occur on other hosts in the same habitat (Zhou & Hyde 2001). Host exclusivity is an exclusive occurrence of a saprobic fungus on a specific host or a restricted host range (Zhou & Hyde 2001). In our study, *Mucoharknessia anthoxanthi* was collected from *Anthoxanthum odoratum* (*Poaceae*) as into the holotype (Li et al. 2016), while another collection was reported from *Dactylis glomerata* (*Poaceae*) (Li et al. 2020) in the same Italian region. We assume *M. anthoxanthi* shows host recurrence on *Poaceae* hosts in this particular region. Additionally, *Ascochyta medicaginicola* is repetitively found on *Medicago* host species from the Emilia–Romagna region, Italy (Hyde et al. 2020b) as well as in different countries such as Canada, the Czech Republic, France, and Minnesota (Farr & Rossman 2022) highlighting its host recurrence nature on *Medicago*. Also, *Melanops* species are assumed to be host exclusive to *Fagales* hosts in different geographic regions in the world (China, Germany, and Italy) (Phillips & Alves 2009, Jiang et al. 2018, this study). However, to confirm these ecological relationships, a broad taxon sampling is required. Exploring fungal ecology provides evidence for an understanding of the biological stability of fungal species on various hosts and habitats.

Although Italy has a rich biodiversity, increasing alteration and destruction of natural habitats can lead to the extinction of many species sharing those ecosystems (Varese et al. 2011). Therefore, strong biodiversity conservation strategies and approaches should be implemented. In order to prioritize conservation efforts, periodic updates of microfungal records and hosts in checklists, and well-organized databases, including their distribution in different Italian habitats, should be of greater concern. On the website of Italian microfungi, Wijesinghe and other curators (https://italianmicrofungi.org), provided a comprehensive checklist for their fungal taxonomic

studies in a single platform. The reported taxonomic, ecological, and geographical data of *Ascomycota* in this study, will be digitized and deposited on the above website, for easy access.
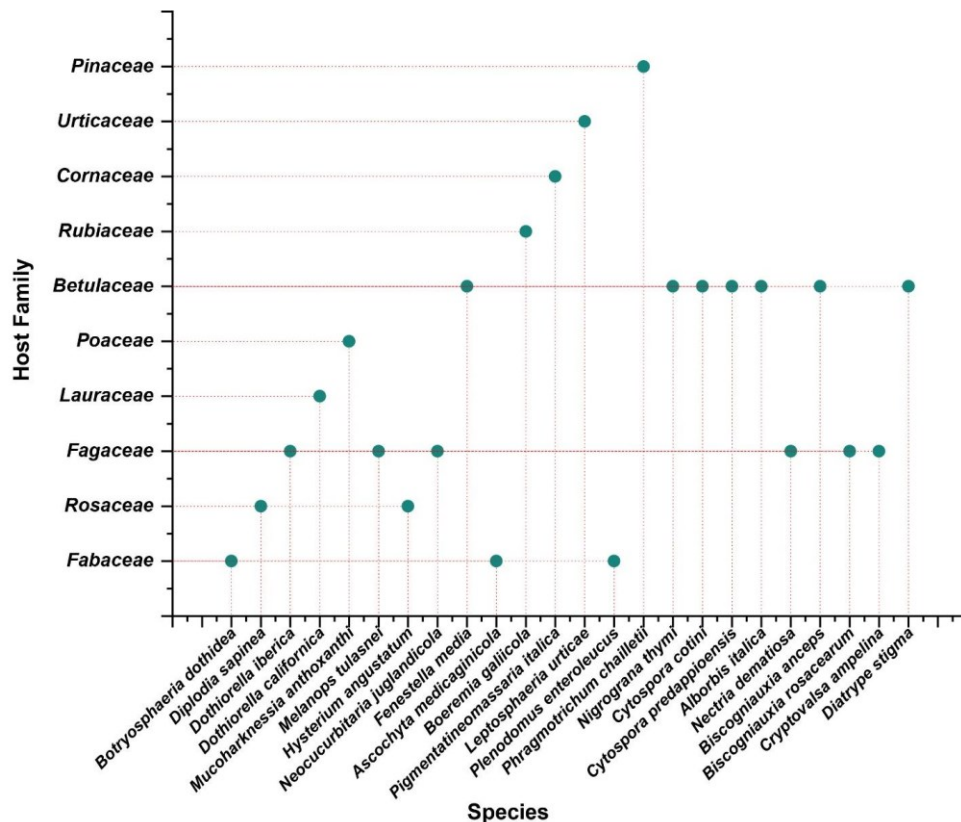

Fig. 53 – Identified fungal species and reported host families in this study.

Researchers (local and foreign) have made significant contributions to the understanding of plant-associated *Ascomycota* which are being processed by the CEFR team. Notable foreign researchers including Anupama Daranagama, Asha Dissanayake, Chayanard Phukhamsakda, Dhanushka Wanasinghe, Hiran Ariyawansa, Indunil Senanayake, Kasun Thambugala, Nalin Wijayawardene, Rungtiwa Phooksamsak, Ruvishika Jayawardena, Saowaluck Tibpromma and Wen-Jing Li (mainly under the guidance of Professor Kevin D. Hyde), along with current Ph.D. researchers have been actively contributing to the update of the global fungal number (Phukhamsakda et al. 2020, Wijesinghe et al. 2021). The fungal collection for this project commenced in 2011 thanks to the efforts of Mr. Erio Camporesi, a remarkable amateur mycologist and collector in Italy. These fungal collections were from more than 300 different host plant species in terrestrial habitats from different provinces in Italy. The hosts (https://italianmicrofungi.org/outline.php) include shrubs, trees, and grasses, with the substrates differentiated as branches (from shrubs and trees), stems (from herbaceous hosts and grasses), and leaves (from shrubs, trees, and grasses). The team is actively contributing by introducing novel fungal species, genera, families, and sometimes novel orders, and novel host and geographical records. All studies are being performed through morphology-based molecular phylogenetic analyses.

We updated the regional *Ascomycota* count (Fig. 54) of Venturella et al. (2011) by adding the regional records from Mr. Erio's collections (2013–2022, September), based on the checklist provided by Wijesinghe et al. (2021). The updated *Ascomycota* list (628 species, 874 records) for the past decade is: from Calabria (1 species), Emilia–Romagna (510 species, 746 records), Marche (4 species), Umbria (1 species), Trentino Alto Adige (26 species, 29 records), and Tuscany (86 species, 92 records). Further fungal studies are required for Abruzzo, Friuli-Venezia Giulia, Molise, and Lazio (Fig. 54, arrowed regions). Studies of lichens and lichenicolous fungi were carried out in

the Molise region by Brackel (2016, 2020) and Nimis (2016), while no significant data was reported from the other regions.
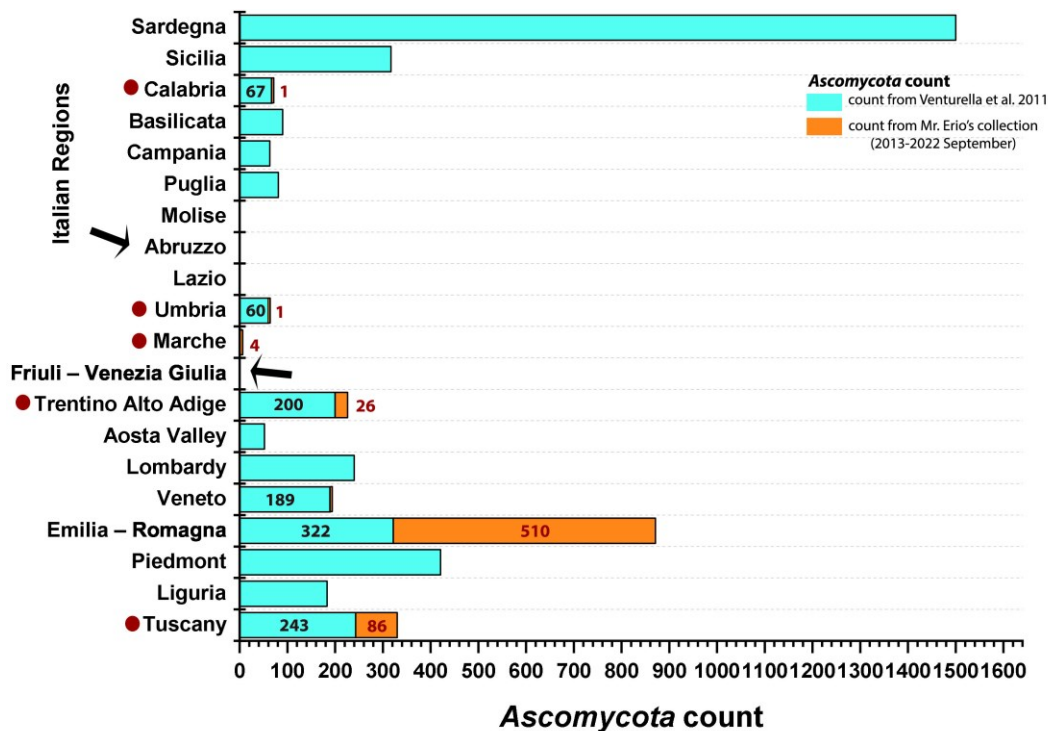
Fig. 54 – The updated *Ascomycota* count based on Venturella et al. (2011) by adding the count from https://italianmicrofungi.org/, 2013–2022, September (Wijesinghe et al. 2021).

The region of Emilia-Romagna, which lists the largest number of *Ascomycota* studies, has become the main collecting site in our study. Through our random collections, we documented a novel fungal genus, *Pigmentatineomassaria* (*Pleosporales)*, two novel species, *Alborbis italica* (*Diaporthales*) and *Pigmentatineomassaria italica* (*Pleosporales*).
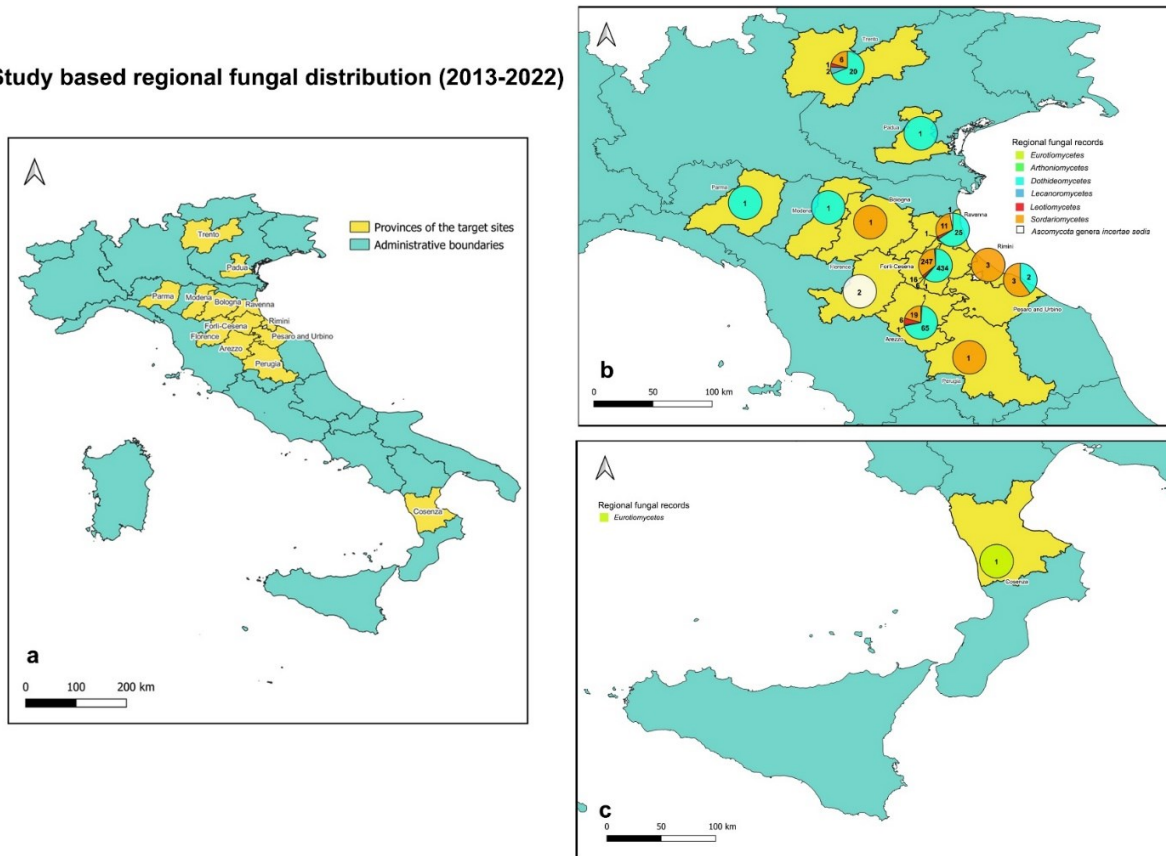
Study-based geographical distribution of *Ascomycota*
Accurate fungal mapping increases our understanding of fungal distribution in different Italian habitats. The distribution of *Ascomycota* was mapped based on data (during 2011–2022 September) from the checklist by Wijesinghe et al. (2021) and in selected Italian habitats (Fig. 55). Forlì-Cesena was the most studied province, with *Dothideomycetes* (434 records) the dominant group, followed by *Sordariomycetes* (247 records), *Leotiomycetes* (16 records), *Ascomycota* genera *incertae sedis* (7 records), and *Lecanoromycetes* (6 records), and only one *Arthoniomycetes*. There was a significant number of *Dothideomycetes* and *Sordariomycetes* in Arezzo (65, 19), Ravenna (25, 11), and Trento (20, 6), in comparison to other reported classes. The vegetation of these provinces consists of savannas, plantations, planted crops, and grasslands (data from Fig. 5). Most of the collections were of saprophytic *Ascomycota*, both sexual and asexual morphs. In forests or vegetative ecosystems, saprobes play an important role in the recycling of nutrients by decaying dead trees, snags, fallen branches, and logs (Saitta et al. 2011). In the future, we suggest further collection efforts in the provinces that received less attention, such as Bologna, Cosenza, Florence, Modena, Padova, Parma, Perugia, Pesaro-Urbino, and Rimini as these areas may have hidden fungal diversity in natural forest ecosystems.

Thus, since ancient times, overall Italian mycological studies have been developed step by step with the active participation of expert mycologists. The historical fungal collections preserved by these experts serve as a precious biorepository for modern mycologists. Furthermore, taxonomic studies based on biphasic or polyphasic approaches greatly contribute to expanding the world's

mycological knowledge. Not only in terrestrial habitats but also in marine ecosystems, fungi are identified as the primary decomposers on woody substrates. Many studies were carried out to understand microfungal diversity in Italian beach ecosystems, including driftwood or submerged woody substrates, sea grasses, rocks, monuments, fish carcasses, and in water (Ciferri 1959, Cuomo & Vanzanella 1983, 1985, Cuomo et al. 1988, Del Frate & Caretta 1983, Grasso et al. 1985, 1990, Garnero 2008, Vezzulli et al. 2009, Fenoglio et al. 2010, Jones 2010, Onofri et al. 2011, Di Piazza et al. 2017, Jones et al. 2019). The first update on Mediterranean lignicolous marine fungi was provided by Garzoli et al. (2015). The majority of the reported taxa are *Ascomycota* (Poli et al. 2018) in comparison with *Basidiomycota*. Jones (2023, in press) further documents studies on marine fungi undertaken by Italian mycologists.

Fig. 55 – The study-based regional *Ascomycota* distribution from the selected provinces in Italy, based on the checklist updated on the online database (https://italianmicrofungi.org/; Wijesinghe et al. 2021). a Provinces of target collecting sites. b–c Distribution of reported *Ascomycota*.

Studies of freshwater fungi and lichens are less developed in Italy (Thüs 2002, Nascimbene & Nimis 2006, 2007, Wirth et al. 2013, and Thüs & Nascimbene 2020). Also, several studies for microfungal richness in extreme environments (Cecchi et al. 2019), and other terrestrial habitats, such as rock inhabiting microfungi on monuments (Onofri et al. 2014, Isola et al. 2016) and microfungi on marble statues (de Leo et al. 2019) were recently discussed by these authors. Coprophilous fungi, including *Ascomycota*, were studied by Caretta & Piontelli (1996), Doveri (2004, 2007, 2011, 2013), and Doveri et al. (2010, 2013), while Lantieri et al. (2009) reported on sabuliculous fungi. However, there are still many natural habitats in Italy that have not been explored by mycologists, and exploring these habitats will contribute to narrowing the gap of unrevealed fungal diversity in unexplored regions. In this study, we encourage more collection efforts on microfungi, the applications of modern taxonomic approaches, updating ecology and mycogeography, as well as annual updating of the *Ascomycota* records, giving priority to biodiversity conservation efforts.

Acknowledgments

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