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Exploring the Genome of the Endophytic Fungus *Botrytis deweyae*: Prediction of Novel Secondary Metabolites Gene Clusters: Terpenes and Polyketides

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Abstract: Fungi have played a pivotal role in human history, from the dangers of fungal toxins to the revolutionary discovery of penicillin. Fungal secondary metabolites (SMs), such as polyketides (PKs) and terpenes, have attracted considerable interest due to their diverse biological activities. *Botrytis deweyae*, an endophytic fungus, exhibits behaviors that are notably distinct from those of its necrotrophic relatives within the genus *Botrytis*. This study explores the importance of terpenes and PK gene clusters and their conservation between species. In addition, new putative biosynthetic gene clusters corresponding to those families were identified. Consequently, the new PKS BdPKS22-26 were also identified in other *Botrytis* species and other fungi. In addition, those new gene clusters identified in this work show differences in the degree of conservation and are phylogenetically closely related to some of the 21 PKSs previously described in the reference strain *Botrytis cinerea* B05.10. Moreover, a new gene cluster related to terpenes in *B. deweyae* B1 and *B. cinerea* B05.10 was also identified that had never been detected before. This new gene cluster is well conserved among other *Botrytis* species in many phylogenetically distant fungal lineages. Understanding the genetic basis and conservation of these putative biosynthetic gene clusters sheds light on the metabolic potential and ecological roles of *B. deweyae* and related fungal species.

Keywords: *Botrytis deweyae*; secondary metabolites; terpenes and polyketides

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1. Introduction

1.1. Fungal Secondary Metabolites

Fungi have a profound connection with humans, highlighted by significant events such as the Turkey X disease outbreak in the 1960s [1] and the discovery of penicillin [2]. The antibiotic era, initiated by penicillin derived from the Penicillium fungus [3,4], transformed healthcare practices worldwide [3].

Fungi exhibit remarkable metabolic adaptability, producing secondary metabolites (SMs) through complex biosynthetic pathways [5,6]. These SMs serve as signaling molecules and defense mechanisms, providing a competitive advantage in natural environments [7]. Their importance extends to applications in medicine, agriculture, and biotechnology [8,9]. Common SM categories include terpenes, polyketides, non-ribosomal peptides, and indoles [10,11].

1.2. The Genus Botrytis

The *Botrytis* genus comprises ubiquitous fungal pathogens that significantly impact agriculture, horticulture, and ecology [12]. Notably, *Botrytis cinerea* is infamous for causing

gray mold disease, affecting various crops like grapes, strawberries, and vegetables, leading to substantial yield and quality losses [13,14]. This pathogen poses challenges for growers as it can infect plants both pre- and post-harvest [15,16].

Ecologically, *Botrytis* serves as a necrotrophic pathogen, contributing to nutrient cycling and decomposition [14,17]. Species within this genus exhibit diverse interactions with plant hosts, ranging from benign to virulent pathogenicity, showcasing their ecological versatility [18]. The genetic diversity and metabolic adaptability of *Botrytis* enable it to evade host defenses and thrive in varying environments [19,20].

Ongoing research into its biology and genetics offers promising strategies for disease management, with advances in genomic tools providing insights into its life cycle and host interactions [14,21].

1.3. Botrytis Deweyae

B. deweyae stands out in the *Botrytis* genus for its combination of endophytic and facultative necrotrophic behaviors, differing from typical necrotrophic traits [18,22]. Initially isolated from *Hemerocallis* plants with "spring sickness", it causes deformities, stunted growth, chlorosis, and necrotic lesions post-winter [22]. While research interest has been limited until recently, reports of its impact date back to the 1970s [22].

Genetic analysis reveals a close relationship between *B. deweyae*, *Botrytis elliptica*, and *Botrytis squamosa* [22]. Although primarily targeting *Hemerocallis*, it has a polyphagy index of 1, indicating a more restricted host range than its relatives [15]. *B. elliptica* is the second most polyphagous species after *B. cinerea* [15]. Recent observations suggest that *B. deweyae* may be expanding its ecological niche, with reports of gray mold on *Polygonatum cyrtonema* in China [23].

1.4. Role of Terpenes and Polyketide Synthases in Secondary Metabolism

1.4.1. Terpenes

Terpenoids, derived from isopentenyl diphosphate (IPP), are synthesized via the mevalonate and deoxyxylulose 5-phosphate pathways [24,25]. The mevalonate pathway, predominant in fungi, converts mevalonate to IPP, which is crucial for terpenoid biosynthesis [25]. Terpenoid biosynthesis begins with IPP and dimethylallyl diphosphate (DMAPP), leading to various terpenoid classes through enzymatic transformations [26–31]. Terpenoids have diverse biological activities, including hormonal functions and mycotoxin production [32–34].

1.4.2. Polyketide Synthases

Polyketides (PKs) are a prominent family of secondary metabolites initiated by the condensation of acetyl-CoA units [35,36]. Polyketide synthases (PKSs) are classified into three major classes based on structure and function: Type I, Type II, and Type III [35,37]. Type I PKSs are multifunctional enzymes resembling fatty acid synthetases, while Type II PKSs are multienzymatic complexes found mainly in prokaryotes [38–43]. Type III PKSs are less diverse and primarily involved in plant defense [44–47]. The structural diversity of PKs is further enhanced by auxiliary enzymes [37].

This study aims to investigate the genomic profiles of *B. deweyae* to identify gene clusters responsible for secondary metabolite biosynthesis, particularly terpenes and polyketides. By comparing with *B. cinerea*, we seek to understand genetic variability and phylogenetic relationships of these clusters, alongside their ecological and functional significance, enhancing our knowledge of metabolic diversity within the genus *Botrytis* and its implications for disease management and biotechnological applications. However, it is important to point out that these results are predictive and, without validation, remain theoretical; therefore, validation would need to be carried out for confirmation.

2. Materials and Methods

2.1. Genome Data Acquisition

The genome data used in this study were sourced from the National Center for Biotechnology Information (NCBI), with accession numbers GCF_000143535.2 for *Botrytis cinerea* B05.10 [48,49] and GCF_014898535.1 for *Botrytis deweyae* B1 [50].

2.2. Genome Set Completeness Assessment

We utilized BUSCO v. 5.7.1 in genome modes, employing the fungi_odb10 dataset, which includes 758 BUSCO groups. This analysis assessed the completeness of the predicted protein set and the genome assembly. We compared the BUSCO scores with those obtained for the *Botrytis cinerea* genome [51]. The alignment of both genomes was visualized by performing a syntenic plot using the TBtools-II tool [52]. The core genes corresponding to the secondary metabolism of *B. deweyae* that showed collinearity with *B. cinerea* were represented in the plot (Supplementary Figure S1).

2.3. Secondary Metabolite Gene Cluster Analysis

Secondary metabolite gene cluster analysis was performed using antiSMASH v. 7.0 (fungal version) software [53]. AntiSMASH is an integrated tool designed to identify putative biosynthetic gene clusters (BGCs) within fungal genomes, providing comprehensive annotations that predict the presence and type of secondary metabolites these clusters may produce (Supplementary Tables S1 and S2). We set up default parameters, which facilitates the identification of potential secondary metabolite gene clusters.

2.4. Domain Analysis

Domain analysis was carried out using the Conserved Domain Database (CDD) alongside the Pfam v. 35 database [54]. Pfam is an extensive collection of protein families, represented by multiple sequence alignments and hidden Markov models (HMMs), facilitating the identification and functional characterization of protein domains. When Pfam did not yield results, the InterPro (https://www.ebi.ac.uk/interpro/ (accessed on 30 July 2024)) database was employed. InterPro aggregates predictive models from multiple sources, including Pfam, PRINTS, PROSITE, and SMART, offering a thorough functional analysis of proteins [55,56].

2.5. Phylogenetic Analysis

Phylogenetic relationships were inferred using sequences from Bd22-26 and XP_038807933.1, which served as queries in a BLASTP search against the NCBI non-redundant protein sequence database [57]. Homologous proteins were selected from the resulting list following specific criteria: percent identity (>50%), coverage (>70%), and the bit-score (>50) [58,59]. The maximum likelihood method was employed to infer evolutionary history [60], utilizing 55 homologous proteins, except where fewer than 50 sequences were identified, in which case all sequences were included. The optimal tree was displayed, and evolutionary distances were computed using the Poisson correction method [60]. This analysis involved 57 amino acid sequences, with ambiguous positions removed for each sequence pair (pairwise deletion option). MEGA v. 11 software was used for these evolutionary analyses [61].

2.6. Homologous Protein Identification

Identification of homologous proteins was performed using a BLASTP search with stringent criteria: percent identity (>50%), coverage (>70%), and bit-score (>50) [58,59]. The search targeted the non-redundant protein sequence database, focusing on *Botrytis* (taxid: 33196), *Botryotinia* (taxid: 40558), and a broader search across fungi (taxid: 4751). This comprehensive approach ensured the identification of homologous proteins not only within the *Botrytis* genus but also across a wide range of fungal species, providing a broader context for comparative analysis.

3. Results

3.1. Genome Completeness Assessment

BUSCO (v5.7.1) analysis (Supplementary Table S3) at the genomic level revealed that 99.1% of the fungi dataset was retrieved in full length. Additionally, 0% of the BUSCO genes for fungi, respectively, were identified as duplicated, indicating that the genome was not assembled in a haploid state with no significant evidence of gene duplications (Supplementary Table S3). *B. cinerea* B05.10 gene content completeness was comparable to that of *B. deweyae* B1 based on BUSCO metrics even though *B. deweyae* B1 was a little bit more fragmented.

3.2. Comparative Analysis of Secondary Metabolite Gene Clusters in B. deweyae and B. cinerea

The comparison of secondary metabolite gene clusters between *B. deweyae* B1 and *B. cinerea* B05.10 reveals distinct differences in their metabolic capabilities. *B. deweyae* B1 contains a greater number of polyketide synthase (PKS) clusters, with 11 identified gene clusters compared to 10 in *B. cinerea* B05.10. Similarly, the number of terpene biosynthesis clusters is higher in *B. deweyae* B1, with six gene clusters, compared to five in *B. cinerea B05.10* (Figure 1).

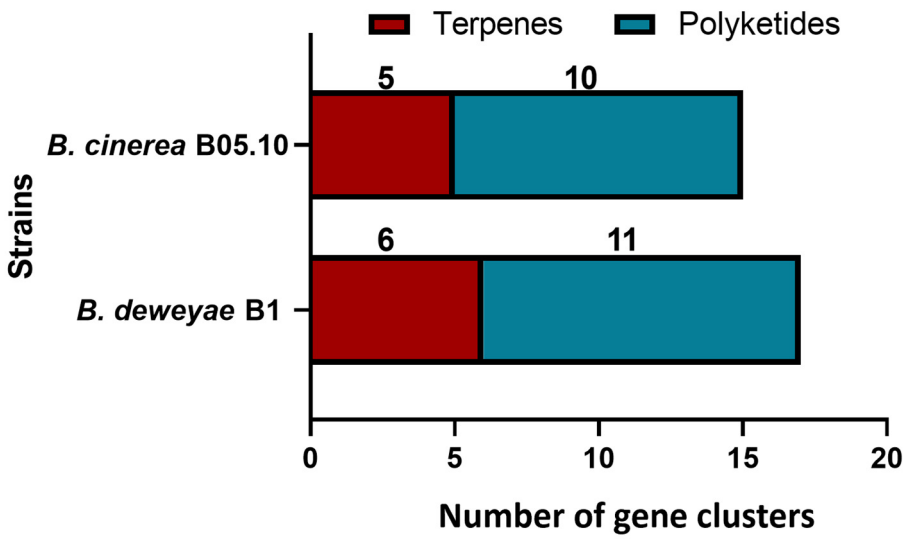

Figure 1. Distribution of different secondary metabolite gene clusters identified in *B. deweyae* and *B. cinerea*.

These differences indicate a higher diversity in the secondary metabolism of *B. deweyae*. The identified PKS clusters in *B. deweyae* include several novel clusters that have not been previously described, suggesting potential new pathways for secondary metabolite production. The analysis of these gene clusters provides insights into the unique metabolic capabilities of *B. deweyae*, which may contribute to its ecological roles and interactions with host plants.

Figure 1 illustrates the distribution of secondary metabolite gene clusters identified in both *B. deweyae* and *B. cinerea*. The increased number of clusters in *B. deweyae* highlights its potential for producing a broader range of secondary metabolites, which could be crucial for its adaptation and survival in diverse environmental conditions.

3.3. Terpene Gene Clusters in B. deweyae

In this section, we focus on the identification and characterization of terpene gene clusters in *B. deweyae*. The analysis revealed several key findings, highlighting the diversity and potential functional roles of these clusters.

Table 1 presents a detailed comparison of the terpene gene clusters identified in *B. deweyae* and *B. cinerea*. The table includes gene IDs, protein IDs, protein lengths, and per-

centage similarities, providing a comprehensive overview of the similarities and differences between these species.

Table 1. Comparison of Terpene Gene Clusters in *B. deweyae* and *B. cinerea*.

GC	Gene ID *B. deweyae*	Protein ID *B. deweyae*	Protein Length in *B. deweyae*	Gene ID *B. cinerea*	Protein ID *B. cinerea*	Protein Length in *B. cinerea*	% Similarity	Annotation
1	EAE98_002948	XP_038813097.1	482	BCIN_06g02400	XP_001560441.1	482	93.57%	*Bcerg*9
2	EAE98_003830	XP_038811923.1	437	BCIN_04g03550	XP_001546971.2	441	79.18%	*Bcstc*4
3	EAE98_006162	XP_038810179.1	278	BCIN_11g06510	XP_024551950.1	321	80.37%	*Bcstc7*
4	EAE98_008016	XP_038807933.1	394	BCIN_14g01170	XP_024552819.1	381	96.33%	Unknown
5	EAE98_008221	XP_038807710.1	399	BCIN_12g06390	XP_024552383.1	399	87.72	*Bcbot*2
6	EAE98_010975	XP_038805111.1	611	BCIN_01g04560	XP_024546243.1	610	90.51%	*Bcphs*1

GC: Gene Cluster. Protein lengths are measured in amino acids (aa). Gene IDs and Protein IDs are sourced from the NCBI database.

This table highlights that *B. deweyae* contains a higher number of unique terpene gene clusters compared to *B. cinerea*, suggesting a more diverse metabolic potential. Notably, the presence of novel gene clusters in *B. deweyae* indicates potential new pathways for secondary metabolite production, which could play crucial roles in the organism's ecological interactions and adaptability.

3.3.1. Description of Terpene Gene Clusters

The identified terpene gene clusters in *B. deweyae* B1 include several well-conserved genes, such as *Bcerg*9, *Bcstc*4, *Bcstc*7, *Bcbot*2, and *Bcphs*1, along with an unannotated gene. *Bcerg*9 encodes for a key enzyme in the ergosterol biosynthesis pathway, essential for maintaining cell membrane integrity [62]. *Bcstc*4 and *Bcstc7* are involved in the biosynthesis of specific sesquiterpenoids: (3*R*,6*E*)-nerolidol and (+)-α-bisabolol (for *Bcstc4* gene) and (+)-4-*epi*-eremophil-9-en-11-ol (for *Bcstc7* gene) [63,64]. *Bcbot*2 is associated with the synthesis of botrydial, a phytotoxic sesquiterpene produced by *B. cinerea* [65]. *Bcphs*1 plays a role in the biosynthesis of retinal, an aldehyde form of vitamin A [66]. These clusters are crucial for the biosynthesis of various terpenoid compounds, which are known for their diverse biological activities.

In *B. cinerea* B05.10, gene clusters corresponding to the sesquiterpene family such as *Bcstc2* (BCIN_08g02350) and *Bcstc5* (BCIN_01g03520) have been detected. However, the gene *Bcstc2* is not present in the genome of *B. deweyae* B1, while the protein BcSTC5 has two homologous proteins, EAE98_005930 and EAE98_005931, with 36% and 63% identity, respectively. The union of these two proteins gives rise to BcSTC5 in *B. cinerea* B05.10, indicating a putative annotation error in the case of *B. deweyae* B1.

Figure 2 illustrates the phylogenetic relationships of all the genes that present the IPR008949—Isoprenoid synthase domain superfamily, terpene synthases identified in *B. deweyae* and their homologs in *B. cinerea*. The phylogenetic tree was inferred using the maximum likelihood method via MEGA 11 software, with bootstrap values from 1000 trials indicated at each branch node.

BdSTC4, BcSTC3, BdBOT2, BcSTC5, BcERG9, and BdSTC7 are closely related, forming part of the same subclade. On the other hand, BcPAX1, BcCOQ1, BcERG20, and BdUnannotated (XP_038807933.1, which forms a cluster in both *B. cinerea* B05.10 and *B. deweyae* B1 according to the antiSMASH cluster prediction tool) are found in another subclade belonging to the same clade 1 (Figure 2). Likewise, for the protein named BcUnannotated the most closely related protein is BcStc2 (Figure 2).

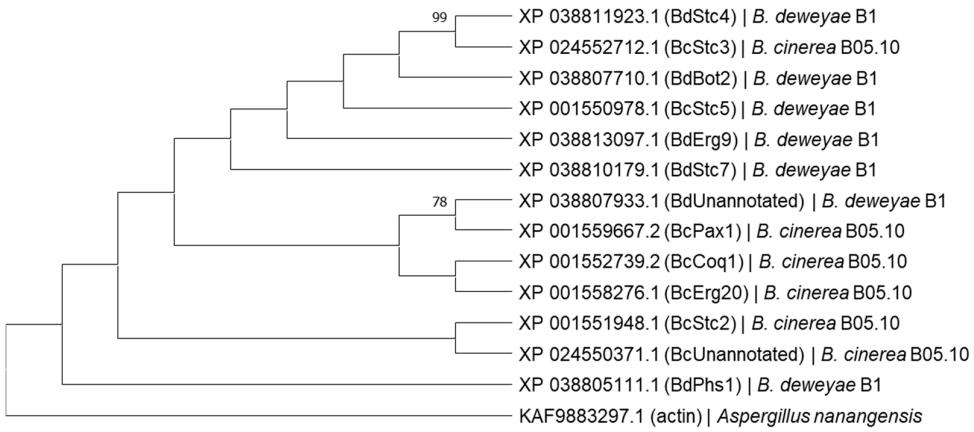

Figure 2. Phylogenetic tree of the amino acid sequences of terpenes from *B. deweyae* B1 together with the amino acid sequences from *B. cinerea* B05.10 that contains the IPR008949 domain. The phylogenetic tree was inferred using the maximum likelihood method via MEGA 11 software and bootstrap values from 500 trials are indicated at each branch node.

The phylogenetic analysis reveals that the terpene synthases in *B. deweyae* are closely related to those in *B. cinerea*, suggesting a shared evolutionary history. However, the presence of unique genes in *B. deweyae* indicates species-specific adaptations and potential new putative biosynthetic capabilities.

3.3.2. Description of EAE98_008016 Gene in *B. deweyae*

The EAE98_008016 gene in *B. deweyae* presents a unique aspect of the species' genomic architecture, differentiating it from *B. cinerea*. This gene, identified via the AntiSMASH tool, is part of a cluster predicted to encode an unannotated terpene (BdUnannotated protein) (Figure 3). Notably, this gene cluster does not contain additional genes in either *B. deweyae* or *B. cinerea* B05.10 (Figure 3).

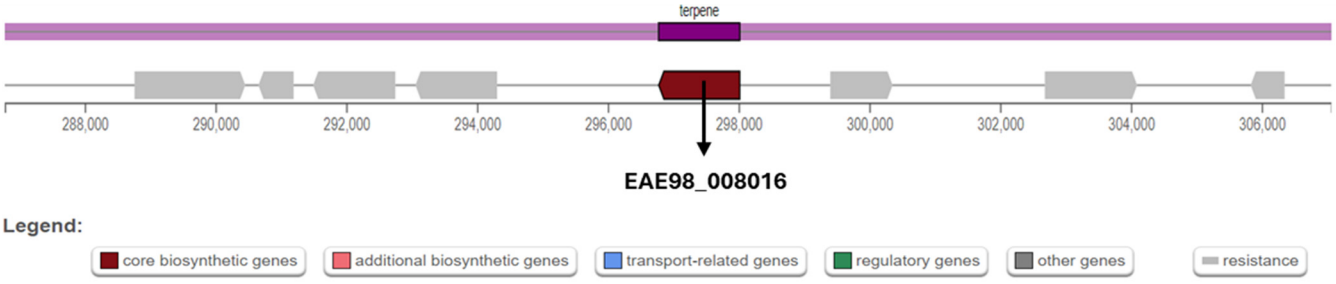

Figure 3. Gene cluster predicted by the AntiSMASH tool for the unannotated terpene EAE08_008016 in *B. deweyae* B1 (Table 1).

The EAE98_008016 gene encodes a protein (XP_038807933.1) with two polyprenyl synthetase domains located between amino acids 316-870 and 858-1097. It shows a 96.33% similarity to the BCIN_14g01170 gene, which encodes the protein XP_024552819.1, in *B. cinerea* (Table 1).

Comparative analysis reveals homologous proteins across various *Botrytis* species, indicating conservation of this gene. This protein has homologs in all *Botrytis* species whose genomes have been sequenced and annotated (Table 2). *B. cinerea*, one of the most studied species in the genus, is represented by multiple strains including T4, BcDW1, Bc448, and B05.10. These strains exhibit high percentages of identity with *B. deweyae*, ranging from 96.33% to 96.70%, suggesting close genetic relationships. *B. elliptica* strain Be9612 shows an even higher percentage of identity (99.75%), indicating a very close genetic similarity to *B. deweyae* B1 (Table 2). Other strains, such as *B. sinoallii* (Bc 23 strain) and *Botrytis hyacinthi*

(Bh0001 strain), also display high levels of identity, with percentages of 99.24% and 98.22%, respectively (Table 2). Several strains from different species exhibit identities above 98%, including *Botrytis aclada* (633 strain), *Botryotinia convoluta* (MUCL 11595 strain), *Botrytis byssoidea* (MUCL 94 strain), and *Botrytis fragariae* (BVB16 strain), among others. In contrast, species such as *Botryotinia calthae* (MUCL 2830 strain), *Botrytis porri* (MUCL 3234 and MUCL 3349 strains), and *B. squamosa* (MUCL 31421 strain) exhibit lower percentages of identity, ranging from 91.42% to 97.11% (Table 2).

Table 2. Distribution of homologous proteins to XP_038807933.1 of *B. deweyae* within the *Botrytis* genus. * means that there are no homologous proteins in this species annotated, but there is a region on its draft genome that could encode the XP_038807933.1 protein in this species.

***Botrytis* Species**	**Strain**	**GeneBank Genome Reference**	**Protein Accession Number**	**Number of Amino Acids**	**Percentage of Identity with *B. deweyae***
B. cinerea	T4	GCA_000227075.1	CCD33657.1	394	96.70%
B. cinerea	BcDW1	GCA_000349525.1	EMR82980.1	394	96.45%
B. cinerea	Bc448	GCA_037039525.1	KAK6598033.1	381	96.59%
B. cinerea	B05.10	GCA_000143535.4	XP_024552819.1	381	96.33%
B. elliptica	Be9612	GCA_014898555.1	KAF7925222.1	394	99.75%
B. sinoallii	Bc 23	GCA_014898435.1	XP_038758944.1	394	99.24%
B. hyacinthi	Bh0001	GCA_004786245.1	TGO37690.1	394	98.22%
B. aclada	633	GCA_014898285.1	KAF7953262.1	381	98.95%
Botryotinia convoluta	MUCL 11595	GCA_004786275.1	TGO54160.1	381	98.95%
B. byssoidea	MUCL 94	GCA_014898295.1	XP_038734128.1	381	98.95%
B. fragariae	BVB16	GCA_013461495.1	XP_037193855.1	381	98.69%
B. tulipae	Bt9001	GCA_004786125.1	TGO06850.1	381	98.69%
B. paeoniae	Bp0003	GCA_004786145.1	TGO30745.1	381	98.43%
Botryotinia globosa	MUCL 444	GCA_014898425.1	KAF7887299.1	381	98.43%
Botryotinia narcissicola	MUCL 2120	GCA_004786225.1	TGO61103.1	381	98.43%
Botryotinia calthae	MUCL 2830	GCA_004379285.1	TEY75198.1	381	97.11%
B. porri	MUCL 3234	GCA_014898465.1	XP_038765908.1	381	95.80%
B. porri	MUCL 3349	GCA_004786265.1	TGO84949.1	381	95.54%
B. squamosa	MUCL 31421	GCA_014898485.2	RCTC02000009.1 *	-	94.40%
B. medusae	B555	GCA_019395255.1	JAHXJL010000116.1 *	-	93.66%
B. pseudocinerea	BP362	GCA_019395245.1	JAHXJK010000109.1 *	-	92.16%
B. fabae isolate	DLY-16-612	GCA_004335055.1	RSAG01000202.1 *	-	91.42%

On the other hand, some strains, such as *Botrytis medusae* (B555 strain), *Botrytis pseudocinerea* (BP362 strain), and *Botrytis fabae* (DLY-16-612 strain), have not been fully characterized in terms of amino acid sequences (Table 2). However, regions on the draft genomes of *B. pseudocinerea*, *B. fabae*, and *B. medusae* homologous to the XP_038807933.1 protein show percentages of identity with the XP_038807933.1 protein of *B. deweyae* ranging from 91.42% to 93.66% (Table 2).

3.3.3. Comparative Analysis of EAE98_008016 Protein with Other Fungal Species

In addition to the comparative analysis within the *Botrytis* genus, Table 3 provides a comprehensive overview of the taxonomic distribution of fungi, focusing on the *phyla Ascomycota*, *Basidiomycota*, *Chytridiomycota*, *Mucoromycota*, *Zoopagomycota*, and *Blastocladiomycota*, in relation to the unannotated gene EAE98_008016 from *B. deweyae*. This breakdown reveals the diversity of fungal species and their distribution across various taxonomic levels, including *phyla*, classes, and orders. Additionally, the table includes the number of proteins and organisms associated with each taxonomic group, providing valuable insights into the functional and ecological diversity of fungi.

Table 3. Distribution of the XP_038807933 protein (EAE98_008016) gene across the *Fungi* Kingdom.

A. Phylum Ascomycota.				
Clade	**Class**	**Order**	**Number of Proteins**	**Number of Organisms**
Sordariomyceta	*Leotiomycetes*	*Heliotales*	75	61
		Unclassified Leotiomycetes	1	1
		Leotiales	1	1
		Leotiomycetes incertae sedis	27	23
		Erysiphales	15	11
	Sordariomycetes	*Togniniales*	3	2
		Diaporthales	32	18
		Magnaporthales	26	7
		Sordariales	93	67
		Coniochaetales	6	5
		Chaetosphaeriales	2	1
		Ophiostomatales	8	5
		Cephalothecales	2	1
		Xylariales	227	122
		Unclassified Xylariomycetidae	2	2
		Xylariomycetidae incertae sedis	4	1
		Hypocreales	546	259
		Glomerellales	159	88
		Microascales	7	6
		Sordariomycetes incertae sedis	2	1
		Lulworthiales	1	1
Leotiomyceta	*Lecanoromycetes*	*Acarosporales*	4	3
		Rhizocarpales	2	1
		Lecanorales	22	12
		Peltigerales	5	4
		Caliciales	3	2
		Teloschistales	47	20
		Unclassified Lecanoromycetidae	1	1
		Ostropomycetidae incertae sedis	2	1
		Umbilicariales	4	3
		Trapeliales	15	12
		Pertusariales	3	3
		Sarrameanales	2	2
		Ostropales	4	4
		Lecanoromycetes incertae sedis	1	1
	Coniocybomycetes	*Coniocybales*	3	2
	Xylonomycetes	*Xylonales*	2	1
	Candelariomycetes	*Candelariales*	4	4
	Sareomycetes	*Sareales*	1	1
	Lichinomycetes	*Lichinales*	4	4
	Xylobotryomycetes	*Xylobotryales*	2	1
	Geoglossomycetes	*Geoglossales*	5	4
	Eurotiomycetes	*Eurotiales*	1116	230
		Onygenales	110	70
		Chaetothyriales	95	41
		Verrucariales	3	2
		Phaeomoniellales	1	1
		Unclassified Eurotiomycetes	1	1

Table 3. *Cont.*

A. Phylum Ascomycota.				
Clade	**Class**	**Order**	**Number of Proteins**	**Number of Organisms**
Dothideomyceta	*Dothideomycetes*	*Mycosphaerellales*	251	45
		Dothideales	138	20
		Myriangiales	7	6
		Cladosporiales	6	4
		Capnodiales	2	2
		Trypetheliales	8	3
		Botryosphaeriales	47	16
		Acrospermales	2	1
		Lineolatales	1	1
		Dothideomycetes incertae sedis	13	8
		Eremomycetales	2	1
		Patellariales	1	1
		Phaeotrichales	1	1
		Mytilinidiales	4	3
		Pleosporomycetidae incertae sedis	2	2
		Pleosporales	216	109
		Aulographales	2	2
		Venturiales	15	5
	Arthoniomycetes	*Arthoniales*	2	2
Saccharomyceta	*Pezizomycotina incertae sedis*	*Thelocarpales*	2	2
		Vezdaeales	2	2
		Pezizomycotina incertae sedis	2	2
	Pezizomycetes	*Pezizales*	34	26
	Orbiliomycetes	*Orbiliales*	35	15
	Saccharomycetes	*Saccharomycetales*	45	23
Taphrinomycotina incertae sedis			3	1
-	*Taphrinomycetes*	*Taphrinales*	3	2

B. Phylum Basidiomycota.			
Class	**Order**	**Number of Proteins**	**Number of Organisms**
Microbotryomycetes	*Leucosporidiales*	1	1
	Sporidiobolales	20	10
	Unclassified Microbotryomycetes	1	1
	Microbotryales	6	4
	Kriegeriales	1	1
	Microbotryomycetes incertae sedis	1	1
Mixiomycetes	*Mixiales*	3	1
Cystobasidiomycetes	*Cyphobasidiales*	1	1
Pucciniomycetes	*Pucciniales*	40	17
Atractiellomycetes	*Atractiellales*	1	1
Agaricomycetes	*Agaricomycetes incertae sedis*	196	135
	Agaricales	233	140
	Jaapiales	1	1
	Boletales	62	34
	Atheliales	2	2
	Amylocorticiales	2	1
	Geastrales	2	1
	Hysterangiales	3	1
	Gomphales	2	2

Table 3. *Cont.*

B. Phylum Basidiomycota.			
Class	**Order**	**Number of Proteins**	**Number of Organisms**
Tremellomycetes	*Filobasidiales*	6	4
	Trichosporonales	18	8
	Tremellales	99	73
	Cystofilobasidiales	3	1
Dacrymycetes	*Dacrymycetales*	2	2
Exobasidiomycetes	*Exobasidiales*	3	2
	Tilletiales	13	5
Ustilaginomycetes	*Ustilaginales*	23	12
	Violaceomycetales	1	1
Malasseziomycetes	*Malasseziales*	14	9
Wallemiomycetes	*Wallemiales*	6	1
C. Phylum Mucoromycota.			
Class	**Order**	**Number of Proteins**	**Number of Organisms**
Mucoromycetes	*Mucorales*	194	57
Umbelopsidomycetes	*Umbelopsidales*	13	5
Endogonomycetes	*Endogonales*	3	2
Mortierellomycetes	*Mortierellales*	88	51
Glomeromycetes	*Glomerales*	21	9
	Diversisporales	18	10
	Paraglomerales	3	2
	Archaeosporales	3	3
	Entrophosporales	12	2
D. Phylum Zoopagomycota.			
Class	**Order**	**Number of Proteins**	**Number of Organisms**
Basidiobolomycetes	*Basidiobolales*	1	1
Entomophthoromycetes	*Entomophthorales*	7	2
Kickxellomycetes	*Kickxellales*	160	137
Dimargaritomycetes	*Dimargaritales*	11	6
Harpellomycetes	*Harpellales*	3	3
Kickxellomycotina incertae sedis	*Ramicandelaberales*	1	1
Zoopagomycetes	*Zoopagales*	4	4
E. Phylum Chytridiomycota.			
Class	**Order**	**Number of Proteins**	**Number of Organisms**
Chytridiomycetes	*Synchytriales*	4	2
	Spizellomycetales	14	8
	Rhizophlyctidales	3	2
	Chytridiomycetes incertae sedis	3	3
	Cladochytriales	3	2
	Chytridiales	7	7
	Lobulomycetales	2	2
	Rhizophydiales	9	5
	Polychytriales	2	1
Monoblepharidomycetes	*Monoblepharidales*	3	3
Neocallimastigomycetes	*Neocallimastigales*	5	5
F. Phylum Chytridiomycota.			
Class	**Order**	**Number of Proteins**	**Number of Organisms**
Blastocladiomycota	*Blastocladiomycetes*	10	5
	Physodermatomycetes	1	1

Ascomycota is the largest *phylum* represented, with 3547 protein sequences across 1419 organisms. The *Sordariomyceta* clade stands out with 1239 identified proteins and 683 organisms (Table 3A). Within this clade, the *Leotiomycetes* class includes significant orders such as *Heliotales*, with 75 proteins in 61 organisms, and *Erysiphales* (Table 3A). The *Sordariomycetes* class shows high protein diversity, especially in orders like *Xylariales*, *Hypocreales*, and *Glomerellales*, which have 227, 546, and 159 proteins, respectively, across 122, 259, and 88 organisms (Table 3A).

The *Leotiomyceta* clade follows closely, with 1462 proteins in 431 organisms. The class *Eurotiomycetes* is prominent, particularly the order *Eurotiales*, which has 1116 proteins in 230 organisms (Table 3A). Other notable orders within *Eurotiomycetes* include *Onygenales* and *Chaetothyriales*, which contribute significantly to the protein diversity with 110 and 95 proteins, respectively (Table 3A). Additionally, less common orders like *Verrucariales* and *Phaeomoniellales* are represented by fewer proteins and organisms (Table 3A).

Lecanoromycetes, another class within *Ascomycota*, includes orders such as *Teloschistales*, *Lecanorales*, and *Trapeliales*, each showing varied protein counts and organism numbers (Table 3A). Other classes such as *Coniocybomycetes*, *Xylonomycetes*, and *Candelariomycetes* exhibit lower but still notable protein counts and organism diversity (Table 3A).

The *Dothideomyceta* clade, with 720 proteins and 232 organisms, represents a diverse group of fungi that includes many plant pathogens, saprobes, and lichenized fungi. The class *Dothideomycetes* covers a wide range of orders with varying protein counts and organism numbers (Table 3A). For instance, *Mycosphaerellales* has 251 proteins in 45 organisms, while *Dothideales* has 138 proteins across 20 organisms. Other orders like *Myriangiales*, *Cladosporiales*, and *Trypetheliales* have fewer proteins and organisms but still contribute to the overall diversity (Table 3A).

Saccharomyceta, the smallest clade in terms of both protein count and organism number, comprises 126 proteins distributed among 73 organisms (Table 3A). This clade, encompassing various orders within the *Pezizomycotina subphylum*, demonstrates diverse protein counts and organism numbers. Notably, *Pezizales* has 34 proteins in 26 organisms, and *Orbiliales* has 35 proteins across 15 organisms (Table 3A). *Saccharomycetales* exhibits 45 proteins in 23 organisms, showcasing the range of diversity within *Saccharomyceta* (Table 3A).

Taphrinomycotina incertae sedis, a class within the *Taphrinomycotina subphylum*, shows limited protein diversity and organism representation, with only three proteins identified in a single organism (Table 3A). In contrast, *Taphrinomycetes* in the order *Taphrinales* demonstrates slightly higher protein diversity with three proteins in two organisms (Table 3A). Despite its modest representation, this class highlights the presence of lesser-known fungal taxa, emphasizing the need for further exploration and study.

Basidiomycota, the second-largest *phylum*, includes classes such as *Microbotryomycetes*, *Agaricomycetes*, and *Tremellomycetes* (Table 3B). *Agaricomycetes* is particularly diverse, featuring orders like *Agaricales*, *Boletales*, and *Tremellales* (Table 3B). *Agaricales* is especially prominent, with a high number of proteins and organisms, indicating its ecological and functional importance within the *phylum* (Table 3B).

Within *Microbotryomycetes*, various orders demonstrate diverse protein counts and organism numbers (Table 3B). *Sporidiobolales* stands out with 20 identified proteins across 10 organisms, showing significant protein diversity (Table 3B). Other orders like *Leucosporidiales*, *Microbotryales*, and *Kriegeriales* exhibit lower protein counts and organism numbers (Table 3B).

The class *Tremellomycetes* includes orders such as *Filobasidiales*, *Trichosporonales*, and *Tremellales*. *Tremellales* shows higher protein diversity with 99 proteins across 73 organisms. Other orders within *Tremellomycetes* exhibit varying protein counts and organism numbers, contributing to the overall diversity within the class (Table 3B).

Other *phyla*, although less represented, still exhibit significant diversity. *Chytridiomycota* and *Mucoromycota* include notable orders like *Mucorales* with 194 proteins in 57 organisms and *Mortierellales* with 88 proteins in 51 organisms (Table 3C,E). *Chytridiomycetes* includes orders such as *Spizellomycetales* and *Rhizophlyctidales*, each contributing to the overall diversity.

Zoopagomycota and *Blastocladiomycota*, though less studied, also show interesting diversity (Table 3D,F). *Kickxellales* within *Zoopagomycota* stands out with 160 proteins in 137 organisms. The order *Blastocladiales* in *Blastocladiomycota* contains 10 proteins in five organisms, adding to the functional variety within these *phyla*.

In summary, the taxonomic analysis of the EAE98_008016 protein across different fungal *phyla* reveals extensive diversity. This diversity reflects the wide range of ecological roles and metabolic capabilities present within the fungal kingdom, providing valuable insights into their functional and ecological significance.

On the other hand, regarding the phylogenetic relationship, the XP_038807933 protein (encoded by the EAE98_008016 gene; Supplementary Table S4) from *B. deweyae* B1 is closely related to proteins of species from the genus *Sclerotinia* and *Stromatinia*, with both proteins located in the same subclade (Figure 4). Additionally, XP_038807933 is phylogenetically related to proteins from fungi of the genera *Ciborinia*, *Monilinia*, *Rustroemia*, *Chlorociboria*, *Bisporella*, *Claussenomyces*, *Coleophoma*, *Amorphotheca*, *Xylogone*, *Hyphodiscus*, *Hyaloscypha*, *Diplocarpon*, *Marssonina*, *Drepanopeziza*, *Rhynchosporium*, *Cadophora*, *Rhexocercosporidium*, *Leptodontidium*, *Lachnellula*, *Glarea*, *Amylocarpus*, *Venustampulla*, *Halenospora*, and *Hymenoscyphus* (Figure 4). Moreover, the top 55 sequences selected for the phylogenetic analysis belong to species from the *Helotiales* order. Among these top hits, one sequence corresponds to an unclassified *Leotiomycetes* species, *Xylogone* sp. PMI_703. Another sequence is from *Leotiomycetes* sp. MPI-SDFR-AT-0126 is closely related to species of the genus *Cadophora*. Lastly, *Claussenomyces* sp. TS43310 is classified within the Leotiales order.

3.4. Polyketide Gene Clusters in B. deweyae

In this section, we focus on the identification and characterization of polyketide gene clusters in *B. deweyae*. The analysis revealed several key findings, highlighting the diversity and potential functional roles of these clusters.

Table 4 presents a detailed comparison of the polyketide gene clusters identified in *B. deweyae* and *B. cinerea*. The table includes gene IDs, protein IDs, protein lengths, and percentage similarities, providing a comprehensive overview of the similarities and differences between these species.

Table 4. Comparison of Polyketide Gene Clusters in *B. deweyae* and *B. cinerea*.

GC	Gene ID in *B. deweyae*	Protein ID in *B. deweyae*	Protein Length in *B. deweyae*	Gene ID *B. cinerea*	Protein ID *B. cinerea*	Protein Length in *B. cinerea*	% Similarity	Annotation
1	EAE98_002293	XP_038813652.1	2542	-	-	-	-	Unknown
2	EAE98_005009	XP_038811280.1	2579	BCIN_05g08400	XP_024549041.1	2571	83.33%	*Bcpks21*
3	EAE98_006691	XP_038809559.1	2575	BCIN_16g05040	XP_024554044.1	2575	88%	*Bcpks16*
4	EAE98_007035	XP_038808800.1	499	BCIN_13g02130	XP_001555327.1	499	93.99%	*Bcchs1*
5	EAE98_009027	XP_038807018.1	2537	-	-	-	-	Unknown
6	EAE98_009190	XP_038806877.1	2346	-	-	-	-	Unknown
7	EAE98_009473	XP_038806567.1	2007	BCIN_05g06220	XP_024548906.1	2126	84.49%	*Bcpks15*
8	EAE98_010322	XP_038805735.1	2415	BCIN_13g01510	XP_001557060.1	2417	94.08%	*Bcpks10*
9	EAE98_010696	XP_038805323.1	2143	BCIN_03g08050	XP_001547095.2	2138	96.08%	*Bcpks13*
10	EAE98_010906	XP_038805042.1	2208	-	-	-	-	Unknown
11	EAE98_010943	XP_038805079.1	2353	-	-	-	-	Unknown

GC: Gene Cluster. Protein lengths are measured in amino acids (aa). Gene IDs and Protein IDs are sourced from the NCBI database.

This table reveals that *B. deweyae* harbors six putative polyketide gene clusters not found in *B. cinerea*, indicating a greater metabolic diversity. These unique clusters (gene IDs: EAE98_002293, EAE98_009027, EAE98_009190, EAE98_010906, and EAE98_010943) suggest the presence of new pathways for secondary metabolite production.

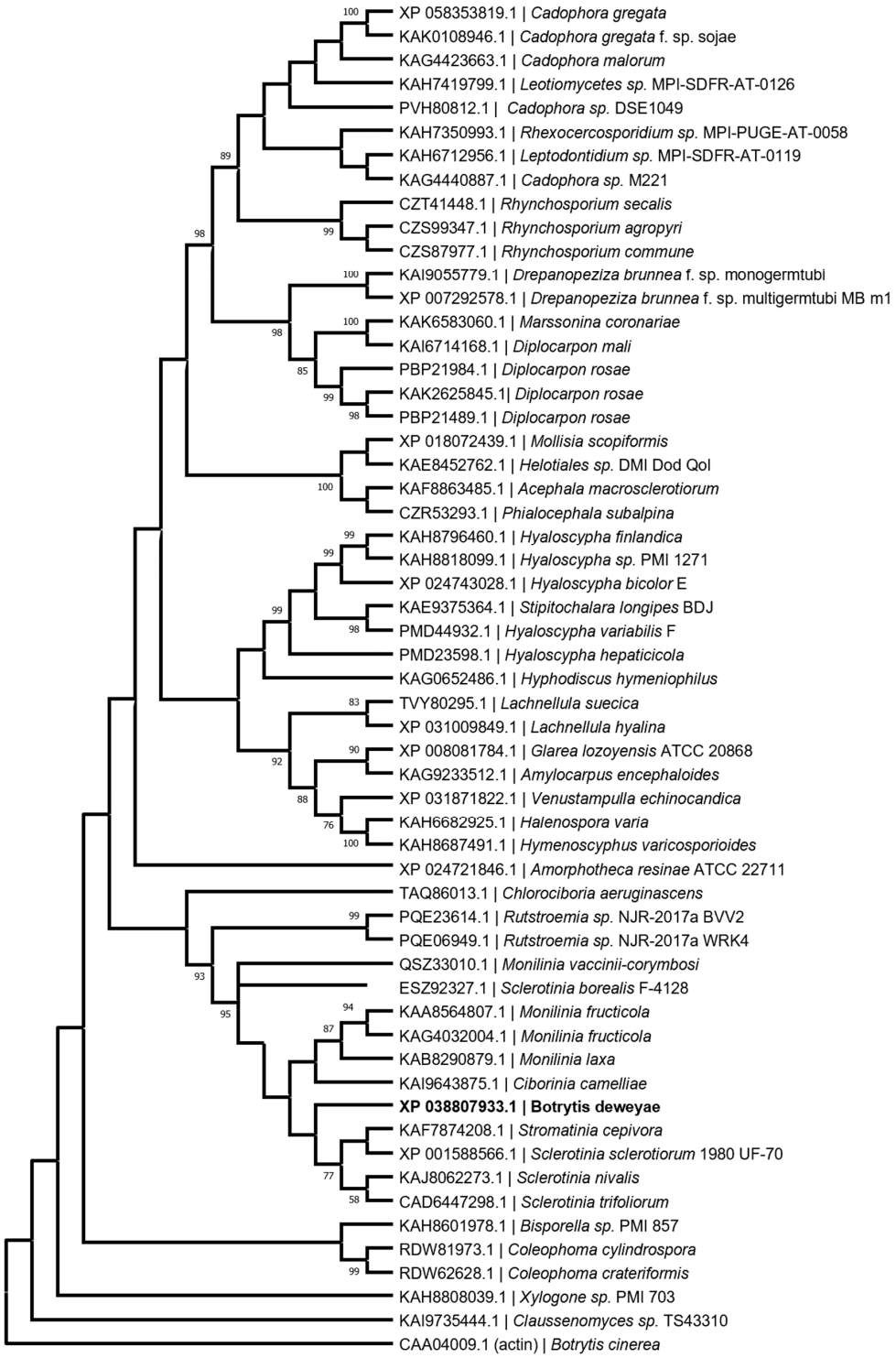

Figure 4. Phylogenetic tree of XP_038807933 protein from *B. deweyae* B1 and homologous protein sequences of other fungal species. The phylogenetic tree was inferred using the maximum likelihood method via MEGA 11 software, with bootstrap values from 500 trials indicated at each branch node. Protein sequences were selected after running similarity search by BlastP using XP_038807933 as query sequence, excluding the *Botrytis* and *Botryotinia* taxids (33196 and 40558, respectively), and filtering the results based on percent identity > 50%, coverage > 70%, and bit-score > 50. The top 55 hits were retrieved for sequence alignment and phylogenetic analysis. The NCBI accession number of each sequence is shown. Actin (CAA04009.1) of *B. cinerea* was used as outgroup. Different clades and subclades are indicated by colored branches (red, green, or violet).

3.4.1. Description of Polyketide Gene Clusters

Previous studies by [20] identified 21 polyketide synthases in *B. cinerea*, of which only BcBOA6/BcBOA9, BcPKS13, BcPKS1, BcPKS21, BcPKS16, BcCHS1, BcPKS10, BcPKS8, BcPKS2, and BcPKS15 form gene clusters [20]. In *B. deweyae*, some of these clusters, specifically BcBOA6/BcBOA9, BcPKS1, BcCHS11, BcPKS8, and BcPKS2, were not identified, indicating differences in gene cluster formation between these species.

For the non-cluster-forming polyketide synthases in *B. cinerea*, no homologous proteins were identified in *B. deweyae* for BcPKS3, BcPKS7, BcPKS9, BcPKS11, BcPKS17, BcPKS19, and BcPKS20. However, although BcPKS5 was not found as a homologous protein, a genomic region in *B. deweyae* suggests the presence of this polyketide synthase, indicating its potential yet unannotated presence. This identifies the possible presence of this gene in the genome of this fungus, but it is not properly annotated for this protein.

Additionally, previous studies by [11] proposed several new potential polyketide synthases (PKSs) in *B. cinerea* B05.10. These genes, including BCIN_09g06350, BCIN_01g00450, BCIN_04g00210, BCIN_03g06470, BCIN_09g06360, BCIN_08g02570, BCIN_08g02560, and BCIN_12g03250, were identified as candidates based on the presence of various indicative domains [11]. However, many of these genes either lack complete PKS domains or are present as fragments. For instance, BCIN_09g06350 contains only the IPR042104 domain with 104 amino acids and lacks other typical PKS domains (Supplementary Table S5). Similarly, BCIN_03g06470, despite having multiple domains such as the zinc-binding dehydrogenase domain, a KR domain, and a phosphopantetheine attachment site domain, does not exhibit the full domain architecture expected of classical PKS genes (Supplementary Table S5). Further analysis reveals that homologous proteins in other fungal species with over 50% identity similarity to these genes often have significantly more amino acids than those in *B. cinerea*, suggesting that these PKS candidates might be incomplete or fragmented (Supplementary Table S6). In contrast, BcFAS2 (BCIN_01g00440) in *B. cinerea* is a well-characterized gene, encoding a protein with 1860 amino acids and containing several complete PKS Type I domains. These include Fas_alpha_ACP, FAS_I_H, ACPS, cond_enzymes superfamily, ketoacyl-synt_C superfamily, and NADB_Rossmann superfamily domains, ensuring its functionality as a full polyketide synthase (Supplementary Table S5). The comprehensive domain structure of BcFAS2, along with its relevance and completeness, justifies its selection for inclusion in the phylogenetic analysis. Homologous proteins from other *Botrytis* species and fungi exhibit similar lengths and domain structures, further supporting the use of BcFAS2 in comparative studies (Supplementary Table S6).

Furthermore, new gene clusters were identified in *B. deweyae* that do not have homologous gene clusters in the genome of *B. cinerea*. These new gene clusters, named BdPKS22-26 (following the numbering after the 21 polyketides previously described in *B. cinerea*), are reported here for the first time, not having been identified in any *Botrytis* genome before (Figure 5).

The phylogenetic distribution of these new polyketide synthases, along with the 21 previously described in *B. cinerea*, reveals their arrangement into two distinct clades (Figure 6).

In the first clade, BdPKS22 can be identified in subclade 1, closely related to BcPKS1 and BcPKS21. BdPKS21 shares a subclade with BcPKS2, BcPKS8, as well as BcPKS10 and BcPKS11, which are phylogenetically related to each other. More distantly related within the same subclade is BcBOA9, which is closely related to BdPKS23, BdPKS24, and BdPKS26. Additionally, subclade 2 of clade 1 shows that BcBOA6 is closely related to BcPKS3 and BcPKS7, while BcPKS4 and BcPKS5 are more distantly related to BcBOA6 within the same subclade. It is noteworthy that the gene clusters of the polyketide synthases BcBOA6 and BcBOA9, responsible for the biosynthesis of botcinins, belong to different subclades. BcBOA6 is closely related to other polyketide synthases described in the genome of *B. cinerea*, while BcBOA9 is closely related to novel gene clusters encoding polyketide synthases in *B. deweyae*.

In the second clade, two subclades are identified. Subclade 1 contains closely related polyketide synthases BcPKS12–BcPKS15. In subclade 2, BdPKS25 is closely related to BcPKS16, which are on the same branch as BcPKS18 and BcPKS19. Additionally, BcPKS17 and BcPKS20 are more phylogenetically distant within the same clade.

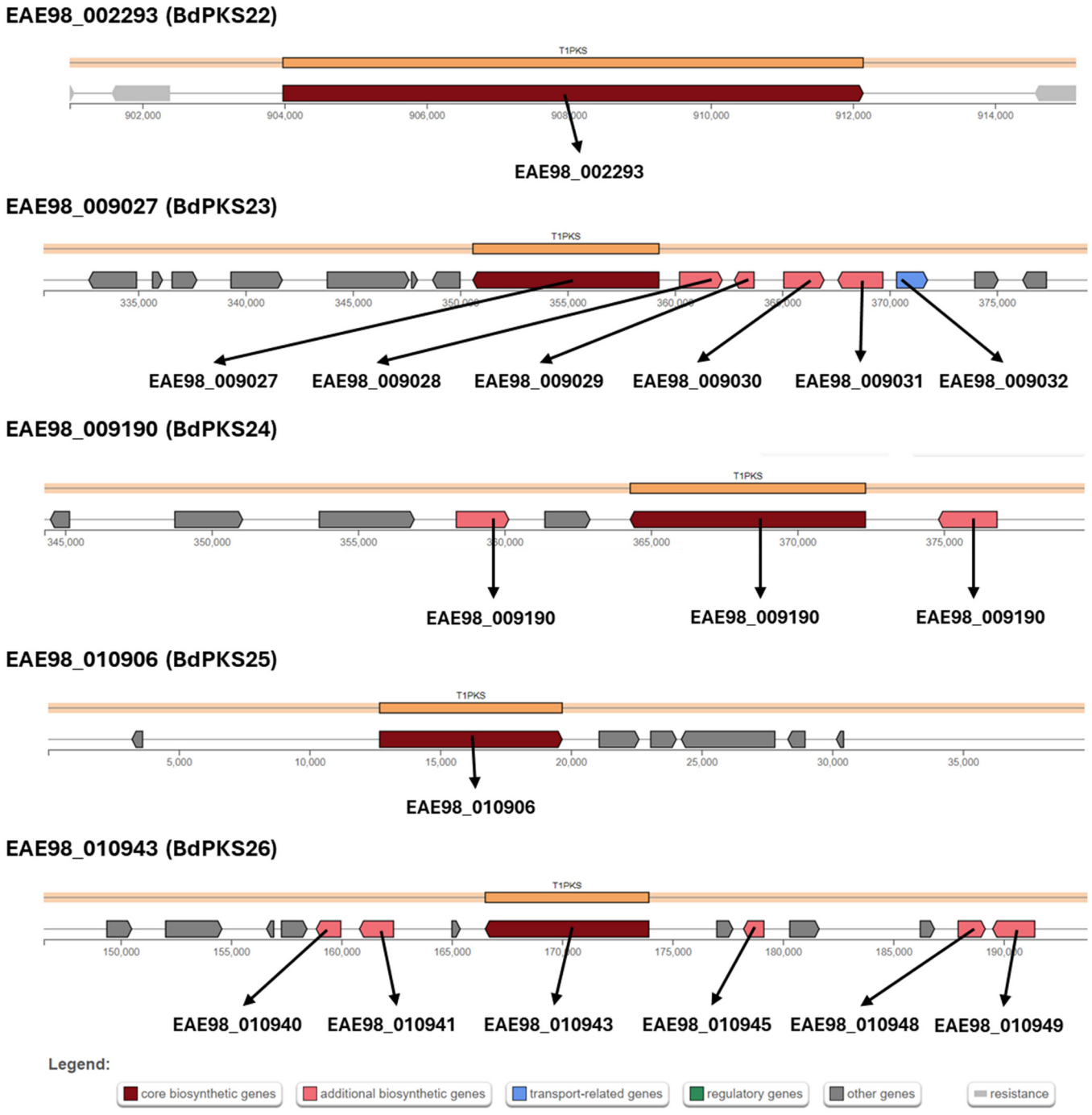

Figure 5. Representation of the gene cluster from BdPKS22-26 in *B. deweyae* predicted by the anti-SMASH fungal version tool.

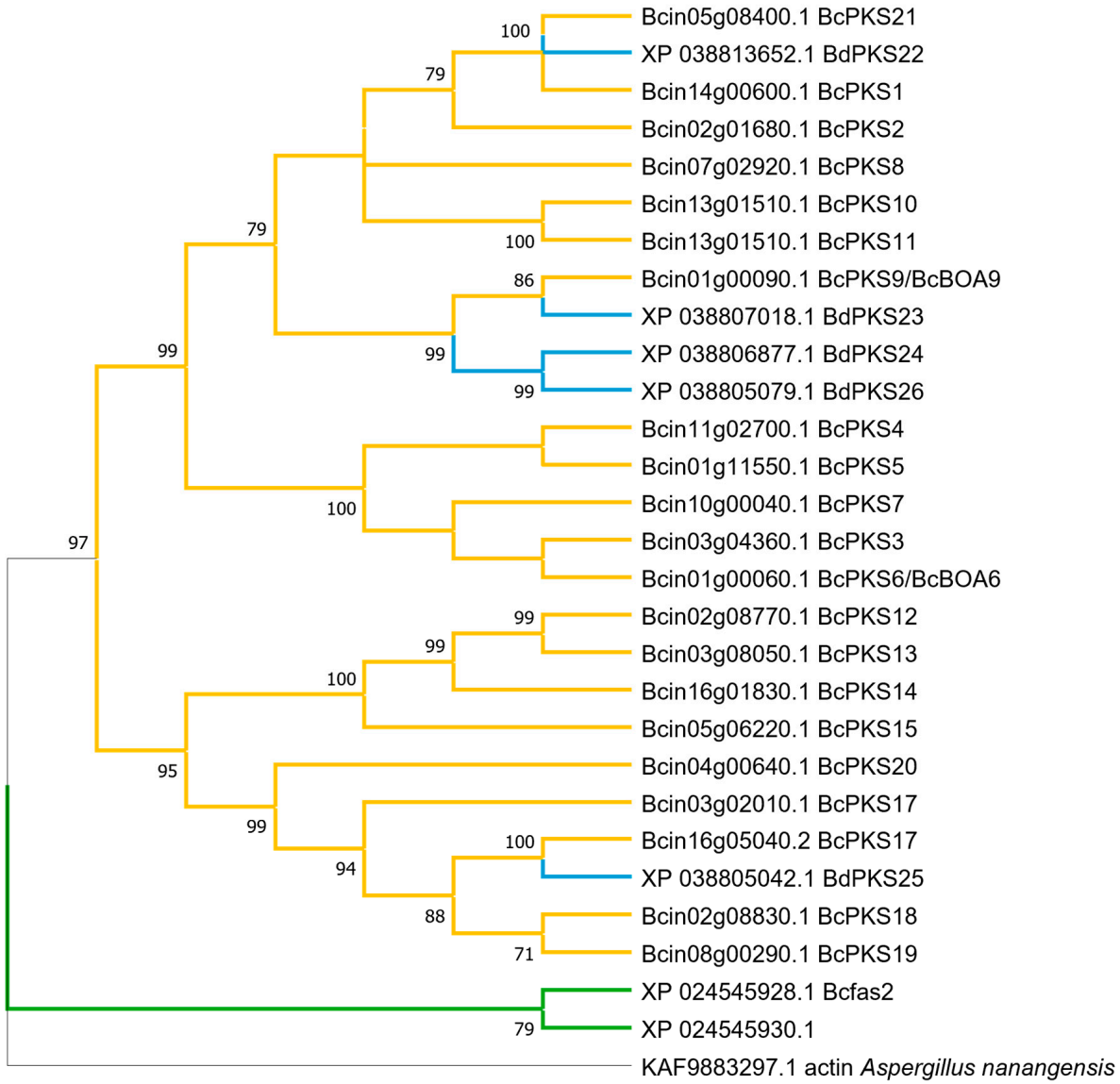

Figure 6. Phylogenetic tree of all the *B. cinerea* genes that showed polyketide domain together with the new putative polyketide synthases identified in *B. deweyae* B1. The evolutionary history was inferred using the maximum likelihood [60]. The bootstrap consensus tree inferred from 500 replicates [60] is taken to represent the evolutionary history of the taxa analyzed [60]. Branches corresponding to partitions reproduced in less than 50% bootstrap replicates are collapsed. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches [60]. The evolutionary distances were computed using the Poisson correction method [60] and are in the units of the number of amino acid substitutions per site. This analysis included 27 amino acid sequences where in the phylogenetic tree were identified in yellow the polyketide synthases previously described in *B. cinerea*, in blue the polyketide synthases of *B. deweyae* and in green the new polyketide synthases identified in *B. cinerea*. All ambiguous positions were removed for each sequence pair (pairwise deletion option). There were a total of 4970 positions in the final dataset. Evolutionary analyses were conducted in MEGA 11 [61].

A point of particular interest is the genetic diversity exhibited by the newly described polyketide synthases in *B. deweyae*. Most of these new PKSs are identified within the same clade and subclade (except for BdPKS22, which belongs to another subclade), with BdPKS25 being the most phylogenetically distant among the newly identified PKSs.

3.4.2. Description of EAE98_002293 Gene in *B. deweyae*—Bdpks22

The gene cluster containing the EAE98_002293 gene, referred to as *Bdpks22* in this study, consists of a single putative biosynthetic gene encoding the protein XP_038813652.1 (Figure 5). The XP_038813652.1 protein, encoded by EAE98_002293, is composed of 2542 amino acids and contains multiple functional domains: beta-ketoacyl synthase N-terminal (aa 21-257, pfam00109), beta-ketoacyl synthase C-terminal (aa 267-382, pfam02801), ketoacyl-synthetase C-terminal extension (aa 386-430, pfam16197), acyl transferase (aa 555-902, cl08282), Hotdog superfamily (aa 956-1242, cl00509), methyltransferase (aa 1453-1558, pfam08242), medium chain reductase/dehydrogenase-like family (aa 1990-2081 and 2044-2165, cl16912), KR domain (aa 2189-2344, pfam08659), and phosphopantetheine attachment site (pfam00550).

BdPKS22 is also found as a homologous protein in *B. elliptica*, *B. convoluta*, *B. galanthina*, *B. porri*, and *B. tulipae*. Strains Be9612 of *B. elliptica* and MUCL 31421 of *B. squamosa* exhibit high conservation percentages of 97.01% and 94.42%, respectively. However, strains MUCL 3234 and MUCL 3349 of *B. porri* show lower conservation percentages of 88.08%. For *B. squamosa*, a specific annotated protein was not identified, but a genomic region (RCTC02000012.1) was found that corresponds to the BdPks22 amino acid sequence (Table 5). This was the only case among the 10 *Botrytis* genomes where a homologous protein to BdPks22 was not found (Table 5).

The distribution of BdPKS22 in other fungi spans the *Sordariomyceta*, *Leotiomyceta*, and *Dothideomyceta* clades. In *Sordariomyceta*, PKS proteins are found in the *Leotiomycete* and *Sordariomycete* orders. Within *Leotiomycete*, *Heliotales* hosts one PKS protein in one organism, and *Xylariales* contains four PKS proteins among three organisms. *Leotiomycetes incertae sedis* also harbors one PKS protein. In *Leotiomyceta*, orders such as *Teloschistales* and *Pertusariales* exhibit PKS proteins. *Teloschistales* displays three PKS proteins across three organisms, while *Pertusariales* contains one PKS protein. In *Eurotiomycetes*, *Eurotiales* includes ten PKS proteins across seven organisms. In *Dothideomyceta*, *Pleosporales* stands out with thirteen PKS proteins identified within two organisms (Table 6).

Table 5. Identification of homologous proteins from BdPKS22-26 in other *Botrytis* species. * The protein accession number refers to the region of the genome that matches the amino acid sequence of the protein obtained from the T-BLAST result.

PKS	*Botrytis* Species	Strain	Protein Accession Number	Number of Amino Acids	Identity with *B. deweyae*
EAE98_002293 (BdPKS22)	*Botrytis elliptica*	Be9601	TGO80363.1	2513	96.73%
	Botrytis elliptica	Be9612	KAF7928242.1	2443	97.01%
	Botrytis convoluta	MUCL 11595	TGO65325.1	2633	90.31%
	Botrytis galanthina	MUCL 435	THV55759.1	2616	89.79%
	Botrytis porri	MUCL 3234	XP_038774872.1	2601	88.08%
	Botrytis porri	MUCL 3349	TGO92077.1	2601	88.08%
	Botrytis tulipae	Bt9001	TGO09113.1	2330	89.98%
	Botrytis paeoniae	Bp0003	TGO26315.1	2527	88.98%
	Botrytis squamosa	MUCL 31421	RCTC02000012.1 *	-	94.42%
EAE98_009027 (BdPKS23)	*Botrytis elliptica*	Be9601	TGO77401.1	2547	96.43%
	Botrytis elliptica	Be9612	KAF7911819.1	2562	95.87%
	Botrytis sinoallii	Bc23	XP_038753118.1	2543	95.77%
	Botrytis fragariae	BVB16	XP_037188393.1	2552	94.07%
	Botryotinia convoluta	MUCL 11595	TGO52589.1	2547	93.87%
	Botrytis hyacinthi	Bh0001	TGO38645.1	2562	92.70%
	Botrytis aclada	633	KAF7946733.1	2544	91.97%
	Botrytis paeoniae	Bp0003	TGO27521.1	2258	84.85%
	Botrytis galanthina	MUCL 435	THV49893.1	1884	86.19%
	Botrytis squamosa	MUCL 31421	RCTC02000008.1 *	-	98.43%

Table 5. *Cont.*

PKS	*Botrytis* Species	Strain	Protein Accession Number	Number of Amino Acids	Identity with *B. deweyae*
EAE98_009190 (BdPKS24)	*Botrytis elliptica*	Be9601	TGO80020.1	2335	97.29%
	Botrytis elliptica	Be9612	KAF7941341.1	2359	94.43%
	Botrytis squamosa	MUCL 31421	RCTC02000005.1 *	-	83.28%
	Botrytis aclada	633	RCSV01000004.1 *	-	78.53%
	Botrytis porri	MUCL 3234	RCTA01000006.1 *	-	72.12%
	Botryotinia globosa	MUCL 444	RCSZ01000002.1 *	-	75.22%
EAE98_010906 (BdPKS25)	*Botrytis paeoniae*	Bp0003	TGO20404.1	2621	91.95%
	Botryotinia globosa	MUCL 444	KAF7896114.1	2528	84.54%
	Botrytis elliptica	Be9601	TGO71777.1	2133	95.90%
	Botrytis elliptica	Be9612	KAF7923671.1	1808	97.11%
	Botrytis squamosa	MUCL 31421	RCTC02000002.1 *	-	84.75%
	Botrytis sinoallii	Bc 23	RCTB01000005.1 *	-	50.05%
EAE98_010943 (BdPKS26)	*Botrytis elliptica*	Be9601	TGO73554.1	2327	98.77%
	Botrytis elliptica	Be9612	KAF7923645.1	2327	98.64%
	Botrytis sinoallii	Bc 23	XP_038763137.1	2073	87.33%
	Botrytis galanthina	MUCL 435	THV54946.1	1623	86.56%
	Botrytis squamosa	MUCL 31421	RCTC02000002.1 *	-	95.29%
			RCTC02000005.1 *		54.23%
	Botrytis medusae	B555	JAHXJL010000007.1	-	89.03%
	Botryotinia globosa	MUCL 444	RCSZ01000001.1	-	86.14%

Table 6. Distribution of homologous proteins from BdPKS22-26 with an identity percentage > 50% and a coverage > 70% in fungi from genera other than *Botrytis*.

PKS	*Phylum*	Clade	Class	Order	Number of Proteins	Number of Organisms
BdPKS22	*Ascomycota*	*Sordariomyceta*	*Leotiomycete*	*Heliotales*	1	1
				Leotiomycetes incertae sedis	1	1
		Leotiomyceta	*Sordariomycete*	*Xylariales*	4	3
			Lecanoromycete	*Teloschistales*	3	3
				Pertusariales	1	1
			Eurotiomycete	*Eurotiales*	10	7
		Dothideomyceta	*Dothideomycetes*	*Pleosporales*	13	2
		TOTAL			**33**	**18**
BdPKS23	*Ascomycota*	*Sordariomycelta*	*Sordariomycete*	*Heliotales*	9	7
				Xylariales	32	25
				Xylariomycetidae incertae sedis	4	1
		Leotiomyceta	*Lecanoromycetes*	*Diaporthales*	4	3
				Lecanorales	2	2
				Teloschistales	5	4
				Peltigerales	1	1
				Pertusariales	1	1
			Eurotiomycetes	*Eurotiales*	3	2
		Dothideomyceta	*Dothideomycetes*	*Dothideomycetes incertae sedis*	1	1
		TOTAL			**62**	**47**

Table 6. *Cont.*

PKS	*Phylum*	Clade	Class	Order	Number of Proteins	Number of Organisms
BdPKS24	*Ascomycota*	*Leotiomyceta*	*Lecanoromycete*	*Sarrameanales*	1	1
				Trapeliales	1	1
				Pertusariales	1	1
				Teloschistales	2	2
			Eurotiomycete	*Onygenales*	1	1
				Eurotiales	2	1
		Sordariomyceta	*Sordariomycete*	*Xylariales*	3	2
		TOTAL			**11**	**9**
BdPKS25	*Ascomycota*	*Sordariomyceta*	*Leotiomycete*	*Heliotales*	8	7
				Leotiomycetes incertae sedis	1	1
			Sordariomycete	*Hypocreales*	6	5
				Microascales	3	2
				Xylariomycetidae incertae sedis	4	1
				Sordariales	6	6
		Dothideomyceta	*Dothideomycetes*	*Dothideomycetes incertae sedis*	5	3
				Pleosporales	4	3
		leotiomyceta	*Arthoniomycetes*	*Arthoniales*	1	1
			Eurotiomycetes	*Eurotiales*	5	4
			Lecanoromycetes	*Lecanorales*	1	1
		TOTAL			**44**	**34**
BdPKS26	*Ascomycota*	*Sordariomyceta*	*Leotiomycete*	*Helotiales*	8	6
			Sordariomycetes	*Hypocreales*	12	9
				Glomerellales	10	5
				Xylariales	53	39
				Sordariales	1	1
		Leotiomyceta	*Lecanoromycetes*	*Diaporthales*	2	1
				Magnaporthales	1	1
				Trapeliales	2	2
				Teloschistales	1	1
			Eurotiomycetes	*Eurotiales*	27	13
				Onygenales	36	2
		Dothideomyceta	*Dothideomycetes*	*Mytilinidiales*	2	1
				Pleosporomycetidae incertae sedis	1	1
				Pleosporales	1	1
				Botryosphaeriales	2	1
		TOTAL			**159**	**84**

Of the 18 homologous hits to BdPKS22 (Figure 7 and Supplementary Table S7), the phylogenetic distribution shows that BdPKS22 is closely related to *Sclerotinia nivalis* within the same subclade (subclade 2 of clade 1). All species in clade 1 belong to *Sordariomyceta*, except *Biscogniauxia marginata* and *Annulohypoxylon truncatum* from *Leotiomyceta*, closely related to *Oidiodendron maius*, *Penicillium occitanis*, and *Talaromyces rugulosus*. Additionally, BdPKS22 shares a clade with species of the genera *Xanthoria*, *Teloschistes*, and *Aspergillus* (Figure 7).

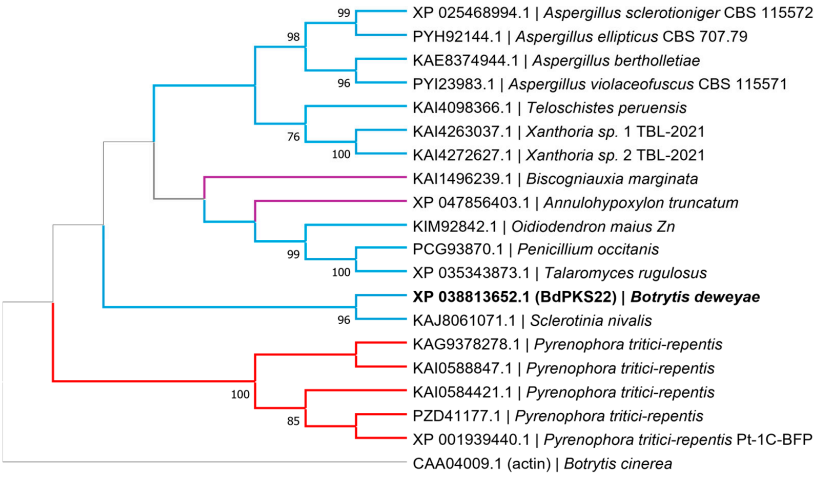

Figure 7. Phylogenetic tree of XP_038813652.1 protein (BdPKS22) from *B. deweyae* and homologous protein sequences from other fungal species. The phylogenetic tree was inferred using the maximum likelihood method via MEGA 11 software, and bootstrap values from 1000 trials are indicated at each branch node. Protein sequences were selected after running a similarity search by BLASTP using XP_038813652.1 as the query sequence, excluding the *Botrytis* and *Botryotinia* taxids (33196 and 40558, respectively), and filtering the results based on percent identity > 50%, coverage > 70%, and bit-score > 50. The top 18 hits were retrieved for sequence alignment and phylogenetic analysis. NCBI accession numbers of each sequence are shown. Actin (CAA04009.1) of *B. cinerea* was used as the outgroup. Taxonomic distribution is highlighted by different colors: *Sordariomycetes* (blue), *Dothideomycetes* (red), and *Leotiomycetes* (violet).

3.4.3. Description of EAE98_009027 Gene in *B. deweyae*—Bdpks23

The gene cluster containing the EAE98_009027 gene, referred to as *Bdpks23* in this study, consists of six putative biosynthetic genes (Figure 5). The gene EAE98_009027 encodes the protein XP_038807018.1, which is composed of 2537 amino acids and contains multiple functional domains: beta-ketoacyl synthase N-terminal (aa 16-266, pfam00109), beta-ketoacyl synthase C-terminal (aa 275-393, pfam02801), ketoacyl-synthetase C-terminal extension (aa 398-528, pfam16197), acyl transferase (aa 556-887, cl08282), polyketide synthase dehydratase (aa 944-1251, pfam14765), methyltransferase (aa 1444-1548, pfam08242), alcohol dehydrogenase GroES-like (aa 1866-1916, cl17172), zinc-binding dehydrogenase (aa 1981-2072, pfam00107), KR domain (aa 2190-2348, pfam08659), and phosphopantetheine attachment site (aa 2467-2525, pfam00550).

Additionally, this cluster includes other genes: EAE98_009028 (543 amino acids, FAD binding domain, aa 101-239, pfam01565), EAE98_009029 (303 amino acids, crotonase/enoyl-CoA hydratase superfamily domain, aa 37-263, cl23717), EAE98_009030 (506 amino acids, FAD binding domain, aa 66-206, cl19922), EAE98_009031 (552 amino acids, cytochrome P450 domain, aa 103-514, cl12078), and EAE98_009032 (438 amino acids, major facilitator superfamily domain, aa 53-391, pfam07690).

The comparative analysis of protein sequence identities among various *Botrytis* species strains relative to the reference protein XP_038807018.1 (BdPKS23) reveals intriguing insights into the genetic diversity within this fungal genus. *B. squamosa* strain MUCL 31421 exhibits the highest identity at 98.43%, indicating a close genetic relationship to the reference protein. Despite the lack of an annotated protein in *B. squamosa*, a genomic region likely involved in the biosynthesis of this protein was identified. *B. elliptica* strains Be9601 and Be9612 follow closely with identities of 96.43% and 95.87%, respectively, suggesting substantial genetic similarity to BdPKS23. *B. sinoallii* strain Bc23 and *B. fragariae* strain BVB16 exhibit identities of 95.77% and 94.07%, respectively. However, *B. paeoniae* strain Bp0003 shows the lowest identity at 84.85%, indicating greater genetic divergence (Table 5).

The distribution of BdPKS23 in other fungi spans the *Ascomycota phylum*, specifically within the *Sordariomyceta*, *Leotiomyceta*, and *Dothideomyceta* classes. In *Sordariomyceta*,

PKS proteins are found in the *Heliotales* (9 PKS proteins in 7 organisms) and *Xylariales* (32 PKS proteins in 25 organisms) orders. *Xylariomycetidae incertae sedis* and *Diaporthales* each contain four PKS proteins found in one and three organisms, respectively. In *Leotiomyceta*, *Lecanorales* contains two PKS proteins found in two organisms, *Teloschistales* exhibits five PKS proteins in four organisms, and *Peltigerales* and *Pertusariales* each contain one PKS protein in one organism. In *Eurotiomycetes*, *Eurotiales* includes three PKS proteins in two organisms. In *Dothideomyceta*, *Dothideomycetes incertae sedis* contains one PKS protein in one organism (Table 6).

The phylogenetic distribution of BdPKS23 is noteworthy as it shows that among the 51 top protein sequences (Figure 8 and Supplementary Table S8), all belong to the *Sordariomyceta* clade except for three *Leotiomyceta* species (genera *Talaromyces* and *Letrouitia*) and one species, *Zopfia rhizophila*, belonging to *Dothideomyceta*. These species are found in a different clade from the one in which BdPKS23 is located, sharing a clade with species of the genera *Lachnellula*, *Hypoxylon*, *Cudoniella*, and *Xylaria*, among others. In clade 2, BdPKS23 is closely related to species of the genera *Mollisia*, *Xylariaceae*, *Icmadophila*, *Teloschistes*, *Mycoblastus*, *Alectoria*, and *Rutstroemia* (Figure 8).

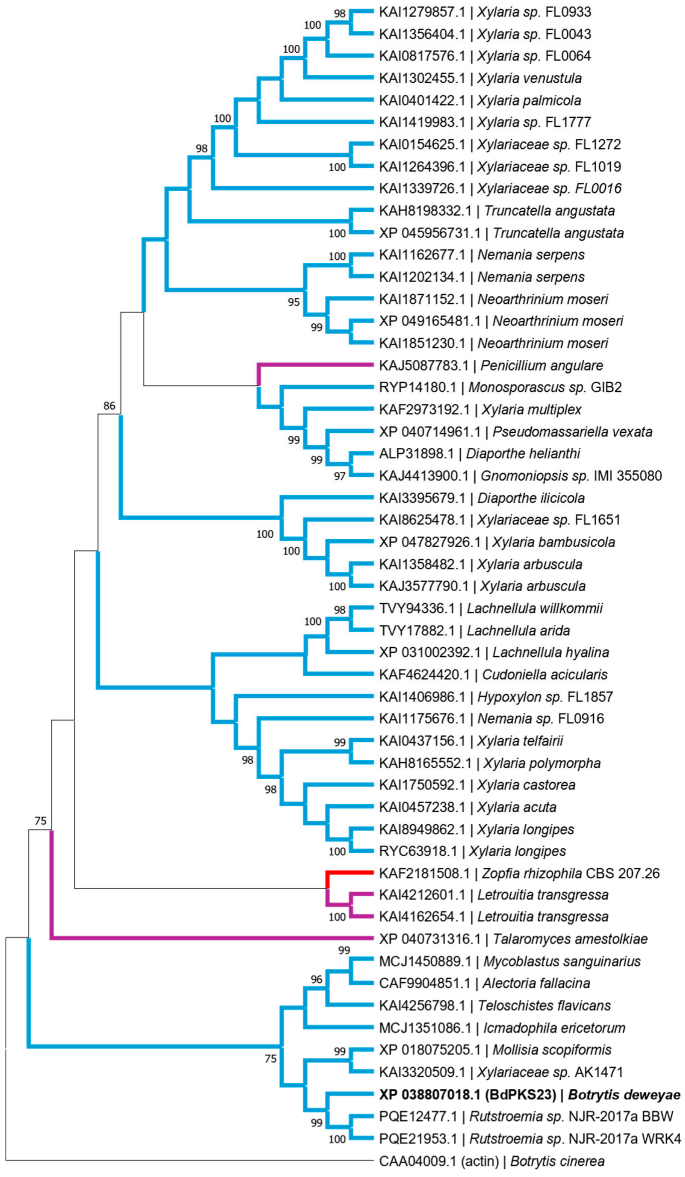

Figure 8. Phylogenetic tree of XP_038807018.1 protein from *B. deweyae* and homologous protein sequences from other fungal species. The phylogenetic tree was inferred using the maximum

likelihood method via MEGA 11 software, and bootstrap values from 1000 trials are indicated at each branch node. Protein sequences were selected after running similarity search by BLASTP using XP_038807018.1 as query sequence, excluding the *Botrytis* and *Botryotinia* taxids (33196 and 40558, respectively), and filtering the results based on percent identity > 50%, coverage > 70%, and bit-score > 50. The top 51 hits were retrieved for sequence alignment and phylogenetic analysis. NCBI accession number of each sequence is shown. Actin (CAA04009.1) of *B. cinerea* was used as outgroup. Different clades and subclades are delimited by color of the branches: *Sordariomycetes* (blue), *Dothideomycetes* (red), *Leotiomycetes* (violet).

3.4.4. Description of EAE98_009190 Gene in *B. deweyae*—Bdpks24

The gene cluster containing the EAE98_009190 gene, referred to as *Bdpks*24 in this study, consists of three putative biosynthetic genes (Figure 5). The gene EAE98_009188 encodes a protein with 311 amino acids, containing the domains metallo-beta-lactamase superfamily (aa 70-232, pfam00753) and hydroxyacylglutathione hydrolase C-terminus (aa 233-310, pfam16123). The gene EAE98_009190 encodes the protein XP_038806877.1, which is composed of 2346 amino acids and contains multiple functional domains: beta-ketoacyl synthase N-terminal (aa 11-249, pfam00109), beta-ketoacyl synthase C-terminal (aa 258-376, pfam02801), ketoacyl-synthetase C-terminal extension (aa 380-514, pfam16197), acyl transferase (aa 543-856, cl08282), polyketide synthase dehydratase (aa 931-1228, pfam14765), alcohol dehydrogenase GroES-like (aa 1644-1696, cl17172), zinc-binding dehydrogenase (aa 1759-1849, pfam00107), KR domain (aa 1991-2163, pfam08659), and phosphopantetheine attachment site (aa 2277-2334, pfam00550). Additionally, the gene EAE98_009191 encodes a protein with 551 amino acids, featuring an amidase domain (aa 81-531, cl18951).

The comparative analysis of protein sequence identities among various *Botrytis* strains highlights the genetic diversity within this fungal genus. *B. elliptica* strains Be9601 and Be9612 show high sequence identities of 97.29% and 94.43%, respectively, compared to the reference protein XP_038806877.1 (BdPKS24). However, *B. squamosa* strain MUCL 31421 exhibits a lower identity of 83.28%, indicating greater genetic divergence. Similarly, *B. aclada* strain 633, *B. porri* strain MUCL 3234, and *B. globosa* strain MUCL 444 display identities of 78.53%, 72.12%, and 75.22%, respectively. These similarities were identified based on draft genome sequences, as no homologous proteins for BdPKS24 were annotated in the genomes of *B. squamosa*, *B. aclada*, *B. porri*, and *B. globosa* (Table 5).

The distribution of BdPKS24 protein across the *Ascomycota phylum*, specifically within the *Leotiomyceta* and *Sordariomyceta* classes, reveals various taxonomic orders harboring these proteins. In the *Leotiomyceta* clade, the orders *Sarrameanales*, *Trapeliales*, *Pertusariales*, and *Teloschistales* each contain one PKS protein in one organism, suggesting a diverse distribution of PKS enzymes within this class, reflecting potential ecological adaptations and metabolic diversity. In the *Eurotiomyceta* clade, the *Onygenales* order contains one PKS protein in one organism, while the *Eurotiales* order harbors two PKS proteins in one organism, indicating a comparatively lower abundance of PKS proteins within *Eurotiomycetes*. In the *Sordariomyceta* clade, the *Sordariomycete* order *Xylariales* exhibits three PKS proteins across two organisms, suggesting a moderate abundance of PKS enzymes within this order (Table 6).

The phylogenetic distribution of BdPKS24 is noteworthy as, among the four top protein sequences (Figure 9 and Supplementary Table S9), all belong to the *Leotiomyceta* clade except for *Monosporascus* sp. mg162, which belongs to the *Sordariomyceta* clade along with *Botrytis deweyae*. BdPKS24 is also related to species of the genera *Loxospora*, *Trapelia*, and *Penicillium* (Figure 9).

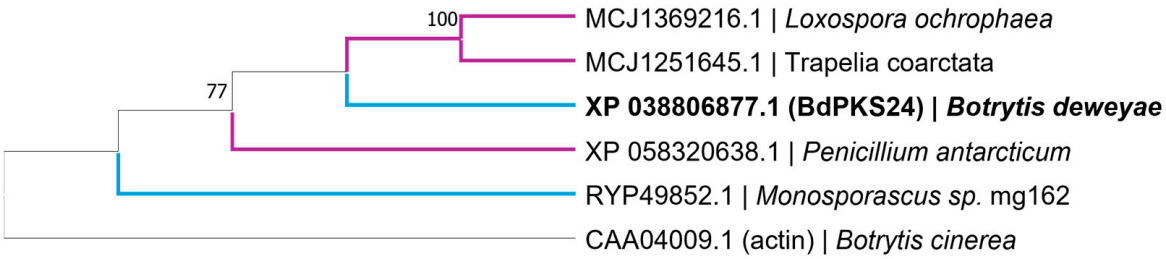

Figure 9. Phylogenetic tree of XP_038806877.1 protein (BdPKS24) from *B. deweyae* and homologous protein sequences from other fungal species. The phylogenetic tree was inferred using the maximum likelihood method via MEGA 11 software, and bootstrap values from 500 trials are indicated at each branch node. Protein sequences were selected after running a similarity search by BLASTP using XP_038806877.1 as the query sequence, excluding the *Botrytis* and *Botryotinia* taxids (33196 and 40558, respectively), and filtering the results based on percent identity > 50%, coverage > 70%, and bit-score > 50. The top 4 hits were retrieved for sequence alignment and phylogenetic analysis. NCBI accession numbers of each sequence are shown. Actin (CAA04009.1) of *B. cinerea* was used as the outgroup. Taxonomic distribution is highlighted by different colors: *Sordariomycetes* (blue) and *Leotiomycetes* (violet).

3.4.5. Description of EAE98_010906 Gene in *B. deweyae*—Bdpks25

The gene cluster containing the EAE98_010906 gene, referred to as *Bdpks25* in this study, consists of a single putative biosynthetic gene, EAE98_010906, which encodes the protein XP_038805042.1 with 2208 amino acids (Figure 5). This protein contains multiple functional domains: beta-ketoacyl synthase N-terminal (aa 122-288, pfam00109), beta-ketoacyl synthase C-terminal (aa 296-411, pfam02801), acyl transferase (aa 565-826, cl08282), Hotdog superfamily (aa 979-1214, cl00509), phosphopantetheine attachment site (aa 1280-1338 and 1384-1439, pfam00550), helix–turn–helix (aa 1497-1580, pfam18558), methyltransferase (aa 1682-1782, pfam08242), and BD-FAE (aa 1894-2155, pfam20434).

The comparison of protein sequence identities among various *Botrytis* strains provides insights into the genetic diversity within this fungal genus. *B. paeoniae* strain Bp0003 exhibits a sequence identity of 91.95% to the reference sequence XP_038805042.1 (BdPKS25), indicating a relatively close genetic relationship. Similarly, *B. globosa* strain MUCL 444 shows a sequence identity of 84.54% to its reference sequence KAF7896114.1. In contrast, *B. elliptica* strains Be9601 and Be9612 demonstrate high sequence identities of 95.90% and 97.11% to their respective reference sequences (TGO71777.1 and KAF7923671.1). *B. squamosa* strain MUCL 31421 displays a sequence identity of 84.75% to its reference sequence RCTC02000002.1. However, in *B. squamosa* and *B. sinoallii*, the similarity was based on draft genome sequences that correspond to the BdPKS25 protein, as no homologous protein for BdPKS25 is annotated in their genomes (Table 5).

The distribution of BdPKS25 protein within the *Ascomycota phylum*, focusing on the *Sordariomyceta* and *Dothideomyceta* clades, demonstrates a diverse array of taxonomic orders harboring these proteins. In the *Sordariomyceta* clade, the *Leotiomycete* order *Heliotales* contains eight PKS proteins across seven organisms, and *Leotiomycetes incertae sedis* hosts one PKS protein in one organism. Within the *Sordariomycete* order *Hypocreales*, six PKS proteins are identified across five organisms, while *Microascales* exhibits three PKS proteins in two organisms. The *Xylariomycetidae incertae sedis* order contains four PKS proteins found in one organism. *Sordariales* contains six PKS proteins across six organisms. In the *Dothideomyceta* clade, *Dothideomycetes incertae sedis* contains five PKS proteins in three organisms, and *Pleosporales* exhibits four PKS proteins in three organisms. Additionally, the *Arthoniomycetes* order *Arthoniales* contains one PKS protein in one organism. In the *Leotiomyceta* clade, *Eurotiomycetes* order *Eurotiales* contains five PKS proteins across four organisms, suggesting significant metabolic diversity. *Lecanoromycetes* of order *Lecanorales* also contains one PKS protein in one organism (Table 6).

Regarding the phylogenetic relationship of BdPKS25, among the top 25 protein sequences (Figure 10 and Supplementary Table S10), all belong to the *Sordariomyceta* clade except for *Bathelium mastoideum*, *Viridothelium virens*, and *Bipolaris maydis*, which belong to the *Dothideomyceta* clade, and *Aspergillus* sp., which belongs to the *Leotiomyceta* clade. These latter three are listed as independent branches within the phylogenetic tree. BdPKS25 is closely related to *Ciborinia camelliae* and shares a subclade with species of the genera *Lachnellula* and *Rutstroemia* sp. NJR-2017a BVV2 (Figure 10).

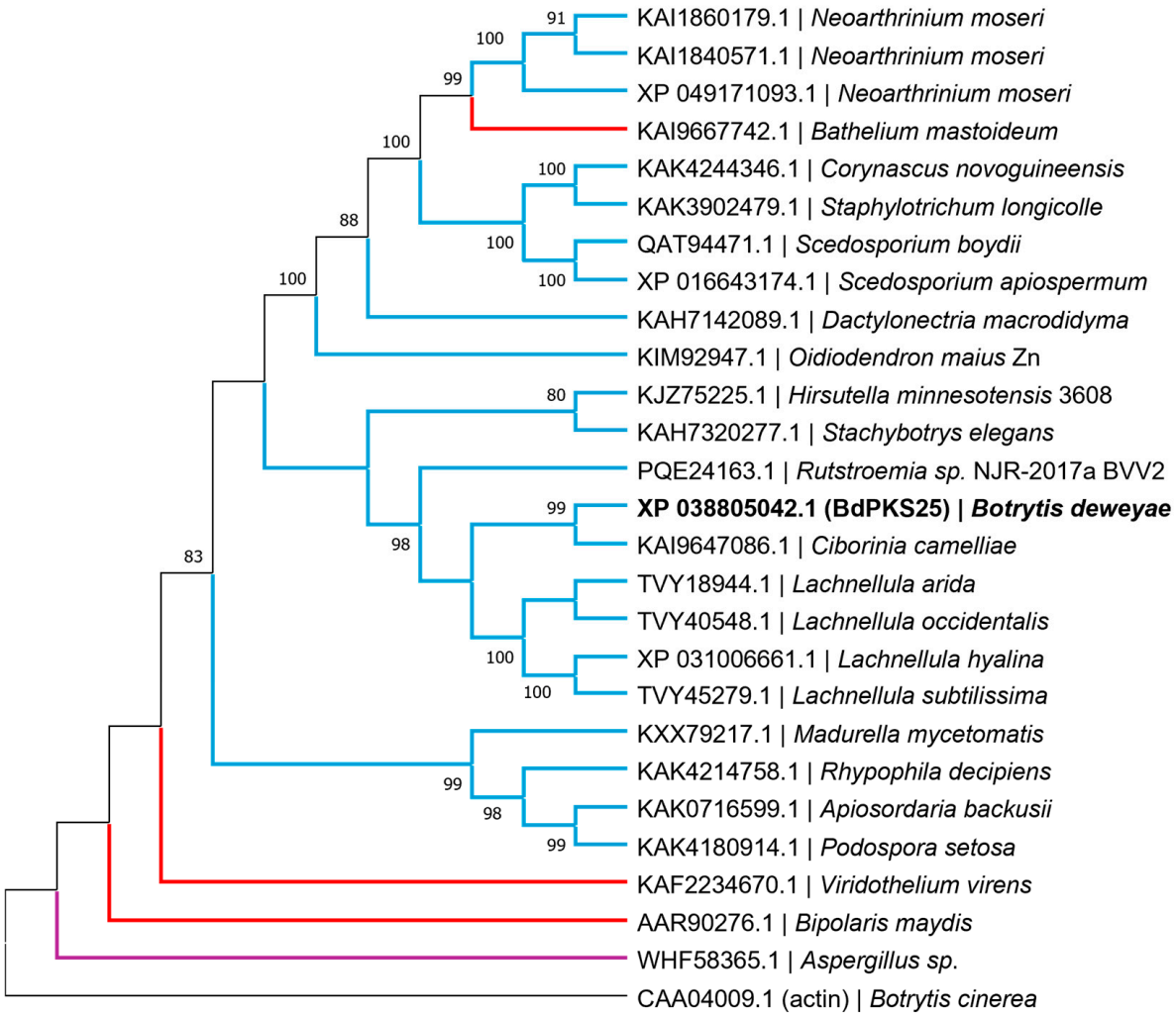

Figure 10. Phylogenetic tree of XP_038805042.1 protein (BdPKS25) from *B. deweyae* and homologous protein sequences from other fungal species. The phylogenetic tree was inferred using the maximum likelihood method via MEGA 11 software, and bootstrap values from 1000 trials are indicated at each branch node. Protein sequences were selected after running a similarity search by BLASTP using XP_038805042.1 as the query sequence, excluding the *Botrytis* and *Botryotinia* taxids (33196 and 40558, respectively), and filtering the results based on percent identity > 50%, coverage > 70%, and bit-score > 50. The top 25 hits were retrieved for sequence alignment and phylogenetic analysis. NCBI accession numbers of each sequence are shown. Actin (CAA04009.1) of *B. cinerea* was used as the outgroup. Taxonomic distribution is highlighted by different colors: *Sordariomycetes* (blue) and *Leotiomycetes* (violet) and *Dothideomycetes* (red).

3.4.6. Description of EAE98_010943 Gene in *B. deweyae*—Bdpks26

The gene cluster containing the EAE98_010943 gene, referred to as *Bdpks26* in this study, consists of six putative biosynthetic genes (Figure 5). The gene EAE98_010940 encodes a protein with 294 amino acids, containing a short-chain dehydrogenase domain (aa 5-191, pfam00106). The gene EAE98_010941 encodes a protein with 513 amino acids, showing an acyltransferase family domain (aa 68-448). The gene EAE98_010943, named in this work as *Bdpks26*, encodes the protein XP_038805079.1, which has 2353 amino acids and contains multiple functional domains: beta-ketoacyl synthase N-terminal (aa 14-265, pfam00109), beta-ketoacyl synthase C-terminal (aa 274-392, pfam02801), ketoacyl-synthetase C-terminal extension (aa 396-517), acyl transferase (aa 546-878, cl08282), polyketide synthase dehydratase (aa 933-1236, pfam14765), KR domain (aa 1967-2145, pfam08659), and phosphopantetheine attachment site (aa 2272-2328, pfam00550). Additionally, the gene EAE98_010945 encodes a protein with 252 amino acids, containing a Rossmann-fold NAD(P)+-binding protein domain (aa 8-200 and 32-246, cl21454), and the gene EAE98_010949 encodes a protein with 576 amino acids, featuring a flavin-binding monooxygenase-like domain (aa 12-554, cl30939).

The comparison of protein sequence identities among various *Botrytis* strains provides valuable insights into the genetic diversity within this fungal genus. *B. elliptica* strains Be9601 and Be9612 exhibit remarkably high sequence identities of 98.77% and 98.64% to their respective reference sequences (TGO73554.1 and KAF7923645.1). *B. sinoallii* strain Bc23 shows a sequence identity of 87.33% to its reference sequence XP_038763137.1. *B. galanthina* strain MUCL 435 displays a sequence identity of 86.56% to its reference sequence THV54946.1. *B. squamosa* strain MUCL 31421 demonstrates a high sequence identity of 95.29% to its reference sequence RCTC02000002.1 and a lower identity of 54.23% to the second reference sequence RCTC02000005.1. However, in *B. squamosa*, the similarity was based on draft genome sequences that correspond to the BdPKS26 protein, as no homologous protein for BdPKS26 is annotated in its genome (Table 5).

The distribution of BdPKS26 protein within the *Ascomycota phylum*, focusing on the *Sordariomyceta*, *Leotiomyceta*, and *Dothideomyceta* clades, reveals a diverse array of taxonomic orders harboring these proteins. In the *Sordariomyceta* clade, within the *Leotiomycete* order *Helotiales*, eight PKS proteins are distributed across six organisms. The *Sordariomycetes* order *Hypocreales* exhibits the highest abundance of PKS proteins, with 12 identified across nine organisms. Additionally, *Glomerellales* contains 10 PKS proteins in 5 organisms, while *Xylariales* exhibits 53 PKS proteins across 39 organisms, indicating its significant role in secondary metabolite production. Other orders such as *Sordariales*, *Diaporthales*, and *Magnaporthales* also contain a smaller number of PKS proteins. In the *Leotiomyceta* clade, *Lecanoromycetes* order *Trapeliales* and *Teloschistales* each contain two and one PKS proteins, respectively, distributed in the same number of organisms. In the *Eurotiomycetes* class, *Eurotiales* contains 27 PKS proteins across 13 organisms, while *Onygenales* exhibits 36 PKS proteins in only 2 organisms. Within the *Dothideomyceta* clade, *Dothideomycetes* contains two PKS proteins in *Mytilinidiales* and one PKS protein each in *Pleosporomycetidae incertae sedis*, *Pleosporales*, and *Botryosphaeriales* (Table 6).

The phylogenetic distribution of BdPKS26 with the top 55 homologous proteins (Figure 11 and Supplementary Table S11) shows a predominance of species belonging to the Sordariomyceta clade, except for *Pleomassaria siparia* (subclade 1 from clade 1), *Mytilinidion resinicola* (subclade 1 from clade 1), and *Glonium stellatum* (clade 2) from the *Dothideomyceta* clade. Additionally, species of the genus *Aspergillus* (subclades 1 and 2 from clade 1) and *Ophidiomyces ophidiicola* (clade 2) belong to the *Leotiomyceta* clade. BdPKS26 is closely related to *Rutstroemia* sp. NJR-2017a BVV2 and *Bisporella* sp. PMI 857 in the same subclade (Figure 11).

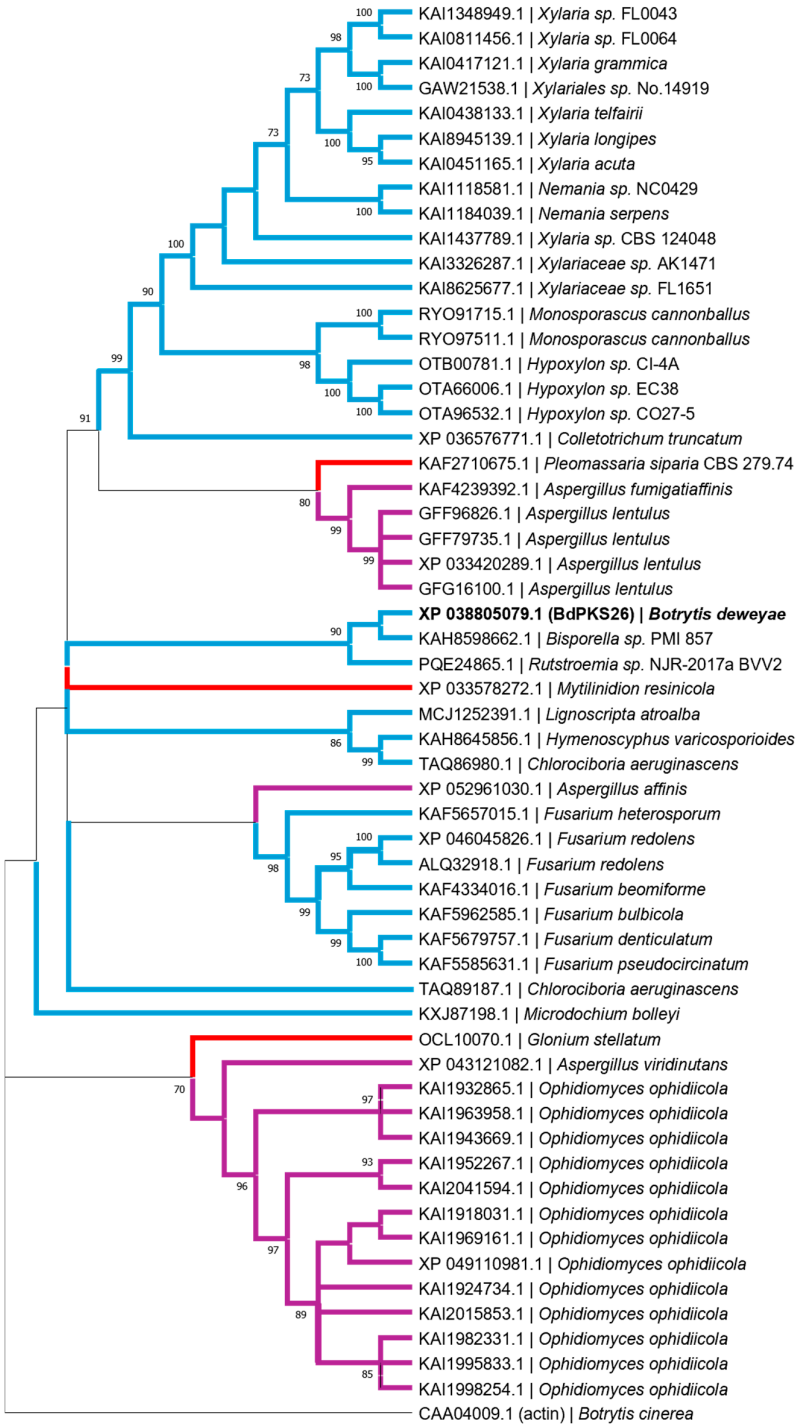

Figure 11. Phylogenetic tree of XP_038805079.1 protein (BdPKS26) from *B. deweyae* and homologous protein sequences from other fungal species. The phylogenetic tree was inferred using the maximum likelihood method via MEGA 11 software, and bootstrap values from 500 trials are indicated at each branch node. Protein sequences were selected after running a similarity search by BLASTP using XP_038805079.1 as the query sequence, excluding the *Botrytis* and *Botryotinia* taxids (33196 and 40558, respectively), and filtering the results based on percent identity > 50%, coverage > 70%, and bit-score > 50. The top 55 hits were retrieved for sequence alignment and phylogenetic analysis. NCBI accession numbers of each sequence are shown. Actin (CAA04009.1) of *B. cinerea* was used as the outgroup. Taxonomic distribution is highlighted by different colors: *Sordariomycetes* (blue) and *Leotiomycetes* (violet) and *Dothideomycetes* (red).

4. Discussion

The comparative analysis of secondary metabolites between *B. deweyae* B1 and *B. cinerea* B05.10 reveals significant insights into their metabolic diversity, especially in polyketide and terpene biosynthesis. *B. deweyae* B1 demonstrates a higher number of polyketide clusters (11 gene clusters) compared to *B. cinerea* B05.10 (10 gene clusters), suggesting potential differences in their secondary metabolite profiles. Similarly, *B. deweyae* B1 exhibits more clusters related to terpene biosynthesis (six gene clusters) compared to *B. cinerea* B05.10 (five gene clusters), indicating a nuanced metabolic variation between these closely related species.

Within the terpene biosynthesis clusters of *B. deweyae* B1, several key genes have been identified, such as *Bcerg*9, *Bcstc*4, *Bcbot*2, and an unannotated terpene gene. While most of these genes have counterparts in *B. cinerea* B05.10, notable differences exist in their regulatory roles. For instance, *Bcstc*7 and *Bcbot*2 in *B. cinerea* B05.10 regulate the synthesis of eremophilane and botryane metabolites, respectively [63,65]. In contrast, *B. deweyae* B1 lacks *Bcstc*2 and *Bcstc*5 but possesses an unannotated terpene gene also identified in *B. cinerea* B05.10.

Further analysis of the relationships between these genes reveals interesting clustering patterns. Genes like *Bdstc*4, *Bcstc*3, *Bdbot*2, and *Bcstc*5 are closely related, forming part of the same subclade, indicating functional similarities in terpene biosynthesis. Conversely, genes like *Bcpax*1, *Bccoq*1, *Bcerg*20, and the unannotated gene form another subclade, suggesting potential functional diversification within the terpene biosynthesis pathway.

The presence of putative unannotated genes in *B. deweyae* B1 suggests the existence of novel metabolic pathways yet to be characterized. The analysis of these unannotated clusters reveals polyprenyl synthetase domains, indicating potential roles in specialized metabolite synthesis. However, validation would need to be carried out for confirmation. These genes have homologs in other *Botrytis* species, suggesting conservation and importance across the genus.

Comparative genomic analysis with other *Botrytis* species highlights the genetic relationships and evolutionary divergence within the genus. Strains like *B. cinerea* T4, BcDW1, and B05.10 exhibit high genetic identity with *B. deweyae* B1, indicating close evolutionary ties. However, strains from different species show varying levels of identity, reflecting the genetic diversity within the *Botrytis* genus.

The taxonomic distribution of fungi, particularly within the *Ascomycota* and *Basidiomycota phyla*, provides a comprehensive overview of fungal diversity and the distribution of the unannotated gene EAE98_008016 from *B. deweyae*. The abundance of proteins and organisms within different classes, orders, and unclassified groups underscores the functional and ecological diversity of fungi. This diversity highlights evolutionary complexity and the potential for discovering novel metabolites with unique biological activities. In addition, this putative unannotated gene is closely related to established terpene biosynthetic pathways, as evidenced by a similarity score of 0.18 to BGC0000673, which is involved in the synthesis of pimara-8(14),15-diene in *Aspergillus nidulans*. It also shows a similarity score of 0.17 to BGC0002320, responsible for producing conidiogenone in *Penicillium rubens*. Furthermore, a score of 0.12 connects it to BGC0002604, associated with sartorypyrone A in *Aspergillus felis*. These connections suggest that the gene may play a significant role in terpene biosynthesis, highlighting its potential importance in the ecological and metabolic dynamics of *B. deweyae*.

The distribution of polyketide synthase (PKS) gene clusters in *B. deweyae* reveals significant genetic diversity and evolutionary history compared to *B. cinerea*. Previous studies identified 21 PKS genes in *B. cinerea*, with only some forming gene clusters [11,20]. These include *Bcboa*6/*Bcboa*9, *Bcpks*13, *Bcpks*1, *Bcpks*21, *Bcpks*16, *Bcchs*1, *Bcpks*10, *Bcpks*8, *Bcpks*2, and *Bcpks*15. However, in *B. deweyae*, clusters corresponding to *Bcboa*6/*Bcboa*9, *Bcpks*1, *Bcchs*11, *Bcpks*8, and *Bcpks*2 were not identified. Additionally, several non-cluster-forming PKS genes in *B. cinerea* had no homologs in *B. deweyae*, except for *Bcpks*5, which showed potential presence but lacked proper annotation.

New potential PKS genes were identified in *B. cinerea* B05.10, including BCIN_09g06350, BCIN_01g00450, BCIN_04g00210, BCIN_03g06470, BCIN_09g06360, BCIN_08g02570, BCIN_08g02560, and BCIN_12g03250 [11]. These genes showed various domain compositions, with some lacking typical PKS domains. Homologous proteins for these genes were identified, showing varying degrees of similarity across different fungal species.

In *B. deweyae*, new putative gene clusters named *Bdpks*22-26 were discovered, not previously found in any *Botrytis* genome. These clusters, along with the 21 described in *B. cinerea*, form two distinct phylogenetic groups. The phylogenetic analysis reveals the presence of novel PKS genes in *B. deweyae*, indicating evolutionary divergence and potential new metabolic pathways. Within the first clade, *Bdpks*22 is closely related to *Bcpks*1, *Bcpks*21, and other PKS genes in *B. cinerea*. This clade also includes *Bcboa*9, *Bdpks*23, *Bdpks*24, and *Bdpks*26, suggesting functional similarities and evolutionary relationships. In the second clade, *Bdpks*25 is closely related to *Bcpks*16, *Bcpks*18, and *Bcpks*19, indicating potential functional similarities. Other PKS genes in this clade, such as *Bcpks*17 and *Bcpks*20, show more distant relationships, suggesting diverse roles in secondary metabolism.

The genetic diversity of PKS genes in *B. deweyae* highlights its varied metabolic functions and adaptive capabilities. High sequence identities were observed in some strains, indicating close genetic relationships, while others exhibited lower identities, suggesting greater genetic divergence. This diversity underscores the potential for varied functions and roles in secondary metabolism, critical for ecological success and adaptability.

The Bdpks22 in question is likely involved in polyketide biosynthesis, as suggested by its similarity to characterized gene clusters. With a score of 0.32 to BGC0002191 (producing prolipyrone B and gibepyrone D in *Fusarium graminearum*), and 0.30 to BGC0002155 (nectriapyrone compounds in *Pyricularia oryzae*), it may contribute to metabolites with diverse biological activities. Additionally, the connection to BGC0001252 indicates potential for producing bioactive polyketides that impact ecological interactions. The gene Bdpks23 is likely involved in polyketide biosynthesis, as indicated by its similarity scores. With a score of 0.33 to BGC0002525, associated with compounds like fusarubin and lucilactaene in *Fusarium* sp., it suggests a potential role in producing bioactive metabolites. Additionally, the scores of 0.33 and 0.32 with BGC0002194 (epipyrone A in *Epicoccum nigrum*) and BGC0002191 (prolipyrone B and gibepyrone D in *Fusarium graminearum*) further support its implication in polyketide biosynthesis. The gene Bdpks24 is likely associated with the biosynthesis of polyketides and non-ribosomal peptides, as indicated by its similarity scores. With a score of 0.29 to BGC0000046, which produces depudecin in *Alternaria brassicicola*, it suggests potential involvement in generating bioactive metabolites. Additionally, there are scores of 0.28 and 0.27 with BGC0002228 and BGC0002227, linked to non-ribosomal peptides in *Colletotrichum incanum* and *Aspergillus luchuensis*, respectively. The gene Bdpks25 is likely involved in polyketide biosynthesis, as suggested by its similarity scores. With a score of 0.20 to BGC0001284, which is associated with alternariol in *Parastagonospora nodorum*, it indicates a potential role in producing bioactive compounds. Additionally, the score of 0.20 with BGC0002175 (YWA1 in *Aspergillus oryzae*) and BGC0000107 (naphthopyrone in *Aspergillus nidulans*) further supports its involvement in diverse metabolic pathways. Finally, the Bdpks26 gene is likely associated with the biosynthesis of polyketides and terpenes, as indicated by its similarity scores. With a score of 0.26 to BGC0001264, linked to betaenones A, B, and C in *Phoma betae*, it suggests a role in producing bioactive polyketide compounds. Additionally, the score of 0.24 with BGC0002266, associated with calidoustene A, B, and C in *Aspergillus calidoustus*, highlights its potential involvement in terpene biosynthesis. However, this analysis is a predictive study where further analyses are necessary to identify the metabolites in which these new putative biosynthetic clusters present in *B. deweyae* are involved.

On the other hand, comparative analysis of PKS sequences among various *Botrytis* strains highlighted genetic diversity within the genus. Strains of *B. deweyae* showed varying degrees of similarity to reference sequences, with some strains exhibiting high sequence identity similarities, indicating close relationships, and others showing lower identity simi-

larities, suggesting greater divergence. The phylogenetic distribution of PKS proteins across different fungal species provided insights into their evolutionary relationships. *Botrytis* PKS proteins were found within the *Sordariomyceta*, *Leotiomyceta*, and *Dothideomyceta* clades, indicating wide distribution across different taxonomic orders. This broad distribution reflects evolutionary complexity and diverse ecological roles of these enzymes.

5. Conclusions

The distribution of PKS and terpene gene clusters in *B. deweyae* provides valuable insights into the genetic diversity and evolutionary history of this fungus. The absence of certain clusters found in *B. cinerea* and the discovery of new putative clusters in *B. deweyae* suggest divergent evolutionary paths in secondary metabolism between these two species. The genetic diversity observed within *B. deweyae* PKS genes highlights the potential for varied metabolic functions and adaptive capabilities within this species.

Comparative analysis and phylogenetic distribution underscore the evolutionary relationships and wide distribution of PKS genes across different fungal taxa. Understanding the genetic basis of secondary metabolism in *Botrytis* species has implications for disease management strategies in agriculture and provides opportunities for the discovery of novel bioactive compounds.

The identification of novel putative PKS and terpene biosynthesis gene clusters in *B. deweyae* highlights its potential for producing unique secondary metabolites. These metabolites could have various applications in agriculture, medicine, and biotechnology. Understanding the biosynthesis pathways of these compounds could lead to the development of new fungicides or pharmaceuticals.

The genetic diversity observed in *B. deweyae* suggests that this species may have a greater capacity for adaptation and survival in diverse environmental conditions. Future research should focus on characterizing the functions of the unannotated genes and novel clusters identified in *B. deweyae*. Functional genomics approaches, such as gene knockouts and overexpression studies, could elucidate the roles of these genes in secondary metabolism. Additionally, comparative genomics with other *Botrytis* species and related fungi could uncover the evolutionary origins and diversification of these metabolic pathways.

Comparative analysis of secondary metabolite gene clusters in *B. deweyae* and *B. cinerea* has revealed significant genetic and metabolic diversity. The discovery of novel PKS and terpene biosynthesis gene clusters in *B. deweyae* underscores its potential for producing unique secondary metabolites. These findings enhance our understanding of the genetic basis of secondary metabolism in *Botrytis* species and provide a foundation for future research into the ecological and biotechnological applications of these compounds.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agronomy14112747/s1, Figure S1: Synteny plot between *B. deweyae* B1 and *B. cinerea* B05.10, showing the BGCs that were collinear with each other. Table S1: BGCs originated by antiSMASH fungal version from *B. deweyae* B1 genome. Table S2: BGCs originated by antiSMASH fungal version from *B. cinerea* B05.10 genome. Table S3: BUSCO analysis for *B. deweyae* B1 and *B. cinerea* B05.10. Table S4: Top 55 homologous proteins to XP_038807933, Table S5: PKS domains of all the putative PKSs identified in *B. cinerea* and *B. deweyae*. Table S6: Distribution of all the putative PKSs described by Suarez et al. in 2024 among other fungi. Table S7: Top 18 homologous proteins to BdPKS22 in other fungi. Table S8: Top 51 homologous proteins to BdPKS23 in other fungi. Table S9: Top 4 homologous proteins to BdPKS24 in other *Fungi*. Table S10: Top 25 homologous proteins to BdPKS25 in other fungi. Table S11: Top 55 homologous proteins to BdPKS26 in other fungi.

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