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Analysis of 21 *Apiospora* genomes reveals a genus with tremendous synthesis potential for carbohydrate-active enzymes and secondary metabolites

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Abstract

The genus *Apiospora* has been relatively understudied in comparison to other filamentous fungi species, despite indications from previous research that this genus could yield valuable discoveries of novel enzymes and secondary metabolites applicable to various practical uses. In this investigation, we conducted sequencing of 16 previously uncharacterized *Apiospora* genomes. Seven are distantly related to previously known genomes and are the first genome assembly available for these species. In conjunction with five existing genome assemblies, we conducted an examination of the genetic composition and diversity within this genus. A deeper analysis of the genomes revealed a genus rich in genes associated with carbohydrate-active enzymes (CAZymes) and secondary metabolites. We identified the synthesis potential of CAZymes to be a core genome feature of the genus. In addition, clustering of polyketide synthase (PKS) and nonribosomal peptide synthetase (NRPS) core genes into gene families, revealed a highly diverse specialized metabolite synthesis potential within the genus. Surprisingly, we also found that certain *Apiospora* species completely lack the gene cluster responsible for the synthesis of 3-nitropropionic acid (3-NPA), a compound previously associated with this genus. Taken together, this study's results elucidate an unusually high potential for the discovery of new enzymes and compounds within the *Apiospora* genus.

Keywords Apiospora, CAZymes, Specialized metabolites, PKS, NRPS, 3-NPA

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Introduction

The genus *Apiospora*, which comprises several filamentous fungi species, is generally understudied, but has gained increasing attention in recent years. Nonetheless, there have still only been a few studies conducted into the genetic contents of the genus [1–7]. This genus has previously, been grouped with *Arthrinium*, as one genus, and taxonomically classified as *Arthrinium*. But in recent years, they have undergone a period of altered phylogenic affiliation [8–13]. Recently, they underwent a taxonomic revision resulting in their division into two distinct phylogenetic clades, primarily falling into the genus *Apiospora*. Additionally, several species were reassigned from the genus *Arthrinium* to *Apiospora*. *Apiospora* fungi have been documented across various geographic and ecological settings, exhibiting a wide distribution spanning all climatic zones [14–20].

A wealth of bioactive secondary metabolites, many of which exhibit intriguing biological and medicinal capabilities, have been found in the fungi kingdom [21, 22]. The genus *Apiospora* has also been demonstrated to have a diverse repertoire of secondary metabolites of potential great importance [23, 24]. Moreover, a diverse array of fungal species produces carbohydrate-active enzymes (CAZymes) aimed at biomass degradation and nutrient production. These enzymes play a crucial role in biological processes such as digestion, metabolism, and cell wall remodeling while also serving a pivotal role in the eco-friendly conversion of lignocellulose into biofuel. Their extracellular secretion from fungal hyphae renders them stable beyond the fungal cell, making them highly attractive for industrial applications [25, 26]. Notably, the genus *Apiospora* exhibits a pronounced capacity for synthesizing such beneficial enzymes [3, 6], thus positioning the *Apiospora* genus as a significant reservoir for the discovery of novel compounds and enzymes with potential utility. It is important to note that *Apiospora* can produce 3-nitropropanoic acid (3-NPA), an irreversible inhibitor of the citric acid cycle and therefore highly toxic [27]. Documented cases of fatal poisoning have been associated with the consumption of coconut and sugarcanes [28].

The development of long-read sequence technology has improved genome assemblies, making it possible to obtain highly contiguous genome assemblies to facilitate comparative genomic studies to describe the full genetic potential of useful genus. Comparative genomic studies can aid in the study of a genus' genetic contents, revealing what genes are shared within a genus as well as what genetic contents are unique to one species.

In this study, we sequenced 16 *Apiospora* species. Seven of these represent species not previously characterized by genome assemblies. We examined the genetic contents across the *Apiospora* genus by facilitating the genome assemblies generated in this study as well as five *Apiospora* genome assemblies available in the public databases and investigated the ability of the *Apiospora* genus to produce CAZymes and secondary metabolites. Lastly, we clustered the secondary metabolite core genes into families and proposed a classification system in agreement with the system used in *Fusarium* [29].

Materials and methods

Fungal strains

In this investigation, whole-genome sequencing was performed on 16 strains of *Apiospora*, of which the following 13 strains were obtained from the Westerdijk Fungal Biodiversity Institute (CBS); *Apiospora arundinis* CBS 124,788, *Apiospora arundinis* CBS 450.92, *Apiospora aurea* CBS 244.83, *Apiospora hydei* CBS 114,990, *Apiospora kogelbergensis* CBS 113,332, *Apiospora kogelbergensis* CBS 117,206, *Apiospora marii* CBS 200.57, *Apiospora marii* CBS 497.90, *Apiospora phaeospermum* CBS 142.55, *Apiospora phragmitis* CBS 135,458, *Apiospora rasikravindrae* CBS 337.61, *Apiospora saccharicola* CBS 334.86 and *Apiospora saccharicola* CBS 831.71. The following three strains were obtained from the IBT Culture Collection of Fungi at the Technical University of Denmark (DTU); *Apiospora arundinis* IBT 11,852, *Apiospora arundinis* IBT 41,787, *Apiospora arundinis* IBT 7813.

DNA extraction and sequencing

High molecular weight (HMW) DNA was extracted from the 16 fungi cultivated in liquid YPG (10 g/L yeast extract, 20 g/L peptone, and 25 g/L glucose) medium at 25 °C for 5 days at 100 rpm, according to The Genomic Buffer Set method described in Petersen et al. (2022) [7]. The HMW DNA was subsequently purified, and small fragments were removed according to the same protocol.

Sequencing libraries were generated from the extracted HMW DNA using the Native barcoding genomic DNA (EXP-NBD104, EXPNBD114, and SQK-LSK109) from Oxford Nanopore Technologies. The libraries were sequenced on either a R9.4.1 or R10.3 flow cell (Additional file 1, Table S1). The raw reads were base called and demultiplexed using Guppy [30] version 3.5.4 in GPU mode using the dna_r9.4.1_450bps_hac.cfg model.

Generation of genome assemblies

Prior to the *de novo* assembly process, the base called reads filtered using Filtlong [31] version 0.2.0 to a quality of either 80 or 90 and a minimum length between 8 kbp and 10 kbp (Additional file 1, Table S1). The genome assemblies were obtained by creating read overlaps using minimap2 version 2.17 [32], whereafter the genome assemblies were created using Miniasm version 0.3 [33]. The genome assemblies were subsequently polished by Racon version 1.3.3 [34] and two rounds of Medaka [35]

version 1.0.1, both with default settings. The completeness of the created genome assemblies was assessed with Benchmarking Universal Single-Copy Orthologs (BUSCO) version 5.0.0 [36] analyses using the Ascomycota version 10 gene set.

Genome assemblies from NCBI

The following already available genome assemblies were obtained from NCBI: *Apiospora phaeospermum* (ASM650353v1) (published as *Arthrinium*), *Apiospora* KUC21332 (ASM1716395v1) (published as *Arthrinium*), *Apiospora malaysiana* (ASM650811v1), *Apiospora pterosperma* (ASM1997648v1), *Apiospora arundinis* AAU 773 (JAPCWZ000000000).

Gene prediction and functional annotation

Genes were predicted in all the *Apiospora* genome assemblies using FunGAP version 1.1.0 [37] supported by 712.6 million reads RNA-reads from *Apiospora arundinis* AAU 773 obtained from NCBI (bio project PRJNA891674). The RNA-seq reads were trimmed using Trim Galore version 0.6.4 with Cutadapt version 2.8 [38] prior to use. Gene prediction for the genome of *Fusarium graminearum* (ASM24013v3) used as outgroup were obtained from NCBI. The predicted genes were functionally annotated by InterProScan version 5 [39] and secondary metabolite genes were predicted by antiSMASH version 5 [40]. CAZyme genes were predicted by dbCAN2 meta server [41] allowing only hits with e-values less than 10^{-15} and a coverage above 0.35.

Orthogroups and phylogenetic analysis

The predicted proteins for all the *Apiospora* genomes were grouped into orthogroups using OrthoFinder version 2.5.4 [42, 43] with default settings. A rooted species tree based on the orthogroups and *Fusarium* as outgroup were constructed by OrthoFinder version 2.5.4 [44, 45] with default settings (Maximum Likelihood).

Orthogroups that contained proteins from at least 20 genomes were defined as the core genome. The dispensable genome was defined as all orthogroups not in the core genomes. Species-specific families were defined as families containing only proteins from the same species. The orthogroups were functionally annotated by blasting the longest protein from each orthogroup against the following databases: the non-redundant database of NCBI, InterProScan, euKaryotic Orthologous Groups (KOG) using BLASTp. Blast results under 10^{-5} e-value were accepted. Blast2GO [46] was used to assign Gene Ontology (GO) terms and calculate enriched features using Fishers exact test (FDR 0.05). CAZyme and secondary metabolite genes were assigned as described above.

Clustering of PKS and NRPS core gene

Clustering of polyketide synthase (PKS) and nonribosomal peptide synthetase (NRPS) core genes was based on a phylogenetic relationship. The PKS and NRPS amino acid sequences were extracted from the antiSMASH prediction. Two multiple alignments between the sequences were created using MAFFT [47]. Subsequently, two phylogenic trees were generated using IQ-TREE [48] with a bootstrapping of 1,000. ModelFinder [49] was employed to identify the best model for the trees. The results obtained were visualized using iTOL [50]. The Multiple Correspondence Analysis (MCA) model was constructed with R [51] version 4.1.1 in RStudio version 2022.7.1 [52] using the version 2.6 package [53].

Compounds resulting from PKS and NRPS genes were predicted using antiSMASH version 5.0 [40] to tentatively assign secondary metabolite biosynthetic gene clusters. Candidate orthologue genes were identified through manual evaluation of the suggested hits in the MIBiG database [54] and through individual BLASTp analyses of key PKS and NRPS genes.

Identification of the 3-NPA gene cluster

To identify gene clusters responsible for the synthesis of 3-NPA in the sequenced *Apiospora* strains, the recently identified cluster from *Aspergillus oryzae* [55] was used as a reference to search for homologous gene clusters in these. Additional orthologous of the FMN-dependent oxidase NpaA from selected strains of *Aspergillus*, *Penicillium*, *Metarhizium* and *Streptomyces* were identified in GenBank by BLASTp analysis using AO090038000030 from *A. oryzae* [55]. The obtained protein sequences were aligned, and a phylogenic tree was created in CLC Main Workbench using Maximum Likelihood Phylogeny with 1000 bootstraps.

Visualization of data

All visualizations were conducted with R version 4.1.1 in RStudio version 2022.7.1; using the R-packages ggplot2 [56], ggtree 3.0.4 [57], and pheatmap version 1.0.12 if nothing else specifically noted.

Results and discussion

High-quality genome assemblies from 16 new *Apiospora* isolates

High molecular weight (HMW) DNA was isolated from each of the 16 *Apiospora* strains to enable whole genome sequencing utilizing Oxford Nanopore Technology. The 16 new *Apiospora* genome assemblies generated in this study were compared to previously sequenced *Apiospora* genomes and a *Fusarium* genome (Fig. 1). Between 1.08 and 3.66 Gb of long reads were used for the *de novo* assembly process (Additional file 1, Table S1).

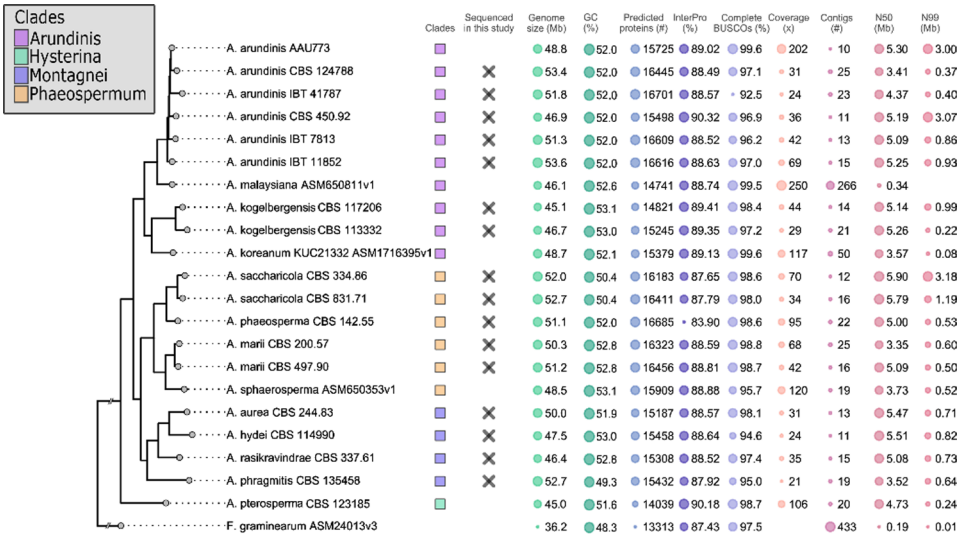

Fig. 1 Phylogenetic relationships within the *Apiospora* genus are based on orthogroups. Squares indicate which clade the isolates belong to according to Pintos and Alvarado [10]. Dots represent genomic features of the assembly

The assemblies had a size ranging from 45.0 to 53.6 Mb and a GC content between 49.3% and 53.1% (Fig. 1), similar to those observed in other studies [3, 5–7, 13]. The genome assemblies based on long-read sequences were observed to be highly contiguous with a low number of large contigs (Fig. 1). Furthermore, complete BUSCO ranged from 92.5% to 98.8%, N50 ranged from 3.35 to 5.9 Mb and N99 ranged from 0.22 to 3.18 all indicative of high completeness and contiguity for all the genome assemblies (Fig. 1). In comparison, the five genome assemblies obtained from the database had similar complete BUSCO (95.7–99.6%) and three were also highly contiguous, whereas one (*A. malaysiana* ASM650811v1) was highly fragmented with 266 contigs. Not surprisingly the fragmented assembly was constructed using short-reads only, whereas the remaining genome assemblies were generated from long-reads.

To elucidate the phylogenetic relationship of the species, a phylogenetic tree was constructed based on the identified orthogroups (Fig. 1). While robust clustering of the *Apiospora* genus into subgenera or sections has not yet been fully conducted, Pintos and Alvarado [10], identified six major phylogenetic clades representing possibly subgenera or sections. Our findings using the orthogroups agrees with those clades suggested by Pintos and Alvarado [10]. The relative placement of the specific species in the tree also corresponds to previous studies [8, 9, 11, 12].

Gene prediction of all the *Apiospora* genome assemblies revealed a total of 14,039 to 16,701 protein-coding genes, with an average of 88.6% predicted to have at least one InterPro domain (Fig. 1). The clade Arundinis was observed to have the highest variation of both predicted proteins in its genomes and genome size. This may stem, however, from the fact that this clade was the most abundant in this study, with six species analyzed. *A. pterosperma* had the lowest number of predicted genes, as well as the smallest genome size. This could indicate that species belonging to the Hysterina clade generally have smaller genomes. To our knowledge, however, this is the first genome from the Hysterina clade, and more species must be sequenced to confirm this.

The core genome is responsible for general housekeeping functions

To investigate the genetic diversity within the *Apiospora* genus, the protein sequences from all the *Apiospora* genome assemblies were divided into orthogroups. Then, the orthogroups were further sorted as belonging to the core genome or the dispensable genome. The core genome was defined as orthogroups containing protein sequences from all genomes. But since average BUCSO completeness was observed to be 97.44%, the chance of observing true orthogroups to be within the core genome is only $0.9744^{21} = 58\%$. Therefore, orthogroups containing proteins shared by at least 20 isolates were used to define the core genome. Orthogroups absent from the core genome were classified as part of the dispensable genome, while proteins found exclusively in a single species were designated as species-specific genes (Fig. 2).

The core genome of the 21 *Apiospora* species consists of 10,016 protein families, representing 69.3% of all genes across the *Apiospora* genomes. A relatively consistent number of core genes was observed across the genomes of all 21 species (Fig. 2), with the lowest count found in *A. pterosperma*, which also has a generally lower total count.

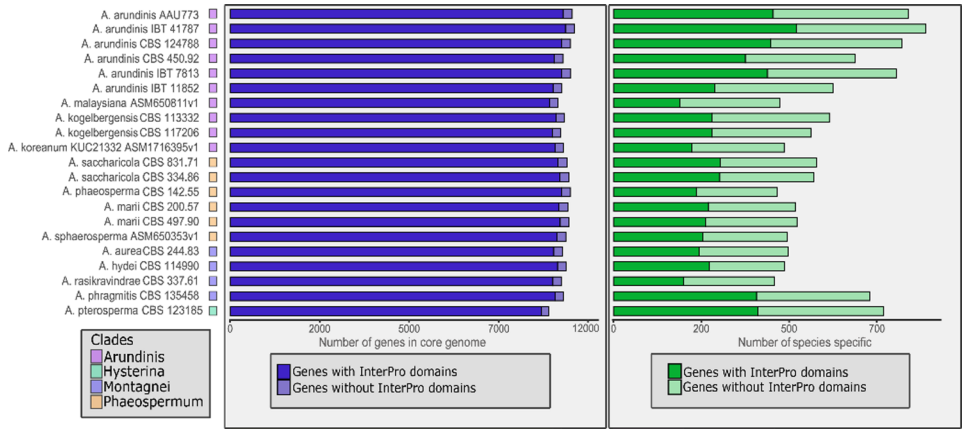

Fig. 2 Core and species-specific genes in the *Apiospora* genus. Squares indicate clade according to Pintos and Alvarado [10]. Blue bars represent core genes, and green bars represent species-specific genes. Shading differentiates genes with at least one InterPro domain from those with no InterPro annotation

On average, 97.4% of the core genes in each genome could be assigned to at least one InterPro domain, in agreement with the core genome primarily comprising genes with conserved functions. It was possible to assign at least one KOG term to 39.4% of the core genes, whereas only 7.8% of the genes in the dispensable genome were assigned a KOG term. The KOG terms "Posttranslational modification, protein turnover, chaperones", "Signal transduction mechanisms", "Energy production and conversion" and "Carbohydrate transport and metabolism" were mostly present in the core genome (Additional file 2, Figure S1). The KOG terms "Secondary metabolites biosynthesis, transport and catabolism" and "Lipid transport and metabolism" mostly defined the dispensable genome (Additional file 2, Figure S1). Furthermore, the core genome contained 2,017 significantly overrepresented Gene Ontology (GO) terms, majority of which are associated with essential housekeeping processes (Additional file 1, Table S2). This agrees with the expectation that the core genome encodes genes necessary for general cellular maintenance, while the dispensable genome contains genes that are not essential for cellular housekeeping. This observation aligns with studies conducted on the genus *Aspergillus* [58, 59].

Between 3.35% and 6.6% of all the genes in each genome were observed to be species-specific genes. This group constitutes 4,160 protein families, and 4.7% of all the genes in all the *Apiospora* genome assemblies. However, a much lower proportion of the species-specific genes could be assigned an InterPro domain compared to the core genes (51% versus 97.4%). This indicates that a higher proportion of the species-specific genes may be pseudogenes, gene fragments, or erroneously predicted genes.

The *Apiospora* genus exhibits high potential for CAZyme production

The production of CAZymes from the *Apiospora* genus is largely unexplored, as only a few studies with *Apiospora* have been published. However, these fungi seem to contain a hitherto uninvestigated useful source of these enzymes [3, 6]. Here, we used the dbCAN2 meta server [41] to predict genes associated with CAZymes.

Between 633 and 741 CAZyme-related genes in the genome assemblies of the 21 *Apiospora* species (Fig. 3B and Additional file 2, Figure S2-S6) could be identified. This indicates that all the inspected species seem able to produce a very high number of CAZymes, and that this number is relatively constant both within and across clades. The CAZyme families AA9, AA7, and AA3_2 were most frequently observed across all the species (Additional file 2, Figure S2). In a separate study, we showed that these enzyme families were highly upregulated in *A. arundinis* AAU 773 when grown on a hay-based medium [13], thus supporting that these enzymes are involved in nutrient acquisition through the degradation of complex carbohydrates. Interestingly, the AA9 family consists of lytic polysaccharide monooxygenases (LPMOs), which have been shown to be of great importance in the production of green biofuel. The AA7 family consists of glucooligosaccharide oxidases and cellooligosaccharide dehydrogenases [60–63]. Whereas the function of the AA3 families is generally to degrade lignocellulose in concert with other AA enzymes, such as the AA9 [60]. Within the GH family, GH18 and GH3 contained the highest number of genes across all species (Additional file 2, Figure S3). However, as GH18 is a chitinase with no role in cellulose degradation, it may be unsurprising that a previous study did not show increased expression of this family in *A. arundinis* AAU 773 when cultivated on a hay-based medium, further supporting its lack of

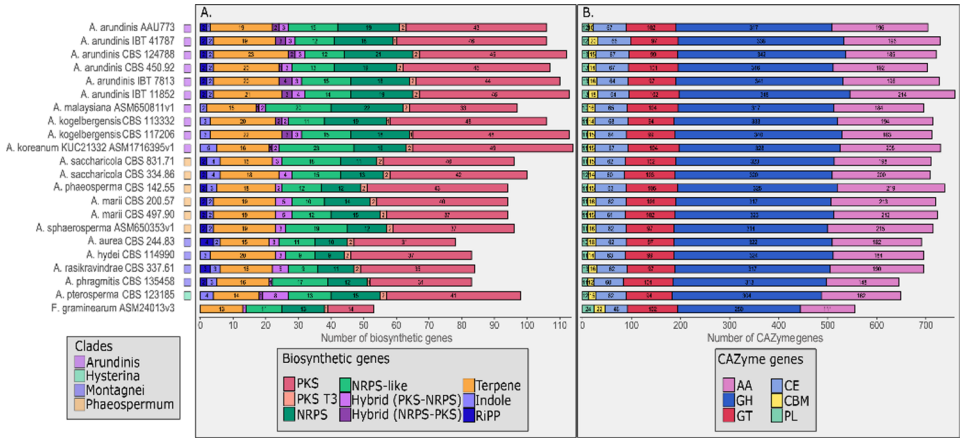

Fig. 3 Number of secondary metabolite core genes and CAZymes in the 21 *Apiospora* genomes. Squares indicate clade according to Pintos and Alvarado [10]. **A.** Secondary metabolite core biosynthetic genes. **B**. CAZyme genes

involvement in lignocellulose breakdown. In contrast, we observed increased expression of the GH3 family which is known to be involved in cellulose and hemicellulose degradation [13]. In addition, the GT2 family was prominently present in all the examined genomes (Additional file 2, Figure S4). The members of the GT families are usually not associated with the degradation of cellulose or hemicellulose, but rather with the formation of glycoside bonds [64, 65]. In agreement with this the GT families was not observed to be upregulated when grown on hay-based media [13]. Finally, genes belonging to the families CE1 and CE5 were observed in high numbers in all genomes (Additional file 2, Figure S5). The function of these families is mostly associated with the degradation of hemicellulose, suggesting that the *Apiospora* genus is also capable of degrading hemicellulose [13].

CAZyme-related genes are a core genome trait for the *Apiospora* genus

To investigate the distribution of CAZyme-related genes across the pangenome groups, one representative protein sequence from each orthogroup was annotated using the dbCAN2 meta server. A total of 726 orthogroups (2.48%) were assigned to at least one CAZyme family. 68.5% were found within the core genome, while only 1.4% were species-specific genes. Additionally, several CAZyme-related genes were significantly overrepresented in the core genome (Additional file 1, Table S3), suggesting that the ability to degrade plant material is a conserved function across all *Apiospora* species and a fundamental characteristic of the genus.

The *Apiospora* genus has a diverse and abundant specialized metabolite potential

The *Apiospora* genus has shown a promising ability to produce several specialized metabolites [23, 24]. Here, we mined the genomes of the 21 *Apiospora* species for specialized metabolite genes. A diverse number of specialized metabolite core genes were observed within this genus, with a total of 2,123 (average 101.1/species) (Fig. 3A). This is more than observed in other filamentous fungi, such as the *Penicillium* genus [66], the *Aspergillus* genus [58, 59] and the *Fusarium* genus [29, 67]. Within a single genome, the highest number of secondary metabolite genes was observed in the clade Arundinis. In contrast, the smallest number was observed in the genome of *A. malaysiana*. However, as discussed previously, this may be due to the fact that this genome assembly is based on short reads only, and highly fragmented, which may cause an underrepresentation of full-length genes. Regardless, the clade Montagnei seems to have a relatively small number of secondary metabolite genes and the genome of *A. pterosperma* harbors a relatively high number of secondary metabolite genes, despite its relatively low gene content and smaller genome size. This species also has the highest number of PKS-NRPS hybrid genes.

To propose a classification systems of the PKS genes from *Apiospora* similar to the system used for *Fusarium* [29], we clustered core PKS genes from all 21 *Apiospora* genomes into families based on their phylogenetic relationship (Fig. 4, Additional file 3 and Additional file 1, Table S4 Only genes observed in more than two different species were included in the analysis, excluding species-specific PKS genes.

A total of 714 PKS genes were included in the analysis, and they were grouped into 92 families (Fig. 4). Within a family, the same, or a highly similar compound is expected to be produced from the gene cluster.

In general, we observed a separate clustering of the non-reducing-PKS (NR-PKS) and the reducing-PKS (R-PKS) in the phylogenic tree based on PKS genes

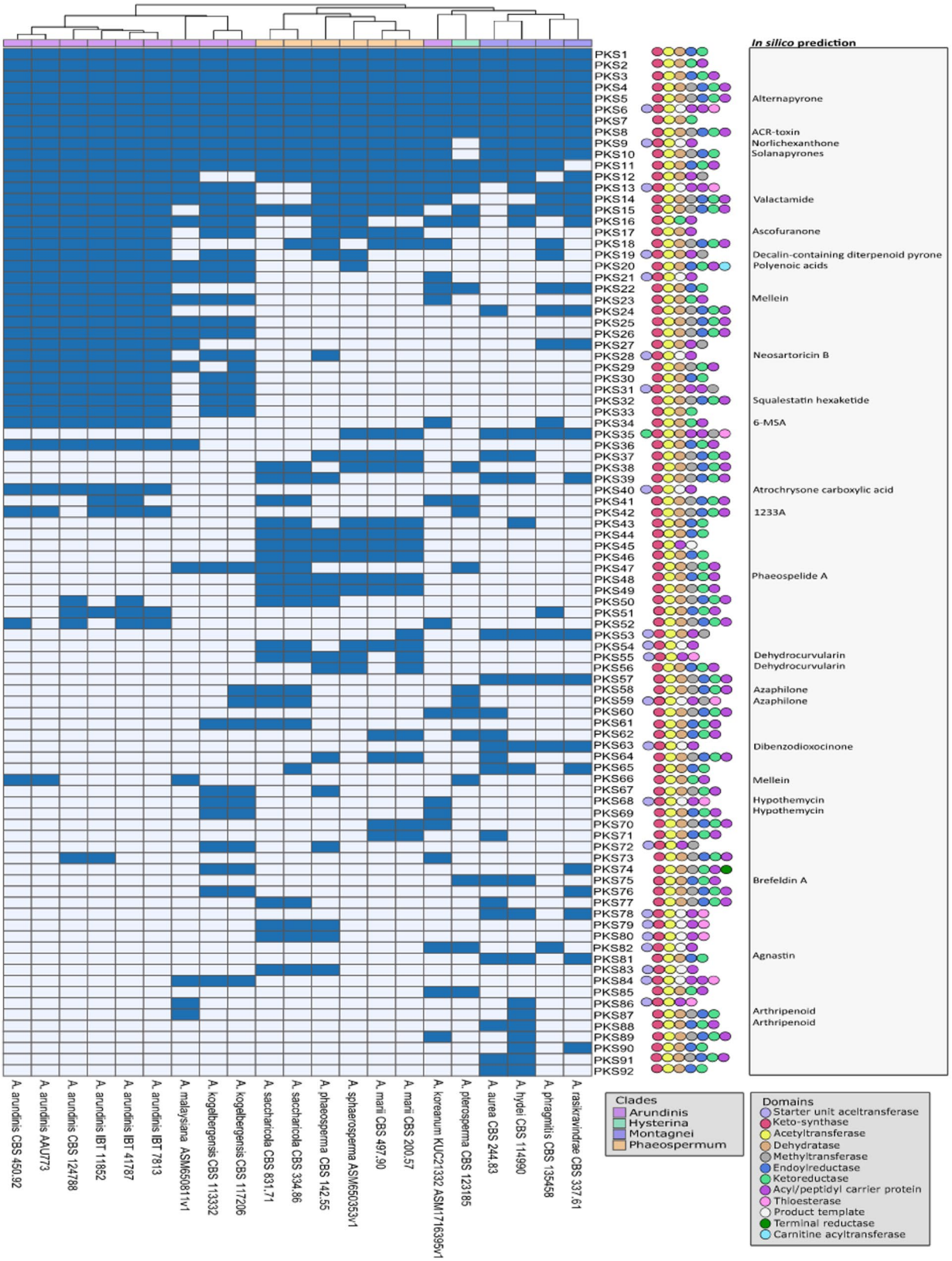

Fig. 4 Core PKS core gene families identified in *Apiospora*. The matrix represents hierarchical clustering of the presence (dark blue) or absence (light blue) of the core PKS genes in the different genomes. Squares indicate clade according to Pintos and Alvarado [10]. Dots represent predicted domains in the core PKS genes. ""*In silico* prediction" shows prediction of resulting compounds produced by the PKS gene clusters

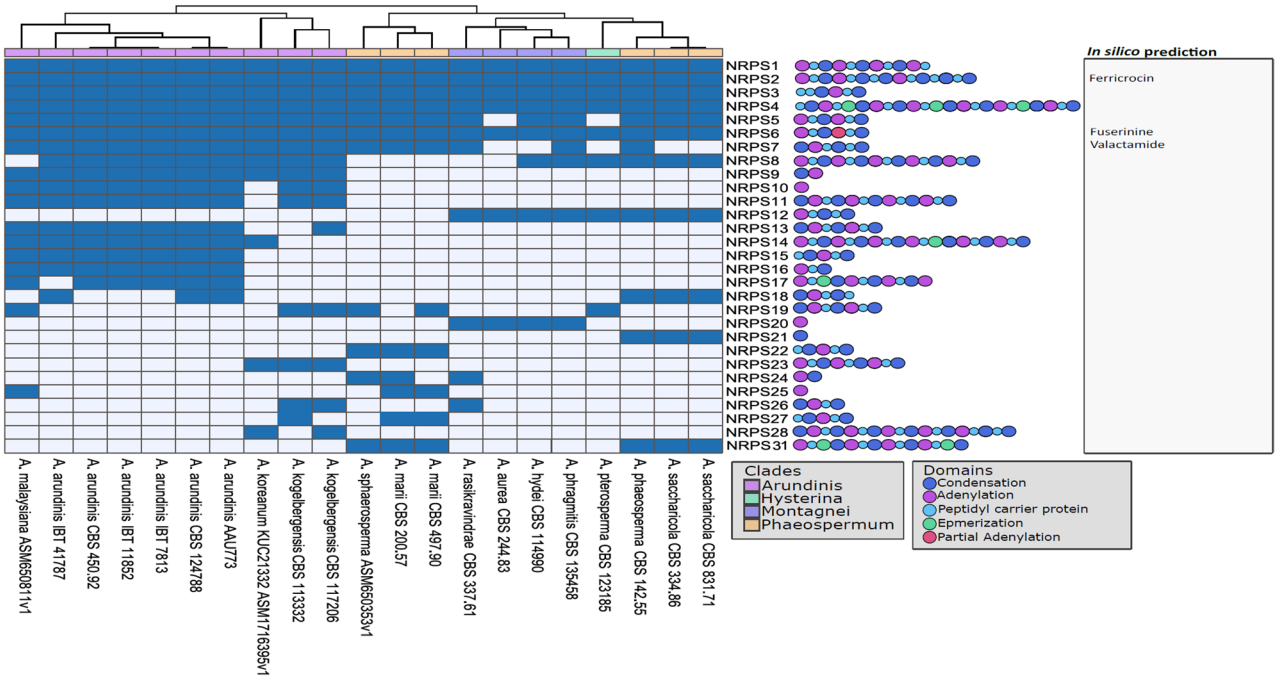

Fig. 5 Core NRPS gene families identified in *Apiospora*. The matrix represents hierarchical clustering of the presence (dark blue) or absence (light blue) of the core NRPS genes in the different genomes. Squares indicate clades according to Pintos and Alvarado [10]. Dots represent the predicted protein domain structure "In silico prediction" shows the prediction of the resulting compounds produced by the NRPS gene clusters

(Additional file 3). PKS gene-based dissimilarity between the genomes was examined using an unsupervised multiple correspondence analysis (MCA) in combination with hierarchical clustering (Fig. 4 and Additional file 2, Figure S9). The MCA model revealed a clustering of the species within the same clades. The hierarchical clustering based on their PKS genes indicates that *A. koreanum* is more closely related to *A. pterosperma* than to the Arundinis clade, which was observed in the phylogenetic tree based on all orthogroups. Of the different PKS families, eight were observed to be conserved across all species, suggesting that these families are fundamentally important for the fungi. Several PKS families were observed to be only present in a single clade.

Within the Arundinis clade, 14 PKS families (PKS21, PKS23, PKS25, PKS26, PKS29, PKS30, PKS31, PKS32, PKS33, PKS36, PKS40, PKS52, PKS69, PKS73, and PKS84) were observed exclusively among the members of this clade. The Phaeospermum clade shared 11 unique PKS families (PKS44, PKS45, PKS46, PKS48, PKS49, PKS54, PKS55, PKS56, PKS79, PKS80 and PKS83). Lastly, eight PKS families (PKS57, PKS63, PKS78, PKS81, PKS88, PKS90, PKS91, and PKS92) were present only in the Montagnei clade. Interestingly, some families (PKS41, PKS42, PKS50, PKS51, PKS52, PKS66, and PKS73) were present in some *A. arundinis* genomes but absent from others. Among *A. kogelbergensis* genomes, three PKS families (PKS29, PKS58 and PKS59) were observed only in one genome. Within *A. saccharicola* genomes two PKS families (PKS18 and PKS65) were observed in only one of them, and finally PKS55 and PKS56 were observed only in one of the *A. marii* genomes. Whether these differences are a result of horizontal gene transfer or gene loss remains to be elucidated.

Parallel to the PKS genes, the core NRPS genes in the 21 Apiospora genomes were analyzed and clustered into NRPS families based on their phylogenetic relationships (Fig. 5, Additional file 3, and Additional file 1, Table S5), and a classification system for the identified NRPS families proposed.

Similar to PKS families, within a single family, the core NRPS genesis is expected to produce the same, or highly similar, compound. A total of 272 NRPS genes were included in the analysis, resulting in 29 NRPS families (Fig. 5). NRPS genes located only in one species were excluded from the analysis. An unsupervised MCA model and a hierarchical clustering were used to detect similarity of the NRPS gene profile across the 21 *Apiospora* genomes (Fig. 5 and Additional file 2, Figure S11). In general, the NRPS profiles for the genome of the same species cluster closely together. However, both MCS and hierarchical clustering show interesting observation, as the Phaeospermum clade was separate in contrast to the phylogenic tree based on all orthogroups. A total of four NRPS families were observed to be conserved across all genomes, again indicating that these NRPS families are highly important for essential processes in these

fungi. Several NRPS families (NRPS9, NRPS10, NRPS11, NRPS13, NRPS14, NRPS15, NRPS16, NRPS17, NRPS23, and NRPS28) were exclusively present in the Arundinis clade, while NRPS21, NRPS22, and NRPS31 were specific to the Phaeospermum clade. The only NRPS family restricted to the Montagnei clade was NRPS20. NRPS18 appeared in only three *A. arundinis* genomes but was also present in the genomes of the two *A. saccharicola* strains and *A. phaeospermum*. NRPS13, NRPS27, and NRPS28 were detected in only one *A. kogelbergensis* genome, whereas NRPS19 and NRPS24 were found in a single *A. marii* genome. Again, these patterns are consistent with both horizontal gene transfer or gene loss during evolution.

Linking secondary metabolite genes to putative compounds by in silico prediction

Out of 121 PKS and NRPS gene families, 25 potential compounds were predicted (Figs. 4 and 5). It is important to note that these are in silico predictions and not experimentally verified. Therefore, the actual compounds produced by these gene clusters may, in some cases, differ in varying degree from those predicted.

PKS5 was predicted to produce alternapyrone (Fig. 4), a compound previously implicated in plant pathogenesis [68], while PKS8 showed similarity to ACR-toxin biosynthetic genes (Fig. 4), which are essential for virulence in *Alternaria alternata* [69]. Although these compounds have not yet been isolated from *Apiospora*, the presence of conserved biosynthetic gene clusters suggests a functional role during host infection. Other PKS families were linked to compounds that have already been isolated from *Apiospora* species (Fig. 4), such as norlichexanthone (PKS9) [24]and mellein (PKS23 and PKS66) [70, 71].

Several predicted biosynthetic gene clusters showed clade-specific distributions (Fig. 4). For instance, PKS34, predicted to produce 6-methylsalicylic acid, and PKS40, linked to atrochrysone carboxylic acid, were mostly restricted to the Arundinis clade, but the PKS34 gene cluster were previously been reported in an *A. saccharicola* isolate [5]. In the present study, however, we did not detect the PKS34 gene cluster in any *A. saccharicola* genome. Similarly, PKS63, predicted to produce dibenzodioxocinone, was specific to the Montagnei clade. Phaeospelide A has previously been isolated from *A. phaeospermum*, and a highly reducing-PKS (HR-PKS) gene from *A. phaeospermum* was shown to be responsible for the production of this compound by heterologous expression in *Aspergillus oryzae* [72]. Here, we identified the PKS48 family as the homologous PKS family, which was present in all species of the Phaeospermum clade.

We also identified PKS and PKS–NRPS combinations homologous to clusters known to require multiple synthases (Fig. 4), including those involved in the production of dehydrocurvularin (PKS55 and PKS56), hypothemycin (PKS68 and PKS69), azaphilone (PKS58 and PKS59), and arthripenoid (PKS87 and PKS88). In some genomes, not all of the genomes containing a PKS55 gene harbored a PKS56 gene, indicating a recent gene loss event.

NRPS2 and NRPS6, predicted to produce two siderophores, ferricrocin and fusarinine, respectively, were conserved across all analyzed genomes (Fig. 5). Ferricrocin has previously been reported from *A. arundinis* [13] whereas fusarinine has not yet been described from any *Apiospora* species. Together, these findings identify both conserved and lineage-specific features of secondary metabolite biosynthesis in *Apiospora*.

In conclusion, while compound predictions can be made for some specific specialized secondary metabolite gene families, the compounds resulting from the majority of the observed families remain unknown. Therefore, Apiospora continues to represent a valuable resource for the discovery and investigation of novel bioactive compounds with potential applications across various critical domains.

The 3-NPA gene cluster is not conserved across *Apiospora* genomes

To investigate the potential of *Apiospora* to produce the highly toxic mycotoxin, 3-NPA, we examined the distribution of the gene cluster responsible for the biosynthesis of 3-NPA, which was recently identified in *Aspergillus* [55], across the sequenced genomes.

Despite the close phylogenetic relationship among the 21 *Apiospora* genomes, our genomic analysis revealed a strikingly uneven distribution of the gene cluster (Additional file 1, Table S5 and additional file 2, Figure S10). We found this gene cluster in 16 of the 21 *Apiospora* genomes (Additional file 1, Table S7 and additional file 2 Figure S10), and interestingly, in only four of the five *A. arundinis* species. The patchy presence of the cluster could be the results of lineage-specific gene loss, horizontal gene transfer, or recent intraspecific diversification events. In any case, the presence/absence polymorphism may reflect ecologically driven selection or strain-specific regulation of secondary metabolism. Further transcriptomic and functional validation will be needed to determine whether the cluster is transcriptionally active in the positive strains and what role it may play in the biology of *Apiospora*.

These findings highlight the importance of sampling multiple strains per species to capture the full scope of biosynthetic diversity within fungal lineages and caution against generalizing from single-reference genomes when assessing secondary metabolic potential. In *Aspergillus*, strains and mutants without the potential to produce 3-NPA have long been sought after, since they are considered safer for use in food production [73].

The increased number of *Apiospora* genome assemblies provides new opportunities for the exploration of this intriguing genus, facilitating future identification of numerous specialized metabolites and enzymes. Future research, including genetic manipulation within these understudied fungi will be of significant interest.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12864-026-12829-1.

Supplementary Material 1.

Supplementary Material 2.

Supplementary Material 3.

Supplementary Material 4.

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Adherence to national and international regulations
Not applicable.

Authors' contributions
TS, LIF, TES and KLN conceived and designed the experiments. TS, LIF, TES and JLS performed all experiments and contributed to the acquisition of the data. TS, JLS, TA and GLH performed all data analysis. TS and TES wrote the original manuscript. TS, CP, LIF, JLS, KLN and TES reviewed and improved the manuscript. All authors read and approved the manuscript.

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Data availability
The sequence reads (fastq) and the resulting genome assemblies are deposited at NCBI under the bio project PRJNA923254. See Additional file 1, Table S1 for specific accession numbers.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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