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Gene synteny analysis of lichen-forming fungi

Comparative Insights from the Fungal Classes Lecanoromycetes, Dothideomycetes, and Eurotiomycetes

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Title: Gene synteny analysis of lichen-forming fungi: comparative insights from the fungal classes Lecanoromycetes, Dothideomycetes, and Eurotiomycetes

Abstract

Lichens are an ancient and successful symbiotic system, formed by a fungus and a photosynthetic partner. This study investigates how the structural organisation of the genome, specifically gene synteny, has been shaped by evolutionary history and ecological demands. A comparative genomic analysis was performed on 33 high-quality fungal genome assemblies from the classes Lecanoromycetes, Eurotiomycetes, and Dothideomycetes. The findings reveal that the level of synteny conservation varies significantly between classes. Eurotiomycetes exhibited the highest average within-class conservation, while Dothideomycetes showed the lowest, reflecting distinct histories of genomic rearrangement.

Functional enrichment analysis showed that genes within conserved syntenic blocks are significantly enriched for core biological processes such as gene expression and protein synthesis. This suggests a strong selective pressure to maintain the order of genes related to fundamental cellular functions across diverse lineages. By integrating phylogenetic and synteny analyses, this research provides a foundational framework for understanding how genome stability is influenced by both deep evolutionary history and the ecological demands of the symbiotic lifestyle.

Key words: Lichen-forming Fungi, Lichen Symbiosis, Gene Synteny, Ascomycota, Comparative Genomics, Phylogenetic Inference, Functional Enrichment.

Dedication and Acknowledgement

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Author’s Declaration

A dissertation submitted to the University of Bristol in accordance with the requirements for the award of the degree of MSc Bioinformatics in the Faculty of Life Sciences.

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Introduction

1.1 Lichens and Symbiosis

Lichens are among the most successful symbiotic systems in nature, formed through the association of a fungal partner (the mycobiont) and one or more photosynthetic partners, typically green algae or cyanobacteria (photobionts) (1). The mycobiont provides structural support and access to water and minerals, while the photobiont contributes fixed carbon through photosynthesis (2). This ancient and highly successful symbiosis has allowed lichens to colonise diverse and often extreme environments, from polar regions to deserts, establishing them as ecological pioneers on barren substrates (3,4).

Beyond their role as colonisers, lichens are critically important to various ecosystems (5). They are widely recognised as bioindicators of air quality due to their high sensitivity to pollutants, particularly sulphur dioxide (6,7). Lichens also provide vital ecosystem services, contributing to nutrient cycling, soil formation, and the prevention of desertification (8,9). The unique metabolic capabilities of this symbiosis have also yielded a wide array of secondary metabolites, including a diverse range of bioactive compounds that have shown promise in industrial and biotechnological applications, such as for UV protection, and as antimicrobial and anticancer agents (10–13).

The repeated, independent evolution of this complex symbiosis in multiple fungal lineages raises fundamental questions about the underlying genetic mechanisms that enable such a life-changing evolutionary transition (14). It is estimated that around 21% of all known fungal species are involved in lichenisation (15,16)**.** Therefore, investigating how genome architecture, specifically gene synteny, which refers to the conserved gene order across species, reflects genome structure over evolutionary time (17), has been shaped by these repeated evolutionary events is crucial for understanding the genetic basis of lichen symbiosis (18).

1.2 Evolutionary Importance of Gene Synteny

Gene synteny refers to the conserved order of genes across different species or lineages, reflecting the structural organisation of genomes through evolutionary time (17). The preservation of gene order strongly indicates shared ancestry, as closely related species tend to maintain larger collinear blocks than those that are more distantly related (19). At

the same time, breaks in synteny provide evidence of genome rearrangements, such as inversions, translocations, and duplications, which can drive diversification and adaptation (20,21).

Beyond its phylogenetic value, synteny is frequently associated with functional relationships (22). Genes involved in the same pathway, biosynthetic cluster, or regulatory network are often maintained together in the genome, facilitating coordinated expression (23). This is particularly important in fungi, where secondary metabolic gene clusters are strongly conserved and linked to traits such as stress tolerance, pathogenicity, and symbiosis (24). By studying synteny, researchers can therefore identify not only patterns of evolutionary history but also genomic regions of ecological or functional significance.

In comparative genomics, synteny analyses have been widely applied to investigate genome stability, lineage-specific rearrangements, and the evolutionary origins of gene clusters (25). Advances in computational approaches, such as MCScanX, a toolkit designed for the detection and evolutionary analysis of gene synteny and collinearity (26), have made it possible to detect syntenic blocks at genome scale by integrating homology searches with gene order information (27). These methods allow the quantification of collinearity within and between lineages, making synteny a powerful lens through which to study both deep evolutionary relationships and adaptive genome dynamics.

Given that lichen symbiosis has emerged multiple times across the fungal tree, synteny offers a framework for disentangling the roles of ancestry, convergence, and ecological pressures in shaping genome organisation (28).

1.3 Current Knowledge and Research Gap in Fungal Synteny & Lichen Genomics

Comparative genomics has provided significant insights into the evolution of fungi, with early studies focusing on model organisms such as *Saccharomyces cerevisiae* and *Neurospora crassa* (29–31). These foundational analyses revealed the dynamic nature of fungal genomes, highlighting frequent lineage-specific gene duplications, gene loss, and chromosomal rearrangements. Within the Ascomycota, the largest phylum of fungi and home to most lichen-forming species, a general pattern of syntenic erosion over long evolutionary timescales has been observed (32,33). However, syntenic blocks are often conserved in closely related species, particularly those containing genes involved in core metabolic pathways and primary cellular functions (34).

Recent advancements in sequencing technologies have spurred a boom in fungal genomics, including the sequencing of several lichen-forming species. This has led to an explosion of new genomic data, with high-quality assemblies becoming available for key species like *Rhizoplaca*, *Letharia*, and *Cladonia* (35–37). These projects have begun to shed light on the genomic basis of the lichen symbiotic lifestyle. Early insights have pointed to the importance of gene family expansions and contractions, particularly in gene families related to secondary metabolism, transporters, and defence mechanisms (38–40). These studies have provided a foundational understanding of the genetic machinery underpinning symbiotic adaptation, identifying key genes involved in nutrient exchange, communication with the photobiont, and stress tolerance.

Despite these advances, synteny-focused analyses remain rare in lichens. Most existing studies have concentrated on gene content and functional annotation rather than the structural organisation of genomes (18,41). This represents a critical gap, since synteny provides a direct means to assess how genome architecture is maintained or reshaped in response to symbiosis (27). Addressing this gap is essential for understanding not only the evolutionary history of lichenisation but also the selective pressures acting on genome stability in symbiotic fungi (42).

Building on this gap, a major opportunity lies in comparative synteny analysis across the three dominant lichen-forming fungal classes: Lecanoromycetes, Eurotiomycetes, and Dothideomycetes. Each of these groups has undergone independent evolutionary transitions to lichenisation, offering a rare chance to ask whether similar genomic signatures have been retained across lineages or whether each class has followed a distinct trajectory (43,44). A cross-class comparison can reveal whether conserved syntenic regions represent parallel adaptations tied to the lichen lifestyle, or whether the structural organisation of genomes instead reflects lineage-specific histories of rearrangement and plasticity (45,46). By approaching the question at this broader scale, synteny becomes not only a tool for reconstructing ancestry but also a framework for disentangling the balance between convergence and divergence in the evolution of lichen symbioses.

1.4 Project aim and objectives

The main goal of this study was to explore the conservation and variation of genome organisation in lichen-forming fungi. Gene synteny was used as a framework to understand how evolutionary history and ecological lifestyle influence fungal genomes. This aim was pursued through the following objectives:

1. Compile and assess genome assemblies of lichen-forming and non-lichenized fungi from public resources in the classes Lecanoromycetes, Eurotiomycetes, and Dothideomycetes.
2. To detect and quantify conserved syntenic blocks across 38 genomes using homology-based methods and the collinearity detection tool.
3. To analyse patterns of synteny conservation within and across classes, assessing the impact of independent lichenisation events on genome organisation.
4. To perform functional enrichment analysis of syntenic genes using Gene Ontology, to identify the biological processes most frequently retained within conserved blocks.
5. To integrate phylogenetic inference with synteny patterns.

Methodology

2.1 Genome Dataset

The genome dataset used in this study consisted of 38 fungal genomes from the phylum Ascomycota, spanning three classes: Lecanoromycetes (10 species), Eurotiomycetes (15 species), and Dothideomycetes (13 species). This classification follows recent taxonomic frameworks for lichen-forming fungi (47). The genomes were downloaded from the National Centre for Biotechnology Information (NCBI) database, which provides publicly available, standardised, and peer-reviewed assemblies widely used in fungal genomics research (48).

The genomes were selected on the following criteria: 1) representation of phylogenetic diversity across orders and families (49) (Table 1); 2) availability of genome, protein, and CDS sequence files; 3) low scaffold number and high assembly contiguity, as reported by NCBI (50) (Table 2) high assembly contiguity, with a low scaffold number, as reported by NCBI (Table 2). It is important to note that a high level of assembly contiguity is crucial for accurate gene synteny analysis, as fragmented genomes can artificially interrupt syntenic blocks, leading to an underestimation of true conservation (51); 4) availability of gene annotations; and 5) inclusion of species that had either retained or lost lichenisation compared to related taxa (2).

2.2 Quality Control

After collating all relevant genome resources, completeness was assessed using Benchmarking of Universal Single-Copy Orthologs (BUSCO) v5.8.2 (52), applied in both genome (nucleotide) and proteome (protein) modes with the *Ascomycota* lineage dataset. BUSCO evaluates assembly quality by detecting highly conserved single-copy orthologs that are expected to occur within a given lineage. To ensure consistency across datasets, all FASTA headers were standardised to a uniform format using a custom Python script.

Most nucleotide assemblies achieved BUSCO completeness scores above 95%, indicating a likely high overall assembly completeness. Protein datasets showed greater variability, with the lowest completeness score observed at 81%.

For this study, assemblies meeting these thresholds (≥90% protein completeness) were defined as high quality (53,54). Assemblies below these cut-offs were excluded from further analyses. This was done in order to avoid interference in results due to incomplete

genome annotations, which would otherwise serve as anchors for identifying collinear regions between genomes.

2.3 Homology Detection

To identify homologous proteins across species, protein–protein sequence comparisons were conducted using the Basic Local Alignment Search Tool for proteins (BLASTp), implemented in NCBI BLAST v2.16.0 (55). For each proteome, a BLAST database was generated and used in an all-against-all search, where every protein sequence was compared to all others. BLASTp was selected as it provides a reliable and efficient heuristic for detecting protein similarity, making it the most widely used tool in comparative genomics (56). This approach generates pairwise similarity data essential for ortholog detection pipelines and is the recommended input for MCScanX, a toolkit for finding and studying the evolution of gene order and gene arrangement similarities across different species. (26), which requires comprehensive homology information to identify collinear gene blocks. To streamline the workflow, custom Python scripts automated the looping of input files, submission of jobs to the HPC, and concatenation of outputs. The resulting BLAST files were merged into a single master.blast file, which served as one of the inputs for MCScanX. The corresponding annotation files for each genome were also concatenated and provided as a second input file for MCScanX.

2.4 Synteny Block Detection

Synteny block detection was performed using MCScanX as mentioned earlier (26). The pipeline was run with default parameters, which help identify collinear gene pairs based on input homology data and genome coordinates. The output generated by MCScanX included lists of collinear blocks, summary statistics on block size, and the percentage of genes assigned to these blocks. MCScanX was chosen as it is one of the most widely used and benchmarked tools for this purpose, providing accurate and scalable detection across diverse taxa (26). Its adoption in numerous comparative genomic studies, such as in plants (25) and fungi (46,57), highlights its reliability and versatility across different lineages.

Following block identification, results were parsed and visualised to enable comparative interpretation. Heatmaps were generated in R v4.4.1 (58) using packages ggplot2 v3.5.2 (59) and pheatmap v1.0.13 (60) to display the proportion of genes retained within syntenic

blocks between species, allowing patterns of conservation and genome plasticity to be readily identified. This integrative approach facilitated the interpretation of both the scale and biological significance of synteny in lichen-forming fungi, providing a key foundation for the subsequent functional enrichment analysis.

2.5 Phylogenetic Tree Construction

To reconstruct the evolutionary relationships among the studied species, a phylogenetic tree was built using BUSCO single-copy orthologs (61). Homologous protein sequences were first identified through BUSCO and then a custom Python script was used to extract only those orthologs present as a single copy in all genomes. Total of 584 single-copy orthologs were compiled into a single dataset and used as input for downstream analyses.

Multiple sequence alignment of the orthologous proteins was performed with MAFFT v7.525 (62), which is widely used for its accuracy and efficiency in handling large genomic datasets. A maximum-likelihood phylogenetic tree was inferred using IQ-TREE (63), which automatically selected the best-fit model of evolution for each gene partition using the ModelFinder Plus approach. The statistical support for the branches was assessed using two methods: the Ultrafast Bootstrap and the SH-aLRT test, both with 1000 replicates.

The resulting tree was visualised using FigTree v1.4.4 (64), enabling a clear representation of the relationships among species. The phylogeny provided an essential framework for placing the synteny results into an evolutionary context, allowing comparisons of conserved genomic blocks to be interpreted in relation to the evolutionary relationships among the species. This integrative approach ensured that synteny patterns could be linked directly to the evolutionary history of lichen-forming fungi (26,65).

2.6 Functional Enrichment Analysis

Functional enrichment analyses were conducted using the topGO package (66) in R v4.4.1 (58). Gene Ontology (GO) annotations were combined with the list of genes located within syntenic blocks to identify overrepresented functional categories. The genes and GO annotations of the lecanoromycete *L. pustulata* were used for the GO enrichment testing. The tests were conducted specifically for gene enrichment among the genes which are located in syntenic blocks in all Lecanoromycetes genomes, Lichenised

Lecanoromycetes species, as well as all lichen-forming fungi from classes Lecanoromycetes, Dothideomycetes, and Eurotiomycetes.

The Gene Ontology database is structured into three categories: Biological Process (BP), Cellular Component (CC), and Molecular Function (MF) (67,68). For this study, BP terms were considered the most biologically relevant, since they capture functional pathways and stress-adaptive processes that are central to the evolution of lichen symbiosis (79). Enrichment testing was performed separately for the three ontologies.

The elim algorithm, which reduces the influence of broader GO categories, was applied in combination with Fisher's exact test to assess statistical significance. Significance was defined at $p \leq 0.01$ based on raw Fisher p-values. Although topGO does not adjust p-values by default, the elim algorithm mitigates false positives by accounting for the GO topology, and the conservative p-value cutoff was chosen to increase robustness (69).

Functional definitions for each GO term were retrieved from the official AmiGO2 Gene Ontology browser (http://amigo.geneontology.org), ensuring consistency with standardised ontology annotations.

2.7 Computational Resources

Computational work was carried out on the BluePebble High-Performance Computer (HPC) at the University of Bristol's Advanced Computing Research Centre (ACRC) (70). Details of the software environments are listed in Appendix 1. Job scripts were customised using Python v2.7.15 and R v4.4.1 in RStudio v2025.05.1+513 (Mariposa Orchid) on Windows 10. Although Python 2.7 reached end-of-life in 2020, it was retained here for compatibility with specific bioinformatics tools available on the HPC environment.

Results

3.1 Genome Dataset Overview

A total of 38 fungal genomes were selected for the analysis from the phylum Ascomycota, spanning three major classes: Lecanoromycetes (n = 10), Eurotiomycetes (n = 15), and Dothideomycetes (n = 13). These assemblies were selected to ensure phylogenetic diversity across orders and families, while also representing both lichen-forming and non-lichenised lineages.

Overall, the majority of assemblies demonstrated high-level of completeness as observed in Table 3, with BUSCO scores ranging from 94.4–98.5% and averages of 97.8% (Dothideomycetes), 96.8% (Lecanoromycetes), and 96.7% (Eurotiomycetes). Most nucleotide assemblies exceeded the 95% completeness threshold. Protein datasets showed greater variation, with mean completeness values of 95.8% (Dothideomycetes), 94.1% (Eurotiomycetes), and 92.5% (Lecanoromycetes).

Based on the thresholds defined earlier a standard of ≥90% protein completeness was set. The majority of assemblies met high-quality standards and were retained for downstream analyses. A small number of proteomes fell below the 90% cutoff and were excluded to minimise potential artefacts in homology searches and synteny analyses. At the end of the analysis we have 33 species ready for analysis. Particularly, 7 Lecaoromycetes, 13 Eurotiomycetes and 13 Dothideomycetes genomes.

3.2 Synteny Block Statistics

The all-versus-all BLASTp search across the 33 fungal proteomes produced 21.2 million pairwise matches, which were subsequently used as input for MCScanX to identify conserved collinear blocks. In total, the program identified 39,379 blocks, which together contained 170,210 genes. Which represents almost half of all annotated genes (48.65%). Block sizes averaged 9.9 genes; most blocks were very small, ranging from the least 6 genes to containing over 600 genes.

When the data were compared across classes, a clear pattern appeared. Within-class comparisons recovered more blocks. This observation was in line with the expectation that gene order is better preserved among closely related species (17). Among the three classes, Eurotiomycetes showed the strongest conservation, with an average within-class synteny value with the mean within-class synteny of about 1605 and a variability

of roughly 1819. Dothideomycetes showed the lowest conservation, averaging just under 900 with a variability of about 1026. Lecanoromycetes were intermediate, with an average of around 1202 and a variability close to 915. This pattern reflects the differing levels of genome stability observed among these fungal groups. Between-class comparisons still detected shared blocks, but these were fewer and generally smaller. Notably, some lichen-forming species from different classes retained shared collinear regions, suggesting that parts of their genomes have been conserved despite long evolutionary separation.

A full breakdown of top species pairs is provided in Table 4 with exact number of syntenic blocks observed as well as the mean number of genes in these blocks.

3.3 Phylogenetic Context and Synteny Heatmap

To provide evolutionary context for the synteny analyses, a phylogenetic tree was first reconstructed from the BUSCO single-copy orthologs (61). As expected, the topology clearly separated the three Ascomycota classes included in this study: Lecanoromycetes, Eurotiomycetes, and Dothideomycetes (Figure 1) . Within each class, the tree recovered well-supported clades that correspond to established taxonomic relationships. This topology served as a scaffold against which pairwise synteny conservation was compared.

The synteny heatmap, aligned with the phylogenetic tree, displays the number of genes that are preserved in syntenic blocks between each pair of genomes. As anticipated, synteny conservation was highest between closely related taxa, but the strength of conservation varied markedly between classes. Eurotiomycetes exhibited the highest overall within-class conservation, with an average of 23.7% of genes retained in syntenic blocks. However, conservation within Eurotiomycetes was highly uneven, ranging from as little as 3.0% in some comparisons to over 80.3% in the most conserved pairs. Lecanoromycetes displayed a slightly lower average of 18.2%, with values ranging between 4.7% and 32.0%, suggesting moderately conserved genome architecture with fewer extreme cases. Dothideomycetes showed the lowest average within-class conservation at 9.7%, although the range was broad (0.5–65.0%), indicating that while some taxa retain very little collinearity, others preserve substantial blocks of gene order.

Pairwise comparisons between Dothideomycetes and Eurotiomycetes averaged only 2.9% of genes in syntenic blocks, while Dothideomycetes and Lecanoromycetes showed similarly low conservation (3.9%). Interestingly, Eurotiomycetes and

Lecanoromycetes retained higher levels of collinearity on average (10.0%, with some comparisons reaching 29.3%), suggesting that certain regions of gene order have been preferentially conserved between these classes. Their close relationship may explain this higher level of conserved gene order.

Overall, the heatmap demonstrates a strong correlation between phylogenetic proximity and synteny conservation. Genomes within the same clade tended to share darker blocks of synteny, whereas comparisons between classes produced much weaker signals (71). Nonetheless, the wide ranges observed within each class highlight that lifestyle, genome architecture, and lineage-specific histories also influence the extent of synteny (72,73). For example, variation in genome size and the proportion of non-coding regions could contribute to the observed differences in synteny conservation, as larger genomes often have more opportunities for chromosomal rearrangements (74). Similarly, unique lineage-specific events may explain the rapid synteny loss observed in certain taxa (75). This demonstrates that while phylogenetic distance is a primary driver of synteny conservation, other biological factors also play a significant role. In particular, the high variability in Eurotiomycetes and Dothideomycetes may reflect both the diverse ecological niches they occupy and their history of genomic rearrangements, whereas Lecanoromycetes show a more consistent pattern of moderate conservation (76).

In summary, combining phylogenetic inference with synteny analysis revealed that while synteny is generally strongest within classes, its extent is highly variable and shaped by both evolutionary distance and lineage-specific factors. The results further suggest that lichen-forming lineages occasionally retain syntenic blocks across class boundaries, hinting at the selective maintenance of functionally important genomic regions (Figure 1)

3.4 Gene Ontology Enrichment

In total, 219 enriched GO terms were identified across the three domains of the Gene Ontology (Biological Process, Cellular Component, and Molecular Function) when applying an enrichment threshold of $p \leq 0.01$. Among the genes in syntenic blocks, a total of 74 enriched GO terms were identified for all Lecanoromycetes, 67 for all lichenised Lecanoromycetes, and 78 across all lichen-forming fungi.

For clarity, detailed results of the enriched Biological Process terms are provided in tabular form for each sub-analysis (Tables 5–7).

3.4.1 GO enrichment among the Lecanoromycetes synthenic genes

In Lecanoromycetes, as a class, GO enrichment of the syntenic genes pointed mainly to basic cellular functions. Among Biological Process terms (Table 5), gene expression, translation, and organelle organisation were strongly represented, together with protein degradation pathways such as the proteasome-mediated ubiquitin-dependent catabolic process. Molecular Function results highlighted electron transfer activity, RNA binding, and ATP binding.

Overall, these findings indicate that conserved gene order in Lecanoromycetes is often linked to essential housekeeping processes, reflecting the need to maintain organisation of core functional modules. This pattern, where syntenic genes are enriched in housekeeping functions, agrees with previous findings that conserved gene order is most often retained for essential cellular processes (77).

3.4.2 GO enrichment among the Lecanoromycetes Lichens synthenic genes

In *Lecanoromycetes* lichens, syntenic genes were strongly enriched for processes linked to cellular organisation and gene regulation. The most significant Biological Process (Table 6) terms included organelle organisation, gene expression, and RNA biosynthetic process. Enriched Cellular Component terms included cytoplasm, nucleus, and cytosol. Within the Molecular Function GO category, terms like RNA binding and electron transfer activity were enriched, suggesting a role for conserved synteny in regulating RNA metabolism and maintaining energy balance. This aligns with genomic findings that lichen-forming fungi harbour expanded gene clusters and transporters to cope with environmental stress (4).

This emphasis on gene expression regulation and stress-adaptive functions is consistent with previous findings that lichen-forming fungi retain conserved biosynthetic and metabolic pathways critical for survival in extreme environments (78).

3.4.3 GO enrichment among the combined lichen-forming species synthenic genes

For all lichen-forming fungi considered together, GO enrichment analysis revealed a strong emphasis on fundamental cellular processes. The most significantly enriched Biological Process (Table 7) terms were macromolecule biosynthetic process and translation, indicating that syntenic conservation in lichens frequently centres around the protein synthesis machinery, as supported by earlier studies (37). This is consistent with the idea that ribosomal and translational genes tend to remain tightly clustered because of their central role in growth and survival (79).

Taken together, these results show that nearly half of the syntenic gene set in lichens is associated with basic biosynthesis, cellular organisation, and regulatory control. The prominence of translational and RNA-modification functions suggests that lichens conserve synteny around modules that secure robust gene expression and stress-adaptive flexibility, a pattern consistent with their ability to thrive in variable and often extreme environments (80).

Discussion

4.1 Overview of Major Findings

This study shows that gene order is variably conserved across fungal classes, with lifestyle and evolutionary history shaping genomic architecture more than phylogenetic distance alone (81). Approximately half of all annotated genes were retained within syntenic blocks, but conservation was uneven between and within classes (82). Eurotiomycetes showed the highest level of similarity within its class, with an average of about 23.7%. However, the values varied widely, ranging from 3% to over 80%. Lecanoromycetes had a more consistent average of around 18.2% (76,76). Dothideomycetes had the lowest average similarity at about 9.7%, but in some pairs, there were still significant blocks of similarity, reaching up to 65%. These results suggest that rearrangement histories and ecological specialisation may influence genome collinearity as much as divergence time (83,84).

Across all analyses, syntenic genes were enriched for translation, macromolecule biosynthesis, RNA regulation, and organelle organisation, indicating that gene order is preferentially conserved in pathways central to metabolism and cellular organisation (34). The enrichment of categories related to macromolecule biosynthesis, RNA regulation, and organelle organization is consistent with the preferential conservation of gene order in pathways central to metabolism and core cellular function. The presence of stress-response and regulatory categories further suggests that there may be additional selective pressure to maintain collinearity in functions relevant to lichen survival under extreme or variable environments (79,85). This functional conservation in the mycobiont complements previously documented photoprotective adaptations in photobionts (11,24), highlighting that survival in extreme environments is a shared genomic and physiological challenge for the entire lichen symbiosis. However, results can vary depending on algorithm choice, significance thresholds, and minimum node sizes. Hence, these results should be viewed in light of the analytical assumptions underlying the method.

In summary, synteny patterns reflect both evolutionary and ecological forces. Conserved gene order tracks phylogenetic relatedness but is also shaped by lineage-specific adaptations, with occasional cross-class conservation suggesting the selective maintenance of functionally important genomic regions.

4.2 Class-Specific Patterns of Synteny Conservation

The three classes included in this study showed very different levels of synteny conservation, which appears to reflect both their evolutionary backgrounds and their ecological roles.

Eurotiomycetes had the highest average conservation (~23.7%), but this was also the most variable group. Some species pairs retained over 80% of their genes in collinear blocks, while others shared only a few genes. Such a wide spread is not surprising given the diversity of Eurotiomycetes lifestyles, which range from common saprotrophs to important human and plant pathogens (86). High conservation within certain genera, such as *Aspergillus* and *Penicillium*, may indicate the selective retention of large biosynthetic clusters, many of which produce secondary metabolites (87,88). On the other hand, frequent rearrangements in other Eurotiomycetes may represent an adaptive response to environmental pressures or host interactions, where genome plasticity could provide a competitive advantage (89).

Lecanoromycetes showed a different pattern, with more consistent levels of synteny across comparisons. On average ~18.2% of genes were conserved, with values falling between 4.7% and 32%. Unlike Eurotiomycetes, there were no extreme outliers, which suggests a more stable genome organisation (76). This relative stability may be connected to the lichen symbiosis, where a balanced relationship with algal or cyanobacterial partners depends on maintaining reliable metabolic and regulatory pathways (90). Large-scale rearrangements could be disruptive in this context, and the more uniform conservation seen in Lecanoromycetes may reflect stabilising pressures acting on their genomes (91).

Dothideomycetes had the lowest mean conservation (~9.7%), though some pairs retained as much as 65%. This group is known for extensive chromosomal rearrangements and variable genome sizes, and many of its members are pathogens or endophytes (84). The low average values observed here are consistent with this history of plasticity, where genome reorganisation is thought to contribute to adaptation across a wide range of hosts and ecological niches (83,92).

4.3 Functional Significance of Conserved Synteny

The GO enrichment analysis makes it clear that syntenic blocks are enriched for genes involved in basic cellular functions. The most common terms included translation, gene expression, RNA biosynthesis, and organelle organisation. These categories reflect

processes that are fundamental to all living cells. It seems likely that keeping genes for these pathways in conserved positions helps maintain the efficiency and stability of the core molecular machinery (93).

Several enriched categories were linked to RNA modification, protein metabolism, and regulatory processes (94). These suggest that conservation also extends to genes that control how information is used and regulated inside the cell. Keeping the structure of these gene modules intact may serve to maintain the integrity and stability of gene regulatory networks, or the interconnected control of gene expression thus reducing the risk of rearrangements that could disrupt a finely tuned expression profile (95). This is especially important in fungi with demanding ecological roles, such as lichen symbionts, where disruption of regulatory balance could compromise the stability of the partnership (90).

From the lichen perspective, the enrichment of genes related to stress response and energy balance is especially significant. Lichens survive in environments that are often dry, cold, nutrient-poor, or highly variable. In these conditions, the ability to regulate energy use and respond quickly to stress is essential (4). The presence of these categories within conserved syntenic blocks indicates that gene order has been maintained in areas crucial for survival. In this way, synteny conservation reflects not only evolutionary history but also the ecological pressures that lichens face, linking genome structure directly to their ability to persist in extreme and fluctuating habitats (2,96).

4.4 Comparison with Previous Studies

The patterns of genome conservation observed in this study both support and expand upon earlier research in fungal and lichen evolution (57). Previous comparative analyses have reported marked differences in genome stability across fungal classes, with some lineages showing extensive conservation of synteny and others exhibiting frequent rearrangements (97). The results are consistent with this general view: Eurotiomycetes displayed the highest mean within-class conservation but also the greatest variability, while Dothideomycetes showed the lowest average conservation, in line with their well-documented genomic plasticity (98). The low synteny values for Dothideomycetes are consistent with a history of chromosomal rearrangement linked to the adaptation of pathogens across a wide range of hosts and ecological niches (92).

For Lecanoromycetes, the findings refine the picture provided by earlier lichen-focused work (94) reported strong synteny among *Cladonia* species and highlighted cases of conservation across class boundaries (37). In contrast, the present study detected a more consistent but moderate level of within-class conservation (~18%) and relatively low levels of inter-class conservation involving Lecanoromycetes. This discrepancy may reflect differences in taxon sampling, since our dataset included a broader range of lichen-forming species rather than focusing on a single genus (99). It may also arise from assembly quality or differences in the stringency of the synteny-detection pipeline (100). Nonetheless, the more uniform pattern observed here suggests that stabilising pressures may be acting within Lecanoromycetes, whereas conservation across classes appears weaker than previously thought. This is supported by studies on evolutionary biology of lichen symbioses (2).

One novel aspect of this analysis is the detection of syntenic blocks conserved between Eurotiomycetes and Lecanoromycetes, with some comparisons retaining nearly 30% of genes in collinear regions. Although limited, this finding suggests that certain genomic regions may be under selective pressure to remain intact across deep evolutionary divides (46). The conservation of these functionally important regions is supported by studies of other lichen-forming fungi (93). These regions may correspond to essential biosynthetic or stress-related modules, consistent with reports that conserved gene clusters in lichens are often functionally important (96).

Overall, this study confirms earlier observations of variable genome stability across Ascomycota but adds new detail by showing that the pattern of variability itself differs by class: the Eurotiomycetes show high species-variability in gene order, the Dothideomycetes generally display high levels of gene order rearrangement, and the Lecanoromycetes show moderate but consistent gene synteny. In doing so, our analysis highlights how both evolutionary history and ecological lifestyle may shape genome architecture (101).

4.5 Limitations of the study and Future Directions

This study provides valuable insights into genome evolution in lichen-forming fungi; however, several limitations should be considered. Firstly, the findings are based on a specific taxon sampling of 38 fungal genomes. While this dataset represents the phylogenetic diversity of Lecanoromycetes, Eurotiomycetes, and Dothideomycetes, the

results may not be broadly generalizable to all species within these classes (99). The inclusion of more or fewer genomes could influence the overall synteny values.

Secondly, the quality of the genome assemblies themselves may have impacted the analysis. The presence of gaps or scaffolds in the assemblies can lead to an underestimation of true synteny, as these breaks can artificially truncate conserved blocks. Future analyses using higher-quality, chromosome-level genome assemblies would provide a more accurate representation of syntenic relationships (102).

Furthermore, the synteny detection pipeline itself can introduce bias (103). Different software and parameter settings can have varying stringencies for defining syntenic blocks, which may lead to discrepancies when comparing results across studies (66,104). It is also important to note that, on average, only about half of all genes in a fungal genome have an associated GO annotation, which means our analyses could only utilise a subset of the total gene data. Finally, while this analysis inferred functional importance from gene annotations, it is limited by the lack of direct experimental evidence. Future research could employ transcriptomics or other functional genomics approaches to validate the roles of these genes in stress response and symbiosis (105,106) .

Conclusions

This study provides key insights into the genomic architecture of lichen-forming fungi, with three main takeaways:

- **Gene Synteny is Dynamic and Class-Specific:** We found that approximately half of all genes are in syntenic blocks, but the level of conservation varies greatly between the Lecanoromycetes, Eurotiomycetes, and Dothideomycetes classes. This confirms that genome stability is shaped by a combination of evolutionary history and lineage-specific factors.
- **Core Functions are Conserved:** Functional enrichment analysis revealed that the most significantly conserved syntenic regions are associated with core cellular processes like gene expression, translation, and organelle organization. This highlights the selective retention of essential housekeeping functions across diverse fungal lineages.
- **Foundation for Future Research:** This work provides a framework for using gene synteny to understand the evolution of lichen symbiosis. Future studies could expand this analysis to more taxa to explore how ancestry and adaptive pressures shape fungal genomes.

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Tables and Figures

Table 1: Genome study dataset- Fungal Species and Lifestyles*

Class	Species	Abbrev.	Lifestyle
Dothideomycetes	*Alternaria dauci*	Adau	Phytopathogen (Thomma 2003)
	Aureobasidium namibiae	Anam	Multi-niche yeast (Gostinčar et al. 2014)
	Aplosporella prunicola	Apru	Phytopathogen (Crous et al. 2016)
	Ascochyta rabiei	Arab	Phytopathogen (Verma et al. 2016)
	Bathelium mastoideum	**Bmas**	**Lichen (Nelsen et al. 2020)**
	Bogoriella megaspora	**Bmeg**	**Lichen (Aveskamp et al. 2009)**
	Diplodia seriata	Dser	Phytopathogen (Phillips et al. 2013)
	Delphinella strobiligena	Dstr	Lichen (Miadlikowska et al. 2016)
	Neodothiora populina	Npop	Phytopathogen (Walker et al. 2016)
	Phyllosticta capitalensis	Pcap	Endophyte (Glienke et al. 2011)
	Venturia effusa	Veff	Phytopathogen (Bock et al. 2016)
	Viridothelium virens	**Vire**	**Lichen (Haridas et al. 2020)**
	Verruconis gallopava	Vgal	Opportunistic zoonotic pathogen (Teixeira et al. 2017)
Eurotiomycetes	*Aspergillus flavus*	Afla	Phytopathogen (Amaike & Keller 2011)
	Aspergillus niger	Anig	Industrial fungus (Pel et al. 2007)
	Blastomyces dermatitidis	Bder	Human pathogen (Muñoz et al. 2015)
	Coccidioides posadasii str. Silveira	Cpos	Human pathogen (Sharpton et al. 2009)
	Cyphellophora europaea	Ceur	Human pathogen (Teixeira et al. 2017)
	Cladophialophora yegresii	Cyeg	Opportunistic pathogen (Badali et al. 2008)
	Exophiala mesophila	Emes	Opportunistic pathogen (Zeng et al. 2021)
	Endocarpon pusillum	**Epus**	**Lichen (Wang et al. 2014)**
	Emydomyces testavorans	Etes	Soil saprotroph (Hubka et al. 2014)
	Lithohypha guttulata	Lgut	Environmental saprotroph (de Hoog et al. 2011)
	Phaeomoniella chlamydospora	Pchl	plant pathogen, grapevine trunk disease (Gramaje et al. 2011)
	Penicillium chrysogenum	Penc	Industrial fungus, penicillin producer (van den Berg et al. 2008)
	Paecilomyces variotii	Pvar	Opportunistic human pathogen (Samson et al. 2011)
	Talaromyces marneffei	Tmar	Human pathogen, AIDS-associated (Boyce & Andrianopoulos 2015)
	Trichophyton rubrum	Trub	Human skin pathogen (Gräser et al. 2006)
Lecanoromycetes	***Agyrium rufum***	**Aruf**	**Lichen (Lücking et al. 2017)**
	Bacidia gigantensis	**Bgig**	**Lichen (Sérusiaux et al. 2018)**
	Cyanodermella asteris	Cast	Endophyte (Wemheuer et al. 2019)
	Cladonia borealis	**Cbor**	**Lichen (Ahti & Hammer 2002)**
	Gomphillus americanus	**Game**	**Lichen (Lücking et al. 2017)**
	Gyalolechia ehrenbergii	**Gehr**	**Lichen (Nash 2008)**
	Lambiella insularis	**Lins**	**Lichen (Resl et al. 2018)**
	Lasallia pustulata	**Lpus**	**Lichen (Armaleo et al. 2019)**
	Pseudocyphellaria aurata	**Paur**	**Lichen (Spribille et al. 2016)**
	Physcia stellaris	**Pste**	**Lichen (Helms et al. 2001)**

*Species known to adopt a lichen lifestyle were highlighted in **bold**

Table 2: Genome study dataset- Taxonomic classification and source information for genome and proteome resources.*

Species name	Accession Number	Class	Order	Family
Lasallia pustulata	**GCA_008636195.1**	Lecanoromycetes	**Umbilicariales**	**Umbilicariaceae**
Cladonia borealis	**GCA_018257855.2**		**Cladoniaceae**	**Lecanorales**
Bacidia gigantensis	**GCA_019456465.1**		**Lecanorales**	**Ramalinaceae**
Gyalolechia ehrenbergii	**GCA_023646125.1**		**Teloschistales**	**Teloschistaceae**
Agyrium rufum	**GCA_022814335.1**		**Pertusariales**	**Pertusariaceae**
Pseudocyphellaria aurata	**GCA_022814125.1**		**Peltigerales**	**Lobariaceae**
Physcia stellaris	**GCA_018902385.1**		**Caliciales**	**Physciaceae**
Lambiella insularis	**GCA_022814265.1**		**Baeomycetales**	**Xylographaceae**
Gomphillus americanus	**GCA_905337335.1**		**Ostropales**	**Graphidaceae**
Cyanodermella asteris **	GCA_900618795.1		Ostropales	Stictidaceae
Endocarpon pusillum	**GCF_000464535.1**	Eurotiomycetes	**Verrucariales**	**Verrucariaceae**
Aspergillus flavus	GCF_009017415.1		Eurotiales	Aspergillaceae
Aspergillus niger	GCF_000002855.4		Eurotiales	Aspergillaceae
Talaromyces marneffei	GCF_009556855.1		Eurotiales	Trichocomaceae
Paecilomyces variotii	GCF_004022145.1		Eurotiales	Thermoascaceae
Penicillium chrysogenum	GCF_028827035.1		Eurotiales	Aspergillaceae
Trichophyton rubrum	GCF_000151425.1		Onygenales	Arthrodermataceae
Emydomyces testavorans	GCA_029449355.1		Onygenales	Nannizziopsiaceae
Coccidioides posadasii str. Silveira	GCF_018416015.2		Onygenales	Onygenaceae
Blastomyces dermatitidis	GCF_000003525.1		Onygenales	Ajellomycetaceae
Exophiala mesophila	GCF_000836275.1		Chaetothyriales	Herpotrichiellaceae
Cladophialophora yegresii	GCF_000585515.1		Chaetothyriales	Herpotrichiellaceae
Lithohypha guttulata	GCF_036872675.1		Chaetothyriales	Trichomeriaceae
Cyphellophora europaea	GCF_000365145.1		Chaetothyriales	Cyphellophoraceae
Phaeomoniella chlamydospora	GCA_001006345.1		Phaeomoniellales	Phaeomoniellaceae
Viridothelium virens	**GCA_010094025.1**	Dothideomycetes	**Trypetheliales**	**Trypetheliaceae**
Bathelium mastoideum	**GCA_026023875.1**		**Trypetheliales**	**Trypetheliaceae**
Bogoriella megaspora	**GCA_026027345.1**		**Trypetheliales**	**Trypetheliaceae**
Aureobasidium namibiae	GCF_000721765.1		Dothideales	Saccotheciaceae
Delphinella strobiligena	GCA_009982845.1		Dothideales	Dothioraceae
Neodothiora populina	GCF_041146345.1		Dothideales	Dothioraceae
Ascochyta rabiei	GCF_004011695.2		Pleosporales	Didymellaceae
Alternaria dauci	GCF_042100115.1		Pleosporales	Alternaria
Aplosporella prunicola	GCF_010093885.1		Botryosphaeriales	Aplosporellaceae
Phyllosticta capitalensis	GCF_038381095.1		Botryosphaeriales	Phyllostictaceae
Diplodia seriata	GCF_021436955.1		Botryosphaeriales	Botryosphaeriaceae
Venturia effusa	GCA_007735645.1		Venturiales	Venturiaceae
Verruconis gallopava	GCF_000836295.1		Venturiales	Sympoventuriaceae

*Species known to adopt a lichen lifestyle were highlighted in **bold**

** Predicted proteins set not currently publicly available, provided by Dr Francisca Segers

Table 3: Genome statistics and BUSCO completeness scores*

Species Name	No. of Scaffold	Scaffold N50	Protein-coding genes	BUSCO Completeness %	
				Genome	Proteome
Lasallia pustulata	43	1.8 Mb	9825	97.1	93.7
Cladonia borealis	48	1.7 Mb	10750	96.4	97.2
Bacidia gigantensis	24	1.8 Mb	18457	97.0	83.7
Gyalolechia ehrenbergii	317	209.3 kb	8050	96.9	89.9
Agyrium rufum	239	240.2 kb	8330	97.3	94.2
Pseudocyphellaria aurata	978	75 kb	9958	97.6	93.8
Physcia stellaris	155	643.7 kb	10218	96.1	87.1
Lambiella insularis	143	574.9 kb	8485	97.9	90.7
Gomphillus americanus	54	685.7 kb	8326	95.4	94.8
Cyanodermella asteris	37	1.8 Mb	10036	95.6	96.7
Endocarpon pusillum	908	178.2 kb	9238	94.4	90.9
Aspergillus flavus	8	4.8 Mb	13707	97.7	97.9
Aspergillus niger	19	2.5 Mb	14058	95.0	94.2
Talaromyces marneffei	8	3.7 Mb	9994	97.3	98.2
Paecilomyces variotii	86	1.7 Mb	9270	97.3	97.6
Penicillium chrysogenum	4	9.5 Mb	11974	97.1	97.8
Trichophyton rubrum	35	2.2 Mb	8616	94.5	81.9
Emydomyces testavorans	5	9.6 Mb	7470	96.5	94.3
Coccidioides posadasii str. Silveira	9	8.1 Mb	8299	96.8	96.7
Blastomyces dermatitidis	25	5.6 Mb	9754	97.2	97.2
Exophiala mesophila	9	5.1 Mb	9181	97.5	97.7
Cladophialophora yegresii	8	4.3 Mb	10118	97.5	98.0
Lithohypha guttulata	142	3.8 Mb	8740	96.6	90.0
Cyphellophora europaea	19	2.4 Mb	11094	97.0	97.2
Phaeomoniella chlamydospora	702	178.6 kb	6907	97.9	82.4
Viridothelium virens	730	324.4 kb	11845	97.7	98.0
Bathelium mastoideum	54	1.3 Mb	10035	97.9	94.4
Bogoriella megaspora	395	253.3 kb	10889	94.7	93.9
Aureobasidium namibiae	47	1.1 Mb	10257	98.3	96.9
Delphinella strobiligena	17	2.3 Mb	10337	98.1	97.6
Neodothiora populina	18	1.9 Mb	7339	98.0	95.3
Ascochyta rabiei	22	1.9 Mb	11525	98.4	90.3
Alternaria dauci	10	3.4 Mb	10026	98.4	96.4
Aplosporella prunicola	334	957.8 kb	12531	97.9	97.9
Phyllosticta capitalensis	14	2.9 Mb	12062	97.1	97.6
Diplodia seriata	21	3.3 Mb	10553	97.9	90.3
Venturia effusa	20	2.5 Mb	10820	98.3	97.8
Verruconis gallopava	367	572 kb	9818	98.5	98.4

*Species denoted in red have been excluded from further analysis due to not meeting the high-quality standards.

Table 4:Summary of synteny block statistics per species pair.

Species A	Species B	Number of Blocks	Mean Block Size
Cyeg	Emes	406	11.33
Bmeg	Vire	382	9.58
Arab	Adau	379	9.04
Bmas	Bmeg	350	9.70
Dstr	Npop	344	13.86
Apru	Pcap	312	9.82
Cbor	Lpus	311	10.15
Pvar	Penc	310	15.29
Tmar	Penc	299	14.59
Cpos	Penc	285	14.31
Tmar	Afla	283	16.17
Bmas	Vire	283	23.04
Bder	Penc	283	13.85
Dstr	Anam	282	8.94
Pvar	Bder	281	15.17
Tmar	Pvar	277	16.70
Afla	Penc	274	20.14
Tmar	Anig	273	15.84
Cyeg	Ceur	272	8.71
Anig	Pvar	271	17.49
Lins	Lpus	267	9.76
Ceur	Emes	267	8.28
Etes	Penc	266	14.68
Afla	Pvar	263	18.47
Afla	Bder	258	16.30
Cpos	Pvar	255	17.01
Anig	Penc	253	20.42
Anig	Bder	248	15.98
Etes	Pvar	248	17.03
Tmar	Cpos	244	16.91
Tmar	Bder	244	15.99
Cpos	Afla	243	17.56
Etes	Tmar	241	16.74
Etes	Lpus	239	9.15
Cbor	Lins	237	8.79
Cpos	Lpus	236	9.23
Cpos	Anig	235	17.15
Etes	Afla	234	17.66
Cbor	Cpos	232	8.55

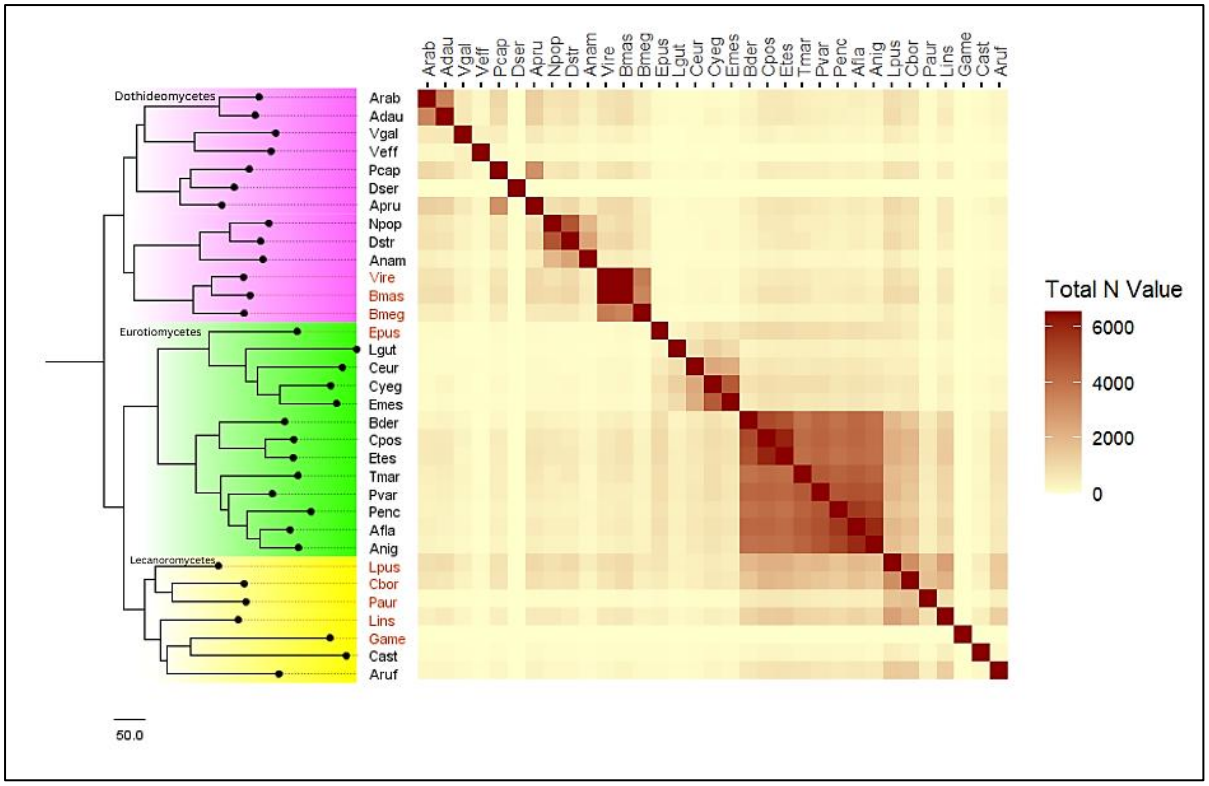

Figure 1. Phylogenetic tree and synteny conservation heatmap of 39 Ascomycota genomes.
A maximum-likelihood phylogenetic tree was reconstructed from conserved single-copy BUSCO orthologs and illustrates the expected separation of the three focal classes: Dothideomycetes (pink), Eurotiomycetes (green), and Lecanoromycetes (yellow). The adjacent heatmap shows the proportion of genes retained in syntenic blocks between each pair of genomes, with darker colours indicating stronger conservation.

Table 5: Top 10 Enriched Biological Processes Gene Ontology (GO) terms for syntenic genes in class Lecanoromyces.

GO.ID	Term	Annotated	Significant	Expected	Fisher	Term Information (AmiGO2)
GO:0008150	Biological_Process	3015	2122	2113.38	0.00000012	Any process carried out by living organisms; the root of the Biological Process ontology.
GO:0010467	Gene Expression	762	605	534.13	0.00054	Conversion of gene information into a functional product (RNA or protein), including transcription and translation.
GO:0006412	Translation	248	196	173.84	0.00054	Formation of a polypeptide by ribosomes using an mRNA (or circRNA) template.
GO:0043161	Proteasome-Mediated Ubiquitin-Dependent ...	34	32	23.83	0.0006	Proteasome-driven breakdown of proteins that have been tagged with ubiquitin.
GO:0006996	Organelle Organization	327	258	229.21	0.00066	Assembly, arrangement, or disassembly of organelles within a cell.
GO:0009451	RNA Modification	44	40	30.84	0.00082	Covalent chemical alteration of RNA nucleotides after transcription.
GO:0016043	Cellular Component Organization	516	410	361.69	0.00087	Assembly, arrangement, or disassembly of cellular structures and complexes.
GO:0008033	Trna Processing	38	35	26.64	0.00098	Conversion of a pre-tRNA into a mature tRNA ready for aminoacylation.
GO:0000466	Maturation Of 5.8S Rrna From Tricistroni...	26	25	18.22	0.00109	Processing step that generates mature 5.8S rRNA from a tricistronic rRNA precursor.
GO:0051247	Positive Regulation Of Protein Metabolic...	26	25	18.22	0.00109	Any process that increases the rate, frequency, or extent of protein metabolism

Table 6: Top 10 Enriched Biological Processes Gene Ontology (GO) terms for syntenic genes of lichen-forming fungi in class Lecanoromyces.

GO.ID	Term	Annotated	Significant	Expected	Fisher	Term Information (AmiGO2)
GO:0008150	Biological_Process	3015	2009	2001.05	0.00000067	Any process carried out by living organisms; the root of the Biological Process ontology.
GO:0006996	Organelle Organization	327	253	217.03	0.0000024	Assembly, arrangement, or disassembly of organelles within a cell.
GO:0010467	Gene Expression	762	567	505.74	0.000079	Conversion of gene information into a functional product (RNA or protein), including transcription and translation.
GO:0051247	Positive Regulation Of Protein Metabolic...	26	25	17.26	0.0003	Any process that increases the rate or extent of protein metabolism.
GO:0032774	Rna Biosynthetic Process	415	315	275.44	0.00057	Synthesis of RNA molecules as part of gene expression regulation
GO:0050789	Regulation Of Biological Process	530	398	351.76	0.00088	Any process that modulates the frequency, rate or extent of a biological process.
GO:0007163	Establishment Or Maintenance Of Cell Pol...	27	25	17.92	0.00151	Any cellular process that results in the specification, formation or maintenance of anisotropic intracellular organization or cell growth patterns.
GO:0033036	Macromolecule Localization	213	169	141.37	0.00152	Any process in which a macromolecule is transported to, or maintained in, a specific location.
GO:0022607	Cellular Component Assembly	222	167	147.34	0.00171	The aggregation, arrangement and bonding together of a cellular component.
GO:0046907	Intracellular Transport	228	171	151.32	0.0019	The directed movement of substances within a cell.

Table 7: Top 10 Enriched Biological Processes Gene Ontology (GO) terms for syntenic genes found in lichen-forming fungi of classes Lecanoromyces, Eurotiomycetes and Dothideomycetes.

GO.ID	Term	Annotated	Significant	Expected	Fisher	Term Information (AmiGO2)
GO:0008150	Biological_Process	3015	2209	2200.86	0.00000028	Any process carried out by living organisms; the root of the Biological Process ontology.
GO:0009059	Macromolecule Biosynthetic Process	820	678	598.58	0.0001	The chemical reactions and pathways resulting in the formation of a macromolecule
GO:0006412	Translation	248	205	181.03	0.00011	Formation of a polypeptide by ribosomes using an mRNA (or circRNA) template.
GO:0006996	Organelle Organization	327	270	238.7	0.00033	A process that is carried out at the cellular level which results in the assembly, arrangement of constituent parts, or disassembly of an organelle within a cell.
GO:0065007	Biological Regulation	592	488	432.14	0.00054	Any process that modulates a measurable attribute of any biological process, quality or function.
GO:0022607	Cellular Component Assembly	222	186	162.05	0.00066	The aggregation, arrangement and bonding together of a cellular component.
GO:0009451	RNA Modification	44	41	32.12	0.00068	The covalent alteration of one or more nucleotides within an RNA molecule to produce an RNA molecule with a sequence that differs from that coded genetically
GO:0050794	Regulation Of Cellular Process	486	394	354.77	0.00074	Any process that modulates the frequency, rate or extent of a cellular process, any of those that are carried out at the cellular level, but are not necessarily restricted to a single cell.
GO:0044237	Cellular Metabolic Process	1491	1181	1088.39	0.00138	The chemical reactions and pathways by which individual cells transform chemical substances.
GO:0016043	Cellular Component Organization	516	427	376.66	0.00198	A process that results in the assembly, arrangement of constituent parts, or disassembly of a cellular component.
GO:0007163	Establishment Or Maintenance Of Cell Pol...	27	26	19.71	0.00205	Any cellular process that results in the specification, formation or maintenance of anisotropic intracellular organization or cell growth patterns.

Appendices

Appendix 1

Supplementary table 1: Summary of Software, Packages, and Databases Used in This Study

Software	Version	Application	Reference
Python	v2.7.15	Custom scripts, job submission	(https://www.python.org/)
R (RStudio)	v4.4.1 (RStudio v2025.05.1)	Figures and enrichment analyses	Team RC (2013); RStudio Team (2020)
BUSCO	v5.8.2	Quality control	Simão et al. (2015); Manni et al. (2021)
QUAST		Quality control	
BLAST+	v2.14.1	All-vs-all protein homology search	Camacho et al. (2009)
MCScanX	2012 release	Synteny block detection	Wang et al. (2012)
MAFFT	v7.525	Phylogenomics	Katoh and Standley (2013)
IQTREE	v1.4.4	Phylogenomics	Minh BQ, et al. 2020; Nguyen LT, et al. 2015
pheatmap (R)	v1.0.13	Heatmap visualisation	Kolde R. *pheatmap* package
ggplot2 (R)	v3.5.2	Heatmap visualisation	Wickham H. *ggplot2: Elegant Graphics for Data Analysis*
topGO	v2.56.0	GO enrichment analysis	Alexa & Rahnenführer (2006)
Database			
NCBI	-	Genome Collection	(https://www.ncbi.nlm.nih.gov/)