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Article

Genome-Scale Phylogenetic Analyses Provide Insights of the Evolutionary History of Species Complexes in the Genus *Fusarium*

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Abstract

Recent developments in next-generation sequencing have improved the reliability of phylogenetic trees, allowing genome-wide phylogenetic analyses. *Fusarium* fungi are important phytopathogenic and clinical fungi. As the identification of species in this genus is conducted on a species complex (SC) basis, evaluating the monophyly of each SC and its evolutionary history are important. Recently, genome-scale phylogenetic analyses using a concatenation approach based on DNA data identified the putative phylogeny of *Fusarium* SCs. However, different data types and analytical approaches often yield different trees, and the possibility of alternative phylogenetic relationships has not yet been discussed. In this study, phylogenetic analyses using concatenation and coalescence approaches as well as polytomy tests based on DNA and amino acid data from 1,190 genes were performed on 23 *Fusarium* SCs. Our results suggest that (i) *F. commune* is independent of *F. nisikadoi* SC; (ii) the branching order among *F. oxysporum* SC, *F. newnwsense* SC, and *F. nisikadoi* SC cannot be determined; and (iii) the phylogenetic position of *F. buxicola* is inconsistent among the analyses. This study provides novel insights into the evolutionary history of *Fusarium* SCs and has implications for their classification and identification.

Keywords: *Fusarium*; species complex; evolutionary history; concatenation approach; coalescence approach; monophyly

1. Introduction

Recent developments in next-generation sequencing technologies, methodologies, and software have enabled analysis of genome-wide phylogenies. Genome-scale phylogenetic trees constructed from the alignment of hundreds to thousands of concatenated genes can significantly improve the reliability of phylogenetic tree branches and provide valuable insights into phylogenetic relationships [1]. Additionally, software for genome-scale coalescent-based phylogenetic analyses, such as ASTRAL [2], has been developed and used to resolve controversial lineages [3]. Furthermore, genome data allows us to predict polytomies, which are useful for discussing evolutionary histories [4,5].

The taxonomy of *Fusarium* Link has long been studied by many researchers because *Fusarium* fungi are internationally recognized as important phytopathogenic and clinical fungi. The genus *Fusarium* was established in 1809, and approximately 400 species are currently phylogenetically recognized. Because of the large number of *Fusarium* species, classification and identification are conducted based on the species complex (SC), a monophyletic group comprising multiple species without synapomorphy, as the operational taxonomic unit. Therefore, the monophyly of each SC must be ensured to accurately classify and identify species.

Since their establishment, the evolutionary history of SCs has been investigated using multiple genes. Geiser et al. [6] inferred phylogenetic relationships based on combined RPB1 (the largest

subunit of the RNA polymerase II gene) and RPB2 (the second largest subunit of the RNA polymerase II gene) nucleotide sequence data. Most phylogenetic relationships among SCs were supported by the results of O'Donnell et al. [7] and Geiser et al. [8], who subsequently analyzed phylogenies based on 19 genes, improving branch reliability. However, differences were observed in the branching order of *F. fujikuroi*, *F. newnesense*, *F. nisikadoi*, and *F. oxysporum* SCs when comparing lineages derived from 559 core genes [9]. In the former, the order was (*F. newnesense* SC, (*F. oxysporum* SC, (*F. nisikadoi* SC, *F. fujikuroi* SC))), whereas in the latter, it was (*F. fujikuroi* SC, (*F. oxysporum* SC, (*F. newnesense* SC, *F. nisikadoi* SC))). Differences were observed in the outer phylogenetic relationships of *Fusarium sensu stricto* (s. str.). Multigene (2- and 19-gene) analyses showed the following SC branching order: ((*F. dimerum* SC, *F. ventricosum* SC), (*F. albidum* SC, ((*F. solani* SC, *F. decemcellulare* SC), (*F. staphyleae* SC, (*Fusarium* s. str., *F. buxicola* SC)))))), whereas the genome-scale phylogenetic analysis showed (*F. ventricosum* SC, (*F. dimerum* SC, (*F. albidum* SC, (*F. staphyleae* SC, ((*Fusarium* s. str., *F. buxicola* SC), (*F. solani* SC, *F. decemcellulare* SC))))) [9]. Both phylogenetic trees based on 19-gene and genome-scale analyses showed high branch support.

However, cases showing discrepancies between nucleotide and amino acid sequences or concatenation and coalescence approaches have been reported in molecular phylogenetic analyses. The coalescent-based method of species phylogeny estimation accounts for the possible effects of incomplete lineage sorting on tree reconstruction [10–13]. In genome-scale analyses, different data types or analysis methods may yield different topologies composed of statistically supported internal branches [10]. In such cases, selecting a single phylogenetic tree to represent the evolutionary history is difficult; therefore, multiple phylogenetic trees that do not fully match should be considered.

Lizcano Salas et al. [14] suggested that there is a conflict regarding the position of the *F. oxysporum* SC between results obtained using concatenation and coalescent approaches based on DNA data. The concatenation approach indicated that *F. oxysporum* SC is sister to a clade consisting of *F. nisikadoi* SC and *F. newnesense* SC, whereas the coalescent approach showed that *F. oxysporum* SC is sister to *F. fujikuroi* SC. This inconsistency highlights an unresolved issue in the phylogenetic placement of SCs within the genus *Fusarium*, and further information is needed to elucidate their evolutionary relationships.

In this study, to evaluate the monophyly and evolutionary history of the current *Fusarium* SCs, we performed genome-scale phylogenetic analyses using both concatenation and coalescence approaches with DNA and amino acid sequences. In addition, to further explore conflicts in evolutionary histories among studies, we conducted a quartet-based polytomy test implemented using ASTRAL (Accurate Species TRee ALgorithm; Mirarab et al. [2]).

Here, we report three conflicting evolutionary histories among the analyses: (i) the phylogenetic position of *F. commune*, a member of the *F. nisikadoi* SC; (ii) the branching order of *F. commune*, *F. newnesense* SC, *F. nisikadoi* SC, and *F. oxysporum* SC; and (iii) the phylogenetic position of the *F. buxicola* SC.

2. Materials and Methods

2.1. Taxon Sampling

The ingroup taxa comprised genomic data from 101 species, representing 23 *Fusarium* SCs. As outgroup taxa, three strains belonging to three species of *Neonectria* (*N. coccinea*, *N. galligena*, and *Nectria* sp.) and *Trichoderma brevicompactum* were selected based on previous studies (Supplementary Table S1 [15–29]).

2.2. Orthology Inference of Genes and Selection

We conducted a reciprocal BLAST search to identify orthologous genes, following the method described by Nozawa et al. [30]. Briefly, gene predictions were performed using Augustus v.3.3.3 [31] with the parameter: “--genemodel=complete --species=fusarium <genomic data>”. Orthology inference was conducted using reciprocal BLAST analysis with BLASTP in BLAST 2.9.0+ software (e-

value $\leq 1 \text{ E-}05$) [32,33], considering the reciprocal best BLAST hits as orthologs. Multiple sequence alignments for each orthologous gene were generated using Clustal Omega v.1.2.2 [34] with default settings at the amino acid sequence level.

Genes longer than 1,000 bp (excluding gap-containing sites after alignment) were selected, as Mirarab et al. [2] demonstrated that species tree accuracy improves when individual gene lengths increase from 500 to 1,000 bp. To obtain gene alignments meeting this criterion, we trimmed the gap-containing sites using trimAl v.1.2 [35] and counted the remaining sites using PhyKIT v.1.2.0 (<https://jlsteenwyk.com/PhyKIT/>; Steenwyk et al. [36]). Sequence statistics for the concatenated datasets were calculated using the Sequence Data Explorer module of MEGA10 [37].

2.3. Phylogenetic Analyses

Phylogenetic trees were constructed using concatenation and coalescence approaches. Concatenated alignments of DNA and amino acid data from all gene sets (1,190 genes) were analyzed using RAxML v.7.0.4 [38] under the GTRGAMMA and PROTGAMMAAUTO models, respectively, with 100 bootstrap replicates.

For the coalescence approach, species trees were inferred using the summary-tree method implemented in ASTRAL III v.4.4.4 [2]. Individual gene trees were first constructed from the DNA and amino acid datasets using MEGA-CC v.10.2.5 [37]. DNA data were analyzed using the maximum likelihood method under a general time-reversible model with gamma distribution, and amino acid data were analyzed using the Poisson distribution model with gamma distribution. Statistical support for each internal branch of the coalescent-based trees was evaluated using local posterior probability (LPP) values [39].

2.4. Gene Tree Discordance Analysis and Polytomy Test

Gene tree support and conflict were evaluated using bipartition analysis implemented in the PhyParts program [40]. The coalescence-based species tree was re-rooted using *T. brevicompactum* (IBT40841), and all individual gene trees were re-rooted accordingly. The program calculated the number of gene trees supporting each bipartition across all the species. The results were summarized using pie charts illustrating the proportions of gene trees that (i) were congruent with the bipartition, (ii) supported most frequent alternative bipartition, and (iii) supported other alternative bipartitions.

The polytomy test was performed using the ASTRAL package (option t 10) [4]. This statistical test is based on the multispecies coalescence model, and evaluates the null hypothesis that a branch in the estimated species tree should be replaced by a polytomy.

3. Results

3.1. Dataset Characteristics

After performing RPB searches, 1,190 orthologous genes were identified. The minimum and maximum alignment lengths for the DNA data were 1,002 and 7,857 bp (mean: 1,688 bp), respectively (Supplementary Table S2). The minimum and maximum proportions of variable alignment were 33.5 and 95.2% (mean: 60.1%), respectively. The minimum and maximum alignment lengths for the amino acid data were 334 and 2,619 sites (mean: 556 sites), respectively. The minimum and maximum proportions of variable alignment sites were 7.5 and 98.3% (mean: 51.6%), respectively.

The alignment length and number of variable and parsimony-informative sites for the 1,190 genes in the DNA data were 1,984,401 bp, 1,184,205 sites (59.7%), and 1,030,213 sites (51.9%), respectively (Supplementary Table S3). For amino acid sequences, the corresponding values were 661,467 sites, 336,905 variable sites (50.9%), and 254,109 parsimony-informative sites (38.4%).

The analysis indicated that the *F. nisikadoi* SC is polyphyletic. In the phylogenetic tree based on the amino acid sequences obtained using concatenation (Figure 1) and coalescent approaches (Figure 2), *F. commune*, previously considered a member of the *F. nisikadoi* SC, was sister to the (*F. foetens* (*F. odoratissimum*, *F. oxysporum*)) clade, which corresponds to an *F. oxysporum* SC, but not to the *F. nisikadoi* SC. In both concatenation and coalescence-based analyses using nucleotide sequences, *F. commune* was found to be the most ancestral lineage within the *F. nisikadoi* SC (Figures 3 and 4).

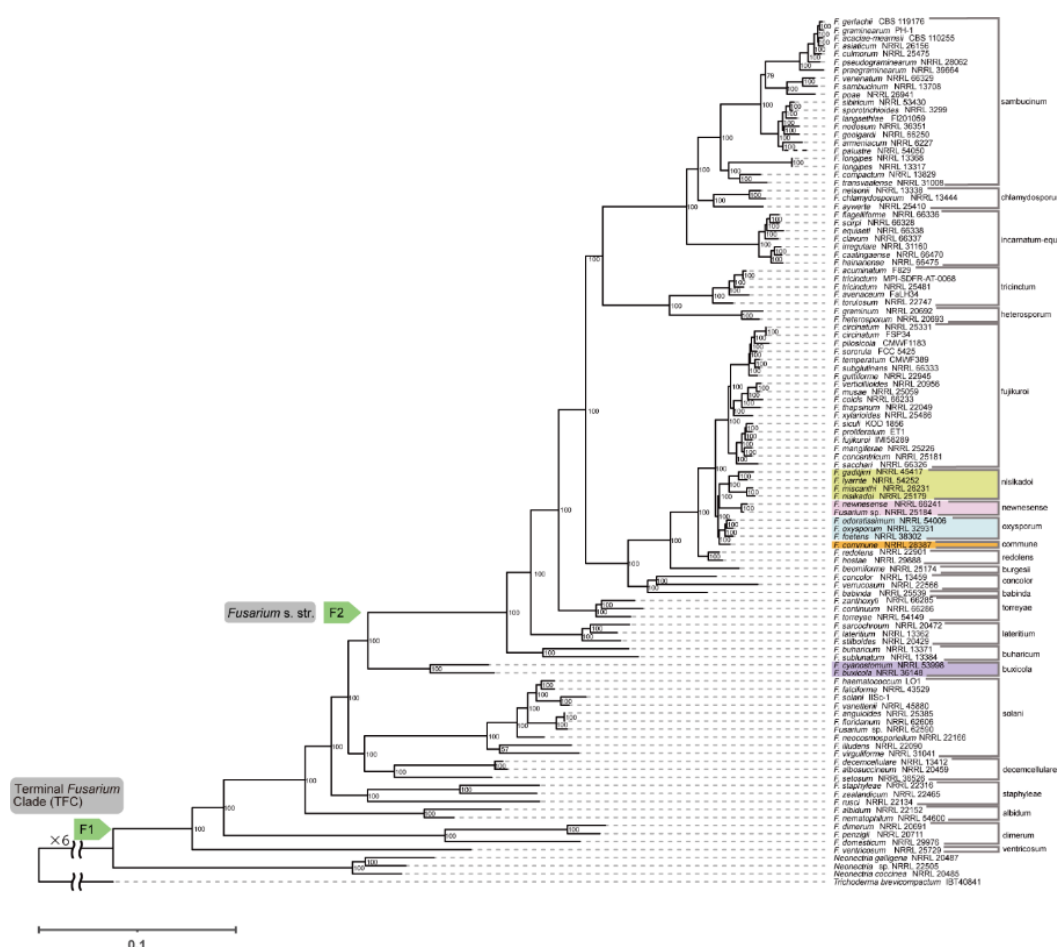


Figure 1. Phylogenetic trees constructed using the concatenation approach in RAxML with the amino acid sequences of 1,190 orthologous genes. Numbers above the internal nodes represent the maximum likelihood of bootstrap (BS) support. Node F1 is for the terminal *Fusarium* clade. Node F2 is for the *Fusarium s. str.* clade.

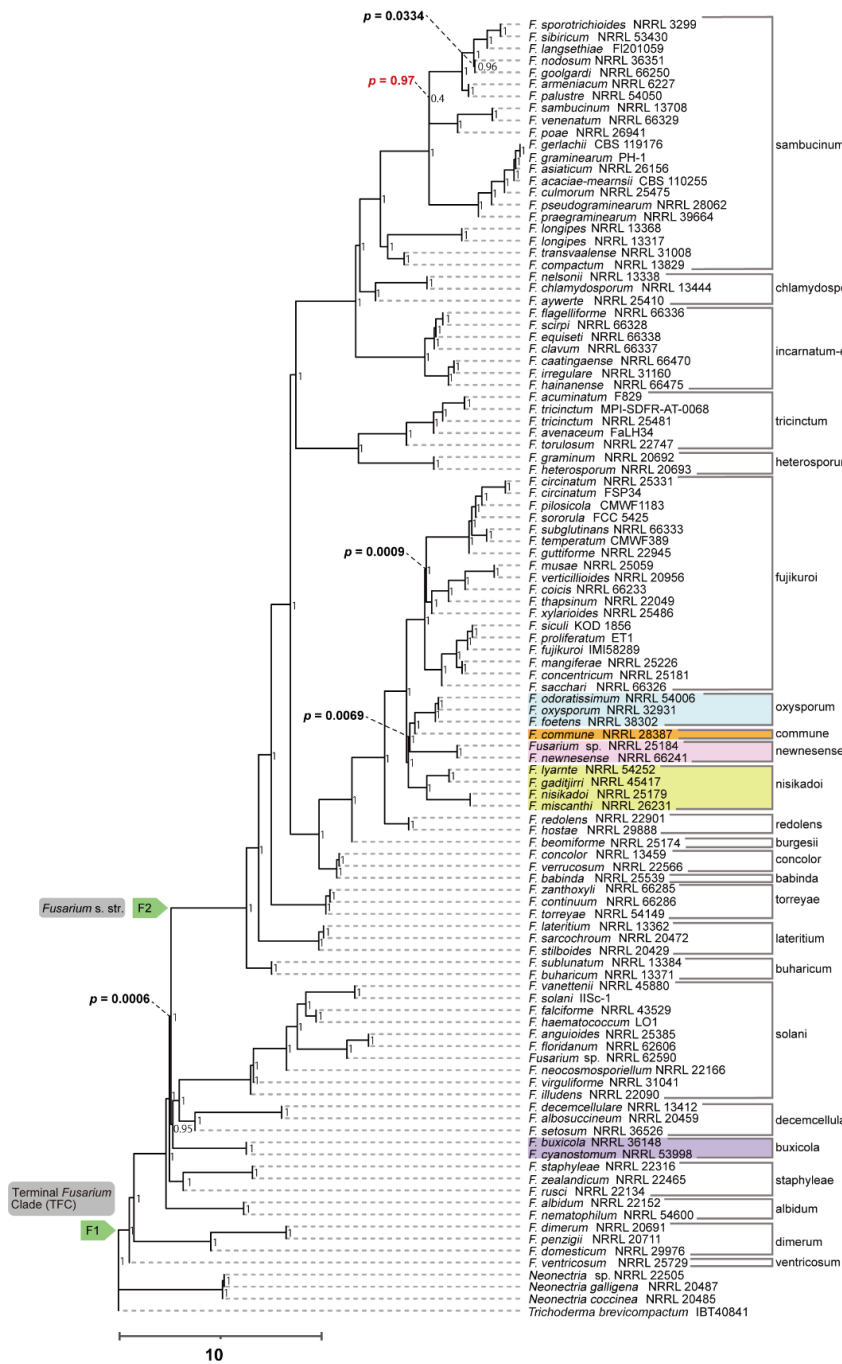


Figure 2. Phylogenetic trees constructed using the coalescent approach in ASTRAL III with the amino acid sequences of 1,190 orthologous genes. Numbers above the internal nodes represent local posterior probability (LPP) support. Node F1 is for the terminal *Fusarium* clade. Node F2 is for the *Fusarium* s. str. clade.



Figure 3. Phylogenetic trees constructed using the concatenation approach in RAxML with the DNA sequences of 1,190 orthologous genes. Numbers above the internal nodes represent the maximum likelihood of bootstrap (BS) support. Node F1 is for the terminal *Fusarium* clade. Node F2 is for the *Fusarium s. str.* clade.

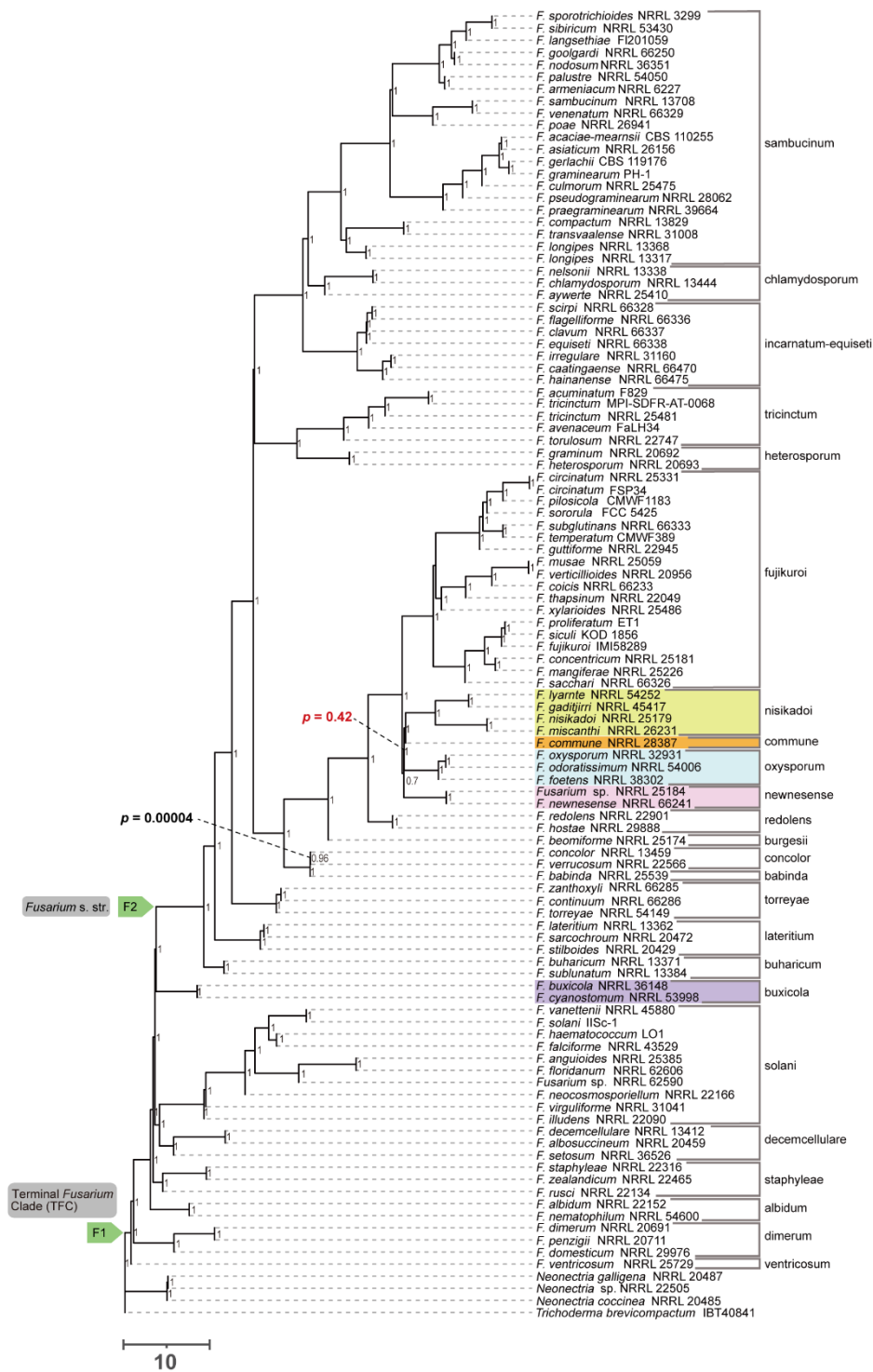


Figure 4. Phylogenetic trees inferred using the coalescent approach in ASTRAL III with the DNA sequences of 1,190 orthologous genes. Numbers above the internal nodes represent local posterior probability (LPP) support. Node F1 is for the terminal *Fusarium* clade. Node F2 is for the *Fusarium s. str.* clade.

The branching order among *F. newnesense*, *F. nisikadoi*, and *F. oxysporum* SCs differed depending on the data type and analytical approach, as follows: the tree based on the concatenation approach using amino acid sequences was ((*F. nisikadoi* SC, *F. newnesense* SC), (*F. oxysporum* SC, *F. commune*)) (Figure 1); the tree based on the coalescent approach using amino acid sequences was (((*F. oxysporum* SC, *F. commune*), *F. newnesense* SC), *F. nisikadoi* SC) (Figure 2); the tree based on the concatenation approach using nucleotide sequences was ((*F. nisikadoi* SC, *F. newnesense* SC), *F. oxysporum* SC) (Figure 3); the tree based on

the coalescent approach using nucleotide sequences was ((*F. nisikadoi* SC, *F. oxysporum* SC), *F. newnesense* SC) (Figure 4). All internal branches were highly supported (bootstrap [BS] = 100; LPP = 1).

In addition, the phylogenetic position of *F. buxicola* differed among analyses. In the tree based on the coalescent approach using amino acid sequences, the *F. buxicola* SC was sister to the monophyletic group comprising *F. solani* and *F. decemcellulare* SCs. In the other analyses, the *F. buxicola* SC was placed between the monophyletic group consisting of *F. solani*, *F. decemcellulare* SC, and *F. buharicum* SC. The phylogenetic relationships of the remaining SCs were consistent across all analyses and the clades of these SCs were strongly supported.

3.3. Gene Tree Discordance

Bipartition analyses were conducted using phylogenetic trees inferred from concatenation- and coalescent-based approaches, based on both DNA and amino acid gene trees. The results confirmed the following phylogenetic uncertainties: 1) *F. commune*, 2) the three-SC group (*F. nisikadoi* SC, *F. newnesense* SC, and *F. oxysporum* SC), and 3) *F. buxicola* SC, which were observed to be incongruent across data types and analytical approaches, as described in the *Phylogenetic Analysis* section. Bipartition analyses focusing on these inconsistent branches are summarized below.

For the phylogenetic tree inferred from the amino acid data, bipartition analysis showed that 17.3% of the gene trees supported the clade comprising *F. nisikadoi* SC and *F. oxysporum* SC (Figure 5). Additionally, the topologies ((*F. newnesense* SC, (*F. commune*, *F. oxysporum* SC)), (*F. nisikadoi* SC, (*F. newnesense* SC, (*F. commune*, *F. oxysporum* SC))), and (*F. buxicola* SC, (*F. decemcellulare* SC, *F. solani* SC)) were supported by 9.8%, 12.4%, and 9.1% of the gene trees, respectively. The concordance rate calculated using ASTRAL III based on quartet trees from the amino acid data supported these results (lanes 3, 8, and 23 in Supplementary Figure S2; lines 3, 8, and 23 of Supplementary Table S4).

For the phylogenetic tree based on DNA data, bipartition analysis revealed that 28.4% of the gene trees supported the clade comprising *F. commune* and *F. nisikadoi* SC (Figure 5). Additionally, the topologies ((*F. commune*, *F. nisikadoi* SC), *F. oxysporum* SC), (*F. newnesense* SC, ((*F. nisikadoi* SC, *F. commune*), *F. oxysporum* SC)), and (*F. buxicola* SC and *Fusarium sensu stricto*) were supported by 15.5%, 25.2%, and 27.8% of the gene trees, respectively. The concordance rate, calculated using the ASTRAL III program based on quartet trees from the DNA data, supported these results (lanes 1, 4, and 7 in Supplementary Figure S3, lines 1, 4, and 7 in Supplementary Table S5).

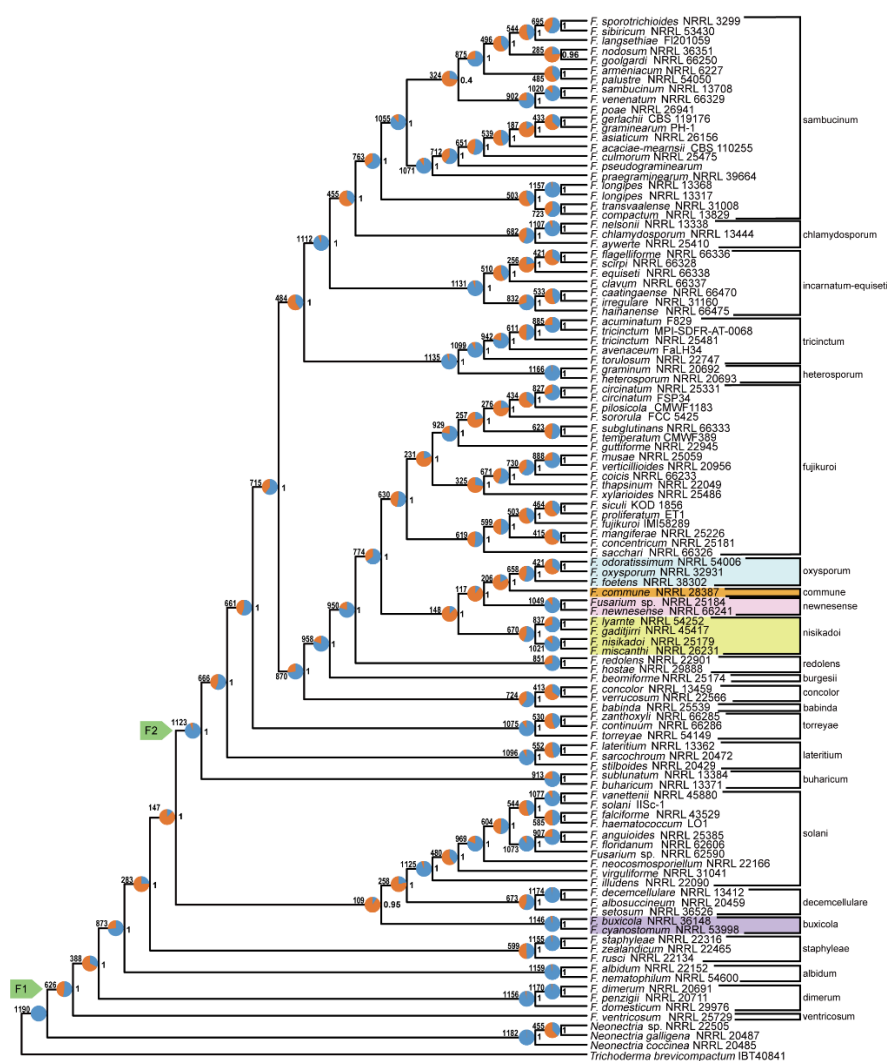


Figure 5. Phylogenetic tree based on amino acid data of 1,190 genes using coalescent-approach analyzed with ASTRAL, with summary of conflicting and concordant homologs. For each branch, the top number indicates the number of homologs concordant with the species tree at that node. The pie charts at each node present the proportion of homologs that support that clade (blue) and the proportion that support the main alternative for that clade (orange). Node F1 is for the terminal *Fusarium* clade. Node F2 is for *Fusarium* s. str. clade.

3.4. Polytomy Test

The polytomy test confirmed an unreliable branching order among the (*F. nisikadoi* SC and *F. commune*), *F. oxysporum* SC, and *F. newnesense* SC clades in the DNA-based coalescent tree. Polytomy was detected among these relationships, with a high p-value of 0.46 (Figure 4).

4. Discussion

4.1. Phylogenetic Reconstruction

Phylogenetic inference using concatenation- and coalescent-based approaches, based on genomic data for various groups of organisms, is now widely used. Concatenation-based phylogenetic analysis is a scaled-up version of traditional multigene analysis, which is automated and utilizes large amounts of data. Therefore, errors, such as paralogous gene sampling and missing data, are typical. However, because these errors occur randomly and infrequently, they tend to be averaged across the dataset, resulting in phylogenetic trees with a high statistical significance.

Nevertheless, recent empirical observations of gene tree discordance, that is, cases in which genes have different evolutionary histories, have shown that the traditional concatenation approach [41] cannot fully account for such discordance among gene trees inferred from phylogenetic data. Consequently, several studies applied coalescent-based methods.

However, because the choice between coalescent- and concatenation-based approaches remains controversial regarding the accuracy of species tree estimation [2,42], both methods were used in the current study. Using both DNA and amino acid data, genome-scale phylogenetic trees were reconstructed to evaluate the monophyly of *Fusarium* SCs and their evolutionary relationships. Comparisons between concatenation- and coalescent-based phylogenetic trees revealed differences in the branching order of certain evolutionary lineages. Likewise, comparisons between DNA- and amino acid-based phylogenetic trees showed topological incongruence (Figures 1–4, Supplementary Figure S1). Both trees had high statistical support for internal branches.

4.2. Evaluation of Monophyly of Species Complexes

Phylogenetic analysis showed that 22 of the 23 SCs were monophyletic (Figures 1–4). *F. nisikadoi* SC was the only polyphyletic SC in the phylogenetic trees, based on both concatenation and coalescence approaches using amino acid data (Figures 1 and 2). Additionally, in trees based on the both approaches using DNA data, *F. commune* was the most ancestral lineage within the *F. nisikadoi* SC (Figures 3 and 4). Bipartition analysis revealed incongruence between the coalescent-based tree and most gene trees at the branch, including *F. commune* and other species of the *F. nisikadoi* SC (Figure 6). To date, no report has addressed the relationship between *F. nisikadoi* SC and *F. commune*, which has been shown to be polyphyletic with *F. nisikadoi* SC species in phylogenetic trees based on the elongation factor α (*tef1 α*) gene [43] and RPB1 and RPB2 [44]. Subsequently, Geiser et al. [8] included *F. commune* in the *F. nisikadoi* SC based on phylogenetic analysis of 19 genes, and Crous et al. [45] included *F. commune* in the *F. nisikadoi* SC based on concatenated data for *tef1 α* , *RPB1*, and *RPB2*. However, *F. commune* appears to be the most ancestral lineage within the *F. nisikadoi* SC in both these reports and in the DNA-based analyses of the current study. Considering these previous findings and our results, *F. commune* should be regarded as being independent of other SCs.

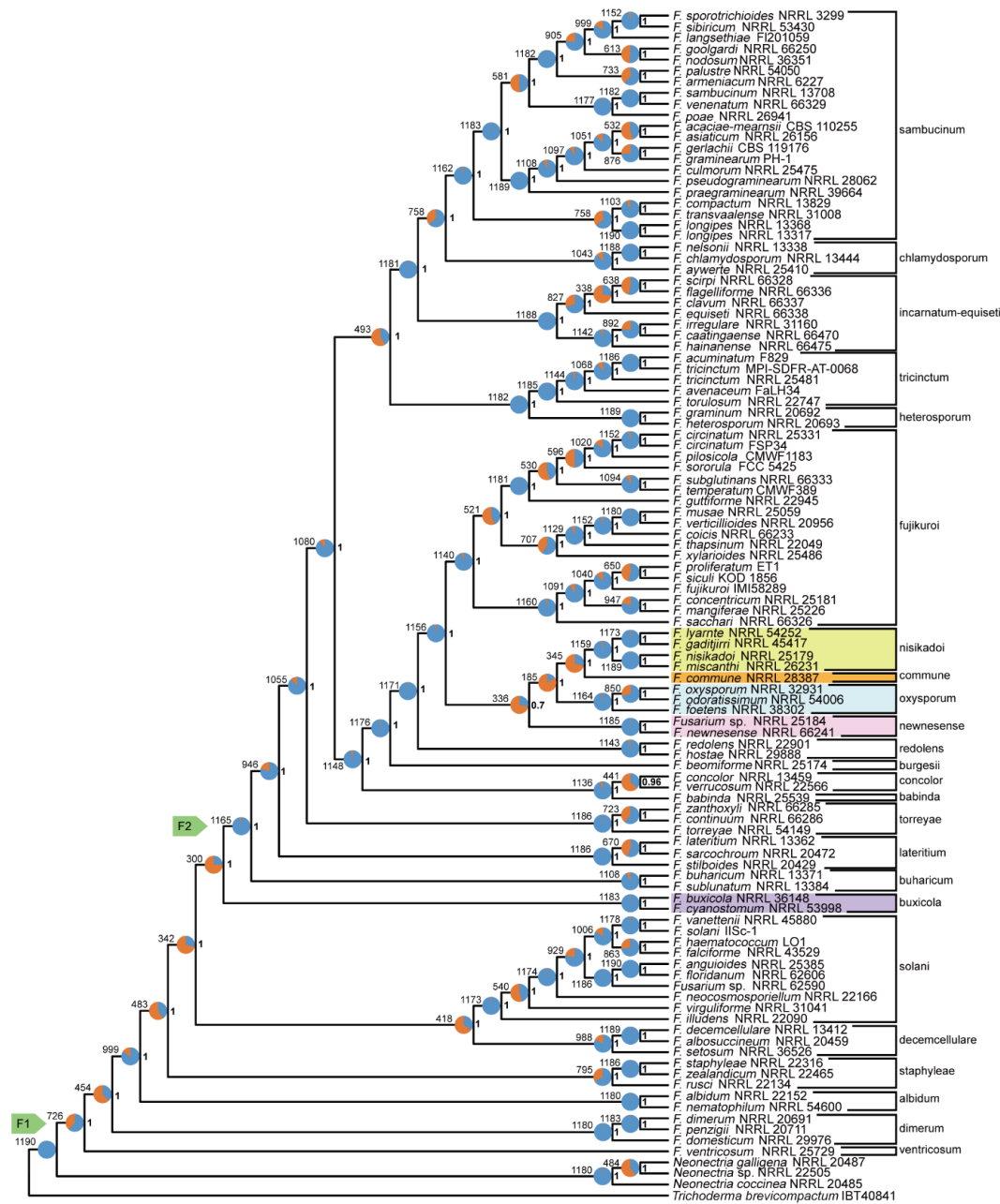


Figure 6. Phylogenetic tree based on DNA data of 1,190 genes using coalescent-approach analyzed with ASTRAL, with summary of conflicting and concordant homologs. For each branch, the top number indicates the number of homologs concordant with the species tree at that node. The pie charts at each node present the proportion of homologs that support that clade (blue) and the proportion that support the main alternative for that clade (orange). Node F1 is for the terminal *Fusarium* clade. Node F2 is for *Fusarium* s. str. clade.

4.3. Evolutionary History of Species Complexes

Phylogenetic analysis showed that the evolutionary histories of 19 of 23 SCs were consistent among all analyses, with high branch support (Figures 1–4). Nevertheless, these phylogenetic relationships differ from those reported in previous studies of concatenation-based DNA data [9]. Regarding the relationships among *F. newnesense*, *F. nisikadoi*, and *F. oxysporum* SCs, Gomez-Chavarria et al. [9] suggested that *F. oxysporum* SC is the most ancestral lineage, followed by (*F. nisikadoi* SC and *F. newnesense* SC) branches with high support. However, the topologies obtained in the current study differed from those of Gomez-Chavarria et al. [9], except for the tree derived from the concatenation approach using DNA data, which used the same data type and method (Figures

1–4, Supplementary Figure S1). Therefore, the branching order among *F. newnesense* SC, *F. nisikadoi* SC, *F. commune*, and *F. oxysporum* SC could not be resolved.

Additionally, the polytomy test based on DNA data indicated that the *F. newnesense* SC, (*F. nisikadoi* SC, *F. commune*), and *F. oxysporum* SC formed a polytomy (Figure 4). This suggests that these three groups diverged within a narrow temporal window, which made it difficult to determine the precise branching sequence. Similar issues have been observed in mbuna cichlids, where different analyses have yielded conflicting divergence patterns, even with whole-genome sequence data [46]. Hard polytomy was detected using the ASTRAL polytomy test, further illustrating the difficulty of inferring the evolutionary order in rapidly radiating groups using molecular data alone. Similar to the findings of Scherz et al. [46] in mbuna cichlids, no definitive solution was observed to resolve the branching order among *F. newnesense* SC, *F. nisikadoi* SC, *F. commune*, and *F. oxysporum* SC. Therefore, maintaining this relationship through polytomy is appropriate.

Regarding phylogenetic relationships outside *Fusarium s. str.*, the branching orders of most SCs were consistent among analyses, except for the position of the *F. buxicola* SC. Three phylogenetic trees constructed using the concatenation approach with both DNA and amino acid data, as well as the coalescence approach with amino acid data, indicated that the *F. buxicola* SC is a sister group to *Fusarium s. str.* clade. However, one tree based on the coalescent approach with amino acid data showed the *F. buxicola* SC as a sister to the (*F. solani* SC, *F. decemcellulare* SC) clade. In both cases, the branches of the clade comprising the *F. buxicola* SC clade and its sister group were highly supported (100%) and LPPs (0.95 and 1.0). Nonetheless, the proportion of branching patterns inconsistent with the coalescent-based tree was higher according to bipartition analysis (Figure 5). Furthermore, the main topology had a lower proportion than the alternative topologies in the quartet tree-based concordance analysis (Supplementary Figure S2 and Supplementary Table S4), confirming the inconsistency between the analyses. Although the majority of evidence supports the placement of the *F. buxicola* SC as a sister group to the *Fusarium s. str.* clade, additional analyses will be necessary to clarify the precise branching order.

4.4. Boundary of the Genus *Fusarium*

In all analyses, both the terminal *Fusarium* and *Fusarium s. str.* clades were monophyletic with high branch support (Figures 1–4; BS = 100%, LPP = 1). No polytomies were observed within these branches (Figures 2 and 4). Bipartition analyses further showed that 1,123/1,190 genes in the ASTRAL tree based on the amino acid data (Figure 5) and 1,165/1,190 genes in the ASTRAL tree based on DNA data (Figure 6) supported the divergence of the *Fusarium s. str.* clade. These results indicate that *Fusarium s. str.* represents an evolutionarily independent. This conclusion aligns with the findings of O'Donnell et al. [7], Geiser et al. [8], and Gomez-Chavarria et al. [9].

Regarding the taxonomic boundary of *Fusarium*, Lombard et al. [47] delimited *Fusarium* to correspond to *Fusarium s. str.* clade, emphasizing the distinct teleomorph morphologies among the independent *Fusarium* genera. Conversely, O'Donnell et al. [7] and Geiser et al. [8] argued that *F. solani* SC should be included in *Fusarium*, as the terminal *Fusarium* clade including *F. solani* SC is well supported, and *F. solani* SC represents an important group of human pathogenic fusaria. Furthermore, the number of teleomorphs identified was insufficient to justify genus-level separation based solely on teleomorphic traits.

To clarify the boundary of *Fusarium*, synapomorphies of terminal *Fusarium* and *Fusarium s. str.* clades should be examined further. If synapomorphy exists for the terminal *Fusarium* clade, the seven groups proposed by Lombard et al. [47] as separate genera should instead be treated as subgenera or species groups, or the terminal *Fusarium* clade may warrant subfamily status. Conversely, if a synapomorphy is found in the *Fusarium s. str.* clade, these seven *Fusarium s. str.* clades should be recognized as a single genus.

5. Conclusions

High-throughput sequencing has enabled the use of large gene sequence datasets for extensive taxon sampling. *F. commune* is independent of the *F. nisikadoi* SC, being polyphyletic based on amino acid data, while *F. commune* represents the most ancestral lineage within the *F. nisikadoi* SC based on DNA data. We observed that the branching order among the *F. newnesense* SC, *F. nisikadoi* SC, *F. commune*, and *F. oxysporum* SC could not be resolved because inconsistent topologies were obtained using different data types and approaches. Our findings provide new insights into the evolutionary history of SCs in *Fusarium*.

Although this study was conducted on a limited number of *Fusarium* species using reliable genome-scale data, future studies incorporating broader taxon sampling will enhance our understanding of the monophyly and evolutionary relationships among *Fusarium* species. Such expanded genomic research will deepen our evolutionary knowledge of this diverse fungal group, and support taxonomic refinement, and provide a stronger foundation for applied studies in agriculture, medicine, and biotechnology.

Supplementary Materials: The following supporting information can be downloaded at the website of this paper posted on Preprints.org, Figure S1: Comparison between (a) concatenation based trees based on DNA and protein data, (b) a concatenation based tree (DNA data) and an coalescent based tree (DNA data), (c) a concatenation based tree (DNA data) and an coalescent based tree (amino acid data), (d) a concatenation based tree (amino acid data) and an coalescent based tree (DNA data), (e) a concatenation based tree (amino acid data) and an coalescent based tree (amino acid data), and (f) coalescent based trees based on DNA and protein data.; Figure S2: Discordance rate of gene trees in a phylogenetic tree inferred by ASTRAL-III based on 1,190 gene trees constructed using MEGA-CC based on protein data. Figure S3: Discordance rate of gene trees in a phylogenetic tree inferred by ASTRAL-III based on 1,190 gene trees constructed using MEGA-CC based on DNA sequence of the protein-coding region.; Table S1: GenBank accessions for genome sequence.; Table S2: Properties of datasets without alignment gaps for concatenation phylogenetic trees based on 1,190 genes in this study; Table S3: Properties of datasets without alignment gaps for genes used for phylogenetic analysis in this study.; Table S4: Discordance rate of gene trees based on amino acid data calculated with ASTRAL program. The column numbers correspond to those in Supplemental Figure S2.; Table S5: Discordance rate of gene trees based on DNA data calculated with ASTRAL program. The column numbers correspond to those in Supplemental Figure S3.

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Abbreviations

The following abbreviations are used in this manuscript:

SC	species complex
LPP	local posterior probability
BS	bootstrap

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