



ORIGINAL ARTICLE

Open Access



Nematode-trapping fungus *Arthrobotrys oligospora* is hungry for iron-chelating agent COQ7 of nematodes

Qunfu Wu^{1†}, Jiao Zhou^{1†}, Donglou Wang^{1†}, Songhan Xue¹, Ling Li¹, Li Wu¹, Junxian Yan¹ and Xuemei Niu^{1*} 

Abstract

Iron homeostasis is critical for the survival of almost all organisms, yet its dysregulation is often caused by a synergistic effect of genetic and environmental factors. Previous studies have shown that trapping devices of the predominant nematode-trapping fungus (NTF) *Arthrobotrys oligospora* serve as an unprecedented iron sequestration system compensatory for lack of the crucial fungal vacuolar iron detoxification mechanism. Here, we found that among the Ascomycota phylum, only NTFs lacked gene *coq7*, which encodes COQ7 responsible for ubiquinol (UQ) biosynthesis and efficient iron chelation. Addition of exogenous UQ₁₀ or heterologous expression of yeast gene *coq7* in *A. oligospora* inhibited the formation of fungal trapping devices. Interestingly, mutant nematodes with disruption of gene *coq7* can greatly reduce nematode-capturing ability of fungal trapping devices. Exogenous COQ7s exhibit significant adsorption effects on fungal trapping devices both in vitro and in vivo. Transcriptional, metabolic, mutational, and phenotypic analyses indicated that *A. oligospora* utilized a chemotaxonomic class of highly oxygenated arthrobotrisins with similar characteristics to UQ₃, instead of UQs, in response to elevated oxygen levels. Loss of arthrobotrisin biosynthesis led to a delayed growth of the mutant Δart but enhanced UQ₈ biosynthesis, trapping device formation, and nematocidal activity. Time-calibrated evolutionary analyses, combined with geological data, indicated that the NTF ancestor lost the *coq7* gene after acquiring the *art* gene cluster during the cold “superoligotrophy” period, characterized by dramatic shifts in global oxygen levels and temperature changes. Our findings indicated that the trapping devices of NTF capture nematodes primarily for iron chelation therapy, rather than solely for food, which addresses the long-standing issue regarding the limited carnivorous ability of trapping devices.

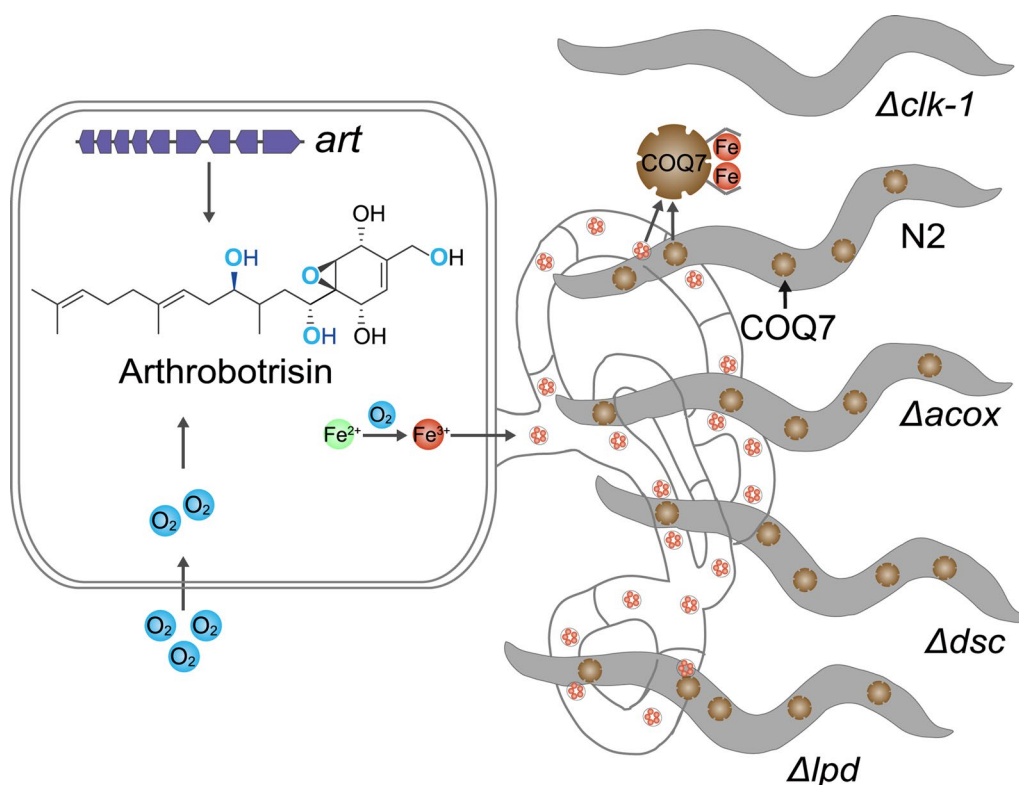
Keywords Nematode-trapping fungi, *Arthrobotrys oligospora*, Iron chelation, Ubiquinone, COQ7, Oxygen, Trapping device, Predatory activity, Metabolic evolution

[†]Qunfu Wu, Jiao Zhou and Donglou Wang have contributed equally to this work.

*Correspondence:
Xuemei Niu
xmniu@ynu.edu.cn

© The Author(s) 2026. **Open Access** This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit <http://creativecommons.org/licenses/by/4.0/>.

Graphical Abstract



1 Introduction

Iron, the most abundant trace metal in living organisms, is essential for oxygen transport, energy metabolism, and DNA synthesis [1–4]. However, iron overload can result from a range of genetic and environmental factors, often leading to parenchymal organ damage [5, 6]. Congenital defects in iron-related genes disrupt absorption, storage, or transport, causing systemic pathologies, such as Caucasians are particularly susceptible to developing iron overload and the associated complications of hemochromatosis due to a higher prevalence of mutations in the homeostatic iron-regulator and iron-storage genes within this population [7, 8]. Among them, *HFE* mutations associated with susceptibility to iron overload are most common in white populations, occurring in 0.3–0.5 percent of white persons of northern European descent [9, 10]. Chelation therapy is the primary treatment for mitigating iron accumulation, particularly from repeated blood transfusions, by promoting iron excretion via urine and/or feces [11, 12]. To date, few studies have investigated the mechanisms of iron overload and chelation in non-human eukaryotes.

A heterogeneous group of fungi within the phylum Ascomycota can differentiate their mycelia into specialized trapping structures under nutrient-deficient conditions in the presence of nematodes, capturing and penetrating them as a primary nutritional source [13]. These fungi, collectively known as nematode-trapping fungi (NTFs), form a monophyletic clade within the class Orbiliomycetes and produce diverse trapping devices, including adhesive networks, adhesive knobs, and constricting rings [14]. The emergence of these trapping structures is widely considered a hallmark of the evolutionary transition from a saprophytic to a predatory lifestyle in NTFs. Nematodes, the most abundant animals on Earth, occupy a wide range of ecological niches and trophic levels within soil food webs [15]. Among them, plant-parasitic nematodes represent highly destructive agricultural pests, infecting more than 4,000 plant species and causing annual crop losses exceeding \$100 billion globally [16]. NTFs are regarded as effective natural antagonists that contribute to biological control of nematode populations in various ecosystems [17]. Paradoxically, despite the widespread availability of nematode prey, NTFs are neither abundant nor widely distributed

in many environments [13]. It has been hypothesized that the evolution of these fungi involved a progressive degeneration of their carnivorous capabilities [18]. However, the molecular and physiological mechanisms underlying the limited predatory efficiency of NTF trapping structures remain largely unknown.

Recent studies have demonstrated that exogenous iron can induce the predominant NTF *Arthrobotrys oligospora* to form trapping devices even in the absence of nematode prey [19, 20]. Chemical and biochemical analyses have revealed that these trapping devices contain significantly more iron than mycelia, as indicated by the presence of iron-rich, electron-dense bodies localized within the trapping devices [19, 20]. Genomic, evolutionary, and functional analyses further suggest that during the Late Paleozoic Ice Age, NTFs lost the CCC1-mediated vacuolar iron detoxification system—a mechanism conserved in most other fungi [20]. Moreover, both the acquisition of the siderophore desferri-ferrichrome and the emergence of trapping structures appear to be closely linked to elevated ambient temperatures, given that intracellular iron content and composition in fungi are inversely correlated with temperature [20]. Collectively, these results suggest that NTFs are prone to iron overload at relatively high temperatures due to loss of the canonical vacuolar iron detoxification pathway, similar to iron overload observed in hemochromatosis caused by mutations in iron-regulatory and storage genes [20]. However, it remains unclear how the iron-rich trapping devices contribute to carnivorous capacity of NTFs.

2 Results

2.1 Only NTFs in Ascomycota lack the gene *coq7*

We hypothesize that NTFs have lost genes encoding iron chelators that are commonly retained in other fungi and nematodes, thereby driving NTFs to prey on nematodes primarily as a source of iron-chelating agents. To investigate this, we first examined 1361 fungal genomes from the phylum Ascomycota, representing 66.16% of the total fungal genomes analyzed. Notably, genomic analysis revealed that among Ascomycota, only nine fungal species, all belonging to NTFs and accounting for 0.66% of ascomycetes, lack the *coq7* gene, which is essential for UQ biosynthesis [21] (Table S1). Importantly, COQ7 is a membrane-bound di-iron enzyme belonging to a unique subgroup of the ferritin superfamily and is implicated in iron binding and chelation [22–24]. We subsequently expanded the analysis to 2057 fungal genomes and conducted an extensive comparative survey, which indicated that only 25 fungal species lack the *coq7* gene, including 9 NTFs from Ascomycota, 12 species from Basidiomycota, 2 from Neocallimastigomycota, and 2 from Zoopagomycota (Table S1). UQs are not only essential electron

carriers in aerobic respiration, serving as redox intermediates in both aerobic bacteria and eukaryotes [25], but also contribute to regulating redox homeostasis in anaerobic microorganisms [26]. Prior studies have shown that most fungi utilize UQs with prenyl side chains of 9 or 10 isoprenoid units (UQ₉ and UQ₁₀) [27, 28]. The biosynthesis of UQ₉ and UQ₁₀ requires at least thirteen proteins, including COQ1–COQ11, YAH1, and ARH1 [28]. Among these, COQ7 is a multifunctional protein involved not only in UQ biosynthesis but also in iron chelation, functioning as a ferritin homolog [22–24].

Analysis of UQ biosynthetic genes across 2057 fungal genomes revealed that, with the exception of *coq9* (56.73%, 1167/2057) and *coq10* (38.89%, 800/2057), all remaining 11 genes—including *coq1* (99.85%, 2054/2057), *coq2* (99.71%, 2051/2057), *coq3* (99.71%, 2051/2057), *coq4* (99.76%, 2052/2057), *coq5* (99.81%, 2053/2057), *coq6* (99.81%, 2053/2057), *coq7* (98.78%, 2032/2057), *coq8* (99.85%, 2054/2057), *coq11* (98.06%, 2017/2057), *arh1* (99.90%, 2055/2057), and *yah1* (99.56%, 2048/2057)—are highly conserved and present in more than 98% of fungal genomes (Fig. 1A and Table S1). These findings suggest that *coq7* is one of the core genes required for UQ biosynthesis and is likely critical for maintaining metabolic function and viability in most fungi [25].

2.2 UQ₁₀ inhibits trapping devices and nematocidal activity of *A. oligospora*

We hypothesized that the absence of *coq7* may disrupt UQ biosynthesis and affect iron sequestration, thereby increasing the susceptibility of NTFs to iron overload and promoting the formation of iron-rich trapping devices. Based on this, it is reasonable to infer that exogenous supplementation with UQ₁₀ could reduce trapping device formation in NTFs. To test this hypothesis, we supplemented *A. oligospora* with UQ₁₀ at concentrations of 10 and 50 μ M under normoxic conditions. These concentrations have been reported to exert no adverse effects on human cells [29], and a solvent-only treatment was used as the control. UQ₁₀ at 50 μ M significantly inhibited the formation of trapping devices in *A. oligospora*, whereas 10 μ M had no observable effect (Fig. 2A, B). Furthermore, UQ₁₀ at 50 μ M markedly impaired the nematode-capturing capacity of *A. oligospora*, consistent with the reduction in trapping device formation (Fig. 2A–C).

2.3 Exogenous *coq7* alleviates fungal trapping devices and nematocidal activity

To evaluate the role of *coq7* in fungal trapping device formation and nematocidal activity in *A. oligospora*, the *coq7* gene (819 bp) from *Saccharomyces cerevisiae* BJ5464 was cloned and introduced into the wild-type (WT) strain of *A. oligospora* to generate the E-*coq7* mutant (Fig. S1A–B).

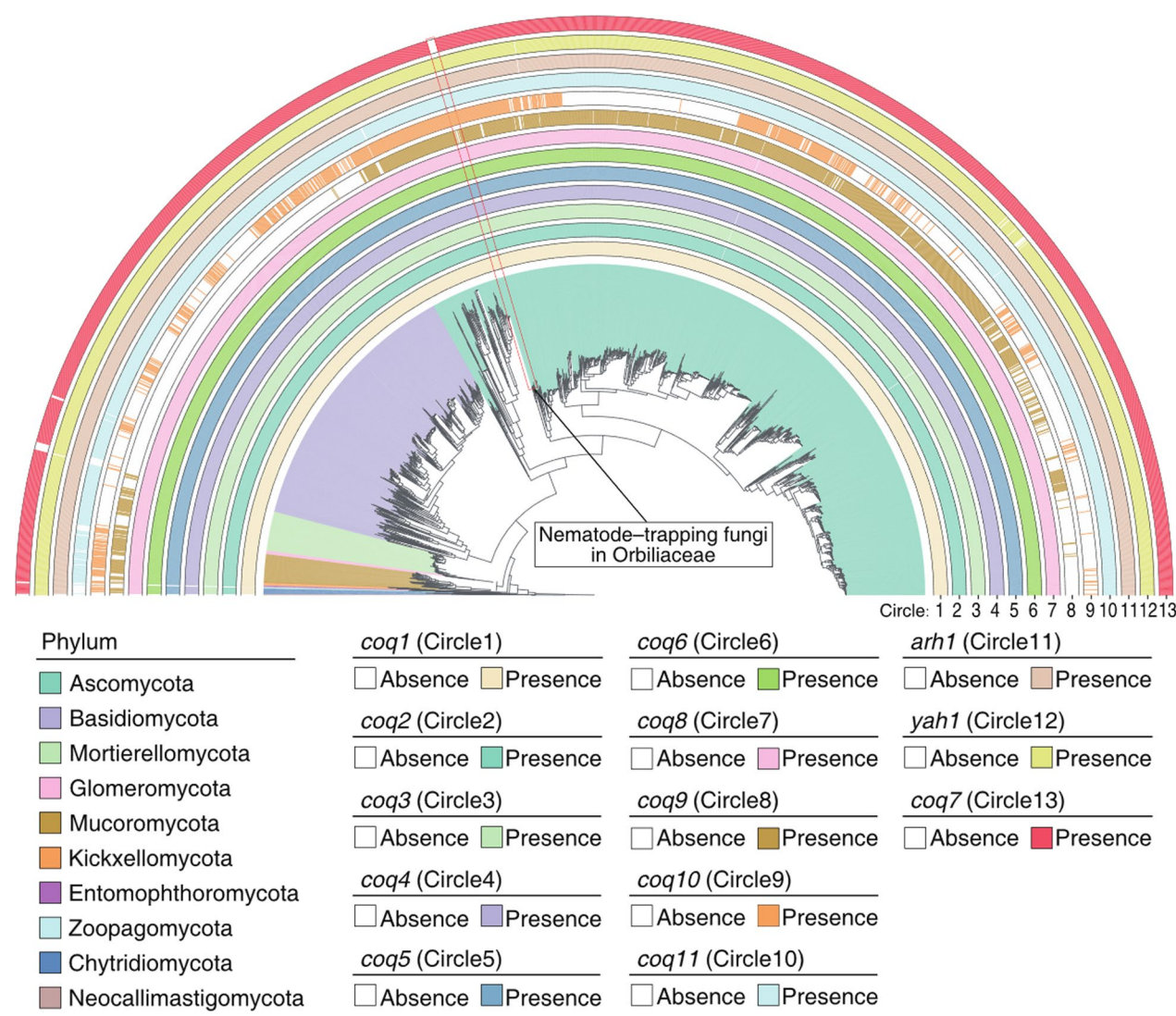


Fig. 1 NTFs lack the gene *coq7* encoding iron chelating COQ7 also for UQ biosynthesis. Distribution patterns of UQ synthesis-related proteins across the fungal kingdom. The phylogenetic tree was constructed using 750 universal single-copy marker genes extracted from 2057 high-quality fungal genomes downloaded from the JGI MycoCosm database. The fungal groups analyzed include Ascomycota (1361 genomes), Basidiomycota (540 genomes), Mucoromycota (57 genomes), Glomeromycota (7 genomes), Mortierellomycota (67 genomes), Kickxellomycota (6 genomes), Chytridiomycota (11 genomes), Neocallimastigomycota (2 genomes), Entomophthoromycota (4 genomes), and Zoopagomycota (2 genomes). The presence or absence of the 13 UQ biosynthesis-related proteins (COQ1–COQ11, YAH1, and ARH1) across various fungal phyla were determined by searching for orthologs of the protein against a custom database containing all predicted protein sequences from 2057 high-quality fungal genomes via BLASTP. The presence or absence of the 13 proteins were mapped onto the tree of fungi kingdom to visualize the distribution patterns of each protein. Each circle (1–13) outside the tree represents the distribution patterns of one of the 13 proteins. Each white block within a circle represents the absence of the corresponding protein in the respective genome and another color represents the presence. Genomes of NTFs in Orbiliaceae are marked in a red box

Transcriptional analysis confirmed successful expression of *coq7* in the E-*coq7* mutant (Fig. S1C). High-performance liquid chromatography (HPLC) analysis revealed that the E-*coq7* mutant exhibited a metabolic profile identical to that of the *A. oligospora* WT strain (Fig. S2), suggesting that exogenous *coq7* expression did not affect the metabolic pathways or UQ biosynthesis in *A.*

oligospora. Remarkably, phenotypic analysis showed that the E-*coq7* mutant formed significantly fewer trapping devices than the WT strain (Fig. 2D, E). Furthermore, the E-*coq7* mutant displayed significantly reduced nematocidal activity compared to the WT strain (Fig. 2D–F). Together, these results suggest that exogenous COQ7 may act in a ferritin-like manner to alleviate iron

accumulation in trapping devices, thereby diminishing the nematocidal activity of *A. oligospora*.

2.4 Disruption of nematode *coq7* did not affect formation of fungal trapping devices

Given that the trapping devices of NTFs are rich in iron and that COQ7 possesses ferritin-like properties, we hypothesized that the predator–prey interaction between NTFs and nematodes may largely result from the natural affinity between ferritin and iron. To investigate whether fungal trapping device efficacy is related to COQ7 in nematodes, the $\Delta clk-1$ mutant and WT (N2) strain of *Caenorhabditis elegans* were employed to assess trapping device formation and nematocidal activity of *A. oligospora*. The *clk-1* gene encodes a 187-residue polypeptide homologous to yeast *coq7*, which is essential for UQ biosynthesis in yeast [30]. Notably, the $\Delta clk-1$ mutant nematodes exhibited a similar effect on fungal trapping device formation compared to the N2 strain and showed a slightly increased effect when *A. oligospora* was treated with 0 and 10 μM UQ₁₀ (Fig. 2A, B), suggesting that the absence of COQ7 in the $\Delta clk-1$ nematode mutant does not significantly affect trapping device formation in *A. oligospora*.

2.5 Disruption of nematode *coq7* reduced nematocidal activity of trapping devices

Many studies have demonstrated that nematodes can induce the formation of fungal trapping devices in NTFs [31, 32]. However, whether nematode components influence the nematode-capturing ability of NTFs remains unreported. Due to the genetic defect, $\Delta clk-1$ mutant nematodes exhibit a general reduction in behavioral rates compared to the N2 nematodes [33], suggesting that $\Delta clk-1$ mutants are less capable of escaping capture by fungal trapping devices than N2 nematodes. Notably, we observed that fungal trapping devices captured nearly three times more N2 nematodes than $\Delta clk-1$

mutants (Fig. 2C). Most $\Delta clk-1$ nematodes were able to evade the traps and maintained a vibrant, curved morphology, in contrast to the stiff, immobilized N2 nematodes (Fig. 2A). The higher capture rate of N2 nematodes indicates a specific attractive interaction between fungal trapping devices and nematode COQ7. It is also possible that the lack of COQ7 in nematodes causes them to also have an iron overload similar to the NTF, resulting in repulsion between the fungal trapping devices and the $\Delta clk-1$ mutant nematodes.

To further investigate the association between nematode COQ7 and the nematocidal activity of fungal trapping devices, we examined three additional nematode mutants deficient in genes encoding Acyl-CoA oxidase (ACOX), paired-like homeobox transcription factor/defecation suppressor (DSC), and bridge-like lipid transfer protein (LPD), respectively. Notably, the *acox* and *lpd* genes are also absent from all NTF genomes (Table S2). Both the $\Delta acox$ and Δlpd nematode mutants exhibited locomotor sluggishness comparable to that of the $\Delta clk-1$ mutant [34, 35]. Phenotypic analysis revealed that the $\Delta clk-1$ and Δlpd mutants had the strongest effects on inducing trapping device formation in *A. oligospora*, whereas the Δdsc mutant had the weakest effect (Fig. 2G, H). Interestingly, the number of Δdsc nematodes captured by the fungal trapping devices was the second highest, surpassed only by the $\Delta acox$ mutant (Fig. 2I). Furthermore, the number of $\Delta acox$ nematodes captured was approximately three times greater than that of the $\Delta clk-1$ mutant (Fig. 2I). These results suggest that among these nematode factors, COQ7 plays a uniquely potent role in enhancing the nematocidal activity of fungal trapping devices.

2.6 *Arthrobotrys oligospora* uses arthrobotrisins instead of UQ to respond to elevated oxygen levels

Because UQs are often associated with oxygen-mediated fungal metabolism, we wondered how *A. oligospora* that

(See figure on next page.)

Fig. 2 Evaluation of the effects of UQ₁₀ and COQ7 on the formation of trapping devices and their nematocidal ability of *A. oligospora*. **A** Pictures of the formation of trapping devices in *A. oligospora* treated with two different nematodes WT N2 and mutant $\Delta clk-1$ in the presence of UQ₁₀ at concentrations of 0, 10 and 50 μM . 0: with solvent; 10 μM : with 10 μM UQ₁₀; 50 μM : with 50 μM UQ₁₀. Red arrow: trapping device is capturing a nematode. **B** Comparison of trapping devices in *A. oligospora* induced by two different nematodes WT N2 and mutant $\Delta clk-1$ in the presence of UQ₁₀, revealed that UQ₁₀ at a concentration of 50 μM strongly inhibited the formation of trapping devices. **C** Comparison of nematocidal ability of the trapping devices in *A. oligospora* induced by two nematodes WT N2 and the mutant $\Delta clk-1$ in the presence of UQ₁₀ indicated that the trapping devices preferred to capture nematodes N2 over mutant $\Delta clk-1$. **D** Pictures of trapping devices and nematocidal activities of WT and the mutant E-*coq7* with harboring an exogenous *coq7* gene from yeast. **E** Quantification of trapping device formation of WT and the mutant E-*coq7*. **F** Quantification of nematocidal activities of WT and the mutant E-*coq7*. **G** Pictures of trapping device formation of *A. oligospora* induced by five different nematodes, including nematode N2, and four nematode mutants $\Delta clk-1$, Δlpd , Δdsc , and $\Delta acox$. **H** Comparison of trapping devices in *A. oligospora* induced by five different nematodes revealed that the nematode mutants $\Delta clk-1$ and Δlpd induced the most trapping devices in *A. oligospora*. **I** Comparison of nematocidal ability of trapping devices in *A. oligospora* induced by five different nematodes indicated that the nematode mutant $\Delta clk-1$ is the last prey the trapping devices prefer to capture. Significance was tested using two-sided unpaired Student's t-test with Bonferroni's correction (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, ns not significant)

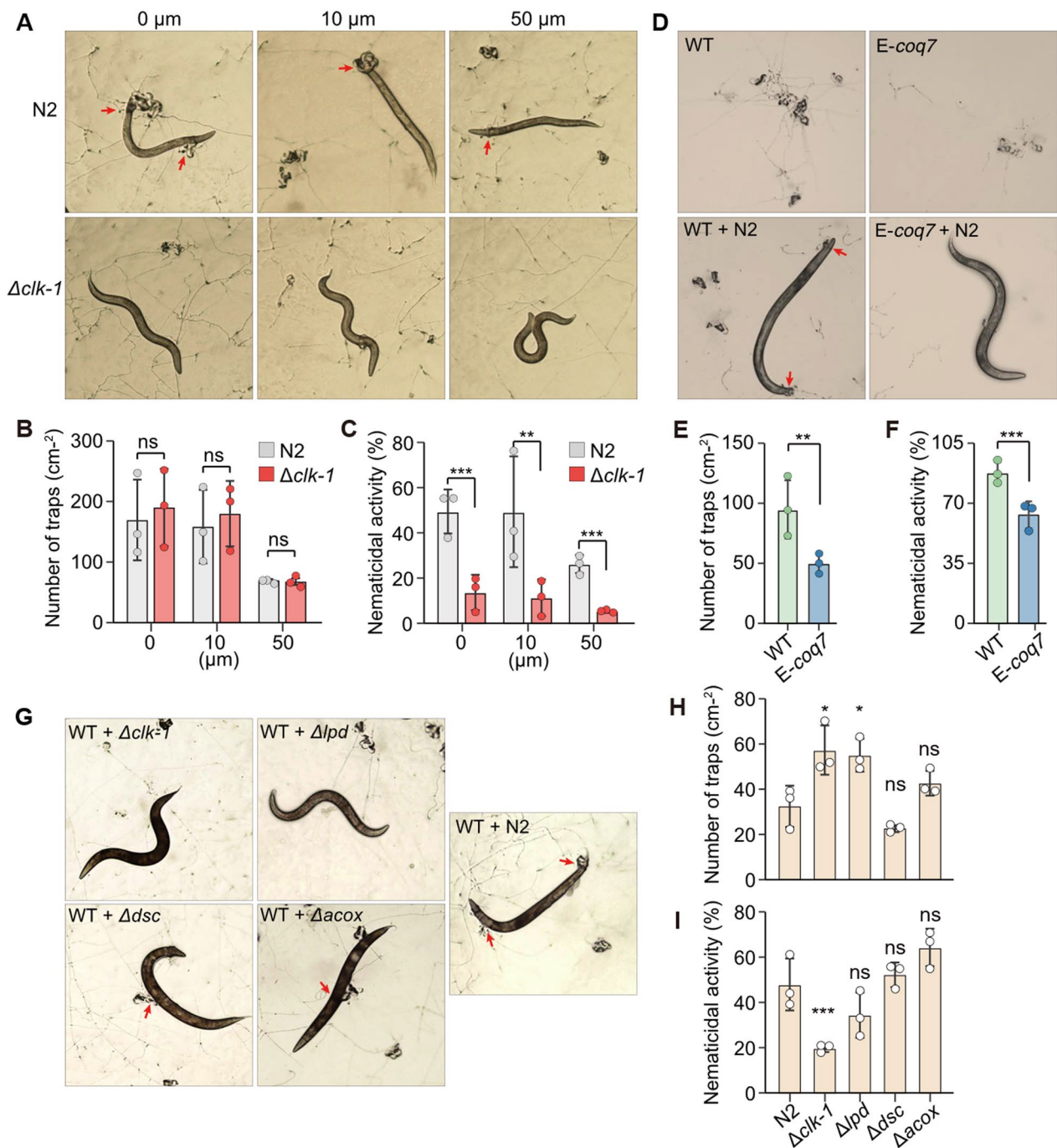


Fig. 2 (See legend on previous page.)

loses the gene *coq7* would be different. Metabolic analysis revealed that the *A. oligospora* WT strain exhibited markedly different metabolic profiles under shaking versus static culture conditions (Fig. 3A). The primary distinction between these conditions is oxygen transfer and mixing: shaking enhances oxygen availability by increasing the surface area between the liquid culture and

the air, leading to improved aeration for more efficient microbial growth, particularly in aerobic organisms [36, 37]. Notably, UQs were not detected in the WT strain under shaking conditions, whereas UQ₈ was present under static conditions (Fig. 3B). The absence of UQ₉ and UQ₁₀ in the WT metabolic profiles aligns with the absence of the *coq7* gene in *A. oligospora*. Furthermore,

transcriptional analysis demonstrated that *coq5*, *coq6*, and *yah1* were significantly downregulated under shaking conditions compared to static conditions (Fig. 3C), consistent with the detection of UQ₈ exclusively under static conditions. Detailed metabolic profiling also revealed that arthrobotrisins, a class of chemotaxonomic meroterpenoids, accumulated significantly in the WT strain under shaking conditions but were nearly absent under static conditions (Fig. 3D). Correspondingly, transcriptional analysis showed that biosynthetic genes within the *art* gene cluster responsible for arthrobotrisin synthesis, including *Ao283–Ao276* and *Ao274–Ao273*, were significantly upregulated under shaking conditions, whereas adjacent non-biosynthetic genes (*Ao275* and *Ao284–Ao286*) exhibited no significant change (Fig. 3E).

To investigate the role of arthrobotrisins in the response of *A. oligospora* to elevated oxygen levels, we constructed a mutant strain Δart , deficient in arthrobotrisin biosynthesis, based on a previous study [38]. We compared the metabolic profiles of the WT and Δart mutant strains under both shaking and static conditions. Principal component analysis (PCA) revealed that under static conditions, the WT and Δart strains clustered closely, whereas they did not cluster together under shaking conditions (Fig. S3). The Δart mutant exhibited nearly identical metabolic profiles under both conditions, contrasting with the pronounced differences observed in the WT strain (Fig. 3A). These results suggest that the absence of arthrobotrisin biosynthesis impairs the fungus's metabolic response to shaking conditions. To further examine the association between arthrobotrisins and oxygen levels, we cultured WT and Δart strains at four shaking speeds: 0, 60, 120, and 180 rpm, as dissolved oxygen levels positively correlate with shaking rates [39]. Metabolic analysis showed that major arthrobotrisin contents were highest in the WT strain at 120 and 180 rpm (Fig. 3F), with contents increasing progressively between 0 and 120 rpm. Conversely, UQ₈ content in the

WT strain decreased as shaking speeds increased from 0 to 180 rpm (Fig. 3G). Moreover, all three functional redox states of arthrobotrisins, corresponding to the fully oxidized, partially reduced, and fully reduced forms of UQ₈, had also been isolated in *A. oligospora* in previous studies (Fig. S4) [40, 41]. These results indicated that the fungus *A. oligospora* used arthrobotrisin biosynthesis in adaptation to the shaking conditions whereas utilized UQ₈ for the static conditions.

In contrast, the Δart mutant exhibited increasing UQ₈ levels over the same shaking speed ranges (Fig. 3G), indicating that the absence of arthrobotrisins may induce UQ₈ production in response to elevated oxygen. Transcriptional analysis confirmed significant upregulation of UQ biosynthetic genes in the Δart mutant relative to the WT strain (Fig. 3H). These results indicated that UQ₈ can functionally substitute for arthrobotrisins in fungal response of the Δart mutant to increased oxygen levels. Collectively, because *A. oligospora* preferentially used arthrobotrisins rather than UQs to cope with oxygen fluctuation, this most likely caused the fungus to lose the gene *coq7*.

2.7 Exogenous COQ7 exhibits adsorption effects on fungal trapping devices both in vitro and in vivo

Two experiments were conducted to evaluate the affinity of exogenous COQ7 for fungal trapping devices. In the first experiment, fluorescently labeled COQ7 and the control protein ACOX were heterologously expressed in *Escherichia coli* BL21. The purified proteins were then incubated with fungal trapping devices of *A. oligospora* WT and its mutant Δart . The results showed that COQ7 adhered to fungal trapping devices of both strains, whereas ACOX did not (Fig. 4A and Fig. S5). In the second experiment, COQ7 was fluorescently labeled within nematodes. Notably, nematode COQ7-derived fluorescence was predominantly localized on the nematode surface (Fig. 4B). Transient losses of fluorescence

(See figure on next page.)

Fig. 3 Evaluation of the roles of UQs and arthrobotrisins of *A. oligospora* in fungal metabolic response to elevated oxygen. **A** Metabolic analysis of *A. oligospora* WT and the mutant Δart under shaking and static conditions. WT strain exhibited markedly different metabolic profiles under shaking versus static conditions, whereas the Δart mutant did not. Additionally, the metabolic profiles of the Δart mutant under both shaking and static conditions display similarity to that of the WT strain under static conditions. **B** HPLC–PDA/MS analysis of the UQs production in *A. oligospora* under shaking and static conditions. Both UQ₉ and UQ₁₀ were absent in the metabolic profiles of the WT strain. Instead, UQ₈ was present in the fungus cultured under static conditions, while its levels were barely detectable under shaking conditions. **C** Transcriptional analysis of 12 UQs biosynthesis-related genes in *A. oligospora* under shaking and static conditions. Significance was tested using wald test with Bonferroni's correction (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$). **D** HPLC–PDA/MS analysis of the major arthrobotrisins in *A. oligospora* under shaking and static conditions. **E** Transcriptional levels of the genes in the *art* gene cluster of *A. oligospora* WT under shaking and static conditions. **F** Comparison of the contents of the major arthrobotrisins in WT and the mutant Δart under four different shaking conditions of 0 rpm – 180 rpm. **G** Comparison of the contents of UQ₈ in WT and the mutant Δart under four different shaking conditions of 0 rpm – 180 rpm. **H** Transcriptional levels of the genes responsible for the UQ biosynthesis in *A. oligospora* in the mutant Δart under shaking and static conditions. Significance was tested using two-sided unpaired Student's *t*-test with Bonferroni's correction. Transcriptional levels significance was tested using Wald test with Bonferroni's correction (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, ns not significant)

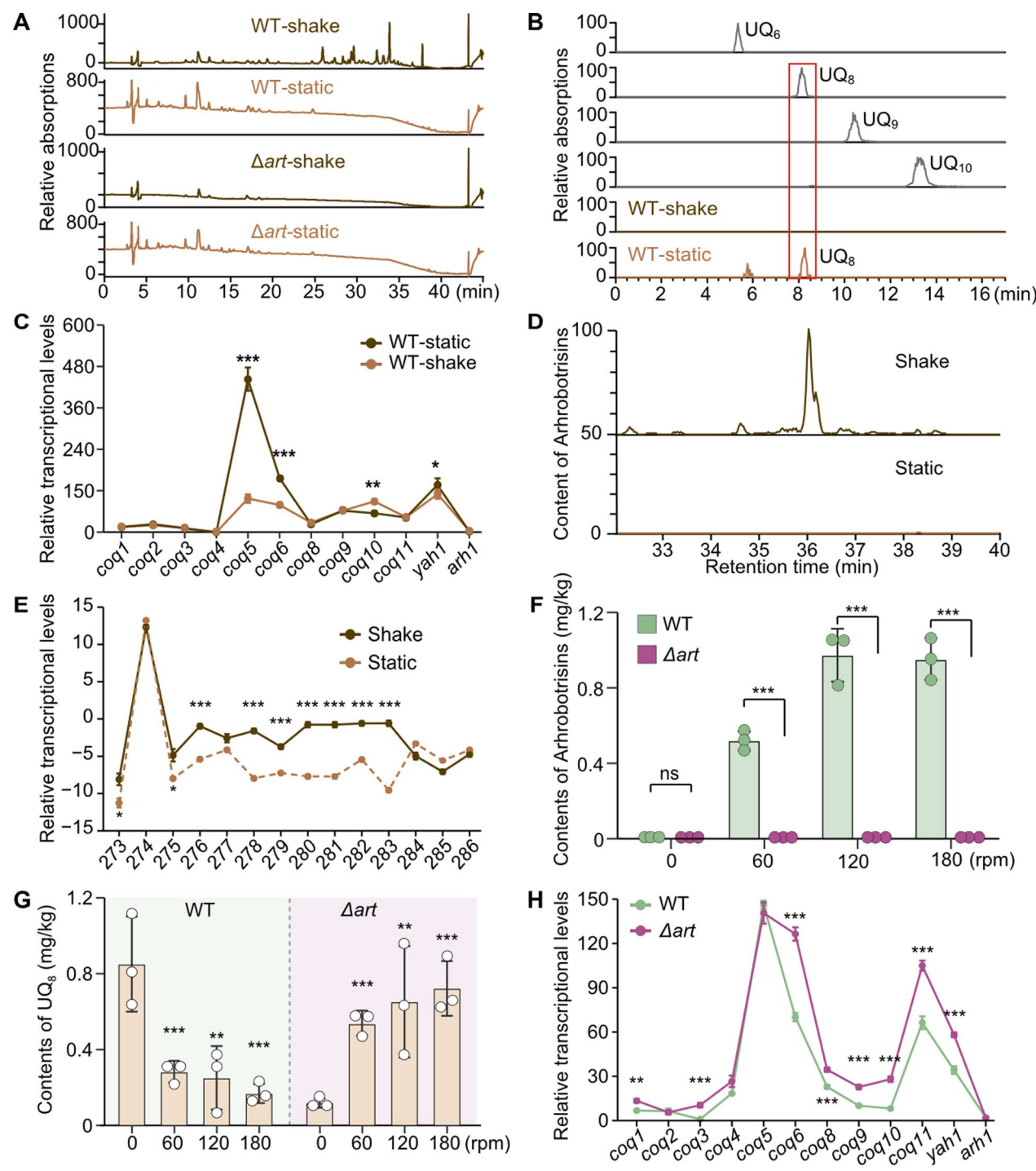


Fig. 3 (See legend on previous page.)

were observed in specific regions of the nematode body surface (Fig. S6), indicating that nematode COQ7 could be shed from the nematode. Recent studies have shown that mitochondria could be exported into surrounding environments since the observed migrasomes are filled with mitochondria directly in peripheral tissue [42–44].

It was most likely that COQ7 (CLK-1) localized in mitochondrion could be also exported from nematode into its surrounding environment. Remarkably, at sites of contact with fungal trapping devices, nematode-associated fluorescence was detected on the surface of fungal trapping devices (Fig. 4B). Collectively, these results provide

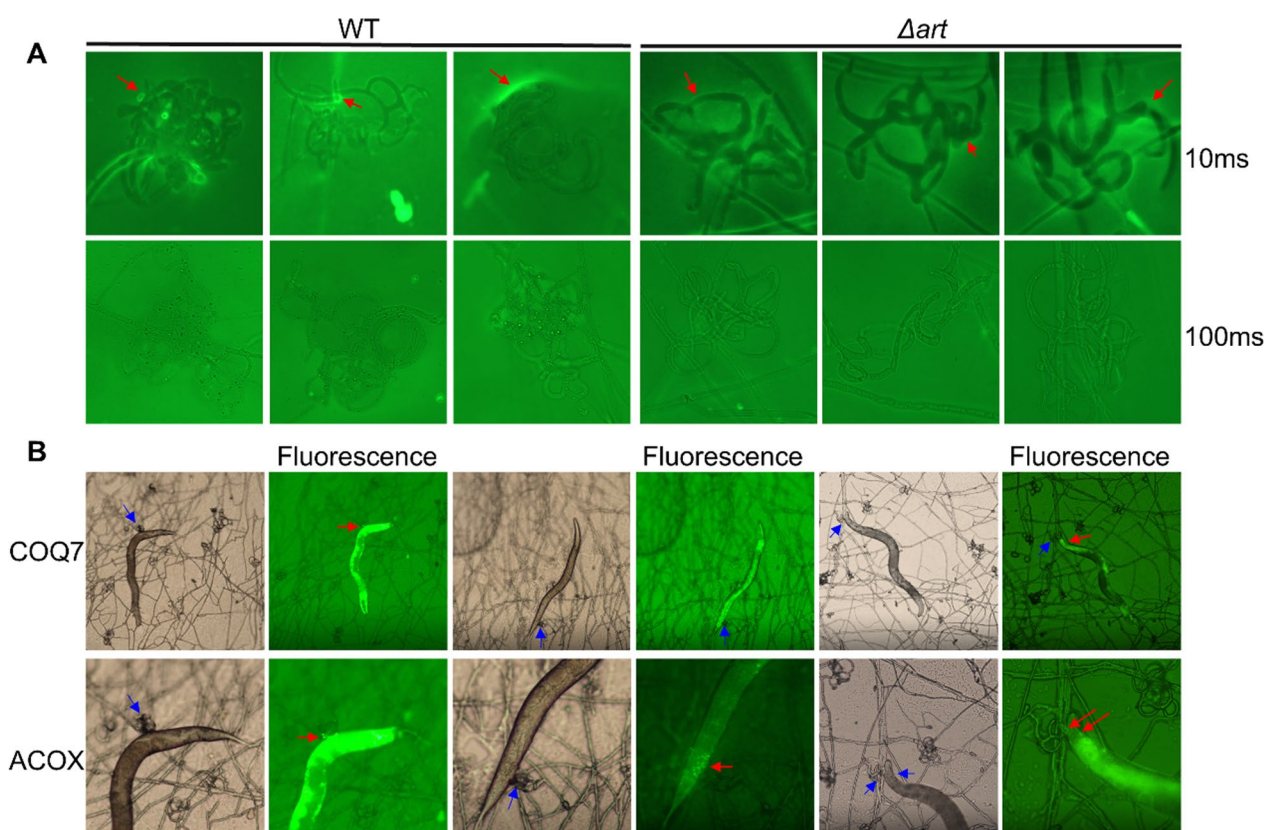


Fig. 4 Illustration of exogenous COQ7 with adsorption effects on fungal trapping devices both in vitro and in vivo. **A** Fluorescently labeled COQ7 protein from genetic engineering *Escherichia coli* displayed strong adsorption effects on fungal trapping devices of *A. oligospora* WT and its mutant Δart while fluorescently labeled ACOX control protein not. Red arrow points to fluorescently labeled COQ7 protein for 10 ms. **B** Nematode with fluorescently labeled COQ7 protein exhibits strong fluorescence on the nematode surface and at the junction with fungal trapping devices. Red arrow points to fluorescently labeled COQ7 protein for 10 ms; Blue arrow points to fungal trapping devices

evidence for nematode COQ7 secretion and surface exposure and demonstrate its affinity for fungal trapping devices.

2.8 Oxygen enhances fungal trapping device formation via increasing iron levels

Numerous studies suggest that oxygen functions as a developmental morphogen in metazoan embryos, driving genomic and metabolic reprogramming during periods of oxygen fluctuation [45, 46]. However, the effects of oxygen fluctuations on fungal morphogenesis, as well as on their metabolic and genomic evolution, remain largely unexplored. Direct evidence linking hyperoxia to the induction of fungal trapping device formation remains lacking, and the roles of specific metabolites in response to hyperoxia are still poorly understood. To investigate the effect of oxygen levels on fungal phenotypes, including growth, trapping device formation, and nematocidal activity, two oxygen conditions, 21% atmospheric oxygen (normal) and approximately 10% atmospheric oxygen (low), were established for agar plate bioassays.

Under low oxygen conditions, the *A. oligospora* WT strain exhibited smaller aerial mycelial colonies and significantly larger hollow areas compared to those grown under normal oxygen levels (Fig. 5A, B), indicating that reduced oxygen inhibited aerial mycelial growth. However, following treatment with *C. elegans* N2 nematodes, the fungal WT strain grown under normal oxygen produced trapping devices, whereas those grown under low oxygen did not (Fig. 5A, C). Additionally, the WT strain under normal oxygen conditions demonstrated strong nematode-trapping activity, while strains grown under low oxygen showed none (Fig. 5D). Previous studies have suggested that mycelial senescence or nutrient deficiency can readily induce trapping device formation [19, 38]. To exclude the possibility that differences in trapping devices resulted from unequal aerial mycelial growth under the two oxygen conditions, we selected cultures with comparable mycelial growth for further trapping device analysis. Specifically, aerial mycelia grown under normal oxygen were one day younger than those grown under low oxygen. Notably, younger aerial mycelia under

normal oxygen developed trapping devices, whereas those under low oxygen did not. Collectively, these results indicate that elevated oxygen levels promote trapping device formation in *A. oligospora* WT.

Since oxygen can oxidize ferrous iron (Fe^{2+}) to ferric iron (Fe^{3+}), resulting in electron loss and Fe^{3+} accumulation. A recent study demonstrated that *A. oligospora* mycelia develop trapping devices to store excess iron, as these structures contain significantly higher iron concentrations than the surrounding mycelia [20]. To evaluate the role of oxygen in regulating iron levels within fungal mycelia, we measured total free iron content under two oxygen conditions. As anticipated, mycelia grown under normal oxygen exhibited significantly higher levels of total free iron—including both Fe^{2+} and Fe^{3+} —compared to those grown under low oxygen (Fig. 5E). The levels of Fe^{2+} , Fe^{3+} , and total free iron under normal oxygen were more than double those under low oxygen, indicating that elevated oxygen substantially increases free iron ion accumulation (Fig. 5E). Meanwhile, the $\text{Fe}^{2+}/\text{Fe}^{3+}$ ratios in mycelia remained similar under both conditions (Fig. 5F), consistent with the biological requirement to maintain redox homeostasis of the $\text{Fe}^{2+}/\text{Fe}^{3+}$ ratio. These results suggest that oxygen is a key factor inducing trapping device formation by elevating free iron content in fungal mycelia.

2.9 Arthrobotrisin deficiency causes spontaneous formation of trapping devices

All arthrobotrisin metabolites in the NTF *A. oligospora* are derived from a key hybrid precursor, farnesyl toluquinol, which shares a distinctive feature with ubiquinol-3 (UQ_3): the farnesyl chain is attached to a benzoquinol moiety [47]. Notably, a series of oxidation reactions are critical for converting farnesyl toluquinol into arthrobotrisins, as each arthrobotrisin incorporates at least four oxygen atoms [41, 47–50]. This oxidative incorporation of molecular oxygen also occurs during ubiquinone biosynthesis, where monooxygenase-catalyzed

hydroxylation reactions introduce three oxygen atoms into the quinone structure [51]. From the perspective of the structural oxidation degree, arthrobotrisin biosynthesis requires more oxygen than ubiquinone biosynthesis. This is consistent with previous research, which indicates that arthrobotrisin production positively correlates with oxygen levels and deficiency in arthrobotrisin biosynthesis results in a significant increase in trapping device formation and nematocidal activity in the mutant Δart [41, 47–50]. Deletion of the genes involved in biosynthesis of highly oxidated arthrobotrisins, specifically the dehydrogenase gene *Ao274* and the cytochrome P450 genes *Ao278*, *Ao280*, and *Ao282* within the *art* gene cluster, markedly enhances nematode-induced trapping device formation in the mutant strains [41, 47, 49, 50]. However, the underlying mechanism by which arthrobotrisin biosynthesis suppresses nematode-induced trapping device formation in *A. oligospora* remains unclear.

The Δart mutant and WT strains were cultivated on plates under normal (21% atmospheric oxygen) and low oxygen conditions (approximately 10% atmospheric oxygen). Notably, the Δart mutant exhibited significantly larger hollow areas at the colony center compared to the WT strain under normal oxygen conditions, whereas both strains showed similar colony growth under low oxygen levels (Fig. 5G, H). This suggests that the arthrobotrisins improve aerial mycelial formation under normal oxygen conditions, consistent with the role of arthrobotrisins in fungal adaptation to elevated oxygen levels. Interestingly, the Δart mutant formed trapping devices without nematode treatment under both oxygen conditions (Fig. 5I, J), while the WT strain did not, indicating that endogenous arthrobotrisin biosynthesis inhibits spontaneous formation of fungal trapping devices. Both Δart and WT strains treated with nematodes produced significantly more trapping devices under normal oxygen conditions compared to low oxygen conditions (Fig. 5I, J), consistent with the above result that elevated oxygen promotes fungal

(See figure on next page.)

Fig. 5 Effects of oxygen on the colony growth, formation of spores and trapping devices and nematocidal activity of *A. oligospora*. **A** Phenotypic analysis of WT under normal (atmospheric oxygen, approximately 21%) and low oxygen (approximately 10%) conditions were performed, including colony growth, trapping device formation, and nematocidal activity. Nor: normal oxygen condition. Low: low oxygen condition. Red arrow: nematode is captured by trapping device. **B** Quantification of aerial mycelial areas of WT under normal and low oxygen conditions. **C** Comparison of trapping devices of WT induced by nematodes under normal and low oxygen conditions. **D** Comparison of nematocidal activity of WT between normal and low oxygen conditions. **E** Comparison of the levels of total free iron, ferrous, and ferric in WT under normal and low oxygen conditions. **F** Comparison of the ferrous/ferric ratios in WT under normal and low oxygen conditions. **G** Pictures of colony growth of *A. oligospora* WT and the mutant Δart under normal and low oxygen conditions. **H** Comparison of hollow area of *A. oligospora* WT and the mutant Δart under normal and low oxygen conditions. **I** Pictures of trapping devices and nematocidal activities of WT and the mutant Δart under normal and low oxygen conditions within 12h. **J** Comparison of trapping devices and nematocidal activities between WT and the mutant Δart under normal and low oxygen conditions within 0h and 12h after nematode treatment. Significance was tested using two-sided unpaired Student's *t*-test with Bonferroni's correction (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, *ns* not significant)

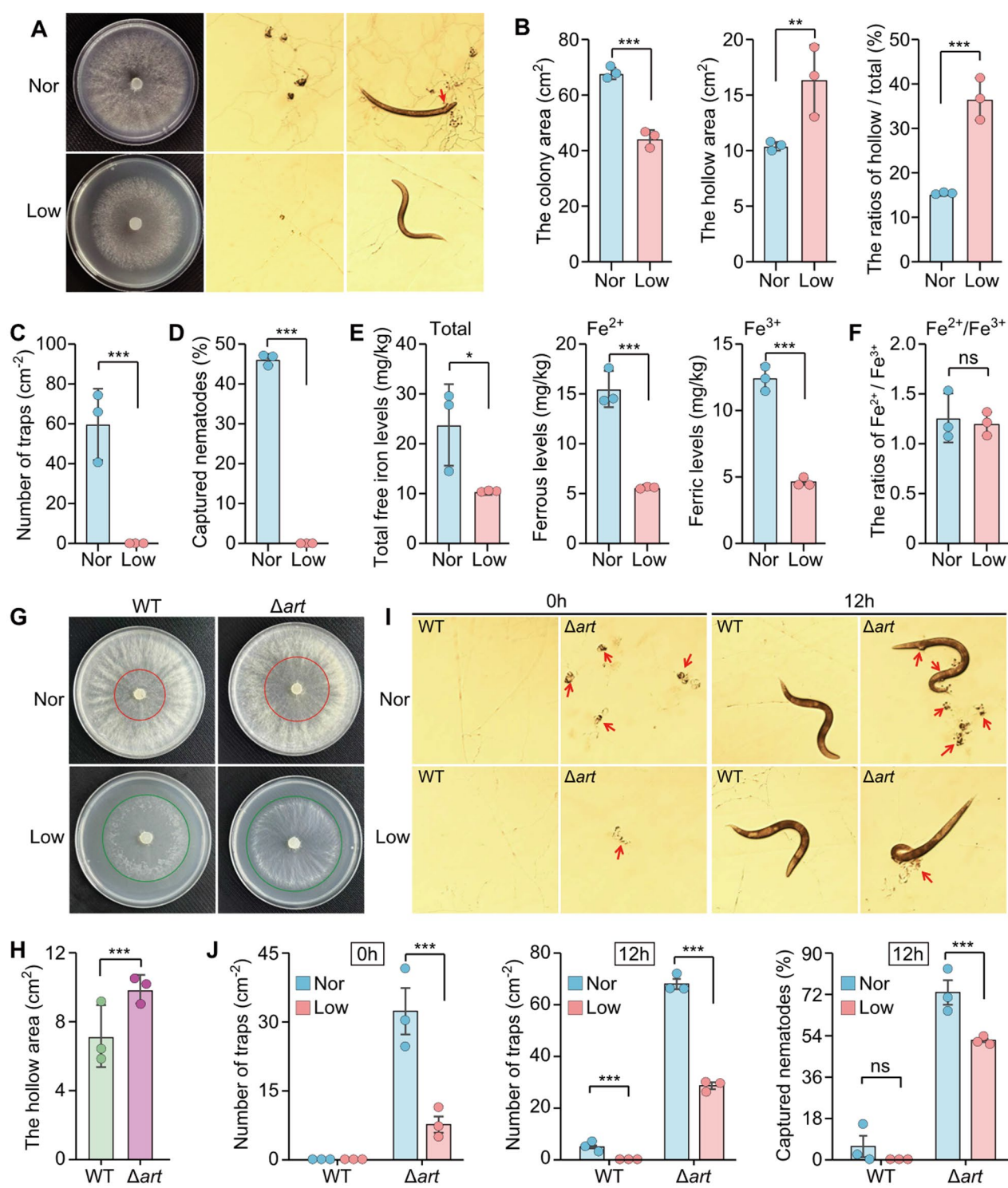


Fig. 5 (See legend on previous page.)

trapping device formation. Importantly, the increase in trapping device numbers attributable to elevated oxygen was substantially greater in the Δart mutant than in the WT strain (Fig. 5I, J). Furthermore, the Δart mutant

exhibited a more pronounced increase in both trapping device formation and nematicidal activity under both oxygen conditions compared to the WT strain (Fig. 5J). Collectively, these results indicate that arthrobotrisin

biosynthesis functions to inhibit oxygen-induced trapping device formation in *A. oligospora*.

2.10 The mutant Δart is much hungrier for UQ₁₀ and COQ7 than WT

Since the Δart mutant, which lacks arthrobotrisins, formed significantly more trapping devices and exhibited stronger nematocidal activity than the WT strain, we hypothesized that the Δart mutant may have a higher demand for UQ₁₀ and COQ7 compared to the WT. To test this hypothesis, the Δart mutant was supplemented with UQ₁₀ at concentrations of 10, 50, and 500 μ M under normal oxygen conditions. Two controls were included: a solvent-only control and an untreated control. Because the Δart mutant can spontaneously develop trapping devices, trapping device formation was induced both in the presence and absence of nematodes. In both scenarios, at all tested UQ₁₀ concentrations, the Δart mutant produced fewer trapping devices than the controls (Fig. 6A–C), with the inhibitory effect of UQ₁₀ on trapping device formation displaying a clear dose-dependent pattern. Notably, the Δart mutant at 10 μ M UQ₁₀ without nematode induction exhibited a slight reduction in trapping device formation (Fig. 6A, B), whereas the WT strain treated with 10 μ M UQ₁₀ in the presence of nematode did not (Fig. 2A, B). These results suggest that the Δart mutant has a higher requirement for UQ₁₀ than the WT strain in inhibiting trapping device formation.

Interestingly, the trapping devices of the Δart mutant treated with 500 μ M UQ₁₀ still exhibited strong nematocidal activity, albeit with a slight reduction compared to other treatments with lower concentrations of UQ₁₀ (Fig. 6D). These results suggest that UQ₁₀ supplementation may not fully satisfy the fungal requirements for iron sequestration despite its inhibitory effect on trapping device development. Subsequently, the nematode

mutant $\Delta clk-1$, along with its WT nematode strain N2, was used to assess trapping device formation and nematocidal activity of the Δart fungal mutant. The $\Delta clk-1$ nematodes induced trapping device formation in the Δart mutant at levels comparable to those induced by N2 nematodes (Fig. 6E, F), confirming that the *coq7* mutation in nematodes does not affect fungal trapping device formation. As expected, with or without UQ₁₀ supplementation, the Δart mutant, like the WT fungal strain, captured significantly more N2 nematodes than $\Delta clk-1$ nematodes (Fig. 6G). Notably, the Δart mutant captured 3.1 times more N2 nematodes than $\Delta clk-1$ nematodes, whereas the WT fungal strain captured 2.6 times more N2 nematodes than $\Delta clk-1$ nematodes, indicating a substantially higher demand for N2 nematodes by the Δart mutant compared to the WT strain (Figs. 6G and 2C). These results suggest that the trapping devices of the Δart mutant have a higher requirement for COQ7 than those of the WT fungal strain.

Three additional nematode mutants, $\Delta acox$, Δdsc , and Δlpd , were employed to evaluate the role of COQ7 in trapping device formation and nematocidal activity of the fungal Δart mutant. Compared to the nematode WT strain N2, the $\Delta acox$ mutant exhibited a slightly stronger effect on inducing trapping device formation in the fungal Δart mutant, whereas the Δdsc , Δlpd , and $\Delta clk-1$ mutants displayed slightly weaker effects (Fig. 6H, I). However, no significant differences in the induction of fungal trapping devices by the Δart fungal mutant were observed among the nematode mutants and N2. Notably, the number of $\Delta clk-1$ nematodes captured by the fungal Δart mutant was the lowest among all nematode mutants tested (Fig. 6J), confirming that nematode COQ7 plays a crucial role in the nematocidal activity of fungal trapping devices, likely due to an attractive interaction between iron-rich trapping devices and the ferritin-like COQ7 protein.

(See figure on next page.)

Fig. 6 Evaluation of the effects of UQ₁₀ and COQ7 on trapping devices and their nematocidal ability in the mutant Δart . **A** Pictures of trapping device development in the mutant Δart treated without (up) and with (down) nematode treatment in the presence of different concentrations of UQ₁₀. Non: control without UQ₁₀ treatment. 0: with solvent only treatment. Red arrow: mature trapping devices. Blue arrow: immature trapping devices. **B** Comparison of trapping devices in the mutant Δart treated with different concentrations of UQ₁₀. **C–D** Comparison of trapping devices (**C**) and nematode-capturing ability (**D**) in the mutant Δart treated with nematodes in the presence of different concentrations of UQ₁₀. **E** Pictures of trapping device development and nematocidal ability in the mutant Δart treated with two different nematodes N2 and the mutant $\Delta clk-1$ in presence of two different concentrations of UQ₁₀. Red arrow: nematode-trapping devices. **F–G** Quantification of trapping device development (**F**) and nematocidal ability (**G**) in the mutant Δart treated with two different nematodes in the presence of different concentrations of UQ₁₀. **H** Pictures of the formation of trapping devices in the fungal mutant Δart induced by five different nematodes, including nematode N2, and the nematode mutants $\Delta clk-1$, Δlpd , Δdsc , and $\Delta acox$. **I** Quantification of trapping devices induced by five different nematodes. **J** Quantification of nematocidal ability of trapping devices in the fungal mutant Δart induced by five different nematodes indicated that the nematode mutant $\Delta clk-1$ is the last prey the trapping devices prefer to capture. Significance was tested using two-sided unpaired Student's *t*-test with Bonferroni's correction (**P* < 0.05, ***P* < 0.01, ****P* < 0.001, *ns* not significant)

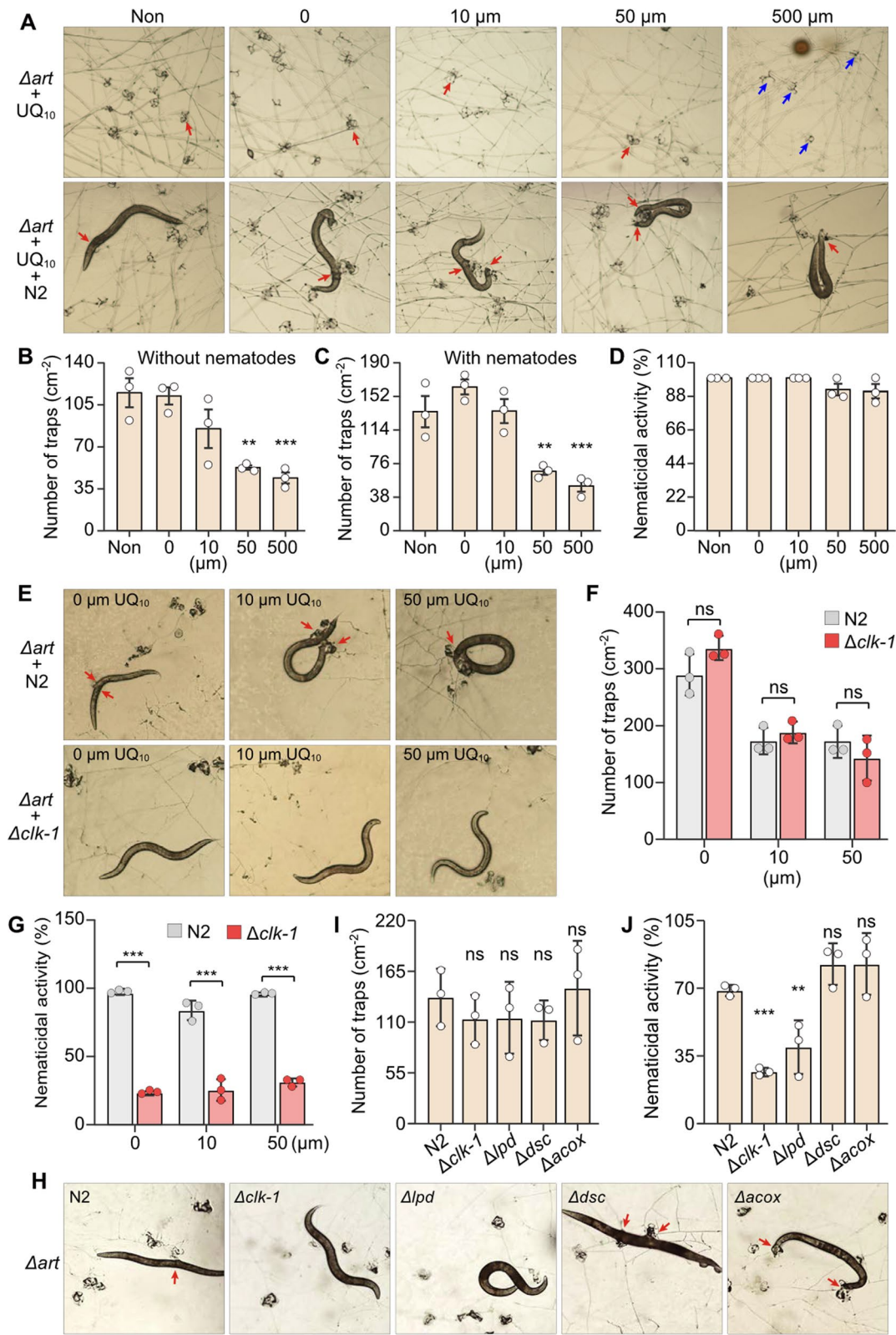


Fig. 6 (See legend on previous page.)

2.11 Occurrence of *art* retainment and *coq7* loss in NTFs during evolution

All eukaryotic organisms must maintain oxygen homeostasis, as oxygen is essential for biological processes but also poses significant risks to cellular function. Traditionally, it has been assumed that biological defenses against hyperoxia are less robust than those against hypoxia, since eukaryotes may not have encountered supraphysiological oxygen levels during evolution [52]. However, geological evidence suggests that rising atmospheric oxygen levels were a key driver in the origin and evolution of aerobic life [53, 54]. Global oxygen levels gradually increased from approximately 1.78% in the early Ordovician period (around 477 million years ago [Mya]) to about 19.88% in the middle Silurian period (around 427 Mya) [55]. At various points in history, atmospheric oxygen levels surpassed the current 21%, reaching approximately 30% during the Carboniferous (around 300 Mya) and Cretaceous (around 100 Mya) periods [55]. To date, limited research has addressed the role of oxygen in the evolution of metabolism, genomes, and morphology in NTFs.

We hypothesized that the loss of *coq7* and the utilization of arthrobotrisins in NTFs under elevated oxygen conditions may be linked to the origin and evolution of trapping devices and their predatory behavior. To investigate this, we analyzed 21 genomes, including nine NTFs (Orbiliomycetes), four fungi from its sister lineage (Pezizomycetes), five fungi from Eurotiomycetes (all harboring the *art* gene cluster), two fungi from Saccharomycetes (which lack the *art* gene cluster), and one fungus from Taphrinomycetes (also lacking the *art* gene cluster) as an outgroup. Using this dataset, we constructed a time-calibrated phylogenetic tree with five calibration points employing MCMCtree. Notably, the loss of the *coq7* gene, together with the loss of *ccc1*, occurred between 413 and 258 Mya, following the acquisition of the *art* gene cluster by the ancestor of NTFs approximately 464 Mya (Fig. 7). This finding suggests that acquisition of the *art* gene cluster may have functioned better in fungal adaptation to fluctuating oxygen levels, rendering the involvement of *coq7* in fungal responses to elevated oxygen redundant or less essential, resulting in the loss of the *coq7* gene in NTFs.

2.12 Temperature and oxygen are key factors driving genomic changes in NTFs

To investigate the evolutionary forces driving the acquisition of the *art* gene cluster and the loss of *coq7* in fungi, we mapped three biogeochemical parameters (atmospheric O₂ levels [55], average global temperature [56], and ironstone occurrences [57]) onto a time-calibrated evolutionary tree. Previous studies have shown that *A.*

oligospora is a temperature-sensitive NTF that fails to grow above 30 °C [20, 58]. Notably, the formation of its trapping devices is positively temperature-dependent, which correlates with the inverse relationship between iron levels and temperature in fungal mycelia [20]. Geological data indicate that around 464 Mya, when the NTF ancestor acquired the *art* gene cluster, atmospheric oxygen levels gradually increased from approximately 1.78% in the early Ordovician (around 477 Mya) to 19.88% in the middle Silurian (around 422 Mya) (Fig. 7). During the same period, Earth's temperatures underwent a pronounced V-shaped fluctuation, with average global temperatures rapidly dropping to 12 °C and subsequently rising to 25 °C between 506 and 420 Mya. A similar, though less pronounced, trend was observed in ironstone levels during the Ordovician (Fig. 7). These findings suggest that the *art* gene cluster may have facilitated fungal adaptation to gradually increasing oxygen levels, consistent with the above finding that the *art* gene cluster functions under elevated oxygen conditions.

Interestingly, species with fruiting bodies in the class Pezizomycetes, which share a common ancestor with NTFs, lost the *art* gene cluster rather than the *coq7* gene around 413 Mya, when atmospheric oxygen levels briefly dropped between 380 and 420 Mya (Fig. 7). This is consistent with the above finding that the *art* gene cluster and *coq7* gene overlap in their function in fungal response to elevated oxygen levels. Additionally, fungal species in Orbiliomycetes and Sordariomycetes lost some genes (*Ao273*, *Ao274*, *Ao276*, *Ao277*, *Ao279*, *Ao281*) within the *art* gene cluster—or the entire cluster—as oxygen concentrations gradually declined from 37.2% to present-day levels after 263 Mya (Fig. 7). This correlation between oxygen levels and the evolutionary dynamics of the *art* gene cluster suggests that environmental oxygen concentration played a crucial role in the gain and loss of genes within this *art* gene cluster. Further analysis of gene gains and losses related to stress responses revealed an enrichment of oxidative stress-related genes at nodes 2, 3, 4, 5, 6, and 11, corresponding to periods of rising oxygen levels (Fig. S7A–B and Table S3).

2.13 Distribution of *art* analogues in *Aspergillus* and *Penicillium*

To better understand the distribution patterns of the *art* gene cluster within the fungal kingdom, we conducted a comprehensive survey of *art* gene cluster homologs across 2057 fungal genomes, using the *A. oligospora* *art* gene cluster as a reference. For further analysis, we retained only those hits that contained *Ao283*, the core biosynthetic gene of the *art* cluster, along with at least four additional *art* cluster genes, including *Ao273*, *Ao274*, *Ao276*, *Ao277*, *Ao278*, *Ao279*, *Ao280*, *Ao281* and *Ao282*.

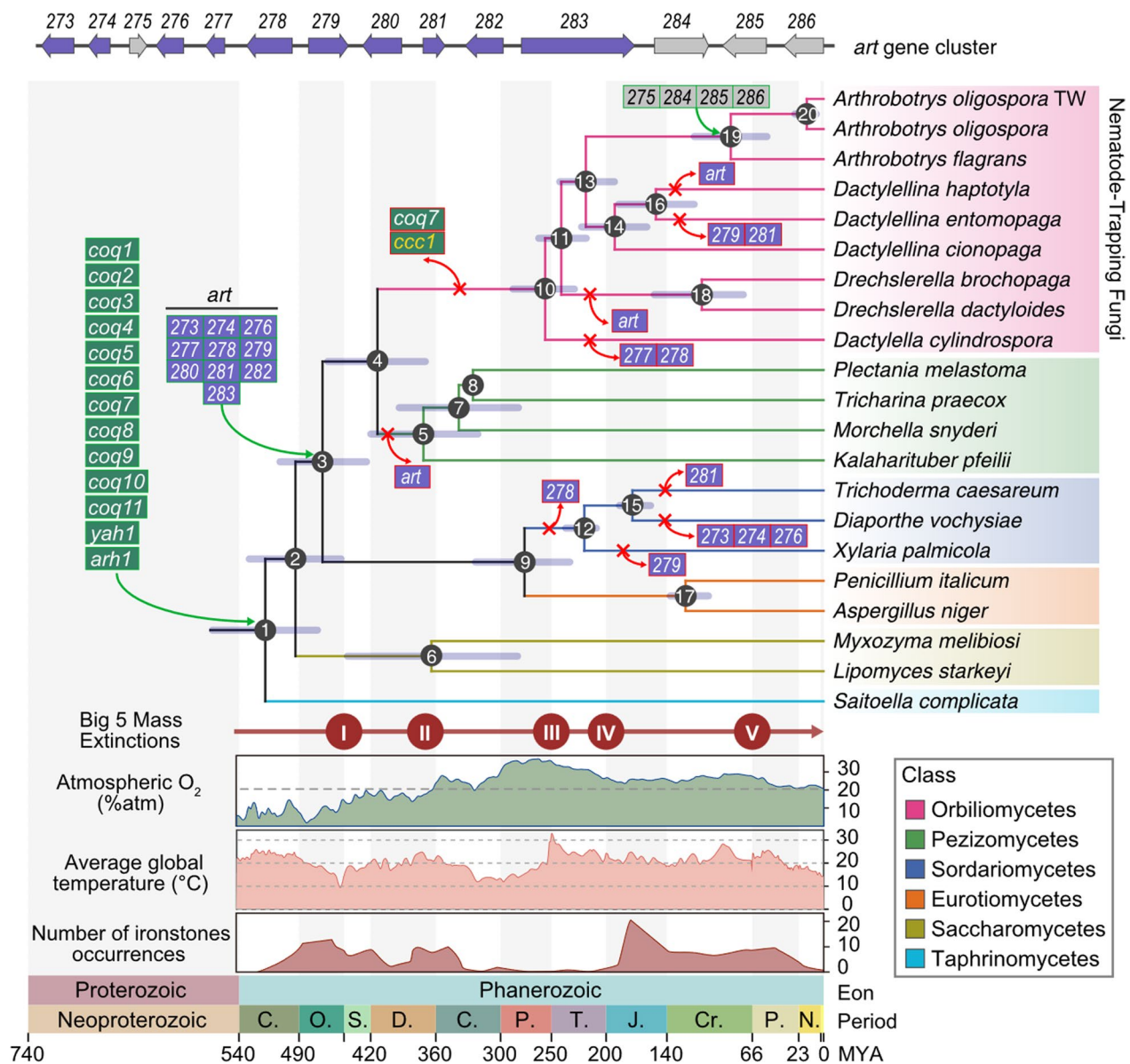


Fig. 7 Dynamic evolutionary landscape of UQs biosynthetic genes and *art* gene cluster. Divergence times of all nodes were estimated by MCMCTree in PAML package with 278,923 fourfold degenerate sites from 1256 single copy orthologous genes and calibrated with one fossil calibration point (A extinct NTF group with ring trapping structure: > 100 Mya) in node 13. To compensate for the availability of limited direct fossil evidence, five phylogenetically deep nodes were calibrated using rigorously validated secondary calibration points derived from highly supported, multi-evidence-based divergence time estimates: node1 (498–571 Mya), node 4 (357–459 Mya), node 12 (216–267 Mya), node 15 (108–181 Mya) and node 17 (64–135 Mya). The error bars represent the 95% highest posterior density (HPD) of a node age; The numbered circles at each node denote the node sequence of the inner nodes; The red cross and an arrow symbol were used to indicate a certain gene loss event. The first panel represents predicted Phanerozoic atmospheric O₂; The second panel represents Phanerozoic global average temperature; The third panel represents the number of occurrences of Phanerozoic ironstones; The x-axis represents geological time periods spanning from the Proterozoic to the Phanerozoic Eon; The letter abbreviations denote: Cambrian (C), Ordovician (O), Silurian (S), Devonian (D), Carboniferous (C), Permian (P), Triassic (T), Jurassic (J), Cretaceous (Cr), Paleogene (P), and Neogene (N) periods

To identify potentially pseudogenized versions of the cluster in fungal genomes lacking a complete set, we performed BLAT searches. This analysis revealed *art* gene cluster analogues in 103 fungal genomes, representing 86

species across 33 fungal genera. These fungal genomes were distributed among several fungal classes, including Eurotiomycetes (13.2%, 52/395), Sordariomycetes (6.6%, 32/488), Dothideomycetes (5.7%, 10/174),

Lecanoromycetes (27.3%, 3/11), and Orbiliomycetes (66.7%, 6/9) (Fig. S8 and Table S4).

Global fungal data from the GlobalFungi database [59] indicates that 67% of the genera (22 of 33) containing the *art* gene cluster are dominant fungal genera, capable of surviving across a broad range of environmental conditions and, in some habitats, becoming the predominant group (Table S5). Moreover, the majority of species (87%, 73 out of 86) belonged to these dominant genera (Table S6). Further analysis of gene turnover within the *art* cluster across the 86 fungal species revealed that only species in *Arthrotrichs*, along with some in *Aspergillus* and *Penicillium*, retained an intact analogue of the cluster. In contrast, other species have lost one or more key biosynthetic genes (Fig. 8). The high occurrence of the *art* gene cluster in dominant fungal genera, together with its intact preservation in *Arthrotrichs*, *Aspergillus*, and *Penicillium*, suggests that this gene cluster may contribute to the ecological success and environmental adaptability of these fungi. This is particularly notable given that *Aspergillus* and *Penicillium* are among the most dominant fungal genera in modern ecosystems.

3 Discussion

Phenotype, defined as the observable characteristics of an organism, arises from the complex interaction between its genetic blueprint (genotype) and environmental factors. Genetic defects, including gene loss or deletions, represent critical perturbations to this genetic blueprint and constitute fundamental causes of phenotypic diversity, influencing both normal variation and disease pathogenesis [60, 61]. Previous studies have demonstrated that the trapping devices of the NTF *A. oligospora* contain numerous iron-rich electron-dense bodies, suggesting that these trapping devices serve as an alternative iron sequestration strategy [20, 62]. This adaptation likely compensates for the loss of the CCC1-mediated vacuolar iron detoxification mechanism, which is otherwise conserved in most fungi [63]. Notably, the CCC1-mediated vacuolar iron storage mechanism was lost in NTFs during the Late Paleozoic Ice Age, and the subsequent formation of fungal trapping devices emerged during the Permian–Triassic mass extinction which is correlated with the significantly elevated global temperatures [20].

In particular, the common desferri-ferrichrome-mediated iron storage pathway, which is widely distributed in fungi, emerged in NTFs during the elevated temperatures of the Cambrian period [20, 64].

This study investigates how the genetic defect—specifically the loss of the *coq7* gene in NTFs—interacts with the environmental factor oxygen to facilitate the fungal iron-rich trapping devices to capture nematodes. Genomic analyses indicate that among the Ascomycota, only nine NTF species lack the essential *coq7* gene, which encodes COQ7, a membrane-bound di-iron protein belonging to a unique subset of the ferritin family and responsible for UQ biosynthesis [22–24]. Time-calibrated evolutionary analyses suggest that the last common ancestor of NTFs within the Orbiliomycetes lost both the *ccc1* and *coq7* genes during the Late Paleozoic Ice Age, a period characterized by cold “superoligotrophy,” marked by extremely low nutrient availability due to cold stress [65]. Previous research has shown that mutation in the *clk-1* gene of *C. elegans* resulted in extended lifespan and generally slowed developmental rates [66]. Thus, the loss of the *coq7* gene in Orbiliomycetes may have conferred a survival advantage during the prolonged cold “superoligotrophy” period.

We observed that the absence of the *coq7* gene in NTFs renders these fungi sensitive to elevated oxygen levels, as increased oxygen availability promotes iron accumulation, potentially leading to iron overload. Phenotypic analyses revealed that the formation of trapping devices and their nematocidal activities were significantly higher in the predominant NTF *A. oligospora* under normoxic conditions compared to hypoxic conditions, suggesting that oxygen is a key driver of trapping device development. Moreover, UQ₁₀ markedly inhibited trapping device formation. The introduction of an exogenous *coq7* gene also significantly suppressed the nematode-induced formation of trapping devices in *A. oligospora*. Importantly, fungal trapping devices exhibited a significantly enhanced ability to capture COQ7-expressing nematodes compared to COQ7-deficient nematode mutant $\Delta clk-1$. Previous studies suggest that nematodes are typically iron-deprived organisms [67, 68]. For example, *C. elegans* can detect pyoverdine, an iron-chelating siderophore secreted by *Pseudomonas aeruginosa*, and preferentially

(See figure on next page.)

Fig. 8 Gene members of *art* gene cluster change among various fungal species containing this gene cluster. The phylogenetic tree delineates evolutionary relationships of 86 fungal species from Eurotiomycetes (n = 43), Lecanoromycetes (n = 1), Dothideomycetes (n = 10), Sordariomycetes (n = 27) and Orbiliomycetes (n = 5). These 86 fungal species were obtained by deduplicating 103 genomes containing *art* (only one genome representing the species was retained when multiple genomes of the same species contained *art*). Heatmap columns correspond to 10 biosynthetic genes (Ao273–Ao274, Ao276–Ao283) for the biosynthesis of arthrotrichin type of meroterpenoids within the cluster; White denotes absence, light pink denotes presence, and dark pink denotes gene expansion

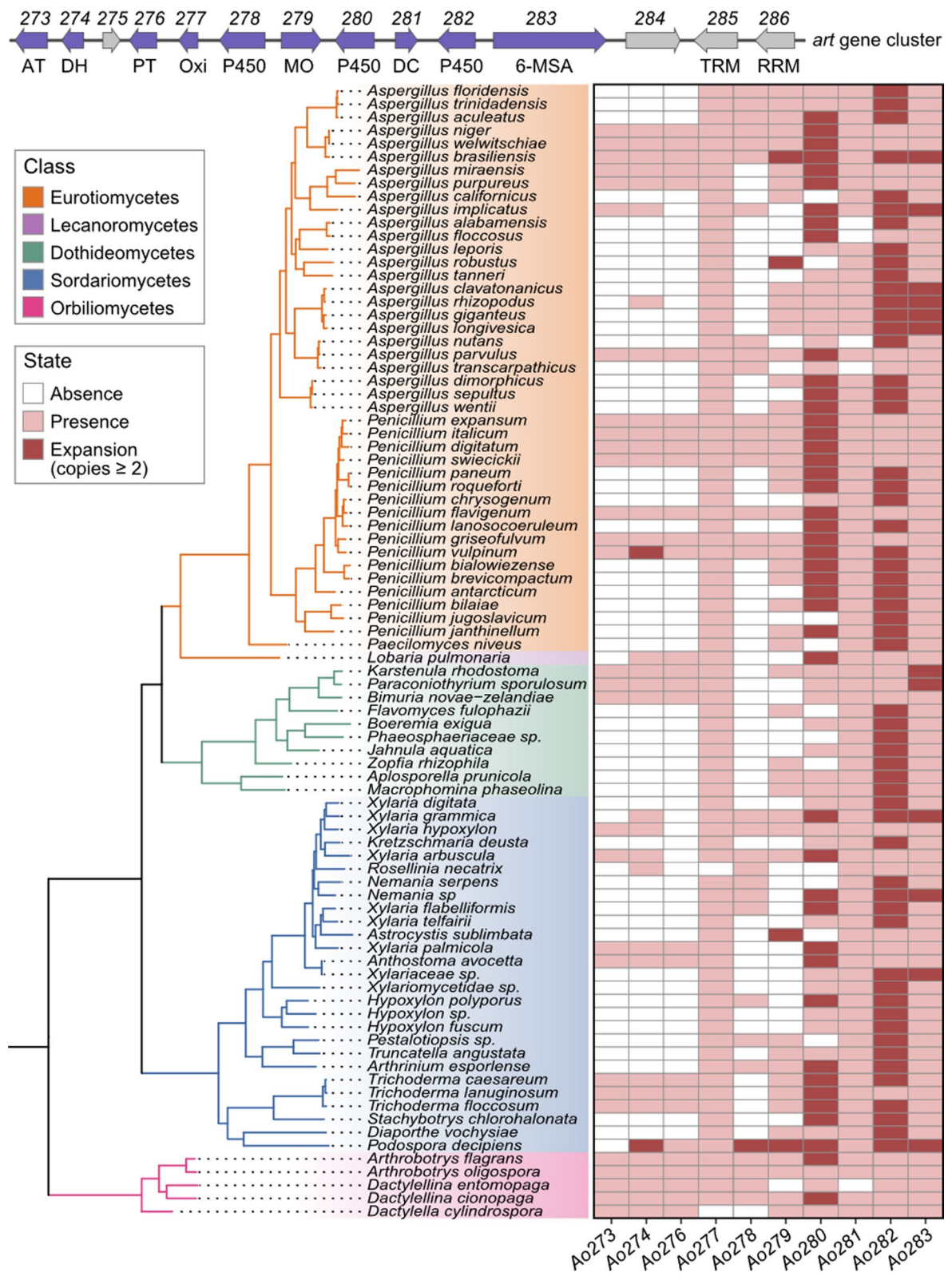


Fig. 8 (See legend on previous page.)

feeds on the bacterial prey [69]. Our further experiments indicated that the fluorescently labeled COQ7 protein from a genetic engineering *Escherichia coli* could adhere to the fungal trapping devices, while the ACOX control protein could not. Further in vivo experiments suggested that nematode COQ7 can be secreted and exposed to the nematode surface, while also indicating the affinity of the nematode COQ7 for the fungal trapping device.

Since the insertion of an exogenous *coq7* gene in *A. oligospora* significantly reduces the formation of fungal iron-rich trapping devices, which is consistent with the previously reported function of COQ7 as an iron chelator [22–24]. Therefore, the deletion of *coq7* in nematodes should also lead to an increase in free iron content in the mutant $\Delta clk-1$ nematodes. Most likely, iron-rich fungal trapping devices may repel iron-rich nematodes, resulting in the failure of *A. oligospora* to capture the mutant $\Delta clk-1$ nematodes without COQ7. In the predator–prey interaction between NTFs and nematodes, the ferritin-like COQ7 protein in nematodes may readily bind to iron-rich fungal trapping devices, potentially triggering fungal predation. This

observation supports the dual function of fungal trapping devices: attracting and capturing nematodes [70].

Remarkably, we found that *A. oligospora* utilizes arthrobotrisins instead of UQ₈ in response to elevated oxygen levels, as UQ₈ is employed only under hypoxic conditions (Fig. 9). Arthrobotrisins that serve as key chemotaxonomic markers for *A. oligospora* possess a triphenyl-phenol backbone structurally similar to that of UQ₃. Triphenyl-phenols and their oxygenated derivatives are common fungal meroterpenoids derived from phenolic precursors [71]. Notable examples include the potent antifungal yanuthones produced by *Aspergillus* and *Penicillium*, as well as the phytotoxic macrophorins from *Macrophoma*, a pathogen responsible for apple fruit rot [72]. However, the biosynthetic pathway of these triphenyl-phenol metabolites has been elucidated only in *A. oligospora*, due to its relatively simple metabolism and reduced number of biosynthetic gene clusters [41, 47–50]. Previous studies have shown that disruption of the arthrobotrisin biosynthetic pathway significantly enhances the formation of trapping

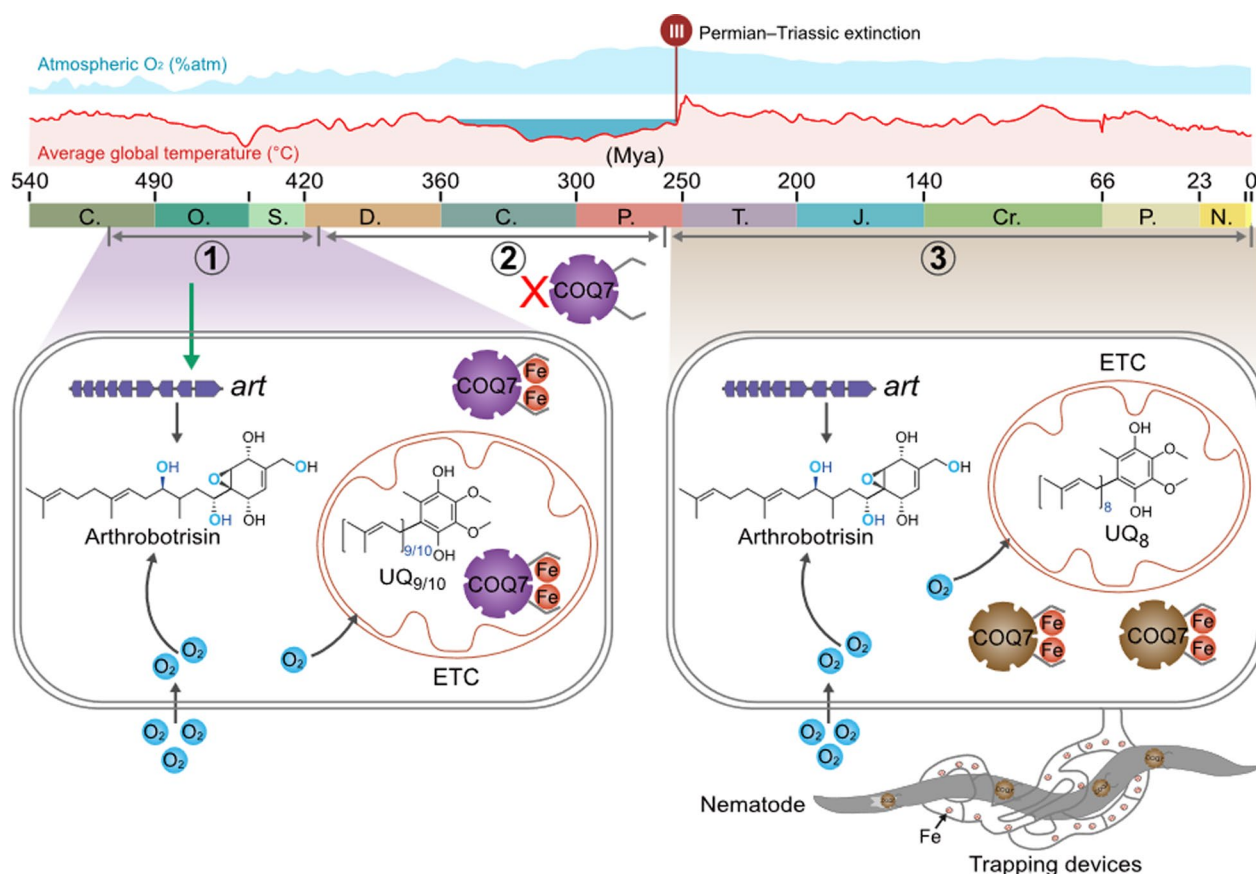


Fig. 9 The association of *coq7* loss in NTF *A. oligospora* with the origin and evolution of nematode-trapping ability of NTF. ETC electron transferring chain

devices and nematicidal activity, but impairs fungal colonization in field soils [48, 50].

In this study, mutation analysis revealed that the Δart mutant lacking arthrobotrisin biosynthesis lost the metabolic response observed in WT caused by elevated oxygen levels and instead significantly promoted the UQ₈ accumulation. Notably, unlike WT, which requires nematode induction to produce traps, the Δart mutant was capable of spontaneously forming trapping devices without nematode induction under both hypoxic and hyperoxic conditions. These results suggest that arthrobotrisin biosynthesis inhibits trapping device formation by modulating oxygen-mediated metabolism in *A. oligospora*. Like the fungal WT strain, the Δart mutant exhibited significantly enhanced nematicidal activity against COQ7-expressing nematode strains compared to COQ7-deficient nematode mutants. These findings suggested that the NTF *A. oligospora* utilizes built-in arthrobotrisin biosynthesis to inhibit oxygen-mediated formation of its own trapping devices, confirming that these trapping devices are physiologically a phenotypic manifestation of iron overload in the fungus. The limited nematicidal activity of these trapping devices is consistent with the therapeutic rationale underlying iron chelation strategies [73], which help explain why NTFs often form more trapping devices but exhibit reduced viability.

Interestingly, fungal species in the class Pezizomycetes, which share a common ancestor with Orbiliomycetes, lost the *art* gene cluster approximately 413 million years ago (Mya), whereas NTFs within Orbiliomycetes lost the *coq7* gene. This divergence in gene loss strategies suggests that *coq7* and the *art* gene cluster may fulfill analogous roles in adapting to fluctuating oxygen conditions, likely due to their structural and functional similarities. Phylogenetic analysis revealed that only the genus *Arthrobotrys* retains an intact *art* gene cluster, while other NTFs have partially or completely lost this cluster. This pattern corresponds with the widespread ecological distribution of *A. oligospora*, the most globally dispersed NTF.

In summary, this study investigates how the loss of *coq7* in *A. oligospora* reshapes fungal metabolic responses to elevated oxygen levels, resulting in the formation of trapping devices that selectively target COQ7-expressing nematodes (Fig. 9). Namely, *A. oligospora* employs two distinct strategies to mitigate iron overload: one involving the acquisition of exogenous iron-chelating agents from nematodes, and the other through endogenous metabolic adaptation. Collectively, these findings further confirm that the formation of fungal trapping devices is a phenotype for iron overload due to a synergistic effect of genetic and environmental factors, highlight the utility of NTFs as a eukaryotic model for studying eukaryotic iron dysregulation or iron overload-related cellular responses.

4 The experimental section

4.1 Gene loss analysis in NTFs

We selected 21 representative genomes including 9 nematode-trapping fungi (Orbiliomycetes), 4 fungi from its sister lineage (Pezizomycetes), 5 fungi (all of these contain *art* gene cluster) evenly sampled across Eurotiomycetes, 2 fungi (without *art* gene cluster) from Saccharomycetes as well as 1 fungus (without *art* gene cluster) from Taphrinomycetes as an outgroup. All-versus-all BLASTP results of 207,036 protein sequences from the 21 fungal genomes were input into OrthoFinder (v.2.5.2) [74] for gene clustering, in which the MCL algorithm was enabled by setting the inflation factor to 1.5. Orthologous gene table were analyzed in Count software [75] to investigate gene repertoire evolution and reconstruct gene family histories for both total and secreted genes. Gene families represented in fewer than three species were excluded from the rate estimation to ensure robustness. Evolutionary rate modeling was performed using a gain–loss–duplication framework, incorporating a Poisson distribution at the root and allowing for lineage-specific rate variation. The optimization procedure was conducted over 100 iterations with a likelihood convergence threshold set at 0.1. Gene content (gain and loss) was inferred using Dollo parsimony, complemented by posterior probability estimation under a birth-and-death process. Each inferred gene loss was validated by conducting BLASTp searches.

4.2 Phylogenetic tree construction of fungal genomes based on BUSCO genes

We systematically collected 2517 publicly available fungal genomes from the JGI MycoCosm database (Access: 2023-6-15) and NCBI genome database. To ensure data quality and completeness, we evaluated all genomes using the Benchmarking Universal Single-Copy Orthologs (BUSCO) [76] tool and retained only those with a completeness score higher than 90%. A total of 2057 high-quality fungal genomes were retained, with Ascomycota (1361 genomes, 66.2%) and Basidiomycota (540 genomes, 26.3%) dominating the dataset. The identified orthologous sequences from each genome were extracted from the respective BUSCO output directories. For each ortholog group, multiple sequence alignments were performed. These alignments were rigorously inspected for quality and subsequently concatenated into a comprehensive alignment matrix. This concatenated dataset provided a holistic view of the conserved genetic information across the sampled genomes. Maximum likelihood (ML) tree with 1000 bootstrap iterations were built.

4.3 Distribution pattern of UQs biosynthesis genes in 1361 Ascomycota genomes

At least 13 proteins (COQ1–COQ11, YAH1, and ARH1) are necessary for the synthesis of UQs. To elucidate the distribution patterns of these proteins in the fungal kingdom, protein sequences of COQ1–COQ11, YAH1, and ARH1 from *Saccharomyces cerevisiae* were used as queries. These sequences were used to search for orthologs via BLASTp (v.2.11.0+) against a custom database containing all predicted protein sequences from 1,361 high-quality fungal genomes. Our criteria for BLASTp were a bit scores greater than 50, minimum alignment length of 50 amino acids and minimum sequence similarity of 30%.

4.4 Distribution pattern of UQs biosynthesis genes in 2057 fungal genomes

The method is consistent with that described above. Presence and absence of each gene were mapped to the phylogenetic tree (containing 2057 fungal genomes) to visualize the distribution of UQs biosynthesis genes in fungal kingdom.

4.5 Fungal strain and culture conditions

The WT and its Δart mutant ($\Delta Ao283$) of *A. oligospora* YMF1.3170 was obtained from the State Key Laboratory for Conservation and Utilization of Bio-Resources & Key Laboratory for Microbial Resources of the Ministry of Education. Δart mutant was constructed according to a previous study [38]. PDA (potato 200 g/L, glucose 20 g/L, agar 15 g/L), water agar (15 g/L agar) media were used for analyzing mycelial growth and related phenotypic traits. For the flask culture, strain was initially cultured on PDA medium at 28 °C for 10 days to obtain conidia, and then the conidia were inoculated in 250 mL of liquid PDB medium (potato 200 g/L, and 20 g of glucose per liter) with a final concentration of 1×10^5 conidia/mL under shaking (180 rpm) and static conditions (0 rpm) at 28 °C.

4.6 Acquisition and cultivation of nematode mutant strains

The *C. elegans* N2 and mutant strains ($\Delta clk-1$, $\Delta acox$, Δlpd and Δdsc) used in this study were obtained from the State Key Laboratory for Conservation and Utilization of Bio-Resources & Key Laboratory for Microbial Resources of the Ministry of Education. Mutants were constructed in previous studies [34, 35]. Both N2 and mutant strains were requested through the standard organization strain request process. Upon arrival, the nematodes were maintained at 20 °C on Nematode Growth Medium (NGM) plates seeded with *Escherichia coli* OP50, as per standard cultivation protocols. The mutation status of the strains was confirmed by PCR amplification, as detailed in the

strain descriptions provided by the repository. Nematode populations were synchronized. In brief, gravid adults were transferred onto fresh NGM plates after growing for about 2 days at 20 °C, and eggs were collected after allowing the adults to lay eggs. The eggs were then allowed to hatch overnight at 20 °C, and synchronized populations of L1 larvae were isolated. These larvae were grown to the desired developmental stage (L4) before being used for experimental purposes.

4.7 Construction of *coq7* heterologous expression strain (E-*coq7*) using *A. oligospora*

To construct a heterologous expression plasmid, the PUC19-1300-D-HYB (PUC19) plasmid was amplified using fusion polymerase chain reaction (PCR) with Phanta Max SuperFidelity DNA Polymerase (Vazyme, Nanjing, China), following the manufacturer's instructions. The primers used in this study are listed in Table S7. The heterologous fragment was amplified from *Saccharomyces cerevisiae* BJ5464 and purified. The *coq7* gene was inserted into the PUC19 plasmid, which contains the strong *P_{trpC}* promoter and *T_{trpC}* terminator from *Aspergillus niger*. The *coq7*-plasmid and blank plasmid transformed into *A. oligospora* protoplasts, respectively. After 2–4 days of incubation at 28 °C, transformation colonies were selected and transferred to new PDA plates. Genomic DNA from putative transformants was extracted after 5 days of incubation at 28 °C, and PCR was performed to confirm the integration of the target gene into the genome. The mutant was identified and verified by PCR and sequencing analysis. The WT strain and E-*coq7* mutant were cultured on PDA medium at 28 °C for 6 days. Vegetative hyphae were harvested from 9-cm Petri dishes, which were then incubated at 28 °C for 3 and 5 days. Following induction with nematode, the hyphae were collected and immediately frozen in liquid nitrogen. Total RNA was extracted from all samples using the AxyPrep Multisource RNA Miniprep Kit (Axygen, Jiangsu, China). This extracted RNA was reverse transcribed into cDNA using the FastQuant RT Kit with gDNase (Takara, Kusatsu, Japan). The cDNA was used as a template to analyze the expression of the *coq7* gene, with cDNA from *S. cerevisiae* BJ5464 serving as the positive control template.

4.8 Prey preference assay of *A. oligospora* on different nematode mutants

To assess the selective preference of *A. oligospora* toward different nematode mutants, nematocidal activity assays were performed using WT (N2) and mutant strains ($\Delta clk-1$, $\Delta acox$, Δlpd , and Δdsc). Fungal mycelia were cultured on standard 9 cm PDA plates at 28 °C for 4 days until mature trapping devices had developed. Approximately

400 synchronized L4-stage nematodes from each mutant were separately suspended in sterile water at equal densities. Three hundred microliters of each suspension were then transferred to independent Petri dishes containing fungal cultures under identical growth conditions. Plates were gently swirled to evenly distribute nematodes, and all dishes were incubated in parallel within the same incubator at 28 °C to ensure uniform environmental conditions. Captured nematodes were monitored at 24 h post-inoculation under a stereomicroscope. For each sample, 6 randomly selected visual fields were examined, and nematodes were considered captured when clearly immobilized within trap rings. Nematode mortality was assessed based on the absence of movement in response to physical stimulation with a fine needle, with straight body posture used as a supporting criterion [19]. All experiments were repeated in triplicate for each mutant.

4.9 Fungal colony growth under normal oxygen and low oxygen conditions

Anaerobic Gas Generator is a disposable oxygen-absorbing and carbon dioxide-generating agent for use in anaerobic jars (C-1, Mitsubishi Gas Chemical Company, Inc.). The mutant strain Δart and the WT were cultured on PDA medium within anaerobic bags. One sachet of anaerobic gas generator is used for a 2.5-L anaerobic jar, and the sachet is discarded after 30 min of reaction to create a low-oxygen environment (approximately 10% oxygen). Smart Sensor (Model: AR8100) oxygen detector was used to detect the oxygen concentration inside the anaerobic jars. Both WT and mutant strains were cultured on PDA at 28 °C in normal oxygen and low oxygen conditions, and their growth rates and colony morphology were observed. Hyphal morphology was examined using 5-day-old PDA cultures grown at 28 °C.

4.10 Trap formation and nematicidal activity assays under normal oxygen and low oxygen conditions

WT and mutant strains were cultured on 9 cm agar plates under normal oxygen and low oxygen at 28 °C for 3–4 days, and then about 400 nematodes (*C. elegans*) were introduced to each culture. After 12 h, traps and captured nematodes per plate were observed under a microscope and counted at specific time-points. Three hundred microliters of the nematode suspension were then transferred to each Petri dish and gently mixed. All dishes were incubated at 28 °C. Nematode mortality was assessed at 12h exposure periods using a normal binocular microscope, and four visions (1cm²/per) in each Petri dish were picked at random for counting. Nematodes were considered to be dead when their bodies were straight, and they failed to move on physical stimuli with a fine needle. Nematicidal activity was evaluated

according to the mean corrected percentage of dead nematodes. All tests were repeated three times, and the data obtained were statistically analyzed.

4.11 LC–MS/MS sample extraction and preparation

The WT and mutant strain were inoculated in 250-ml PDB flasks cultured at 28 °C under shaking (180rpm, 120rpm, 60rpm) and static conditions (0 rpm) for 7 days prior to analysis by high performance liquid chromatography with diode array detection MS (UPLC-DAD/MS). The fermentation broths were extracted with an equal volume of ethyl acetate, and the organic layers were evaporated and dissolved in 1 ml of menthol and filtered through a 0.22 µm filter membrane. All samples were subsequently analyzed using an HPLC system coupled with a Q Exactive Focus Orbitrap mass spectrometer (Thermo Fisher) and equipped with an Agilent Zorbax ODS 4.6 by 250 mm column (Agilent, Santa Clara, CA) in the electrospray ionization (ESI) mode. The data were acquired in both positive and negative ion modes. To ensure the accuracy of compound identification and quantification, QC samples were injected periodically throughout the analysis to monitor instrument stability and data reproducibility. The metabolites extracted from WT and mutant strains *A. oligospora* were analyzed by LC–MS following the established methods in the literature.

4.12 LC–MS/MS data processing and analysis

Raw MS data files generated from the LC–MS/MS system were processed using Thermo Scientific Xcalibur software for data acquisition and processing. Raw MS data were also imported into Compound Discoverer (version 3.3) software for comprehensive data analysis. Peak detection was performed using the built-in algorithm. The raw data were processed to identify and align chromatographic peaks across all samples using a reference sample. A list of features, including accurate mass, retention time, and ion intensities, was generated for each sample. Feature Finder module was used to extract relevant features from the chromatograms. The extracted features were matched to compounds using the mzCloud databases within Compound Discoverer. The software utilized both the exact mass of detected ions and their fragment ion patterns to propose possible compound identities. The ion intensities of identified compounds were normalized against total ion count (TIC). Quantitative analysis was performed to determine the relative abundance of each metabolite in the different sample groups. Statistical analysis was conducted using the software's built-in tools, including principal component analysis (PCA) and hierarchical clustering, to visualize group

separation and identify significantly different compounds across mutant Δart under shaking and static conditions.

4.13 Quantitative real-time PCR analysis

The WT and mutant strain were inoculated in 250-ml PDB flasks cultured at 28 °C under shaking (180 rpm) and static conditions (0 rpm) for 7 days. After induction, hyphae were collected and frozen immediately in liquid nitrogen. Total RNA was extracted from the mycelia of each sample using an RNA extraction kit (Axygen, Jiangsu, China) and then reverse transcribed into cDNA using the PrimeScriptHRT reagent kit (with genomic DNA (gDNA); TaKaRa, Kusatsu, Japan). The resulting cDNA was used as a template for RT-qPCR to analyze the mRNA expression of *Ao273-286* with specific paired primers (Table S8) and the LightCycler 480 with SYBR green I master mix (Roche, Basel, Switzerland). β -Tubulin was used as an internal standard. All RT-qPCR experiments were performed in triplicates. The relative transcript level (RTL) of each gene was calculated as the difference of the cycle threshold (CT) value for the gene β -Tubulin and *Ao273-286* under static condition and shaking condition.

4.14 RNA-seq data analysis

Raw reads from both WT and Δart samples were examined using FastQC. To obtain clean data, fastp (v.4.10.0) [77] was used with default parameters to filter adaptor sequences and remove low-quality reads. The reference genome and gene model annotation files for *A. oligospora* ATCC 24927 were downloaded from NCBI's Genome Browser. Genome indexing was performed using 'hisat2-build' in Hisat2 (v.2.2.1) [78], incorporating splice sites and exon information. The filtered paired-end reads were aligned to the reference genome using Hisat2. Gene abundance for each sample was estimated from the aligned BAM files. Differential expression analysis was conducted. Resulting p-values were adjusted using the Benjamini–Hochberg approach to control the false discovery rate.

4.15 Assay for iron contents

The WT and mutant strain were inoculated in 250 mL potato dextrose broth (PDB) flasks and cultured at 28 °C (180 rpm or 0 rpm) for 7 days. The fermentation broth was extracted with the equal volume of ethyl acetate, the mass of mycelium was weighed and recorded, then dissolved in 30 mL of distilled water and homogenized to obtain a well-ground mixture. Subsequently, 1 mL of the mixture was centrifuged at 12,000 rpm for 5 min to remove the supernatant for the following experiments. An iron assay kit (#ab83366, Abcam) was used for evaluation of the intracellular ferrous iron level (Fe^{2+} and

Fe^{3+}) in fungi. First, mycelia (20–80 mg) were collected in phosphate-buffered saline (PBS) and homogenized in iron assay buffer using a dounce homogenizer sitting on ice with 50–100 passes. Iron reducer was then added into the collected supernatant, thoroughly mixed, and incubated. Finally, an iron probe was added, mixed, and incubated for 1 h. The resulting solution was immediately measured on a microplate reader at OD=593 nm.

4.16 Identification homologue of art gene cluster in the fungi kingdom

Biosynthetic gene clusters for each genome were annotated by antiSMASH 7.0 (v.7.0.0) [79] via command line version with default parameters, except for the argument 'taxon fungi'. In total, 84,476 secondary metabolism gene clusters were annotated from 2057 fungal genomes. A local database was generated based on all the gene clusters using the 'cblaster makedb' module (v.1.3.18) [80]. To identify *art* gene cluster homologous in fungal kingdom, the search function of cblaster was used to query amino acid sequences of 14 genes in *art* gene cluster (*Ao273–Ao286*) against the local database constructed above. Only resultant hits containing *Ao283*, the core biosynthetic gene of *art*, as well as at least four other genes in this cluster, were considered as putative *art* homologues. We found 103 fungal genomes containing *art* gene clusters homologues. We further conducted additional analyses to strengthen the functional annotation of the core biosynthetic genes within the *art*-like clusters identified in different species. The predicted protein sequences of the core genes in each *art*-like cluster were submitted to the NCBI Conserved Domain Database (CDD). This analysis revealed that all identified core genes share the same conserved domain architecture, including PksD, KR_FAS_SDR_x, PKS_PP and hot_dog superfamily, consistent with the functional modules typically found in *Ao283* (Table S9). The distribution pattern of presence and absence of *art* gene cluster was mapped to the phylogenetic tree (containing 2057 fungal genomes) to visualize the distribution of the gene cluster in the fungi kingdom.

4.17 Gene members of art gene cluster among various fungal species

The 103 fungal genomes containing the *art* gene cluster were used for this analysis. When a species had multiple genomes containing *PKS-TPS* gene cluster, only the genome with the highest number of gene members in this gene cluster was retained as the representative of that species. We identified 86 fungal species containing the *PKS-TPS* gene cluster. Evolutionary relationships of these 86 fungal species were extracted from the phylogenetic tree, which included 2057 fungal genomes. A heatmap

was generated to illustrate the presence, absence, or expansion (gene copies > 2) of these genes across these fungal species.

4.18 Phylogenetic tree construction

for nematode-trapping fungi in Ascomycota

In the section of gene loss analysis, 1256 sets of 1:1 orthologous protein encoding genes were identified among the 21 fungi species. Amino acid sequences of each orthogroups were aligned with the 'auto' parameter, and PAL2NAL (v14) [81] was used under default parameters to create a codon alignment from MAFFT-aligned amino acids. The aligned orthogroups amino acid sequences and its codon alignment were concatenated and used to construct an amino acid (AA) and a nucleotide (NT) data matrix, respectively. Based on AA data matrix, we determined the best model using the "-m TEST" parameter, which automatically estimates the best fitting model of substitutions according to Bayesian Information Criterion values (BIC) using IQ-TREE2 (v. 2.2.2.7) [82]. A maximum likelihood (ML) tree with 1000 bootstrap iterations was built based on the best fitting model.

4.19 Function annotations of homologous groups

To assign function annotations to each homologous group (OG), we selected a representative sequence for each group. First, we ordered the sequences within each OG by their lengths. Next, we employed the UCLUST algorithm to cluster the sequences within each OG based on 30% amino acid sequence similarity. The sequence with the highest abundance in each OG was designated as the representative sequence. In cases of equal abundance, the longest sequence was chosen as the representative. For each representative sequence, gene function and gene ontology (GO) were predicted using the PANNZER2 web server [83].

4.20 Estimating divergence times of nematode-trapping fungi in Ascomycota

To estimate the divergence times, fourfold degenerate (4D) sites were extracted using get4foldSites (<https://github.com/brunonevado/get4foldSites>) and concatenated into a single supermatrix. The Bayesian relaxed molecular clock approach was employed to estimate species divergence time using MCMCTREE in PAML (v.4.10.0) [84] (clock=independent rates, burn-in=10,000, sample number=100,000, sample frequency=10, model=JC69) based on 278,923 fourfold degenerate sites from 1256 single copy orthologous genes and calibrated with one fossil calibration point (A extinct NTF group with ring trapping structure: > 100 Mya) [85]. To compensate for the availability of limited direct

fossil evidence, five phylogenetically deep nodes were calibrated using rigorously validated secondary calibration points derived from highly supported, multi-evidence-based divergence time estimates: node1 (498–571 Mya) [86], node 4 (357–459 Mya) [87], node 12 (216–267 Mya) [88], node 15 (108–181 Mya) [88] and node 17 (64–135 Mya) [88]. We used Tracer (v.1.7.1) [89] to visually check convergence of parameters across replicate MCMC chains in report of the MCMC runs. All the resulting effective sample sizes (ESS) ranged from 291 to 1306, suggesting that the parameters across replicate MCMC chains reached convergence (The effective sample size (ESS) should be higher than 200).

4.21 Dynamic landscape of gene members in art gene cluster during the evolutionary timeline

Based on the time-calibrated phylogenetic tree, we mapped three representative biogeochemical properties—atmospheric O₂ [55], average global temperature [56], and the number of ironstone occurrences [57] during the Phanerozoic eon onto the tree. Gene members of *art* gene cluster (Ao273–Ao286) and UQs synthesis-related genes of 21 genomes in phylogenetic tree were also mapped onto the tree to display their loss and gain at different nodes.

4.22 Expression, purification, and feeding of recombinant fluorescently labeled COQ7-EGFP/ACOX-EGFP fusion proteins

The complete coding sequences of *acox* and *coq7* from *Saccharomyces cerevisiae* were amplified by PCR using gene-specific primers and cDNA as the template. The purified PCR products were individually cloned into the pCold II expression vector, which contains an N-terminal His tag and a cold-shock promoter, to generate C-terminal fusions with enhanced green fluorescent protein (EGFP). The resulting vector designated pCold II-His-ACOX-EGFP and pCold II-His-COQ7-EGFP, were verified by DNA sequencing to confirm the accuracy of the insert sequences and reading frames. The validated recombinant plasmids were transformed into the *E. coli* expression strain BL21 (DE3). A single colony was inoculated into LB medium containing ampicillin (100 µg/mL) and cultured at 37 °C with shaking at 200 rpm until the OD₆₀₀ reached 0.5–0.8. Protein expression was induced by adding isopropyl β-D-1-thiogalactopyranoside (IPTG) to a final concentration of 0.5 mM, followed by incubation at 16 °C with shaking at 200 rpm for 16 h. After induction, bacterial cells were harvested by centrifugation at 4 °C and 8000 rpm for 5 min. The pellet was resuspended in ice-cold lysis buffer and lysed by sonication on ice. Cell debris was removed by centrifugation at 4 °C and 8000 rpm for 30 min, and the supernatant was collected

as the crude extract. The supernatant was applied to a Ni-NTA affinity column pre-equilibrated with lysis buffer. After sample loading, the column was washed with 10 column volumes of wash buffer to remove non-specifically bound proteins. Bound proteins were eluted with 4 column volumes of elution buffer. Eluted fractions containing the target proteins were pooled and concentrated using an Amicon Ultra-15 centrifugal filter device. During concentration, the buffer was exchanged three times with desalting buffer to remove residual imidazole completely. The purity, molecular mass, and specificity of the purified proteins were evaluated by SDS-PAGE, and protein concentrations were quantified using the BCA assay. Fluorescence activity was confirmed by fluorescence microscopy to verify the structural integrity and functionality of EGFP. For feeding assays, spores of *A. oligospora* were spread onto water agar plates and incubated for 3–4 days to allow trap formation. Purified protein solution was then added to the medium, and the plates were immediately examined under a microscope.

4.23 Construction of *C. elegans* strains expressing CLK-1:EGFP fusion protein

Genomic DNA from *C. elegans* WT (N2) was used as template to amplify a fragment containing approximately 2.0 kb of the upstream promoter region and the complete coding sequence (CDS) of the *clk-1* gene via PCR with gene-specific primers. Homology arms (15–25 bp) complementary to the ends of the linearized vector were added to the 5' ends of the primers. The vector pCFJ97-75, which carries the dominant selection marker *rol-6*, the EGFP reporter gene, and an ampicillin resistance gene, was linearized by inverse PCR. The *clk-1* promoter fragment was seamlessly inserted into the linearized vector using homologous recombination, resulting in the fusion expression construct p[clk-1p::CLK-1::EGFP; rol-6]. The ligation product was transformed into *E. coli* DH5 α competent cells and plated onto LB agar containing ampicillin. Positive clones were selected and confirmed by colony PCR and sequencing to verify the correct insertion, orientation, and in-frame fusion. The validated reporter plasmid was mixed with the co-injection marker plasmid pCFJ104, which expresses a red fluorescent protein specifically in body wall muscle cells to facilitate visual screening under a fluorescence microscope. The final injection mixture contained the reporter vector at 50 μ g/mL and pCFJ104 at 1 μ g/mL, diluted in sterile TE buffer or standard nematode injection buffer. This DNA mixture was microinjected into the gonad of young adult N2 hermaphrodites. Injected P0 animals were transferred to fresh NGM plates and maintained at 20 °C. After 3–4 days, F1 progeny were screened under a stereomicroscope equipped with fluorescence optics, and

individuals exhibiting both red fluorescence in the body wall muscle and broad green fluorescence were selected.

4.24 Statistical analysis

Statistical analyses and related plots were performed using the R software environment (version 4.4.2). Significance tests of all quantitative measurements in experimental groups were determined using two-sided unpaired Student's t-test. Differential gene expression analysis was performed through Wald test, following normalization of RNA-seq data. *P*-values were adjusted using the Bonferroni correction method. Statistical significance thresholds were defined as **P* < 0.05, ***P* < 0.01, and ****P* < 0.001, with corresponding asterisks denoting these significance levels in all graphical representations. Data was obtained from three biological replicates unless stated otherwise. Graphical data are presented as mean $\pm$ s.d.

Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1007/s13659-026-00590-7>.

Supplementary Material 1.
Supplementary Material 2.
Supplementary Material 3.
Supplementary Material 4.
Supplementary Material 5.
Supplementary Material 6.
Supplementary Material 7.
Supplementary Material 8.

Author Contributions

Qunfu Wu and Jiao Zhou conceived and designed the experiments; Jiao Zhou, Donglou Wang, Songhan Xue, Ling Li, Li Wu and Junxian Yan performed the experiments; Qunfu Wu and Jiao Zhou analyzed the data; Qunfu Wu and Jiao Zhou wrote the paper. All authors reviewed the results and approved the final version of the manuscript.

Funding

This work was sponsored by the Central Guidance Fund for Local Science and Technology Development (grant no. 202407AB110021), Projects 202201BF070001-012 from "Double tops" Program from Yunnan Province and Yunnan University, 202201AT070172 from Yunnan Province and Scientific Research Fund of Education Department of Yunnan Province and Graduate Research Innovation Fund of Yunnan University (KC-24249015).

Data availability

Our sequences files of transcriptome are accessible from the National Center for Biotechnology Information (NCBI) under BioProject accession: PRJNA1195634 (<https://www.ncbi.nlm.nih.gov/bioproject/PRJNA1195634>).

Declarations

Competing interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Author details

¹State Key Laboratory for Conservation and Utilization of Bio-Resources in Yunnan and Yunnan Key Laboratory of Basic Research and Innovative Application for Green Biological Production, Key Laboratory for Microbial Resources of the Ministry of Education, School of Life Sciences, Yunnan University, Kunming 650091, People's Republic of China.

Received: 26 September 2025 Accepted: 5 January 2026

Published online: 02 March 2026

References

- Theil EC, Goss DJ. Living with iron (and oxygen): questions and answers about iron homeostasis. *Chem Rev*. 2009;109:4568–79.
- Hirota K. An intimate crosstalk between iron homeostasis and oxygen metabolism regulated by the hypoxia-inducible factors (HIFs). *Free Radic Biol Med*. 2019;133:118–29.
- Zhang S, Xin W, Anderson GJ, Li R, Gao L, Chen S, et al. Double-edge sword roles of iron in driving energy production versus instigating ferroptosis. *Cell Death Dis*. 2022;13:40.
- Paul BT, Manz DH, Torti FM, Torti SV. Mitochondria and iron: current questions. *Expert Rev Hematol*. 2017;10:65–79.
- Shander A, Cappellini MD, Goodnough LT. Iron overload and toxicity: the hidden risk of multiple blood transfusions. *Vox Sang*. 2009;97:185–97.
- Gulec S, Anderson GJ, Collins JF. Mechanistic and regulatory aspects of intestinal iron absorption. *Am J Physiol Gastrointest Liver Physiol*. 2014;307:G397–409.
- Adams PC, Reboussin DM, Barton JC, McLaren CE, Eckfeldt JH, McLaren GD, et al. Hemochromatosis and iron-overload screening in a racially diverse population. *N Engl J Med*. 2005;352:1769–78.
- Motulsky AG, Beutler E. Population screening in hereditary hemochromatosis. *Annu Rev Public Health*. 2000;21:65–79.
- Bacon BR. Hemochromatosis: diagnosis and management. *Gastroenterology*. 2001;120:718–25.
- Pietrangelo A. Hereditary hemochromatosis—a new look at an old disease. *New Engl J Med*. 2004;350:2383–97.
- Kohgo Y, Ikuta K, Ohtake T, Torimoto Y, Kato J. Body iron metabolism and pathophysiology of iron overload. *Int J Hematol*. 2008;88:7–15.
- Entezari S, Haghi SM, Norouzkhani N, Sahebnaaz B, Vosoughian F, Akbarzadeh D, et al. Iron chelators in treatment of iron overload. *J Toxicol*. 2022;2022:4911205.
- Yang E, Xu L, Yang Y, Zhang X, Xiang M, Wang C, et al. Origin and evolution of carnivorous in the Ascomycota (fungi). *Proc Natl Acad Sci USA*. 2012;109:10960–5.
- Yang Y, Yang E, An Z, Liu X. Evolution of nematode-trapping cells of predatory fungi of the Orbiliaceae based on evidence from rRNA-encoding DNA and multiprotein sequences. *Proc Natl Acad Sci USA*. 2007;104:8379–84.
- van den Hoogen J, Geisen S, Routh D, Ferris H, Trautspurger W, Wardle DA, et al. Soil nematode abundance and functional group composition at a global scale. *Nature*. 2019;572:194–8.
- Dayi M. Evolution of parasitism genes in the plant parasitic nematodes. *Sci Rep*. 2024;14:3733.
- Zhang Y, Li S, Li H, Wang R, Zhang KQ, Xu J. Fungi–nematode interactions: diversity, ecology, and biocontrol prospects in agriculture. *J Fungi*. 2020;6:206.
- Kozia ATE, Cheng KC, Thorn RG. Phylogenetic analyses of Nematoconus and Hohenbuehelia (Pleurotaceae). *Can J Bot*. 2007;85:762–73.
- Zhou J, Wang DL, Wu QF, Jiang Y, Yan JX, Wu L, et al. Rare NRPS gene cluster for desferriochromate biosynthesis controls the conflict between trap formation and nematocidal activity in *Arthrobotrys oligospora*. *J Agric Food Chem*. 2024;72:3560–71.
- Zhou J, Wu Q, Wu L, Yan J, Cheng Q, Wang D, et al. Trapping devices of carnivorous fungus *Arthrobotrys oligospora* can isolate heat-triggered excess irons. *bioRxiv*. 2024. <https://doi.org/10.1101/2024.07.25.605209>.
- Manicki M, Aydin H, Abriata LA, Overmyer KA, Guerra RM, Coon JJ, et al. Structure and functionality of a multimeric human COQ7:COQ9 complex. *Mol Cell*. 2022;82:4307–23.
- Jonassen T, Proft M, Randez-Gil F, Schultz JR, Marbois BN, Entian KD, et al. Yeast CLK-1 homologue (COQ7/CAT5) is a mitochondrial protein in coenzyme Q synthesis. *J Biol Chem*. 1998;273:3351–7.
- Andrews SC. The ferritin-like superfamily: evolution of the biological iron storeman from a rubrerythrin-like ancestor. *Biochim Biophys Acta Gen Subj*. 2010;1800:691–705.
- Behan RK, Lippard SJ. The aging-associated enzyme CLK-1 is a member of the carboxylate-bridged diiron family of proteins. *Biochemistry*. 2010;49:9679–81.
- Pierrel F, Burgardt A, Lee JH, Pelosi L, Wendisch VF. Recent advances in the metabolic pathways and microbial production of coenzyme Q. *World J Microbiol Biotechnol*. 2022;38:58.
- Wang Y, Lilienfeldt N, Hekimi S. Understanding coenzyme Q. *Physiol Rev*. 2024;104:1533–610.
- Lenaz G, Fato R, Formiggini G, Genova ML. The role of coenzyme Q in mitochondrial electron transport. *Mitochondrion*. 2007;7:S8–33.
- Kawamukai M. Biosynthesis of coenzyme Q in eukaryotes. *Biosci Biotechnol Biochem*. 2016;80:23–33.
- Hoppe U, Bergemann J, Diembeck W, Ennen J, Gohla S, Harris I, et al. Coenzyme Q10, a cutaneous antioxidant and energizer. *BioFactors*. 1999;9:371–8.
- Fox BW, Ponomarova O, Lee YU, Zhang G, Giese GE, Walker M, et al. *C. elegans* as a model for inter-individual variation in metabolism. *Nature*. 2022;607:571–7.
- Saxena G, Dayal R, Mukerji KG. Interaction of nematodes with nematophagous fungi: induction of trap formation, attraction and detection of attractants. *FEMS Microbiol Lett*. 1987;45:319–27.
- Youssar L, Wernet V, Hensel N, Yu X, Hildebrand H-G, Schreckenberger B, et al. Intercellular communication is required for trap formation in the nematode-trapping fungus *Duddingtonia flagrans*. *PLoS Genet*. 2019;15:e1008029.
- Bansal A, Zhu LJ, Yen K, Tissenbaum HA. Uncoupling lifespan and healthspan in *Caenorhabditis elegans* longevity mutants. *Proc Natl Acad Sci USA*. 2015;112:E277–86.
- Zhang SQ, Box AC, Xu N, Le Men J, Yu J, Guo F, et al. Genetic and dietary regulation of lipid droplet expansion in *Caenorhabditis elegans*. *Proc Natl Acad Sci USA*. 2010;107:4640–5.
- Lu TT, Lee SJ, Apfel UP, Lippard SJ. Aging-associated enzyme human clock-1: substrate-mediated reduction of the diiron center for 5-demethoxyubiquinone hydroxylation. *Biochemistry*. 2013;52:2236–44.
- Heyman B, Lamm R, Tulke H, Regestein L, Büchs J. Shake flask methodology for assessing the influence of the maximum oxygen transfer capacity on 2,3-butanediol production. *Microb Cell Fact*. 2019;18:78.
- Klößner W, Büchs J. Advances in shaking technologies. *Trends Biotechnol*. 2012;30:307–14.
- He ZQ, Wang LJ, Wang YJ, Chen YH, Wen Y, Zhang KQ, et al. Polyketide synthase–terpenoid synthase hybrid pathway regulation of trap formation through ammonia metabolism controls soil colonization of predominant nematode-trapping fungus. *J Agric Food Chem*. 2021;69:4464–79.
- Pretzner B, Maschke RW, Haiderer C, John GT, Herwig C, Sykacek P. Predictive monitoring of shake flask cultures with online estimated growth models. *Bioengineering*. 2021;8:177.
- Xie H, Huang M, Zheng Y, Wu Q, Dai G, Wang D, et al. Transcription levels of SDR gene *AO274* in nematode-trapping fungus *Arthrobotrys oligospora* regulates configurations and oxidation patterns of arthrobotrisins and formation of trapping devices. *J Agric Food Chem*. 2025;73:5947–60.
- Teng LL, Song TY, Xu ZF, Liu X, Dai R, Chen YH, et al. Selected mutations revealed intermediates and key precursors in the biosynthesis of polyketide–terpenoid hybrid sesquiterpenyl epoxy-cyclohexenoids. *Org Lett*. 2017;19:3923–6.
- Lee CS, Song J. Migrating cells dispose of damaged mitochondria into the surrounding environment: mitochondria quality control coupled with cell migration in mammalian in vivo model. *Mol Cells*. 2021;44:781–3.
- Liu Y, Li S, Rong W, Zeng C, Zhu X, Chen Q, et al. Podocyte-released migrasomes in urine serve as an indicator for early podocyte injury. *Kidney Dis*. 2020;6:422–33.
- Zhang Y, Guo W, Bi M, Liu W, Zhou L, Liu H, et al. Migrasomes: from biogenesis, release, uptake, rupture to homeostasis and diseases. *Oxid Med Cell Longev*. 2022;2022:4525778.
- Hansen JM, Jones DP, Harris C. The redox theory of development. *Antioxid Redox Signal*. 2019;32:715–40.

46. Dunwoodie SL. The role of hypoxia in development of the mammalian embryo. *Dev Cell*. 2009;17:755–73.
47. Teng LL, Song TY, Chen YH, Chen YG, Zhang KQ, Li SH, et al. Novel polyketide-terpenoid hybrid metabolites from a potent nematocidal *Arthrobotrys oligospora* mutant $\Delta AOL_s00215g278$. *J Agric Food Chem*. 2020;68:11449–58.
48. He ZQ, Tan JL, Li N, Zhang HX, Chen YH, Wang LJ, et al. Sesquiterpene epoxy-cyclohexenoids and their signaling functions in nematode-trapping fungus *Arthrobotrys oligospora*. *J Agric Food Chem*. 2019;67:13061–72.
49. Song TY, Xu ZF, Chen YH, Ding QY, Sun YR, Miao Y, et al. Potent nematocidal activity and new hybrid metabolite production by disruption of a cytochrome P450 gene involved in the biosynthesis of morphological regulatory arthrospores in nematode-trapping fungus *Arthrobotrys oligospora*. *J Agric Food Chem*. 2017;65:4111–20.
50. Xu ZF, Chen YH, Song TY, Zeng ZJ, Yan N, Zhang KQ, et al. Nematocidal key precursors for the biosynthesis of morphological regulatory arthrospores in the nematode-trapping fungus *Arthrobotrys oligospora*. *J Agric Food Chem*. 2016;64:7949–56.
51. Alexander K, Young IG. Three hydroxylations incorporating molecular oxygen in the aerobic biosynthesis of ubiquinone in *Escherichia coli*. *Biochemistry*. 1978;17:4745–50.
52. Alva R, Gardner GL, Liang P, Stuart JA. Supraphysiological oxygen levels in mammalian cell culture: current state and future perspectives. *Cells*. 2022;11:3123.
53. Canfield DE. The early history of atmospheric oxygen: homage to Robert M. Garrels. *Annu Rev Earth Planet Sci*. 2005;33:1–36.
54. Ostrander CM, Heard AW, Shu Y, Bekker A, Poulton SW, Olesen KP, et al. Onset of coupled atmosphere–ocean oxygenation 2.3 billion years ago. *Nature*. 2024;631:335–9.
55. Mills BJW, Krause AJ, Jarvis I, Cramer BD. Evolution of atmospheric O₂ through the Phanerozoic, revisited. *Annu Rev Earth Planet Sci*. 2023;51:253–76.
56. Scotese CR, Song H, Mills BJW, van der Meer DG. Phanerozoic paleotemperatures: the earth's changing climate during the last 540 million years. *Earth-Sci Rev*. 2021;215:103503.
57. Van Houten FB, Arthur MA. Temporal patterns among Phanerozoic oolitic ironstones and oceanic anoxia. *Geol Soc Lond Spec Publ*. 1989;46:33–49.
58. Belder Ed, Jansen E. The influence of temperature, nutrition, light and the growth time of the mycelium on capture and infection of *Meloidogyne hapla* by *Arthrobotrys oligospora*. *Fundam Appl Nematol*. 1994;17:57–66.
59. Větrovský T, Morais D, Kohout P, Lepinay C, Algora C, Awokunle Hollá S, et al. Globalfungi: a global database of fungal occurrences from high-throughput-sequencing metabarcoding studies. *Sci Data*. 2020;7:228.
60. Rahim NG, Harismendy O, Topol EJ, Frazer KA. Genetic determinants of phenotypic diversity in humans. *Genome Biol*. 2008;9:215.
61. Exposito-Alonso M, Booker TR, Czech L, Gillespie L, Hateley S, Kyriazis CC, et al. Genetic diversity loss in the Anthropocene. *Science*. 2022;377:1431–5.
62. Veenhuis M, Van Wijk C, Wyss U, Nordbring-Hertz B, Harder W. Significance of electron dense microbodies in trap cells of the nematophagous fungus *Arthrobotrys oligospora*. *Antonie Van Leeuwenhoek*. 1989;56:251–61.
63. Aghabi D, Sloan M, Gill G, Hartmann E, Antipova O, Dou Z, et al. The vacuolar iron transporter mediates iron detoxification in *Toxoplasma gondii*. *Nat Commun*. 2023;14:3659.
64. Kosman DJ. Molecular mechanisms of iron uptake in fungi. *Mol Microbiol*. 2003;47:1185–97.
65. Gurung K, Field KJ, Batterman SA, Goddérís Y, Donnadiou Y, Porada P, et al. Climate windows of opportunity for plant expansion during the Phanerozoic. *Nat Commun*. 2022;13:4530.
66. Miyadera H, Amino H, Hiraishi A, Taka H, Murayama K, Miyoshi H, et al. Altered quinone biosynthesis in the long-lived *clk-1* mutants of *Caenorhabditis elegans*. *J Biol Chem*. 2001;276:7713–6.
67. Rao AU, Carta LK, Lesuisse E, Hamza I. Lack of heme synthesis in a free-living eukaryote. *Proc Natl Acad Sci USA*. 2005;102:4270–5.
68. Wu B, Novelli J, Jiang D, Dailey HA, Landmann F, Ford L, et al. Interdomain lateral gene transfer of an essential ferrochelatase gene in human parasitic nematodes. *Proc Natl Acad Sci USA*. 2013;110:7748–53.
69. Hu M, Ma Y, Chua SL. Bacterivorous nematodes decipher microbial iron siderophores as prey cue in predator–prey interactions. *Proc Natl Acad Sci USA*. 2024;121:e2314077121.
70. Yu X, Hu X, Pop M, Wernet N, Kirschhöfer F, Brenner-Weiß G, et al. Fatal attraction of *Caenorhabditis elegans* to predatory fungi through 6-methylsalicylic acid. *Nat Commun*. 2021;12:5462.
71. Jiang M, Wu Z, Liu L, Chen S. The chemistry and biology of fungal meroterpenoids (2009–2019). *Org Biomol Chem*. 2021;19:1644–704.
72. Geris R, Simpson TJ. Meroterpenoids produced by fungi. *Nat Prod Rep*. 2009;26:1063–94.
73. Salimi Z, Afsharinasab M, Rostami M, Eshaghi Milasi Y, Mousavi Ezmareh SF, Sakhaei F, et al. Iron chelators: as therapeutic agents in diseases. *Ann Med Surg*. 2024;86:2759–76.
74. Emms DM, Kelly S. OrthoFinder: phylogenetic orthology inference for comparative genomics. *Genome Biol*. 2019;20:238.
75. Csűös M. Count: evolutionary analysis of phylogenetic profiles with parsimony and likelihood. *Bioinformatics*. 2010;26:1910–2.
76. Simão FA, Waterhouse RM, Ioannidis P, Kriventseva EV, Zdobnov EM. BUSCO: assessing genome assembly and annotation completeness with single-copy orthologs. *Bioinformatics*. 2015;31:3210–2.
77. Chen S, Zhou Y, Chen Y, Gu J. fastp: an ultra-fast all-in-one FASTQ preprocessor. *Bioinformatics*. 2018;34:i884–90.
78. Kim D, Paggi JM, Park C, Bennett C, Salzberg SL. Graph-based genome alignment and genotyping with HISAT2 and HISAT-genotype. *Nat Biotechnol*. 2019;37:907–15.
79. Blin K, Shaw S, Augustijn HE, Reitz ZL, Biermann F, Alanjary M, et al. antiSMASH 7.0: new and improved predictions for detection, regulation, chemical structures and visualisation. *Nucleic Acids Res*. 2023;51:W46–50.
80. Gilchrist CLM, Booth TJ, van Wersch B, van Grieken L, Medema MH, Chooi Y-H. cblaster: a remote search tool for rapid identification and visualization of homologous gene clusters. *Bioinformatics Adv*. 2021;1:vbab016.
81. Suyama M, Torrents D, Bork P. PAL2NAL: robust conversion of protein sequence alignments into the corresponding codon alignments. *Nucleic Acids Res*. 2006;34:W609–12.
82. Minh BQ, Schmidt HA, Chernomor O, Schrempf D, Woodhams MD, von Haeseler A, et al. IQ-TREE 2: new models and efficient methods for phylogenetic inference in the genomic era. *Mol Biol Evol*. 2020;37:1530–4.
83. Törönen P, Medlar A, Holm L. PANNZER2: a rapid functional annotation web server. *Nucleic Acids Res*. 2018;46:W84–8.
84. Yang Z. PAML 4: phylogenetic analysis by maximum likelihood. *Mol Biol Evol*. 2007;24:1586–91.
85. Schmidt AR, Dörfelt H, Perrichot V. *Palaeoanellus dimorphus* gen. et sp. nov. (Deuteromycotina): a Cretaceous predatory fungus. *Am J Bot*. 2008;95:1328–34.
86. Berney C, Pawłowski J. A molecular time-scale for eukaryote evolution recalibrated with the continuous microfossil record. *Proc R Soc Lond B Biol Sci*. 2006;273:1867–72.
87. Beimeforde C, Feldberg K, Nylander S, Rikkinen J, Tuovila H, Dörfelt H, et al. Estimating the Phanerozoic history of the Ascomycota lineages: combining fossil and molecular data. *Mol Phylogenet Evol*. 2014;78:386–98.
88. Shen XX, Steenwyk JL, LaBella AL, Opulente DA, Zhou X, Kominek J, et al. Genome-scale phylogeny and contrasting modes of genome evolution in the fungal phylum Ascomycota. *Sci Adv*. 2020;6:eabd0079.
89. Rambaut A, Drummond AJ, Xie D, Baele G, Suchard MA. Posterior summarization in Bayesian phylogenetics using Tracer 1.7. *Syst Biol*. 2018;67:901–4.

Publisher's Note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.