INTERNATIONAL SOCIETY FOR MEDICINAL MUSHROOMS

国际药用菌学会

International Society for Medicinal Mushrooms （ISMM）was founded in Vancouver, Canada. As a global non-profit organization, ISMM promotes the development of research, education, production, transportation, marketing and cultivation of medicinal mushrooms to have people to work towards common aspirations and goals. The integration will increase the impact of the international medicinal mushroom industry and benefit the health of people in the world.

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国际药用菌学会（International Society for Medicinal Mushrooms），简称ISMM，在加拿大温哥华注册成立，由从事药用菌产业的科研、教学、生产、流通、市场、文化及相关产业链的单位、团体和个人自愿组成的为实现共同意愿的非营利性国际组织。本学会致力于促进国际药用菌产业各个领域的融合与发展，以提升药用菌行业在全球的影响力，造福人类健康。

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NEWSLETTER OF THE INTERNATIONAL SOCIETY FOR MEDICINAL MUSHROOMS

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News Reports

SpaceX astronauts aim to grow edible mushrooms in space on Fram2 mission

By Jano Gibson

A small box containing the material to grow mushrooms and the root-like structure of fungus will be stowed on board a SpaceX Crew Dragon capsule. (Reuters: NASA)

In short:

Scientists are trying to identify the best crops and farming techniques for future space travel to the Moon and Mars.

They believe mushrooms could be the perfect crop as they can be grown rapidly in confined spaces and provide many health and culinary benefits.

What's next?

Astronauts will try to grow oyster mushrooms in space for the first time when SpaceX's Fram2 mission launches next week.

When SpaceX launches its next mission in the coming days, it will include an Australian-led experiment that could have significant ramifications for the future of space exploration.

The Fram2 mission, scheduled to blast off from Cape Canaveral in Florida next Monday local time, is already aiming to make history by becoming the first human space flight to orbit the Earth's polar regions.

But the four-person team of amateur astronauts, including Australian Eric Philips, will also be attempting another novel achievement.

"We're attempting to 'fruit' mushrooms in space for the very first time," Dr Flavia Fayet-Moore said.

The nutrition scientist, who heads Australian company FoodiQ Global, submitted a successful expression-of-interest to undertake the project on the upcoming mission.

Under the plan, a small box containing substrate — the material used to grow mushrooms — and mycelium — the root-like structure of fungus — will be stowed on board SpaceX's Dragon capsule.

Dr Flavia Fayet-Moore says the aim is to create a "sustainable, nutiritious and delicious food supply" for exploration missions. (Supplied: FoodiQ Global)

Part-way through the four-day mission, the box will be inspected to see whether the tiny "pins" of fungus have fruited into oyster mushrooms while flying 450 kilometres above the Earth.

"I will monitor how the fruiting bodies grow, documenting development rate, signs of contamination, and various other properties," said Mr Phillips, who is an experienced polar explorer.

Mushroom substrate block, which will be sent to space aboard SpaceX Dragon. (Supplied: FoodiQ Global)

"As an advocate for exploration, this is an exciting opportunity to push the boundaries and play a role in creating sustainable food solutions for space — something I never imagined I would explore".

Once the team returns to Earth, the oyster mushrooms will be sent to a lab to assess the effects of space on their growth, biochemistry, genetics and nutritional value.

The information gleaned from "Mission MushVroom" will add to previous experiments that focused on the medical and material benefits of fungi in space.

"The reason why we're researching this is to really create a sustainable, nutritious and delicious food supply for the exploration missions to the Moon and Mars,"

Dr Fayet-Moore said.

The Fram2 mission will also involve about 20 other experiments, including:

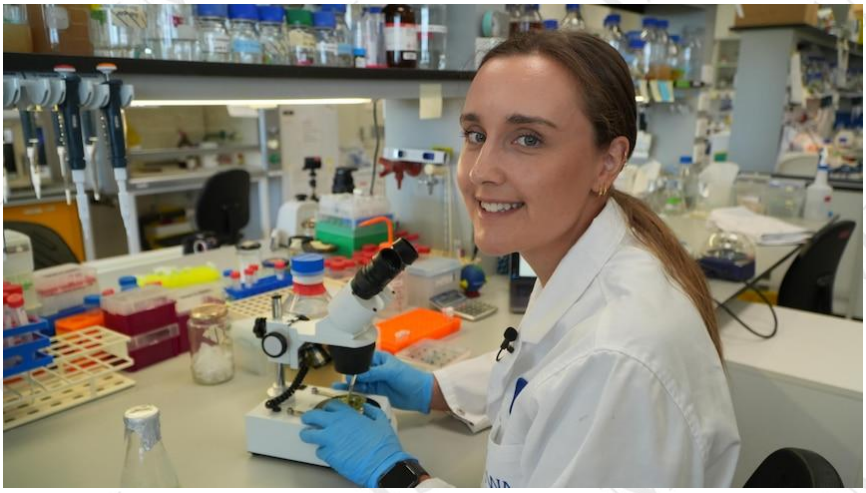
- the first X-rays of humans in space
- performing exercise studies to maintain muscle and skeletal mass
- exiting the Dragon capsule without assistance after returning to Earth
- using a mobile MRI device to assess how spaceflight impacts brain anatomy
- analysing how female reproductive hormones are impacted by microgravity and radiation

'The perfect space crop'

Future space travel will require crops that can be grown in confined spaces, harvested quickly and provide nutritional and culinary benefits to astronauts.

Redesigning plants to allow astronauts to grow food in space

Photo shows Plants for Space researcher Leni Campbell-Clause turns away from the microscoope to look at the camera.



When astronauts are orbiting earth, it's often not chocolate they crave but a fresh, crunchy salad. That's largely been a pipe dream, but a team of scientists is now working to re-design plants to enable that to happen.

"In space, NASA is only prioritising crops that you can literally pick and eat because we don't have the capabilities to process food in microgravity yet," Dr Fayet-Moore said.

Mushrooms have the potential to meet those requirements, she said.

"They are the perfect space crop.

"Many plants take over 100 days to get [to] harvest, whereas mushrooms can have an end-to-end cycle of only 30 days.

"And they can supply the astronauts with that food, but uniquely, what's really exciting from a nutrition perspective,

is that they have nutrients found across the food groups."

Dr Flavia Fayet-Moore says mushrooms are the "perfect space crop". (Supplied: NASA/Kjell Lindgren)

The oyster mushroom experiment on Fram2 is part of a global initiative examining the best way to support long-term human exploration in space.

"Mushrooms are a really critical part of thinking about that whole circular system of how we feed and support people long term," Jenny Mortimer, a professor at the University of Adelaide, said.

Duckweed sent to space

Photo shows A net in a body of water with a hand above it holding little pieces of an aquatic plant.

Duckweed is an abundant plant in many parts of the world and researchers are testing if it can be grown in space.

To the Moon and beyond

Professor Mortimer is also a chief investigator at the Australian Research Council's Centre of Excellence in Plants for Space.

Her team is involved in a separate experiment that will include several plant species being sent to the Moon during NASA's Artemis III mission, which is scheduled to launch in 2027.

The mission involves a plan for astronauts to spend a week on the Moon's surface to conduct scientific experiments.

In one of those projects, duckweed and two other plants will be cultivated in a specially-designed growth chamber on the Moon.

Samples will then be returned to Earth to analyse how the "hostile" space environment affects them.

Professor Jenny Mortimer and her team are involved in a separate experiment that will include several plant species being sent to the Moon in 2027. (Supplied)

"There's radiation, there's the lunar gravity, there's lots of things we can't really replicate very easily here on Earth to understand how they grow," Professor Mortimer said.

She said identifying the best crops and farming techniques for space travel was critical for extended, self-sufficient trips to places such as Mars, which could take several years to complete.

"It's really hard to plan for everything you need on these types of missions," she said.

"So you need some ability to make the things that you might need."

The lessons learned from the experiments on Fram2 and Artemis III could also lead to improved farming techniques on Earth, she said.

Source: www.abc.net.au

Medicinal Mushrooms Show Promise for Combating Insulin Resistance

By Angelika Erdélyi

Edible mushrooms could be used as a natural intervention to regulate blood sugar and improve metabolic health, a new study from Semmelweis University suggests. With global rates of type 2 diabetes (T2DM) on the rise, the researchers shed light on how bioactive compounds in medicinal mushrooms, such as polysaccharides and terpenoids, may help prevent or alleviate insulin resistance (IR).

Common mushrooms like white button (*Agaricus bisporus*), shiitake (*Lentinula edodes*), and oyster (*Pleurotus ostreatus*), now widely consumed as part of the Western diet, could serve as preventative or complementary treatments for managing insulin resistance, according to a comprehensive review recently published in the *International Journal of Molecular Sciences*.

IR occurs when the body's cells fail to respond properly to insulin, causing glucose to build up in the bloodstream. Over time, this can lead to T2DM, a condition that affects an estimated 830 million people worldwide, as data from the World Health Organization shows.

The bioactive components in mushrooms include polysaccharides, terpenoids, phenolic compounds, and bioactive proteins, along with essential vitamins and minerals. These compounds interact with key metabolic processes to promote better insulin sensitivity and overall metabolic health.

For example, indigestible polysaccharides, a major fibre component of mushrooms, contribute to metabolic balance by feeding beneficial gut bacteria. These bacteria support nutrient absorption, strengthen the intestinal barrier, and lower chronic inflammation – an important factor in insulin resistance. Additionally,

polysaccharides reduce sugar and fat absorption, which aids in weight management, crucial for both preventing and managing diabetes.

“Recent research has highlighted the significant impact of gut health on conditions like obesity and type 2 diabetes,” explains Dr Zsuzsanna Németh, a biologist at Semmelweis University’s Department of Internal Medicine and Oncology and the study’s lead author.

By nurturing gut bacteria through diet, we can improve insulin sensitivity and overall metabolic health.

Other ways of how bioactive components in mushrooms could improve IR:

- **Modulating glucose absorption:** Mushroom compounds regulate intestinal enzymes, slowing the rate at which glucose enters the bloodstream, thereby preventing sharp blood sugar spikes.
- **Enhancing glucose uptake:** By improving insulin signaling pathways, bioactive components help transport glucose more efficiently into muscle and fat cells.
- **Balancing insulin production:** Mushrooms can stimulate insulin secretion by pancreatic β-cells and protect them from cell death by increasing the expression of the GLP-1 hormone, helping to maintain healthy blood sugar levels.
- **Optimising lipid metabolism:** By promoting the use of free fatty acids as an energy source, mushrooms reduce harmful fat accumulation and support healthier muscle function.
- **Improving adipose tissue function:** Pathologically enlarged fat tissue produces pro-inflammatory substances, contributing to insulin resistance. Medicinal mushrooms may help restore optimal fat storage and release while promoting the production of anti-inflammatory adiponectin, thereby improving metabolic balance.

“Our study reveals the remarkable potential of natural compounds in mushrooms to address key metabolic pathways,” adds Dr Németh. “As interest grows in non-pharmacological approaches to disease prevention, this opens up exciting possibilities for using edible medicinal mushrooms as complementary agents in diabetes management.”

However, Dr. Németh advises that in cases of illness, supplemental mushrooms should be consumed as part of a balanced diet and under the guidance of healthcare professionals.

Edible mushrooms have been part of diets worldwide and used in medicine for thousands of years, particularly in Asia. Extracts from these mushrooms exhibit numerous health benefits, including anti-diabetic, anti-inflammatory, cardioprotective, and antioxidant properties. They are also known for their high tolerability among cancer patients undergoing treatment. The Semmelweis study highlights the importance of the natural habitat and growing conditions to maximise the nutrient content and minimise environmental contamination in medicinal mushrooms.

Photo: Balint Barta – Semmelweis University; Cover photo (illustration): Envato Elements – Olena Rudo

Source: https://semmelweis.hu/

Up-coming Events

Russian Mushroom Days 2025 in Moscow

Russian Mushroom Days is the main annual event for Russian mushroom growers. In terms of importance, it is comparable to the Olympic Games for athletes.

In 2025, the Moscow Olympics - 1980 will mark 45 years.

And, at the same time, the main event of 2025 of the Russian Mushroom Industry will take place on May 13-14 at the capital's Radisson Blu Olympiyskiy Hotel near the renovated Olympic sports complex, which is scheduled to open at the end of April 2025.

Venue

Radisson Blu Olympiyskiy Hotel will be glad to welcome participants of Russian Mushroom days 2025!

Location: Moscow, Samarskaya street, 1

Venue of Russian Mushroom Days 2025 - Radisson Blu Olympiyskiy Hotel

Conference

Speakers annually include specialists and experts from various areas of the mushroom community: mushroom and mushroom product producers, sellers and buyers, processors and chefs, doctors and nutritionists. Learn about the latest achievements first-hand!

Topics

- Mushroom industry of Russia
- Management in mushroom growing
- Marketing in mushroom growing
- Research and development of new technologies
- Nutritional and medicinal properties of cultivated mushrooms

The sections will feature stars in the fields of management, marketing, engineering, production technologies, dietetics, sports nutrition, processing and preparation of mushrooms. Specialists from Russian and foreign mushroom companies, scientists and start-ups.

Exhibition

The exhibition traditionally features industry leaders, those who have something to show and tell! Teams of producers of mushrooms and mushroom products, equipment, compost, mycelium, casing soil, nutritional supplements, packaging materials.

Contact

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Dutch Mushroom Days

Block the calendar

The Board of the Mushroomdays Foundation is pleased to inform you that the date of the next edition of the Mushroom Days has been set for **April 22-24, 2026**. The event will again take place in the **Brabanthallen** in **'s-Hertogenbosch**. This meets the preference of the exhibitors for a frequency of "*every 3 years*". Also, for the same reason, the Mushroomdays Foundation has placed an option with the Brabanthallen for an edition on June 13-15, 2029.

After a very successful edition in 2023, there is no reason for the Mushroom Days Committee to opt for a substantially different format for the event, but (as always) to look for further optimization on a detailed level.

The Mushroom Days Committee plans to send out the first mailing for participation and registration in the 2nd quarter of 2025. We are very much looking forward to welcoming you all again in order to shape together this great global trade fair. We will keep you informed via our website www.mushroomdays.com.

Kind regards,
Piet Lempens
Chairman Mushroomdays Foundation.

Source: https://champignondagen.nl/home-eng/

First Announcement of the 11th International Conference on Mushroom Biology and Mushroom Products (ICMBMP11）

The 11th International Conference on Mushroom Biology and Mushroom Products

Oct. 13-17, 2026

Accra, Ghana.

We are delighted to announce that the 11th International Conference on Mushroom Biology and Mushroom Products (ICMBMP 11) will be held from 13th to 17th October 2026, in Accra, Ghana. This prestigious conference, organized once every four years, serves as a dedicated platform to showcase the myriad benefits of mushrooms and their contributions to global health.

The 11th ICMBMP aims to provide an opportunity for researchers, business professionals, mushroom practitioners, and enthusiasts to converge and share the latest research findings on mushrooms. It serves as a vital platform to discuss how mushrooms can positively impact sustainable food systems, human health, and environmental sustainability. Through various planned activities, participants will gain insights into cutting-edge research, innovative mushroom-based products, and their role in addressing global challenges.

Conference Highlights:

Dates: 13th – 17th October 2026
Venue: Accra, Ghana
Format: In-person

Registration for the conference will open shortly. Please stay tuned for further updates and instructions on how to register.

For any inquiries or further information, please contact the secretariat of the 11th ICMBMP 2026 at: Tel: +233-207930703; Email: icmbmp11@foodresearchgh.org

We look forward to welcoming you to ICMBMP 11, where we can collectively explore the vast potential of mushrooms in nutrition, medicine, and sustainable development.

Best regards,

Secretariat of the 11th ICMBMP 2026

Research progress

Dectin-1-Dependent Activation of Flt3 Ligand-Induced Dendritic Cells by the Caterpillar Medicinal Mushroom *Cordyceps militaris* (Ascomycetes) Fruiting Body

Takashi Kanno [a], Rui Tada [a], Toyokazu Nakasone [b], Shigemi Okamatsu [b], Yoichiro Iwakura [c], Kazuhiro Tamura [d], Hiroaki Miyaoka [e], Yoshiyuki Adachi [a]

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[e] *Department of Biomolecular Organic Chemistry, School of Pharmacy, Tokyo University of Pharmacy and Life Sciences, Tokyo, Japan*

Abstract: *Cordyceps militaris*, an entomopathogenic fungus traditionally used in East Asian medicine, contains 1,3-β-glucans with well-known immunomodulatory properties. Our previous research has demonstrated that both fruit body powder and hot water extract of *C. militaris* can activate bone marrow-derived dendritic cells through dectin-1 signaling. However, the immunological effects on Fms-like tyrosine kinase 3 ligand-induced dendritic cells (fDCs), which closely resemble steady-state conventional dendritic cells *in vivo*, remain unexplored. In this study, we investigated the expression of dectin-1 in fDCs and its response to *C. militaris* fruit body powder (RK). Flow cytometric analysis revealed that conventional and plasmacytoid dendritic cells within the fDC population expressed dectin-1, with conventional dendritic cells showing particularly robust expression. Similar expression patterns were observed in freshly isolated splenic DCs. Importantly, RK induced significant tumor necrosis factor-α production in wild-type fDCs, whereas this effect was completely abolished in dectin-1-knockout fDCs. These findings demonstrate that *C. militaris* fruit-body powder activates fDCs through a dectin-1-dependent pathway, providing new insights into its immunomodulatory mechanisms and potential therapeutic applications.

Keywords: Beta-glucan, *Cordyceps militaris*, dectin-1, Flt3, dendritic cells, fruit body, medicinal mushrooms

International Journal of Medicinal Mushrooms, Volume 27, Issue 6, 2025, pp. 13-22

DOI: 10.1615/IntJMedMushrooms.2025058213

Cultivation and utilization of edible mushrooms: From extraction of active components to effective substrate utilization

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Abstract: Edible mushrooms are a group of fungi that hold significant nutritional and economic value, widely distributed across the globe. Mushrooms are valuable not only for their direct uses but also for the extraction of bioactive compounds such as polysaccharides, proteins, and secondary metabolites, which possess antioxidant, antimicrobial, and anticancer properties, making them important in pharmaceutical and nutraceutical applications. Additionally, the by-products of mushroom cultivation, such as spent mushroom substrate and mushroom waste, can be repurposed for various sustainable uses, including animal feed, organic fertilizers, and even as substrates in bioremediation, contributing to waste reduction and resource efficiency in food and agricultural systems. This review focuses on mushroom cultivation, extraction of bioactive compounds, and the sustainable use of cultivation by-products. It also aims to offer insights into future studies, including strategies for optimizing the extraction of functional compounds and promoting the recycling of mushroom waste and cultivation substrates, thereby enhancing their applications in food, agriculture, and environmental sustainability.

Graphical Abstract

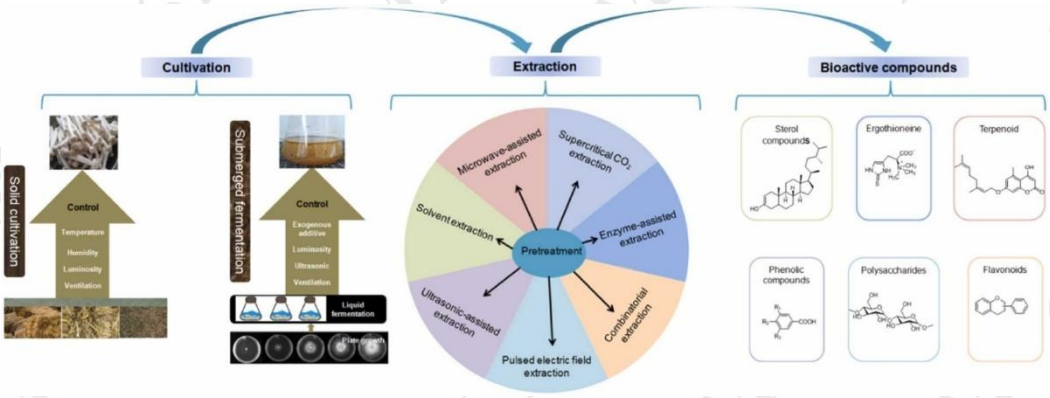

Figure Review of mushroom cultivation, extraction and bioactive components

Journal of Food Composition and Analysis, Volume 140, April 2025, 107224

https://doi.org/10.1016/j.jfca.2025.107224

Decoding the difference of four species of Cordyceps based on polysaccharides and immunomodulation activity

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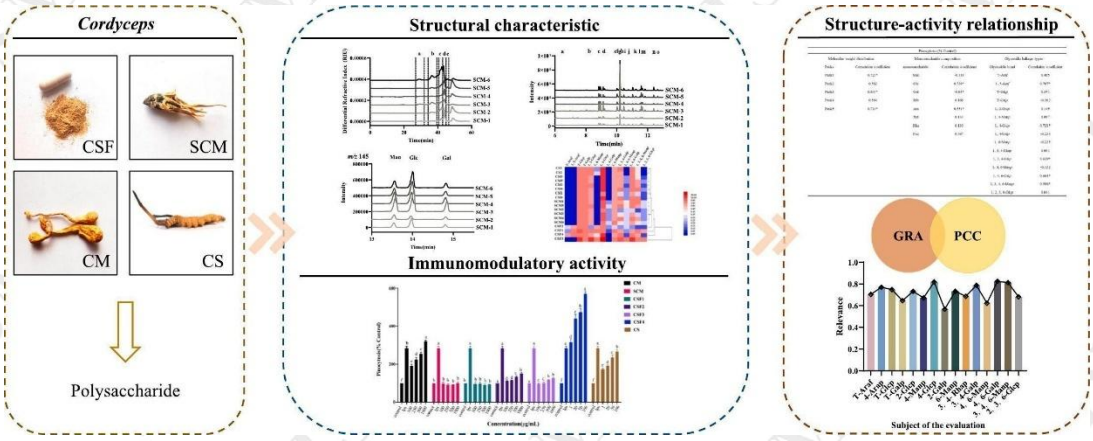
Abstract: Nucleosides and polysaccharides are the main bioactive ingredients of *Cordyceps* genus. Nucleosides shows significant differences in different *Cordyceps* species. However, the differences of polysaccharides have not been decoded. Here, the structure characters of polysaccharides including molecular weight (Mw) distribution, compositional monosaccharides and glycosidic linkage types were compared in *C. sinensis* (CS)*, C. militaris* (CM)*,* silkworm-hosted *C. militaris* (SCM) and *Cordyceps* fermented products (CSF). Compositional monosaccharides including mannose, glucose and galactose, and 1,4-Glc*p* glycosidic linkage were found abundant in *Cordyceps* species. Chemometric analysis showed that *Cordyceps* exhibit significant differences in structural information especially glycosidic linkage types. Besides, polysaccharides in CS and CSF-4 had obviously strong capacity of stimulating phagocytic, NO production and cytokines secretion. Gray relational analysis and Pearson correlation analysis were performed to further investigate the relationship between key polysaccharide structure and immunomodulatory activities. The results indicated that polysaccharides with relatively large number of 1, 4-Glc*p* and Mw in range of 7.16×10^6 Da-7.99×10^7 Da and 1.43×10^4 D-6.94×10^5 Da probably contributed to its immunomodulatory activities. The chemical and biological evaluation of natural and various cultured *cordyceps* in this study is useful for understanding and regulating the quality of cultured *Cordyceps.*

Graphical abstract



International Journal of Biological Macromolecules, Volume 294, March 2025, 139424

https://doi.org/10.1016/j.ijbiomac.2024.139424

Unlocking the potential of edible mushroom proteins: A sustainable future in food and health

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Abstract: Drawing protein from animals, plants, and microorganisms to build a diversified food supply system meets people's needs for food variety, nutrition, and health. It also reflects the comprehensiveness, diversity, and sustainability

of agricultural development. With the growing interest in the development and utilisation of new protein resources, edible mushroom proteins have attracted widespread attention. Edible mushroom proteins are nutritionally rich, possess various bioactivities and functionalities, and are produced with higher efficiency and are healthier compared to animal and plant proteins. At present, edible mushroom proteins hold great potential for application in various fields, including food, medicine and biological control. This article discusses the research progress in the development and utilisation of edible mushroom proteins, covering their composition, nutritional value, extraction and detection methods, functionalities, applications, and provides prospects for future development directions. The aim is to provide a reference for further exploration and utilisation of edible mushroom proteins.

Food Chemistry, Volume 481, 30 July 2025, 144026

https://doi.org/10.1016/j.foodchem.2025.144026

Approaches and challenges for a sustainable low-carbon mushroom industry

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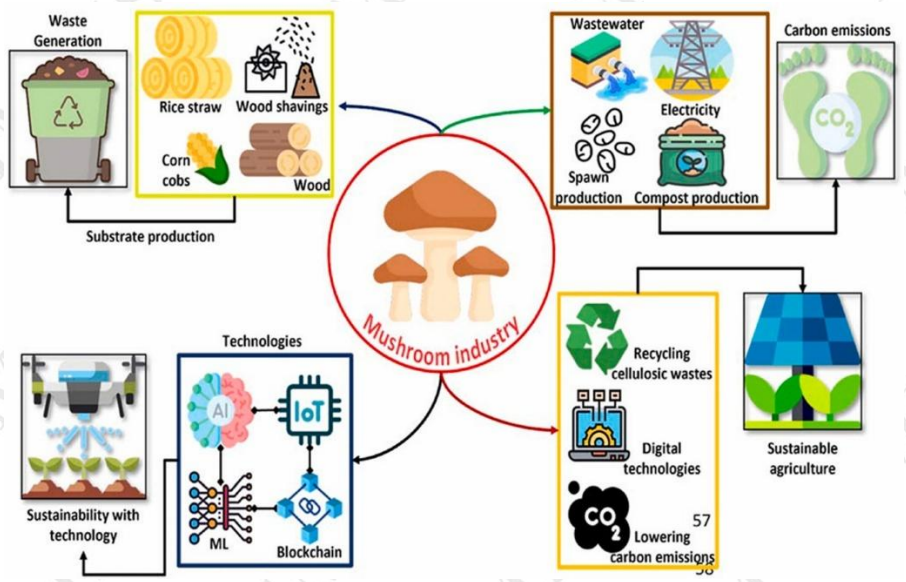
Abstract: Sustainable agriculture holds a key role in attaining a balanced approach to increase productivity, especially for growing industries like mushroom production. Producers like China, the USA, and the UK lead the race for global mushroom production, while India lags with 0.18 million tonnes of output. However, ensuring the sustainability of the mushroom industry is needed for environmental conservation, long-term economic viability, and the overall well-being of communities dependent on this agricultural sector. This review focuses on the recent trends in waste and carbon footprint generation from the mushroom industry, emphasizing spent mushroom substrate and utilities like electricity. Moreover, this review extensively covers recent advancements in research concerning incorporating innovative technologies such as AI and precision agricultural technologies like Internet-of-Things (IoT) and big data and

contemporary approaches, such as solar energy in mushroom farming. The challenges the mushroom industry faces, and policies to tackle them and promote sustainable agriculture are also thoroughly explored. The review concludes that the carbon footprint generation and waste release from mushroom production can be mitigated using AI, IoT, big data, machine learning, integrated sensors, etc., by increasing production efficiency and optimizing processes. Conventions such as circular economy, conventional energy substitution, carbon credit, and carbon capturing can also alleviate carbon emissions and carbon footprint. Therefore, this will allow the mushroom industry to align with Sustainable Development Goals 7 (affordable and clean energy) and 13 (climate action). Moreover, there is an urgent need to refine the schemes and provisions to make mushroom cultivation a sustainable agricultural sector.

Graphical abstract



Renewable and Sustainable Energy Reviews, Volume 212, April 2025, 115338

https://doi.org/10.1016/j.rser.2025.115338

An Examination of Cholinergic Symptoms Produced by the Fly Agaric Mushroom *Amanita muscaria* (Agaricomycetes): Revisiting the Role of Muscarine

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Abstract: The English mycological and toxicological literature has, for decades, asserted that muscarine concentrations in *Amanita muscaria* are insignificant based on a study from the 1950s that demonstrated muscarine levels in fresh *A. muscaria* mushrooms at a meager 0.0003%. This position has been maintained despite frequent reports of cholinergic symptoms following consumption of this mushroom and despite the dated study upon which this position is based. To update the literature on *A. muscaria's* pharmacology and to address disparities between the current scientific consensus on the role of muscarine, a cholinergic compound, in *A. muscaria* poisonings and the frequent reports of cholinergic

symptoms following its ingestion, four steps were taken: (1) surveys were collected from 53 individuals who experienced cholinergic symptoms following ingestion of *A. muscaria*; (2) mushroom samples were procured for HPLC-MS/MS analysis from three survey participants; (3) mushrooms were collected independently for HPLC-MS/MS analysis; and (4) commercial analyses of *Amanita muscaria* were compiled to illustrate a range of muscarine concentrations. Survey results demonstrated that mild-to-moderate cholinergic symptoms were experienced at doses that reflect common use of the mushroom for recreational, therapeutic, and spiritual purposes (1−20 g dried). Results of HPLC-MS/MS analyses demonstrated muscarine concentrations ranging from 0.004% up to 0.043%, significantly exceeding the consensus value. Study findings demonstrate that current understandings of muscarine concentrations in *A. muscaria* are inaccurate, and that the occurrence of muscarine in *A. muscaria* must be understood as a broad range, one that ranges from the insignificant up to physiologically significant levels.

Keywords: *Amanita muscaria*, cholinergic syndrome, isoxazoles, microdosing, muscarine, mushroom toxins, psychoactive mushrooms

International Journal of Medicinal Mushrooms, Volume 27, Issue 7, 2025, pp. 1-15

DOI: 10.1615/IntJMedMushrooms.2025058603

Investigating the therapeutic potential of *Ganoderma lucidum* in treating optic nerve atrophy through network pharmacology and experimental validation

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[e]*The First Affiliated Hospital of Guizhou University of Traditional Chinese Medicine, China*

[f]*Chengdu Coma Ren Far Technology Co., LTD, China*

Abstract:

Objective

The aim of this study is to employ network pharmacology to identify potential therapeutic targets for Ganoderma lucidum in the treatment of optic atrophy, and elucidate the underlying pharmacological mechanism.

Methods

This study is mainly divided into two parts. In the first part, the chemical composition and Target of Ganoderma lucidum compound were predicted by TCMSP and Swiss Target Prediction, and the crossover gene between OA and Ganoderma

lucidum target gene was screened based on GeneCards and OMIM database. Then, the target genes were enriched and the main pathways of action were analyzed to discover the possible mechanism of action for the treatment of optic atrophy. Finally, the selected core compounds and core targets were interfaced to understand the main binding patterns and affinity. The second part mainly verifies whether Ganoderma lucidum polysaccharide has protective effect on RGC. Firstly, CCK8 method was used to detect the proliferation and virulence analysis of RGC-5 cells with different concentrations of Ganoderma lucidum polysaccharide, and then RGC-5 cells were cultured in subgroups for 12 h, and then put into anaerobic encapsulation to make molds. After 24 h of continuous culture, cells were removed and collected for subsequent RT-PCR and WB detection.

Results

Through screening target genes of Ganoderma lucidum and OA, 85 potential therapeutic targets were obtained by intersection. Through PPI network analysis of 85 potential targets, it was found that the degree values of TP53, TNF, CASP3, IL6, EGFR, MTOR, ESR1 and other targets were higher. (+)-Ganoderic acid Mf, (+)-Methyl ganolucidate A, epoxyganoderiol A, Ergosta-4,7, 22-Trien-3, 6-Dione and other compounds play a key role in the whole network. It may be the key compound of *Ganoderma lucidum* in treating OA. Through enrichment pathway analysis, it was found that the number of genes was enriched in AGE-RAGE signaling pathway, cAMP signaling pathway, inflammation and cancer pathways, and the structure of TP53, TNF, CASP3, and IL6 binding to the above compounds was stable and the binding activity was high.

Conclusions

The findings suggest that Ganoderma lucidum may exert its therapeutic effects on optic atrophy by targeting TP53, TNF, CASP3, and IL6. Additionally, it may also be involved in the AGE-RAGE signaling pathway and cAMP signaling pathway. These results provide reference for the clinical application of *Ganoderma lucidum* in the treatment of OA.

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Immunomodulatory effects of bioactive polysaccharides from *Pleurotus pulmonarius* on LPS-stimulated THP-1 human macrophages

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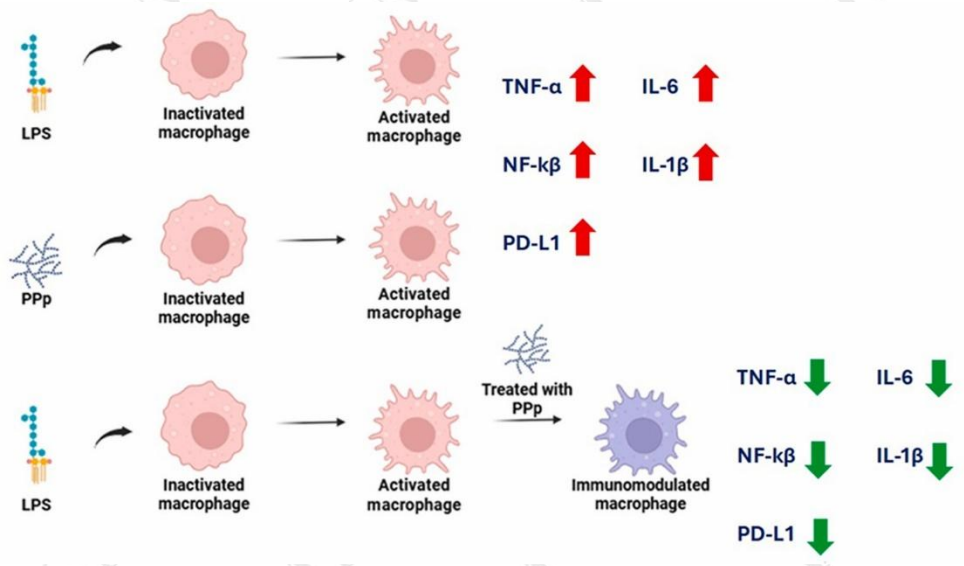
Abstract: Edible mushrooms represent valuable reservoirs of bioactive compounds with significant potential for pharmaceutical and nutraceutical applications. Their immunomodulatory effects have been linked to various nutraceutical and therapeutic benefits. This study investigates the immunostimulatory effects of soluble polysaccharides from *Pleurotus pulmonarius* (PPp) on THP-1 macrophages. Initial assays revealed high carbohydrate and β-glucan content in PP soluble extracts. Isolated soluble *Pleurotus pulmonarius* polysaccharides (PPp) induced dose-dependent (10–80 μg/mL) proliferation of THP-1 cells and upregulated expression of TNF-α, IL-1β, and IL-6 genes. Additionally, PPp inhibited nitric oxide (NO) production (15%–45%), downregulated inducible Nitric Oxide Synthase (iNOS) gene expression (33%–83%), and suppressed Programmed Cell Death-Ligand 1 (PD-L1) gene expression (69–95%) in LPS-stimulated THP-1 human macrophages. These findings suggest that soluble *Pleurotus pulmonarius* polysaccharides (PPp) modulates signaling pathways, balancing NO generation and iNOS expression in LPS-stimulated macrophages, signifies the ability of soluble *Pleurotus pulmonarius* polysaccharides (PPp) to regulate immunity level by polarizing LPS-stimulated macrophages. This study significantly contributes to advancing our understanding of *Pleurotus pulmonarius* polysaccharides' immunomodulatory properties, offering insights into the nutraceutical value of *Pleurotus pulmonarius* (PP) as a promising functional food.

Graphical abstract



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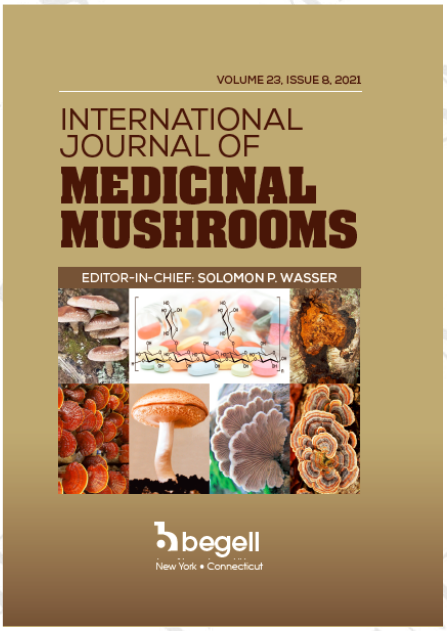
International Journal of Medicinal Mushrooms Call for Papers



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Points and Reviews

Three-Dimensional Structural Heteromorphs of Mating-Type Proteins in *Hirsutella sinensis* and the Natural *Cordyceps sinensis* Insect–Fungal Complex

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Abstract: The MAT1-1-1 and MAT1-2-1 proteins are essential for the sexual reproduc- tion of *Ophiocordyceps sinensis*. Although *Hirsutella sinensis* has been postulated to be thesole anamorph of *O. sinensis* and to undergo self-fertilization under homothallism orpseudohomothallism, little is known about the three-dimensional (3D) structures of themating proteins in the natural *Cordyceps sinensis* insect–fungal complex, which is a valuable therapeutic agent in traditional Chinese medicine. However, the alternative splicing anddifferential occurrence and translation of the *MAT1-1-1* and *MAT1-2-1* genes have been revealed in *H. sinensis*, negating the self-fertilization hypothesis but rather suggesting the occurrence of self-sterility under heterothallic or hybrid outcrossing. In this study, theMAT1-1-1 and MAT1-2-1 proteins in 173 *H. sinensis* strains and wild-type *C. sinensis* isolates were clustered into six and five clades in the Bayesian clustering trees and belonged to 24 and 21 diverse AlphaFold-predicted 3D structural morphs, respectively. Over three-quarters of the strains/isolates contained either MAT1-1-1 or MAT1-2-1 proteins but notboth. The diversity of the heteromorphic 3D structures of the mating proteins suggestedfunctional alterations of the proteins and provided additional evidence supporting theself-sterility hypothesis under heterothallism and hybridization for *H. sinensis*, Genotype #1 of the 17 genome-independent *O. sinensis* genotypes. The heteromorphic stereostruc-tures and mutations of the MAT1-1-1 and MAT1-2-1 proteins in the wild-type *C. sinensis*isolates and natural *C. sinensis* insect–fungi complex suggest that there are various sources of the mating proteins produced by two or more cooccurring heterospecific fungal species in natural *C. sinensis* that have been discovered in mycobiotic, molecular, metagenomic, and metatranscriptomic studies, which may inspire future studies on the biochemistry of mating and pheromone receptor proteins and the reproductive physiology of *O. sinensis*.

Keywords: heteromorphic stereostructures of MAT1-1-1 and MAT1-2-1 proteins; Bayesian clustering; AlphaFold-predicted 3D protein structures; *Hirsutella sinensis*; reproductionof *Ophiocordyceps sinensis*; sexual life of the natural *Cordyceps sinensis* insect–fungal complex

1. Introduction

The natural *Cordyceps sinensis* insect–fungal complex is one of the most expensive therapeutic agents in traditional Chinese medicine and has a rich history of clinical applications for centuries in health maintenance, disease amelioration, post-illness and post-surgeryrecovery, and antiaging therapy [Zhu et al., 1998 [1], 2011 [2]]. As defined by the *Chinese Pharmacopoeia*, natural *C. sinensis* is an insect–fungal complex containing the *Ophiocordyceps sinensis* fruiting body and the remains of a *Hepialidae* moth larva (an intact, thick larval body wall with numerous bristles, an intact larval intestine and head tissues, and fragments of other larval tissues) [Ren et al., 2013 [3]; Zhang et al., 2014 [4]; Lu et al., 2016 [5]; Liet al., 2022 [6], 2023 [7]]. Studies of natural *C. sinensis* have demonstrated its multicel- lular heterokaryotic structures of hyphal and ascosporic cells and genetic heterogeneity,including at least 17 genomically independent genotypes of *O. sinensis* fungi and >90 other fungal species spanning at least 37 fungal genera and larval genes [Jiang & Yao 2003 [8];Zhang et al., 2010 [9], 2018 [10]; Xia et al., 2015 [11]; Guo et al., 2017 [12]; Li et al., 2016 [13], 2020 [14], 2022 [6], 2023 [7], 2023 [15]; Zhong et al., 2018 [16]; Kang et al., 2024 [17]]. Among the numerous heterogeneous fungal species, *Hirsutella sinensis* was postulated by Wei et al., 2006 [18] to be the sole anamorph of *O. sinensis*; however, 10 years later, the key authorsreported a species contradiction in an artificial cultivation project conducted in a product-oriented industrial setting between anamorphic inoculates of three GC-biased *H. sinensis*strains on *Hepialidae* moth larvae and the sole AT-biased teleomorph (Genotype #4 of *O.sinensis*) in cultivated *C. sinensis* [Wei et al., 2016 [19]]. Notably, the Latin name *Cordyceps sinensis* has been used indiscriminately since the 1840s for both the teleomorph/holomorph of the fungus *C. sinensis* and the wild insect–fungal complex, and the fungus was renamed *Ophiocordyceps sinensis* in 2007 [Sung et al., 2007 [20]; Zhang et al., 2012 [21]; Ren et al.,2013 [3]; Yao & Zhu 2016 [22]; Li et al., 2022 [6]]. Zhang et al., 2013 [23] proposed improper implementation of the “One Fungus=One Name” nomenclature rule of the InternationalMycological Association [Hawksworth et al., 2011 [24]] while disregarding the presence of multiple genomically independent genotypes of *O. sinensis* fungi and inappropriately replacing the anamorphic name *H. sinensis* with the teleomorphic name *O. sinensis*. Thus, we continue using the anamorphic name *H. sinensis* for Genotype #1 of the 17 *O. sinensis*genotypes in this paper and refer to the genomically independent Genotypes #2–17 fungi as *O. sinensis* before their systematic positions are taxonomically determined, regardless of whether they are genetically GC- or AT-biased. We continue the customary use of the name *C. sinensis* to refer to the wild or cultivated insect–fungal complex because the renaming of *C. sinensis* to *O. sinensis* in 2007 did not involve the indiscriminate use of the Latin name for the natural insect–fungal complex, although this practice will likely be replaced inthe future by the differential use of proprietary and exclusive Latin names for the multiple genome-independent *O. sinensis* genotypic fungi and the insect–fungicomplex.

The sexual reproductive behavior of ascomycetes is controlled by transcription factors encoded at the mating-type (MAT) locus [Debuchy et al., 2006 [25]; Jones & Bennett2011 [26]; Zheng & Wang 2013 [27]; Wilson et al., 2015 [28]]. Holliday et al., 2008 [29], Stone et al., 2010 [30], and Hu et al., 2013 [31] reported failures when trying to induce the development of *C. sinensis* fruiting bodies and ascospores via the use of pure *H. sinensis* cultures as inoculants. Zhang et al., 2013 [23] summarized the failures over 40 years of academicexperience in research-oriented academic settings. Hu et al., 2013 [31] and Bushley et al.,2013 [32] hypothesized that *H. sinensis* undergoes self-fertilization under homothallism or pseudohomothallism; however, [Zhang et al., 2009 [33], 2011 [34] and Zhang and Zhang2015 [35] reported the differential occurrence of the *MAT1-1-1* and *MAT1-2-1* genes innumerous wild-type *C. sinensis* isolates and hypothesized that *O. sinensis* underwent facultative hybridization. Moreover, Li et al., 2023 [36], 2024 [37] reported

the alternative splicing, differential occurrence, and differential transcription of mating-type and pheromone receptor genes in *H. sinensis* and natural *C. sinensis*, suggesting the occurrence of self-sterilityin *H. sinensis* under heterothallism or hybridization and the demand of sexual partnersduring the sexual life of the natural *C. sinensis* insect–fungicomplex.

Sequences of the *MAT1-1-1* and *MAT1-2-1* genes and proteins of *H. sinensis* are available in the GenBank database, but little is known about the polymorphic stereostructures of the proteins in *H. sinensis* strains and wild-type *C. sinensis* isolates, which are extremely crucial to the sexual reproduction of *O. sinensis* and for the maintenance of the natural ecological population volume of the Level II endangered authentic traditional Chinesemedicinal "herb" [China Ministry of Agriculture and Rural Affairs 2021 [38]], which is anatural *C. sinensis* insect–fungi complex. In this work, we analyzed and correlated the statis- tical clustering of the primary structures and AlphaFold-predicted 3D structural models of the MAT1-1-1 and MAT1-2-1 proteins from 173 *H. sinensis* strains and wild-type *C. sinensis* isolates and correlated the heteromorphic structures of the protein sequences encoded by the genome, transcriptome, and metatranscriptome assemblies of *H. sinensis* and natural *C. sinensis*.

2. Materials and Methods

2.1. C. sinensis Isolates and Accession Numbers of the MAT1-1-1 and MAT1-2-1Proteins

The AlphaFold database (Cambridgeshire, UK) lists the accession numbers of the MAT1-1-1 and MAT1-2-1 proteins and the 3D protein structures, which were derived from 173 *H. sinensis* strains and wild-type *C. sinensis* isolates that were collected from variousproduction areas on the Qinghai–Tibet Plateau [Zhang et al., 2009 [33], 2011 [34]; Hu et al., 2013 [31]; Zhang & Zhang 2015 [35]; Tunyasuvunakool et al., 2021 [39]].

2.2. Genome, Transcriptome, and Metatranscriptome Assemblies of H. sinensis Strains and the Natural C. sinensis Insect–Fungal Complex

The genome assemblies ANOV00000000, JAAVMX000000000, LKHE00000000, LWBQ00000000, and NGJJ00000000 of the *H. sinensis* strains Co18, IOZ07, 1229, ZJB12195, andCC1406-20395, respectively, were used for mating protein analysis [Hu et al., 2013 [31]; Li et al., 2016 [40]; Jin et al., 2020 [41]; Liu et al., 2020 [42]; Shu et al., 2020 [43]].

The transcriptome assembly GCQL00000000 for the *H. sinensis* strain L0106 and the metatranscriptome assembly GAGW00000000 for the natural *C. sinensis* samples collected from Kangding County, Sichuan Province, China, were also used for mating protein analysis [Liu et al., 2015 [44]; Xiang et al., 2014 [45]].

Another metatranscriptome assembly was derived from mature natural *C. sinensis* sam- ples collected from Deqin, Yunnan Province, China (*cf*. the Appendix of [Xia et al., 2017 [46]]). The metatranscriptome assembly sequences were uploaded to a repository database, www. plantkingdomgdb.com/Ophiocordyceps_sinensis/data/cds/Ophiocordyceps_sinensis_CDS. fas (accessed from 18 May 2017 to 18 January 2018), which is currently inaccessible, but apreviously downloaded cDNA file was used for mating protein analysis.

2.3. Statistical Clustering Analysis for the MAT1-1-1 and MAT1-2-1 Protein Sequences

Multiple protein sequences of the *H. sinensis* strains and wild-type *C. sinensis* isolates were analyzed via the auto mode of MAFFT (v7.427). Bayesian clustering trees of theMAT1-1-1 and MAT1-2-1 protein sequences were then inferred via MrBayes v3.2.7 software (Markov chain Monte Carlo [MCMC] algorithm, New York, NY, USA) with a samplingfrequency

of 100 iterations after discarding the initial 25% of the samples from a total of 1 million iterations [Huelsenbeck & Ronquist 2001 [47]; Ronquist et al., 2012 [48]; Li et al.,2022 [6], 2023 [49], 2024 [37]]. Clustering analysis was conducted at Nanjing Genepioneer Biotechnologies Co. (Nanjing, China).

2.4. AlphaFold-Based Prediction of 3D Structures of Mating Proteins

The 3D structures of the MAT1-1-1 and MAT1-2-1 proteins of the 173 *H. sinensis* strains and wild-type *C. sinensis* isolates were computationally predicted from their amino acid sequences via the artificial intelligence (AI)-based machine learning technology AlphaFold (https://alphafold.com/ (Cambridgeshire, UK), accessed from 18 October 2024 to 31 December 2024) and downloaded from the AlphaFold database for structural polymorphism analysis [Jumper et al., 2021 [50]; David et al., 2022 [51]; Rettie et al., 2023 [52]; Abramson et al., 2024 [53]; Varadi et al., 2024 [54]]. The heteromorphic 3D structures of the MAT1-1-1 and MAT1-2-1 proteins were grouped based on the results of AlphaFold structural andBayesian clustering analyses.

The AlphaFold database provides per-residue model confidence, the prediction of its score in the local distance difference test (pLDDT), between 0 and 100, a per-residue score that is assigned to each individual residue [Mariani et al., 2013 [55]; Jumper et al., 2021 [50]; David et al., 2022 [51]; Monzon et al., 2022 [56]; Xu et al., 2023 [57]; Abramson et al., 2024 [53]; Varadi et al., 2024 [54]]. Model confidence bands are used to color-code the residues in the 3D structure: very high confidence (pLDDT > 90) residues are shown in dark blue, high (90 > pLDDT > 70) in light blue, low (70 > pLDDT > 50) in yellow, and very low (pLDDT < 50) in orange [Mariani et al., 2013 [55]; Wroblewski & Kmiecik 2024 [58]]. Note that a protein region that is assigned a low pLDDT score does not necessarily indicate that this region is the most variable region in the protein sequence; in contrast, a substantially variable region of a protein may be assigned a high pLDDT score. The AlphaFold database provides an average pLDDT score for each of the predicted 3D structure models of mating proteins, representing the overall model confidence in the predicted 3D structures.

2.5. Alignment Analysis of Protein Sequences

The amino acid sequences of the MAT1-1-1 and MAT1-2-1 proteins of *H. sinensis* and natural *C. sinensis* were aligned and compared via the GenBank Blastp program (https://blast.ncbi.nlm.nih.gov/ (Bethesda, MD, USA), accessed from 18 October 2024 to 1 December 2024).

2.6. Amino Acid Properties and Scale Analysis

The amino acid components of the mating proteins were scaled based on the general chemical characteristics of their side chains (*cf*. Supplementary Table S1) and plotted se-quentially with a window size of 21 amino acid residues for the α-helices, β-sheets, β-turns, and coils of the MAT1-1-1 and MAT1-2-1 proteins via the linear weight variation model of the ExPASy ProtScale algorithm (https://web.expasy.org/protscale/ (Basel, Switzerl;and), accessed from 18 October 2024 to 1 December 2024) [Deleage & Roux 1987 [59]; Gasteiger et al., 2005 [60]; Peters & Elofsson 2014 [61]; Simm et al., 2016 [62]; Li et al., 2024b [37]]. The plotting topologies and waveforms of the ProtScale plots for the proteins were compared to explore alterations in the 2D structures of the mating proteins.

3. Results

3.1. Diversity of the MAT1-1-1 and MAT1-2-1 Proteins in H. sinensis Strains and Wild-Type C. sinensis Isolates on the Basis of the AlphaFold-Predicted 3D Structures

A prior publication [Li et al., 2023 [36], 2024 [37]] reported the differential occurrence, alternative splicing, and differential transcription of mating-type genes in *H. sinensis* and natural *C. sinensis*. The current paper focuses on the diverse stereostructures of thetranslation products, namely, the MAT1-1-1 and MAT1-2-1 proteins of *O. sinensis*.

The AlphaFold database lists the accession numbers for 138 MAT1-1-1 proteins and 79 MAT1-2-1 proteins, which were derived from 173 *H. sinensis* strains and wild-type*C. sinensis* isolates [Zhang et al., 2009 [33], 2011 [34], 2013 [23]; Bushley et al., 2013 [32]; Hu et al., 2013 [31]; Zhang & Zhang 2015 [35]]. Among the 173 strains/isolates, 42 (24.3%) had records of AlphaFold-predicted 3D structures for both the MAT1-1-1 and MAT1-2-1proteins. A majority (75.7%) of the strains/isolates presented 3D structure records for either the MAT1-1-1 or MAT1-2-1 protein, suggesting differential cooccurrences of the two mating proteins essential for the sexual reproduction of *O. sinensis*. In addition, strains CS68-2-1229 and CS2 have duplicated accession numbers for either the MAT1-1-1 or MAT1-2-1 protein, unlike the 171 other strains/isolates (i.e., 173 = 138 + 79 – 42 − 2).

Strain CS68-2-1229 has two accession numbers for the MAT1-1-1 protein, namely, AGW27560 and AGW27528, which share 100% sequence identity. However, AGW27560is a full-length protein containing 372 amino acids, whereas AGW27528 is an N- andC-terminally truncated protein containing 301 amino acids with 80.9% query coverage.

Strain CS2 has two accession numbers for the full-length MAT1-2-1 protein, namely, AEH27625 and ACV60400, which contain 249 amino acids and share 100% sequenceidentity; however, the sequences were released by GenBank 6 years apart, on 03-JUN-2010 and 25-JUL-2016, respectively.

Table 1. GenBank accession numbers (in green, in parentheses) for the full-length MAT1-1-1 proteins of the *H. sinensis* strains and wild-type *C. sinensis* isolates under the corresponding AlphaFold UniProt codes.

AlphaFold UniProt Code (Bayesian Cluster/Branch *)	Strain/Isolate Number (GenBank Accession Number)
U3N942 (A1)	CS68-2-1229 (AGW27560) (AGW27528), GS09_111 (ALH24945), GS09_131 (ALH24947), ID10_1 (ALH24954), IOZ07 (KAF4512729), NP10_1 (ALH24955), NP10_2 (ALH24956), QH07_188 (ALH24957), QH07_197 (ALH24958), QH09_37 (ALH24968), QH09_46 (ALH24969), QH09_56 (ALH24970), QH09_66 (ALH24971), QH09_78 (ALH24972), QH09_93 (ALH24973), QH09_122 (ALH24959), QH09_131 (ALH24960), QH09_151 (ALH24961), QH09_20L (ALH24965), QH09_33L (ALH24967), QH10_1 (ALH24974), QH10_4 (ALH24975), QH10_7 (ALH24976), SC09_21 (ALH24987), SC09_36 (ALH24988), SC09_37 (ALH24989), SC09_47 (ALH24990), SC09_57 (ALH24991), SC09_77 (ALH24993), SC09_107 (ALH24978), SC09_117 (ALH24979), SC09_128 (ALH24980), SC09_147 (ALH24981), SC09_157 (ALH24982), SC09_167 (ALH24983), SC09_180 (ALH24984), SC09_190 (ALH24985), SC09_200 (ALH24986), SC10_18 (ALH24996), SC10_21 (ALH24997), SC10_4 (ALH24998), XZ05_3 (ALH25002), XZ05_7 (ALH25004), XZ05_12 (ALH25000), XZ06_124 (ALH25006), XZ06_152 (ALH25007), XZ07_108 (ALH25009), XZ07_133 (ALH25010), XZ07_154 (ALH25011), XZ07_166 (ALH25012), XZ07_176 (ALH25013), XZ07_180 (ALH25014), XZ08_4 (ALH25018), XZ08_10 (ALH25015), XZ08_24 (ALH25016), XZ08_26 (ALH25017), XZ08_56 (ALH25019), XZ08_59 (ALH25020), XZ08_A1 (ALH25021), XZ08_B1 (ALH25022), XZ09_4 (ALH25029), XZ09_46 (ALH25030), XZ09_48 (ALH25031), XZ09_59 (ALH25032), XZ09_71 (ALH25033), XZ09_80 (ALH25055), XZ09_106 (ALH25024), XZ09_113 (ALH25025), XZ09_118 (ALH25026), XZ09_15 (ALH25027), XZ09_32 (ALH25028), XZ10_7 (ALH25038), XZ10_15 (ALH25035), XZ10_17 (ALH25036), XZ10_23 (ALH25037), XZ12_1 (ALH25056), XZ12_33 (ALH25058), XZ12_43 (ALH25059), YN07_6 (ALH25039), YN07_8 (ALH25040), YN09_3 (ALH25044), YN09_72 (ALH25049), YN09_81 (ALH25050), YN09_85 (ALH25051), YN09_89 (ALH25052), YN09_96 (ALH25053),YN09_101 (ALH25041), YN09_140 (ALH25042)

Table 1. *Cont.*

AlphaFold UniProt Code (Bayesian Cluster/Branch *)	Strain/Isolate Number (GenBank Accession Number)
A0A0N9QMM1 (A1)	GS09_121 (ALH24946), GS09_201 (ALH24949), GS09_225 (ALH24950), SC09_1 (ALH24977)
T5A511 (A1)	**Co18** (EQK97643) (KE657544 410←1519 and ANOV01017390 410←1519)
A0A0N9R5B3 (A2)	SC09_65 (ALH24992)
A0A0N7G849 (A2)	SC09_97 (ALH24995)
A0A0N9QUF3 (A3)	GS09_143 (ALH24948)
A0A0N9R4V2 (A3)	YN09_61 (ALH25047)
A0A0N9QMS9 (B)	YN09_6 (ALH25046), YN09_22 (ALH25043), YN09_51 (ALH25045), YN09_64 (ALH25048)
A0A0N7G845 (C)	GS09_229 (ALH24951), GS09_281 (ALH24952), GS09_311 (ALH25054), GS10_1 (ALH24953), QH09_164 (ALH24962), QH09_173 (ALH24963), QH09_201 (ALH24964), QH09_210 (ALH24966), SC09_87 (ALH24994)
A0A0N9QUK2 (D1)	XZ05_8 (ALH25005)
A0A0N9QMT4 (D2)	XZ07_H2 (ALH24999), XZ12_16 (ALH25057)
A0A0N9QMR3 (E1)	XZ06_260 (ALH25008), XZ09_100 (ALH25023)
A0A0N9QMS4 (E2)	XZ09_95 (ALH25034)
A0A0N7G850 (E3)	XZ05_6 (ALH25003)
A0A0N9R4Q4 (E4)	XZ05_2 (ALH25001)

Note: The names of the pure *H. sinensis* strains are highlighted in bold and underlined, whereas those of the wild-type *C. sinensis* isolates are not. * Branch 1 is shown in red, Branch 2 in pink, Branch 3 in purple, and Branch 4 in brown, with the cluster codes (English letters) in parentheses determined via the Bayesian analysis (*cf*. Figure 1 below). The "←" arrows indicate sequences in the antisense strands of the genome of the *H. sinensis* strain Co18.

The 138 MAT1-1-1 proteins belong to diverse 3D structural models or morphs under 24 UniProt codes in the AlphaFold database, 118 of which are full-length proteins belonging to 3D structural morphs under 15 AlphaFold UniProt codes and are listed in Table 1.Among the 118 full-length proteins, 89 (75.4%) are under the UniProt code U3N942 and are considered "likely authentic" proteins. The remaining 20 of the 138 MAT1-1-1 proteins are truncated and belong to 3D structural morphs under nine other UniProt codes.

The 79 MAT1-2-1 proteins belong to diverse 3D structural morphs under 21 UniProt codes in the AlphaFold database, 74 of which are full-length proteins containing 249 amino acids belonging to 3D structural morphs under 17 AlphaFold UniProt codes and are listed in Table 2. Among the 74 full-length MAT1-2-1 proteins, 38 (51.4%) are under the UniProt code D7F2E9 and are considered "likely authentic" proteins. The remaining 5 of the 79 MAT1-2-1 proteins are truncated and belong to 3D structural morphs under four other UniProt codes.

Table 2. GenBank accession numbers (in green and in parentheses) for the full-length MAT1-2-1proteins of the *H. sinensis* strains or wild-type *C. sinensis* isolates under the corresponding AlphaFold UniProt codes.

AlphaFold UniProt Code (Bayesian Cluster/Branch **)	Strain/Isolate Number (GenBank Accession Number)
D7F2E9 (I-1)	**CS2** (AEH27625) (ACV60400), **CS26-277** (AGW27541), **CS36-1294** (AGW27538), **CS37-295** (AGW27539), SC-2 (ACV60395), SC-4 (ACV60396), SC-5 (ACV60398), SC-7 (ACV60397), SC09-37 (AFH35019), SC09_47 (AFX66423), SC09_57 (AFX66424), SC09_77 (AFX66426), SC09_97 (AFX66428), XZ05_7 (AFX66442), XZ05_12 (AFX66444), XZ06_152 (AFX66445),XZ07_11 (AFX66447), XZ07_46 (AFX66448), XZ09_106 (AFX66464), XZ09_15 (AFX66455), YN09_101 (AFX66482), YN09_72 (AFX66477), XZ09_113 (AFX66465), XZ-LZ06-1 (ACV60369), XZ-LZ06-7 (ACV60370), XZ-LZ06-21 (ACV60371), XZ-LZ06-108 (ACV60373), XZ-LZ07-30 (ACV60377), XZ-LZ07-108 (ACV60379), XZ-ML-191 (ACV60376),
D7F2E9 (I-1)	YN-1 (ACV60390), YN-5 (ACV60392), YN-6 (ACV60393), YN-8 (ACV60394), YN09_81 (AFX66478), YN09_85 (AFX66479), YN09_89 (AFX66480)
T5AF56 (I-1)	**Co18** (EQL04085) (ANOV01000063 9329→10182)
V9LW10 (I-2)	SC09_200 (AFX66437)
D7F2H1 (I-2)	YN-4 (ACV60391)
D7F2F2 (I-2)	XZ-LZ06-61 (ACV60372)
A0A0A0RCF5 (II-1)	XZ12_16 (AIV43040)
D7F2J7 (II-2)	XZ05_8 (AFX66443), XZ06-124 (AFH35020), XZ-LZ07-H2 (ACV60418), XZ-LZ07-H1 (ACV60417)
D7F2F5 (III)	XZ05_2 (AFX66441), XZ06_260 (AFX66446), XZ09_80 (AFX66461), XZ09_95 (AFX66462), XZ09_100 (AFX66463), XZ-LZ05-6 (ACV60415), XZ-SN-44 (ACV60375),
V9LWC9 (IV-1)	YN09_64 (AFX66476)
V9LVS8 (IV-2)	YN09_6 (AFX66472), YN09_22 (AFX66473), YN09_51 (AFX66474)
D7F2E3 (V-1)	GS09_111 (AFX66388), CS560-961 (AGW275424), QH09-93 (AFH35018), XZ-NQ-154 (ACV60363), XZ-NQ-155 (ACV6036)
D7F2G5 (V-2)	QH-YS-199 (ACV60385)
D7F2H9 (V-2)	SC-3 (ACV60399)
V9LW71 (V-2)	QH09_11 (AFX66401)
V9LVU8 (V-2)	YN09_61 (AFX66475)
V9LWG5 (V-2)	ID10_1 (AFX66484)
U3N6V5 (V-2)	CS6-251 (AGW27537)
‡	NP10_1 (AFX66485), NP10_2 (AFX66486), YN09_3 (AFX66471), YN09_96 (AFX66481), YN09_140 (AFX66483)

Note: The names of the pure *H. sinensis* strains are highlighted in bold and underlined, whereas those of the wild-type *C. sinensis* isolates are not. ** Branch 1 is shown in red and Branch 2 in pink, with the cluster codes(Roman numerals) in the parentheses determined via the Bayesian analysis, as shown in Figure 2 below. ‡, The5 MAT1-2-1 protein sequences are included in the GenBank database but not in the AlphaFold database (*cf*.Supplementary Figure S1). The "→" arrow indicates the sequence in the sense strand of the genome of the *H. sinensis* strain Co18.

3.2. Bayesian Analysis of the MAT1-1-1 and MAT1-2-1 Proteins

Figure 1 shows the Bayesian clustering tree for 40 protein sequences covering the diverse structural morphs of MAT1-1-1 proteins under 24 UniProt codes. The sequencesALH24945, ALH24947, and AGW27560 represent a group of 89 sequences under UniProt code U3N942, which were clustered into Branch A1 of Cluster A, as shown in red alongside the tree in Figure 1. Branch A1 in Figure 1 also includes the full-length MAT1-1-1 proteins under UniProt codes A0A0N9QMM1 and T5A511. Cluster A includes other full-lengthMAT1-1-1 protein sequences with very similar 3D structures, which were clustered intoBranch A2 in pink and Branch A3 in purple alongside the tree. The full-length MAT1-1-1 protein sequences with significantly variable 3D structures were clustered within Clusters B–E in Figure 1,

either branched or unbranched, under various UniProt codes in red forBranch 1, in pink for Branch 2, in purple for Branch 3, or in brown for Branch 4.

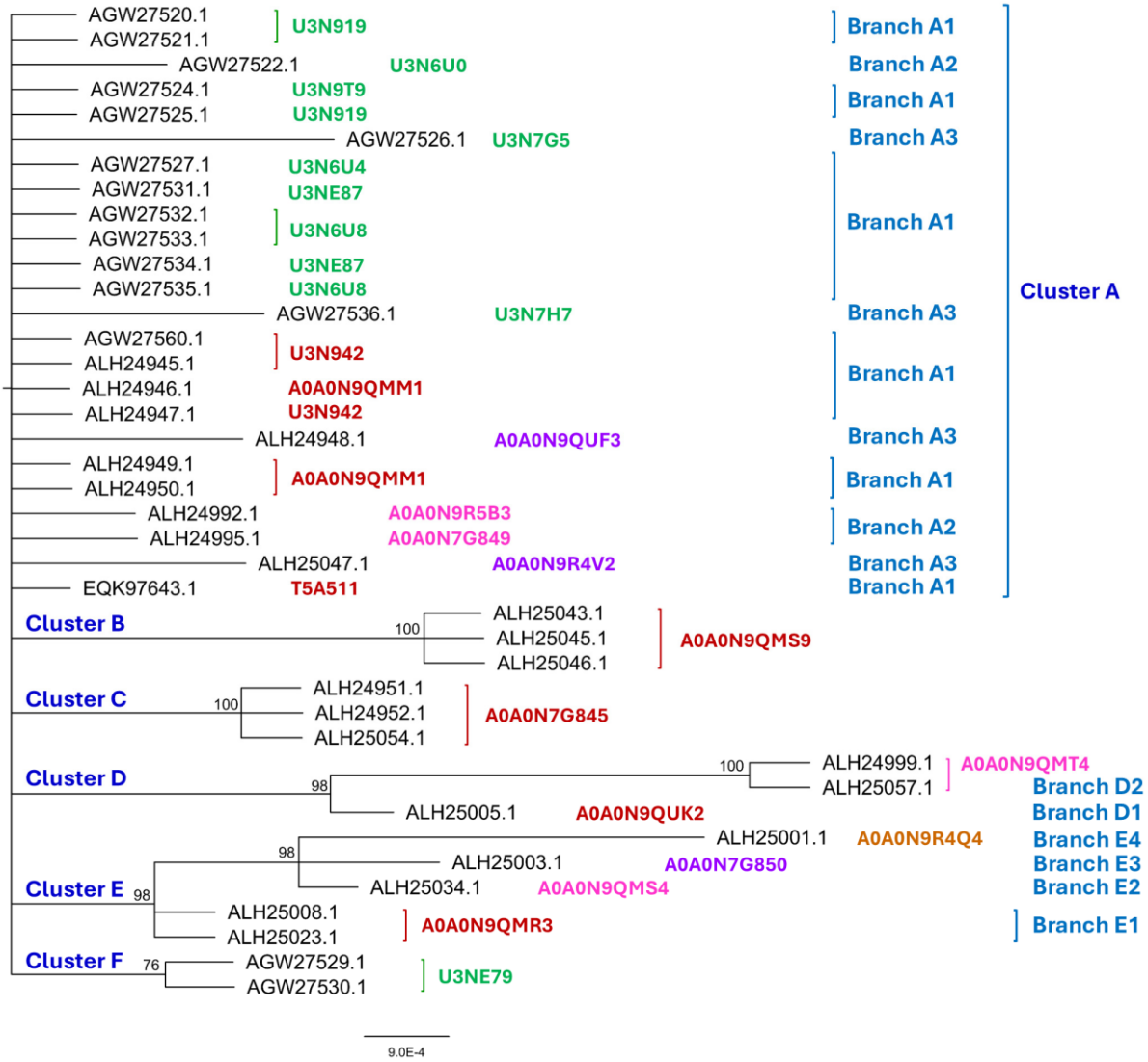


Figure 1. The Bayesian majority rule consensus clustering tree was inferred via MrBayes v3.2.7 software for the 40 full-length and truncated MAT1-1-1 proteins of the *H. sinensis* strains and wild- type *C. sinensis* isolates. The clusters and their branches (in blue) are shown alongside the tree. The AlphaFold UniProt codes for the 3D structures of the full-length proteins are shown in redalongside the tree for Branch 1 of the clusters, in pink for Branch 2, in purple for Branch 3, and inbrown for Branch 4. The AlphaFold UniProt codes in green indicate the N-/C-terminally truncated MAT1-1-1 proteins.

Many truncated MAT1-1-1 proteins were found under UniProt codes U3N919, U3N6U0, U3N9T9, U3N7G5, U3N6U4, U3NE87, U3N6U8, and U3N7H7, which are shown in green alongside the tree in Figure 1 and were clustered into Branches A1–A3 of Cluster A. In addition, Cluster F contains the truncated MAT1-1-1 proteins under the UniProt code U3NE79 in green alongside the tree in Figure1.

The 79 MAT1-2-1 proteins have various 3D structural morphs under 21 UniProt codes in the AlphaFold database (*cf.* Table 2), among which 32 representative sequences weresubjected to Bayesian clustering analysis, as shown in Figure2.

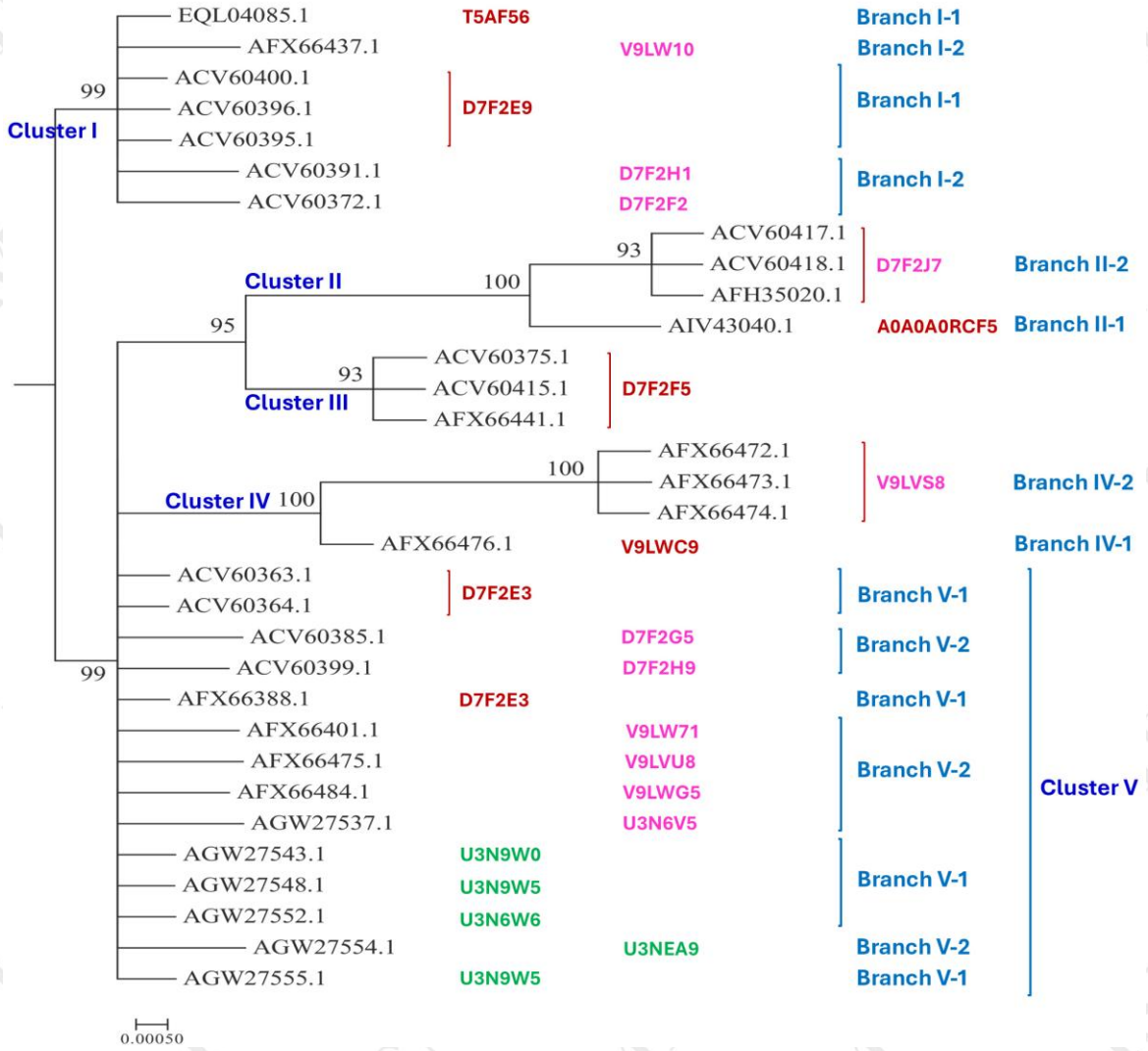


Figure 2. The Bayesian majority rule consensus clustering tree was inferred via MrBayes v3.2.7 software for the 32 full-length and truncated MAT1-2-1 proteins of the *H. sinensis* strains and wild- type *C. sinensis* isolates. The clusters and their branches (in blue) are shown alongside the tree. The AlphaFold UniProt codes for the 3D structures of the full-length proteins are shown in red alongside the tree for Branch 1 of the clusters and in pink for Branch 2 of the clusters. The AlphaFold UniProt codes in green indicate the C-terminally truncated MAT1-2-1 proteins.

Among a total of 79 MAT1-2-1 proteins, 74 are full-length proteins, containing 249 amino acids and belonging to diverse 3D structural morphs under 17 AlphaFoldUniProt codes. The remaining five MAT1-2-1 proteins are truncated and belong to 3Dstructural morphs under four other UniProt codes. Among the 74 full-length MAT1-2-1proteins, 39 (52.7%) proteins under the UniProt codes D7F2E9 and T5AF56 were clustered into Branch I-1 of Cluster I of the Bayesian tree shown in Figure 2 and Table 2. Branch I-2 of Cluster I includes three MAT1-2-1 protein sequences with very similar 3D structures be- longing to 3D structural morphs under the UniProt codes V9LW10, D7F2H1, and D7F2F2, as shown in Figure 2. Twenty-seven other full-length MAT1-2-1 proteins with significantly variable 3D structures were within Clusters II–V under various UniProt codes. BranchesV-1 and V-2 of Cluster V also include five truncated MAT1-2-1 proteins under differentUniProt codes, which are shown in green alongside the tree.

The GenBank database contains five other MAT1-2-1 protein sequences, namely, AFX66471, AFX66481, AFX66483, AFX66485, and AFX66486, which were derived from wild-type *C. sinensis* isolates YN09_3, YN09_96, YN09_140,

NP10_1, and NP10_2, respectively, with predicted 3D structure records in the AlphaFold database for the MAT1-1-1 proteins but not for the MAT1-2-1 proteins (*cf*. Tables 1 and 2). The five MAT1-2-1protein sequences are 100% identical to the reference sequence ACV60363 of Branch V-1 in theBayesian clustering tree (*cf*. Figure 2 and Supplementary Figure S1), indicating that the five protein sequences likely belong to Branch V-1 of Cluster V, together with five other Branch V-1 proteins, including the reference protein ACV60363, as listed in Table 1 and Figure 2.

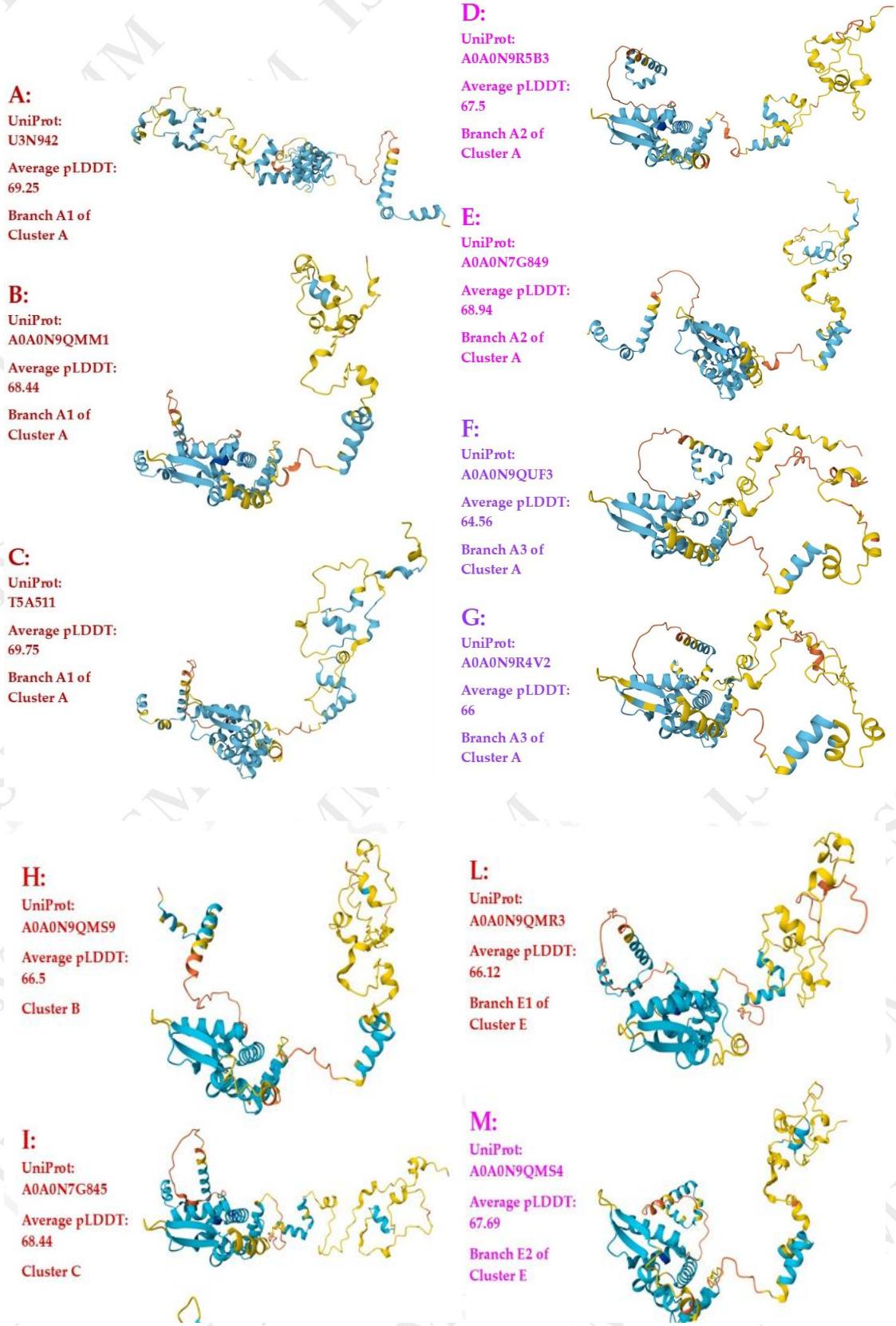


Figure 3. *Cont.*

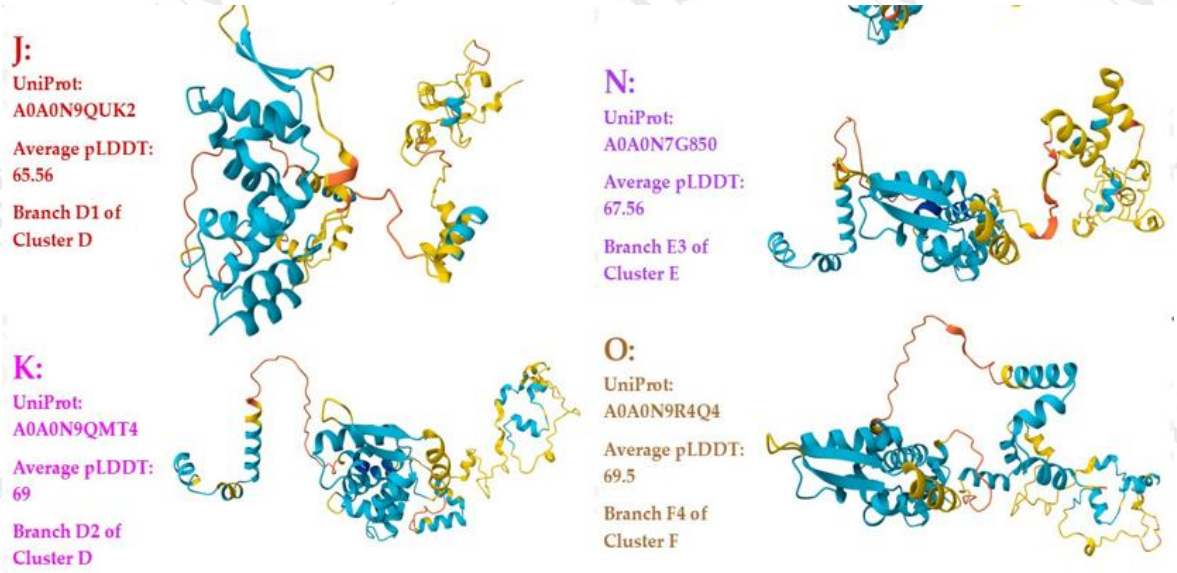


Figure 3. Fifteen 3D structural morphs (Panels **A**–**O**) for the 118 full-length MAT1-1-1 proteins of *H. sinensis* strains and wild-type *C. sinensis* isolates. The UniProt codes in red are for Branch 1 of each cluster shown alongside the Bayesian tree in Figure 1, while those in pink are for Branch 2, thosein purple are for Branch 3, and those in brown are for Branch 4. An average pLDDT score for each predicted 3D structural model was computed via AlphaFold technology and is shown in each of the 3D structural panels (**A**–**O**), indicating model confidence (*cf*. Section 2.4): ▮ very high (pLDDT >90), ▮ high (90 > pLDDT > 70), ▮ low (70 > pLDDT > 50), and ▮ very low (pLDDT < 50).

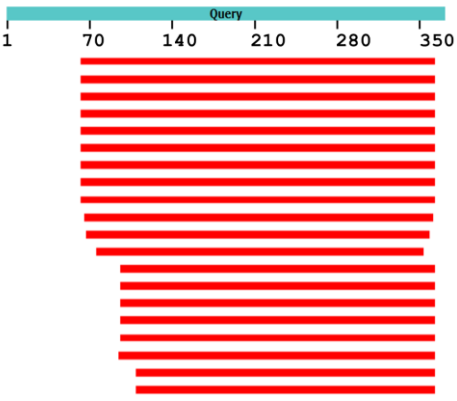
3.3. Heteromorphic AlphaFold-Predicted 3D Structures of the MAT1-1-1 Proteins

Figure 3 shows the AlphaFold-predicted 3D structures of the 118 full-length MAT1-1-1 proteins under 15 structural morphs (Panels A–O), which are also listed in Table 1. Among the 118 full-length proteins, 89 (75.4%) are under the UniProt code U3N942, as predicted by AlphaFold technology (Panel A of Figure 3). This 3D structure model most likely represents the authentic protein structure with full mating functionality.

As shown in Table 1 and Figures 1 and 3, 94 (79.9%) of the 118 full-length MAT1-1-1 proteins are under the UniProt codes U3N942, A0A0N9QMM1, and T5A511 and clustered into Branch A1 of Cluster A in the Bayesian tree belonging to the 3D structure morphs A–C. The 94 full-length MAT1-1-1 proteins are most frequently detected and are likely authentic with full matingfunctionality.

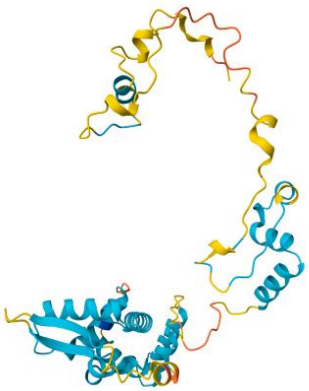
Figure 4 shows the sequence distributions (Panel A) and predicted 3D structures of 20 other MAT1-1-1 proteins, which are truncated at the N- and C-termini; these structures constitute the remaining nine diverse morphs of 3D structures (Panels B–J).

A. Distribution of 20 N-/C-terminal truncated MAT1-1-1 protein



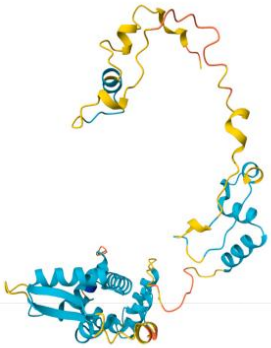
B:
UniProt: U3N9T9
Branch A1 of Cluster A

Strain	Accession:
CS18_266	AGW27520
CS26_277	AGW27521
CS34_291	AGW27524
CS36_1294	AGW27518
CS_37-295	AGW27519
CS6-251	AGW27517
CS68-2-1229	AGW27528
CS91-1291	AGW27523



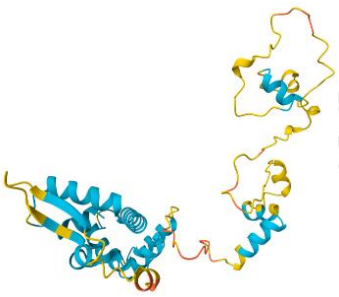
C:
UniProt: U3N919
Branch A1 of Cluster A

Strain	Accession:
CS76-1284	AGW27525



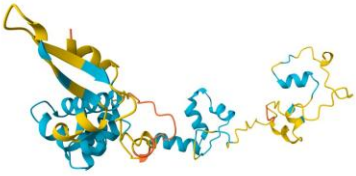
D:
UniProt: U3N6U4
Branch A1 of Cluster A

Strain	Accession:
CS25-273	AGW27527



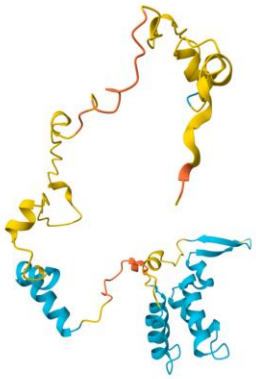
I:
UniProt: U3N7H7
Branch A3 of Cluster A

Strain	Accession:
CS68-2-1228	AGW27536



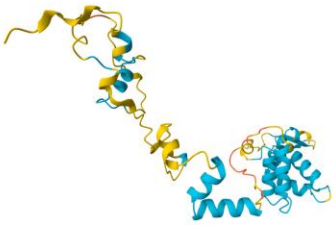
E:
UniProt: U3NE87
Branch A1 of Cluster A

Strain	Accession:
CS70-1212	AGW27531
CS71-1219	AGW27534



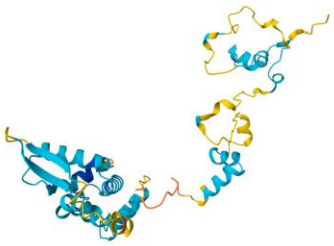
F:
UniProt: U3N6U8
Branch A1 of Cluster A

Strain	Accession:
CS68-5-1216	AGW27532
CS71-1218	AGW27533
CS71-1220	AGW27535



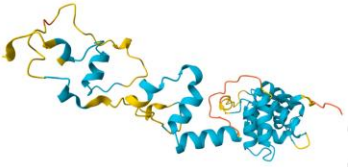
G:
UniProt: U3N6U0
Branch A2 of Cluster A

Strain	Accession:
CS560-961	AGW27522



H:
UniProt: U3N7G5
Branch A3 of Cluster A

Strain	Accession:
CS561-964	AGW27526



J:
UniProt: U3NE79
Cluster F

Strain	Accession:
CS70-1208	AGW27529
CS70-1211	AGW27530

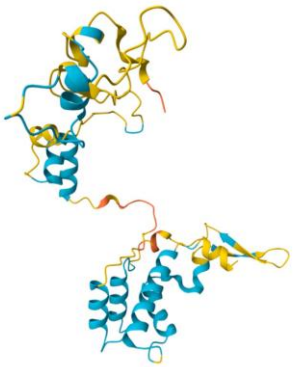


Figure 4. The sequence distribution (Panel **A**) and 9 diverse 3D structure morphs (Panels **B**–**J**) of the 20 N-/C-terminally truncated MAT1-1-1 proteins of *H. sinensis* strains and wild-type *C. sinensis* isolates.

Model confidence: very high (pLDDT > 90), high (90 > pLDDT > 70), low (70 > pLDDT > 50), and very low (pLDDT < 50).

3.4. Heteromorphic AlphaFold-Predicted 3D Structures of the MAT1-2-1 Proteins

Among the 79 MAT1-2-1 protein sequences belonging to the 21 diverse 3D structural morphs, 74 are full-length proteins, 69 of which belong to diverse structural morphs under 17 UniProt codes and are shown in Panels A–Q of Figure 5.

As shown in Table 2 and Figures 2 and 5, 39 (52.7%) of the 74 full-length MAT1-2-1 proteins are under the UniProt codes D7F2E9 and T5AF56 and clustered into Branch I-1 of Cluster I in the Bayesian tree belonging to 3D structural morph A of the MAT1-2-1 proteins. The 39 full-length MAT1-2-1 proteins are frequently detected and are likely authentic with full mating functionality.

As shown in Supplementary Figure S1, the five other full-length MAT1-2-1 protein se- quences (AFX66471, AFX66481, AFX66483, AFX66485, and AFX66486) exhibit 100% sequence identity with the Branch V-1 reference protein ACV60363. Thus, the five protein sequences without AlphaFold-predicted 3D structure records likely belong to the 3D structural morph K, together with the Branch V-1 reference protein ACV60363 (*cf*. Figures 2 and 5).

Figure 6 shows the sequence distribution (Panel A) and the AlphaFold-predicted 3D structures of the C-terminally truncated MAT1-2-1 proteins, which constitute the remaining four diverse morphs of 3D structures (Panels B–E).

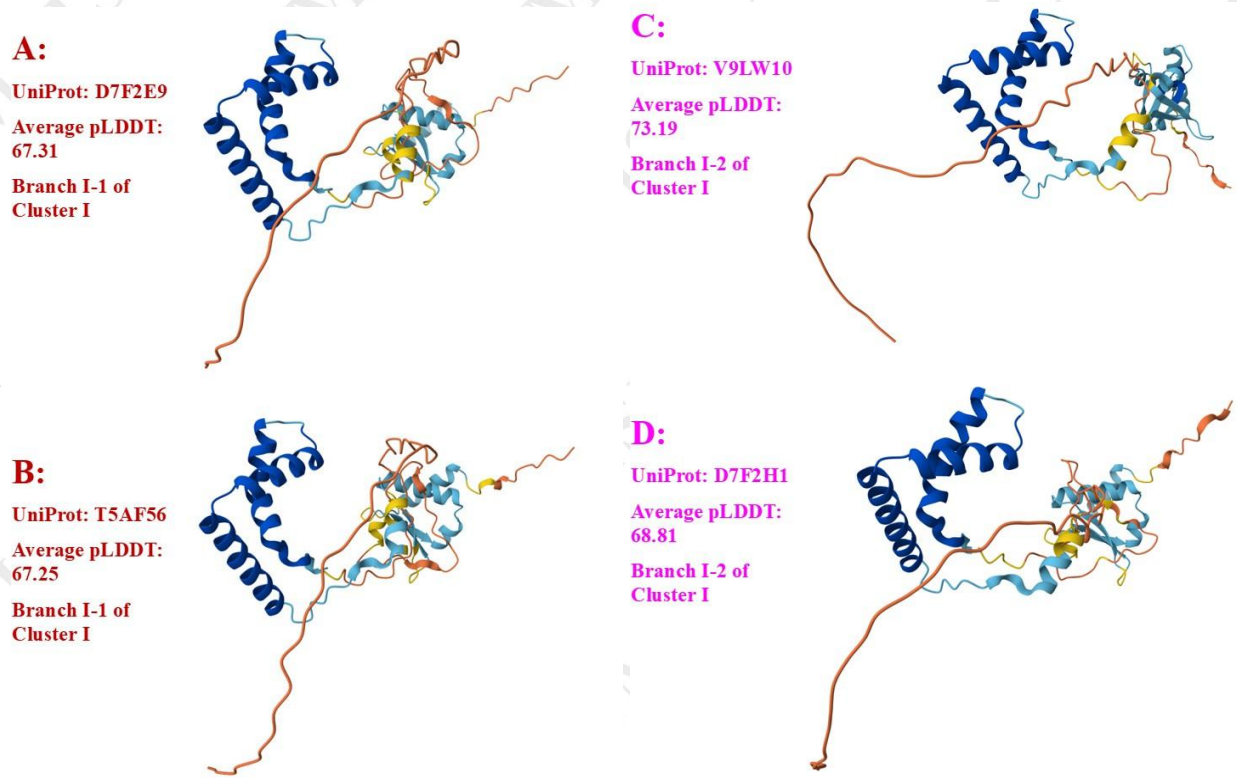


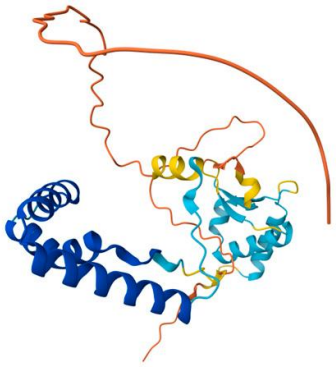
Figure 5. *Cont.*

E:

UniProt: D7F2F2

Average pLDDT: 66.44

Branch I-2 of Cluster I

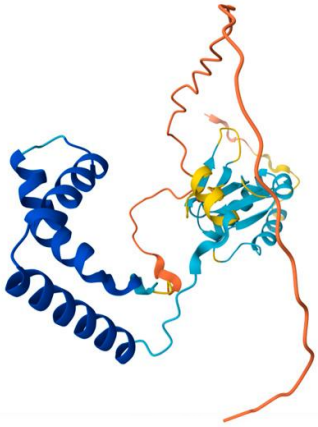


F:

UniProt: A0A0A0RCF5

Average pLDDT: 71.44

Branch II-1 of Cluster II

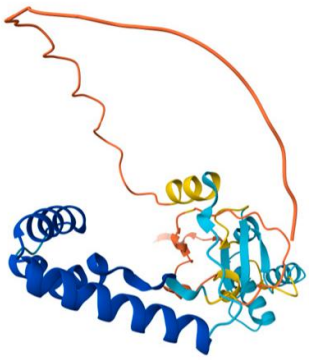


G:

UniProt: D7F2J7

Average pLDDT: 66.31

Branch II-2 of Cluster II

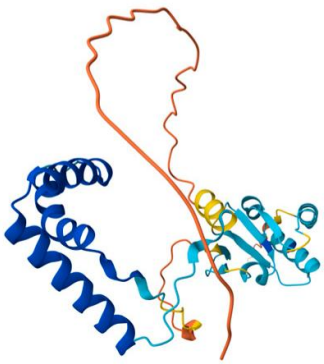


H:

UniProt: D7F2F5

Average pLDDT: 72.44

Cluster III

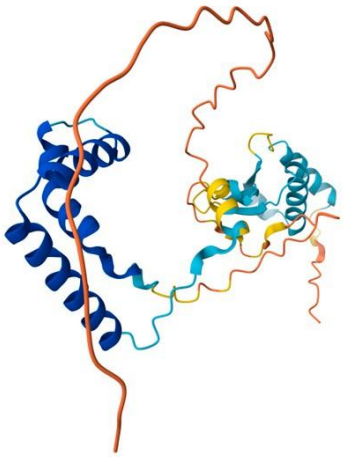


I:

UniProt: V9LWC9

Average pLDDT: 68.56

Branch IV-1 of Cluster IV

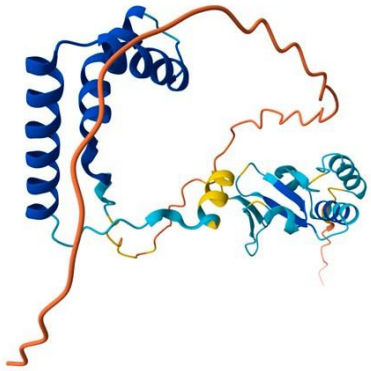


J:

UniProt: V9LVS8

Average pLDDT: 74.44

Branch IV-2 of Cluster IV

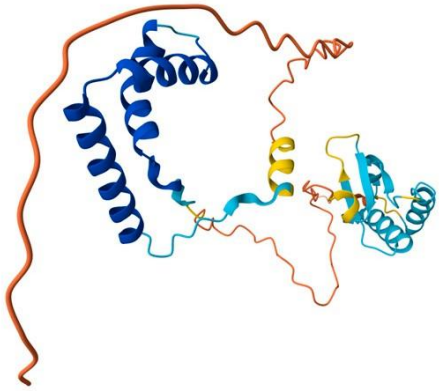


K:

UniProt: D7F2E3

Average pLDDT: 68.56

Branch V-1 of Cluster V



L:

UniProt: D7F2G5

Average pLDDT: 73.75

Branch V-2 of Cluster V

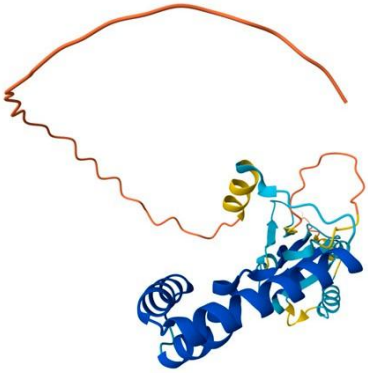


Figure 5. *Cont.*

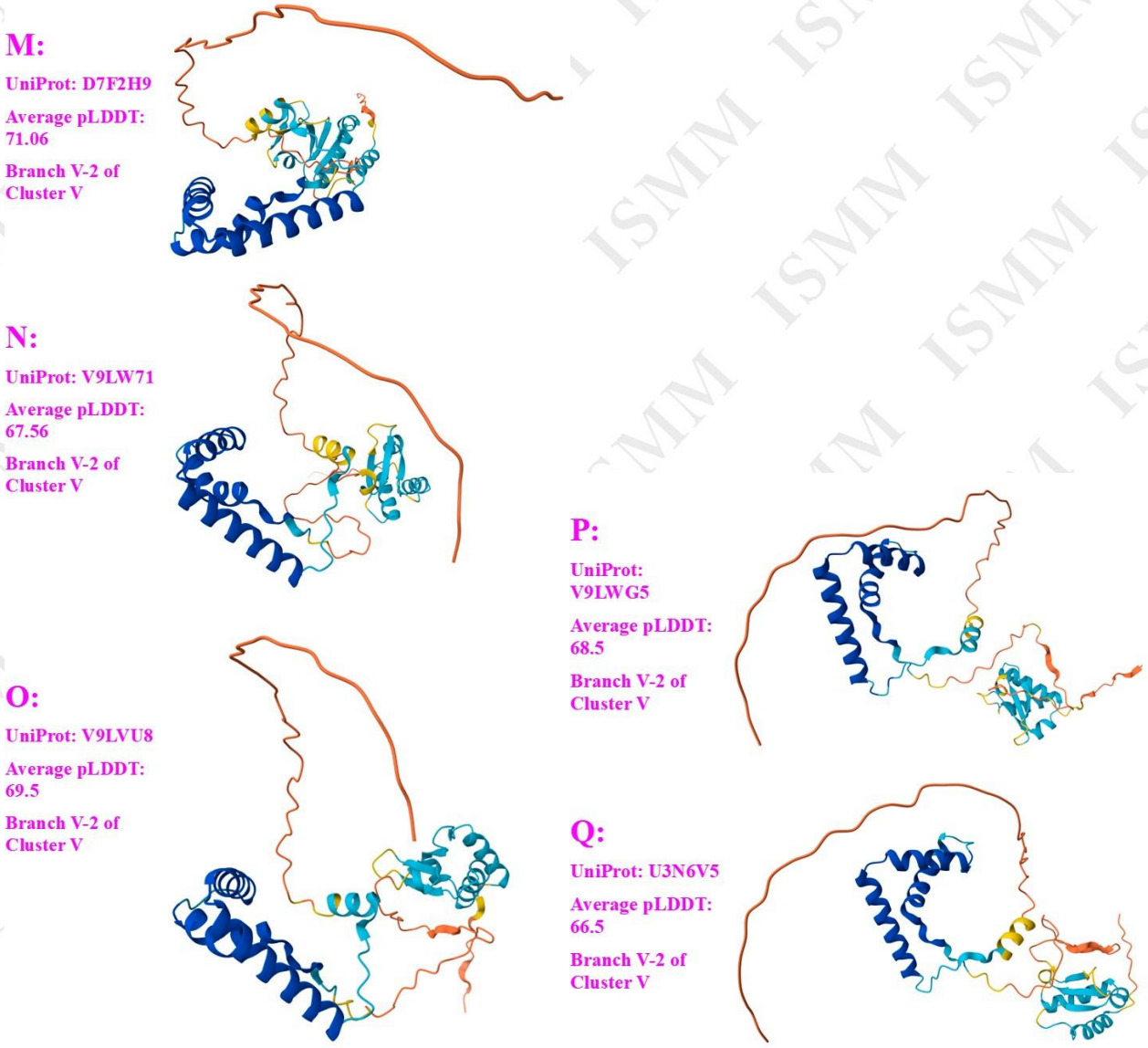


Figure 5. Seventeen 3D structural morphs (Panels **A**–**Q**) for the full-length MAT1-2-1 proteins of *H. sinensis* strains and wild-type *C. sinensis* isolates. The UniProt codes in red are for Branch 1 of each cluster shown in the Bayesian tree in Figure 2, and those in pink are for Branch 2. Model confidence: very high (pLDDT > 90), high (90 > pLDDT > 70), low (70 > pLDDT > 50), and very low (pLDDT < 50).

3.5. Primary Structures of the MAT1-1-1 Proteins

Because of the diversity of the 3D structures of the MAT1-1-1 proteins, the variations in their primary amino acid sequences were then analyzed. The 118 full-length MAT1-1-1 proteins (*cf*. Table 1) consisted of 372 amino acids and contributed to 15 diverse 3D structural morphs (*cf*. Figure 3). Among the 118 full-length proteins, 89 shared 100% sequence identity with the query protein sequence (AGW27560), whereas 20 other proteins shared 98.1–99.6% sequence similarity with the query sequence, as they contained various conservative and nonconservative substitutions of amino acid residues at isolated sites, which may havean impact on mating function. Figure 7 shows the alignment of the full-length MAT1-1-1protein sequences covering five Bayesian clusters, A–E (Branches A1, A2, A3, B, C, D1,D2, E1, E2, E3, and E4), as shown in Figure 1 and Table 1, and 15 AlphaFold 3D structural morphs, A–O (*cf*. Figure 3), as well as the

MAT1-1-1 protein sequences encoded by the genome assemblies of *H. sinensis* and the metatranscriptome assemblies of the natural *C.sinensis* insect–fungal complex.

The MAT1-1-1 protein contains a MATalpha_HMGbox domain, which is found in high-mobility group (HMG) proteins involved in DNA binding [Hu et al., 2013 [31]]. This domain is located in segment 51→225 of the query sequence AGW27560, as highlightedin blue and underlined in Figure 7. Some nonconservative residue substitutions in other MAT1-1-1 proteins were found within this domain, as shown in red in Table 3.

The full-length MAT1-1-1 protein sequence EQK97643 (372 aa) was derived from *H. sinensis* strain Co18 under the AlphaFold UniProt T5A511 and published in GenBank on 22-MAR-2015 (*cf*. Table 1; Figure 3). A segment of the genome assembly ANOV01017390 (410←1519), which was also annotated as KE657544 (410←1519) in GenBank, was derived from the same *H. sinensis* strain but published in GenBank on 20-AUG-2013 and was found to be C-terminally truncated (352 aa; 95.1% query coverage vs. EQK97643).

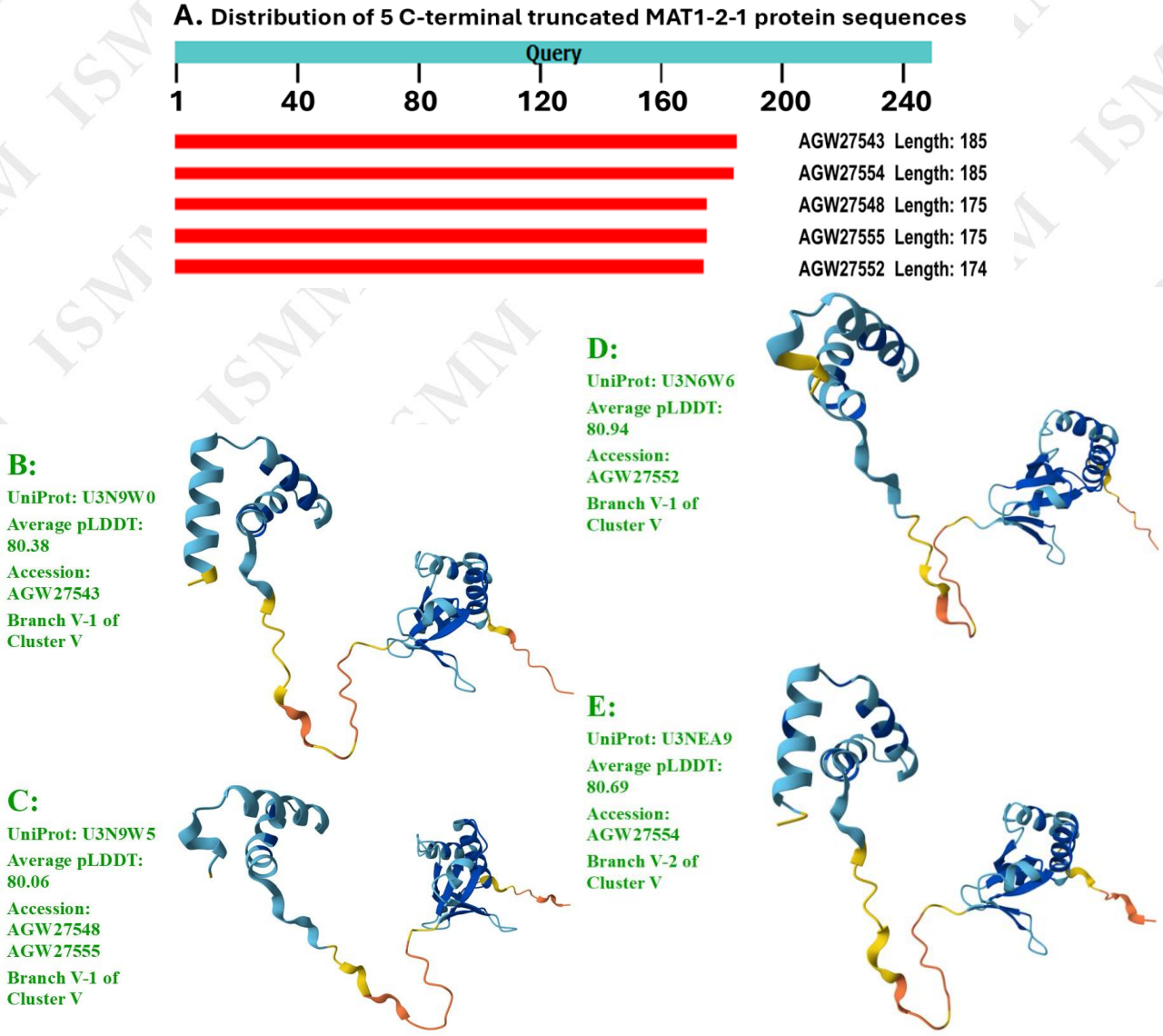


Figure 6. The sequence distribution (Panel **A**) and 3D structures (Panels **B–E**) of 5 C-terminally truncated MAT1-2-1 proteins of *H. sinensis* strains and wild-type *C. sinensis* isolates belonging to 4 diverse 3D structural morphs. Model confidence: very high (pLDDT > 90), high (90 > pLDDT > 70), low (70 > pLDDT > 50), and very low (pLDDT < 50).

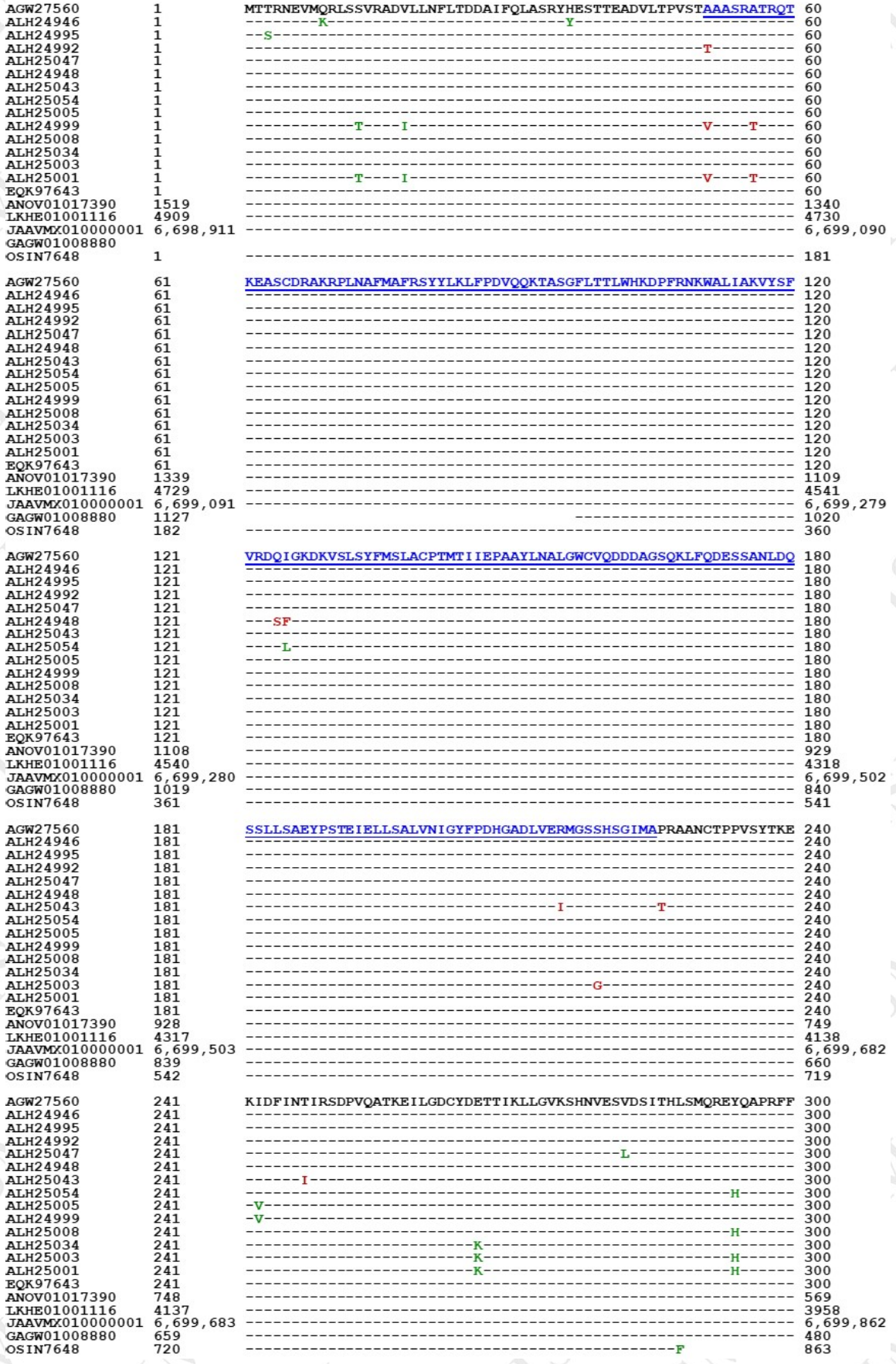

AGW27560 1 MTTRNEVMQRLSSVRADVLLNFLTDDAIFQLASRYHESTTEADVLTPVSTAAASRATRQT 60
ALH24946 1 --------K----------Y---------- 60
ALH24995 1 --S---------- 60
ALH24992 1 ----------T---------- 60
ALH25047 1 ---------- 60
ALH24948 1 ---------- 60
ALH25043 1 ---------- 60
ALH25054 1 ---------- 60
ALH25005 1 ---------- 60
ALH24999 1 ----------T----I----------V----T---- 60
ALH25008 1 ---------- 60
ALH25034 1 ---------- 60
ALH25003 1 ---------- 60
ALH25001 1 ----------T----I----------V----T---- 60
EQK97643 1 ---------- 60
ANOV01017390 1519 ---------- 1340
LKHE01001116 4909 ---------- 4730
JAAVMX010000001 6,698,911 ---------- 6,699,090
GAGW01008880
OSIN7648 1 ---------- 181
AGW27560 61 KEASCDRAKRPLNAFMAFRSYYLKLFPDVQQKTASGFLTTLWHKDPFRNKWALIAKVYSF 120
ALH24946 61 ---------- 120
ALH24995 61 ---------- 120
ALH24992 61 ---------- 120
ALH25047 61 ---------- 120
ALH24948 61 ---------- 120
ALH25043 61 ---------- 120
ALH25054 61 ---------- 120
ALH25005 61 ---------- 120
ALH24999 61 ---------- 120
ALH25008 61 ---------- 120
ALH25034 61 ---------- 120
ALH25003 61 ---------- 120
ALH25001 61 ---------- 120
EQK97643 61 ---------- 120
ANOV01017390 1339 ---------- 1109
LKHE01001116 4729 ---------- 4541
JAAVMX010000001 6,699,091 ---------- 6,699,279
GAGW01008880 1127 ---------- 1020
OSIN7648 182 ---------- 360
AGW27560 121 VRDQIGKDKVSLSYFMSLACPTMTIIEPAAYLNALGWCVQDDDAGSQKLFQDESSANLDQ 180
ALH24946 121 ---------- 180
ALH24995 121 ---------- 180
ALH24992 121 ---------- 180
ALH25047 121 ---------- 180
ALH24948 121 ---SF---------- 180
ALH25043 121 ---------- 180
ALH25054 121 ----L---------- 180
ALH25005 121 ---------- 180
ALH24999 121 ---------- 180
ALH25008 121 ---------- 180
ALH25034 121 ---------- 180
ALH25003 121 ---------- 180
ALH25001 121 ---------- 180
EQK97643 121 ---------- 180
ANOV01017390 1108 ---------- 929
LKHE01001116 4540 ---------- 4318
JAAVMX010000001 6,699,280 ---------- 6,699,502
GAGW01008880 1019 ---------- 840
OSIN7648 361 ---------- 541
AGW27560 181 SSLLSAEYPSTEIELLSALVNIGYFPDHGADLVERMGSSHSGIMAPRAANCTPPVSYTKE 240
ALH24946 181 ----------PRAANCTPPVSYTKE 240
ALH24995 181 ---------- 240
ALH24992 181 ---------- 240
ALH25047 181 ---------- 240
ALH24948 181 ---------- 240
ALH25043 181 ----------I----------T---------- 240
ALH25054 181 ---------- 240
ALH25005 181 ---------- 240
ALH24999 181 ---------- 240
ALH25008 181 ---------- 240
ALH25034 181 ---------- 240
ALH25003 181 ----------G---------- 240
ALH25001 181 ---------- 240
EQK97643 181 ---------- 240
ANOV01017390 928 ---------- 749
LKHE01001116 4317 ---------- 4138
JAAVMX010000001 6,699,503 ---------- 6,699,682
GAGW01008880 839 ---------- 660
OSIN7648 542 ---------- 719
AGW27560 241 KIDFINTIRSDPVQATKEILGDCYDETTIKLLGVKSHNVESVDSITHLSMQREYQAPRFF 300
ALH24946 241 ---------- 300
ALH24995 241 ---------- 300
ALH24992 241 ---------- 300
ALH25047 241 ----------L---------- 300
ALH24948 241 ---------- 300
ALH25043 241 ------I---------- 300
ALH25054 241 ----------H------ 300
ALH25005 241 -V---------- 300
ALH24999 241 -V---------- 300
ALH25008 241 ----------H------ 300
ALH25034 241 ----------K---------- 300
ALH25003 241 ----------K----------H------ 300
ALH25001 241 ----------K----------H------ 300
EQK97643 241 ---------- 300
ANOV01017390 748 ---------- 569
LKHE01001116 4137 ---------- 3958
JAAVMX010000001 6,699,683 ---------- 6,699,862
GAGW01008880 659 ---------- 480
OSIN7648 720 ----------F 863

Figure 7. *Cont.*

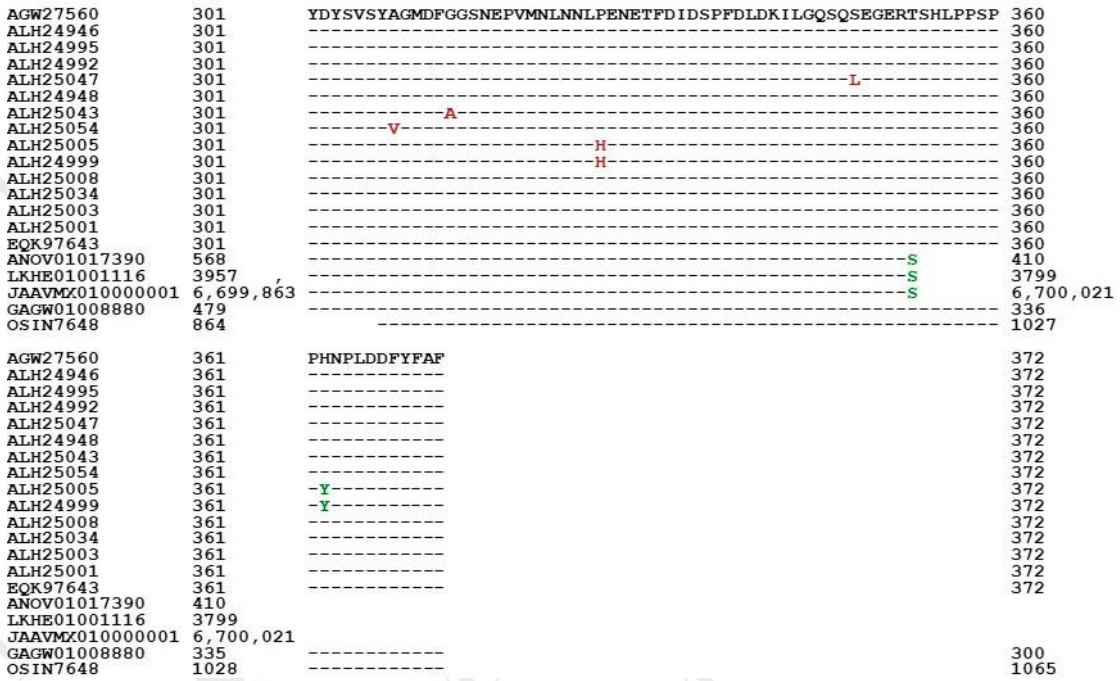


Figure 7. Alignment of the full-length sequences of representative MAT1-1-1 proteins of 15 structural morphs and corresponding translated sequence segments of the genome and metatranscriptome assemblies of *H. sinensis* strains and natural *C. sinensis*. The MATalpha_HMGbox domain is highlighted in blue and underlined in the query protein sequence AGW27560 (51→225). The residues shownin green indicate conservative amino acid substitutions, and those in red indicate nonconservativeamino acid substitutions. The hyphens indicate identical amino acid residues, and the spaces denote unmatched protein sequence gaps.

Table 3 summarizes the protein sequence alignment results, including mutant amino acid residues and the percent similarity vs. the sequences of the "likely authentic" full- length MAT1-1-1 protein AGW27560, which are correlated with the statistical and structural analytical results obtained from Bayesian clustering and 3D structure prediction (including the stereostructure morphs and the associated AlphaFold UniProt codes) (*cf*. Table 1,Figures 1, 3 and 7). The correlations shown in Table 3 indicate that the minor sequencedifferences within or outside the MATalpha_HMGbox domain of the MAT1-1-1 proteinsequences may have had an impact on the diverse 3D structures.

Among the 138 MAT1-1-1 protein sequences, 20 are truncated at both the N- and C-termini, showing 68–80% query coverage and belonging to nine diverse 3D structural morphs (*cf*. Figure 4). Eighteen of the twenty truncated proteins presented 100%sequence identity with the representative full-length MAT1-1-1 protein AGW27560 under the UniProt code U3N942 (*cf*. Table 1). Among the 18 truncated proteins, 17 were clustered into Branch A1 of Cluster A in the Bayesian clustering tree (*cf*. Figure 1); however, theprotein AGW27526 under the UniProt code U3N7G5 showed the longest Bayesian clustering distance vs. other truncated proteins and was clustered into Branch A3 of ClusterA (*cf*. Figure 1). Two other truncated proteins (AGW27522 and AGW27536) under theUniProt codes U3N6U0 and U3N7H7 shared 99.6–99.7% sequence similarity with the query sequence AGW27560 with either a nonconservative P-to-L substitution or an L residuedeletion and were clustered into Branches A2 and A3 of Cluster A,respectively.

Table 3. Summary of the full-length MAT1-1-1 protein sequence alignment results (mutant aminoacids and the percent

similarity vs. the likely authentic protein AGW27560), correlating with the Bayesian branches/clusters and the 3D structural models and associated with the AlphaFold UniProt codes.

Accession Number	% Similarity to AGW27560	Amino Acid Residue Substitution		Bayesian Cluster		3D Structure Model	AlphaFold UniProt Code
		Conservative	Nonconservative	Branch	Cluster		
AGW27560 ALH24946 EQK97643	100% 99.4% 100%	Q-to-K, H-to-Y		A1	A	A B C	U3N942 A0A0N9QMM1 T5A511
ALH24992 ALH24995	99.7%	T-to-S	A-to-T	A2	A	D E	A0A0N9R5B3 A0A0N7G849
ALH25047 ALH24948	99.4%		S-to-L	A3	A	F G	A0A0N9R4V2 A0A0N9QUF3
ALH25043	98.9%		R-to-I, P-to-T, T-to-I, G-to-A,		B	H	A0A0N9QMS9
ALH25054	99.4%	I-to-L	A-to-V		C	I	A0A0N7G845
ALH25005	99.2%	H-to-Y	P-to-H	D1	D	J	A0A0N9QUK2
ALH24999	98.1%	S-to-T, I-to-V, H-to-Y	A-to-V, A-to-T	D2	D	K	A0A0N9QMT4
ALH25008	99.7%	Y-to-H		E1	E	L	A0A0N9QMR3
ALH25034	99.4%	E-to-K, Y-to-H		E2	E	M	A0A0N9QMS4
ALH25003	99.2%	E-to-K, Y-to-H	S-to-G	E3	E	N	A0A0N7G850
ALH25001	98.4%	S-to-T, V-to-I, E-to-K, Y-to-H	A-to-V, A-to-T	E4	E	O	A0A0N9R4Q4

Note: The amino acid residue substitutions shown in red indicate nonconservative changes within the MATalpha_HMGbox domain of the MAT1-1-1 proteins. Other residue substitutions shown in black are located within or outside the MATalpha_HMGbox domain.

Figure 7 also shows the C-terminally truncated MAT1-1-1 proteins encoded by the genome assemblies ANOV01017390, LKHE01001116, and JAAVMX010000001 of *H. sinensis* strains Co18, 1229, and IOZ07, respectively [Hu et al., 2013 [31]; Li et al., 2016 [40], 2024 [37]; Shu et al., 2020 [43]]. The truncated MAT1-1-1 proteins encoded by the genome assemblies had a deletion of 19 amino acid residues (SHLPPSPPHNPLDDFYFAF) at the *C-termini* and contained a nonconservative T-to-S substitution (*cf*. Figure 7). The MAT1-1-1 protein encoded by the metatranscriptome assembly GAGW01008880 of natural *C. sinensis* is truncated by 96 amino acids at the N-terminus (MTTRNEVMQRLSSVRADVLLNFLTDDAIFQLASRHESTTEADVLTPVSTAAASRATRQTKEASCDRAKRPLNAFMAFRSYYLKLPDVQQ-QKTASG) partially with and outside the MATalpha_HMGbox domain [Hu et al., 2013 [31]; Xiang et al., 2014 [45]; Li et al., 2024 [37]]. The MAT1-1-1 protein encoded by the meta- transcriptome assembly OSIN7648 features midsequence truncation with a deletion of 18 amino acids (SMQREYQAPRFFYDYSVS) outside the MATalpha_HMGbox domain and a nonconservative L-to-F substitution within the exon II-encoding region of the *MAT1-1-1* gene [Xia et al., 2017 [46]; Li et al., 2024 [37]].

To be continued

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Call for Papers

Aiming to build the relationship between the members and the Society, the publication of the newsletters was proposed before the launching of the Society. The newsletters represent one of the key official publications from the Society. Contents of the newsletters will include notifications of the decisions made by the committee board, reviews or comments contributed by ISMM committee members, conferences or activities to be organized, and the status updated in research, industrialization, and marketing for medicinal mushrooms. The newsletters will be released quarterly, by the first Monday of every January, April, July, and October, with possible supplementary issues as well. The Newsletter is open to organizations or professionals to submit news, comments, or scientific papers relating to medicinal mushroom research, marketing, or industry.

Contact information

For any inquiry in membership enrollment, subscribing to ISMM newsletters, upcoming activities and events organized by ISMM, or submitting news reports, statements, or manuscripts to the Society, please contact the secretariat's office in Beijing, China.

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