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.

Honorable President: Prof. S.T.Chang, Prof.S.P. Wasser
President: Academician Li Yu
Executive President: Mr. Chen Hui
Secretary General: Mr. Liu Ziqiang

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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News Reports

A Tribute to Professor Shu-Ting Chang on his 95th Birthday

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On September 30th, 2025, Professor Shu-Ting Chang, S-T to many of his friends and acquaintances, editor of the *International Journal of Medicinal Mushrooms*, celebrated his 95th birthday (Fig. 1). Here, we look at Professor Chang's academic accomplishments, including his contribution to the development of culinary-medicinal mushrooms in China, Africa, South America; provide a review of his academic career; master principles; his pyramid model for edible mushrooms; and his wishes for the development of mushroom industry.

Professor Chang has spent well over 65 years promoting the cultivation of mushrooms as food and medicine. He has over 240 published scientific papers and has authored 23 books. He is also one of the initiators and editors at the *International Journal of Medicinal Mushrooms*. Prof. Chang is 95 years of age but remains in relatively good health and always has a smile on his face.

The name Shu-Ting Chang occupies a prominent place in the world of mycology because of his outstanding input into the field of mushroom biology, mushroom cultivation, and medicinal mushrooms. He is a world leader in mushroom biology and medicinal mushroom science. During his 65-year career in research on basic and applied aspects of mushrooms, he has published 10 papers in the *International Journal of Medicinal Mushrooms*.

Professor Chang is also a great teacher. Many of his students have received both doctoral and master's degrees. He is not only an academic teacher, but he is a practical teacher for mushroom growers and farmers around the world, including China and some African and South American countries. And he is a great scientific organizer. He is one of the main organizers of all 10 International Medicinal Mushroom Conferences. Professor Chang was one of the initiators of publishing our special *International Journal of Medicinal Mushrooms*. He has been an editor of our journal from its inception (in 1999) until now.

Professor Chang was born in Yuanping, Shanxi, China, but he is Australian by nationality since 1973. He is married to Judy Li-Ju Chang (nee Lee). Their children are David Ming-Tsan, Barbara Ming-Wai, Judy Ming-Sze, Ernest Ming-Cheng, and Jennifer Ming-Jing.

Professor Chang graduated from the National Taiwan University and received a bachelor of science degree in 1953. He received his master of science from the University of Wisconsin in 1958. He stayed at the University of Wisconsin to pursue his Ph.D., which he received in 1960. After receiving his Ph.D., he started his professional career at the Chinese

University of Hong Kong (CUHK). He began as an assistant lecturer in biology and worked his way up to emeritus professor.

FIG. 1: *Professor Shu-Ting Chang*

Throughout his scientific career, over a span of 50 years, Professor Chang has been connected to CUHK in one way or another. He also influenced administrative changes in the Department of Biology at CUHK. For 11 years (from 1983 to 1994), he served as chairman of the Department of Biology. From 1974 to 1977, he was the dean of the Faculty of Science. From 1979 to 1981, he served as the director of the Office of Student Affairs. From 1975 to 1985, he was the director of the Research Laboratory for Food Protein Production. From 1983 to 1994, he was director of the Marine Science Laboratory. From 1985 to 1992, Professor Chang was director of the Institute of Science and Technology. In 1980, he was the head of the Division of Biology in the graduate school. From 1983 through 1994, he was chairman of the Board of Studies in Biology. Professor Chang became chairman of the Science Center Management Committee, which he served on for 3 years. From 1991 to 1992, he was director of the Chinese Medicinal Material Research Center. From 1993 to 1995, he was chairman of the Science Engineering Complex Management Committee.

Since 1960, Professor Chang's main field of research has been mushroom biology, mushroom technology for cultivation, and mushroom biotechnology for tonic and medicinal products. The main philosophy and drive behind this line of research for the past 65 years can be summarized as follows.

Modern technology for human civilization is expanding every day. However, human beings still face and will continue to face three basic problems capable of causing crises: shortage of food, pollution of the environment, and diminishing quality of human health resulting from a continuously increasing world population.Mushrooms (macrofungi) not only can convert the huge lignocellulosic biomass waste into rich protein food, but can also produce notable nutriceutical and pharmaceutical products that have many health benefits. The most significant aspect of mushroom cultivation is to create pollution-free or zero emission environments.

In addition, mushroom-based farming and industry could provide employment to the youth and women, particularly in rural areas in less developed countries.

Mushrooms are relatively fast-growing organisms. Some tropical mushrooms can be harvested and consumed within 10 days after spawning. By using different varieties, mushrooms can be cultivated all year round.

Professor Chang's main research areas are: (1) the life cycle of mushrooms using genetic and cytological techniques; (2) improvement of mushroom strains by genetic manipulation including molecular markers and protoplast fusion techniques; (3) nutritive values and medicinal effects of mushrooms; (4) development and improvement of cultivation technology; (5) development of new concepts of mushroom biology, mushroom science (concerned with mushroom production), and mushroom biotechnology (concerned with mushroom products); and (6) introduction and development of the terms and concepts: "what is a mushroom?," "mushroom biology," "mushroom nutriceuticals," and the "non-green revolution" concept. This research has improved the supply of nutritious food for human consumption, the quality of life for humanity, and the condition of polluted environments.

As the result of Professor Chang's 65 years of research on the basic and applied aspects of mushrooms, the following major contributions have already had and will continue to have an impact at national, regional and global levels.

In 1969, cotton wastes from cotton textile industry were used for the first time to grow straw mushrooms (*Volvariella volvacea*) in Hong Kong. Protoplast techniques were used to breed high-temperature strains of shiitake culinary-medicinal mushroom (*Lentinus edodes*). The new disciplines of mushroom biology and mushroom biotechnology were established, leading to the First International Conference on Mushroom Biology and Mushroom Products, held at the Chinese University of Hong Kong, August 23–26, 1993, and also leading to the formation of the World Society of Mushroom Biology and Mushroom Products (WSMBMP). The term and concept "mushroom nutriceuticals" was established with Professor J.A. Buswell in 1996. Mushroom cultivation and mushroom products were named as the non-green revolution" for the first time in 1998 because of the positive effects of cultivation and development of edible and medicinal mushrooms on equitable economic growth and human welfare. The benefits of mushrooms include their use as food, health tonics, medicine, animal feed, and fertilizer, and for protecting and regenerating the environment, for promoting sustainable development, and for contributing positively to economic and social conditions.

Professor Chang is an author or co-author of 23 books. Special attention must be drawn to his latest book with Professor Ph.G. Miles, published in 2004, entitled *Mushrooms: Cultivation, Nutritional Value, Medicinal Effect, and Environmental Impact*. This book is an encyclopedic review of mushroom biology, including cultivation, nutritional value, medicinal value, and environmental impact, and it emphasizes worldwide trends and developments in mushroom biology from an international perspective. It is highly recommended for medicinal mycologists, mushroom growers, botanists, plant pathologists, and professionals and scientists in related fields. The book shows that mushroom cultivation has and will continue to have a positive global impact on long-term food nutrition, healthcare, environmental conservation, regeneration, and economic and social change, and its value cannot be overstated. The potentially extensive use of this book can be recognized in universities, classrooms, laboratories, etc., as it is useful for beginning students through PhD studies and beyond. No past or current book comes close to covering all of the areas concerning mushrooms considered in this book.

Professor Chang is Honor President of the International Society of Medicinal Mushrooms. He has served as the Vice-President of the World Society for Mushroom Biology and Mushroom Products. He was a member of the Executive Committee of International Society for Mushroom Science (ISMS) from 1996 to 2004. He was also the President of the International Mushroom Society for the Tropics from 1981 to 1995, National-Point-of-Contact Representative of Hong Kong for the UNESCO Regional Network of Microbiology in Southeast Asia from 1981 to 1993, and Executive Secretary of the Headquarters of the UNESCO Regional Network of Microbiology in Southeast Asia from 1984 to 1987. He was

elected to be a member of the Executive Board of International Union of Microbiological Societies (IUMS) from 1990 to 1994, and is a member of the Standing Committee on Membership, Structure and Status of International Council of Scientific Unions (ICSU) from 1993 to 1996.

Professor Chang was one of the initiators of our special *International Journal of Medicinal Mushrooms*. He is and has been an editor of the journal since its inception in 1999. He has been a critical and proficient reviewer of several articles. He has also been an author himself. During this time, he published very important keynote papers in our journal. They are:

- Global impact of edible and medicinal mushrooms on human welfare in the 21st century: nongreen revolution (*Int. J. Med. Mushrooms* 1999; 1;1–8).
- World production of cultivated edible and medicinal mushrooms in 1997 with emphasis on *Lentinus edodes* (Berk.) Sing. in China (*Int. J. Med. Mushrooms* 1999; 1:291–300).
- *Ganoderma lucidum* (Curt.:Fr.) P.Karst. (Aphyllophoromycetidae) – a mushrooming medicinal mushroom (*Int. J. Med. Mushrooms* 2000; 2:139–46).
- A 40-year journey through bioconversion of lignocellulosic wastes to mushrooms and dietary supplements (*Int. J. Med. Mushrooms* 2001; 3:299–310).
- The world mushroom industry: trends and technological development (*Int. J. Med. Mushrooms* 2006; 8:297–314).

The need for scientific validation of culinary-medicinal mushroom products (*Int. J. Med. Mushrooms* 2006; 8:187-95). In this paper, Professor Chang suggested a very important proposal adopting the G" guidelines to produce quality mushroom products, where GLP is Good Laboratory Practice, GAP is Good Agriculture Practice, GMP is Good Manufacturing Practice, GPP is Good Production Practice, and GCP is Good Clinical Practice.

Development of the culinary-medicinal mushrooms industry in China: past, present, and future (*Int. Med. Mushrooms* 2006; 8:1-17). In this paper, Prof. Chang brought attention to the world's mycological and mushroom growers society's achievements in the culinary-medicinal mushroom industry in China. In 1978, Professor Chang was invited to conduct the first mushroom training workshop in China. At that time, production of cultivated mushrooms in China was only 60,000 tons and after 25 years, in 2003, China mushroom production had increased to 10.4 million tons (approximately 70% of the world's total production). Now, China is the largest producer, consumer and exporter of mushrooms in the world. China produced 43.4 million mushroons in 2023, and produces over 80% of world's shiitake culinary-medicinal mushrooms. Chang mentioned in his paper that the reason why China was so successful in the development of the mushroom industry and underlined these reasons: (1) strong leadership and initiatives of central and institutions, (2) there are a lot of innovations in mushroom cultivation local governments, and (3) strong scientific support from academic technology by mushroom farmers. The growth of domestic markets because of a strong national economy, which has been a key factor in the expansion of China's mushroom cultivation. In this breakthrough paper Chang also elucidated possibilities for future development in the medicinal mushroom industry and suggested to Chinese colleagues to pay attention to some unsolved problems in production of Chinese mushrooms and in cultivation improvement. The last part of his paper was dedicated to how successful China was in development of its mushroom

industry and should serve as an example for what is possible for other countries, especially for developing countries.

- Development of the world mushroom industry: Applied mushroom biology and international mushroom organizations (*Int. J. Med. Mushrooms* 2008; 10:195–208).

- The role of culinary-medicinal mushrooms on human welfare with a pyramid model for human health (*Int. J. Med. Mushrooms* 2012; 14(2):95–134).

- Current and future research trends in agricultural and biomedical applications of medicinal mushrooms and mushrooms products (*Int. J. Med. Mushrooms* 2018; 20(11):1034–48).

In 2017, we published together with Professor Chang in the *Oxford Research Encyclopedia of Environmental Science* (Oxford University Press; DOI: 10.1093/acrefore/9780199389414.013.231) a chapter dedicated to the cultivation and environmental impact of mushrooms. We highlight that mushrooms can be used as food, tonics, medicines, as cosmeceuticals, and natural biocontrol agents in plant protection with insecticidal, fungicidal, bactericidal, herbicidal, nematoticidal, and antiphytoviral activities. The multidimensional nature of the global mushroom cultivation industry, its role in addressing critical issues faced by humankind and its positive contributions are presented below. Furthermore, mushrooms can serve as agents for promoting equitable economic growth in society. Because lignocellulose wastes are available in every corner of the world, they can be properly used in the cultivation of mushrooms and therefore could pilot a so-called white agricultural revolution in less developed countries and in the world at large. Mushrooms demonstrate a great impact on agriculture and the environment and have great potential for generating a great socio-economic impact on human welfare on local, national, and global levels.

Since 1977, as an eloquent and prominent speaker, Professor Chang has been associated with agents of the UN, e.g., UNESCO, UNDP/UNOPS, UNU, UNIDO, and FAO as a consultant on mushroom cultivation and mushroom products. Since 2000, Professor Chang has been invited twice by the Commonwealth Secretariat in London to serve as a consultant on mushroom farming in Namibia. He has also been invited to conduct many training workshops/courses on mushroom biology and mushroom biotechnology all over the five continents, most recently in African and Latin American countries, sponsored by UNDP/UNOS. He has also been invited to serve as scientific advisor and Honorary Professor for over 30 research institutes and universities in China. Professor Chang has also been honored in receiving a lot of different awards. The diversity of Chang's research programs, scientific, organizational, and pedagogical activities and his significant achievements in various fields of mycology represent him as a scientist of a wide scope of interests, a brilliant science organizer, a world scientific leader of the school of mushroom biology, whose achievements and accomplishments became incorporated into the world of science.

Professor Chang was the honor President and main organizer of the 5th International Medicinal Mushrooms Conference (September 2009, Nantong, China) and, 7th International Medicinal Mushrooms Conference in August 2013, Beijing, China) and 10th International Medicinal Mushrooms Conference (September 2019, Nantong, China) (Fig. 2).

Professor Chang has an ability to learn languages and easily adapts to various cultures, and is currently living in Canberra, Australia, with his family. He is an extraordinary, vibrant man in the broadest sense of the word. People are attracted to him like a magnet because of his charm, wit, and erudition. Chang's wide circle of interests, his clarity of scientific vision, his enormous energy, and his enthusiasm and honesty are just a few of his many great personal attributes.

His hobbies are traveling, reading, golf, cycling, and walking. From the first encounter with him, you sense the warmth of his personality and his outgoing, unconventional nature. Professor Chang is not only a creative and gifted scientist, but also an intellectual with a broad education. As Professor Chang celebrates his 95th birthday, we friends, colleagues, collaborators, and students wish him good health (Fig. 3).

FIG. 2: *Prof. S.T. Chang with Prof. S.P. Wasser and Mr. Hui Chen, during the 10th International Medicinal Mushroom Conference, September 2019, Nantong, China*

FIG. 3: *Prof. S.T. Chang at Jiangsu Alphay Biological Technology Co. Ltd. in China*

Source: International Journal of Medicinal Mushrooms. 27(10):1-5 (2025)

The 2025 International Mushroom Days

The 2025 International Mushroom Days themed "Live in the Present, Focus on the Future" was held form 14th to 16th April, 2025, at Xiamen Fliport Hotel C&E Center, Fujian, China.

This expo marks the 4th edition of the premier international event dedicated to the entire mushroom industry chain since 2020, covered an exhibition area of approximately 17,000 square meters, featuring 251 participating enterprises, with 7 overseas exhibitors from the Netherlands, France, Germany, and Japan. Nearly 500 guests from 35 countries of the world attended the event.

The 2025 International Mushroom Days opening ceremony

Mr. Xu Xiaohu, Vice President of China Chamber of Commerce for Import & Export of Foodstuffs, Native Produce & Animal By-Products (CFNA), said "China is now the world's largest producer and consumer of edible fungi, accounting for over 70% of global output for consecutive years. In 2024, the industry's value exceeded RMB 380 billion, with exports reaching more than 150 countries and regions, generating nearly USD 2 billion in annual foreign exchange revenue."

Mr. Xu Xiaohu, Vice President of CFNA

As a vital sector embodying the macro-agriculture and macro-food perspectives, China's edible fungi industry has achieved leapfrog development in recent years with broad prospects, yet faces multifaceted challenges. This expo's

theme 'Live in the Present, Focus on the Future' aims to pool industry wisdom—seizing opportunities amidst challenges and pioneering innovation while upholding core principles."

Mr. Shi Wei, Chairman of Hongzhen Bio-Tech Group, highlighted the company's R&D said: "After 12 years of research, we established China's first fully independent mechanized production line for cultivated porcini mushrooms, securing 80 core patents to fill the domestic gap in premium edible fungi strains. Notably, we achieved reverse technology transfer to seed powerhouses like South Korea and Japan—a first for Chinese edible fungi enterprises. With strategic investments from six state-owned funds, we will amplify R&D investment, accelerate industrial upgrading, and enhance global brand influence to propel China's edible fungi industry onto the world stage."

Mr. Shi Wei, Chairman of Hongzhen Bio-Tech Group

Advancing toward global fungi leadership requires breakthroughs in strain breeding, deep processing, and international trade. The expo integrates resources to build collaborative platforms driving technological advancement. Dr. Huang Chenyang, Chief Scientist of China's National Edible Fungi Industrial Technology System, emphasized: "This event provides critical insights, knowledge exchange, and market expansion opportunities. We advocate establishing it as an international brand to boost global competitiveness and open a new chapter of high-quality industry development."

Dr. Huang Chenyang, Chief Scientist, Institute of Agricultural Resources and Regional Planning, CAAS

Dr. Guo Liangdong, Director-General of the Mycological Society of China, remarked: "China's edible fungi industry exhibits vigorous growth, becoming an essential and dynamic agricultural sector that boosts rural incomes and revitalization. Our Society remains committed to uniting mycologists nationwide—enhancing education, advancing research, and scaling scientific frontiers."

Dr. Guo Liangdong, Director-General of Mycological Society of China, Institute of Microbiology, CAS

Mr. Zheng Zhi

Mr. Zheng Zhi, Vice Chairman of the Asian Convention and Exhibition Association Alliance, Chairman of the China Urban Convention and Exhibition Association Alliance, and Lifetime Founding President of the Xiamen Convention and Exhibition Association, noted: "Since launching in Xiamen in 2020, the expo has driven high-quality industry growth through innovation and global collaboration. Over five years, it has facilitated over 100 partnerships, advanced international standards integration, and injected momentum into China's dual-circulation development paradigm."

Mr. Liu Ziqiang, Secretary-General of CFNA's Edible Fungi Committee, and Ms. Lv Wenjing, Director of China National United Advertising Corporation., signed an agreement to accelerate China's "Edible Fungi Brand Cultivation Initiative."

Signing Ceremony between CFNA and China National United Advertising Corporation

Mr. Zhao Dongming, Deputy Secretary-General of CFNA's Edible Fungi Committee, and Mr. Lin Wanhe, Director of Haha Online, inked a pact to collaborate on brand globalization, industrial innovation, talent development, and multi-sector integration—advancing rural revitalization and employment through mushroom-centric cultural tourism.

Signing Ceremony between CFNA and Haha Online

Professor Li Yu, Academician of the Chinese Academy of Engineering, President of the International Society for Medicinal Mushrooms, National Poverty Alleviation Model, and Chair of Jilin Agricultural University's Academic Committee, officially declared the event open.

Professor Li Yu

Up-coming Events

Asian Mycological Congress

The Asian Mycological Congress (AMC), organized by the Asian Mycological Association (AMA), serves as a significant international scientific forum held every two years. It facilitates the exchange of ideas and advancements in mycological research among scientists from various nations. In 2025, AMC will take place in Guangzhou, China, from November 23rd to 26th, 2025. The event is hosted by Zhongkai University of Agriculture and Engineering, in collaboration with the Chinese Society of Mycology and the Asian Mycological Association.

AMC 2025 will feature a diverse range of topics covering both theoretical and applied aspects of mycology. It aims to bring together researchers, educators, and industry professionals to present their latest findings, foster collaboration, and explore innovative solutions in the field. The upcoming congress in Guangzhou highlights a strong commitment to advancing mycological research and strengthening international cooperation. We warmly invite colleagues from academia, industry, and related fields to join us for this prestigious event.

Scientific Program

Main Sessions:

The Asian Mycological Congress 2025 offers a comprehensive scientific program designed to bring together researchers, students, and professionals to explore the latest developments and challenges in mycology. The program includes a wide range of sessions covering fundamental and applied aspects of fungal biology.

1. Taxonomy and Nomenclature

2. Fungal Metabolites and Bioactive Compounds
3. Clinical Mycology
4. Genetics and Breeding of Edible Fungi
5. Fungal Systematics and Diversity
6. Mycotoxins and Toxicogenic Fungi
7. Population Genetics and Genomics
8. Edible Fungal Resources and Cultivation
9. Recent Advancements in Fungi-like taxa
10. Fungal Plant Pathology
11. Freshwater and Marine Fungi
12. Ecology and Environmental Mycology
13. Interactions with other Microorganisms
14. Fungal Plant Interactions
15. Fungi in Green Agriculture
16. Mycorrhiza and Endophytic Fungi
17. Evolution and Diversification
18. Entomogenous Fungi
19. Medicinal Fungi
20. Mycology in Asia-the future

Each session will feature plenary addresses, oral presentations, and poster discussions to provide a platform for knowledge exchange and networking among experts and early-career researchers. Join us to explore the dynamic world of mycology and its potential to address global challenges.

Special Graduate Student Sessions:

Graduate students are encouraged to showcase their research and participate in focused sessions. These include:

1.Fungal Systematics and Diversity
2.Applied and Environmental Mycology
3.Fungal Pathology

Registration Process

Online Registration:

o All participants must register online through the official AMC 2025 website: https://www.amc2025.cn

o Complete the registration form with accurate personal and professional details.

Payment:

o Registration is confirmed only after full payment is received.

o Payment methods include credit/debit cards, bank transfers, or other options as specified on the registration page.

o A confirmation email will be sent upon successful payment.

o The bank information for receiving the payment is as follows:

BENEFICIARY: wu han yi gu wang luo gu fen you xian gong si

ACCTOUNT NO: 3202105819100377683

SWIFT CODE: ICBKCNBJHUB

RECEIVING BANK: INDUSTRIAL ANDCOMMERCIALBANKOF CHINAHUBEI PILOT FREE TRADE ZONE WUHAN BRANCH

BANKADDRESS: NO.780, HIGH-TECHROAD, HONGSHANDISTRICT, WUHAN, HUBEI, CHINA

Early Bird Registration:

o Take advantage of discounted rates by registering before the **early bird deadline: [August 31th, 2025]**.

o Standard rates apply after this deadline.

On-site Registration:

o On-site registration will be available during the conference. However, pre-registration is strongly encouraged to ensure access to all sessions and conference materials.

Registration Categories and Fees

Category	Early Bird Fee	Standard Fee
Regular Participant	$350	$400
Student (with valid ID)	$300	$320
Accompanying Person	$180	$220
On-site Registration	-	$450

Note: Accompanying persons will not have access to scientific sessions but can attend social events.

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; website: https://www.saas.sh.cn/wsmbmp/about

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

Support for mushroom picking policy in forests: the role of values and awareness

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Abstract: Mushrooms are of great importance to the well-being of forest eco-systems and of humans. Extensive mushroom picking may threaten the long-term conservation of mushrooms in forests. Regulations governing the amount and types of mushrooms collected in forests may serve as an effective means of combatting the dangers of extensive mushroom picking. Such regulations restrict pickers' ability to collect mushrooms. Hence, it is important to understand the drivers for supporting such policies. Utilizing a national representative survey in Israel, the present study examined the role of values, environmental awareness, and environmental attitudes in explaining public support for mushroom picking regulations. The results show that environmental awareness, and environmental worldview positively predict support for mushroom regulation, whereas hedonistic values are associated with lower levels of support. The two regulations that received the highest support were picking mushroom species that are not threatened by extinction and picking in areas where there are no mushrooms in danger of extinction. Research findings highlight the importance of intra-personal factors in explaining individual support for such policy as well as the importance of integrating social science research in the study of ecological science. Furthermore, research findings highlight the importance of education and of enhancing public awareness of the mutual connections between mushroom and forests ecology.

Keywords: Environmental awareness, Personal values, Environmental policy support, Pro-environmental behavior, Forest ecology

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Mushrooms: Potential Agents for the Prevention and Slowdown of Alzheimer's Disease: A Review

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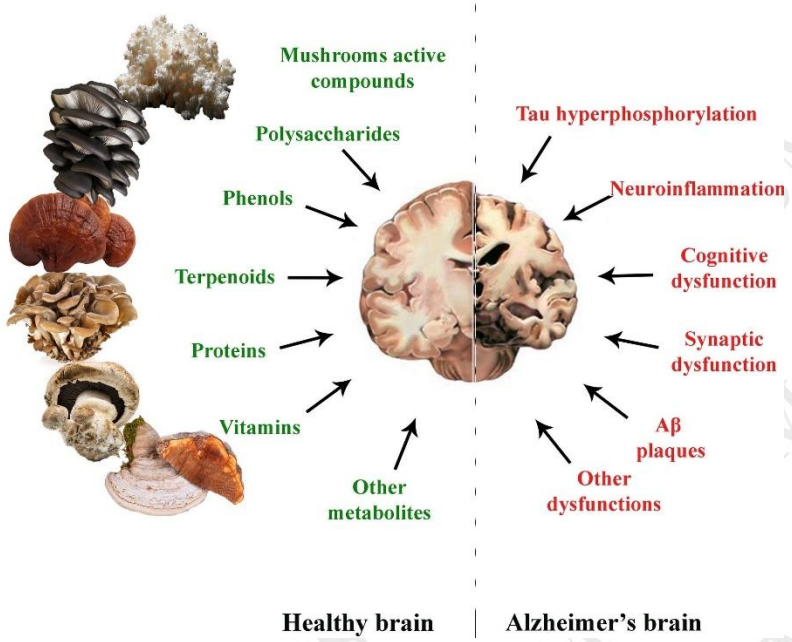
Abstract: Alzheimer's disease as a neurodegenerative disorder is characterized by a decline in cognitive abilities that makes it difficult or impossible to perform ordinary tasks. It is the most common form of dementia and its exact causes are still unknown. Approximately 45.0 million people are affected by this disease, which is the leading cause of death worldwide. Although numerous commercial drugs are available on the world market, many of them have mutagenic, toxic, carcinogenic and other side effects. Therefore, today the world's trend is use of natural products without any harmful effects. Edible and medicinal mushrooms as producers of numerous biologically active compounds, such as polysaccharides, proteins, sterols, terpenoids, etc., could be a safe and effective neuroprotective agents and a promising therapy for patients with Alzheimer's disease. Mushrooms are highly valued functional foods and diet supplementation with them could significantly reduce the risk of apparence of Alzheimer's disease or slow down its development. The results of numerous studies have shown that the addition of mushrooms to the diet not only increases the effectiveness of conventional drugs but also reduces their harmful effects. However, despite numerous studies on mushrooms' medicinal properties, much more *in vivo* research and clinical trials are still needed to fully understand the potential of mushrooms for the prevention and treatment of Alzheimer's disease, as well as to determine their optimal administration. Reviewing all the results so far and considering future necessary studies were the main aims of this review article.

Figures



Keywords: Alzheimer's disease, medicinal mushrooms, mushrooms` metabolites, neuroprotective activity

International Journal of Medicinal Mushrooms, Volume 27, Issue 10, 2025, pp. 7-19

DOI: 10.1615/IntJMedMushrooms.2025059428

Molecular Identification Technologies in Authentication of Chinese Caterpillar Mushroom *Ophiocordyceps sinensis* (Ascomycota) and Related Species: A Review

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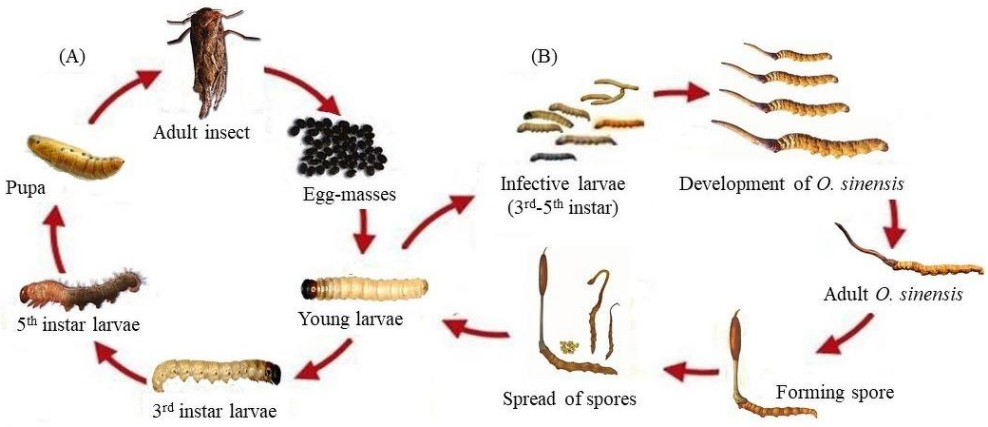
Abstract: *Ophiocordyceps sinensis* fruit bodies, an insect-fungi complex, is a renowned ingredient in traditional Chinese medicine. Although numerous previous reviews have concentrated on the bioactive components and pharmacological properties of *O. sinensis* fruit bodies, there remains a notable lack of literature regarding the development of novel methods for authenticating these fruit bodies, particularly in the context of applying molecular identification techniques. The authentication of *O. sinensis* fruit bodies poses significant challenges due to the widespread contamination of these ingredients with counterfeit products. This article first provides an overview of the life cycle, biological characteristics, and habitat of *O. sinensis.* It then summarizes the importance of molecular identification techniques for *O. sinensis* and outlines the main techniques related to DNA molecular manipulation for the identification of medicinal plants, including *Cordyceps* and its related species. The article concludes by emphasizing the application of these identification techniques in the study of *O. sinensis* over the past decade. Additionally, the review suggests the potential of using molecular biology and multi-omics techniques to elucidate differences among biological individuals in complex environments and to construct microbial fingerprint maps for verifying the authenticity of *Cordyceps* and its related species. This review provides a scientific reference for the development of new detection methods for rapid and accurate authentication of *O. sinensis* and its related species.

Figures



Keywords: *Ophiocordyceps sinensis, Cordyceps,* molecular identification, authenticity, related species, medicinal mushrooms and fungi, the insect-fungi complex

International Journal of Medicinal Mushrooms, Volume 27, Issue 11, 2025, pp. 21-35

DOI: 10.1615/IntJMedMushrooms.2025059733

A review of edible mushroom-mediated green synthesis of nanoparticles and their emerging biomedical potentials

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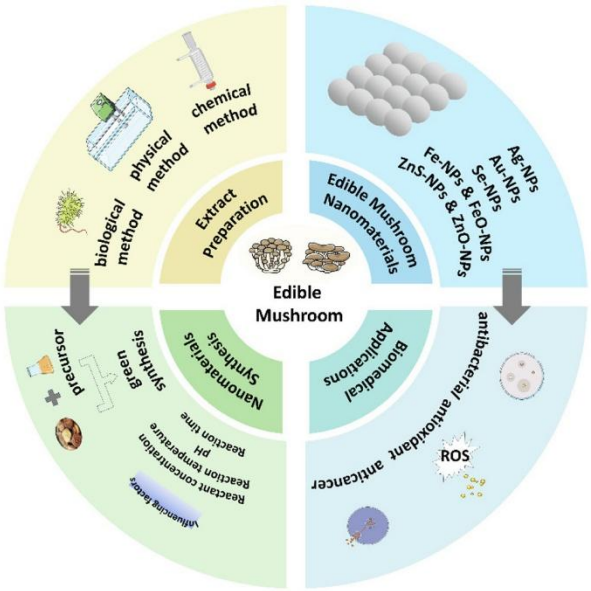
Abstract: Edible mushrooms are large fungi that are loved for their unique flavor and texture. The rich nutrient content of edible mushrooms is also considered to be an important source of medicinal value and bioactive components. Nowadays edible mushroom extracts have penetrated into the field of nanotechnology and their main role is to act as reducing, capping and stabilizing agents for the synthesis of nanoparticles. This green synthesis of nanoparticles has been used with several applications due to its high stability, low toxicity and environmental friendliness. This review will systematically describe the various aspects of nanoparticles synthesis from edible mushrooms, mainly including the preparation of edible mushroom extracts, steps of nanoparticles synthesis, influencing factors and characterization techniques. To collate so far various nanoparticles synthesized by edible mushrooms, such as Ag, Au, Se, etc. And highlight the current status of the application of edible mushroom synthesized nanoparticles in the field of medical research such as antibacterial, antioxidant and anticancer.

Graphical abstract



Inorganic Chemistry CommunicationsNovember 2025

https://doi.org/10.1016/j.inoche.2025.115129

Unveiling the glucan profile: a comparative study of Lion's Mane and Shiitake mushrooms

C. S. Keerthana [a ǂ], Rozy Kumari [b ǂ], Muskan Beura [a], Sumit Sharan [c], Susheel Kumar Sharma [d], Ananta Ganjoo [e], Anil Dahuja [a], Veda Krishnan [a]

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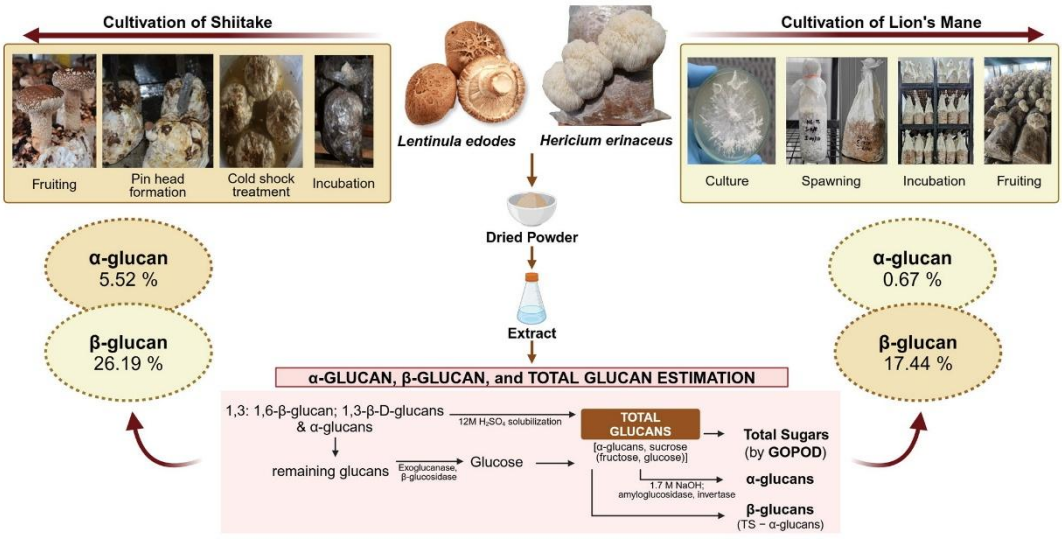
Abstract: Mushrooms are rich in bioactive polysaccharides, particularly α- and β-glucans, renowned for their immunomodulatory properties. The present study optimised the cultivation methods and quantified α-, β-, and total glucan from *Hericium erinaceus* (Lion's Mane) and *Lentinula edodes* (Shiitake) mushrooms. Shiitake exhibited an α-glucan content ranging from 0.806% to 5.521%, with the highest at 5.521% (DMRO-356), approximately 8.2 times higher than Lion's Mane (HE-TC), which had 0.673%. For β-glucans, Shiitake strains showed a range of 13.433–26.190%, with the highest value at 26.190% (DMRX-2022) which was 1.5-fold higher than HE-TC at 17.442%. The findings emphasise Indian Shiitake strains' remarkable α and β-glucan content and their potential as immune-boosting supplements. Incorporating these mushrooms into commercial products and health supplements requires more research on their bioavailability and health advantages.

Graphical Abstract



Keywords: Lion's mane mushroom, Shiitake mushroom, β-glucan, α-glucan, functional foods

Natural Product ResearchAvailable online 25 May 2025

https://doi.org/10.1080/14786419.2025.2509142

Structure characterization, simulated digestion, and microbial modulation of *Pleurotus ostreatus* polysaccharides

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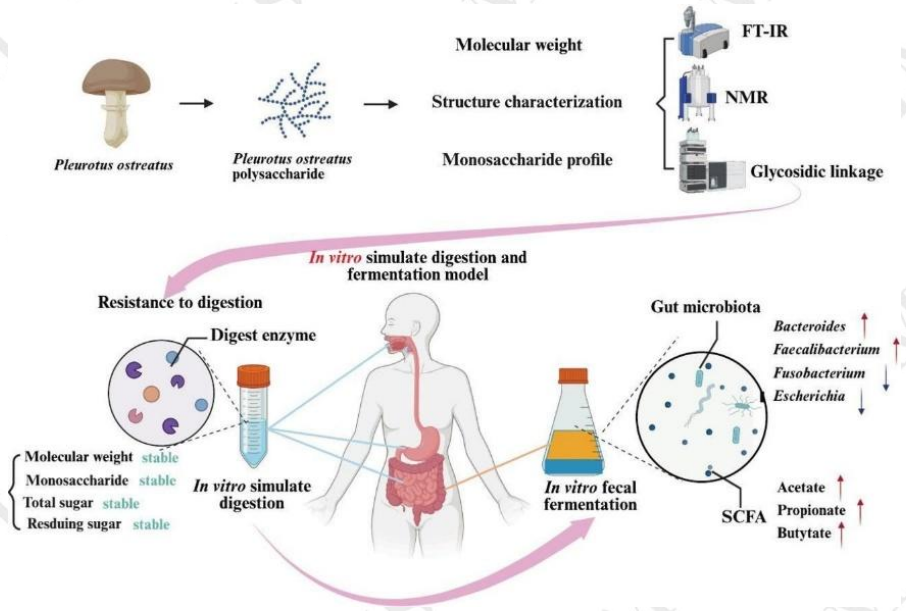
Abstract: Edible mushrooms polysaccharides are widely recognized for their biological activities, yet the prebiotic potential of *Pleurotus ostreatus* polysaccharides (POP) remains underexplored. In this study, POP was isolated, structurally characterized, and evaluated for digestibility and impact on gut microbiota. Structural analysis revealed that POP comprises →4)-α-Glcp-(1→, →3)-β-Glcp-(1→, →4,6)-α-Glcp-(1→ and →3,4)-α-Glcp-(1→ linkages as the backbone, with side chains of 1,6-α-Galp and α-Manp residues. POP was resistant to simulated gastrointestinal digestion. During fecal fermentation, POP significantly increased microbial diversity, with Chao1 and Shannon indices rising by 26.95 % and 32.67 %. Furthermore, POP promoted the relative abundance of beneficial genera like *Prevotella* (8.1-fold increase), *Bacteroides* (3.8-fold), and *Faecalibacterium* (4.4-fold), while reducing opportunistic pathogens including *Fusobacterium* and *Escherichia*. Total short-chain fatty acid (SCFA) production reached 54.7 mM at 24 h, particularly acetate, propionate, and butyrate. These results highlight the prebiotic potential of POP and support its application as a functional food ingredient for improving gut health.

Graphical abstract



Food Chemistry, Volume 493, Part 2, 30 November 2025

https://doi.org/10.1016/j.foodchem.2025.145849

Anti-cancer effects of *Cordyceps sinensis*, *C. militaris* and *C. cicadae* and their mechanisms of action

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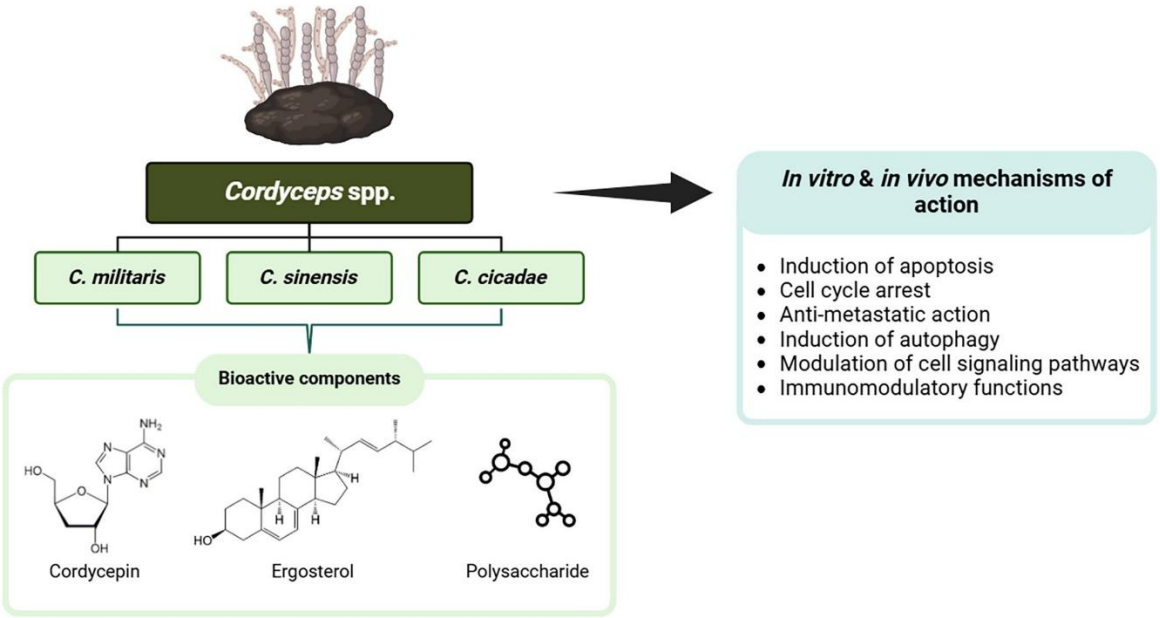
Abstract: Over the past decade, species of *Cordyceps*, particularly *C. sinensis, C. militaris*, and *C. cicadae*, along with their bioactive components cordycepin, ergosterol and polysaccharides, have shown significant anti-cancer potential. These effects are mediated through mechanisms including apoptosis induction, cell cycle arrest, and modulation of key signalling pathways, as reviewed here. Notably, the bioactive components exhibit greater potency than crude extracts, with cordycepin being a promising chemotherapeutic lead. Future investigations into the *in vivo* efficacy and safety profiles of *Cordyceps* extracts and their bioactive components are greatly encouraged. Additionally, structural modification of cordycepin may offer opportunities to enhance its therapeutic potential.



Keywords: Cordycepin, ergosterol, polysaccharides, PI3K/Akt, MAPK/ERK, Wnt/β-catenin

Journal of Asian Natural Products ResearchAvailable online 22 May 2025

https://doi.org/10.1080/10286020.2025.2497282

Impacts of global climate change on mushroom productions: challenges and opportunities

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[b]*Department of Biology, McMaster University, Hamilton, Ontario, L8S 4K1, Canada*

Abstract: Climate change has been linked to a wide range of adverse impacts on human health and socioeconomic well-being. While mushrooms have been suggested as potential agents for mitigating climate change, the effects of climate change on mushroom production remain largely unexplored. Here, we review and analyze the potential impacts of climate change on the production of both cultivated and wild edible and medicinal mushrooms, with a focus on the impacts of changing temperature and precipitation. First, we review global temperature and precipitation scenarios projected by 2100. Most climate scientists predict that global temperature will continue to rise gradually and precipitation distribution will become increasingly uneven in the future. Second, our analyses suggest that these environmental shifts will negatively impact the productivity of most cultivated mushrooms in multiple ways, including restricted substrate supplies, damage from pests and pathogens, and high electrical expenditures, etc. Third, some wild mushrooms may benefit from warmer and more humid conditions in certain regions, leading to increased mycelial growth and fruiting. However, hotter and drier environments in other regions, as projected for the future, will likely suffer decreasing yields for most mushrooms. For many wild gourmet mushroom species, their suitable habitats may change, with the majority predicted to experience habitat shrinkage, resulting in an overall decrease in productivity. Aside from challenges, we also discuss opportunities, including incorporating smart technologies for monitoring environmental factors, utilizing artificial intelligence for predictive analytics, and automating tasks such as irrigation and cooling/heating. We note that long-term monitoring across multiple ecological zones is needed to accurately quantify the impacts of global climate change on mushroom production and fine-tune strategies for sustainable mushroom cultivation.

Keywords: Climate change, Cultivated mushrooms, Wild mushrooms, Substrate supplies, Mushroom pests and pathogens, Global temperature, Precipitation pattern, Smart technologies

Agriculture Communications, Available online 14 August 2025

https://doi.org/10.1016/j.agrcom.2025.100091

The research progress of Ganoderma as a medicinal and edible product

Meilin Wang, Ding Li, Xu Gao, Xinze Wang, Li Zhang, Jiaxin Ding, Ke'er Xuan, Ge Wang, Yan Bai, Jian Gong

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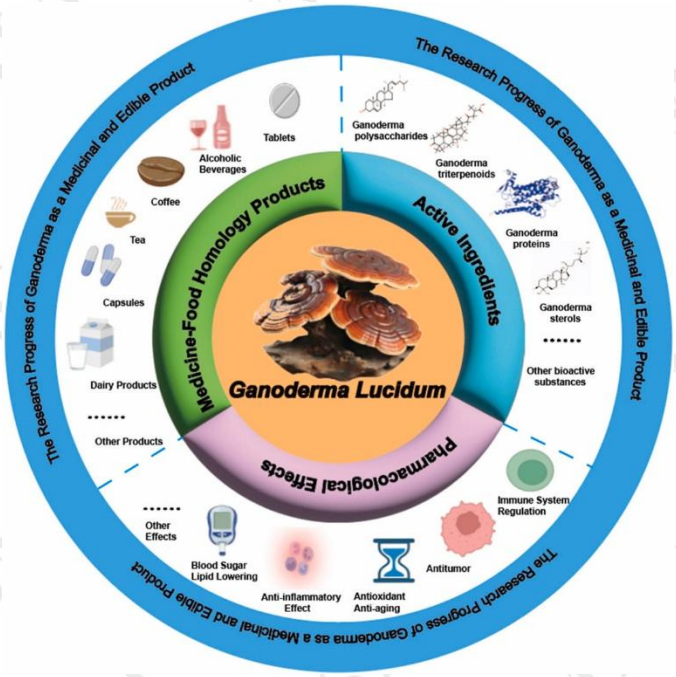
Abstract: Ganoderma, as a representative resource of both food and medicine, has held a significant place in traditional medicine since ancient times. It is rich in various bioactive compounds such as polysaccharides, triterpenoids, sterols, and proteins, and exhibits a wide range of pharmacological activities including immunomodulatory, anti-tumor,

antioxidant, and hypoglycemic effects. This review focuses on the chemical characteristics and molecular mechanisms of the major active constituents of *G. lucidum*, and briefly outlines the development trends of its product types, with particular emphasis on its potential applications in the prevention and management of chronic diseases and in health promotion. Based on this, the current trends in *G. lucidum* product development are further summarized. Future research should concentrate on elucidating the synergistic mechanisms of active components, accumulating clinical evidence, and establishing standardized evaluation systems to promote the scientific application of *G. lucidum* as a medicinal and edible product.

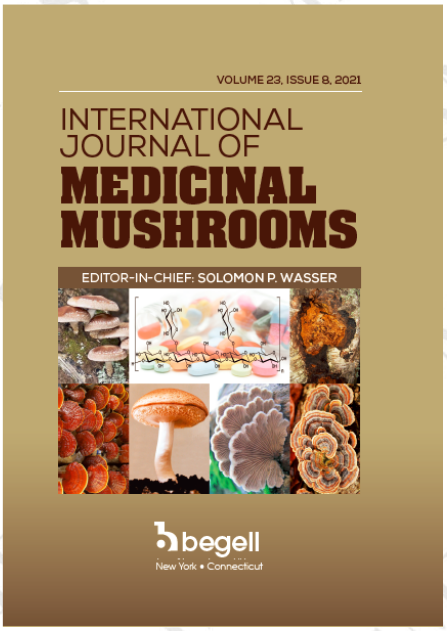
Graphical abstract



Food Bioscience, Volume 71, September 2025

https://doi.org/10.1016/j.fbio.2025.107055

International Journal of Medicinal Mushrooms Call for Papers



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Medicinal Mushrooms

2025, Vol. 27, Issue no.10

International Journal of
Medicinal Mushrooms

2025, Vol. 27, Issue no.11

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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Orgainal Published on Journal of Fungi 2025, 11, 244

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

Continued from previous issue:

3.6 Primary Structures of the MAT1-2-1 Proteins

Among the 74 MAT1-2-1 proteins available in the AlphaFold database, 69 are full- length proteins containing 249 amino acids and are attributed to diverse 3D structuralmorphs under 17 UniProt codes (*cf*. Figure 5; Table 2). Among the 69 full-length proteins, 39 are 100% identical to the query sequence AEH27625 and clustered into Branch I-1 of Cluster I of the Bayesian tree (*cf*. Figure 2). The remaining 30 full-length proteinsshare 97.6–99.6% sequence similarity with the query protein sequence, containing various conservative and nonconservative substitutions of amino acid residues at isolated sites, which may have an impact on the mating function.

Figure 8 shows the alignment of the full-length MAT1-2-1 protein sequences covering five Bayesian clusters (Branches I-1, I-2, II-1, II-2, III, IV-1, IV-2, V-1, and V-2; *cf*. Figure 2) and 17 AlphaFold 3D structural morphs (*cf*. Figure 5 and Table 2), as well as the MAT1-2-1 protein sequences encoded by the genome and transcriptome assemblies of *H. sinensis* and the metatranscriptome assembly of the natural *C. sinensis* sample that was collected from Deqin, Yunnan Province, China. According to GenBank, both the MAT1-2-1 protein sequence EQL04085 and the genome assembly ANOV01000063 (9329→10,182) were derived from *H. sinensis* strain Co18 and submitted to GenBank by the same group of authors. However, the genome assembly ANOV01000063 (9329→10,182) contains a conservative S-to-A substitution, whereas EQL04085 does not contain this substitution. Note: the arrows "→" and "←" indicate sequences in the sense and antisense strands of the genomes, respectively. The MAT1-2-1 protein contains an HMG-box_ROX1-like domain, which binds the minor groove of DNA in a sequence-specific manner [Hu et al., 2013 [31]]. This domain is located in segment 127→197 of the query sequence AEH27625, which is shown in blue and underlined in Figure 8. Some conservative residue substitutions in other MAT1-2-1 proteins were found within this domain, as shown in red in Table 4.

```
AEH27625          1       MANPINMIPNPQWNATDYEAIWKGLEAQVNPFSQILCLEGDFFRQLDDAAKLFIARKLME 60
AFX66437          1       ------------------------------------------N----------------- 60
ACV60391          1       ------------------------------------------------------------ 60
ACV60372          1       ----------------------------------L------------------------- 60
AIV43040          1       --------------I----------------------------------------------- 60
ACV60417          1       --------------I----------------------------------------------- 60
ACV60415          1       ------------------------------------------------------------ 60
AFX66476          1       ---------------------------------------------G-------------- 60
AFX66472          1       ---------------------------------------------G-------------- 60
ACV60363          1       ------------------------------------------------------------ 60
ACV60385          1       ----------------------------------V------------------------- 60
ACV60399          1       ------------------------------------------------------------ 60
AFX66401          1       ------------------------------------------I----------------- 60
AFX66475          1       ----------------------------------T------------------------- 60
AFX66484          1       ------------------------------------------------------------ 60
AGW27537          1       ------------------------------------------------------------ 60
EQL04085          1       ------------------------------------------------------------ 60
ANOV01000063 9329         ------------------------------------------------------------ 9508
LWBQ01000021 239,726      ------------------------------------------------------------ 239,547
NGJJ01000619 23,883       ------------------------------------------------------------ 23,704
LKHE01001605 14,713       ------------------------------------------------------------ 14,534
GCQL01020543 1143         ------------------------------------------------------------ 964
OSIN7649          1       ------------------------------------------------------------ 60

AEH27625          61      HVQESVLYVNDGNGPDRVYLGAPRHFVVGGGMILQISGYAPYWIRRSVSKVVTATVLAPP 120
AFX66437          61      ------------------------------------------------------------ 120
ACV60391          61      ------------------R----------------------------------------- 120
ACV60372          61      ------------------------------------------------------------ 120
AIV43040          61      ------------------------------------------------------------ 120
ACV60417          61      ------------------------------------------------------------ 120
ACV60415          61      ------------------------------------------------------------ 120
AFX66476          61      ---------------------------------------------A-------------- 120
AFX66472          61      ---------------------------------------------A-------------- 120
ACV60363          61      ------------------------------------------------------------ 120
ACV60385          61      ------------------------------------------------------------ 120
ACV60399          61      ------------------------------------------------------------ 120
AFX66401          61      ------------------------------------------------------------ 120
AFX66475          61      ------------------------------------------------------------ 120
AFX66484          61      ------------------------------------------------------------ 120
AGW27537          61      ----------------I------------------------------------------- 120
EQL04085          61      ------------------------------------------------------------ 120
ANOV01000063 9558         ------------------------------------------------------------ 9740
LWBQ01000021 239,506      ------------------------------------------------------------ 239,315
NGJJ01000619 23,666       ------------------------------------------------------------ 23,472
LKHE01001605 14,496       ------------------------------------------------------------ 14,302
GCQL01020543 963          ------------------------------------------------------------ 784
OSIN7649          61      ------------------------------------------------------------ 120
```

Figure 8. *Cont.*

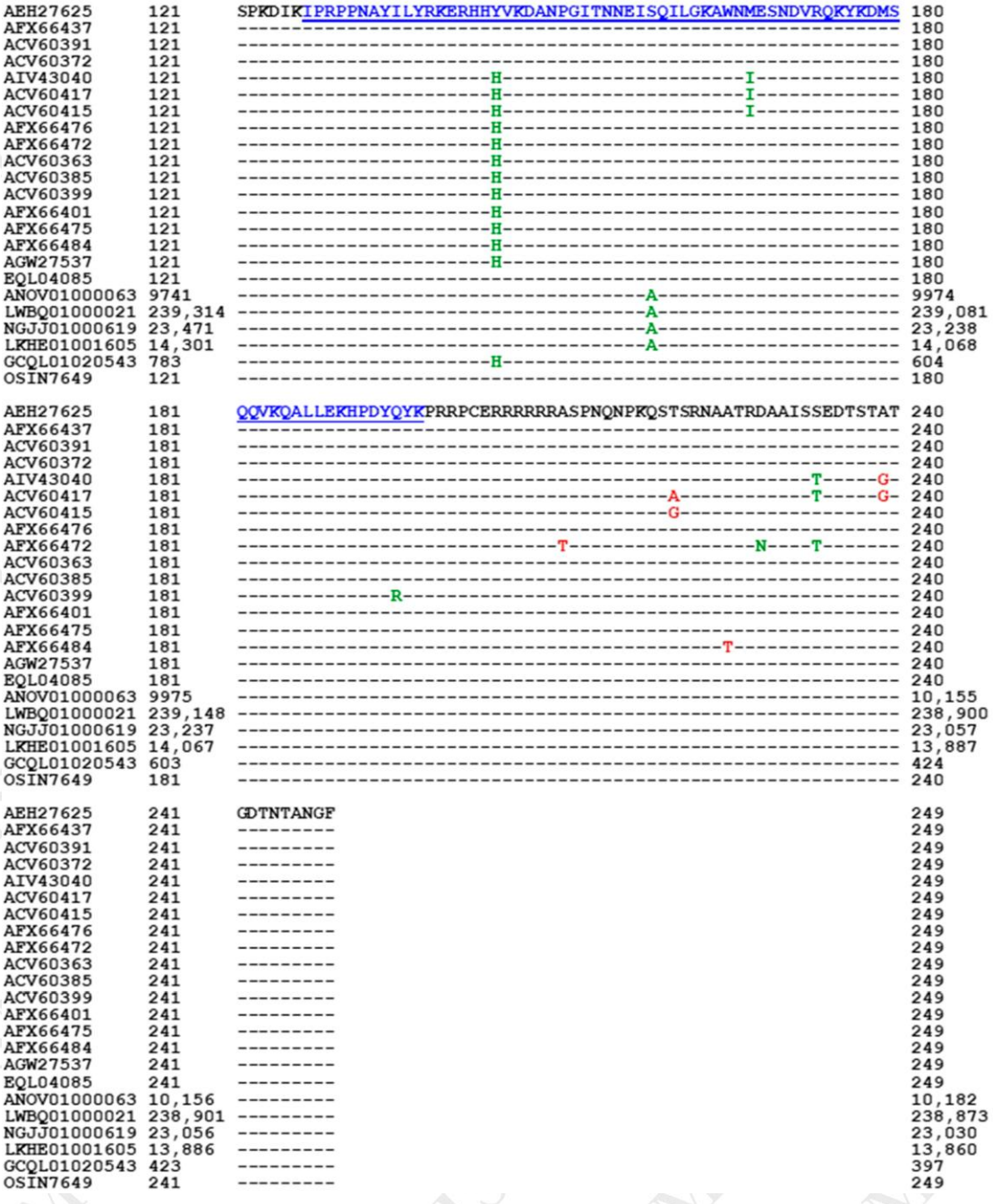


Figure 8. Alignment of the full-length sequences of representative MAT1-2-1 proteins of 17 diverse 3D structural morphs and the translated segments of the corresponding genome, transcriptome, and metatranscriptome assemblies of *H. sinensis* strains and natural *C. sinensis*. An HMG-box_ROX1-like domain is highlighted in blue and underlined in the query protein sequence AEH27625 (127→197). The residues shown in green refer to conservative amino acid substitutions, and those in red indicate nonconservative amino acid substitutions. The hyphens indicate identical amino acid residues.

Table 4 summarizes the protein sequence alignment results, including mutant amino acid residues and percent similarities relative to the "likely authentic" full-length MAT1-2-1 protein AEH27625. The sequence alignment results are correlated with the statistical and structural analytical results obtained from the Bayesian clustering and 3D structure prediction (including the stereostructure models and the associated AlphaFold UniProt codes) (*cf*. Table 2, Figures 2, 5 and 8). The correlations shown in Table 4 indicate that the minor sequence differences within or outside

the HMG-box_ROX1-like domain of the MAT1-2-1 protein sequences may have an impact on the diverse 3D structures.

Table 4. Summary of the full-length MAT1-2-1 protein sequence alignment results (mutant aminoacids and percent similarity vs. the likely authentic protein AEH27625), correlating with the Bayesian branches/clusters and the 3D structural models and associated with the AlphaFold UniProt codes.

Accession Number	% Similarity to AEH27625	Amino Acid Residue Substitution		Bayesian Cluster		3D Structure Model	AlphaFold UniProt Code
		Conservative	Nonconservative	Branch	Cluster		
AEH27625 EQL04085	100% 100%			I-1	I	A B	D7F2E9 T5AF56
AFX66437 ACV60391 ACV60372	99.6%	D-to-N	G-to-R Q-to-L	I-2	I	C D E	V9LW10 D7F2H1 D7F2F2
AIV43040	97.6%	Y-to-H, M-to-I, Q-to-R, S-to-T	T-to-I, A-to-G	II-1	II	F	A0A0A0RCF5
ACV60417	97.6%	Y-to-H, M-to-I, S-to-T	T-to-I, T-to-A, A-to-G	II-2	II	G	D7F2J7
ACV60415	98.8%	Y-to-H, M-to-I	T-to-G		III	H	D7F2F5
AFX66476	98.8%	Y-to-H	D-to-G, V-to-A	IV-1	IV	I	V9LWC9
AFX66472	97.6%	Y-to-H, D-to-N, S-to-T	D-to-G, V-to-A, A-to-T	IV-2	IV	J	V9LVS8
ACV60363	99.6%	Y-to-H		V-1	V	K	D7F2E3
ACV60385	99.2%	I-to-V, Y-to-H		V-2	V	L	D7F2G5
ACV60399	99.2%	Y-to-H, Q-to-R		V-2	V	M	D7F2H9
AFX66401	99.2%	Y-to-H	D-to-I	V-2	V	N	V9LW71
AFX66475	99.2%	Y-to-H	I-to-T	V-2	V	O	V9LVU8
AFX66484	99.2%	Y-to-H	A-to-T	V-2	V	P	V9LWG5
AGW27537	99.2%	V-to-I, Y-to-H		V-2	V	Q	U3N6V5

Note: The amino acid residue substitutions shown in red indicate conservative changes within the HMG- box_ROX1-like domain of the MAT1-2-1 proteins. Other residue substitutions shown in black are located within or outside the HMG-box_ROX1-like domain.

In addition to the 74 full-length MAT1-2-1 proteins, five other protein sequences are C-terminally truncated and were clustered into Branches V-1 and V-2 of Cluster Vin the Bayesian tree (shown in green in Figure 2), exhibiting 69–74% query coverageand 99.4–99.5% protein sequence similarity to the reference full-length MAT1-2-1 protein AEH27625 under the UniProt code D7F2E9. The five truncated MAT1-2-1 proteins contain a single conserved Y-to-H substitution with the HMG-box_ROX1-like domain, belonging to different 3D structural morphs under the 4 AlphaFold UniProt codes (*cf*. Figure 6).

Figure 8 also shows a conservative S-to-A substitution with the HMG-box_ROX1-like domain in the MAT1-2-1 proteins encoded by the genome assemblies ANOV01000063, LKHE01001605, LWBQ01000021, and NGJJ01000619 of *H. sinensis* strains Co18, 1229, ZJB12195, and CC1406-20395, respectively [Hu et al., 2013 [31]; Li et al., 2016 [40], 2023 [36], 2024 [37]; Jin et al., 2020 [41]; Liu et al., 2020 [42]]. A conservative Y-to-H substitution with the HMG-box_ROX1-like domain was found in the transcriptome assembly GCQL01020543 of the *H. sinensis* strain L0106 [Liu et al., 2015 [44]; Li et al., 2024 [37]]. No mutation was detected in the MAT1-2-1 protein encoded by the metatranscriptome assembly OSIN7649 of natural *C. sinensis* [Xia et al., 2017 [46]; Li et al., 2024 [37]], indicating no variation in the 3D structures and mating functionality of the MAT1-2-1 protein.

3.7. Differential Genomic Occurrence of the MAT1-1-1 and MAT1-2-1 Proteins in H. sinensis

Table 5 lists the differential occurrence of the MAT1-1-1 and MAT1-2-1 proteins encoded by the genome assemblies ANOV01017390/ANOV01000063, JAAVMX010000001, LKHE01001116/LKHE01001605, LWBQ01000021, and NGJJ01000619 of the *H. sinensis* strains Co18, IOZ07, 1229, ZJB12195, and CC1406-20395, respectively [Hu et al., 2013 [31]; Li et al., 2016a [40]; Jin et al., 2020 [41]; Liu et al., 2020 [42]; Shu et al., 2020 [43]]. The genome assemblies LWBQ00000000 and NGJJ00000000 of the *H. sinensis* strains do not contain the genes encoding the MAT1-1-1 proteins, and the genome assembly JAAVMX000000000 does not contain the gene encoding the MAT1-2-1 protein.

Table 5. Percentage similarity between the sequences EQK97643 and AEH27625 for the MAT1-1-1and MAT1-2-1 proteins, respectively, and the mating protein sequences encoded by the genome assemblies of *H. sinensis* strains.

H. sinensis Strain	Genome Assembly Segment	Percentage Similarity	
		MAT1-1-1 (vs. EQK97643)	MAT1-2-1 (vs. AEH27625)
Co18	ANOV01017390 (410←1519) ANOV01000063 (9329→10,182)	99.7%	99.6%
1229	LKHE01001116 (3799←4909) LKHE01001605 (13,860←14,713)	99.7%	99.6%
IOZ07	JAAVMX010000001 (6,698,911→6,700,021) JAAVMX000000000	99.7%	—
ZJB12195	LWBQ00000000 LWBQ01000021 (238,873←239,726)	—	99.6%
CC1406-20395	NGJJ00000000 NGJJ01000619 (23,030←23,883)	—	99.6%

Note: The "→" and "←" arrows indicate sequences in the sense and antisense strands of the genomes, respectively.

Although repetitive copies of many genes have been identified in the *H. sinensis* genome assemblies and the mutant repetitive genome sequences have normal transcrip-tional abilities and encode mutant proteins with altered functional specificities [Li et al.,2024 [63]], no repetitive copies of the *MAT1-1-1* or *MAT1-2-1* genes were identified inthe *H. sinensis* genome assemblies, proving that the mutant mating proteins with diversestereostructures may be encoded by mutant mating-type genes of certain *H. sinensis* strains or heterospecific fungal species other than *H.sinensis*.

3.8. Differential Transcriptomic and Metatranscriptomic Occurrences of the MAT1-1-1 and MAT1-2-1 Proteins in H. sinensis and the Natural C. sinensis Insect–FungalComplex

Table 6 shows the differential occurrence of the MAT1-1-1 and MAT1-2-1 proteins encoded by the transcriptome assembly of the *H. sinensis* strain L0106 and the metatran-scriptome assemblies of natural *C. sinensis* specimens [Xiang et al., 2014 [45]; Liu et al.,2015 [44]; Xia et al., 2017 [46]]. The transcriptome assembly GCQL00000000 does not contain the gene encoding the MAT1-1-1 protein [Liu et al., 2015 [44]], and the metatranscriptome assembly GAGW00000000 of natural *C. sinensis* does not contain the gene encoding theMAT1-2-1 protein [Xiang et al., 2014 [45]]. Worth mentioning, Bushley et al., 2013 [32] and Li et al., 2023 [36], 2024 [37] reported disrupted translation of the MAT1-2-1 transcriptdue to alternative splicing of the gene with unspliced intron I, which contains three stopcodons, in *H. sinensis* strain 1229. The alternative splicing of the *MAT1-2-1* gene eventually produces a largely truncated protein lacking the C-terminal portions (including the entire HMG-box_ROX1-like domain) of the protein encoded by exons II

and III of the gene and significantly alters the 3D structure of the protein with dysfunctionality.

Table 6. Percentage similarity between the sequences EQK97643 and AEH27625 for the MAT1-1-1 and MAT1-2-1 proteins, respectively, and the mating proteins encoded by the transcriptome assembly of *H. sinensis* strain L0106 and the metatranscriptome assemblies of natural *C. sinensis*.

H. sinensis Strain or Natural *C. sinensis*	Transcriptome or Metatranscriptome Assembly Segment	Percentage Similarity	
		MAT1-1-1 (vs. EQK97643)	MAT1-2-1 (vs. AEH27625)
H. sinensis strain L0106	GCQL00000000 GCQL01020543 (397←1143)	—	99.6%
Mature natural *C. sinensis* (Collected at Deqin, Yunnan)	OSIN7648 (1→1065) OSIN7649 (1→397)	94.9%	100%
Natural *C. sinensis* * (Collected at Kangding, Sichuan)	GAGW01008880 (300←1127) GAGW00000000	100%	—

Note: *, Natural *C. sinensis* samples of unknown maturation stage. The "→" and "←" arrows indicate sequences in the sense and antisense strands of the genomes, respectively.

3.9. Diverse Secondary (2D) Structures of the MAT1-1-1 Proteins Encoded by the Genome of H. sinensis and the Metatranscriptome of Natural C. sinensis Insect–Fungal Complex

The predicted 3D structures of the truncated MAT1-1-1 proteins encoded by the genome and metatranscriptome assemblies are not available in the AlphaFold database. Figure 9 shows changes in the 2D structures, α-helices (Panel A), β-sheets (Panel B), β-turns (Panel C), and coils (Panel D) of the truncated MAT1-1-1 proteins that were analyzed via ExPASy ProtScale technology. EQK97643 (372 aa) of the *H. sinensis* strain GS09_111 was used as the reference for the authentic MAT1-1-1 proteins for the 2D analysis shown in the upper plots in all panels of Figure 9, which was clustered into Branch A1 of Cluster A (*cf*. Figure 1) and belongs to 3D structural morph A under the UniProt code U3N942 (*cf*. Figure 3).

The upper-middle plots in all panels show the 2D structures of the MAT1-1-1 protein encoded by the genome assembly ANOV01017390 of the *H. sinensis* strain Co18, which also represents two other MAT1-1-1 sequences within the genome assemblies LKHE01001116 and JAAVMX010000001 of the *H. sinensis* strains 1229 and IOZ07, respectively. The lower-middle and lowest plots in each panel of Figure 9 present the 2D structures of the MAT1-1-1 proteins encoded by the metatranscriptome assemblies GAGW01008880 and OSIN7648 of natural *C. sinensis*, respectively.

The open boxes shown in red in the EQK97643 plots indicate the C-terminal truncation region occurring in the genome assembly ANOV01017390 of *H. sinensis* strain Co18, which also represents two other genome assemblies LKHE01001116 and JAAVMX010000001 of *H. sinensis* strains 1229 and IOZ07, respectively.

The N-terminal truncation region of the MAT1-1-1 protein encoded by the metatran- scriptome assembly GAGW01008880 of natural *C. sinensis* is indicated with open boxes shown in blue in the reference protein EQK97643 plots in Figure 9.

The open boxes shown in green in the OSIN7648 plots, as well as the corresponding region in the reference EQK97643 plots for the authentic MAT1-1-1 protein for structural comparison, outline the changes in the topology and waveform

of the α-helix, β-sheet,β-turn, and coil in the midsequence truncation region in the MAT1-1-1 protein encoded by the metatranscriptome assembly OSIN7648. The topology and waveform changes appear to be more dramatic in the α-helix and β-turn plots in the midsequence truncation region of the protein OSIN7648 than in the β-sheet and coil plots. The midsequence truncationand apparent changes in the 2D structures imply significant alterations in the variable protein folding and 3D structures of the MAT1-1-1 proteins encoded by the genome and metatranscriptome assemblies and their mating functionalities.

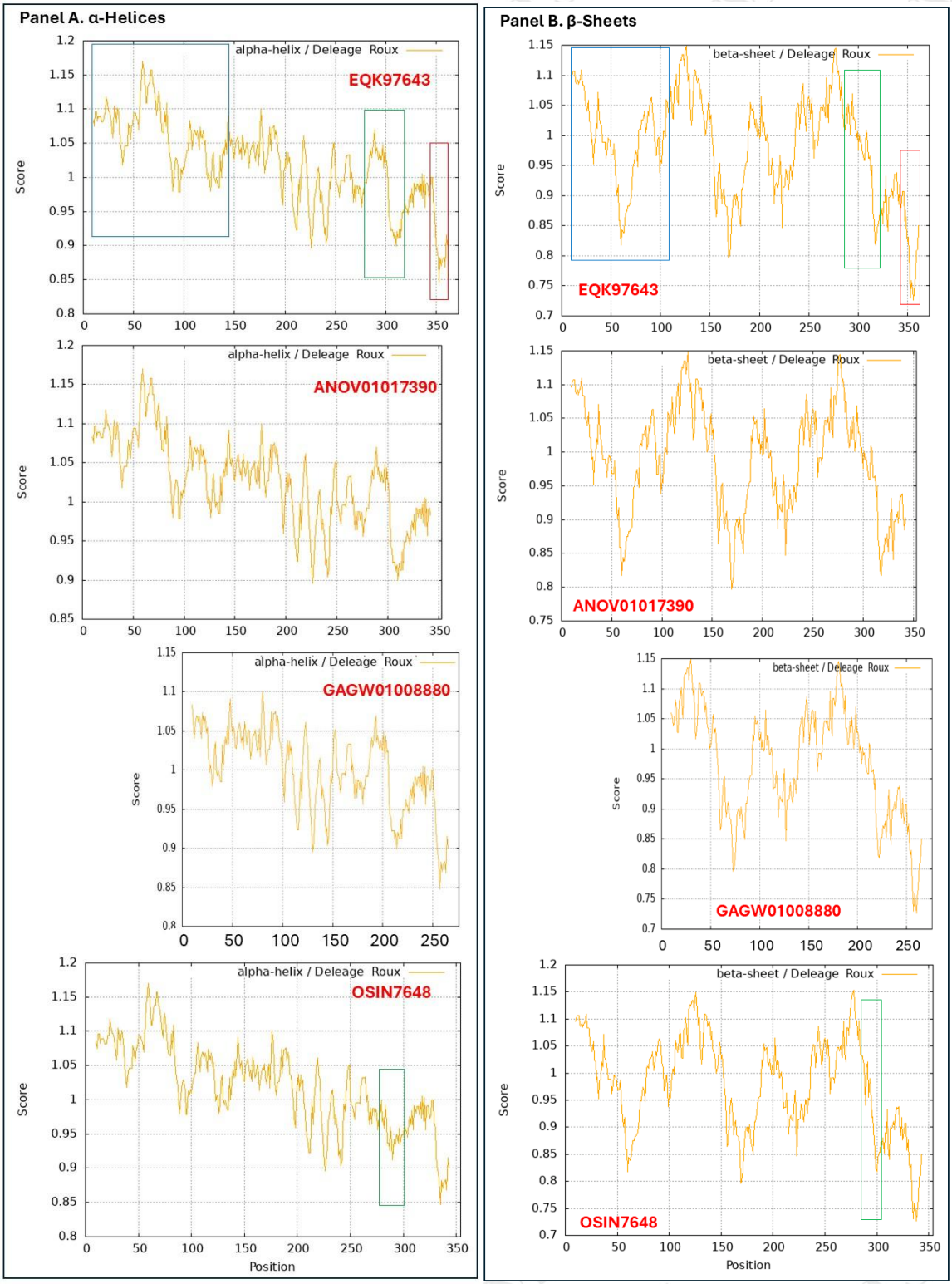


Figure 9. *Cont.*

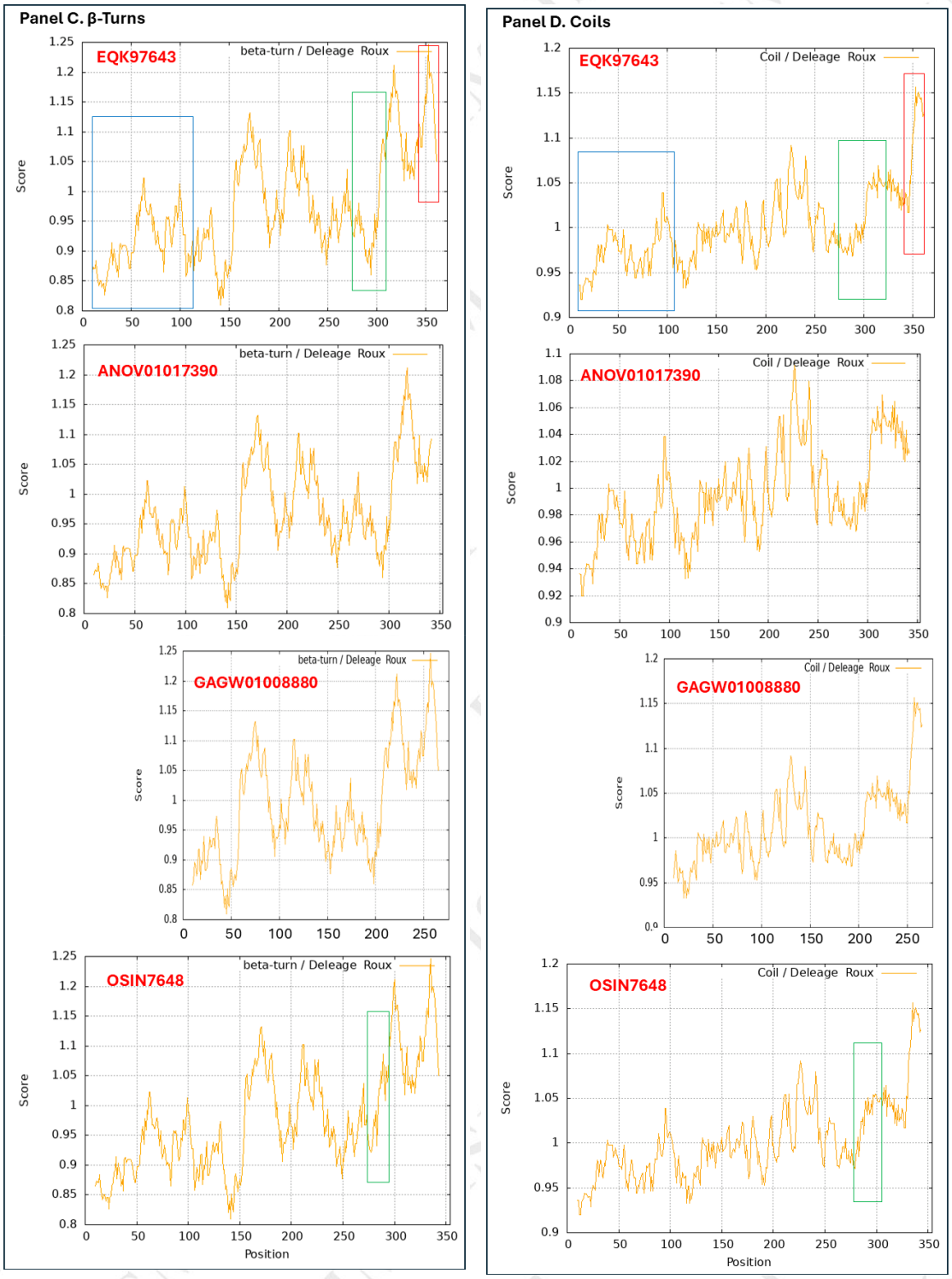
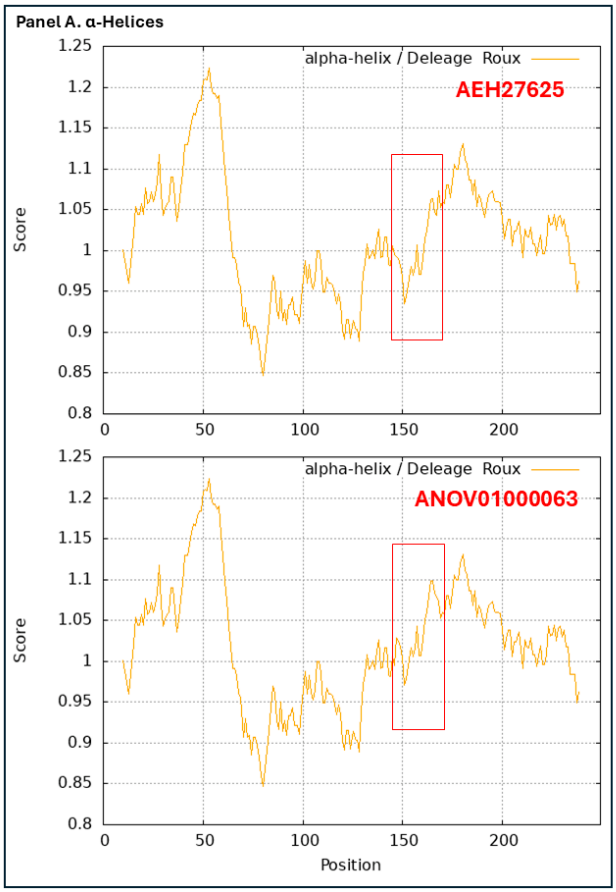


Figure 9. ExPASy ProtScale plots for the α-helices (Panel **A**), β-sheets (Panel **B**), β-turns (Panel **C**), and coils (Panel **D**) of the MAT1-1-1 proteins. Each panel contains 4 ProtScale plots for the 4 MAT1-1-1 proteins. The open boxes in blue in all EQK97643 plots indicate the N-terminal truncation region occurring in the MAT1-1-1 protein encoded by the metatranscriptome assembly GAGW01008880. The open boxes in red in all the EQK97643 plots indicate the C-terminal truncation region occurring in the genome assembly ANOV01017390. The open boxes in green in all the OSIN7648 plots, as well as in the corresponding region in all plots for the MAT1-1-1 protein EQK97643 for topology and waveform comparisons, indicate the midsequence truncation region occurring in the MAT1-1-1 protein encoded by the metatranscriptome assembly OSIN7648.

3.10. Diverse 2D Structures of the MAT1-2-1 Proteins Encoded by the Genomes, Transcriptomes, and Metatranscriptomes of H. sinensis and the Natural C. sinensis Insect–Fungal Complex The predicted 3D structures of the mutant MAT1-2-1 proteins encoded by these genome, transcriptome, and metatranscriptome assembly sequences are unavailable in the AlphaFold database. Figure 10 shows the 2D structures of the MAT1-2-1 proteins for theα-helices (Panel A), β-sheets (Panel B), β-turns (Panel C), and coils (Panel D). Each panelof Figure 10 contains two ProtScale plots for two MAT1-2-1 proteins. AEH27625 (249 aa) of the *H. sinensis* strain CS2 was used as the reference for the full-length MAT1-2-1 proteinshown in the upper plots of all panels. The lower plots in all panels of Figure 10 represent the MAT1-2-1 protein encoded by the genome assembly ANOV01000063 (9329→10,182),also representing three other genomic sequences, namely, NGJJ01000619 (23,030←23,883), LWBQ01000021 (238,873←239,726), and LKHE01001605 (13,860←14,713). Figure 10 shows slight changes in the topology and waveforms of the α-helices, β-turns, and coils in the MAT1-2-1 protein sequences encoded by the genome assembly ANOV01000063. Thevariation regions are outlined with the open boxes shown in red in the ANOV01000063plots in Panels A, C, and D, respectively, as well as in the corresponding region in thereference MAT1-2-1 protein AEH27625 plots for topology and waveform comparisons.No apparent 2D changes were found in the topology or waveforms of the β-sheets of theMAT1-2-1 proteins encoded by the genome sequences.



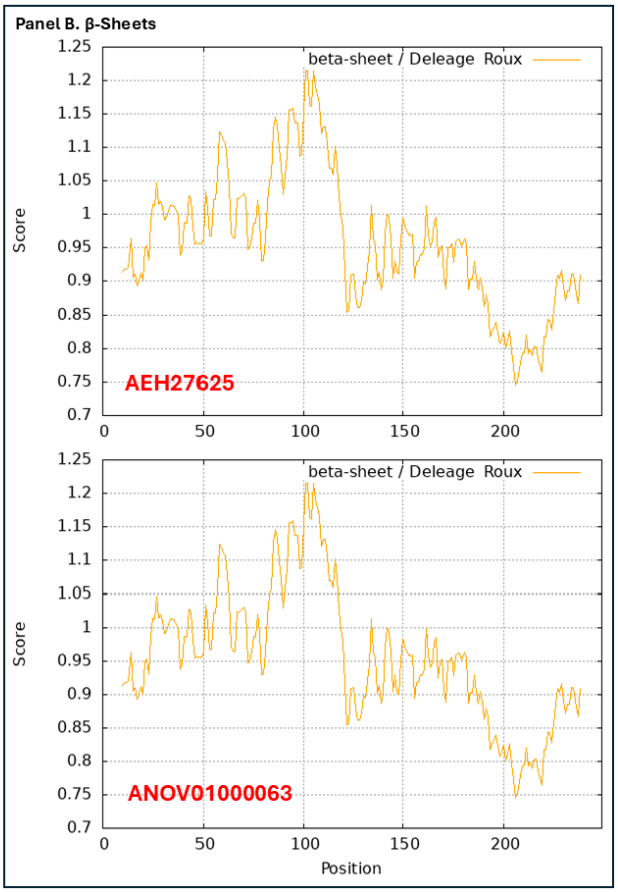


Figure 10. *Cont.*

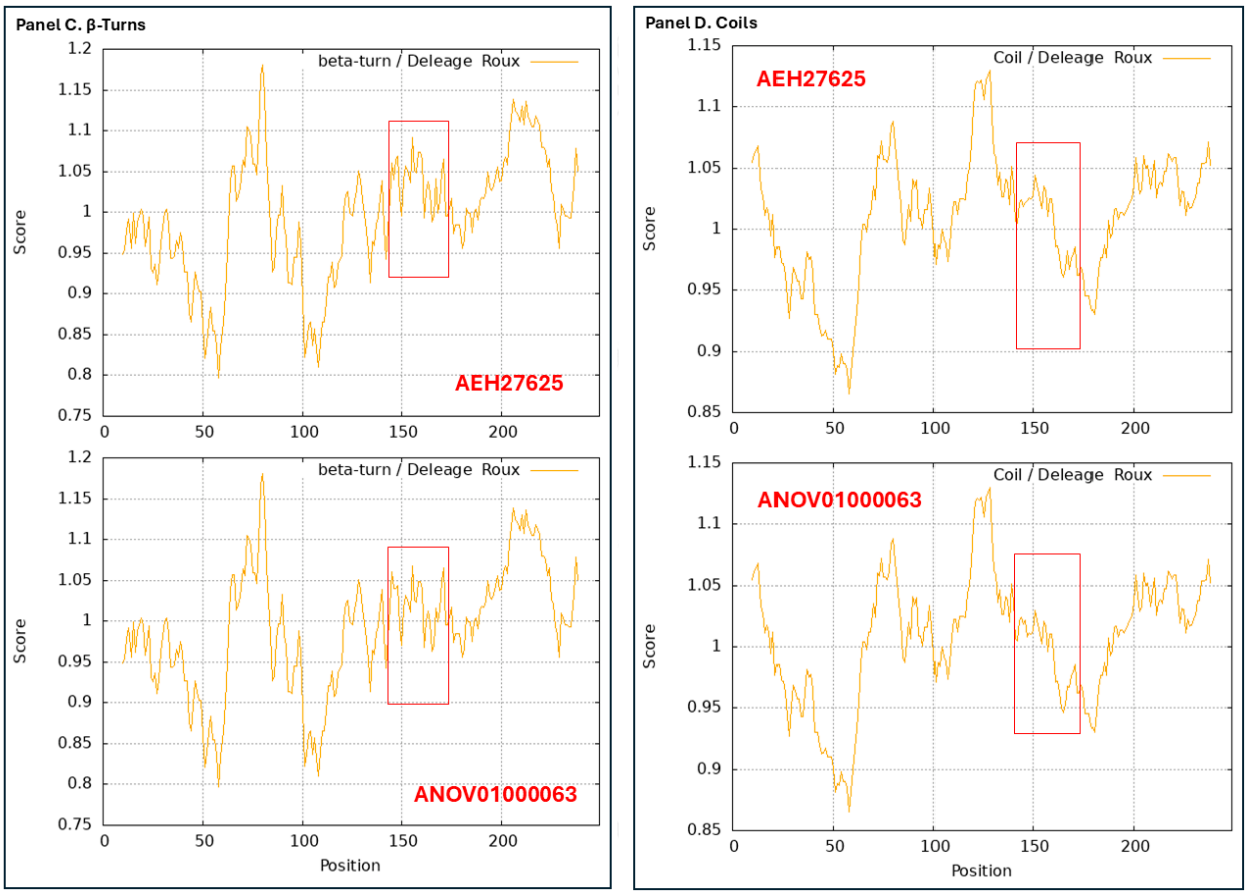


Figure 10. ExPASy ProtScale plots for the α-helices (Panel **A**), β-sheets (Panel **B**), β-turns (Panel **C**), and coils (Panel **D**) of the MAT1-2-1 proteins. Each panel contains 2 ProtScale plots. The open boxes in red in the ANOV01000063 plots in Panels (**A**,**C**,**D**) for the α-helices, β-turns, and coils, as well as in the corresponding region in the AEH27625 plots for the authentic MAT1-2-1 protein for topology and waveform comparisons, indicate the variation region occurring in the genome assembly.

In addition, no apparent changes were observed in the topology and waveforms in the ProtScale plots for the MAT1-2-1 proteins encoded by the transcriptome assemblyGCQL01020543 (397←1143) of *H. sinensis* strain L0106 and the metatranscriptome assembly OSIN7649 (1→249) of natural *C. sinensis*, as compared with those for the reference protein AEH27625 [Liu et al., 2015 [44]; Xia et al., 2017 [46]; Li et al., 2024 [37]]. Thus, these fully functional MAT1-2-1 proteins potentially belong to 3D structural morph A under theUniProt code U3N942 (*cf*. Figure 3) and are included within Branch I-1 of Cluster I in the Bayesian tree (*cf*. Figure 2).

4. Discussion

4.1. Protein 3D Structure Analysis via the AI-Based AlphaFold Prediction System in Combination with Statistical Bayesian Clustering Technology to Stratify 3D Structure Models

The AI-based AlphaFold 3D structure prediction technology displayed extremely refined and powerful abilities in exploring and uncovering the subtle sequence differences of the proteins and in revealing the impact of these subtle sequence differences on 3Dstructure predictions. The AlphaFold database contains 15 diverse 3D structural models

for the full-length MAT1-1-1 proteins (excluding the truncated proteins) and 17 diverse 3D structural models for the full-length MAT1-2-1 proteins (*cf*. Figures 3 and 5).

As shown in Figures 1–6 and Tables 1 and 2, the complexity of the 3D structure heteromorphs predicted using AlphaFold technology was clarified and organized after the combined use of statistical Bayesian clustering analysis. This combination of the two powerful technologies helped stratify the 3D structural heteromorphic groups. As a result, the 15 and 17 stereostructure heteromorphs for the full-length MAT1-1-1 and MAT1-2-1proteins, respectively, were stratified into five Bayesian clusters for each of the matingproteins, regardless of whether the clusters were branched or unbranched. Thus, different 3D structure models within a special Bayesian branch are closely structurally related, whereas other models may be structurally and statistically distant.

4.2. Heteromorphic 3D Structures of the MAT1-1-1 and MAT1-2-1 Proteins in H. sinensis Strains and Wild-Type C. sinensis Isolates

The study presented in this paper demonstrated the heteromorphic 3D structures of the MAT1-1-1 and MAT1-2-1 proteins in 173 *H. sinensis* strains and wild-type *C. sinensis*isolates. Appropriate interaction of the MAT1-1-1 and MAT1-2-1 proteins with functional stereostructures is essential for the sexual reproduction of *O. sinensis* during the lifecycle of the natural *C. sinensis* insect–fungi complex. However, 75.7% of the strains/isolatescontained either MAT1-1-1 or MAT1-2-1 proteins but did not generate correspondingpairing mating proteins. These strains/isolates were harvested from scattered production locations on the Qinghai–Tibet Plateau. The harvesting locations and protein accessioninformation are available in both the GenBank and AlphaFold databases.

A total of 6 Bayesian clusters with clustering branches and 24 AlphaFold-predicted 3D structural morphs were demonstrated for the heteromorphic stereostructures of 138 MAT1- 1-1 proteins (*cf*. Figures 1, 3 and 4). The full-length and truncated MAT1-1-1 proteinsbelonged to 15 and 9 diverse morphs of 3D structures, respectively. The most frequentlydetected "authentic" MAT1-1-1 proteins were clustered into Branch A1 of Cluster A inthe Bayesian tree (*cf*. Figure 1 and Table 1), belonging to 3D structural morph A of theMAT1-1-1 proteins (*cf*. Figure 3). Figure 7 and Table 3 show that the scattered amino acid residue substitutions, conservative or nonconservative, are located within or outside theMATalpha_HMGbox domain of MAT1-1-1 protein sequences [Hu et al., 2013 [31]].

A total of five Bayesian clusters with clustering branches and 21 AlphaFold 3D struc- tural morphs were demonstrated for the heteromorphic stereostructures of 79 MAT1-2-1proteins (*cf*. Figures 2, 5 and 6). The full-length and truncated MAT1-2-1 proteins belonged to 17 and 4 diverse morphs of 3D structures, respectively. Many (52.7%) of the MAT1-2-1 proteins were clustered into Branch I-1 of Cluster I in the Bayesian tree (*cf*. Figure 2 andTable 2), belonging to 3D structural morph A of the MAT1-2-1 proteins (*cf*. Figure 5). Figure 8 and Table 4 show that the dispersed amino acid residue substitutions, conservative or nonconservative, are located within or outside the HMG-box_ROX1-like domain ofMAT1-2-1 protein sequences [Hu et al., 2013 [31]].

Zhang and Zhang [2015 [35]] reported 4.7% and 5.7% variations in the exon sequences of the *MAT1-1-1* and *MAT1-2-1* genes, respectively, in numerous wild-type *C. sinensis*isolates. Exon variations may disrupt the translation of coding sequences, in additionto alternative splicing and differential occurrence and transcription of mating genes, asreported by Li et al., 2023 [36], 2024 [37]. The mutation rates reported by Zhang and Zhang 2015 [35] appeared to be much greater than those present in the GenBank database, which includes numerous variable sequences of the MAT1-1-1 and MAT1-2-

1 proteins of *C. sinensis* isolates [Li et al., 2024 [37]]. Presumably, Zhang and Zhang [2015 [35]] might not have uploaded all the variable sequences to the GenBank database to truthfully represent the natural diversity of the variable stereostructures of the mating proteins.

Although the AlphaFold database does not include the predicted 3D structures for the mating proteins encoded by the genome and transcriptome assemblies of *H. sinensis* strains and the metatranscriptome assemblies of the natural *C. sinensis* insect–fungi complexes,the 2D structures of the mutant MAT1-1-1 and MAT1-2-1 proteins (including those with truncation of large protein segments) that were translated from the nucleotide sequences were analyzed to explore the variations in the α-helices, β-sheets, β-turns, and coils via ExPASy ProtScale technology (*cf*. Figures 7–10). The apparent changes in the 2D structures of the mating proteins indicate altered 3D structures and subsequent dysfunction and even complete deactivation of the mating proteins.

Research has confirmed that the MAT1-1-1 and MAT1-2-1 proteins can interact with each other [Jacobsen et al., 2002 [64]; Rams & Kück 2022 [65]]. The interaction of mating proteins with functional stereostructures is essential for the recognition of compatible mating partners and plays a crucial role in regulating sexual reproduction by controlling gene expression related to mating compatibility within a fungal species. The MAT1-1-1 protein contains a MATalpha_HMGbox domain, and the MAT1-2-1 protein contains an HMG-box_ROX1-like domain [Hu et al., 2013 [31]]. Both domains are involved in DNA binding, binding to specific DNA sequences in the genome to regulate the transcription of genes involved in mating processes. We highlighted the domains in the sequences of the mating proteins in Figures 7 and 8 and summarized the conservative and nonconservative amino acid residue substitutions within or outside the domains in the sequences of the mating proteins of the wild-type isolates in Tables 3 and 4.

The heteromorphic stereostructures of the mating proteins might explain, at least partially, why efforts made in the past 40–50 years to cultivate pure *H. sinensis*, Genotype #1 of the 17 *O. sinensis* genotypes, in research-oriented academic settings to induce the production of fruiting bodies and ascospores have consistently failed, as reported and summarized previously [Holliday & Cleaver 2008 [29]; Stone 2010 [30]; Hu et al., 2013 [31]; Zhang et al., 2013 [23]]. Tables 5 and 6 of this paper confirmed the differential occurrence of the mating proteins encoded by the genome and transcriptome assemblies of the *H. sinensis* strains and by the metatranscriptome assemblies of the natural *C. sinensis* insect–fungi complex. Bushley et al., 2013 [32] and Li et al., 2024 [37] reported alternative splicing of the *MAT1-2-1* gene with unspliced intron I, which contains three stop codons, in *H. sinensis* strain 1229. Consequently, the C-terminally truncated MAT1-2-1 protein fragment lacked the major portion of the protein encoded by exons II and III of the gene.

4.3. Differential Occurrences of MAT1-1-1 and MAT1-2-1 Proteins with Heteromorphic Sereostructures in H. sinensis Strains and C. sinensis Isolates

Uploaded to the GenBank and AlphaFold databases by many researchers, 131 (75.7%) of the 173 *H. sinensis* strains and wild-type *C. sinensis* isolates generated either the MAT1-1-1 or MAT1-2-1 protein but not both. This phenomenon was confirmed by the differential occurrence of the mating proteins encoded by the genome, transcriptome, and metatranscriptome assemblies of the *H. sinensis* strains and the natural *C. sinensis* insect–fungi complex samples (*cf*. Tables 5 and 6).

However, 42 other strains/isolates (24.3%) produced both MAT1-1-1 and MAT1-2-1 proteins, the sequences of which were derived from genomic DNA isolated from *H. sinensis* strains or wild-type *C. sinensis* isolates but, unfortunately, not

detected directly via genetranscription assays or biochemical examinations. In addition to the intracellular biological processes of the MAT1-1-1 and MAT1-2-1 proteins, such as differential transcription andalternative splicing of the genes, as reported by Li et al., 2024 [37], it needs to be considered whether the mating proteins with heteromorphic 3D structures are capable of expressing their mating functions to accomplish the sexual reproduction of *O. sinensis* during thelifecycle of the natural *C. sinensis* insect–fungi complex.

The MAT1-1-1 proteins from 35 of the 42 strains/isolates were clustered into Bayesian Cluster A (*cf*. Figure 1), accompanied by one of the twenty MAT1-2-1 proteins that were clustered into Bayesian Cluster I or one of the fifteen MAT1-2-1 proteins that were clustered into Bayesian Cluster V (*cf*. Figure 2). These results will be meaningful and useful for the future design of biochemical protein research and reproductive physiological research to explore the functionalities of mating proteins that are clustered into different Bayesian clusters and have diverse 3D structural morphs. The most challenging aspect of biochemical examinations is determining the stereostructures and molecular dynamics of fully functioning proteins under native conditions or after renaturation [Zhu & Gray 1994 [66]]. Figures 7 and 8 show the amino acid residue substitutions in the mating protein sequences of the *H. sinensis* strains and wild-type *C. sinensis* isolates, which cause the alterations of 3D structures shown in Figures 1 and 6 and Tables 1 and 2. Table 3 summarizes the correlation between the altered primary structures and the diverse stereostructures. Some amino acid residue substitutions occur within the MATalpha_HMGbox domain and HMG-box_ROX1-like domain of the MAT1-1-1 and MAT1-2-1 protein sequences, respectively. In addition to the single-residue substitutions, Figures 7 and 8 show more pronounced mutations in the mating proteins encoded by the genome, transcriptome, and metatranscriptome assemblies of *H. sinensis* strains and natural *C. sinensis* insect–fungi complexes, which caused dramatic changes in the 2D structures of the mating proteins (*cf*. Figures 9 and 10). These findings will inspire further refined research to accurately locate the causal relationship between specific variations in amino acid sequences and protein 3D structural changes, as well as the potential impact on mating protein functionality.

4.4. Heteromorphic 3D Structures of the Mating Proteins and Sexual Reproductive Behavior of H. sinensis, Genotype #1 of O. sinensis

Sexual reproduction of *O. sinensis* is crucial for maintaining the natural population volume of the *C. sinensis* insect–fungi complex, which is endangered at Level 2 of National Key Protected Wild Plants [China Ministry of Agriculture and Rural Affairs 2021 [38]]. The following hypotheses have been previously proposed for *H. sinensis*, the sole anamorph of *O. sinensis* postulated by Wei et al., 2006 [18]: (1) homothallism [Hu et al., 2013 [31]], (2) pseu- dohomothallism [Bushley et al., 2013 [32]], and (3) facultative hybridization [Zhang & Zhang 2015 [35]]. These hypotheses are based on nucleotide data derived from molecular, genome, and transcriptome studies of *H. sinensis*. In theory, self-fertilization under homoth- allism and pseudohomothallism in ascomycetes becomes a reality when the interaction of MAT1-1-1 and MAT1-2-1 proteins with appropriate stereostructures exhibit full mat- ing functions within a single fungal cell [Turgeon & Yoder 2000 [67]; Debuchy & Turgeo 2006 [25]; Jones & Bennett 2011 [59]; Zhang et al., 2011 [34]; Bushley et al., 2013 [32]; Hu et al., 2013 [31]; Zheng & Wang 2013 [27]; Wilson et al., 2015 [28]; Zhang & Zhang 2015 [35]]. However, after thoroughly analyzing genetic and transcriptional data for *H. sinensis* in the literature, Li et al., 2023 [36], 2024b [37] reported differential occurrences, alternative splicing, and differential transcription of the mating-type genes of the MAT1-1 and MAT1-2 idiomorphs and the pheromone receptor genes in 237 *H. sinensis* strains. Thus, the evi- dence indicated that *H. sinensis*, which is Genotype #1 of the 17 genomically independent *O. sinensis* genotypes, is self-

sterilizing and incapable of completing self-fertilization butrequires sexual partners to accomplish *O. sinensis* sexual reproduction under heterothallism or hybridization.

Because of the absence of repetitive copies of the *MAT1-1-1* or *MAT1-2-1* genes in the *H. sinensis* genome assemblies, the alternative splicing and differential occurrence and transcription of the mating-type genes and the diversity of heteromorphic 3D structuresof the mating proteins with altered functionalities indicate that there may be two or more *H. sinensis* populations, either monoecious or dioecious. The different *H. sinensis* popu-lations may participate as sexual partners capable of producing either the functioningMAT1-1-1 or the functioning MAT1-2-1 protein with proper stereostructures for reciprocal pairing and interaction during successful physiological heterothallism crossing. Thus, a- and α-pheromones and corresponding α- and a-pheromone receptor proteins play critical roles in the communication of sexual signals between sexual partners. If this assumption is correct, the sexual partners might possess indistinguishable *H. sinensis*-like morphological and growth characteristics, as elucidated previously [Kinjo & Zang 2001 [68]; Zhang et al., 2009 [33]; Chen et al., 2011 [69]; Li et al., 2013 [70], 2016 [13]; Mao et al., 2013 [71]]. For instance, the indistinguishable *H. sinensis* strains 1229 and L0106 produce complementary transcripts of the mating-type genes and proteins of the MAT1-1 and MAT1-2 idiomorphs [Bushley et al., 2013 [32]; Liu et al., 2015 [44]; Li et al., 2023 [36], 2024 [37]].

Even if the physiological heterothallism hypothesis is incorrect for *O. sinensis*, one of the mating proteins might be produced by genome-independent, heterospecific fungal species, which would result in plasmogamy and the formation of heterokaryotic cells(*cf*. Figure 3 of [Bushley et al., 2013 [32]]) to ensure successful sexual hybridization or even parasexual reproduction if the heterospecific species are capable of breaking interspecific reproduction isolation, similar to many cases of fungal sexual hybridization and parasexual reproduction that promote adaptation to the extremely adverse ecological environmenton the Qinghai–Tibet Plateau [Pfennig 2007 [72]; Seervai et al., 2013 [73]; Nakamura et al., 2019 [74]; Du et al., 2020 [75]; Hénault et al., 2020 [76]; Samarasinghe et al., 2020 [77];

Mishra et al., 2021 [78]; Steensels et al., 2021 [79]; Kück et al., 2022 [80]]. Alternatively, to complete physiological heterothallism or hybridization for reproduction, mating part-ners that produce the MAT1-1-1 and MAT1-2-1 proteins with functional stereostructuresmight exist in adjacent hyphal cells, which might determine their mating choices, and they may communicate with each other through a mating signal-based transduction system of pheromones and pheromone receptors and form “H”-shaped crossings of multicellularhyphae [Hu et al., 2013 [31]; Bushley et al., 2013 [32]; Mao et al., 2013 [71]]. In fact, Maoet al., 2013 [71] illustrated the “H”-shaped morphology in *C. sinensis* hyphae that genetically contained either AT-biased Genotype #4 or #5 of *O. sinensis* without cooccurrence of the GC-biased Genotype #1 *H. sinensis* and that the genome-independent AT-biased *O. sinensis* genotypes shared indistinguishable *H. sinensis*-like morphological and growth character-istics. To date, no study has reported the identification of a- and α-pheromone genes in the genome or transcriptome assemblies of *H. sinensis* strains or in the metatranscriptome assemblies of natural *C. sinensis*; however, α- and a- pheromone receptor genes were found to differentially occur in the genome and transcriptome assemblies of *H. sinensis* and inmetatranscriptome assemblies of the natural *C. sinensis* insect–fungi complex [Hu et al.,2013 [31]; Li et al., 2024 [37]]. Thus, the mating signal transduction between sexual partners to ensure appropriate interaction between MAT1-1-1 and MAT1-2-1 proteins with functional stereostructures remains a mystery in *O. sinensis*, which may inspire further research.

4.5. Heteromorphic 3D Structures of the Mating Proteins and Sexual Reproduction Strategy During the Lifecycle of the

Natural C. sinensis Insect–Fungi Complex

In addition to the intensive attention given to *H. sinensis*, the N-terminally and midsequence-truncated MAT1-1-1 proteins and the variable MAT1-2-1 proteins encoded by the metatranscriptome assemblies of the natural *C. sinensis* insect–fungi complex exhibitalterations in the 2D structures of the proteins (*cf*. Figures 7–10). These results suggest heteromorphic 3D structures of the mating proteins and dysfunctional or anomalous fungal mating processes during the lifecycle of the natural *C. sinensis* insect–fungi complex.

Li et al., 2016 [13] reported the genetic heterogeneity of the wild-type *C. sinensis* isolates CH1 and CH2, which were isolated from the intestines of healthy living larvae of *Hepialus lagii* Yan, based on their *H. sinensis*-like morphology and growth characteristics.The *C. sinensis* isolates CH1 and CH2 contained GC-biased Genotype #1 (*H. sinensis*) andAT-biased Genotypes #4–5 of *O. sinensis*, as well as *Paecilomyces hepiali* [Dai et al., 1989 [81]], which was renamed *Samsoniella hepiali* in 2020 [Wang et al., 2020 [82]]. The impure wild-type *C. sinensis* isolates exhibited 15–39-fold greater inoculation potency on the larvae of *Hepialus armoricanus* than did pure *H. sinensis* (n = 100 for each inoculant; $p < 0.001$), indicating the symbiosis of multiple intrinsic fungi during the lifecycle of natural *C. sinensis*, at least inthe larva infection stage [Li et al., 2016 [13]].

We found that no repetitive copies of the *MAT1-1-1* or *MAT1-2-1* genes were identified in the *H. sinensis* genome assemblies. Thus, the genetic heterogeneity of the wild-type *C. sinensis* isolates suggests that the coexisting MAT1-1-1 and MAT1-2-1 proteins withvaried amino acid sequences and diverse 3D structures detected in 24.3% of the wild- type *C. sinensis* isolates might have been derived from genome-independent heterospecific fungi, which may pair complementarily and reciprocally to ensure proper interactions and accomplish mating processes during the heterothallic or hybrid reproduction of *O.sinensis*. The different fungal sources for the cooccurring MAT1-1-1 and MAT1-2-1 proteins are evidenced by the species contradiction between the inoculants (3 purified GC-biased *H. sinensis* strains) and the genome-independent teleomorphic AT-biased Genotype #4,which is reportedly the sole teleomorphic fungus present in the fruiting body of culti-vated *C. sinensis* [Wei et al., 2016 [19]]. These authors reported that successful artificialinoculation-based cultivation projects under product-oriented industrial settings used a special cultivation strategy without pursuing strict fungal purification by adding soil that was transported from natural *C. sinensis* production areas on the Qinghai–Tibet Plateauinto the industrial cultivation system [Wei et al., 2016 [19]].

The AMT1-1-1 and MAT1-2-1 proteins with diverse stereostructures may originate from heterogeneous fungal sources in single hyphal and ascosporic cells. Li et al., 2013 [70] reported the genetic heterogeneity of 15 cultures of two groups derived from monoas-cospores, the reproductive cells of natural *C. sinensis*, after 25 days of in vitro incubation at 18 °C. The first group included seven homogeneous clones (1207, 1218, 1219, 1221, 1222,1225, and 1229), containing only *H. sinensis* (GC-biased Genotype #1 of *O. sinensis*). Thesecond group included eight heterogeneous clones (1206, 1208, 1209, 1214, 1220, 1224, 1227, and 1228), containing both GC-biased Genotype #1 and AT-biased Genotype #5 of *O. sinensis*. The sequences of the GC- and AT-biased *O. sinensis* genotypes reside in in-dependent genomes and belong to independent fungi [Xiao et al., 2009 [83]; Zhu et al., 2010 [84]; Li et al., 2022 [6], 2024 [37]]. Bushley et al., 2013 [13] The collaborators of Li et al., 2013 [70], observed multicellular heterokaryotic hyphae and ascospores of natural *C. sinensis* with mononucleated, binucleated, trinucleated, and tetranucleated structuresand reported the PCR results for 22 clones, which included 7 additional clones (1210, 1211, 1212, 1213, 1216, 1223, and 1226) forming the third group of

ascosporic clones in addition to the aforementioned first and second groups of clones (*cf*. Figures 2 and 3 of [Bushley et al., 2013 [32]]). However, neither Bushley et al., 2013 [32] nor Li et al., 2013 [70] reported thegenetic features of the third group of ascosporic clones. However, there is no doubt from the two literature reports that the ascosporic clones from the third group were genetically distinct from those in the homogeneous first group or heterogeneous second group andapparently belonged to taxonomically different fungal species or fungal complexes.

Zhang and Zhang [2015 [35]] commented that the nuclei of binucleated hyphal and ascosporic cells (as well as mononucleated, trinucleated, and tetranucleated cells) of natural

C. sinensis likely contained different genetic materials. These multicellular hyphal andascosporic cells of natural *C. sinensis* might contain two or more sets of genomes of inde-pendent fungi, which might be responsible for the production of complementary matingproteins for sexual reproductive outcrossing. Thus, the monoascospores of natural *C. sinen- sis* might be characterized by more complex genetic heterogeneity, coexisting with moreheterospecific fungal species than the cooccurring GC-biased Genotype #1 and AT-biased Genotype #5, which were reported by Li et al., 2013 [70].

Unlike culture-dependent experiments, which are apparently unable to detect noncul- turable fungal species, Li et al., 2023 [49], 2023 [15] reported culture-independent studiesand demonstrated the genetic heterogeneity of *C. sinensis* ascospores and the stromalfertile portion (SFP), which is densely covered with numerous ascocarps, which are the reproductive cells and organs of natural *C. sinensis*. These authors observed semi-ejectedand fully ejected ascospores of natural *C. sinensis* and reported the cooccurrence of theGC-biased *O. sinensis* Genotypes #1 and #13/14, AT-biased *O. sinensis* Genotypes #5–6 and #16 within AT-biased clade A in the Bayesian phylogenetic tree, *S. hepiali* (≡*P. hepiali*), and an AB067719-type fungus. In addition, the *C. sinensis* SFP contained another fungal group, namely, AT-biased Genotypes #4 and #15 of *O. sinensis*, which were clustered into AT-biased clade B in the Bayesian phylogenetic tree [Li et al., 2023 [15]]. Genotypes #4 and #15 were absent in the ascospores, which is consistent with the results of culture-dependent studies [Li et al., 2013 [70]]. The abundance of fungal components exhibited marked dynamicalterations in a disproportional and asynchronous manner in the *C. sinensis* SFP before and after ascospore ejection and in the two types of ascospores [Li et al., 2023 [49]]. Thus, thecoexistence of the MAT1-1-1 and MAT1-2-1 proteins detected from the wild-type *C. sinensis* isolates might have been derived interindividually from fungi that might serve as mating partners of *O. sinensis* to accomplish heterothallic or hybrid reproduction.

Li et al., 2023 [36], 2024 [37] summarized prior scientific evidence regarding the sexual reproduction of *O. sinensis*. Based on genetic heterogeneity with multiple heterospecificfungal species in the natural *C. sinensis* insect–fungi complexes and multicellular het-erokaryotic structures of ascospores and hyphae, the scientific evidence may be dividedinto two aspects: (1) the 17 cooccurring genome-independent genotypes of *O. sinensis* indifferent combinations and (2) the taxonomically heterospecific fungal species, which are based on the mycobiota of >90 co-colonizing fungi belonging to at least 37 fungal genera in the stromata and caterpillar bodies of natural *C. sinensis* insect–fungi complexes [Zhanget al., 2010 [9], 2018 [10]; Xiang et al., 2014 [45]; Meng et al., 2015 [85]; Xia et al., 2015 [11], 2017 [46]; Guo et al., 2017 [12]; Wang et al., 2018 [86]; Zhong et al., 2018 [16]; Li et al., 2019 [87]; Zhao et al., 2020 [88]; Yang et al., 2021 [89]; Kang et al., 2024 [17]].

(1) Evidence for the differential cooccurrence of multiple genotypes of *O. sinensis* in the compartments of the

natural *C. sinensis* insect–fungi complex is as follows:

(1-a). Differential occurrence of AT-biased Genotype #4 or #5 of *O. sinensis* with- out the cooccurrence of GC-biased *H. sinensis* in natural *C. sinensis* samples collected from different production areas in geographically remote locations [Engh 1999 [90]; Kinjo and Zang 2001 [68]; Stensrud et al., 2005 [91], 2007 [92]; Mao et al., 2013 [71]];

(1-b). Multiple cooccurring GC- and AT-biased genotypes of *O. sinensis* have beenobserved differentially in different combinations in the stroma, caterpillar body, ascocarps, and ascospores of natural *C. sinensis* [Xiao et al., 2009 [83]; Zhu et al., 2010 [84]; Li et al., 2013 [70], 2022 [6], 2023c [49], 2023d [15]; Mao et al., 2013 [71]]. The abundances of the *O. sinensis* genotypes underwent dynamic alterations in an asynchronous, disproportional manner in the caterpillar bodies and stromata of *C. sinensis* specimens during maturation, with a consistent predominance of the AT-biased genotypes of *O. sinensis*, not the GC- biased *H. sinensis*, in the stromata, indicating that the sequences of *O. sinensis* genotypes were present in independent genomes of different fungi [Xiao et al., 2009 [83]; Zhu et al., 2010 [84]; Hu et al., 2013 [31]; Li et al., 2013 [70], 2016a [40], 2020 [14], 2022 [6], 2023c [49]; Jin et al., 2020 [41]; Liu et al., 2020 [42]; Shu et al., 2020 [43]];

(1-c). The GC-biased Genotypes #1 and #2 of *O. sinensis* cooccur in the stromata of natural *C. sinensis*. The abundance of the GC-biased genotypes was dynami- cally altered during *C. sinensis* maturation [Zhu et al., 2010 [84]];

(1-d). The cooccurrence of GC-biased genomically independent Genotypes #1 and #7 of *O. sinensis* was detected in the same specimen of natural *C. sinensis* [Chen et al., 2011 [69]];

(1-e). A species contradiction between the anamorphic inoculants (GC-biased Geno- type #1 *H. sinensis* strains) and the sole teleomorph of AT-biased Genotype #4 of *O. sinensis* was detected in the fruiting body of cultivated *C. sinensis* in a product-oriented industrial setting [Wei et al., 2016 [19]];

(1-f). Discovery of Genotypes #13 and #14 of *O. sinensis* in semi-ejected and fully ejected multicellular heterokaryotic ascospores, respectively, collected from the same *C. sinensis* samples [Li et al., 2023 [15]];

(1-g). The genetic heterogeneity of ascospores and SFP, the reproductive cells andorgans of natural *C. sinensis*, involves multiple GC- and AT-biased *O. sinensis* genotypes in different combinations [Li et al., 2013 [70], 2022 [6], 2023 [49], 2023 [15]].

(2) Evidence for the differential cooccurrence of heterospecific fungal species in different compartments of the natural *C. sinensis* insect–fungi complex is as follows:

(2-a). Mycobiota findings for differential cooccurrence of >90 fungal species of atleast 37 fungal genera in the caterpillar bodies and stromata of natural *C. sinen- sis* [Zhang et al., 2010 [9], 2018 [10]; Xia et al., 2015 [11]; Guo et al., 2017 [12];

Zhong et al., 2018 [16]; Kang et al., 2024 [17]];

(2-b). A good number of *C. sinensis* isolates contained mutant MAT1-1-1 and MAT1-2- 1 proteins, especially those proteins with C-and/or N-terminal truncations that belong to nine and four diverse 3D structural morphs (*cf*. Figures 4 and 6), re- spectively. The mutant proteins were either clustered into a separate Bayesian clade or clustered within the main clustering branches in the Bayesian trees (*cf*. Figures 1 and 2). The MAT1-1-1 and MAT1-2-1 proteins

encoded by meta- transcriptome assemblies of natural *C. sinensis* also exhibited either large- segment truncation or sequence variations similar to those observed in wild- type *C. sinensis* isolates (*cf*. Figures 7–10). Some of the mutant proteins might be produced by heterospecific fungi in impure wild-type *C. sinensis* isolates and in natural *C. sinensis* insect–fungi complexes;

(2-c). Discoveries of the formation of the heterospecific *Cordyceps-Tolypocladium* com- plex in natural *C. sinensis* [Engh 1999 [90]; Stensrud et al., 2005 [91], 2007 [92]] and the dual anamorphs of *O. sinensis*, involving psychrophilic *H. sinensis* and mesophilic *Tolypocladium sinensis* [Li 1988 [93]; Chen et al., 2004 [94]; Leung et al., 2006 [95]; Barseghyan et al., 2011 [96]];

(2-d). A close association of psychrophilic *H. sinensis* and mesophilic *S. hepiali*(≡*P. hepiali*) was found in the caterpillar body, stroma, ascospores, and stromal fertile portion, which was densely covered with numerous ascocarps of nat- ural *C. sinensis*, and in the wild-type *C. sinensis* complexes, which appeared to be difficult to purify [Dai et al., 1989 [81]; Jiang & Yao 2003 [8]; Chen et al., 2004 [94]; Zhu et al., 2007 [97], 2010 [84]; Yang et al., 2008 [98]; Li et al., 2016 [13], 2023 [15]];

(2-e). Although Genotypes #13–14 are among the 17 genotypes of *O. sinensis*, these 2 GC-biased genotypes feature precise reciprocal cross substitutions of large DNA segments among two heterospecific parental fungi, namely, *H. sinensis* and an AB067719-type fungus. The taxonomic position of the AB067719-type fungus is undetermined to date, and more than 900 heterospecific fungal se- quences, which are highly homologous to AB067719, have been uploaded to GenBank [Li et al., 2023 [15]]. Chromosomal intertwining and genetic material recombination may occur after plasmogamy and karyogamy of the heterospe- cific parental fungi under sexual reproduction hybridization or parasexuality, which is characterized by the prevalence of heterokaryosis and results in concerted chromosome loss for transferring–substituting genetic materials without conventional meiosis [Bennett & Johnson 2003 [99]; Sherwood & Ben- nett 2009 [100]; Bushley et al., 2013 [32]; Seervai et al., 2013 [73]; Nakamura et al., 2019 [74]; Mishra et al., 2021 [78]; Kück et al., 2022 [80]; Li et al., 2023 [15]].

The selection of different genotypes of *O. sinensis* or heterospecific fungal species as sexual partners depends on their mating choices for hybridization or parasexuality and their ability to break interspecific isolation barriers to adapt to extremely harsh ecolog- ical environments on the Qinghai–Tibet Plateau and the seasonal climate changes from extremely cold winters, when *C. sinensis* is in its asexual growth phase, to warmer springs and early summers, when *C. sinensis* switches to the sexual reproduction phase [Pfennig 2007 [72]; Du et al., 2020 [75]; Hénault et al., 2020 [76]; Samarasinghe et al., 2020 [77]; Steensels & Gallone 2021 [79]].

5. Conclusions

The analysis of the MAT1-1-1 and MAT1-2-1 proteins in the 173 *H. sinensis* strains and wild-type *C. sinensis* isolates revealed heteromorphic stereostructures of the mating proteins, which were clustered into multiple Bayesian clustering clades and branches. In addition to the evidence of alternative splicing and differential occurrence and translation of the *MAT1-1-1* and *MAT1-2-1* genes in *H. sinensis* [Li et al., 2023 [36], 2024 [37]], the diversity of heteromorphic mating proteins suggested stereostructure-related alterations in the mating functions of proteins and provided additional evidence supporting the self- sterility hypothesis under heterothallic and hybrid reproduction for *O. sinensis*, including

H. sinensis, Genotype #1 of the 17 genome-independent *O. sinensis* genotypes. The hetero- morphic stereostructures of the mutant MAT1-1-1 and MAT1-2-1 proteins discovered inwild-type *C. sinensis* isolates and the natural *C. sinensis* insect–fungi complexes may suggest diverse sources of the mating proteins produced by two or more cooccurring heterospecific fungal species in natural *C. sinensis* that have been discovered in mycobiota, molecular,metagenomic, and metatranscriptomic studies, regardless of whether culture-dependent or culture-independent research strategies wereused.

Supplementary Materials: The following supporting information can be downloaded at https: //www.mdpi.com/article/10.3390/jof11040244/s1. Table S1. Amino acid scales based on the general chemical characteristics of their side chains for ProtScale analysis (https://web.expasy.org/protscale/, accessed from 18 October 2024 to 1 December 2024) to predict the secondary structures (α-helices, β-sheets, β-turns, and coils) of proteins; Figure S1. Alignment of the full-length sequences of reference MAT1-2-1 proteins AEH27625 of structural morph Branch I-1 of Cluster I, ACV60363 of structural morph Branch V-1, and five MAT1-2-1 proteins.

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