【附件三】成果報告(系統端上傳 PDF 檔)

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教育部教學實踐研究計畫成果報告

Project Report for MOE Teaching Practice Research Program

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腐朽木材真菌與城市林樹木安全性

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腐朽木材真菌與城市林樹木安全性

一. 本文 Content (3-15 頁)

1. 研究動機與目的 Research Motive and Purpose

Fungi are the most important organisms in degradation of wood and recycling of the organic molecules cellulose and lignin, because fungi produces the largest amounts of effective enzymes. The process of wood degradation can start in living trees and continues after fall of the stem for many years. The detailed degradation of the different types of cell walls and tissues in the tree follows very specific patterns depending on the fungal species and tree species. The specificity of this interaction is hardly known outside central Europe and northern America, but is highly important for estimating or predicting the safety of trees infected with fungi, particularly in urban areas, such as roadsides, parks and schools, where trees are planted. The public institutions in Taiwan allocate a lot of funding for monitoring tree safeties and for precautions against uncontrolled tree fall, but scientific data and public awareness are both very low.

2. 文獻探討 Literature Review

(1) The situation of mycology in Taiwan

Over 120,000 species of fungi have been described worldwide, but this is estimated to be less than 10% of the estimated species number (Hawksworth 2001, Hawksworth & Lücking 2017). The fact that the majority of fungal species is unknown is the major obstacle in any kind of mycological research. The number of mycologists focusing their research on investigating fungal diversity has been low, but stable in Taiwan, but in the last years increased considerably in mainland China (Dai et al. 2015) and other Asian countries. The exploration of Taiwanese fungi is particular, because its foundations have been laid by

Japanese mycologists who also contributed studies of Taiwanese fungi even after the World War II. The most prominent researcher was Kaneyoshi Sawada who described and recorded several hundred species (Sawada 1959).

Based on the efforts of Japanese mycologists and subsequent generations of Taiwanese mycologists, we now know about 6000 species of fungi in Taiwan (Wang et al. 1999). Many records have been compiled in the Mycobiota Taiwanica (2015). Plant parasitic fungi have been included in the list of plant diseases by Hsu et al. (2002). Records from mycologists in Taiwan are also generally cited in mycofloristic publications from mainland China (particularly in the series Flora Fungorum Sinicorum, Science Press, Beijing).

The grounds around universities and schools with active mycologists working there are potentially the mycologically best explored areas, because they are most convenient for collecting specimens for teaching mycology and supervising mycological theses of students. Schools and universities usually provide an environment for supporting an atmosphere of learning by planting trees erecting woody constructions. Such trees and constructions like wooden fences are spontaneously colonized by wood-decaying fungi.

Many first records and new species of fungi in a country were discovered on the grounds of elementary and high schools and universities. The planting of numerous foreign trees and regular maintenance such as mowing and removing of plant litter and rotting wood on school ground are adverse to the growth of fungi which need a less disturbed environment. Many records of fungi do not mean that these environments would be ideal for the occurrence of fungi, but rather of researchers of fungi. The records of fungi, therefore, mainly depend on the presence of mycologists who deal with certain systematic groups. Hence, particularly many records were published from universities with a large park-like campus and strong mycological research teams. Master and PhD students and their supervisors naturally often included their next surrounding when studying fungal diversity in Taiwan. Such theses and publications are, therefore, particularly rich in first records and even new species. Examples are fungi from the campus of National Taiwan University, such as anamorphic Ascomycota (Chen 1997), Basidiomycota (Wu 1990), Myxomycota (Chung & Liu 1998, Liu & Chang 2011, and references given therein), which all include species on dead wood. New discoveries from university campi of the project applicant was the new species *Corynesporopsis acaciae* R. Kirschner on dead wood of *Acacia confusa* from the campus of National Central University (Kirschner 2015). Other species on dead wood were first records from Taiwan, *Hyalosynnema multiseptatum* Matsushima and *Physalacria corticola* Corner at NTU (Kirschner & Chen 2008, Kirschner et al. 2001).

This potential, however, can be more explored than it is often the case. Particularly NTU has experimental agricultural and forest stations in the mountains providing particular opportunities to involve students in research on mushrooms in remote mountainous areas by a combination of practical and theoretical training (Tschen et al. 1999, 2000, 2001, 2002, 黃靜芬等 2011). Our project will not only document a part of the fungal diversity occurring at the campus of National Taiwan University, but may also be of assistance in searching particular species for research and teaching typical for urban regions at low elevations in Taiwan.

(2) The situation of knowledge and application of wood decay fungi in Taiwan

In the past, wood decay fungi in Taiwan were morphologically described and identified so that several books and articles mainly in Chinese are available on this topic, which can mainly be credited to the scientific merits of Dr. Tun-Tschu Chang (張東柱) from the Taiwan Forest Research Institute (e.g. Chang et a. 1999) and Dr. Sheng-Hua Wu (吳聲華) from the National Museum of National Science in Taichung (e.g. Wu et al. 2002). Since both researchers are retired and because of the major new taxonomical progress in mycology due to molecular phylogenetic techniques, the progress of this discipline in Taiwan urgently needs further support. Particularly the species taxonomy of the important genus *Ganoderma* (靈芝) has progressed rapidly through molecular analyses, which is not yet reflected in the books in Taiwan.

(3) Germany as model for research of wood decay fungi in Taiwan

Since sustainable forestry has been developed first in Germany and became a model for forestry worldwide, Germany is also among the leading countries for developing scientific standards for diagnosing and maintaining wood decay and tree safety. Particularly, Prof. Dr. Francis W.M.R. Schwarze formerly from the University of Freiburg, Germany, now the head of Empa - Swiss Federal Laboratories for Materials Science and Technology, Switzerland, has dedicated decades of research in this field, which is documented in numerous international scientific journals and two major textbooks on wood decay fungi in English and German: “Fungal Strategies of Wood Decay in Trees” and “Diagnosis and Prognosis of the Development of Wood Decay in Urban Trees”. These books introduce the laboratory techniques and basic and applied research results about the major wood decay fungi in Central Europe. In a unique form these books illustrate the specific wood decay patterns of different fungal species in different tree species with focus on living infected trees and provide examples for application in tree safety prognosis and developing new products. Hitherto there is no other comparably comprehensive book in this field so that the two books serve as model for the study of wood decay in other countries. Since the tree and wood decay fungi species in Taiwan are largely different from those investigated by Schwarze and study of wood decay fungi has hitherto focused on the identification of wood decay fungi on common trees. Combining the approach of wood decay pattern research from Schwarze with a molecular update of the fungi identified by Chang seems to be the next necessary step for progress in the field in Taiwan. For these reasons, it is important to introduce to the students the traditional taxonomical knowledge of wood decay fungi in Taiwan as well as with modern molecular and biodiversity informatics approaches and a sound background of wood decay patterns as exemplified by the research in Germany.

3. 研究問題 Research Question

Our hypothesis is that the complex connection between scientific research and application be implemented in a course for students by the example of wood decay by fungi. Although this topic is complex by the diversity of methods and highly specific interactions, its inherent potential for didactical reduction and its close connection to all-day life are highly beneficial for teaching purposes.

(1) Diversity of approaches:

The species number of wood decay fungi on the university campus is limited so that it is realistic to apply diverse approaches on these species. Field collection brings the participants of the course in contact with nature outdoor and enhances scientific and intuitive insight in nature. Illustration of the fungi in the field by photographing nurtures esthetic values and conform to scientific standards. Demonstration of lab work for microscopy and interpretation of DNA sequences for species identification introduce practical research. Cultivating fungal strains is a method for training how to deal with living organisms in an appropriate way and is also a way for appreciating biological resources for research and potential application. Our first results from courses at NCU and NTU have found that some common species are involved in wood decay in Taiwan which have never been recorded for the country.

(2) Didactical reduction:

The advantage of selecting wood-decay fungi is that the most competent wood-decomposing species belong to limited sets of systematic groups (e.g. Basidiomycota: Agaricales, Gloeophyllales, Hymenochaetales, and Polyporales; Ascomycota: Xylariales). This limitation allows a simplified introduction into basic concepts of fungal systematics and general biological taxonomy. In contrast to many other fungi, the fruit-bodies of most wood decay fungi persist for several weeks or even years so that they can be encountered during the courses independent from weather conditions.

(3) Interdisciplinary connections:

The interaction in wood decay fungi can be classified as white rot, brown rot and soft rot, depending on which structural wood elements are principally degraded. For understanding the specificity of these interactions, students have to understand the connection between mycology, ecology and botany, particularly anatomy and taxonomy of trees. The methods include molecular biology and biodiversity informatics for understanding the principles of species identification and geographic and host distribution. Certain wood decay fungi have narrow preferences for specific plants while other fungi do not have. Some fungi are aggressive pathogens, whereas other species are merely saprobic decomposers. Different fungi compete for the same wood substrate. Although these interdisciplinary connections are complex in detail and poorly investigated, the basic principles are easy to learn from the above examples.

(4) Establishing balance between digital (virtual) reality and natural reality:

A present problem in education is a decline in outdoor activity compared to increasing online activity. Particularly, in the present digital era dominated by pictures, there is a danger of confusing the virtual representation with reality. In the virtual representation, the actual size of objects cannot be as clearly recognized as in the direct perception. Demonstrating fresh specimens in addition to the pure optical perception from indirect media also provides important other sensual clues, such as touch, smell, and even taste. In lab courses, students actively collect fungi on the campus which are used for microscopy and molecular identification in the classroom. By this process, students are encouraged to increase their awareness about fungi that can be encountered regularly on the campus and appreciate the diversity of real organisms. This awareness is especially important for children since they have a high capacity and motivation to know living beings around them, but this capacity is largely devoted to virtual “synthetic species” such as Pokémon instead of native wildlife (Balmford et al. 2002).

4. 研究設計與方法 Research Methodology

Observation of fungi is done throughout the year on the campus with the opportunistic strategy (Mueller et al. 2011) during the term. Fresh samples are photographed directly in their habitat or occasionally in the lab. Samples are dried on an electrical drier and preserved for later deposition at TNM. Prior to drying of some specimens, strains are isolated and deposited at BCRC. Giving the exact locations of fungi follows the campus map of NTU and GPS.

(1) Cultivation of strains

Wood decay fungi usually form macroscopically visible fruit-bodies which can be used for cultivation with the spore-print method and by transferring hyphae from fruit-bodies. In the spore-print method, a small area of the spore-bearing layer (hymenophore) is cut out from the fruit-body and fixed top-down beneath the lid of a Petri dish with glue. The spores are liberated usually overnight and fall onto the below agar, where they germinate and produce hyphae. Fresh fruit-bodies usually contain no or few other microbes inside. Under clean conditions (laminar flow), fruit-bodies are cut open with a sterile knife. Hyphae can be extracted from the clean exposed interior of the fruit-bodies and transferred to agar media for growth. Conventional agar media such as malt extract agar, potato dextrose agar etc. are all suitable for growing saprobic fungi. For preventing contamination with bacteria, antibiotics such as chloramphenicol are added.

(2) Morphology

Mushrooms (macrofungi) are identified with color guides such as Chou & Chang (2005), Tschen et al. (1999 – 2002), and Wu et al. (2002), followed by consulting more specialized books such as Seifert et al. (2012), Sutton (1980), Yang (2015), and special articles in international and local scientific journals. Fungal material is sectioned by hand or squashed for light microscopy. Light microscopic mountings are made in 5–10% (w/v) aqueous KOH solution, with or without staining with phloxine or cotton blue and observed at 1000x magnification (oil immersion). Most photographs will represent the fungus in its habitat on the campus; exceptionally, photographs made with a dissecting or light microscope are added, particularly for microscopic fungi and slime molds with small fruit-bodies. Preferably, different stages of development are shown in the photographs for indicating the intraspecific variability.

(3) Molecular identification of fungi:

For molecular identification of fungi, genomic DNA is extracted with Genomic DNA Spin Kit (Plant), (Bioman Scientific Co., Ltd., Taiwan). The methods according to the manufacturer's protocol, modified are followed. The PCR products are amplified with different primers to identify species was based on published phylogenetic analyses of specific genera or species complexes of fungi. DNA sequences of the internal transcribed spacer regions (ITS) and/or the ribosomal large subunit RNA gene (LSU rDNA) are generated for a preliminary identification. For most wood decay Basidiomycota, these two regions are sufficient for species identification. Occasionally, an additional barcode is required for better species resolution. For example, the primer pair TUB2Fd/TUB4Rd is used for amplification of the beta tubulin gene, particularly for the identification of Xylariales. For the primer sequences, see the below table. The PCR mixture consists of 12.5 µl 2X GoTaq® Green Master Mix (Promega, Genelabs Life Science Corp., Taiwan), 1 µl of primer pairs (for the primer sequences see the following references for the amplification), ddH2O (up to 25 µl) and 5 µl genomic DNA. The ITS/LSU sequences amplification conditions are used as described by Gardes & Bruns (1993), Kurtzman & Robnett (1997), White et al. (1990). The TUB sequences amplification conditions follow Groenewald et al. (2013), Woudenberg et al. (2009). The HIS3 sequences amplification conditions are as in Bills et al. (2009). The TEF sequences amplification conditions are according to Carbone & Kohn 1999. Additional primers and amplification protocols may be applied according to the taxonomic standards in further genera of fungi.

The amplified products are checked with 2% agarose gel electrophoresis subsequently stained with SafeView DNA Stain (GMbiolab Co., Ltd., Taiwan) and visualized under UV light (312 nm).

DNA sequencing is performed by Mission Biotech (Nankang, Taipei) with the same primers used for the PCR. The DNA sequences are edited using CodonCode Aligner version 4.0.1 (CodonCode Corp., USA) and submitted to GenBank and the DNA Data Bank of Japan. The sequences are submitted to BLAST searches at GenBank (https://blast.ncbi.nlm.nih.gov/). Depending on the similarities of the used barcode and verifiability by morphology, the strains are identified to species or genus. For reproducibility by published vouchers and scientific quality, only sequences cited in publications are considered for identification.

(4) Sources used for identification and assessing distribution of the wood decay fungi in the project:

Color guides of Taiwanese mushrooms (e.g. Chou & Chang 2005, Mycobiota Taiwanica 2015, Wu et al. 2002)

Online database with illustrations and descriptions of fungi from Taiwan hosted by the Bioresource Collection & Research Center http://www.bcrc.firdi.org.tw/fungi/index.jsp

Species of fungi of Taiwan: http://web2.nmns.edu.tw/TFungi/chinese/fungi1.php

Biodiversity database of Taiwan - TaiBif: http://taibif.tw/en/node/140

Plant parasitic fungi worldwide with search options for Taiwan: https://nt.ars-grin.gov/fungaldatabases/fungushost/fungushost.cfm

Catalogue of living strains: BCRC https://catalog.bcrc.firdi.org.tw/

Catalogue of fungal specimens at the National Museum of Natural Science, Taichung: http://collection.nmns.edu.tw/scripts/fungi.dll

DNA sequences: http://www.mycobank.org/BioloMICSSequences.aspx, GenBank https://www.ncbi.nlm.nih.gov/nucleotide/

Scientific fungus names: Index Fungorum http://www.indexfungorum.org/names/names.asp

Scientific plant names: TROPICOS http://www.tropicos.org/

5. 教學暨研究成果 Teaching and Research Outcomes

Fifteen students (including a foreign student and one from Hong Kong) joint the course, provided weekly oral reports and attended weekly written self-tests and a written exam. Because of the covid-19 situation, parts of the lectures and self-tests, as well as the end term report were conducted online, while other lectures and tests and a demonstration of wood-decay fungi on the campus were onsite. The course was given bilingual (English, Chinese). The written teaching materials were in English and deposited in an online repository of the university. Parallel to the lectures, the students formed groups, collected and characterized a wood-decay fungus by host tree, morphology and molecular data, made conclusions about the safety of the infected tree, and presented the results in a report at the end of the term in English. The evaluation of tree safety was to be done with the traffic light colors green for safe, yellow for problematic, and red for immediate danger. The fungi identified and presented by the student teams were *Aleurodiscus* sp. with too poor samples for final conclusions, the potentially aggressive wood decay fungi *Ganoderma gibbosum* (2x) and *Ganoderma orbiforme* (2x) with tree safety concern, and the saprobic wood decay fungi *Microporus* sp., *Hexagonia tenuis*, *Ranadivia modesta*, and *Trametes hirsuta* with least concern about tree safety.

The Taiwanese students profited from the dual language so that could train their English listening abilities more effectively than in a purely English course. The language of the weekly reports was chosen by the students (Chinese or English), the final report was in English. The English talking abilities were, therefore, also trained. The foreign student did not complain about the Chinese reports, since several students voluntarily presented in English, the reports were brief and focused on the repetition from last week. During the practical part of the course, the student encountered the difficulties of collecting adequate specimens and that not all research questions could be fully resolved within one term. The evaluation of tree safety revealed to be somewhat subjective due to the lack of data.

6. 建議與省思 Recommendations and Reflections

Within the promotion of sustainability of resources and environment (SDGs), education and basic research about biodiversity seem to be underrepresented, in spite of the high educational efficacy through the spontaneous affinity and curiosity of people to living beings in their environment. Digital approaches are naturally implemented in basic research as principal tools, compared to some artificial attempts of doing something "digital" in teaching and evaluating the outcome of class activities. Maybe teachers in the fields of natural resources & environment and biodiversity could be more encouraged to apply for support for their courses. The time for preparation and conducting the project is sufficient and the support from MOE is generous. The young people seem to be able and willing to accept the challenges of English in teaching in order to enhance their English capabilities, if there is some guidance in written and oral instructions.

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三. 附件 Appendix (請勿超過 10 頁)