# The Workshop on Science and Technology Cooperation

**Biodiversity: Global Awareness and Sustainability** 

**Organized** by The Thailand Research Fund (TRF) Chinese Academy of Sciences (CAS) November 6 - 8, 2017 Chiang Mai, Thailand









#### The Workshop on Science and Technology Cooperation Biodiversity: Global Awareness and Sustainability

With mutual interest and strong intention to promote scientific exchange and cooperation between China and Thailand, a series of Workshop on Science and Technology Cooperation have been continuously organized with the first one hosted by the Ministry of Science and Technology (MOST) in Bangkok, Thailand in June 2006. The second workshop was held in Chengdu, China in June 2007 and hosted by the Chinese Academy of Sciences (CAS) followed by the Mini-workshop on Science, Technology and Innovation Cooperation hosted by MOST in Bangkok, Thailand in September 2011. The third workshop was hosted by MOST together with the Thailand Research Fund (TRF) in Bangkok, Thailand in August 2015. The workshops have provided unique opportunity for scientists from both countries to exchange their knowledge and research findings so thus a number of cooperative research programs have been initiated. The forth workshop recently held in November 2016 in Xishuangbanna, China and jointly hosted by CAS and TRF, in particular, focusing on biodiversity conservation and bio-resources development was quite successful and has led to a number of new collaborative research projects.

In order to continue and strengthen the cooperation, TRF together with CAS are once again proud to jointly organize the Workshop on Science and Technology Cooperation under the theme "Biodiversity: Global Awareness and Sustainability" during November 6-8, 2017 in Chiang Mai, Thailand. The purpose of the workshop is to provide a platform for scientists from both countries to strengthen the existing collaboration, to share the knowledge, experiences and practices on the research and development in the areas of biodiversity conservation and bio-resources sustainable use, to build up new collaborations as well as to cultivate even more enduring friendship.

### The Workshop on Science and Technology Cooperation Biodiversity: Global Awareness and Sustainability

#### November 5, 2017 (Sunday)

Arrival of delegates at Kantary Hills, Chiang Mai

#### November 6, 2017 (Monday)

07.00-08.00	Breakfast	
08.00-08.30	Registratio	n
08.30-08.45	Welcome A Director, T	Address by <b>Prof. Suthipun Jitpimolmard</b> , MD FRCP he Thailand Research Fund
08.45-09.00	Opening sp Director, X	beech by <b>Prof. Dr. Chen Jin</b> Iishuangbanna Tropical Botanical Garden, CAS
09.00-09.35	Keynote L Documenti integration	<b>ecture: Prof. Dr. Jiang Zhigang</b> , Institute of Zoology, CAS ng the species diversity of China's mammals: Inventory and of biodiversity
09.35-10.10	Keynote L Khon Kaer Biodiversit Mekong R	<b>Acture: Prof. Dr. La-orsri Sanoamuang</b> , Faculty of Science, In University by of freshwater calanoid copepods in the floodplain of the iver Basins
10.10-10.20	Group Pho	to
10.20-10.25	Announcer	nent
10.25-10.40	Coffee/Tea	break/Informal discussion
Session 1	Chair: Sa	vitree Limtong
10.40-11.00	Lecture 1	Mapping Asia plants: Initiative and progress <i>Keping Ma</i>
11.00-11.20	Lecture 2	A decade of advances in understanding the biodiversity of fungi in the greater Mekong subregion and their biotechnological potential <i>Kevin D Hyde</i>
11.20-11.40	Lecture 3	World data centre for microorganisms: the cooperation on big data for microbial resources worldwide <i>Juncai Ma</i>
11.40-12.00	Lecture 4	Thai biodiversity databases: From collections of scientific materials and literatures <i>Lily Eurwilaichitr &amp; Supawadee Ingsriswang</i>
12.00-13.00	Lunch/Info	ormal discussion

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Session 2	Chair: Cl	ien Jin
13.00-13.20	Lecture 5	Genetic diversity and phylogenetic relationships of long-tailed macaques ( <i>Macaca fascicularis</i> ) in Thailand in relation to their morphological characters, fertility and infectious disease susceptibility <i>Suchinda Malaivijitnond</i>
13.20-13.40	Lecture 6	Speed up DNA taxonomy on wild bees in Southeast Asia Chao-Dong Zhu
13.40-14.00	Lecture 7	Combining molecular and morphological data for species identification of amphibians in Doi Inthanon National Park, Chiang Mai, Thailand: A conservation and resource management effort <i>Chatmongkon Suwannapoom</i>
14.00-14.20	Lecture 8	Out of the sino-himalayas: Evidence for historical biogeography and spatio-temporal diversification of the old world babblers <i>Fumin Lei</i>
14.20-16.30	Poster session + Coffee/Tea break/Informal discussion	
15.30-16.30	TRF/CAS executives/administrative staff meeting	
16.30-17.15	Leave Kantary Hills Hotel for Tweechon Botanical Garden	
17.15-18.00	Sight-seeir	ng in Tweechon Botanical Garden
18.00-20.00	Welcome p	party in Northern Thai Traditional-Style Market (Kad Mua)
		November 7, 2017 (Tuesday)
08.00-09.00	Breakfast	
Session 3	Chair: Na	apavarn Noparatnaraporn
09.00-09.20	Lecture 9	Spatial planning for protected areas in response to climate change (SPARC): An update <i>Richard T. Corlett</i>
09.20-09.40	Lecture 10	Sargassum plagiophyllum population in Southern Thailand: A potential bio-resource Anchana Prathep
09.40-10.00	Lecture 11	Developing priorities for karst conservation <i>Alice C. Hughes</i>
10.00-10.20	Lecture 12	Uncovering the diversity of herpetofauna in karst ecosystem in Southern Thailand Anchalee Aowphol
10.20-10.35	Coffee/Tea	break/Informal discussion

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Session 4	Chair: Alice C. Hughes
10.35-10.55	Lecture 13 Vespid wasp venom hyaluronidases: Purification and specific detection tools for hyaluronic acid <i>Sakda Daduang</i>
10.55-11.15	Lecture 14 Peptide diversity in amphibian skin secretions <i>Ren Lai</i>
11.15-11.35	Lecture 15 Natural product discovery from actinomycetes by physicochemical and genome analysis <i>Arinthip Thamchaipenet</i>
11.35-11.55	Lecture 16 Studies on biodiversity and its applications in synthetic biology for natural products <i>Zhihua Zhou</i>
11.55-13.00	Lunch/Informal discussion
Session 5	Chair: Saisamorn Lumyong
13.00-13.20	Lecture 17 Open biodiversity and health big data (BHBD) initiative <i>Yiming Bao</i>
13.20-13.40	Lecture 18 eDNA-based technologies for biodiversity study Maslin Osathanunkul
13.40-14.00	Lecture 19 Different community assembly mechanisms of trees and herbs along elevational gradient in a hyperdiverse subalpine forests <i>Yahuang Luo</i>
14.00-14.20	Lecture 20 Visitors and pollinators of some Thai native banana species (Musaceae) Sasivimon Swangpol
14.20-14.40	Lecture 21 Research on fig and fig wasp coevolution and effective conservation <i>Yan-Qiong Peng</i>
14.40-16.00	Round Table Discussion
16.00-16.15	Closing Ceremony
16.15-17.15	Working Group Discussion (Individual)
17.45	Leave Kantary Hills Hotel for Dinner
18.00-20.00	Dinner at Galae Restaurant

November 8, 2017 (Wednesday)			
06.30-07.30	Breakfast		
07.30-16.00	Kew Mae Pan Nature Trail Study		
17.00	Dinner at Khum Vieng Yong Restaurant		
November 9, 2017 (Thursday)			
07.00-09.00	Breakfast		

10.00 Departure from Kantary Hills Hotel

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### ORAL SESSION

#### Documenting the species diversity of China's mammals: Inventory and integration of biodiversity

Jiang Zhigang<sup>1,6</sup>, Liu Shaoying<sup>2</sup>, Wu Yi<sup>3</sup>, Jiang Xuelong<sup>4,6</sup>, Zhou Kaiya<sup>5</sup>

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China has entered a golden age of biodiversity research and conservation. On the one hand, the Chinese government actively fulfills the United Nations Convention on Biological Diversity, and inputs more resource for biodiversity conservation and research; on the other hand, Chinese scientists have carried out large-scale biodiversity investigation and field research in the areas which were not surveyed before. Chinese scientists discovered new species and recorded a new occurrence of species in the country. Jiang et al. (2015) reported that China has 673 species of mammals (1). Due to the discoveries of mammalogy in China and the recent modification in mammalian taxonomy and conservation needs in the country, the inventory of China's mammal diversity is urgently needed to be updated. Therefore, we collected the new species and new records of mammal species in China from literature since March 2015, adopted the new taxonomy for ungulates, then incorporated our own research, added the mammalian species in the Zangnan Region, China and renewed the inventory the mammal diversity in the country. The new inventory of China's mammals has one super order, 13 orders, 56 families, 248

genera and 693 species. Compared with that reported by Jiang et al. (2015), there are one super order, one order, one family, three genera and 20 species more in the new China's mammalian diversity inventory. Of the mammalian species in country, the statuses of 18 species, mostly rodents, are still disputing among mammalogists. There are 146 endemic mammalian species in China, which accounted for 21% of the total mammal species in the country. Of those endemic species by order, the highest endemic rate is found in Lagomorpha (37%), followed by Eulipotyphla (34%) and Artiodactyla (25%). China is the country in the world with the richest mammal diversity. Documenting the species diversity of China's mammals is the first step of inventory and integration of the biodiversity in the country. Cooperation and new research resolves the some puzzles in taxonomy. Though we cannot end the anarchy in taxonomy (2), instead the data provides information for scientific decision. The inventory is the base for assessing the status of mammalian species and for taking conservation action for rescuing endangered species.

- 1. Jiang Z, Ma Y, Wu Y, Wang YX, Feng Z, Zhou K, Liu S, Luo Z, Li C. China's mammalian diversity. Biodiversity Science, **2015**, 23, 351-364.
- 2. Garnett ST. and Christidis LES L. Taxonomy anarchy hampers conservation. Nature 2017, 546:25-27.



Zhigang Jiang received his Ph.D. in Wildlife Ecology and Management from the University of Alberta, Canada in 1993. He is the Professor of Conservation Biology in University of Chinese Academy of Science (UCAS) and the Executive Director of Endangered Species Scientific Commission of China (National CITES Scientific Authority of China). His main interests include endangered mammal conservation, species inventory and extinction risk assessment.

## Biodiversity of freshwater calanoid copepods in the floodplain of the Mekong River Basins

La-orsri Sanoamuang

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Copepods are a group of micro-crustaceans living in nearly every freshwater habitats. Some species are planktonic and benthic forms, living in wetlands, such as lakes, ponds, swamps, reservoirs, and many live underground in freshwater caves<sup>1</sup>. Copepods are sometimes used as biodiversity indicators in freshwater ecosystem<sup>2</sup>. The updated list of freshwater, calanoid copepods recorded from the Mekong River Basins (Thailand, Laos and Cambodia) during 1993-2016 is presented. Of the 45 taxa identified, 41, 19 and 21 have to date been recorded from Thailand, Laos and Cambodia, respectively. Among these, 11 species are new to science and members of genera Arctodiaptomus and Paradiaptomus are reported for the first time from Southeast Asia. Mongolodiaptomus pectinidactylus has never been found previously since its discovery from China in 1964. Eodiaptomus sanoamuangae has been found only in Thailand and China. Seven species (Dentodiaptomus siamoindosinensis, Eodiaptomus

phuphanensis, E. phuvongi, Heliodiaptomus phuthaiorum, Phyllodiaptomus christineae, P. praedictus and M. mekongensis,) are endemic to Thailand, Laos and Cambodia. Ten species (Arctodiaptomus munensis, Mongolodiatptomus phutakaensis, M. rarus, Neodiaptomus siamensis, N. songkhramensis, Phyllodiaptomus roietensis, P. surinensis, P. thailandicus, Tropodiaptomus sp.1 and Tropodiaptomus sp.2) are endemic to Thailand. While Allodiaptomus nongensis has been recorded only in Laos and M. cambodiaensis and *Phyllodiaptomus* sp. have been found only from Cambodia. The peculiarity of the Mekong floodplain Calanoida is a high degree of co-occurrence of several species in the same habitats.3 About 90% of the recorded species from Laos and Cambodia correspond with those found in northeast Thailand, documented an affinity of the calanoid communities between these neighboring countries.<sup>4</sup> Due to limited numbers of samples from Laos and Cambodia, it is likely that more species await further investigations.

- 1. Boxshall, G. A.; Defaye, D. Hydrobiologia 2008, 595, 195-207.
- 2. Brancelj, A.; Boonyanusith, C.; Watiroyram, S.; Sanoamuang, L. J. Limnol. 2013, 72(s2), 327-344.
- 3. Sanoamuang, L.; Athibai, S. Hydrobiologia 2002, 489, 71-82.
- 4. Proongkiat, K.; Sanoamuang, L. Crustaceana 2008, 81, 177-189.



La-orsri Sanoamuang, Khon Kaen Univ. (B.Sc., 1977), Chulalongkorn Univ. (M.Sc., 1981), Univ. of Canterbury (Ph.D., 1992, Vida Stout), Univ. of Gent (Postdoc, 1993, Henri Dumont), Lecturer Khon Kaen Univ. (1977-2003), Prof., Khon Kaen Univ. (2004-present). Research fields: Biodiversity of freshwater zooplankton (rotifers, cladocerans, copepods, clam shrimp and fairy shrimp); aquaculture and applications.

#### Mapping Asia Plants: Initiative and Progress

Keping Ma<sup>1,2</sup>, Bo Liu<sup>1</sup>, Hongfeng Wang<sup>1</sup>, Maofang Luo<sup>2</sup>, Zheping Xu<sup>1</sup>, Xuehong Xu<sup>2</sup>, K. N. Ganeshaiah<sup>3</sup>

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Because of the huge population and rapid growth of economy, biodiversity including plants are being seriously threatened in Asia. Both conservation and sustainable use of biodiversity need species distribution data. Asia is the continent with highest diversity of plants. However, the information of plant taxonomy and distribution in Asia are not available at continental scale and more scattered, compared with other continents such as Europe, America and Africa. So, a better infrastructure for plant distribution in Asia is important. In 2015, Asia Biodiversity Conservation and Databases Network (ABCDNet) started a project entitled Mapping Asia Plants for cataloguing species of plants and collecting distribution data from the following data sources: 1) specimens, 2) observations, 3) species checklists, 4) expert maps, 5) inventories, 6) vegetation plots, 7) literatures and 8) on line datasets. Major achievements so far are as follows. 1) for South Asia, datasets for 40 thousands species and 1 million specimens collected; 2) for Southeast Asia, 120 thousands species names, 1.3 million records of occurrence; 3) for Central Asia, a dataset for 8,500 species, belonging to 162 families and 1,329 genera; 4) for Northeast Asia, a species checklist including 9673 species, belonging to 262 families and 2,782 genera; 5) for North Asia, 6900 species of higher plants and their distribution in 13 sub-regions; 6) for China, national higher plant checklists including 35835 species(including infrataxa), 9 million records of occurrence. Some of the data collected are shared with the websites of ABCDNet (www.abcdn.org/).

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#### A Decade of Advances in Understanding the Biodiversity of Fungi in the Greater Mekong Subregion and their Biotechnological Potential

#### Kevin D Hyde

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Fungi are an understudied, but essential, fascinating and biotechnologically useful group of organisms. We have been studying the fungi of northern Thailand at Mae Fah Luang University since January 2008. During this time, we have grown from a single researchers to a large team and have published more than 500 SCI papers. Our studies have been diverse, from phylogeny and taxonomy of microfungi, to growing novel mushrooms, and correctly identifying plant pathogens. In this presentation, I will discuss the importance of fungi and the advances we have made in the Centre of excellence in understanding the biodiversity of fungi in the region. We have made huge advances in the understanding of the fungi at the higher levels and are leading the world. We have inventoried a large number of new fungi for the region but a huge amount is still to be done. For example, in the edible genus Agaricus, we have introduced more than 20 new species, and many more are waiting description. In these relatively well-known genera we are finding about 40% of species we collected

are new to science. In the microfungi, which are relatively poorly studied, the percentage is much larger.

At the same time, we have been finding ways to exploit these fungi. Our work has resulted in the discovery of at least ten new species which are being developed as novel industrial mushrooms. We have isolated at least ten novel medicinal compounds from Thai fungi and are also looking at ways to exploit them in biocontrol. Selected examples from this study will be given.

Our work is just a beginning. Fungi have been generally neglected over time, despite the fact that they provided Penicillin, Lovastatin and various important medicines. Fungi have been poorly exploited and yet have a huge potential in biocontrol, bioremediation, novel compound discovery as well as basic industrial organisms (mushroom, fertilizers and cosmetics.



4

Science 🏠

Kevin D Hyde, Cardiff University, UK (BS.C., 1979), Portsmouth University (M.Sc., 1980), Portsmouth University (Ph.D., 1987, Gareth Jones), University of Wales, Cardiff (D.Sc., 2001, Tropical Microfungi), Queensland Department of Primary Industries, (Principal Investigator., 1989-1992), University of Hong Kong (1992-2007), Mae Fah Luang University (2008-present). Research fields: Biodiversity, taxonomy, phylogeny and bio-exploitation of fungi.

### World Data Centre for Microorganisms: the cooperation on big data for microbial resources worldwide

Juncai Ma

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WFCC-MIRCEN World Data Centre of Microorganisms (WDCM), which was established 50 years ago as the data center of the World Federation for Culture Collection (WFCC)-Microbial Resource Center (MIRCEN). It aims to provide integrated information services by fully utilizing hyperlinked big data technology for microbial resource centers and microbiologists all over the world.

In order to provide an informative system to culture collections and their academic and user communities of microbial resources. WDCM constructed a comprehensive data platform and established several databases for specific applications. CCINFO serves as a metadata recorder of on 726 culture collections from 75 countries and regions. It provides a unique identity for each culture collection and lists of species name of collection's holdings. Using the strain numbers and species names, we developed Analyzer of Bioresource Citation (ABC), a data mining tool to extract information from public resources such as Pubmed, WIPO, GOLD database and NCBI nucleotide sequence database. After online or offline data submission from individual culture collection, WDCM automatically links the catalogue information of each collection to knowledge available of each strain extracted by ABC and provides this information to the public through the Global Catalogue of Microorganisms (GCM). GCM gathers strain catalogue information and provides a data retrieval, analysis, and visualization system of microbial resources. Currently, GCM includes 400,813 strains from 113 culture collections in 44 countries and regions. The Reference strain database is a special subject database provides information on the strains used in certain international or regional standards. WDCM provides free access to all these services at www.wdcm.org.

WDCM hope to further develop these platforms to be an integrated data server by cutting-edge information technology for culture collections and microbiologists all over the world. Future developments such as "BIG DATA" technology including semantic web or linked data will allow the system to provide more flexible data integration broader data sources. Linking WDCM strain data to broader data sets such as environmental, chemistry and research literature can add value to data mining and targeting microorganisms as potential sources of new drugs or industrial products.

#### Thai Biodiversity Databases: From Collections of Scientific Materials and Literatures

Lily Eurwilaichitr and Supawadee Ingsriswang

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ThaiSciBiodiversity is an online information resource of biological collections including type culture collections, type specimen collections, seed banks, plant germplasm, genetic stocks, and other biological resources collected in Thailand. It is initiatively developed by the Ministry of Science and Technology (MOST) in collaboration with three agencies under MOST, namely the National Science and Technology Development Agency (NSTDA), the Thailand Institute of Scientific and Technological Research (TISTR), and the National Science Museum (NSM) to strengthen research towards the sustainable conservation and utilization of biodiversity in Thailand. The development of ThaiSciBiodiversity focuses on two aspects: (1) a mechanism to digitize and integrate to ensure the high-quality, wellannotated data of biological resources, and (2) the information aggregation capabilities of thematic collection/research networks. A software called iCollect has been freely distributed to registered collection/institutional members to facilitate sharing of information with ThaiSciBiodiversity via Web services. To enhance data quality, iCollect also supports features to capture, verify, validate and transform data records into a ThaiSciBiodiversitysupported standard data format (an extension of the Darwin Core standard). Integrated data and information from different sources will then be curated and made accessible through the websites, www.thaiscibiodiversity.org, and www.thai2bio.net.

Moreover, an automated updating system of taxonomical databases and the species checklist of Thailand has been developed under the Thai-Species Watch project, supported by Biodiversity-Based Economy Development Office (BEDO), Thailand. Thai-Species Watch employs two parts in the text mining process to extract the information from the literatures: Named Entity Recognition (NER) and the classification of relation between entities: Species and Location. Species and location entities appeared in the literature are recognized and tagged using dictionary-based pattern-matching approaches. In order to keep Thai Species Watch database automatically up-to-date, data entries are daily retrieved from several RSS services of new scientific publication via Internet. Results from the extraction, new species and location where the species discovered, are recorded into the database and made available on the website at www.thaispecieswatch.org. The website keeps track on the vast biodiversity in Thailand and provides search capabilities and analytical reports on the newly discovered species in Thailand. Both ThaiSciBiodiversity and Thai-Species Watch present the utilization of digital technology to enable the capabilities for the advancement of taxonomy and exploration of species diversity.



Lily Eurwilaichitr, Chulaongkorn University. (B.Sc., 1993), University of Kent, UK. (Ph.D., 1996), Deputy Executive Director, National Center for Genetic Engineering and Biotechnology (BIOTEC) (2010-present), Director, Thailand Bioresource Research Center (TBRC). Research fields: Microbial Resource Management, Enzyme Discovery, Microbial Biotechnology

#### Genetic diversity and phylogenetic relationships of long-tailed macaques (*Macaca fascicularis*) in Thailand in relation to their morphological characters, fertility and infectious disease susceptibility

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Long-tailed macaque (Macaca fascicularis; Mf) is one of the popularly used non-human primate (NHP) model for biomedical research. This is not only because they are commercially available and commonly encountered, but their genetic characteristics are also closely related to those of humans. Currently, Mf are classified into 10 different subspecies, and three of them, common (M. fascicularis fascicularis; Mff), Burmese (*M. f. aurea; Mfa*), and dark-crowned (*M. f. atriceps*) long-tailed macaques are reported subspecies in Thailand. Mff can be seen throughout Thailand and were reported to hybridize with rhesus macaques (M. mulatta; interspecific hybridization) and with Mff (intraspecific hybridization), and their offspring are fertile. The interspecific hybridization was proposed at 15-20°N and the intraspecific hybridization was at the Isthmus of Kra (10° 15' N, 99° 30' E) and vicinity. Recently, Zhang et al. (2017) reported that Mff with different degree of genetic admixture from Mm show the different

response to malaria susceptibility across the experiments.

In 2012, Chulalongkorn University established the National Primate Research Center of Thailand (NPRCT). This center aims to provide a good quality in health and genetic of NHPs and to provide research services for both in-country and aboard. The first NHPs being bred are Thai origin long-tailed macaques. Thus, this project aims to assess the genetic diversity of long-tailed macaques throughout Thailand and the degree of genetic admixture with rhesus macaques in relation to their morphological characters, fertility and susceptibility to malaria infection. The genetic admixture between Mfa and Mff will also be determined. The specimens of known origin rhesus and long-tailed macaques throughout Thailand and vicinity will be collected and analyzed. Thus, known genetic long-tailed macaques which can be used as laboratory NHP models for malaria research at the NPRCT will be the ultimate goal of this project.



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#### Speed Up DNA Taxonomy on Wild Bees in Southeast Asia

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Wild pollinating bees provide key ecosystem service. However, wild bees have not yet been well studied, while many might disappear without naming because of climate change, intensive land use and pesticides etc. We estimate there could be more than 3,000 species in China, with around 1,200 catalogued recently. For adjacent countries in Southeast Asia, there could be even more undescribed wild bee species in tropical and subtropical areas.

DNA taxonomy helps to delimit bee species even with very rare reference collection available in



Malaise Trapping in Northeast China

this region. It also facilitates our understanding of associations between bees and host plants by sequencing pollens they carry. We developed protocols to integrate different types of DNA data from both public databases and laboratory. Besides, regular sampling techniques, including Malaise Traps and Nest Traps help to collect fresh bee samples for large datasets.

Our bee projects start from DNA taxonomy, describe more bee species and try to understand their diversity, distribution and biology on the tree of bees.



Sweeping in Northeast China

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Research Interests: Integrative systematics on parasitoid wasps and pollinator bees; Species Interactions among tri-trophic interactions involving wasps and bees; Insect diversity monitoring network.

#### Combining molecular and morphological data for species identification of amphibians in Doi Inthanon National Park, Chiang Mai, Thailand; A conservation and resource management effort

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Biological diversity and distribution patterns are the foundations of taxonomical research and conservation. As part of the Indo-Burma diversity hotspot, Thailand harbors one-third of the amphibian species of the region. Several new amphibian species have been described from Thailand, which suggests an underestimated biodiversity in the region. Despite this, only few documented studies on the diversity pattern of amphibians in northern Thailand have been conducted. In particular, Doi Inthanon National Park, where the country's highest peak is located, warrants a comprehensive study on species diversity of amphibians.

This group of animal has been identified to include several cryptic species. With formal morphological approach alone, cryptic diversity cannot be uncovered, especially for morphologically conservative amphibians. In contrast, molecular marker is a good method to identify biodiversity by using a short fragment of mitochondrial genes or nuclear genes. The method compares unknown gene sequences to known ones, which can quickly and correctly detect species diversity in studied areas.

In this study, the aims are 1) to combine molecular with morphological data based on geographical location database of the amphibians from Doi Inthanon National Park in order that a network and amphibian calendar for distribution of each season can be developed 2) to perform region cross-species spatial analyses to explain patterns of species richness and distinctiveness across Thailand, 3) to identify conservation priority areas and develop a plan for conservation of endangered species as well as a guideline for sustainable utilization of amphibians in Doi Inthanon National Park, and 4) to identify species that are of least concern which can serve as protein source for consumption by local populations and possibly provide the local with an alternative source of income.

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### Out of the Sino-Himalayas: Evidence for Historical Biogeography and Spatio-temporal Diversification of the Old World Babblers

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The biogeographic and diversification dynamic analyses in large clades can reveal evolutionary mechanisms underlying large temporal and spatial scales. The species rich and morphologically diverse group of the Old World Babblers are regarded as an ideal model to reveal this process but lack of a comprehensive study. Here, we provide a multilocus and time-calibrated phylogeny of babblers with 404 species (90%) to access their biogeographic history and diversification dynamics. Our results supported an out of Sino-Himalayan Mountains pattern with several independent dispersals to other regions. In addition, we found white-eyes underwent a long distance colonization via Trans-Indian Ocean route into Africa. Diversification analyses indicated babblers had diversified with an increasing rate at 2 Ma, driven by white-eyes invasion to previously unexploited islands in Australasia and Africa. But diversification decelerating occurred at other regions indicating a diversity-dependent process due to competition of available sources. GeoSSE and DR analyses showed diversification rate was not accelerated in the Sino-Himalayan Mountains. Thus, diversity hotspot of babblers in the Sino-Himalayan Mountains is result of time-for-speciation effect and niche conservatism, while species-pump and multiply immigrations hypotheses play a limited role in this process. Our results provide a better understanding of babblers biogeographic history and spatiotemporal diversification.



The Workshop on Science and Technology Cooperation Biodiversity: Global Awareness and Sustainability

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### Spatial Planning for Protected Areas in Response to Climate Change (SPARC): an update

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The Spatial Planning for Protected Areas in Response to Climate Change (SPARC) project aims to assess the risks from climate change to existing protected areas across the tropics and propose strategies by which these risks can be reduced. SPARC is funded by the Global Environment Facility (GEF), a multi-national fund that addresses global environmental problems, and it is administered by Conservation International. SPARC-Asia is looking at the area from subtropical Asia through to New Guinea. Threats to protected areas are being assessed by a variety of methods, including the predicted climate change and its velocity, modeled changes in ecosystem function, and the modeled climate-change response of species for which we have good enough data on their current distributions. Initial results suggest that the severity of the impacts will depend strongly on the success of the recent Paris Climate Agreement. If global warming is held below the Paris target of 1.5°–2°C, then the main additional threat to biodiversity is likely to come from an increased frequency and intensity of extreme events, particular short periods of exceptionally high temperatures, but possibly also droughts and/or floods. If this target is substantially exceeded, however, much of the current protected area system will become very vulnerable before the end of this century, with major changes in vegetation and in the suitability of individual protected areas for the species they were designated to protect.

## Sargassum plagiophyllum population in Southern Thailand, a potential bio-resource

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Sargassum is the largest brown algae commonly found in the tropics and subtropics, it is the richest genus of the Fucales and there are around 334 species of Sargassum worldwide. There are around 17 species of Sargassum reported in Thai waters, there are over 4 species reported at the upper Gulf of Thailand, namely Sargassum baccularia (Mertens) C.A. Agardh, S. binderi Sonder, S. oligocystum Montagne, and S. polycystum C.A. Agardh. Sargassum, produces family of sulfated fucoidan and other polysaccharide such as focoidan, they also have a high antioxidant activity. These suggested a very high potential bio-resource of Sargassum as they are rich in diversity as well as chemical compounds.

Here, we attempt to have a better understanding of diversity, population structure, growth, reproduction, biomass and % coverage of *Sargassum*, and how environments can influence the population as well as their chemical compounds as such knowledge would provide an important information for further uses and applications of *Sargassum*.

The populations are monitored monthly for at least a year at two locations of Ko Lanta, Krabi province, where *Sargassum* are common and abundant. The canopy coverage, density, length, biomass and development stage of *Sargassum* are examined from January 2017, which a total of 7 stages of *Sargassum* are defined: 1) Holdfast, 2) Juvenile, 3) non-fertile Adult Plant, 4) Fertile Adult Plant, 5) Fertile Plant-Senescent, 6) Senescent and 7) Holdfast-Senescent; and closely monitored in permanent plots. Growth, recruitment and mortality rate as well as size structure and inequality will be examined. For the chemical compounds, phlorotannin concentrations will be investigated by looking into different plant parts and seasons. Those biological and chemical information will be examined further with the environmental conditions (light intensity and temperature). The study is now on going and the findings will be presented and discussed.



Figure 1 Dynamics and Canopy coverage of S. plagiophyllum population at Klong Hin, Koh Lanta, Krabi province during January, February and April 2017; a) January b) February and c) April



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#### **Developing priorities for karst conservation**

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Karsts represent a challenging ecosystem for conservation. With almost unparalleled levels of endemism, and an estimated 90% of cave fauna undesribed in some countries they represent priorities for further research. However growing demand for cement in many regions, and especially parts of Southeast Asia where accelerating cement demand caused China to use 6.6 gigatonnes in just a two year period, exceeding US historical construction and leading to an exponential increase in the demand for cement and consequently the increasing destruction of limestone karsts to meet that cement demand.

Thus to ensure karst diversity is adequately protected, we require both an understanding of hotspots of biodiversity, and how karst landscapes can be effectively managed to maintain karst ecosystems. Here we review a recently initiated project which sets to do that. By initially developing methods to map karsts, then develop stratified inventories of karst biodiversity, hotspots of endemism and also evaluate how landscape configuration changes the microclimate and species present on karst ecosystems.

We discuss how such stratified approaches can be implemented and scaled across the landscape to generate standardized information on karst biodiversity and understand how to manage karts and develop effective priorities to account for diversity and endemism.

Given the almost unparalleled endemism in these systems it is essential that we rapidly develop priorities that most effectively protect biodiversity in these challenging systems.

#### Uncovering the Diversity of Herpetofauna in Karst Ecosystem in Southern Thailand

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Karst ecosystem has been referred as biodiversity reservoirs which contains high level of animal endemism. In Thailand, many endemic vertebrates in karst ecosystem have been reported, including reptiles and amphibians. Species diversity of herpetofauna in karst ecosystem in southern Thailand was investigated between 2015-2016. Among reptile species, high species diversity of geckos was found in karst ecosystem such as Bent-toed Gecko Cyrtodactylus and Rock Gecko Cnemaspis. The combination of morphological and molecular analyses was used to clarify taxonomic problem and explore phylogenetic relationships of genus Cnemaspis in the chanthaburiensis and siamensis groups. The morphological and molecular data provided the evidence for describing three new species of Rock Geckos Cnemaspis (C. lineogularis, C. phangngaensis, and C. thachanaensis) from the Thai portion of the Thai-Malay Peninsula. Unlike reptiles, a small number of amphibians was reported for their occurrence in karst ecosystem. Stream toads in genus Ansonia mainly live in lotic environments, but the first limestone cave-dwelling toad Ansonia khaochangensis was recently discovered from a limestone cave in southern Thailand. The finding of A. khaochangensis in Thailand has provided the knowledge on historical biogeography of genus Ansonia which is distributed in a narrow range in Southeast Asian rainforests. However, karst biodiversity in Southeast Asia are threatened by human activities and conservation planning of endemic species and their unique habitats in karst ecosystem are urgently needed.

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### Vespid Wasp Venom Hyaluronidases: Purification and Specific Detection Tools for Hyaluronic acid

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Vespid wasps are ones of the most dangerous venomous animals. Their venom are mixtures of proteins, enzymes, toxins, etc., even causing fatalities in serious cases in humans and animals whom are envenomated. The major biologically active protein are protease, phospholipase, hyaluronidase, allergen, antigen 5 and mastoparan [1]. Hyaluronidase (HAase), well known as a "spreading factor", is a glycoside hydrolase that hydrolyses  $\beta$ -1, 4-glycosidic bonds between N-acetylglucosamine and D-glucuronic acid of hyaluronic acid (HA), a primary component of the extracellular matrix of tissues facilitating venom toxin diffusion into the tissue and blood circulation of prey. Venom of two vespids, Vespa affinis and V. tropica are focused. The higher in potency of V. tropica than V. affinis corresponded to the higher proportion of HAase in V. tropica than V. affinis [2]. That was suggested to be the direct effects of HAase to the potency of the venom. Primary structures of V. tropica HAase had been clarified. There are at least 2 isoforms: type A (an active form) and type B (an inactive form). They shared about 70% of similarity [3]. The catalytic amino acid residues of type A (Asp and Glu) were substituted by Asn in type B, resulting in the loss of activity. However, the binding

activity is predicted to be unaffected cause of the binding region still being conserved. The recombinant HAase type A and B are genetically produced in *E.coli*. Surprisingly, type B showed one-third of activity lower than type A. The substitution of catalytic residues from Asp or Glu (in type A) to Asn (in type B) is not completely inactive, as being predicted.

For hyaluronic acid (HA), it is well known for medical and pharmaceutical applications including cosmetics and neutraceuticals. The biological sources for HA is quite limited. HA-rich animal sources such as bovine synovial fluid, rooster combs need suitable technology to purify and detection of HA.

The HAase of both types are under investigation for trying to use for applications. One of the most possible one is to develop them as a ligand as a purification tool for HA. Another application is for high specific detection of HA in various sources. However, the mutation will be necessary for completely remove the enzyme activity. The substitution may be form Asp, Glu or Asn to Ala, the smallest amino acid without any charges and steric hindrances. The studies are under investigation.

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#### Peptide diversity in amphibian skin secretions

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Amphibian naked skins are directly exposed to various living environments. They encounter multiple biotic or non-biotic factors such as microorganisms, parasites, predators, radiation or other physical injuries. For their surviving, a large amount of functional molecules have been identified from their skins to treat these environment factors. Amphibian skins are a treasury to prospect bioactive substance. Most of these bioactive molecules are peptides. More than ten families of peptides including about 1,000 members have been identified from amphibian skins. High level of biochemical diversity with various pharmacological functions make amphibian skins attractive subjects for chemical prospecting and screening peptide candidates with medicalpharmaceutical significance.

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### Natural Product Discovery from Actinomycetes by Physicochemical and Genome Analysis

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Searching for novel natural products from the famous antibiotic producers, actinomycetes, particularly Streptomyces are always of interest for drug industry. Previous conventional screening such as biological assay has been quite a limited method to discover novel compounds. Recently, novel bioactive compounds have been characterised employing physicochemical property. In this work, maklamicin<sup>1</sup>, linfuranone A<sup>2</sup>, and bisoxazolomycin<sup>3</sup> have been identified from Micromonospora sp. GMKU 326, Microbispora sp. GMKU 363, Actinomadura sp. K4S16, and Streptomyces subflavus subsp. irumaensis AM-3603, respectively. Recent sequencing of a large number of actinobacterial genomes has revealed the presence of numerous cryptic/silent gene clusters with the potential to direct the biosynthesis of new specialised metabolites.

In this study, a cryptic Type I-Type III polyketide hybrid biosynthetic gene cluster in genome of Streptomyces venezuelae was transcriptionally activated in its *AbldM* mutant. Constitutive expression of *vemR*, a transcriptional activator of the large ATP-binding LuxR like (LAL) family, in the  $\Delta bldM$ mutant led to the production of a novel compound, venemycin<sup>4</sup>. Co-expression of the venemycin biosynthetic gene cluster and *vemR* in the newly constructed heterologous host Streptomyces coelicolor M1317<sup>5</sup> also resulted in venemycin production. Further genome sequence analysis of endophytic Streptomyces sp. GKU 8956 and Microbispora sp. GKU 8237 revealed diversity of biosynthetic gene clusters as promising sources for potential new natural product discovery.

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#### Studies on biodiversity and its applications in synthetic biology for natural products

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Natural compounds play an irreplaceable role for human to face the health challenges. Many drugs and health products are derived from natural compounds or their derivates.

On one hand, there are unique, unexplored and diverse plant and microbial resources in nature, which produce a large amount of natural compounds and other active substances to help them adapt to the environment or host. However, we are currently knowing about some metabolic pathways related to the natural compound biosyntheses from a small amount of plant and microbial species, which is completely too small comparing with the high biodiversity. Because more than 99% of the microorganisms in nature are uncultured at present, while less novel natural products could be isolated from the culturable microorganisms. Besides, we also know less about the natural compound compositions and their biosynthetic pathways for the most of plants in nature. The studies on the microbiome and the genome of wild plants (especial for the medicine plants) would bring big chances to elucidate the biosynthetic pathways for more and more plant species and special microbiome, as well as to collect the diverse key bioparts for synthetic biology for natural products.

On the other hand, investigation demonstrated that the natural compounds would usually be modified by the intestinal microbiome after taking it and then demonstrate the therapeutic effects. By contrast, recently more and more studies have also shown that natural compounds could significantly regulate the intestinal microbial composition, which reach the therapeutic cure by take effects on the intestinal microbial microflora. For example, metformin could restore the microbial group producing short-chain fatty acids in the intestinal microflora of type II diabete patients, and thereby alleviating the symptoms of diabetes. Our recent research also shows that ginsenoside CK by intraperitoneal injection can significantly regulate the mouse intestinal microbial community structure. It seems more detail studies on the variations of the diversity in the intestinal microbial microflora would help us to understand the mechanism to turn a natural product to be an ideal drug.

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Biological research has entered into an era of big data including a wide variety of biodiversity data from all life forms on earth and covering a broad range of health data from multiple omics levels as well as from clinics, physiology, lifestyle, and environment. In addition, such data are being generated at evergrowing rates and distributed throughout the world with heterogeneous standards and diverse limited access capabilities. Thus, providing open access to biodiversity & health big data is essential for an expedited translation of big data into applied knowledge and is becoming increasingly vital in advancing scientific research and promoting human healthcare and precise medical treatment.

In response to the appeal "Open Data in a Big Data World" by the International Council for Science, Dr. Yongbiao Xue, director of Beijing Institute of Genomics (BIG), recently proposed an initiative of Open Biodiversity and Health Big Data (BHBD) to the International Union of Biological Sciences (IUBS), of which he is an executive member. This proposal was approved by IUBS.

Global sharing of BHBD is able to advance scientific research and promote the fair distribution of benefits throughout the world, which yet cannot be accomplished without engagement of the entire global communities on the following three issues:

• To build the principles and mechanisms for global sharing of BHBD in accordance with laws and ethics of member countries.

• To develop a big data platform for BHBD integration, translation and sharing that is publicly accessible to worldwide communities.

• To promote the level of participation and influence of IUBS in global biological research.

The BHBD initiative will be built based on BIG Data Center (BIGD) at BIG, Chinese Academy of Sciences (1). BIG has participated and accomplished several major research projects with remarkable success, such as "Construction of the Genomics Data Platform" supported by the National High Technology R&D Program of China, "CAS Precision Medicine Initiative (CAS-PMI)" supported by the CAS Key Research Program, "Global Health Data Sharing" supported by the CAS International Partnership Program, "Public Service Platform and Innovative Application Demonstration for Scientific Big Data" supported by National Development and Reform Commission.

In January 2016, BIG launched BIGD to establish the platform for data archiving, integration, and mining, aiming to facilitate the collection, sharing, and application of big omics data, with the focus on precision medicine projects and strategic bioresources. In December 2016, BIGD released Genome Sequencing Archive (2), a multi-layer database for archiving and international sharing of genomic data. Since its establishment, BIGD has been continuously promoting the international research cooperation and overseas scientific exchange with experts in big data sharing. BIGD has initiated collaborations with surrounding countries such as Russia, Saudi Arabia, Pakistan, and Singapore, with the aim to build a Global Union for Omics Data Sharing.

We are hoping to establish collaboration with Thailand in the areas of biodiversity conservation and bio-resources sustainable use, by providing a platform for hosting, analyzing and sharing data generated from all TRF-CAS programs.

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Biodiversity practically important for the welfare of human. The study of plant biodiversity is continuously gaining importance in the environmental sciences in order to monitor ecosystem trends. Despite the uninterruptedly emerging technology, biological diversity has been still progressively deteriorated. Thailand is situated in a hot and humid climatic zone supporting a variety of tropical ecosystems which make Thailand be one of the world's richest countries in terms of plant biodiversity. The conservation of plants has received considerably less attention than animals, although plants are also important for us. Forests are globally important in regulating climate and locally important in sustaining communities and supporting biodiversity. However, forest ecosystems are among the most threatened habitats globally due to severe threats.

In a rapidly changing world we need methods to efficiently assess biodiversity. Conventional methods rely on direct observation or specimen collection are expensive and labour-intensive that hinder frequent monitoring. Recently, an alternative approach for monitoring biodiversity is that of environmental DNA (eDNA), genetic material obtained directly from environmental samples (soil, sediment, water, etc.) without any obvious signs of biological source material1, is becoming popular. It is now possible to recover an unpreceded number of DNA sequences directly from environmental samples. Many studies have proven that the eDNA-based approach is an effective, sensitive and convenient method. Plant communities aboveground are easier to assess but in contrast monitoring belowground plants are impractical or almost impossible2. The most powerful aspects of eDNA analysis is the ability to sample biodiversity that is not easily sampled by other means especially in cases where traditional sampling strategies may fall short and thus the rapidly expanding study of eDNA has generated ability to detect species and conduct genetic analyses for conservation, management, and research.

Although, the number of studies demonstrating successful eDNA detection has increased rapidly in recent years3-5, less research has explored the application of such technology in tropical regions including Thailand. Therefore, evaluation of eDNAbased technologies for assessing plant biodiversity is the main focus here. Two ways of the investigations are 1) using eDNA barcodes for species-specific monitoring of a rare and/or endangered plants species and 2) using eDNA metabarcoding for providing a comprehensive view of plant diversity in the studied site. Reliable survey eDNA-based methods for monitoring plants would greatly advance assessment and help us in understanding of threats to them and success in conservation management.

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### Different community assembly mechanisms of trees and herbs along elevational gradient in a hyperdiverse subalpine forests

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Determining how the relative strength of community assembly processes varies along elevational gradient is elemental for understanding community responses to environmental change. However, little is known about effects of environmental variables on maintaining the diversity of trees and herbs along elevational gradient. The assembly processes of different life forms communities (trees and herbs) were inferred by using functional traits data in 15 0.1-ha plots across 1200 m elevational gradients in Yulong Mountain of Yunnan, Southwest China. We used null model to detect the importance of abiotic filtering and biotic interactions along elevational gradient. We also analyzed the effect of environmental variables on species, functional diversity within community (alpha diversity), across communities (beta diversity). Our results revealed that tree species richness was highest and functional tend to be divergent at medium elevations, while convergent functional structure was detected at low and high elevations. The elevation, and soil variables (e.g. soil water content, available potassium content) might influence the species richness and functional

diversity. Whereas the understory herb species exhibited decreased richness along elevation dramatically. Interestingly, the maintenance of functional diversity was observed along elevational gradient. Clustering structure was detected in herbaceous assemblages and traits were more convergent at high canopy cover plots. Environmental filtering (e.g. elevation, canopy cover, soil pH, carbon and nitrogen content) might shape the understory herbaceous species and functional diversity. Faster species turnover than functional turnover indicated that both tree and herbaceous communities were likely structured by a stronger dispersal limitation. The nestedness and turnover components together driven the functional beta diversity. The findings here suggest the importance of abiotic stress or biotic interactions in shaping the diversity of different group communities at different elevations. Neutral processes may also be a predominant in structuring species composition along elevational gradient. Our study also highlights trait-based approaches to understand the species coexistence and community assembly in different groups in the subalpine forests.

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## Visitors and pollinators of some Thai native banana species (Musaceae)

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Recent discoveries of new wild banana taxa (Musaceae) in Thailand have inspired curiosity in their relationships with visitors and pollinators. In contrast to familiar commercial cultivars with parthenocarpic fruits, palatable fresh, and aborted seeds, fruits of wild bananas have thin fresh with dozens of stony seeds. Common inflorescence appearance of the bananas is pendant with red purple bracts, while the newfound ones possess various inflorescence directions and bract colours. These inflorescences can be erect then curve down or crawl, horizontal then slanting upward or curve like an S-shape with shiny orange red, pinkish orange, vellow or white bracts. In natural habitats in Thailand, we have been observing the animal visitations to some taxa in the banana family e.g. Musa nanensis, M. acuminata and M. rubra. We found that the relations between these bananas and animals are not

species-specific; bats, birds, bees, and rodents visit several banana species and on the other hand, several animal species were seen drop in on each individual plant. Diurnal visitors included birds, often spiderhunters and sunbirds, flies (Diptera) and stingless bees (Trigona), were seen with banana inflorescences, while, nocturnal visitors recorded were rats (Rattus) and bats. For these animals, the bananas not only provide food, nectar and fruits, but also shelters. Although some birds are stealers of the nectar, several animals are pollinators and seed dispersers. Since wild bananas are pioneer species at the forest edges and flower all year round, they provide requisites for animals and environment. These banana-animal mutualisms are essential for the web of life in fertile forests and their habitats are needed to be protected due to heavy deforestations in the areas.

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## Research on fig and fig wasp coevolution and effective conservation

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Fig trees are of great ecological significance in the tropics, with fruits that are fed on by more birds and mammals than any other plants. The relationship between fig and pollinating fig wasp is a striking example of an obligate plant-insect mutualism, and molecular evidence suggests that both partners coexist about 75 Ma. Obviously, maintaining fig and fig wasp coevolutionary stabilization is important for biodiversity conservation in tropics. How should we effectively conserve them? It is known that fig trees are very diversity, are distributed in different habitats, have various life forms and have different population sizes among species. Fig trees and pollinating fig wasps have coevolved to show morphological matching, physiological adaption, phenological synchronization and so on. Both partner reproduce offspring each other, the wasps must quickly find female phase syconia once they leave male phase syconia because of short-lived adults. More exactly, there is a available receptive tree in the population when the wasps emerge. The needs of the pollinators drive the phenology of fig trees, leading to the all-year-round flowering. In general, syconia that produce more seeds also reproduced more pollinating fig wasps. However, the mismatching phenologies could lead to produce few or zero numbers of seeds and wasps. In contrast, fig wasps showed higher species diversity than fig trees, but some wasp species are very rare and could disappear with environmental change. The species and population dynamics need to be monitored for a long term. In order to effectively conserve fig trees and fig wasps, we face similar questions and suggest to (1) conserve natural and diverse habitats; (2) put them in suitable place; (3) ensure enough individuals; (4) monitor population dynamics, (5) conserve species, genetic and functional diversity; and (6) correctly utilize them.

### **POSTER SESSION**
## Species interactions and biodiversity: Unpicking the mechanisms

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Ecological communities are the natural arenas where (co)evolution shapes the nature and strength of species interactions, and where their outcomes help regulate local levels of diversity<sup>1</sup>. Additionally, the modification of these processes by climatic and biogeographic factors subsequently shape biodiversity outcomes at larger geographic scales<sup>2</sup>. Within communities, species interactions unfold along two major axes: competitive processes dominate within trophic levels, whilst associations such as predator-prey, host-parasite and plant-pollinator characterise interactions between trophic levels. My ongoing research often focuses on the obligate brood pollination mutualisms between plants and insects (e.g. fig-fig wasps, leafflower-moths, yucca-moths) and the parasitic insect communities

that exploit them. Thus, I hope to understand how species interactions, and the abiotic factors that affect them, influence the structure of global biodiversity. The host plants that underpin these systems exhibit great diversity in south-east Asia and are often key drivers of ecosystem function. Such model communities make ideal systems for investigating some key mechanisms that determine global biodiversity patterns<sup>3, 4</sup>. These include: (i) determinants of specialist vs. generalist strategies; (ii) the role of niche process in community assembly; (iii) patterns of host-switching; (iv) the importance of coevolution in diversification; (v) the impact of abiotic heterogeneity on community dynamics; and (vi) the incidence of shared biogeographic histories.

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In China, the area with karst landform covers a total area of 3.44 million km2, accounting for one third of the whole land area of the country (Li Wenhua 2015). High soil calcium is an important factor influencing physiological characteristics of plants in karst areas. Without special adaptations these soil conditions can inhibit photosynthesis, growth and phosphorus metabolism, thus limiting the number of species which can survive in karst areas (Li Wenhua 2015). Consequently many species have evolved in situ and karst systems have large numbers of endemic species, with many species only known from a single karst.

As karsts have such high site specific endemism, that the loss of even a single karst may cause the extinction of species endemic to that site, and damage the population of regionally endemic species. Thus developing an infrastructure for the development of exsitu collections of vulnerable species and developing approaches to allow the survival of karst plants within an artificial karst may represent an important conservation tool for many species. The horticulture department is setting up a research group that will help the living collection becoming a botanical collection that is used for study in taxonomy (cataloguing, identifying and describing plants), as well as exsitu conservation and ecological landscaping. Thus, we would like to recreate gardens that mimic natural habitats where natural sourced collections can be displayed, studied and conserved for a number of karst endemic taxa.



## Towards an inventory of the biodiversity of aquatic fungi in Thailand

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Over the past 15 years, the systematic exploration of fungal diversity from aquatic habitats, freshwater and marine, of Thailand have been conducted by BIOTEC mycologists. Freshwater fungi (FF) are fungi that occur on submerged or partially submerged substrata in freshwater ecosystems. They play an important role in the decomposition of dead organic material in streams, breaking down lignocelluloses and releasing nutrients in the ecosystem (Shearer et al. 2007). A number of publications document the collections of FF from naturally substrata in Thailand starting by Tubaki et al. (1983). Subsequently, more extensive reports of FF for Thailand have been made and these provide insight into the diversity of FF present in Thailand and current total stands as 130 species. Additionally, marine fungi are those that have the ability to germinate and form mycelia under natural marine conditions. Since 1998, Thai marine mycologists have expanded the knowledge on Thai marine fungal diversity. To date there are 188 species

of marine fungi discovered for the country (Suetrong et al. 2017). Marine fungi have been revealed to produce a vast diversity of new chemical structures with active bioactivities. They can be used as the quorum sensing inhibitor for regulation of bacterial pathogens in aquaculture. Additionally, thraustochytrids, the marine protist, have attracted attention as a proven alternative source of polyunsaturated fatty acids (PUFAs) that can be used as feed supplement. This paper shows the great diversity of aquatic fungi to be found from freshwater and marine ecosystems in Thailand and aims to produce a checklist for the country. Recently, there has been increasing disturbance to Thai aquatic habitats due to natural and human activities. These effects will decrease the amount availability of substrate substrata available for colonization of microbes and their evenness. Therefore, there is a need to examine the fungal composition and their biodiversity as well as their impact towards human well-being.

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## Taxonomy, molecular phylogeny and DNA barcode of Lichenized Fungi of Thelotremoid Graphidaceae (Ascomycota: Ostropales) in Thailand

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Thelotremoid lichen is large group of family Graphidaceae, subfamily Graphidoideae. The thelotremoid Graphidaceae belong to two tribe: Ocellularieae and Thelotremateae, both can distributed in tropical rainforest in Thailand. Various phenotype characters have been used for classification at the genus and species levels, including thallus, trentepohlioid photobiont, apothecioid, solitary ascomata lacking both a columella and lateral paraphyses, and having non-amyloid ascospores as well as secondary chemistry with depsidones group. The molecular phylogeny data was used the mitochondrial small subunit rDNA (mtSSU), the nuclear large subunit rDNA (nuLSU), and the second largest subunit of the DNA-directed RNA polymerase I and II (RPB1, RPB2), as well as ITS

for DNA barcode. Thelotremoid Graphidaceae in Thailand were found 81 species and 16 genera: Astrochapsa (4 species), Chapsa (5 species), Chroodiscus (6 species), Compositrema (1 species), Cruentotrema (2 species), Glaucotrema (1 species), Leucodecton (3 species), Melanotrema (2 species), Myriotrema (3 species), Nitidochapsa (2 species), Ocellularia (37 species), Pseudochapsa (2 species), Pseudotopeliopsis (1 species), Rhabdodiscus (1 species), Stegobolus (1 species) and Thelotrema (8 species). Most of all belong to tribe Ocelluarieae (53%). The thelotremoid lichen was investigated 10 new species and 15 new records from Thailand. The molecular studies in this project was help to fill gaps in the molecular data set from a previous project as well as the database can be used for the future.

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## Diversity of Bacteria from Namdokmai Mango Plantation in Bangkachao Area and Their Plant Growth Promoting Traits

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Bangkachao area is located in Samutprakarn province of Thailand. The area comprises of six sub-districts and is recognized as the best producer of the Nam Dok Mai mango which has been registered as a geological index since 2013. Leaves and barks of the mango tree were collected from three Nam Dok Mai mango plantations in each six sub-districts of Bangkachao area. Direct isolation was used to obtain bacterial isolates from fresh samples. Two hundred and forty one bacterial isolates were collected from a total of 18 sample sites. Results on morphological and physiological characteristics showed that, the rod and cocci shaped bacteria were mostly Gram-positive whereas Gram-negative was found as a minority. The 16S rDNA sequence analysis showed 76 closest species within 18 families. Identification results indicated that the genus Bacillus was found as a majority among 241 bacterial species associated with mango tree. Three new bacterial species or genera i.e. BBB1-04, BBB3-04 and LBS2-06 will be proposed as a result of low similarity (98.32, 96.56 and 98.03% similarity) to

their closest type strains which were Pantoea cypripedii ATCC29267<sup>T</sup>, Azotobacter vinelandii DJ and Achromobacter anxifer LMG26857<sup>T</sup>, respectively. One hundred and five isolates were subjected to characterization on IAA production. Results showed that, among all, the isolate BSN2-02, identified as Micrococcus aloevera, was the best IAA producer by producing 445.25 mg IAA/L. The isolate LBB2-02 was the best phosphate solubilizing bacteria whereas the isolate LSN2-06 which was identified as Pseudomonas stutzeri was the most effective strain for siderophore producer. The bacteria with ability to produce siderophore were further investigated on their capabilities to inhibit the growth of Mango's pathogenic fungi, Colletotrichum gloeosporioides which causes the mango anthracnose. Only 2 isolates, LBB1-04 and LBB3-03, inhibited the fungal growth and the isolate LBB3-03 showed greater ability. Based on the results of plant growth promotion traits and antagonistic activity, LBB3-03 was selected and identified as Pseudomonas oryzihabitans by 16S rDNA sequence analysis.



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## Actinobacteria from Neglected Habitats: Diversity and Biotechnological Applications

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Actinobacteria are large group of Gram-positive bacteria with high %G+C content in their genomes. They are prolific producers of useful bioactive metabolites in particular antibiotics. In the search of beneficial microbes for biotechnological applications, actinobacteria are of priority choice due to their unbeaten track records. They are widely distributed in soils but can be found in many other environments including caves and marine sediments. We focus our research on the discovery of novel actinobacteria and believe that there are higher chance to be found in rare and under explored environments. These novel actinobacteria are likely to have novel properties useful for biotechnology. The studies of actinobacterial diversity in caves of Thailand are very limited. Our previous work showed that some actinobacteria

especially members of the genus *Streptomyces* are dominant in caves. Some of them showed promising antimicrobial activities. We have described one new species from Thai cave soil, *Microbispora thailandensis*. Another under research biome is mangrove. Our work with Chinese collaborators also showed high degree of actinobacterial diversity. These bacteria showed potential in bioactive compounds production and plant growth promotion. Two new species have been proposed, *Jiangella mangrovi* and *Streptomyces ferrugineus*. Our results support the view that rare or underexplored environments such as caves and mangrove are rich reservoirs of novel actinobacterial taxa with biotechnological potential to be exploited.

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## Biotyping of microbial strains isolated in Thailand and application of thermotolerant yeast as a cell factory for protein production

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Thailand has a rich source of biodiversity, especially microorganisms including bacteria, yeasts, and fungi. Currently, Thailand Bioresource Research Center (TBRC) and BIOTEC Culture Collection (BCC) hold a large collection of these microorganisms, including >15,000 strains of bacteria, >6,000 strains of yeasts, and >38,000 strains of filamentous fungi, reflective of the great biodiversity in Thailand, both taxonomically and ecologically and implicative of mostly untapped resource of valuable microbes capable of producing high-valued products.

Biotyping of microorganisms of interest has been employed for clinical diagnostics (analysis of the origin of a human, animal, or plant disease), detecting food-born pathogens, classifying microorganisms used in industry and biotechnology (Fraser et al., 2016). The most effective strategy using MALDI-TOF MS has been shown to be reliable for identification and classification of microorganisms. The method was first employed mainly for bacterial analysis, especially for clinical diagnostics. Currently, it has been expanded to analysis of yeasts and fungi in clinical medicine, biotechnology, and industry. Due to the advantage of relatively fast and robust nature, MALDI-TOF MS is an attractive tool for biotyping yeasts and other microorganisms with desirable characteristics. We aim to identify microbial strains isolated in Thailand to verify its ability to differentiate closely related strains. The acquired MS dataset will provide information on the robustness of MALDI-TOF MS biotyping identification. In order to confirm the identity of the microbial strains, the identification by MALDI-TOF MS will be compared with the identification based on rDNA sequences.

Among microbial diversity identified in Thailand, thermotolerant yeast, *Ogataea thermomethanolica* TBRC656, has been studied for its potential as a host for protein production. Its characteristic and potential application for industrial enzyme production especially at high temperatures has been reported (Tanapongpipat et al., 2012, Harnpicharnchai et al., 2014; Promdonkoy et al., 2014)

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## Screening and Phylogenetic Analysis of Carotenoid-Rich Dunaliella spp. in Thailand

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 $\beta$ -carotene has been widely used for antiradiation and eye protection due to its strong antioxidant property. Marine unicellular microalgae *Dunaliella salina* can accumulate large amount of  $\beta$ -carotene when cultivated under adverse conditions. In this work, we attempted to identify and screen carotenoid producing strains of *Dunaliella* spp. from saltern pond and salt soil resources collected from 24 provinces of Thailand. To date, over 35 single-cell clones unicellular microalgal *Dunaliella* spp. have been isolated in our laboratory. Among them, 22 isolates were identified and further compared based on total carotenoid content. From the morphological characteristics, 18S rDNA and ITS1-5.8S-ITS2 sequences, these isolates were identified as belonging to *D. salina* and *D. viridis*. The total carotenoid content of these isolates vary from 0.44 pg/cell to 22.00 pg/ cell. Among these 20 isolates, *D. salina* NUAC10, *D. salina* NUAC09 and *D. salina* NUAC13 could accumulate high amount of total carotenoid up to 19.90 $\pm$ 1.94, 20.98 $\pm$ 0.55 and 22.00 $\pm$ 1.14 pg/cell, respectively. Thus, further researches are studied on optimal condition of cultivation and investigated the profiles of  $\beta$ -carotene isomers from different *Dunaliella salina* strains.

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## The enhancement of hydrogen photoproduction in Chlorella sorokiniana exposed to simultaneous nitrogen limitation, sulfur- and/or phosphorus-deprivation

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Hydrogen  $(H_2)$  is considered to be one of the best candidates for clean and renewable energy. Previous studies have revealed that some microalgae were able to convert solar energy into H, energy through the biophotolysis of water. In this study, H<sub>2</sub> photoproduction and PSII photochemical activities were investigated in Chlorella sorokiniana exposed to simultaneous nitrogen limitation, sulfur (S-) and/ or phosphorus (P-) deprivation. The C. sorokiniana pre-cultured in regular TAP medium produced about ~48.2 ml/L of H<sub>2</sub> under S-deprivation with 7 mM NH<sub>4</sub>Cl. However, cells pre-grown in a low concentration of NH<sub>4</sub>Cl generated a large amount of H<sub>2</sub> after transfer to simultaneous nitrogen limitation, sulfur- and/or phosphorus-deprivation. The maximum  $H_2$  outputs of ~77.3, ~98.1 and ~125.1 ml/L were obtained in the N-limitation cultures exposed to TAP-P (0.7 mM NH<sub>4</sub>Cl), TAP-S

(0.7 mM NH<sub>4</sub>Cl) and TAP-S-P (0.7 mM NH<sub>4</sub>Cl), respectively. The average rate of H<sub>2</sub> productivity for the N-limited culture exposed to TAP-P (0.7 mM NH<sub>4</sub>Cl), TAP-S (0.7 mM NH<sub>4</sub>Cl) and TAP-S-P  $(0.7 \text{ mM NH}_{A}\text{Cl})$  was ~1.07, ~1.36 and ~1.50 ml/L/h, respectively. The above result clearly indicated a new and most interesting finding of the mutual effect of simultaneous N-limitation, S- and/or P-deprivation on H<sub>2</sub> photoproduction in this alga. The photosynthetic inhibitors,3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU) and 2,5-dibromo-3-methyl-6-isopropylpbenzoquinone (DBMIB) repressed the H, production in TAP-S-P (0.7 mM NH<sub>4</sub>Cl) medium by 68.04% and 98.65%, respectively. The results indicated that two-thirds electrons were generated from the splitting of H<sub>2</sub>O in PSII and the remaining one-third electrons possibly came from other substrate catabolism.



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## Three Interesting Macrofungi from Bamboo Forests in Loei and Petchabun Provinces during Rainy Season

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Numerous macrofungi were found associated with tropical woody bamboos (Bambuseae) from bamboo forests in Loei and Petchabun provinces during rainy season in 2016-2017 and three of them had some promising characteristics. On the bamboo forest floor, an interesting bioluminescent mushroom belonging to the genus Mycena (Pers.) Roussel, 1806 (Basidiomycota, Agaricomycetes, Mycenaceae) was found on rotten bamboo calms, sheaths and fallen leaves after a few consecutive precipitations. The wet forest ground was illuminated during the dark night by several glowing fruiting bodies, and glowing mycelium on rotten leaves and sheaths. Two species of fungi producing big-dark ascostromata were always found hanging on node of bamboo calms or twigs. One called Kao-Niew-Ling was identified recently as

Gelatinomyces siamensis N. Sanoamuang, W. Jitjak, S. Rogtong & A.J.S. Whalley, 2013 (Ascomycota, Leotiomycetes, incertae sedis). The Kao-Niew-Ling fungus produced lot of red substances naturally inside the fruiting body which were finally identified as Oosporein. The second species was seen as a grey-to black matter with pores on the surface and was always associated with a population of black ants. Inside its fruiting body, there was a cavity simultaneously providing food and functioning as a nest for the black ants. Detailed morphology and a few DNA sequences analysis showed that this ant-fungus associated species was well placed in the Ascomycota, Dothideomycetes, Capnodiaceae and it is new to science.

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## Tree species diversity along the '101°E' forest transect from Southwest China to the Indo-China Peninsula

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Large forest plots (16-50 ha) can be used as platforms to explore tree species diversity and its maintaining mechanisms at local scales (<1 km<sup>2</sup>). However, little is known about how these local-scale patterns and processes vary at regional scale. We propose the '101°E' forest transect from Southwest China to the Indo-China Peninsula (hereafter '101 transect') to act as a platform to explore how local-scale patterns and processes change along an environmental gradient at regional scale.

The '101 transect' consists of ten large forest plots, from five of which we have collected key

functional traits for nearly twenty thousand tree individuals of more than 1,000 tree species. We compare the differentiation between common and rare species in intraspecific trait variation to distinguish how common and rare species co-exist at local-scale tree assemblages and how this varies with the latitudinal environmental changes. Based on results from above analysis, we want to test stress-gradient hypothesis at regional scale, namely the strength of environmental filtering increases with habitat harshness, while the strength of similarity limitation decreases with habitat harshness.



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## Molecular Phylogenetic Analyses Unravel Cryptic Generic and Species Complexes: Examples from The Pantropical Flowering Plant Family Annonaceae

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Molecular phylogenetics is nowadays an indispensable discipline to understand the evolutionary relationships among organisms. In addition, it is used as a framework for (re)classification. Classical classification, i.e. a classification based solely on morphology, is often artificial because similar elements tend to be grouped together regardless of whether they share a common ancestor. This is due to convergent evolution or the lack of obvious synapomorphies. Molecular phylogenetic analyses can shed light on the more plausible hypotheses on relationships and classification. This can be well exemplified in the pantropical flowering plant family Annonaceae. The genus Polyalthia Blume has been shown to formerly consist of disparate elements and a new genus, Huberantha Chaowasku (1), was established to accommodate a strongly supported clade with unique pollen morphology [1]. Further, another new genus, Winitia Chaowasku (2), was

erected based on thorough macromorphological and pollen morphological studies coupled with phylogenetic branch lengths [2]. Similarly, an additional new genus (3) is being proposed for a strongly supported clade that is unable to assign to any existing genera [3], and one more new genus (4) is on its way to becoming formally characterized [4].

The above-mentioned phenomena can also be well applied to species level. Extensive molecular phylogenetic inferences of two sister genera, *Marsypopetalum* Scheff. and *Trivalvaria* (Miq.) Miq. (5), revealed that distantly related species have been misclassified under a single species [5]. It is concluded that the more powerful 'molecular phylogenetics' will be fully replacing the classical methods of classification in the near future, with aid of advancing technology in molecular biology.



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## Biogeography and biodiversity of southeast Asian tropical pine savannas and coniferous forests under past and future climates

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Environmental change in the Anthropocene will impact some biomes more severely than others. Savannas, which consist of C<sub>3</sub> woody plants and C<sub>4</sub> grasses, are threatened by increases in both rainfall and atmospheric CO<sub>2</sub> levels that are expected to tilt the competitive balance in favour of C3 trees, and hence shift savannas towards forests. However, model predictions of vegetation change are uncertain and more data is required to understand how savannas have been impacted by past climate change and how they might respond in the future. Pine stands of Pinus kesiya and Pinus merkusii occur as isolated patches across Southeast Asia, from China to Indonesia, and often have a distinct savanna physiognomy. They are tolerant of fire, a disturbance commonly associated with savannas. The two pine species have different distributions, suggesting their ecological niches are different, but no comparative study has yet been attempted.

In this project, we are comparing these two pine species and their associated savanna communities across their geographical range in Southeast Asia. We use genetic analyses to understand how their populations expanded and contracted in the past, and their capacity for climatic adaptation. We use tree ring isotope analyses to understand their growth responses under enriched CO<sub>2</sub> and their fire history. We measure their floristic and functional diversity to identify drivers and assess differences between the two communities. These analyses will then help parameterize dynamic vegetation models to predict future changes to the distribution of savannas and test possible management strategies. These assessments will inform conservation priorities and management needs in Southeast Asia.

## Isolation and characterization of polysaccharides and lignin from plant resources for biotechnological application

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Lignocellulose is an excellent source of valuable compounds that can be used in pharmaceutical, food, and cosmetics in addition to being a source of sugars for bioethanol industry. To earn more income and balance the ethanol production cost, we optimized and developed pretreatment processes as a means to modify structure for improved enzymatic saccharification and to extract biomass components for specific application. In this study, different alkaline pretreatment methods were used to remove lignin, thus leaving the solid rich in cellulose and hemicellulose. The alkaline pretreatment parameters, including solid:liquid ratio and alkali concentrations were optimized using surface response methodology (RSM) to reduce water consumption and reduce acid use in lignin precipitation process. The treated solid was further processed in two ways: 1) using a sequential step of pretreatment techniques (acid or ionic liquid) to separate cellulose from hemicellulose; 2) introducing to xylan digestion by enzymes to generate xylo-oligomers, and subjecting the residual solid (cellulose) after xylan digestion to ethanol fermentation. All extracted components as well as products were comprehensively characterized using SEM, FTIR, XRD, TGA/DSC, and biological tests. In addition, lignin in pretreated liquid fraction, which is generally regarded as waste, was acidified to obtain lignin powder and was evaluated its biological activity potential. This work provides basic information of extracted biomass components, which can be applied in several industries. This work extends knowledge on diverse plant-based bio products and sheds light on the integration of ethanol industry and hemicellulose and lignin utilization to make biofuel production more economic viable.



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## The Pollination Network Structure in Mixed Fruit Orchards in Southern Thailand

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Pollination is a main ecosystem service. Currently, this service is considered under threat due to habitat loss and fragmentation. Mixed fruit orchards are common in Southeast Asia. The reproductive success of some plant species is dependent on the distance to the forest edge, because forest remnants can be important sources of pollinator. The response of reproductive biology and pollination ecology of single plants to habitat changes is inadequate; however, we need to understand pollination interaction the community level. This study was conducted to determine whether pollination network structure and reproductive success in mixed fruit orchards depend on distance from forest edge. Ten pairs of mixed fruit orchards at near and far from big forest patches were selected to study in 2012. The number of flowers and capitulums were counted. The number of flower-visiting animals were focally observed and counted for 15 minutes from each plant. Camera traps were applied for nocturnal animals. Fruit and seed set were observed after two weeks of pollination, and compared between both study sites. Network structures, including interaction density, nestedness, number of compartments, evenness were calculated. The species number of plants and animals from the orchards that near to forest are higher than orchards that far from forest. The most species-rich of visitors was bee (Trigona and Apis). The connectance, nestedness, robustness, and evenness of pollination network from study sites near to the forest tend to be higher than study sites far from the forest. In conclusion, the structure of pollination network in mixed fruit orchards tend to change negatively with the distance from the forest edge. However, this study was conducted only in Southern Thailand and real landscapes are more complex and heterogeneous. Thus our future studies of landscape effect on pollination network should include more areas in Southeast Asia and the perspective of landscape functional heterogeneity.

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## Invitation to forest canopy ecology: global scale collaboration opportunities

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The canopy is the defining components of any forest ecosystem and is known to harbour a large proportion of global biodiversity. The canopy community is vitally important for the essential roles in ecosystem functioning and services. Over the last several decades there have been significant advances in canopy science, and extensive education programs have been successful in disseminating information about the importance of forests and their canopies in promoting human well-being. There remain many challenges in understanding canopy systems in order to make predictions about the consequences of global-scale human disturbances and their impact on forest biodiversity and ecosystem functioning. Much further research is needed to understand fully forests and their canopy ecosystems. Moreover, canopy science is undergoing an exciting, radical change of approach from descriptive studies to experimental manipulation. Here we examine recent progress in canopy science, and invite you to join global canopy networks which can be incorporated into multilateral, collaborative efforts to further develop our understanding of canopy ecosystems.





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## Diversity of fishborne zoonotic trematodes in Asia: potential and limitation

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Fishborne zoonotic trematode (FZT) infection affects the health of more than 18 million people around the world, particularly in Asian countries. Humans are mainly infected with FZT when eating raw or inadequately cooked fish contained the infective larva (metacercaria). The information on the biodiversity of FZT is beneficial for planning parasite control in the food supply and also reflect the current environmental conditions as the indicators of environmental change. Recently, twelve families are in the list of FZT. Among them, liver fluke, Opisthorchis viverrini, has been recognized as the group I carcinogen inducing cholangiocarcinoma. This species distributes in the lower Mekong basin. In the past, the cryptic species of O. viverrini was not considered until O. lobatus was discovered in the overlapping area distribution of O. viverrini. The morphological similarity of two parasites causes the misidentification, that may affect to report of prevalence of O. viverrini. Not only the study in Indochina, we also studied the diversity of several FZT in Japan. The diversity of intestinal fluke belonging genus Metagonimus was revealed. The result showed that morphological characters of the Japanese representatives investigated in this study seem to be homoplastic. Morphological adaptations among

species of *Metagonimus*, which may have diverged within a small region of central Japan. Although, the study of the diversity of FZT in Asia has still been limited and most studies focused only on the well-known species. Our studies indicated the potential of the high diversity of FZT in Asia that needs to be discovered.



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### **Bdelloid Rotifers in Thailand**

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Bdelloids are a group of rotifers in phylum Rotifera. The distinguished characteristics of this organism are obligated asexual reproduction by parthenogenesis and able to resist in unsuitable environments such as drying habitat in which they can remove water from their body. For above reasons, they can disperse in both moist terrestrial habitats and water bodies. Although bdelloids are well studied in foreign countries especially Europe, but they have a few recorded in Southeast Asia including Thailand because the limitations in the studying bdelloids such as the important features can be observed only when they are alive. Thus, there are only five bdelloid species have been recorded from Thailand. Therefore the present study aims to explore the diversity of freshwater bdelloid rotifers in Thailand. The specimens were collected in 25 freshwater habitats

throughout Thailand. A total of 13 species, namely Dissotrocha aculeata, D. macrostyla, Macrotrachela pinnigera<sup>\*</sup>, Otostephanos donneri<sup>\*</sup>, Philodina megalotrocha<sup>\*</sup>, Rotaria mento<sup>\*</sup>, R. neptunia<sup>\*</sup>, R. neptunoida<sup>\*</sup>, R. ovata<sup>\*</sup>, R. rotatoria, R. tardigrada<sup>\*</sup>, R. megarostris\*\* and Rotaria sp.\* were found. Of which, nine species are new records for Thailand (one asterisk) and one is new to science (two asterisks). Moreover, the morphological features of Rotaria sp. are different from other members in the genus and we are working on it. For this preliminary survey, the species diversity of bdelloid rotifers in Thailand has increased from 5 species to 13 species. It indicated that freshwater habitats in Thailand have high diversity of this fauna. Thus, intensively study can be reveal actual species diversity of bdelloids rotifers in this region.

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## Species Diversity of Epikarst-Dwelling of Copepods in Caves from Thailand

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Most of the studies on freshwater zooplanktons (cladocerans, rotifers, brachiopods, ostracods, and copepods) in Thailand are based exclusively on epigean faunas while cave or any subterranean faunas have been neglected thus far. The results of recent studies on cave faunas in the northern, western, southern, and some parts of northeastern Thailand, revealed there are many interesting species of microcrustaceans which are new genus, new species, and new records for this country. I have mainly focused on species communities in dripping water, drained out from cutaneous caves (epikarstic zones) which are rich in species diversity. The main groups of microcrustaceans found in our sampling during 2010-2017 are Syncarida, Amphipoda, Isopoda, and Copepoda. Amongst animal communities, copepod is the dominant group in both species richness and its occurrence.

The knowledge gained from studying cavedwelling Copepoda in Thailand has been greatly improved, especially for harpacticoids and cyclopoids. To date, 10 new species from Thailand were described and named by our research team including 6 *Elaphoidella* (Harpacticoida), 2 *Bryocyclops* and 2 *Fierscyclops* (Cyclopoida). These numerous interesting species are under taxonomic study or in the process of being published. Most new species are found particularly in single caves or in narrow areas, with some having a stygobite distribution characteristic and thus more sampling in both studied and non-studied areas are expected to yield greater knowledge on regional diversity. As a result of these studies, an identified key for the genus *Elaphoidella* Chappuis, 1929, harpacticoids, is provided for SEA species, which is useful for the future study in adjacent areas. The number of described cyclopoid and calanoids species are also increased and current studies aim to provide additional identified keys for other genus. Because of this recent morphological taxonomy on copepods others can use these keys as tools for species diversity and species monitoring studies in the future.

Another cave-dwelling microcrustacean also found and named from Thailand is Syncarida; *Siambathynella laorsriae* (new genus and species). Some species of syncarids as well as amphipoda and Isopoda are still undescribed yet.

At present, I am still working on a morphological taxonomy for undescribed copepods in both epigean and subterranean samples, and I plan to take samples in non-studied areas in Thailand and nearby countries in SEA. Molecular taxonomy and ecological studies on recently-known species are required for future study. Based on a goal of our research team, we are also interested in the study on a different group of inland microcrustaceans or zooplanktons in terms of biodiversity and taxonomy.

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### **Millipede Diversity of Myanmar**

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The Republic of the Union of Myanmar, formerly known as Burma, is a globally recognized biodiversity hotspot, because it supports a very high number of species, many of which are unique to the region and of global importance. At present, the millipede fauna of Myanmar comprises 92 species from 34 genera, 13 families and 8 orders. Most of the diversity, including 66 new species and further 4 new records, were described in 1889-1896 by Pocock 1. That material was mainly collected by Leonardo Fea during his several trips across Myanmar (1885), as well as Eugene William Oates, who was a civil servant in the Public Works Department in India and Myanmar from 1867-1899. The bulk of millipede diversity in Myanmar belongs to the order Polydesmida which includes 4 families, 17 genera and 45 species (48.9%), followed by Spirostreptida with 2 families, 7 genera and 19 species (20.6%); Sphaerotheriida with 1 family, 3 genera and 12 species (13%) and Spirobolida with 2 families, 2 genera and 10 species (10.9%). The family Paradoxosomatidae (Polydesmida) contains the greatest proportion of species (37), followed by Harpagophoridae (Spirostreptida) (14) and Zephroniidae (Sphaerotheriida) (12). The four most species-rich genera are Zephronia

(10 species), Orthomorpha (8), Antheromorpha (6) and Gonoplectus (5), which combined represent 31.5% of the indigenous species diversity. Most of the known species (70 of 92) have only been recorded in Myanmar and for the time being are to be regarded as endemic. Four of 34 genera present in Myanmar are also endemic: Ctenorangoon, Tuberogonus, Alogolykus and Cryptodesmoides (although the latter genus is still dubious). There are five widespread, synanthropic, anthropochore species, as well as 13 shared between Myanmar and Thailand such as, e.g., Zephronia viridescens, Tonkinbolus caudulanus, Eudasypeltis setosus, Orthomorpha insularis, O. karschi or Tylopus doriae. Altogether, there are 57 collecting localities in Myanmar, mostly those visited by Fea 2 together with the neighboring rivers or islands. The localities that support most of the species recorded in Myanmar are Yangon with 14 species (11 type localities), followed by Palon in Pegu with 13 species (9 type localities) and Malewoon with 12 species (5 type localities). The number of the places in Myanmar whence Diplopoda have been taken is quite low because of the limited exploration of the country, coupled with no local research in this group.



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## Systematic Investigation on Centipede Diversity in Mainland Southeast Asia: A Case Study in Order Scolopendromorph (Myriapoda, Chilopoda)

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Scolopendromorph centipedes are primordial soil macro-arthropod predators which are commonly distributed along tropical region. Several nominated species were reported with ambiguous information such as scattered distribution records, taxonomic problems that caused by morphological and genetic variations. The unreliable taxonomic character used in the past also caused the species delimitation problem in several genera. In this study, centipede specimens were collected thorough mainland SE Asia were compare morphologically with the historical collections to investigate geographical variation. All representative taxa within Scolopendromorph genera were sampled to constructs phylogenetic tree based on standard gene for myriapod phylogenetic study. Taxonomic revision demonstrate three SE-Asian Scolopendromorphs families; Scolopendridae, Cryptopidae and Scolopocryptopidae. Family Scolopendridae represents vast diversity and exhibits high variation in both species and population levels. The problems on species complex were critically revised and the differentiation of colouration patterns among geographical populations were recorded as changes limited to local populations. Two blind centipede families showed lower diversity, although their morphological character such as body length and size seem to relate with some ecological habitats. In phylogenetic result, the phylogenetic tree depicts

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monophyly of each centipede family. The discordance of genetic relationship within members was found due to the sensitive changes during tree optimization. A geometric-morphometric analysis was initially assigned based on classical landmark method to evaluate species boundary in some scolopendromorphs. The shape variability on three selected characters; the coxosternite, the cephalic plate, and the tergite 21 are showing significantly informative signal for species identification in Scolopendra centipede. However, the diversity of order Scolopendromorpha in SE-Asia is still underestimate so far as indicated by morphological and genetic evidences. Further information is needed especially from neighboring fauna such as Indian subcontinent and East Asia to provide the evidences of genetic transfer and fauna diversity exchange along transitional zone of this region. The past and recent ecological and geological records in this territory are also required to adjust the evolutionary history of these living fossil-like arthropod group.





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## Phylogeography of the river basins in Indochina: a case study of freshwater mussel species

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Phylogeographic data from freshwater aquatic taxa can be contributed significantly understanding on past geological history of the river systems. When the topology of a drainage network has remained stable enough for long periods of evolutionary time, the retained genetic structure would be seen and general predictions can be made regarding to that structure. The freshwater resources in Indochina are normally supported by two principal river systems, the Chao Phraya and the Mekong river basins. Various studies have examined genetic variation, and showed general trend of high genetic differentiation between freshwater taxa from these two basins. However, phylogeography and the study on correlated dimension of molecular entity and ecosystem characteristics of freshwater fauna in the region are still limited to the invertebrate species, and never expanded such investigation to freshwater mussels, even the mussels are one of the most important fauna, and likely to have similar distribution patterns to the fish hosts due to the unique parasitic co-evolution. The freshwater mussel genus Contradens, which is occurring throughout Indochinese waters with several morphologically different species/subspecies, was selected for phylogeographic study. The results revealed that the mussels were separated into at least seven clades, reflecting the river basins in the region, namely Chaopraya Clade, Malay Clade, Tonle Sap Clade, Korat Plateau Clade, Lanxang Clade, Huai Luang Clade, and Sonkram Clade. This biogeographic partitioning suggested that the middle Mekong river basin probably evolved from at least four historically independent drainage systems, and each drainage was probably isolated from each other for an extensive period of evolutionary time. This study shed new light on drainage evolution of Indochina, and become very useful basic information practicing for further biodiversity and conservation managements.



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## Genetic diversity, population dispersal and current status of edible amphibians in the Family Microhylidae from northeastern Thailand

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Amphibians are regarded as good ecological indicators because they have high degree of sensitivity and respond to very slight changes in the environment. In addition, they also play an essential role in ecosystem as secondary consumers in many food chains. Therefore, decline or extinction of their population has significant impact on other organisms along with them. One of enigmatic amphibian group is the Family Microhylidae that inhabit a wide variety of habitats, with most species living within terrestrial, fossorial, arboreal or freshwater aquatic ecosystems. Microhylids in Thailand are commonly abundant throughout the country and very popular for consumption and trade, especially in the northeastern region or "Isan" where approximately twelve species are found, i.e., Glyphoglossus guttulata, G. molossus, Kaloula mediolineata, K. pulchra, Microhyla berdmorei, M. butleri, M. fissipes, M. heymonsi, M. pulchra, M. mukhlesuri, Micryletta inornata and Kalophrynus interlineatus (Khonsue and Thirakhupt, 2001; Chuaynkern and Chuaynkern, 2012; Hasan et al., 2014). Most of them are affected by overharvesting for food during the breeding seasons while some species such as G. molossus are categorized as near threatened by IUCN (2017). Sustained overexploitation can lead to the destruction of amphibians as the population (numbers) may drop and the specie may become extinct. Population dispersal and current status of these edible amphibians are critical indeed and have to be studied urgently. The objective herein is to determine the genetic variation of edible microhylids frogs in northeastern Thailand based on mitochondrial DNA sequence in order to understand their genetic diversity and population structure. In the meantime, their systematic and taxonomic status are expected to revises. Lastly, field survey in different topologies of the Khorat Plateau, e.g., river systems and mountain ranges as well as data from interviews will be collected in order to evaluate population dispersal and current status.

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Off Donsak, one of the major habitats for various marine mammal species in southern Gulf of Thailand, the habitat status is currently impacted by ferry transportation, fishing gears, marine debris and pollutions from sewages. These impacts are concurrently impacting population viability of three major coastal cetaceans inhabiting these habitats, including the Indo-pacific humpback dolphin (Sousa chinensis), Irrawaddy dolphin (Orcaella brevirostris) and Indo-Pacific finless porpoises (Neophocaena phocaenoides) (1). Khanom is the adjacent area of Donsak District where have the most famous in dolphin watching tourism. The past few years Donsak have been started dolphin watching tourism in Nang Gum beach and Rat Island. Without the control of related government office, the recently new fishermen villages who changed their livelihood from artisanal fishery to wildlife coastal cetacean tourism (2). Wildlife watching tourism affecting to coastal cetacean species and being in conflict between fishermen community benefits and the conservation (3). Department of Marine and Coastal Resources (DMCR) who responses in the cetacean monitoring directly have been conducted to study and investigated in cetacean population and habitat in the middle Gulf of Thailand. DMCR Chumporn province (The middle gulf of Thailand Center) continuously monitored population status of the Indo-Pacific humpback dolphin in Donsak Water and adjacent area (Khanom water, Nakhon Si Thammarat through photo-identification tasks based on opportunistic surveys. Khanom water is the famous place in wildlife cetacean watching tourism (4). The lack of systematic design and limited budget resources, nonetheless, potentially restricted the efficiency of dolphin population monitoring. Development and applications of novel techniques become more urgent than ever to overcome and reduce the budget limitation, and improve current dolphin monitoring efficiency. This study will develop techniques for monitoring dolphin occurrence based on systems integrating surveillance camera and automatic water quality sensors, which should be applied in the Donsak River Estuary. By this system, instantaneous abundance, spatial distribution and habitat utilization of the dolphins, as well as the habitat condition will be recorded and reported continuously in a fine temporal resolution. Dolphin occurrence, habitat core area and spatial distribution were reported precisely. These results will provide real-time (or quasi-real time) information for immediate conservation actions for local governments.



Figure 1. Minimum Convex Polygon of three coastal cetacean from opportunistic survey

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## Late Middle Pleistocene diversity of vertebrates in Khok Sung (Northeastern Thailand)

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The terrace deposit of Khok Sung, Nakhon Ratchasima province, has vielded the richest Pleistocene vertebrate fauna of Thailand, where abundant fossil mammals and reptiles (skulls, isolated teeth, and postcranial remains) were recovered. The mammalian fauna consists of at least 15 recognized species in 13 genera, including a primate, proboscideans, carnivores rhinoceroses, suids, bovids, and cervids, characterized by mostly extant elements associated to some extinct (Stegodon cf. orientalis) and extirpated (Crocuta crocuta ultima, Rhinoceros unicornis, Sus barbatus, and Axis axis) taxa. Ten reptilian species (9 genera) are also identified. The mammalian assemblage comprises megaherbivores (> 1000 kg) of approximately 19% of the species (including proboscideans, rhinoceroses, water buffaloes) and other large species of about 37% (including artiodactyls, primates, and carnivores) of the vertebrate fauna. The most abundant mammal group of the locality is represented by the artiodactyls (9 species). The non-mammalian species consists of about 44% of the total vertebrate fauna. The order Testudines is the most diverse group of non-mammalian taxa in the locality (22% of the fauna). In addition, other vertebrates such as birds and fish are tentatively observed. A single fragmentary cervical vertebra of the bird order Galliformes is also present. The Khok Sung mammalian taxa characterize the Pleistocene Ailuropoda-Stegodon faunal complex found throughout

the subtropical to tropical forested regions of South China and mainland Southeast Asia. A chital, Axis axis, whose distribution is today restricted to the Indian Subcontinent, is reported here for the first time in Southeast Asia during the Pleistocene. The age of the Khok Sung fauna is tentatively attributed to the late Middle Pleistocene as either 188 or 213 ka, based on the paleomagnetic data and on the faunal comparisons. Compared to other Southeast Asian Pleistocene and extant faunas, the Khok Sung mammal assemblage yields most of mainland Southeast Asian taxa that migrated to Java during the latest Middle Pleistocene, supporting the hypothesis that Thailand was a part of the Sino-Malayan migration route from South China to Java. The Sunda shelf, forming when the sea-levels dropped during glacial stages, is supposed to provide the only possible route of mammalian dispersal between Southeast Asian mainland and islands.



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## A Potential Wound Healing Ability of Plasma from Crocodylus siamensis

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*Crocodylus siamensis* share their aquatic living environments with a variety of opportunistic pathogens and microbes. Although wounds resulting from fighting with other crocodiles or different species occurring frequently, they appear to heal rapidly and without any infection, despite the harsh environment. This anecdotal evidence suggests the probably that *C. siamensis* plasma might be exhibited would healing property. Therefore, to determine the role of *C. siamensis* plasma during wound healing, the *in vitro* human kertinocytes cell line (HaCaT) was firstly focused on functional analyzed regarding to



proliferation and migration effects. Additionally, *in vivo* mouse excisional skin wound healing and wound infection healing models were used to study the cutaneous regeneration during skin tissue recovering. The collected data from scratch wound experiment demonstrates that crocodile plasma was be able to decrease the gap of wounds in a dose-dependent manner. Consistent with *in vitro* results, remarkably enhanced wound repair was also observed in a mouse excisional skin wound model after treatment with plasma. The size of skin wounds after treatment with plasma was more rapidly decreased and almost

> complete wound closure was observed after 9 days. The effects of *C. siamensis* plasma on wound healing were further elucidated by treating wound infections by *Staphylococcus aureus* ATCC 25923 on mice skin. The results indicate that crocodile plasma promotes the prevention of wound infection. Therefore, this work strongly supports the utilization of *C. siamensis* as injured skin therapeutic products.

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## A soluble protein expression and characterization of *Crocodylus siamensis* hemoglobin in Pichia pastoris

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The function of crocodile hemoglobin (cHb) is transporting oxygen from lungs to tissues of the body, but it also contains a source of antimicrobial peptides on their molecule. In the present study, the eukaryotic expression system was chosen. Like yeast, crocodiles are eukaryotic cell, so the metabolic pathway is the same as yeast. The Pichia pastoris expression system is being successfully used for the production of various recombinant heterologous proteins. It can further be grown to high cell densities and therefore constitutes a fast and cost-effective platform allowing high protein expression yields and success rates for a variety of recombinant proteins. A 15-kDa of soluble protein was succeeding from P. pastoris expression after 72 h at 25°C. The authenticity of recombinant cHb was confirmed using LC/MS-MS, resulting in a-globin chain. The characteristic of recombinant hemoglobin was confirmed using heme insertion method. The heme content of recombinant  $\alpha$ -globin was calculated to be 0.2 mol of heme/mol of globin. In addition, the quantitative analysis of the iron in recombinant protein revealed that recombinant  $\alpha$ -globin contained 0.031 g of atom iron/mol. Additionally, the UV-VIS profile presented a specific signal pattern at 415 nm, indicating that the recombinant protein formed a heme complex identical

to active oxyhemoglobin (540 nm and 580 nm). Moreover, the extinction coefficient of heme was determined by converting the recombinant  $\alpha$ -heme to the pyridine hemochrome as described above. By pyridine hemochrome assay, the extinction coefficient of heme-containing protein ( $\epsilon$ 415) was 378.6 mM<sup>-1</sup> cm<sup>-1</sup>. Taken collectively, the derived results of this work could therefore be applied in further expression studies of heme-bound proteins. Moreover, the presented methodology may serve as an attractive option for protein production and purification on a large-scale, which is deemed important for further investigation and characterization of the structural features of the cHb protein.



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## Studying on Anticancer Activity of KT2, Antimicrobial Peptide Analogs from Siamese crocodile Leucrocin I

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Cancer represents one of the most significantly threat to human health on a global scale; hence, the development of effective cancer prevention strategies, as well as the discovery of novel therapeutic agents against cancer are urgently required. In light of this challenge, the present study aimed to investigate the effect of KT2, a cationic Leucrocin I analogs from *Crocodylus siamensis* against HCT-116 human colon cancer cell lines. Using MTT assay, results demonstrated that KT2 at the concentrations of 37.5, 75 and 150  $\mu$ g/mL exhibited strong cytotoxic effect against HCT-116 cell lines after 24 h of treatment (IC50 75  $\mu$ g/mL). The mechanism involved in cancer cell cytotoxicity of this peptide was shown to be associated with the induction of apoptosis, as evidenced by annexin V-FITC/PI staining flow cytometry analysis. Moreover, the significantly inhibited S-G2/M transition in the cancer cell, leading to the arrest of cell population growth was observed when treated with KT2. These collected data clearly demonstrated that KT2 has great potentially in the treatment of human colon cancer cells.



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