







THE 13th CONFERENCE OF





PRTRG 13 Online Proceeding





PRTRG 13

Talenta 13

2020 12-13 FEB





Taipei | Taiwan 2020



PRTRG 13 raiwan 2020 12-13 FEB







THE 13th CONFERENCE OF



February 12th-13th, 2020

Taipei Zoo Taipei, Taiwan

Organizers: PACIFIC RIM TERMITE RESEARCH GROUP



Co-organizers:









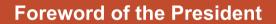
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Dear participants,

It is with great pleasure to welcome you to participate the 13th Conference of the Pacific Rim Termite Research Group (PRTRG 13) held in Taipei, Taiwan, February 12 - 13, 2020.

This conference is a well-established biennial event at the PRTRG. PRTRG continues a tradition of bringing together eminent speakers and scholars engaged in termite researches, young researchers and students, academics and professionals from pest control industries. We devote to provide a forum for knowledge exchange and future research collaboration. As we also encourage the interac-

tions between all stage of researchers, we provide travel fund awards to support young scientists/students to take participation in the PRTRG conference and challenge themselves to learn and to gain invaluable scientific experience.

This year, over 60 papers were submitted, and 46 of them will be made on the conference. We are honored to have invited Dr. Nan-Yao Su to be our Keynote speaker. Dr. Su is an authority on termites and is known for his innovative approach for their population management. The outstanding Dr. Thomas Bourguignon, Dr. Thomas Chouvenc and Dr. Chun-I Chui are invited to deliver in the Kunio Tsunoda Memorial Lectures.

I would like to express our gratitude to the following local organizers: Taipei Zoo, Department of Entomology, National Chung Hsing University, Taiwan Entomological Society, Chung Hsi Chemical Plant, Ltd., as well as the editor, Dr. Partho Dhang for his hard work in reviewing submissions. I would also like to thank all the participants for your presentations and the contributions, and the sponsors for their support and participation. I wish you all a successful and fruitful conference.

President of PRTRG 2016-2020



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Taiwan is composed of the main island, Taiwan Island and 22 smaller islands, with a total of 36,188 km². Taiwan Island is 394 km in length and 144 km in width at widest point. Taiwan locates in southeastern Asia and lies on the Tropic of Cancer (22-25°N latitude). More than two thirds area of Taiwan Island are mountainous. The northern and central regions are subtropical, whereas the south is tropical and the mountainous regions are temperate. The islands provide various habitats for termites, such as the forests of high mountains and the seashore vegetations. More than twenty termite species from five families were recorded. Termite research



in Taiwan began in early 1900s, and several major termite pests were named after Taiwan, historical known as Formosa, including the Formosan subterranean termite, *Coptotermes formosanus*, and Formosan fungus-growing termite, *Odontotermes formosanus*.

Population of Taiwan is over 23.7 million, and most people live in lowland area of western Taiwan. Han Taiwanese is the major ethnic group (> 95%). Taiwanese aborigines comprise \sim 2% of the population. More than 96% of population can read and speak the national langue, Taiwanese Mandarin, and 70% Taiwanese can also speak Hokkien and 15% Taiwanese can also speak Hakka.

Taipei is Taiwan's largest city as well as its economic, political, and cultural center. It is a modern cosmopolitan metropolis with a lively and diversified cultures. Taipei is part of a major high-tech industrial area. Railways, highways, airports, and bus lines connect Taipei with all parts of the island. It is also home to various world-famous architectural or cultural landmarks, which include Taipei 101, Lungshan Temple of Manka. The Taipei Fine Arts Museum, National Taiwan Museum, National Palace Museum, Presidential Office Building, and several night markets dispersed throughout the city. Natural features such as Maokong, Yangmingshan, and hot springs are also well known to international visitors.



Venue & Transportation

International Lecture Hall, Xinguang Special Exhibit House (Giant Panda House) 3F, Taipei Zoo, TAIWAN

Participants may take the Taipei Metro (MRT) "Brown Line" to MRT Taipei Zoo Station, then take a 10-minute short walk from the front gate to the Giant Panda House.



- 10 mins walk from the entrance to the PRTRG 13 venue (Giant Panda House)
- 3 mins walk from the venue to the Lecture Hall for welcome party



Keynote Speech

Time: 09:00-09:30, February 12, 2020

Tunneling Behavior and Foraging Strategy of Subterranean Termites



*Prof. Nan-Yao Su*University of Florida, Distinguished Professor

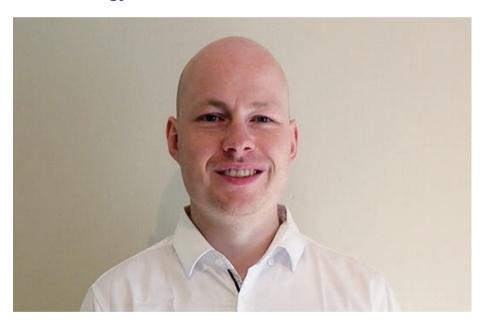
Prof. Nan-Yao Su is recognized as an international authority on termites. His innovative approach, termite colony elimination system, has been commercialized and marketed to 18 countries, which made a great contribution to termite management. Moreover, he authored and co-authored over 260 peer-reviewed articles and book chapters on termite biology and control.



Kunio Tsunoda Memorial Lectures

Time: 09:30-10:00, February 12, 2020

Coptotermes Hybrids as a Model to Study Core Aspects of Termite Biology



Dr. Thomas Bourguignon

Okinawa Institute of Science and Technology Graduate University

Dr. Thomas Bourguignon is an ecologist and evolutionary biologist with special interests on termites. In his earlier academic career, Dr. Bourguignon was well-known for his publication on Science, titled "Explosive backpack in old termite workers". Now he focuses on using next generation sequencing (NGS) technology to investigate the evolutionary ecology of termites, and has become the leading figure of termite biodiversity and phylogenetic researches.



Kunio Tsunoda Memorial Lectures

Time: 10:30-11:00, February 12, 2020

Diet and Cocladogenesis Shape Termite Gut Bacteria Communities



Dr. Thomas Chouvenc

University of Florida

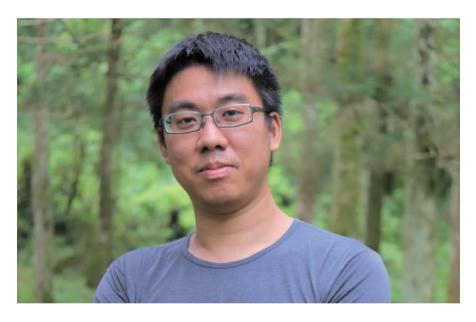
Dr. Thomas Chouvenc's research focuses on subterranean termite biology, with a particular interest in the field of termite-microorganism associations, termite ecology and the evolution of insect societies. He is well-known for his review article on biological control of termites, which is cited more than ten times per year. Recently, Dr. Chouvenc's studies on hybridization of *Coptotermes* species boosted the researches on termite biology and their economic impacts in US.



Kunio Tsunoda Memorial Lectures

Time: 11:00-11:30, February 12, 2020

Termite Fungal Cultivation as a Ruminant-Like Digestive System?



Dr. Chun-I Chiu

National Chung Hsing University

Dr. Chun-I Chiu's research focuses on the nutritional ecology, behavioral ecology and fungus-termite mutualism of fungus-growing termites. His researches proved fungus-growing termites perform eat-and-run foraging strategy and pleometrosis colony-founding, both are features rarely reported in termites. Dr. Chiu's research on fungus-termite mutualism titled "Fungal nutrition allocation enhances mutualism with fungus-growing termite", is a key breakthrough of understanding both fungi and termite ecology. He currently studies to incorporate the knowledge of fungus-termite mutualism in the baiting system.



Forum

Time: 10:40-12:00, February 13, 2020

Termite management innovation inspired from insights of termite biology

Host:



Prof. Chow-Yang Lee

Professor and Endowed Presidential Chair in Urban Entomology, University of California, Riverside

Research Areas: Urban & Industrial Entomology, Insect Toxicology

Speakers:



Dr. Charunee Vongkaluang

Director, Royal Forest Department, Thailand (retired)
Research Areas: Termite Biology and Timber Pest Management,

Timber Protection



Prof. Nan-Yao Su

Distinguished Professor, University of Florida, Entomology and Nematology Department

Research Areas: Urban Entomology



Dr. Partho Dhang

Entomologist, Consultant, Author & Editor with CABI

Research Areas: Urban Pest Management



Dr. Chun-I Chiu

National Chung Hsing University

Research Areas: Fungi and Termite Ecology

PRTRG 13 EXCURSION

Yehliu Geopark & Yangmingshan National Park

12:50-13:00 Departure from Taipei Zoo

13:00-13:50 Sightseeing through the beautiful northern coastline of Taiwan

13:50-15:30 **Yehliu Geopark**

The Yehliu Geopark (Fig. 1) is one of Taiwan's most well-known natural attractions. It consists of plenty of amazing stones formed by wind and sea erosion, such as the elegant "Queen's Head," "Fairy's Shoe," "Honeycomb Rocks," "Tofu Rocks" and ocean ditches.

In fact, the Yehliu Geopark is mainly made up by sedimentary rocks, and over years and years of weathering, the erosion by the wind and waves and the exposure to the atmosphere, the rocks in Yehliu has become the natural wonders standing at the north coast, showing the power of nature.







Fig. 1. Yehliu Geopark

Fig. 2. Flower Clock

Fig. 3. Xiaoyoukeng

16:30-17:00 Yangmingshan Flower Clock

The Flower Clock (Fig. 2) is a landmark of the Yangmingshan National Park. It is a large clock made of flowers with a diameter of 22 feet. It's the first stop that the visitors often make while exploring the Park. The chinoiserie garden, pavilions kiosks, streams, fountains and the elegant buildings bring the visitors back to 1960s, seeing the trend and fashion of the era.

17:20-18:00 Xiaoyoukeng

Xiaoyoukeng (Fig. 3) is a post-volcanic geological landscape located on Mt. Qixing's north-western corner. It is approximately 805 meters above sea level and is famed for the fumaroles, sulfur crystals, hot springs and spectacular "landslide terrain" formed by post-volcanic activity. It also offers good views of the northeast coast outside Jinshan town area, and you can overlook the volcanic cones of Mt. Zhuzi, Mt. Datun and Mt. Xiaoguanyin.

18:00-19:00 Heading to the Howard Plaza Hotel Taipei for Gala Dinner. End of the excursion.

Program at a Glance

Wednesday, February 12, 2020				
Time	Schedule			
08:00-08:50	Registration			
08:50-09:00	Welcome Speech			
09:00-09:30	Keynote Speech: Prof. Nan-Yao Su			
	Moderator: Prof. Sulaeman Yusuf			
09:30-10:00	Kunio Tsunoda Memorial Lectures: Dr. Thomas Bourguignon			
	Moderator: Dr. Hou-Feng Li			
10:00-10:30	Photography Session & Tea Break			
10:30-11:00	Kunio Tsunoda Memorial Lectures: Dr. Thomas Chouvenc			
11:00-11:30	Kunio Tsunoda Memorial Lectures: Dr. Chun-I Chiu			
11:30-12:30	Presentation Section 1: Biodiversity and systematics of termite (4 presentations)			
	Moderator: Mr. Su-Chart Leelayouthyotin			
12:30-13:30	Lunch			
13:30-15:00	Presentation Section 2: Ecology and behavior of termite (6 presentations)			
	Moderator: Dr. Kok Boon Neoh			
15:00-15:30	Tea Break & Poster			
15:30-16:30	Presentation Section 3: Termitophile (4 presentations)			
	Moderator: Dr. Foong Kuan Foo			
16:30-17:30	Presentation Section 4: Termite management I (4 presentations)			
	Moderator: Dr. Khoirul Himmi Setiawan			
17:30-18:30	Biennial General Meeting and Honorary Membership Conferring Ceremony			
18:30-20:00	Welcome Dinner @ Taipei Zoo			

Thursday, February 13, 2020			
Time	Schedule		
08:30-09:00	Registration		
09:00-10:15	Presentation Section 5: Termite management II (5 presentations)		
	Moderator: Dr. Su Yee Lim		
10:15-10:40	Tea Break & Poster		
10:40-12:00	Forum: Termite management innovation inspired from insights of termite biology		
	Host: Prof. Chow-Yang Lee		
	Speakers: Dr. Charunee Vongkaluang, Prof. Nan-Yao Su, Dr. Partho Dhang, Dr. Chun-I Chiu		
12:00-13:00	Lunch		
13:00-18:00	Excursion: Yehliu Geopark and Yangmingshan National Park		
18:00-20:30	Gala Dinner @ The Howard Plaza Hotel Taipei		

Program Agenda

08:00-08:50 Registration 08:50-09:00 Welcome Speech
08:50-09:00 Welcome Speech
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Keynote speech Moderator: Prof. Sulaeman Yus
09:00-09:30 O_01 Tunneling Behavior and Foraging Strategy of Subterranean Termites
Nan-Yao Su
Kunio Tsunoda Memorial Lectures Moderator: Dr. Hou-Feng L
09:30-10:00 O_02 Diet and Cocladogenesis Shape Termite Gut Bacteria Communities
Thomas Bourguignon
10:00-10:30 Photography Session & Tea Break
10:30-11:00 O_03 Coptotermes Hybrids as a Model to Study Core Aspects of Termite Biology
Thomas Chouvenc
11:00-11:30 O_04 Termite Fungal Cultivation as a Ruminant-Like Digestive System?
Chun-I Chiu
Presentation Section 1: Biodiversity and systematics of termites Moderator: Mr. Su-Chart Leelayouthyoti
11:30-11:45 O_05 Morphological Characters Conundrum in Termite Species Identification: The
Challenges and Effort to Address the Problems
Anugerah Fajar, S Khoirul Himmi, Astuti Latif, Mochammad Fadholi, Didi Tarmadi, Ikhsan
Guswenrivo, Sulaeman Yusuf, and Tsuyoshi Yoshimura
11:45-12:00 O_06 Supplement of some Species of <i>Glyptotermes</i> (Isoptera: Kalotermitidae)
Collected in Quang Nam Province, for the Termite Fauna of Vietnam
Nguyen Thi My, Nguyen Quoc Huy, and Nguyen Van Quang
12:00-12:15 O_07 Taxonomic Revision of Coptotermes spp. in East Malaysia Based on
Morphometric and Genetic Analyses
Norsyarizan Jamil, Wan Nurainie Wan Ismail, and G Veera Singham
12:15-12:30 O_08 Termite Taxonomy in Taiwan
Chia-Chien Wu and Hou-Feng Li
12:30-13:30
Presentation Section 2: Ecology and behavior of termites Moderator: Dr. Kok Boon Neo
13:30-13:45 O_09 Outbreak of Timber Pests in Philippines: As a Consequence of Frequent
Typhoons
Partho Dhang
13:45-14:00 O_10 The Genome of <i>Prorhinotermes flavus</i> : a Termite Species with High Salinity
Tolerance
Chung-Yen Lin, Shu-Hwa Chen, Meiyeh Jade Lu, Yi-Chen Huang, Ping-Heng Hsieh, Guan-Yu Cher
Yi-Ning Chiu, Po-Ying Fu1, I-Hsuan Lu, Yung-Hao Ching, Chun-I Chiu, Chia-Ying Liu, Hou-Feng
and Ming-Der Lin

14:00-14:15	O_11 Quantitative Analysis of "Termite Frass" and "Termite Droppings" Using					
	Decayed and Undecayed Food					
	Kumiko Kihara, Takafumi Nakagawa, Akinori Yamada, Shigeharu Moriya, Yuichi Hongoh, and					
	Tsuyoshi Yoshimura					
14:15-14:30	O_12 Distribution of Odontotermes hainanensis (Insecta: Isoptera) on Three River					
	Dike Systems in the Northern Vietnam					
	Ngo Truong Son and Nguyen Quoc Huy					
14:30-14:45	O_13 How to Confine Reproductives Using an Excluder for the Study of					
	Subterranean Termites					
	Sang-Bin Lee, Thomas Chouvenc, and Nan-Yao Su					
14:45-15:00	O_14 Behavioral Patterns and Building Material of Termite Shelter Tube					
	Construction					
	Bo-Ye Chen, Chun-I Chiu, Fang-Chi Chang, and Hou-Feng Li					
15:00-15:30	Tea Break & Poster					
Presentation Section	on 3: Termitophile Moderator: Dr. Foong Kuan Foo					
15:30-15:45	O_15 Exploring the Cryptic Life-History of the Rove Beetles Associated with					
	Formosan Subterranean Termite					
	Wei-Ren Liang and Hou-Feng Li					
15:45-16:00	O_16 Fungal Biotrophic Parasites of Taiwanese Termite: The First Record of					
	Termitaria, Mattirolella, Antennopsis, Laboulbeniopsis in Taiwan					
	Kuan-Chih Kuan, Jie-Hao Ou, Wen-Jun Lin, Chi-Yu Chen, and Hou-Feng Li					
16:00-16:15	O_17 Seasonal Feeding Ecology of an Obligate Myrmecophagous Mammal, Chinese					
	Pangolin					
	Nick Ching-Min Sun, Chun-Chieh Liang, Chung-Chi Lin, Kurtis Jai-Chyi Pei, and Hou-Feng Li					
16:15-16:30	O_18 The predator of ants and Termites: pangolins					
	Hsuan-Yi Lo, Nick Ching-Min Sun, Bo-Ye Chen, Hsuan-Ya Yu, Chun-Chieh Liang, Chung-Chi Lin,					
	Chen-Yen Lien, Hou-Feng Li, and Shih-Chien Chin					
	on 4: Termite management I Moderator: Dr. Khoirul Himmi Setiawan					
16:30-16:45	O_19 Replacing Some Myths by Truths about Termites					
4.5.4.7.4.7.00	Eric Cheng					
16:45-17:00						
	Dioxide					
17.00.17.15	C.M. Garcia and M.R. San Pablo					
17:00-17:15						
	Aboveground Protection of Malaysian Native Hardwoods Against Subterranean					
	Termite Threats in Buildings and Outdoors					
17.15 17.20	Daouia Messaoudi, Andrew H.H. Wong, Carlson A.D. Tawi, Nebil Bourguiba, and Olivier Fahy					
17:15-17:30	· · · · · · · · · · · · · · · · · · ·					
	Subterranean Termites at Different Temperature Mobil Forming Mobil Poshid and Abdul Hofiz Ab Mojid					
17.20 19.20	Mohd Fawwaz Mohd Rashid and Abdul Hafiz Ab Majid Pionnial Concret Macting and Honorary Membership Conferring Coromony					
17:30-18:30	Biennial General Meeting and Honorary Membership Conferring Ceremony Welcome Dinner @ Teinei Zoo					
18:00-20:00	Welcome Dinner @ Taipei Zoo					

Program Agenda

Thursday, February 13, 2020						
08:30-09:00	Registration					
Presentation Sec	tion 5: Termite management II	Moderator: Dr. Su Yee Lim				
09:00-09:15	0-09:15 O_23 Reliable Laboratory Trial for Testing Innovative Anti-Termite Barriers					
	Marie-France Thévenon, Luc Martin, Luc Pignolet, Kévin Candelier, Alban Guyot, Sylvain Lotte, and					
	Daouïa Messaoudi					
09:15-09:30	O_24 Household Termite Infestation Survey and S	Swarming Season Estimation with				
	Synergy of Citizen Science Project					
	Shih-Ying Huang, Wei-Jie Li, Yi-Ying Tsai, and Hou-Feng L	i				
09:30-09:45	O_25 Termite infestation survey and baiting Form	osan subterranean termite				
	(Blattodea: Rhinotermitidae) of protected old tree	s in urban areas				
	Yu-Yi Lai and Hou-Feng Li					
09:45-10:00	O_26 Smartphone Image-based Identification of I	Four Major Termite Pest Species by				
	Using Deep Learning					
	Jia-Hsin Huang, Yu-Ting Liu, Hung Chih Ni, Bo-Ye Chen, S	hih-Ying Huang, Huai-Kuang Tsai, and Hou-				
	Feng Li					
10:00-10:15	O_27 Furfurylation Treatment on Mangium and Pine Woods Extremely Reduces					
Subterranean Termite Feeding Activities						
	Yusuf Sudo Hadi, Elis Nina Herliyana, Imam Busyra Abdilla	h, Gustan Pari, and Wa Ode Muliastuty				
	Arsyad					
10:15-10:40	Tea Break & Poster					
10:40-12:00	Forum: Termite Management Innovation Inspired	l from Insights of Termite Biology				
	Host: Prof. Chow-Yang Lee					
	Speakers: Dr. Charunee Vongkaluang, Prof. Nan-Yao Su, Dr.	Partho Dhang, and Dr. Chun-I Chiu				
12:00-13:00	Lunch					
13:00-18:00	Excursion: Yehliu Geopark and Yangmingshan Nationa	l Park				
18:00-20:30	Gala Dinner @ The Howard Plaza Hotel Taipei					

List of Poster Presentations

15:00-15:30, Wednesday, February 12, 2020 10:15-10:40, Thursday, February 13, 2020

P_01	Evolution of Termite Symbiosis Informed by Transcriptome-Based Phylogenies Aleš Buček, Jan Šobotník, Shulin He, Mang Shi, Dino P. McMahon, Edward C. Holmes, Yves Roisin, Nathan Lo, and Thomas Bourguignon
P_02	Characterizing the feeding behaviour of a generalist inquiline termite Simon Hellemans, Martyna Marynowska, Thomas Drouet, Gilles Lepoint, Denis Fournier, Magdalena Calusinska, and Yves Roisin
P_03	Intestinal Protists of the Enigmatic Termite Family Stylotermitidae (Insecta: Blattodea) and its Phylogenetic Position Kai-Yuan Liu, Cheng-Lung Tsai, Li-Wei Wu, and Hou-Feng Li
P_04	The Assembly of Fungal Communities in Termite Guts is Affected by Diet and Taxonomy Lucia Zifcakova, Crystal Clitheroe, Yukihiro Kinjo, and Thomas Bourguignon
P_05	Genus Coptotermes (Rhinotermitidae) in Sumatra and West Java: Morphological and Phylogenetic Studies Bramantyo Wikantyoso, Shu-Ping Tseng, S. Khoirul Himmi, Sulaeman Yusuf, and Tsuyoshi Yoshimura
P_06	Termites Investigation on Forest Plantation Area in Riau, Sumatera Indonesia Fadjar Sagitarianto, Saripah Ulpah, Wagner Morais, and Budi Tjahjono
P_07	De novo Development and Polymorphism Analysis of Microsatellite Markers in Prorhinotermes flavus (Blattodea: Rhinotermitidae) Yung-Hao Ching, Vani Vennela, Yi-Ning Chiu, Ming-Der Lin, and Hou-Feng Li
P_08	Worldwide Historical Biogeography of Termites (Blattodea: Isoptera) Menglin Wang and Thomas Bourguignon
P_09	Plasticity of Reproductive Caste Development of <i>Prorhinotermes flavus</i> (Blattodea: Rhinotermitidea) Yi-Ning Chiu, Chun-I Chiu, Chia-Ying Liu, Ming-Der Lin, and Hou-Feng Li
P_10	Does Body Size Variation of Formosan Fungus-Growing Termite Fit Bergmann's Rule? Wen-Jun Lin and Hou-Feng Li
P_11	Transport of Microplastics from Surface into Soil by Termites Hiroki Yabumoto, S. Khoirul Himmi, and Tsuyoshi Yoshimura
P_12	Monitoring the Hybridization of Two Notorious Termite Pests in Taiwan Guan-Yu Chen, Shih-Ying Huang, Yung-Hao Ching, Ming-Der Lin, and Hou-Feng Li
P_13	Identification of Termite – Ectoparasitic fungi Relationship Ikhsan Guswenrivo, Tomoya Imai, Deni Zulfiana, Titik Kartika, Sulaeman Yusuf, and Tsuyoshi Yoshimura

P_14	A Nematode Species Associated with Termitophilic beetle, Ziaelas formosanus – A Study of its Highly Derived Morphological/Structural Characters Natsumi Kanzaki, Wei-Ren Liang, Chun-I Chiu, and Hou-Feng Li
P_15	Detection of Potential Compound from Wood-Decay Fungi for Bio-attractant of Termite Deni Zulfiana, Titik Kartika, Anis Sri Lestari, Ni Putu Ratna Ayu Krishanti, Apriwi Zulfitri, Anugerah Fajar, Sulaeman Yusuf, Nobuhiro Shimizu, Aya Yanagawa, and Tsuyoshi Yoshimura
P_16	Patented Biobased Microemulsion-Based Pyrethroid Solutions provide Permanent Wood Protection against <i>Coptotermes</i> termites Daouia Messaoudi, Andrew H.H. Wong, Carlson A.D. Tawi, Nebil Bourguiba, and Olivier Fahy
P_17	Monitoring Uptake and Penetration of Pesticide during Impregnation of an easily Impregnable (<i>Pinus sylvestris</i>) and a Refractory (<i>Picea abies</i>) Wood Species with Bio-Based Emulsion Gel Industrial Formulations Daouïa Messaoudi, Katia Ruel, and Jean-Paul Joseleau
P_18	Developing Evaluation Methods for Termiticide used in Taiwan Guan-Hon Chen, Shang-Ho Yang, and Hou-Feng Li
P_19	Termite Infestations in Important Wooden Cultural Properties of Korea Hyean-Cheal Park and Sang-Bin Lee
P_20	Features of Semiautomatic and Fully Automatic Systems for Monitoring Termite Activity using Electromagnetic Induction with Non-Looping Method Junfeng Shen, Jing Yao, Mei Zhang, and Dayu Zhang
P_21	Phylogeny of the Subfamily Rhinotermitinae (Blattodea: Rhinotermitidae) Based on Mitochondrial COII and 16SrRNA Sequences Yunling Ke, Fusheng Huang, Wenjing Wu, and Zhiqiang Li
P_22	Molecular Mechanism of Soldier Differentiation in the Formosan Subterranean Termite He Du, Xueyi Huang, Bingrong Liu, Runmei Huang, and Zhiqiang Li
P_23	Hybridization Between the Two Sympatric Termite Reticulitermes Species Under Laboratory Condition Jia Wu and Qiuying Huang
P_24	Proteomic Quantification of Lysine Acetylation in the Termite <i>Reticulitermes chinensis</i> Yutong Liu and Qiuying Huang
P_25	GC-MS Analysis of Metabolites Produced by Coptotermes formosanus Bred on Bagasse Wenjing Wu, Zhiqiang Li, and Shijun Zhang
P_26	Differential Response of Cellulose and Hemicellulose Hydrolysis System to Laccase Inhibition of Coptotermes formosanus Wenhui Zeng, Bingrong Liu, and Zhiqiang Li



ABSTRACT BOOK





Oral Presentations



 O_01

Tunneling Behavior and Foraging Strategy of Subterranean Termites

by

Nan-Yao Su

Entomology and Nematology Department
Ft. Lauderdale Research and Education Center
University of Florida

Abstract

Studies of the geometric pattern of subterranean termite tunnels revealed rules dictating their tunneling behavior which can be described by an algorithm and duplicated by simulations. Laboratory studies with 2-dimensinal arenas showed that subterranean termites construct a predictable tunnel geometry pattern by using simple local rules such as dead reckoning and Global Away Vector (GAV), and their tunnels are shaped by traffic congestion due to individuals in queue immediately behind the excavator at the tunnel tip. Computer simulation studies demonstrated that termite tunnel geometry is optimized to encounter food in clumped distribution, and their primary/branch tunnel length ratio of 0.1 is optimized to intercept more food particles. Moreover, the exponent of branch length distribution (α =0.15) is optimized for foraging efficiency, i.e. encounter more foods at the shortest distance to transport them back to the nest.

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Diet and Cocladogenesis Shape Termite Gut Bacteria Communities

by

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Abstract

Termites are one of the few insect groups feeding on wood and soil, which they digest in association with the microorganisms in their gut. Termites are believed to acquire some of their gut microbes from their food, and inherit others from their parents and nestmates through proctodeal trophalaxy, or coprophagy. However, the influence of food and vertical inheritance on termite whole gut bacterial communities has never been properly tested. In this paper, we used 16S rRNA metagenomic data of 94 termite species to test the influence of diet and vertical inheritance in a statistical framework. Using phylogenetic ANOVA, we found a significant effect of diet on gut bacterial community composition and species richness. The gut of soil-feeders hosted more bacterial species than that of wood-feeders, possibly because soil hosts more microbes than wood. Using host-parasite cospeciation tests, we found a cocladogenesis signal for 93 bacterial lineages, out of the 272 gut bacterial lineages analyzed, which make up 48.13% of the bacterial sequences analyzed. We also analyzed wood- and soil-feeding Termitidae separately and found a cophylogenetic signal for 59.18% and 21.52% of the bacterial sequences, respectively. Our analyses show that a large proportion of the gut bacteria are vertically inherited and that this is more so for wood-feeding termites than for soil-feeding termites. Our study identified the lineages which, at least partly, coevolve with termites, and are therefore good target for future studies aiming to determine the function of symbiotic termite gut bacteria.

Keywords: endosymbionts, Isoptera

Introduction

Some 170 Million years ago, termites and their related *Cryptocercus* roaches evolved the capacity to feed on lignocellulose (Bourguignon et al. 2015, Bucek et al. 2019), one of the most abundant biomolecule on Earth (Breznac and Brune 1994). Although termites secrete their own cellulases (Watanabe and Tokuda 2010), they also associate with the microorganisms in their gut to degrade lignocellulose. The most ancient of these associations has been with cellulolytic flagellates, which largely assist lower termites, and their related *Cryptocercus* roaches, in lignocellulose digestion (Brune and Ohkuma 2011). These eukaryotic protists are also the hosts of many symbiotic bacteria, which, for some, help digesting lignocellulose and recycling nitrogen (Hongoh et al. 2008a,b). Despite their importance in lower termites, flagellates have been lost and replaced by bacteria or fungi in Termitidae about 50 Million years ago (Bourguignon et al. 2015, Bucek et al. 2019), triggering the dietary diversification of the Termitidae along the wood-soil humification gradient (Bourguignon et al. 2011). Among the Termitidae, the basalmost Macrotermitinae domesticated the cellulolytic fungi *Termitomyces*, while other Termitidae are dependent on prokaryotes only for thriving on their food (Brune 2014, Brune and Dietrich 2015).

Some of the bacteria associated with termites lost the ability to live outside their host. This is well illustrated by the reduced genomes of some endosymbionts of termite flagellates, which lack key biosynthetic genes present in the genome of their free-living relatives (Hongoh et al. 2008a, b). These endosymbionts are transferred with flagellates among nestmates and across generations by proctodeal trophallaxis, during which termite individuals swallow faecal fluid provided by their nestmates, and the microorganisms they contain (Nalepa 2015). Consequently, termites, their gut protists, and the ecto- and endosymbionts of termite gut protists, all cospeciate and their phylogenies largely concur (Noda et al. 2007, Ikeda-Ohtsubo and Brune 2009, Desai et al. 2010). However, beside few case

studies, the extent to which the bacterial communities are vertically transmitted by proctodeal trophallaxis remains uncertain, and no studies have investigated cocladogenesis for termites and their free-living gut bacteria, thence for bacteria of Termitidae.

Another factor structuring gut bacterial communities is the diet of the termite host. Recently, studies analysing whole bacterial communities using 16S rRNA metagenomic have flourished, and some supported the hypothesis that diet of the termite host is the preponderant factor structuring the bacterial communities of termite gut (Dietrich et al. 2014, Mikaelyan et al. 2015), albeit with modest sampling of the termite diet diversity. However, analyses of 16S rRNA metagenomic dataset also suggested that vertical inheritance is a prime factor shaping gut bacterial communities (Otani et al. 2014, Abdul Rahman et al. 2015, Tai et al. 2015), although these inferences were based on whole community analyses and did not test for cocladogenesis with the termite host (Brune and Dietrich 2015).

Distinguishing between the effect of vertical inheritance and host diet is complex for diet present phylogenetic autocorrelation, so that related termite species often share diet (Bourguignon et al. 2011). Here, we present the first attempt to disentangle the effect of diet and phylogeny on termite gut bacterial communities, taking into account phylogenetic autocorrelation. We used a cultivation-independent approach and sequenced a 16S rRNA gene fragment with Illumina MiSeq to characterize the bacterial communities of 94 termite species, representative of the termite diet and phylogenetic diversity. We tested whether bacteria are vertically inherited using a cocladogenesis approach, comparing phylogenies of related bacterial species inferred from 16S rRNA to the termite phylogeny based on full mitochondrial genomes. We also tested for the influence of diet as a factor shaping bacterial communities, taking into account the phylogenetic autocorrelation of diet.

Materials and methods

Samples

We used 95 samples representing 94 species of termites distributed across the termite phylogenetic tree (see Bourguignon et al. 2018 for a complete list). All samples were collected in the field, preserved in RNA-later® and stored at -80°C until DNA extraction.

Microbial 16S rRNA sequencing

Whole genomic DNA was extracted from dissected digestive tracts of five to ten workers using the NucleoSpin® Soil kit of Macherey-Nagel according to manufacturer protocol. We used the primers 343Fmod (TACGGGWGGCWGCA) and 784Rmod (GGGTMTCTAATCCBKTT) for PCR amplifications (Köhler et al. 2012). We conducted PCR amplifications using GoTaq® with the same condition as described in Köhler et al. (2012), that is initial denaturation (3 min at 95°C), 26 cycles of amplification (20 s at 95°C, 20 s at 48°C, and 30 s at 72°C), and a terminal extension (3 min at 72°C). Multiplexing and subsequent paired-end sequencing with Illumina MiSeq were carried out through commercial service (BGI Tech. Solutions Co., China).

Termite mitochondrial genome sequencing

Whole genomic DNA of termites was extracted using the phenol-chloroform extraction procedure. The complete mitochondrial genomes were amplified with TaKaRa LA Taq in two long PCR amplicons using the primers designed by Bourguignon et al. (2016). Both fragments were then mixed in equimolar concentration. Multiplexing and subsequent paired-end sequencing with Illumina HiSeq2000 was done through commercial service (BGI Tech. Solutions Co., China).

Termite phylogenetic analyses

We assembled separately 88 base pairs paired-end reads using the CLC suite of programs, as described in Bourguignon et al. (2015). In all polymorphic base cases, we selected the base with the highest representation. We omitted control regions of the mitochondrial genomes from the final matrix, as they are generally poorly assembled with short reads. We annotated the 22 tRNAs, the 13 protein-coding genes and the two ribosomal RNAs by eye, aided by previously published sequences that we

aligned on each mitochondrial genome using Muscle algorithm implemented in MEGA 5.2.1 (Tamura et al. 2011). Protein-coding genes were aligned as codon and tRNA and ribosomal RNA genes were aligned as DNA. Resulting alignments were concatenated with SequenceMatrix (Vaidya et al 2011).

We determined the partitioning scheme with PartitionFinder (Lanfear et al. 2012). We used a GTR model with gamma-distributed rate variation across sites for all partitions. Phylogenetic analyses were carried out without the third codon position. The partitioning scheme comprised 12 partitions.

We analysed the concatenated DNA sequence alignment with a relaxed molecular-clock model using BEAST 1.8.0 (Drummond and Rambaut 2007). Rate variation was modelled among branches using uncorrelated lognormal relaxed clocks (Drummond and Rambaut 2007), with a single model for all genes, that allows for a different relative rate for each partition. A Yule speciation process was used for the tree prior (Gernhard 2008) and posterior distributions of parameters, including the tree, were estimated using MCMC sampling. We performed two replicate MCMC runs, with the tree and parameter values sampled every 10,000 steps over a total of 100 million generations. A maximum clade credibility tree was obtained using Tree Annotator within the BEAST software package with a burn-in of the first 10 million generations as determined with Tracer 1.5 (Rambaut and Drummond 2007). Acceptable sample sizes and convergence to the stationary distribution was also checked using Tracer 1.5. The molecular clock was calibrated using 12 minimum age constraints. Minimum age constrains were determined based on the fossil record, and we systematically selected the youngest possible age for each fossil as mentioned on the Paleobiology Database (PaleoBioDB, www.paleobiodb.org last accessed on July 15, 2015).

Testing for diet influence of whole gut bacteria communities

We selected reads longer than 350 base pairs and dismissed others from subsequent analyses. We identified chimera using UCHIME (Edgar et al. 2011) against the DictDb bacterial reference database (Mikaelyan et al. 2015), using a score threshold of 0.5 and a minimum divergence of 1.5. We sorted reads into operational taxonomic units (OTUs) (3% sequence dissimilarity) using UPARSE (Edgar 2013). We excluded OTUs represented by singletons from subsequent analyses. For each OTU, the most abundantly represented sequence was selected as reference. We used the naïve Bayesian classifier from MOTHUR (Schloss et al. 2009, Wang et al. 2007) implemented in QIIME (Caporasa et al. 2010a) for taxonomic assignment using DictDb as a reference database (Mikaelyan et al. 2015).

All samples were subsampled at 3000 sequences, which was the size of the smallest library. Using the QIIME toolkit (Caporaso et al. 2010a), we aligned OTU reference sequences with PyNAST (Caporaso et al. 2010b), reconstructed a phylogenetic tree with FastTree (Price et al. 2009), and calculated the weighted and unweighted Unifrac metrics (Lozupone and Knight 2005), which we used as measures of beta diversity to compute Principal Coordinate Analysis (PCoA) and investigate differences in termite gut bacterial communities. Eight diets were recognized: FGI, –Wood-feeding– lower termites; FGII, Wood-feeding Termitidae; FGIIe, Epiphyte-feeders; FGIIf, Fungus-growers; FGIIg, Grass-feeders, FGIIlit, Litter-feeders; FGIII, Wood-soil interface-feeders; FGIV, Soil-feeders (Donovan et al. 2001). We tested the influence of termite diet on bacterial communities using scores along the two main principal components. To take into consideration phylogenetic autocorrelation, we carried out phylogenetic ANOVA using the function phylANOVA from the R package phytools (Revell 2012), using 10,000 simulations. Using phylogenetic ANOVA, we also tested the influence of diet on bacterial species diversity using the abundance-based richness estimators ACE, Chao1, phylogenetic distance (whole tree), and the diversity indices of Shannon and Simpson, all implemented in QIIME.

Testing for cophylogeny and diet influence of bacterial groups

Clean reads longer than 350 base pairs were clustered in 3% OTUs for each species separately using UPARSE (Edgar 2013). We dismissed all OTUs that were represented by less than 5 reads in order to reduce the analyses to putatively significant bacterial species, and to eliminate any possible foreign contamination. We also dismissed the chimera, which we identified with UCHIME (Edgar et al. 2011), using a score threshold of 0.5 and a minimum divergence of 1.5. The most abundantly represented sequence was selected as a reference for each 3% OTUs. Each reference sequence of 3% OTUs of

each termite species were then pulled together and clustered in groups of 12% similarity with uclust (Edgar 2010), implemented in QIIME (Caporasa et al. 2010a). We assign taxonomy to each group using MOTHUR (Schloss et al. 2009, Wang et al. 2007) implemented in QIIME (Caporasa et al. 2010) and DictDb (Mikaelyan et al. 2015), and randomly selected one sequence as representative of each group of 12% similarity. We then mapped all reference sequences of 3% OTUs to the reference sequences of each group of 12% similarity using the function map read to reference from the QIIME package, with the method bwa-sw (Li and Durbin 2010) and minimum identity of 12%. We used the observation map generated to extract the reference sequences of 3% OTUs from each group of 12% similarity. All groups including less than ten reference sequences of 3% OTUs were dismissed from further steps. We aligned the reference sequences of 3% OTUs from each group with Muscle (Edgar 2004) and reconstructed a phylogenetic tree with FastTree (Price et al. 2009). We then tested for cophylogeny using the phylogenetic trees of each bacterial group and the phylogenetic tree of their termite hosts, using the permutation test for host-parasite cospeciation of Hommola et al. (2009) implemented in R with 10,000 permutations. We carried out these analyses for all species of termites, for soil-feeding and wood-feeding termites separately, and for lowe termites, Termitidae and woodfeeding Termitidae separately.

Results

Testing for diet influence of whole gut bacteria communities

We obtained an average of 12,115 high-quality sequences from the V3-V4 region of the bacterial 16S rRNA. This dataset was previously used by Bourguignon et al. (2018). We clustered the combined dataset into operational taxonomic units (OTUs) with a distance below 3%, from which we removed singletons. The smallest library contained 3,269 sequences, so we subsampled 3000 sequences from each library. We carried out a Principal Coordinate Analysis (PCoA) using the unweight Unifrac metric (Lozupone and Knight 2005), in order to compare the influence of diet and taxonomy. Eight diets were recognized: FGI, -Wood-feeding-lower termites; FGII, Wood-feeding Termitidae; FGIIe, Epiphyte-feeders; FGIIf, Fungus-growers; FGIIg, Grass-feeders, FGIIlit, Litter-feeders; FGIII, Woodsoil interface-feeders; FGIV, Soil-feeders (Donovan et al. 2001). PCoA showed clustering according to diet and taxonomic position (Fig. 1). We carried out phylogenetic ANOVA to test whether termite diet influenced gut bacteria communities and found significant differences among diets along the first (F = 40.95; p < 0.001) and second axes (F = 24.74; p < 0.001). Post hoc comparisons showed that most of the feeding groups significantly differ along at least one of the two main axes (Table 1). We carried out the same analyses with the weighted Unifrac metric and found no significant differences along the first axis (F = 6.22; p = 0.152), while the second axis revealed significant differences (F = 11.41; p = 0.035), FGII differing from FGIII and FGIV (Table 1).

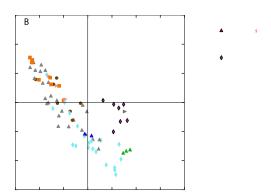


Fig. 1. Community structure of the gut microbiota of different termite species. Community similarities were calculated on the distribution of OTUs 3%, using Unweighted Unifrac, and visualized with Principal Correspondence Analyses. A. Scatter plot showing the diet of termite species: FGI, —Woodfeeding—lower termites; FGII, Wood-feeding Termitidae; FGIIe, Epiphyte-feeders; FGIIf, Fungus-

growers; FGIIg, Grass-feeders, FGIIlit, Litter-feeders; FGIII, Wood-soil interface-feeders; FGIV, Soil-feeders. B. Scatter plot showing the taxonomic positions of each termite sample. Phylogenetic trees show the position of each category.

We also tested the influence of diet on bacterial species diversity using the abundance-based richness estimators ACE, Chao1, phylogenetic distance (whole tree), and the diversity indices of Shannon and Simpson, all implemented in QIIME. Phylogenetic ANOVA showed that, globally, termite species feeding on soil possessed more bacterial species in their gut than species feeding on wood, with significant differences between feeding groups for ACE (F = 20.37; p = 0.004; FGIII \neq FGII = FGIIe = FGIIg; FGIV \neq FGII = FGIIe = FGIIg = FGIIlit)), Chao1 (F = 21.34; p = 0.003; FGIII \neq FGII = FGIIe = FGIIg = FGIIg), phylogenetic distance (whole tree) (F = 17.98; p = 0.007; FGIII = FGIV \neq FGII = FGIIg), but not for Shannon (F = 9.23; p = 0.061) and Simpson indices (F = 1.85; p = 0.653).

	FGI	FGII	FGIIe	FGIIf	FGIIg	FGIII	FGIIlit	FGIV
FGI	-	**	*	NS	**	**	NS	**
FGII	NS	-	NS	NS	NS	**	NS	**
FGIIe	NS	NS	-	NS	NS	*	NS	**
FGIIf	NS	NS	NS	-	NS	**	NS	**
FGIIg	NS	NS	NS	NS	-	**	NS	**
FGIII	NS	**	NS	NS	NS	-	*	NS
FGIIlit	NS	NS	NS	NS	NS	NS	-	*
FGIV	NS	**	NS	NS	NS	NS	NS	-

Table 1. Results of the phylogenetic ANOVAs comparing the influence of termite diet on bacterial communities. Scores of the Principal Coordinate Analyses along the first two components were used as values. Upper right values are the results with unweighted Unifrac, and lower left values are the results with weighted Unifrac. Groups are considered to differ when significant differences were found along one of two main components. NS: non-significant; *p < 0.05; **p < 0.01. FGI, —Wood-feeding—lower termites; FGII, Wood-feeding Termitidae; FGIIe, Epiphyte-feeders; FGIIf, Fungus-growers; FGIIg, Grass-feeders, FGIIlit, Litter-feeders; FGIII, Wood-soil interface-feeders; FGIV, Soil-feeders.

Testing for cophylogeny between termites and their gut bacteria

We clustered the sequences from each of the 94 termite species separately into 3% OTUs, determined one reference sequence for each 3% OTU, and pulled them into a single dataset from which we clustered 3% OTUs into groups of 12% sequence similarity. We obtained 622 groups with 12% sequence similarity, among which 279 groups were represented by more than 10 OTUs and made up 89.4% of the reads. We computed the phylogenetic tree of these 279 groups and tested for their congruence with the phylogenetic tree of termites, which we inferred from 88 full mitochondrial genomes, using the permutation test of host-parasite cospeciation of Hommola et al. (2009). Out of the 279 groups, we found that the phylogenetic trees of 93 bacterial groups were significantly congruent with that of termites (43 groups with p <0.001, 65 groups with p <0.01, and 93 groups with p <0.05). 37.72%, 42.48% and 48.13% of the bacterial sequences analyzed were found to coevolve with termites at p < 0.001, p < 0.01 and p < 0.05, respectively (Fig. 2, Table S1).

Although these results support cophylogeny between many bacterial lineages and termites, the success of gut microbes horizontally acquired depends on selective conditions, such as host diet, which are themselves strongly conserved across the host phylogeny, so that phylogenetic correlation can be observed even in the absence of vertical transmission (Sanders et al. 2014). To confirm that the significant cocladogenesis observed earlier is the result of vertical transmission of bacterial symbionts and not of phylogenetic autocorrelation of diet, we carried out the Hommola et al.'s cospeciation test on wood-feeding and soil-feeding termites separately. We analyzed 116 and 222 groups of bacteria

with 12% sequence similarity for wood-feeding (FGI + FGII) and soil-feeding (FGIII + FGIV) termites, respectively. Other groups were not analyzed because they contained less than 10 OTUs 3%. We found that the phylogenetic trees of 49 bacterial groups were significantly congruent with that of wood-feeding termites (17 groups with p <0.001, 28 groups with p <0.01, and 49 groups with p <0.05, making up 30.43%, 33.78% and 43.10% of the analyzed bacterial sequences, respectively); while 32 bacterial groups show significant cophylogeny with soil-feeding termites (8 groups with p <0.001, 10 groups with p <0.01, and 32 groups with p <0.05, making up 12.00%, 12.09% and 21.52% of the analyzed bacterial sequences, respectively) (Fig. 2).

Although gut protists and their endosymbionts were shown to be vertically transmitted in lower termites (Noda et al. 2007, Ikeda-Ohtsubo and Brune 2009, Desai et al. 2010), our results suggest that the wood-feeding Termitidae coevolved with a higher proportion of their gut bacteria than the lower termites (Fig. 2). To confirm these results, we tested for cospeciation of gut bacteria with lower termites, Termitidae and wood-feeding Termitidae, that we analyzed separately with Hommola et al.'s cospeciation test. We analyzed 85, 266 and 108 groups of bacteria with 12% sequence similarity for all lower termites, Termitidae and wood-feeding Termitidae, respectively. We found that the phylogenetic trees of 17 bacterial groups were significantly congruent with that of lower termites (1 group with p <0.001, 5 groups with p <0.01, and 17 groups with p <0.05, making up 6.39%, 9.44% and 17.60% of the analyzed bacterial sequences, respectively); while 63 bacterial groups show significant cophylogeny with Termitidae (20 groups with p <0.001, 34 groups with p <0.01, and 63 groups with p <0.05, making up 21.44%, 27.68% and 41.72% of the analyzed bacterial sequences, respectively), and 26 bacterial groups show significant cophylogeny with wood-feeding Termitidae (10 groups with p <0.001, 15 groups with p <0.01, and 26 groups with p <0.05, making up 43.59%, 48.14% and 59.18% of the analyzed bacterial sequences, respectively).

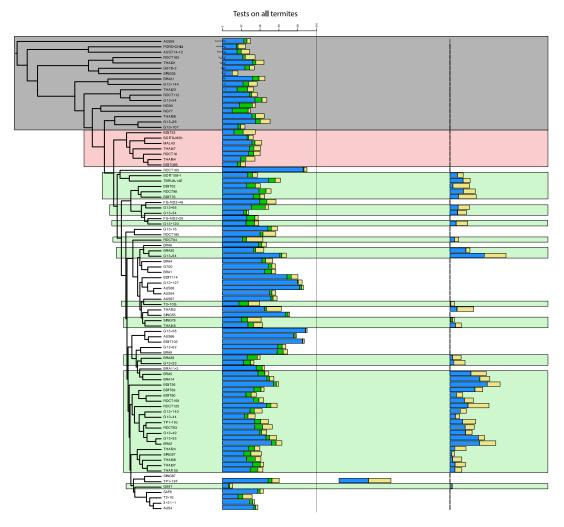


Fig. 2. Proportion of bacteria coevolving with termites. Grey background shows lower termites, red background shows fungus-growing termites, green background shows soil-feeding termites.

We carried out a phylogenetic ANOVA to test whether the proportion of bacteria coevolving with termites varies between feeding-groups, as suggested by Figure 2. We found that a smaller proportion of bacteria coevolve with lower termites (FGI) and soil-feeding termites (FGIII + FGIV) compared to wood-feeding Termitidae (FGII) (F = 50.27; p = 0.006; FGI vs FGII: p = 0.005; FGII vs FGIII + FGIV: p < 0.001).

Discussion

In this study, we confirmed that the gut bacterial communities of termites are both structured by the diet and by the taxonomy of their termite hosts (Fig. 3) (Dietrich et al. 2014, Otani et al. 2014, Mikaelyan et al. 2015, Abdul Rahman et al. 2015, Tai et al. 2015). Accounting for phylogenetic autocorrelation, we found that termite diet influence bacterial community composition and species richness. Termites feeding on wood had significantly less species of bacteria in their gut than termite species feeding on soil. We also found significant cocladogenesis for 48.13% of the bacterial sequences analyzed, without distinction between diets and presence of gut protists (lower or higher termites). Analyses restricted on species having the same diet, or sharing the presence/absence of gut protists, show that both diet and gut protists influence the proportion of coevolving bacteria.

Significant cocladogenesis between termites and their gut bacteria reached 17.60% in –wood-feeding–lower termites, 59.18% in wood-feeding Termitidae, and 21.52% in soil-feeders.

Many insects lack dependable transmission mechanisms for their gut bacteria, which prevents the evolution of intimate associations, and favor widely distributed bacteria, acquired from the environment (Engel and Moran 2013). Termites, as other social insects, can transmit their gut bacteria to nestmates during social interactions, which are for termites through coprophagy or proctodeal trophallaxis (Brune 2014, Brune and Dietrich 2015, Nalepa et al. 2015). Previous studies showed that proctodeal trophallaxis is responsible of cocladogenesis between termites, their gut protists, and the endosymbionts of their gut protists (Noda et al. 2007, Ikeda-Ohtsubo and Brune 2009, Desai et al. 2010). Our results confirm this scenario, and show that not only endosymbionts of gut protists are largely vertically inherited, but that ectosymbionts, freely living in the gut, such as those of Termitidae, are also vertically transmitted. However, horizontal transfers are also likely to occur in bacterial lineages showing cocladogenesis with termite hosts, the prevalence of which should be investigated in future studies. In any case, our results confirm that termite sociality, and proctodeal trophalaxis, is an efficient mechanism of bacterial heritability, paralleling that of symbionts associated to specialized host cells, called bacteriocytes, and maternally transmitted through oocytes (Moran et al. 2008).

Our analyses show that a smaller proportion of the gut bacteria coevolved with lower termites compared with wood-feeding Termitidae. Both groups feed on wood, but lower termites differ by the presence of symbiotic protists which participate to wood digestion (Brune and Ohkuma 2011). Although the presence of cellulolytic protists could leave fewer functions available for bacteria of lower termites, reducing opportunities for coevolution, these results might also be caused by an incomplete sampling of lower termites. We used lower termite species from distinct genera, all distant by 20 to 150 Mya, while the higher termite species we used were distant by 5 to 50 Mya. This possibly explains why we failed to detect any significant coevolution with lower termites for *Endomicrobia* and *Candidatus Armantidilum*, which are known to coevolve with congeneric termite species (Noda et al. 2007, Ikeda-Ohtsubo and Brune 2009). Future studies, with a more comprehensive sampling of lower termites are required to validate our results. Similar studies focusing on Macrotermitinae are also welcome, as the proportion of bacteria found coevolving with them was equally small, perhaps because Macrotermitinae, assisted by the fungus *Termitomyces* to degrade wood, require no association with bacteria for wood digestion (Poulsen et al. 2014).

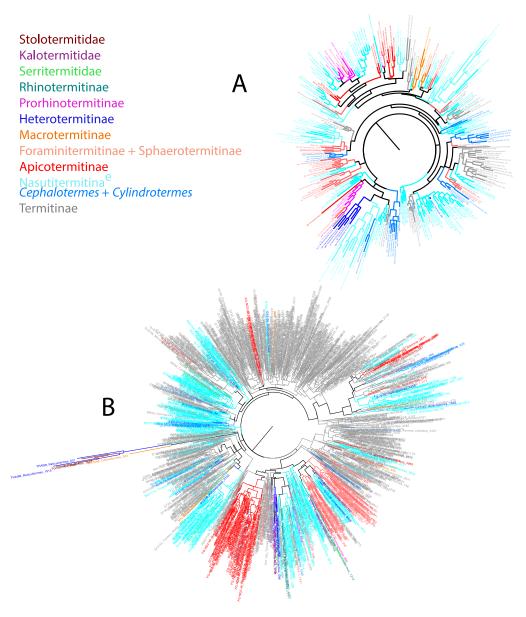


Fig. 3. Phylogenetic tree of two bacterial clusters found to significantly coevolve with termites (p < 0.001). A. *Treponema* Subcluster f; B. *Treponema* Subcluster a. Colors indicate the termite taxonomic group in which they were found.

Our results show that the proportion of bacteria coevolving with wood-feeding Termitidae was significantly higher in comparison to that coevolving with soil-feeders (All Termitidae) (Fig. 2). Bacteria associated with wood-feeders play two important roles: they contribute to lignocellulose digestion and they provide their host with nitrogenous nutrients, scarce in wood (Breznac and Brune 1994, Brune and Ohkuma 2011, Engel and Moran 2013, Brune 2014, Brune and Dietrich 2015). In contrast, soil-feeders require no association with bacteria for their nitrogen metabolism, as they mobilize and digest the peptidic component of soil organic matter (Ji et al. 2000, Ji and Brune 2001), but could be associated with bacteria for the hydrolysis of peptides and possibly other humus compounds (Brune and Ohkuma 2011). Nevertheless, soil-feeding termites probably do not require association with bacteria in the same extent as wood-feeding termites, and, as they evolved soil feeding, vertical inheritance of gut bacteria decreased and horizontal inheritance increased.

Our results confirm that diet significantly influences the composition of gut bacterial communities, supporting previous studies (Dietrich et al. 2014, Mikaelyan et al. 2015). Diet was also found to significantly influence the diversity of bacterial communities, which were richer in soil-feeders compare to wood-feeders. One possible explanation is that soil-feeders pick up more bacteria from their environment. Our results also confirm that the bacterial community of fungus-growers, which participate to the final digestion of lignocellulose (Poulsen et al. 2014), is markedly modified.

Previous studies have investigated relationships between bacterial communities and their termite hosts using a 16S rRNA metagenomic approach (Dietrich et al. 2014, Otani et al. 2014, Mikaelyan et al. 2015, Abdul Rahman et al. 2015, Tai et al. 2015), but none of them have tested for cocladogenesis. In consequence, they did not prove vertical inheritance per se and, because they analyzed whole bacterial communities, they failed to determine the bacterial lineages coevolving with termites, so the lineages having intimate associations and providing important services to termites. Our approach unambiguously identifies the bacterial lineages coevolving with their host using 16S rRNA metogenomic data, providing clear targets for future bacterial genome sequencing. Our approach could be applied to other 16S rRNA metagenomic dataset, which are now available for a variety of organisms, and similarly identify the microbes coevolving with their hosts, and thence likely contributing to their host fitness.

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Coptotermes Hybrids as a Model to Study Core Aspects of Termite Biology

by

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Abstract

The emergence of hybrid termites between *Coptotermes formosanus* and *Coptotermes gestroi*, provides new avenues for termite research and for the understanding unique evolutionary processes. Aspects such as hybrid introgression, hybrid vigor, transmission mechanisms of protozoans in incipient colonies, role of the host and recombined protozoa lineages in colony vigor, and chemical communication across species, are all research subjects currently being investigated. This presentation will provide a summary of these aspects already known about hybrid termites, and will also provide a general overview of the current research using hybrids as a model for unique evolutionary questions.

Introduction

Coptotermes is one of the most important termite pest genera globally, as they are responsible for potentially more than \$32 billion annual damage to structures (Rust and Su 2012). In addition, Coptotermes is known to impact live trees and alter tree canopy especially in area where they can be invasive and in forestry crops (Chouvenc and Foley 2018, Evans et al. 2019). Among the suspected 21 Coptotermes species (Chouvenc et al. 2016a), two species have strong invasive ability: Coptotermes formosanus Shiraki and Coptotermes gestroi (Wasmann). Coptotermes formosanus, a subtropical species, is native to mainland China and Taiwan and has spread to Japan, Hawaii, and most of the southern United States. Coptotermes gestroi, a tropical species, is native from Southeast Asian, and has spread to most of the tropical areas around the world. The two species have similar soldier morphology, but they can be identified by the number of pair of setae on both sides of the fontanelle (Figure 1, Chouvenc et al. 2014).

The two species evolved in allopatry for ~17Ma (Bourguignon et al. 2016), however, owing to an increasing global human activity, both species were able to establish in many non-native areas. Such species establishment has resulted in cases of new sympatry in very few limited geographic areas: Taiwan, Hainan, Southeast Florida, and Hawaii (Figure 2, Chouvenc et al. 2015).

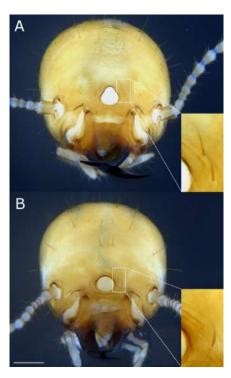


Figure 1: Soldier morphology of A: *Coptotermes formosanus*, B: *Coptotermes gestroi* (From Chouvenc et al. 2014)

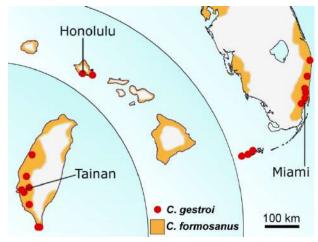


Figure 2: Overlapping distributions of *Coptotermes gestroi* and *Coptotermes formosanus* (from Chouvenc et al. 2015).

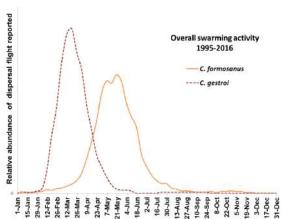


Figure 3: Dispersal flight season overlap between *Coptotermes gestroi* and *Coptotermes formosanus* in Southeast Florida (from Chouvenc et al. 2017).

The two invasive termite species can be in direct competition for resources (Li et al. 2010) and can both impact structures and trees. The two species also historically had different dispersal flight seasons (Scheffrahn and Su 2005), however, since 2013, the two species populations established in Southeastern Florida displayed overlapping seasonal dispersal flights (Figure 3, Chouvenc et al. 2017).

During simultaneous flights of the two *Coptotermes* species in South Florida, interspecific mating behaviors were observed in the field between male *C. gestroi* and female *C. formosanus*. When brought back into the laboratory, the paring were able to established hybrid colonies and some levels of hybrid vigor were observed within the first year of growth (Figure 4, Chouvenc et al. 2015). Since 2013, hybrid colonies have been established in the laboratory every year and such colonies have been used as a model to answer a series of fundamental questions concerning basic termite biology and evolutionary processes during hybrid introgression. The presentation will cover the different avenues of research this hybrid termite model can provide for termite research, and our understanding of evolutionary processes.



Figure 4: Established hybrid colony between a *Coptotermes formosanus* queen and *a Coptotermes gestroi* male (Chouvenc et al 2015).

1- Hybrid establishment and introgression

It is currently unknown if hybrid colonies have the ability to produce fertile alates, which would allow for the establishment of hybrid population and gene introgression in hybrid zones. After all, it remains possible that this hybrid termite is a "mule", and will not be a source of a new potential termite threat in such areas. However, in the potential case that such introgression occurs, it is necessary to determine what would be the potential distribution range of such hybrid termites. Patel et al. (2019a, 2019b) revealed that hybrids have the ability to tolerate temperature ranges from tropical and subtropical conditions, implying that it may be able to establish in the combined ranged of the two parental species. In addition, the two species have different foraging patterns (Uchima and Grace 2009) and it is currently unknown if the hybrid termites would be able to be a pest as much as their parental species. Such aspect needs to be investigated further.

2 - Conflict between two symbiotic lineages

The hybrid termite provides a unique model to study the transmission mechanisms of protozoa in lower termite. In order to digest wood, lower termites rely on a community of symbiotic protist species in their gut. The protists are highly specialized to the hindgut environment and cannot survive outside of their host. In termites, both the king (male parent) and the queen (female parent) pass hindgut protists to their young in newly established colonies. Each termite species harbors a unique and characteristic community of protist species, but virtually nothing is known about how these communities are maintained, i.e., by competition or cooperation. More importantly, very little is known about different protist species' impacts on host fitness. The discovery of a hybrid termite resulting from the mating between *C. formosanus* and the Asian subterranean termite, *Coptotermes gestroi*, which harbor different species of protists, provides a unique opportunity to investigate protist interactions and community inheritance. There is a need to characterize and compare the protist hindgut communities of *C. gestroi*, *C. formosanus*, and their hybrids. This will reveal patterns of coexistence and/or competitive exclusion among the protists, as well as any potential effect of parental gender on symbiont community composition.

3 - Hybrid vigor: termite host genetic recombination, symbiont lineage, or both?

As hybrid termites exhibited higher growth rates and fitness than either parental species, this termite hybrid model allows for the evaluation of host vs. symbiont contributions to hybrid vigor. This can be critical for evaluating the potential destructiveness and generating informed pest control strategies. Such approach can cast light on which aspects of termite biology contribute most to the observed vigor of the hybrids by profiling the symbiont communities of chimeric colonies. Chimeric colonies, in which parents are hybrids but symbiont communities derive from a single host species, and vice versa, will be established by swapping primary reproductives between hybrid and conspecific colonies after they have lost their protists, but before the colonies mature sufficiently to reject intruders. The concept of "transhostation" is here explored.

4 - The source of mating confusion between the two *Coptotermes* species

Despite their major economic impact and wide distribution, and being among the most studied termite species (Chouvenc et al. 2016), the chemical communication involved in mating behavior of *Coptotermes* species remains unclear (Bland et al. 2007). Unlike many other termite species, *Coptotermes* females display minimal calling behavior and it was suggested that during their large and chaotic dispersal flight events, the high density of potential mates in the direct surrounding rendered the need for a long distance volatile sex pheromone unnecessary, as males would randomly run into females and engage in tandem behavior. Alates would aggregate on trees or around artificial lights, then chaotically and randomly run into a potential mate. Both females and males possess a sternal gland (ventral, 5th abdominal segment) that produces (3Z,6Z,8E)-dodeca-3,6,8-trien-1-ol, which is known as the trail pheromone in the *Coptotermes* worker caste and potentially the reproductive caste. Females also possess a couple of tergal glands (dorsal, 9th and 10th abdominal segment), which were suggested to produce a contact sex pheromone that helps males initiate a tandem pair (Raina et al. 2003, Bland et al. 2004). However, DTE was not detected and instead, a heavier compound identified as trilinolein was isolated from female tergal glands (Bland et al. 2004), but was never confirmed as a

contact sex pheromone in *Coptotermes*. As the role of tergal glands and the functional chemistry involved in *Coptotermes* mating behavior remains unclear, heterospecific mating behavior between two *Coptotermes* species may provide an opportunity to answer a series of questions regarding the specific chemical cues, or absence thereof, involved in the formation of tandem pairs in *Coptotermes*, which would explain why male *C. gestroi* may prefer to mate with females *C. formosanus* instead of females from their own species.

Conclusion

The emergence of hybrid termites provides a model to study unique aspects of termite biology. This presentation will provide an overview of the current research performed to answer a series of questions, and open new questions concerning termite biology and unique evolutionary processes.

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Termite Fungal Cultivation as a Ruminant-Like Digestive System?

by

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Abstract

The digestive process of fungus-growing termites was recently hypothesized as a ruminant system. The digestive process of these termites has acquired the following ruminant-like features: (1) Fermentation sites, which includes fungus garden and the termite intestine; (2) A feature where dietary proteins are predominantly assimilated by symbiont microbes, and termites dependence on microbial protein. Ruminant system was suggested to benefit mammals by transforming the dietary protein into high-quality microbial proteins, degrading the unpalatable or toxic compounds in diets, or functioning as a food-preserving system. Similar hypothetical functions are also supported in fungus-growing termites.

Keywords: Nutritional symbiont, fungus-growing termite, ruminant mammal, forestomach fermentation

Introduction

Fungus-growing termites (Termitidae: Macrotermitinae) decompose wood- and foliage-litters with the help of their obligatory symbiont fungi, *Termitomyces* spp. Fungus-growing termites collect litters, shortly mixed the fragments of litters with spores of *Termitomyces* fungi in their intestine, and then excrete it as a material for constructing fungus garden in less than 3.5 hours (Li et al. 2017). Termites continuously consume the aged part of fungus garden, which contains cellulose, hemi-cellulose, sugar (Li et al. 2017), amino acids (Chiu et al. 2019), enzymes (da Costa et al. 2018), and fungal tissues (Hyodo et al. 2003), and harvest the fungal nodules growing on the surface of fungus garden as diet (Nobre et al. 2010, Nobre and Aanen 2012). The undigested fiber maybe fermented for the second time in termite intestine (Li et al. 2015). Each single substrate of fungus garden is entirely consumed by termites in around 45 days (Li et al. 2017). The digestive process of fungus-growing termites and the products of fungal cultivation are summarized in Fig. 1A.

The functions of fungal cultivation by termite is hypothesized to (1) providing enzyme to facilitate the digestion of lignocellulose by termites (Martin and Martin 1978); (2) degrading lignin prior to termite digestion (Hyodo et al. 2003); (3) producing protein-rich diets (fungal nodules) for termites (Nobre et al. 2010); and (4) functioning as a ruminant (Nobre and Aanen 2012). In comparison to the non-ruminants, the ruminant hypothesis assumes that fungal cultivation helps fully ferments the plant materials prior to termite digestion. This paper presents a comparison between the digestive process of fungus-growing termites with that of non-ruminant and ruminant animal. Further functions of a termite ruminant-like digestive system are discussed.

Ruminant and non-ruminant digestive system of mammals

Herbivores mammals have two types of fermentation system to digest their fiber-rich and protein-poor diets, one in forestomach and the other in the hindgut fermentation systems (Chivers and Langer 1994a, Langer 1994). The forestomach fermenters including all ruminant mammals, (e.g. cow, goat, and sheep), accommodate a fermentation structure prior to their true stomach (Chivers and Langer 1994a, Langer 1994). The forestomach fermentation continuously converts cellulose in ingesta to fatty acids as energy for mammals, and the digesta pass to the true stomach when the food particle is reduced to a specific size (Chivers and Langer 1994b). Proteins in the ingesta are primarily hydrolyzed and

assimilated by the microbes in forestomach (McDonald 1953, Janis 1976, Wolin 1979). Since the major digestion and transformation of amino acid is done by microbes, ruminant mammals largely depend on microbial proteins (60-80% from microbes) (National Research Council 1985). The digesta is again fermented for the second time in hindgut for the production of short-chain fatty acids (Hofmann 1989, Ørskov and Ryle 1990, Chivers and Langer 1994b, Chivers and Langer 1994a). The digestive process of ruminant mammals and their fermentation products are summarized in Fig. 1B. The hindgut fermenters of non-ruminant animals, (e.g. elephants, horses, and rabbits), do not have forestomach (Chivers and Langer 1994a). The ingesta of hindgut fermenters directly pass to true stomach for digestion, so they do not depend on microbial protein (National Research Council 1989). The digesta pass to hindgut for fermentation. Similar to forestomach, the hindgut fermentation chamber also converts cellulose to fatty acids as a source of energy (Janis 1976, Hintz et al. 1978, Demeyer 1981).

Termite fungal cultivation: ruminant or non-ruminant?

The digestive process of non-ruminant mammals includes one single fermentation sites, the hindgut. In contrast, the digestive process of fungus-growing termites and ruminant mammals include two fermentation sites; the first fermentation site (fungus garden or forestomach) is prior to the stomach digestion; and the second fermentation site (hindgut) is posterior to the stomach digestion (Fig. 1AB). In non-ruminant mammals, the dietary protein is digested in true stomach and assimilated in small intestine (National Research Council 1989). In contrast, dietary protein ingested by fungus-growing termites and ruminant mammals are primarily assimilated by microbes (National Research Council 1985, Chiu et al. 2019) (Fig. 1AB). With dual fermentation sites and dependency on microbial protein, the digestive process of fungus-growing termites is closer to that of ruminant mammals, rather than that of non-ruminant mammals.

The function of the first fermentation site is partially different in between termites and ruminant mammals. The first fermentation site continuously releases fatty acids as the major energy source for ruminant mammals (Fig. 1B). Different to ruminant mammals, the first fermentation site of termite releases amino acids by producing fungal nodules (Fig. 1A). Fungus-growing termites are more likely to obtain energy from assimilating the final product of fungus garden, majorly sugar, hemi-cellulose, cellulose, and hemi-cellulose (Fig. 1A).

Hypothetical functions of ruminant-like digestive system in termite

Both non-ruminant and ruminant mammals efficiently obtain energy with their respective fermentation systems. However, because the microbial metabolism is in forestomach, this leads to a loss of energy and delay in digestion, ruminant mammals are not competitive to the non-ruminant mammals when nutritious diets are available (Kinnear et al. 1979, Kinnear and Main 1979). Nevertheless, ruminant mammals are able to utilize diverse low-quality proteins and non-protein nitrogen, with the advantage of their forestomach (Moir 1965, National Research Council 1985, Moir 1994). For example, cattle, a ruminant mammal, could survive without the supplement of protein and amino acids (Virtanen 1966, Oltjen 1969). The wastes of nitrogen metabolism, such as ammonia, are recycled in small intestine, and supply to microbes to synthesize more amino acids (Reynolds and Kristensen 2008). Forestomach fermentation also decreases the toxicity of plant material to the host (Freeland and Janzen 1974, Janis 1976, Chivers and Langer 1994b). Forestomach is also considered as a food preserving organ, which allows the ruminant mammals to ingest a large amount of diet and quickly leave the feeding site to escape from enemies (Colbert 1966).

The functions of ruminant system may benefit the animals which have the following features: (1) Performing eat-and-run foraging strategy to lower the predation stress; (2) high variation of dietary protein quality; (3) feeding on toxic or unpalatable diets. Interestingly, all of these features were supported in fungus-growing termites. For example, the fungus-growing termite *Odontotermes formosanus* (Shiraki) was observed to perform an eat-and-run foraging strategy in the hot and wet seasons (Chiu et al. 2018). *O. formosanus* was observed foraging on bark, dead branches, rotten woods, leaf litter, fruits, algae, fungi, and cattle faeces (Chiu et al. 2019), which predictably contains proteins of different qualities. The composition of α -amino acids in fresh fungus garden of *O. formosanus* was different among localities (Chiu et al. 2019), also shows that the dietary protein quality is variable.

High concentration of laccase, an enzyme act on the unpalatable phenolic compounds, was found concentrating in the fungus gardens of termites (da Costa et al. 2018).

Conclusions

In conclusion, the digestive process of fungus-growing termites is a ruminant-like system. As an external ruminant, the fungus gardens may benefit fungus-growing termites by transforming the dietary protein to microbial protein, degrading the unpalatable phenolic compounds, or functioning as a food-preserving system.

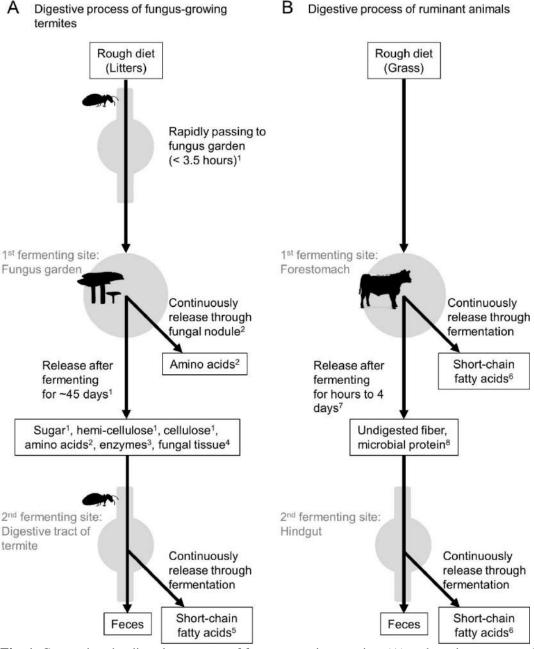


Fig. 1. Comparing the digestive process of fungus-growing termites (A) and ruminant mammals (B). Termites and ruminant animals both ferment the diets prior to their own digestion (1st fermenting site). The 1st fermenting site continuously release amino acids for termites through the fungal nodules, and release short-chain fatty acids as energy source for ruminant animals. Both termites and ruminant

animals assimilate microbial protein or amino acids from the fermentation products, and obtain additional energy by fermenting the undigested fiber, cellulose, or hemicellulose passed to the 2nd fermenting site. References cited: ¹, Li et al. (2017); ², Chiu et al. (2019); ³, da Costa et al. (2018); ⁴, Hyodo et al. (2003); ⁵, Li et al. (2015); ⁶, Janis (1976), Hintz et al. (1978), Demeyer (1981); ⁷, Krishnamoorthy et al. (1983), Grajal and Parra (1995), Vanhatalo et al. (1996); ⁸, National Research Council (1985).

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Morphological Characters Conundrum in Termite Species Identification: The Challenges and Effort to Address the Problems

by

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Abstract

Species determination using morphological characters has been a convoluted process to handle, especially for non taxonomists who try to ascertain the identity of their model organisms. This study demonstrated the difficulties in utilizing morphological characters to pinpoint the species identity of termites collected from the nine years old teak plantation in Cibinong Science Center-Botanical Garden, Indonesia. The study also built an algorithm of branching choices build upon web-based platform as the tool to help ease the workload and hasten the identification process. The results showed that the incomplete range of morphological measurements, and vague descriptive-words are the main hurdles for the species fixation for termites. Other than that, in some cases we were led to a species identity which only has minuscule descriptive information, most of them describe the species as the larger or the smaller specimen without additional characters, which can leave some doubt for researcher once the specimen need to be validated by other characters. We identified the hurdles and developed a program toolbased on Python programming language to aid researcher on the determination of termite species. The program tool is expected to simplify and to fasten the identification process of termite specimens.

Keywords: Termite identification, species determination, morphological characters, program tool, Python programming language

Introduction

Termite species identification definitively has been a problematic issue for years especially because of difficulties to find key morphological characters. Some studies found how the morphological guideline, although powerful to the level of families, is reaching its end when trying to delineate the species (Chouvenc et al., 2016). The guideline has also fallen short at giving visual aids since most of the collection that was used to describe the species in the first place has been difficult to access or to identify further (Korb et al., 2019; Short et al., 2018). A study reported that hazy description in the guidelines, especially for uncommon species, can yield up to 90% of misattribution, even for the taxonomist who had identified the exact specimen previously (Korb et al., 2019). These problems actually can also happen to prominent taxonomists as the study reported its experimental results (Korb 2019). Such results showed how unstable human-based observation, especially when the guideline used has been dated with a lot of rarely used descriptive-words which makes the meaning skewed to some extent (Korb 2019).

Another concern is the misattribution of species names. For instance, Kirton (2005) revealed the nature of misattribution of Asian Subterranean Termite (*Coptotermes gestroi*), which was until recently classified as *C. havilandi*, while the real *C. havilandi* was categorized as *C. travians*. Such situation is

not an isolated case. There are many instances across academia which reflect the same situation. Another study erected two new cryptic species of leech (*Hirudo medicinalis*) which were also identified as *H. medicinalis* in the past. Such condition renders past researches that used *H. medicinalis* as the model organism becomes questionable since the validity of the result needs to be checked whether it was on *H. medicinalis* or not (Siddall et al., 2007; Wägele et al., 2011). In fact, A study compiled how often the original work on the model organisme and how they were quoted by others as a plus point on the certainty of organism identity since it is related to the credibility of the effect observed on the designated species (Wägele et al., 2011).

In the process to curb the trend of misattribution, the problems plaguing the guideline need to be addressed. Furthermore, the current state of the guideline needs to be made into more user-friendly to let researcher identify species in a more relaxed situation. Hence, this study is going to demonstrate the difficulties in using the current guideline and also showed how to hasten the process of species identification by employing algorithms of branching choices build upon web-based platform. The usage of logic function is expected to ease the work of researcher since most of the time the researcher needs to keep returning to the guideline for every new character observed. Such routine can lead to unfocused mind that can lead researcher astray during species identity elucidation.

Materials and methods

Termite specimens

The specimens were collected from a teak plantation that was established nine years ago in the area of Cibinong Science Center – Botanical. The sampling method used was the belt-transect method (Davies, 1997; Jones & Eggleton, 2000). The transect was laid out in 100 m x 2 m format, which was divided into 20 sections; each section stands for 5 m x 2 m. For each designated section, two experience collectors will search the entire section ranging from 10 cm below the ground up to 2 meters above the ground, with the collections done under half an hour for each section. For each specimen found, the specimen was secured in a vial coded for the section. A solution containing 70% ethanol was used as preservative.

Morphological characters observation

All of the collected specimens were examined under digital microscope Leica S8 APO B (Leica Microsystems CMS GmbH, Germany) where each specimen morphological character and dimensional measurements were noted down and compared to the guideline. The note was ordered based on branching points (1 to 15 branching point), with each branching point to be categorized into five different types of morphological characters (Table 1).

All of the observed characters were analyzed and marked for their peculiarity on the case by case basis. For the morphological characters on the guideline that were found to be questionable such as the measurement was overlapped; in half-fulfilled state; below/beyond the range of measurement, or unclear shape of the character; they were tallied together. The recorded data then presented into hit number data and accuracy data. The hit number is the amount of the character used to find species, tallied together into two group, a normal group that is a character that is well defined and easy to matched and a questionable group that is difficult to ascertain. The accuracy data is the percentage of normal group out of the total character used in a morphological character type. The questionable percentage is 100% minus the percentage of accuracy. The information can be used to judge morphological character type merits in determining termite species identity.

The primary guideline used was *Key to the Indomalayan Termites* (Ahmad, 1958) especially termite soldiers key to families and species section. The comparison also used *On the identity of Odontotermes* (*Isoptera, Termitidae*) (Ahmad, 1949), *Termites (Isoptera) of Thailand* (Ahmad, 1965), *Termites of Peninsular Malaysia* (Tho, 1992) and *A Systematic Key to Termites of Thailand* (Sornnuwat et al., 2004) as additional references.

Table 1. Description of the type of morphological characters in termite spesies identification

Morphological Character	Type's Description	Example of Character
Type I	The presence/absence of a character	 Is the tooth presence in the left mandible? Is the median suture presence?
Type II	The number of repeated character	 How many segmens are in the antennae?
Type III	The general form and direction and color of the characters	 Is the labrum broadly or narrowly rounded? Is the labrum tongue-shaped or lancet-shaped? Is the inner margin below the tooth concave or convex? Is the mandible very strongly or slightly hooked?
Type IV	Dimensional measurement of the characters: The length, width, diagonal, angle measurement of the characters.	 What is the length of the head with or without mandible? What is the length of the left mandible? What is the width of the head at its various points such as at its narrowest point, widest point, at its post antenna foveal? What is the angle between the tooth and the inner margin?
Type V	The comparison or ratio of the dimensional measurement of two or three characters or subcharacters:	 Is the pronotum narrower or equal in width with the head? Is the left mandible length more than a half of the head without mandible? What is the length of the second articles in terms of the third, and fourth articles?

Building termite morphological defining characters hierarchy

We developed termite morphological hierarchy based on the guideline provided by Ahmad (1958). The hierarchy tree was made based on morphological commonness across various termite species as the differentiation key. The hierarchical tree was made in the spreadsheet processor program as the simplified guideline. The generated information then turned into a logical branching function with the evaluator as decision maker, while the program will generate the best possible species based on the database. Two other possible species that closely resemble the queries will also be presented together with their parameters. Thus, the information can be used by the evaluator to decide which species is the observed specimen. The program was made in phyton and deployed on the website https://idenrayap.herokuapp.com/#/ to broaden the access.

Results and discussion

Accuracy issue in determining the morphological characters

The transcet in the teak plantation site had recovered four termite genera. The identification process of termit genera is not difficult, since the morphological key that lead to each genus is very distinctive. Therefore, the identification of termite species will be the focus of present study. The summary of the

characters' accuracy from the pooled four genera (*Macrotermes*, *Microtermes*, *Odontotermes*, and *Pericapritermes*) is presented on Figure 1.

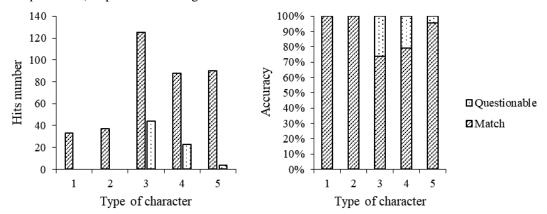


Figure 1. Type of termite-morphological character used during identification and each character's level of accuracy.

The results showed that while Type 1-5 are used during the identification, the usage intensity among the characters has been varied across five different types (Figure 1). It is noteworthy that Type 3-5 are heavily used during the identification. While it was heavily used, its level of accuracy is quite problematic which ranging between 75% to 95%. Such accuracy can lead to differences in interpreting species identity, since some character's interpretation is still up for debate (indicated by lighter bar on Figure 1). On the other hand, Type 1 and Type 2 relied heavily on the distinct characters which makes them easier to recognize, and therefore, they are more dependable characters to be used in the identification process.

The problem from Type 3 is mainly stemmed from its keyword boundary which depends on the evaluator interpretation. Some keyword such as convex and concave; notched and emarginate; rounded and pointed with qualifier detail such as slightly, moderately, broadly, narrowly, and others can lead to the confusing conclusion, which most of times will yield different understanding from one evaluator to another (Korb et al., 2019). Several past studies cited the fact that scant availability of the visual aid of the voucher specimen as the reason for the differences in evaluator's perception regarding the true graphics of the voucher specimen (Kirton, 2005). Even if we decided to look for the model specimen that was used by pioneering taxonomist as source material for the classification in the past, a long time had passed since then, and most of the specimen would be in not so satisfactory state to be measured properly (Chouvenc et al., 2016; Short et al., 2018).

As a side note, some observations showed that Type 3 could be vague even for a clear character once the magnification coming to play. For instance, how to define whether the tip of labrum is pointed or not pointed? This is challenging because when the picture was magnified to certain level, the pointedness of the labrum seemed more difficult to ascertain.

Next is the character of Type 4, which was different from Type 3 as Type 4 tended to fail when the specimen measurement was in-between of the two species; or barely outside the range of measurement, while the identified specimen exhibited various characters that appear in the other measurement range. As a side note, some species only have some dimensional measurements as their marker, especially for those species that were in the end line of the branching line (Figure 2). The lack of additional information on the morphological characters makes the validation of species identity for the corresponding specimen is difficult to conduct.

Type 5 character is the most complicated one since it employed the relation between two observed characters or sub-characters in terms of measurement. The interplay that caused the differences in interpretation was when the measurement was so close to the boundary of the relation, therefore, it

can tilt the species choice to the other option. The issue is the derivation of the problems observed in Type 4.

To finally solved the morphological characters is nonetheless a herculean work. Only by coming up with a new reorganization with various new supplemental information or by turning all of the entry into mathematical equation (see Type 1, 2 and 5 morphological characters in Table 1), a faster and more exact identification process can be done. A guideline that rely on mathematical equations can give us a better chance to get a robust result compared to that of conventional identification method.

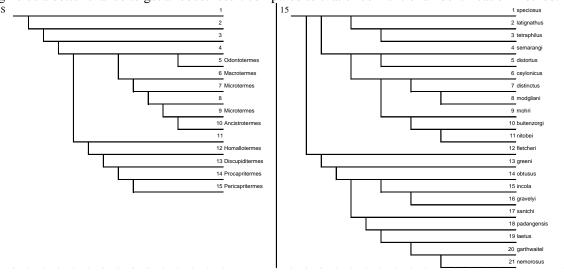


Figure 2. Left image: Branching line for key to genera of four identified termite specimens (5,6,9,15). Right image: An example of a branching line for key to species from Pericapritermes. Keys to other species are not displayed because of lack of space in this publication. A blank after number in key to genera (left image) indicates the genera that are not discussed in the present study.

Addressing the determination of termite species

In addressing species determination, especially in the light of difficulties to interpreting the observed morphological characters, we developed a set of algorithm of branching choices which was build upon the web-based platform. The set of branching line design to find four genera and its corresponding species are shown on Figure 2. Generally, the tool is a deterministic model with fixed pathway that is in the form of hierarchical tree (Figure 2). Each intersection (branching point) of the tree consisted of choices that can be chosen by the evaluator, which can lead further down in the hierarchy till finally reach to a species that matched the queries. The choices in each branching line are a morphological character or a combination of it that belong to one of five Types (Table 1).

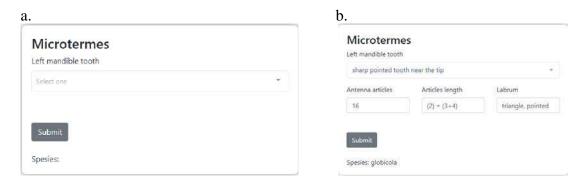


Figure 3. a. User interface for Microtermes; and b. User interface when choices started appearing after the previous query was filled

These branching line (Figure 2) was then turned into set of command or a program in which the general user interface is presented on Figure 3. Based on the experience of using the program, it is easier for the user to employ the guideline compared to using the guideline directly. Since the program took hold most of the process in switching the clue directly, the user will have a better focused in accessing the species identity, especially the interpretation issue of Type 3-5 morphological characters.

Conclusion

The difficulty of using morphological character has been discussed and it was found that Type 1 and Type 2 are reliable types of character in identifying termite specimen. It is also important to note the shortcoming of Type 3-5 characters and a need to solve them. The developed program tool also has been made to let the evaluator to be more aware of mistakes in interpreting Type 3-5 characters. While the tool is a deterministic model, its ability to show the big picture while identifying would help the evaluator to generate a better judgment during the identification process of termite species.

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Author Contributions. A Fajar and S K Himmi are equally contributed in research design, experiment execution, and manuscript writing; S K Himmi, A Latif, D Tarmadi and I Guswenrivo contributed in termite specimens collection; A Fajar, S K Himmi, A Latif contributed in termite specimens identification; M Fadholi contributed in developing the program tool and user interface; S Yusuf, T Yoshimura contributed in research design and discussion for manuscript preparation.

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Supplement of some Species of *Glyptotermes* (Isoptera: Kalotermitidae) Collected in Quang Nam Province, for the Termite Fauna of Vietnam

by

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Abstract

Glyptotermes Froggatt, 1896 is a large genus of family Kalotermitidae. This genus is widely distributed in the world, and up to 74 species have been recorded in the Oriental region. The record of species belonging to this genus in Vietnam is very few, mainly in the Northern Vietnam. In this study, we present the result of identyfication of 9 termite samples of Glyptotermes collected in Quang Nam province, Vietnam. From these samples, 5 species were found, of them 3 species newly recorded for the termite fauna in Vietnam including Glyptotermes fujianensis, Glyptotermes coorgensis, Glyptotermes dilatatus and the remaining two species have not yet been scientifically named. Specimens of these species were also described in morphology.

Keywords: *Glyptotermes*, termites, identify, biodiversity,

Introduction

Glyptotermes Froggatt, 1896 is a termite genus with large body size in the family Kalotermitidae. The soldiers and imagoes have specific characteristics. Soldiers: Head-capsule generally subrectangular, cylindrilcal, with frons sloping infront and concave to form two prominent humps; antennae with 10 to 15 segments; mandibles is broaden at the base and has some clear teeth; posmentum is long, broaden in the anterior half and has a narrow waist in the posterior half; pronotum is narrower than or, as wide as or slightly wider than head- capsule; legs with 3 apical tibial spur on each tibia; tarsi 4- segmented; abdomen elongate. Imagoes: the same shape as Kalotermes; but the midrib is parallel to the lateral (ala tendon); there is no tendon connecting the midrib to axillary tendon; the antennae with from 10 to 15 segments; tibia spur formula is 3:3:3; tarsi 4- segmented; abdomen elongate (Chhotani 1975; Huang et al, 2000; Tho 1982). The species of this genus are usually found in hard or rotten wood in the moist forests or in the scars of living trees (Scheffrahn et al, 2001). In the world, 127 species have been recorded, including 6 species in Australia, 8 species in Papuan, 74 species in Oriental, 5 species in Palaeactic, 12 species in Ethiopian, 26 species in Neotropical and 4 common species (present in two regions) (Krishna et al. 2013). In this genus, 36 species have been recorded and described in China (Huang et al. 2000), 14 species have been recorded and described in India (Chhotani, 1975; Roonwal & Chhotani 1989; Bose 1999), 11 species have been recorded and described in Malaysia (Tho 1982, Thapa 1981), 3 species in Japan (Takematsu, 1997).

In Vietnam, only a few species of genus *Glyptotermes* were recorded, mainly from the Northern Vietnam. Nguyen Van Quang et al (2005) recorded 3 species belonging to *Glyptotermes* in Luong Son district, Ha Tinh province, inluding *Glyptermes fucus*, *Glyptotermes satsumensis* and *G. tsaii*. Nguyen Duc Kham et al. 2007 found *Glyptermes fucus* in Lang Son province, *Glyptotermes satsumensis* in Phu Tho and Thanh Hoa provinces and described in detail these species. In 2008, three new species of this genus were recorded by Nguyen Van Quang et al., including *G. longnanensis*, *G. succineus* in Xuan Son National Park, Phu Tho province and *G. guizhouensis* in Cat Ba National Park, Hai Phong province. Nguyen Van Quang et al. (2012) recorded 5 species of *Glyptotermes* in mountain area of Hanoi, in which 3 species (*G. almorensis*, *G. montanus and*, *G. ceylonicus*) were new records for the termite fauna of Vietnam.

Quang Nam is the central province of Vietnam. The area has a rugged terrain and has a very large area of natural forest, with forest covering level more than 40% area of the province. Factors such as geographical position, natural conditions and climate contribute to the high biodiversity in Quang Nam, where is transitional zone between northern and southern flora of Vietnam. There were many researches on biodiversity conducted in this province, in which many rare and precious animals have been found in the nature reserves, protective forests and other special-use forests. However, the research on termites is still very limited in the area. Within the framework of implementing the task "Research on species composition, distribution of termites (Isoptera) in Quang Nam province", we collected over 800 samples of termites in the research area. Among them, 9 termite samples were collected belonging to *Glyptotermes*. In this paper, the scientific names of species of *Glyptotermes* from the samples collected in Quang Nam, Vietnam are listed and the samples which have not yet been identified are described.

Materials and methods

Quang Nam is a province in the South-Central Coastal region of Vietnam. Quang Nam's topography has a gradually inclined direction from the West to the East, forms three distinct regions, including the Western mountainous region, the midland in the middle and the coastal plain region, of which the hill and mountainous regions account for 72% of the total area of the province. Quang Nam's natural forest area is still very large, with the forest covering level of more than 40% and mainly in the West. Especially in the mountainous region, complex terrain, high separating level. The average height is from 700m to 800m, the slope is 25 - 30°, some places are over 45°. The area belongs to a typical tropical climate, with only two seasons: dry and rainy seasons; the average temperature in the rainy season is 20°C and the dry season is 29°C; the humidity is below 55%; the average rainfall is 2,000 - 2,500mm but unevenly distributed over time and space, the mountainous region is about from 3,200mm - 4,000mm.

Sample collection method

On the survey routes or in the standard survey plots, the signs of termites such as galleries, feces ... on the dry tree trunks, rotten wood section fallen in the forest were observed... Then, the wood sections with marks of termite were split, cleaved to collect termites. During the periods of sample collection, we tried to collect as much as possible the quantity of soldiers in each colony encountered. Once the termites are found, soft forceps is used to pick up termites into plastic small vials containing 75% alcohol, then labeled, and brought to the laboratory of the Institute of Ecology and Works Protection for analysis. The features of the locations where 9 termite samples of *Glyptotermes* were collected in the natural forests of Quang Nam province, Vietnam from 2016-2019, are shown in table 1.

Table 1. Samples of Glyptotermes in Quang Nam province

No	Sign of sampling	Location of sampling	Coordinate	Habitat	Altitude (m above sea level)
1	QN.040718.777	Tra Cang, Nam Tra My, Quang Nam, Viet Nam	15°06'08.6"N 108°02'19.1"E	Wet, hard wood	1,192
2	QN.050718.853	Tra Mai, Nam Tra My, Quang Nam, Viet Nam	15°12'00.7"N 108°06'47.8"E	Dry brank of live tree	433
3	QN.050718.840	Tra Nam, Nam Tra My, Quang Nam, Viet Nam	14°59'13.12"N 108° 3'15.43"E	Rotten wood	1,568
4	QN.010819.04	Lang, Tay Giang, Quang Nam, Viet Nam	15°51'23"N 107°26'42"E	Wet, hard wood	1,150
5	QN.030819.34	Ca Dy, Nam Giang, Quang Nam, Viet Nam	15°39'39"N 107°49'15"E	dry, hard wood	351
6	QN.020819.18	Lang, Tay Giang, Quang Nam, Viet Nam	15°51'17.24"N 107°26'38.55"E	dry, hard wood	998

7 QN.010819.113	A Xan, Tây Giang,	15°48'29"N	Wet, hard	1,322	
/	QN.010619.113	Quang Nam, Viet Nam	107°19'52"E	wood	1,322
0	QN.040819.14	Phuoc Hoa, Phuoc Son,	15°26'07"N	dry, hard	354
0	QN.040619.14	Quang Nam, Viet Nam	107°54'06"E	wood	334
0	ON 040910 45	Phuoc Hoa, Phuoc Son,	15°25'54"N	dry, hard	505
9	QN.040819.45	Quang Nam, Viet Nam	107°54'04"E	wood	525

Sample identification: The main equipment used in termite identification is the double-lens magnifier with measuring rule Leca MZ6 of Germany. The other necessary tools are analytical needles, soft forceps, petri dishes, etc. Because the termites are preserved in alcohol, so they are often hard and that make it difficult to measure dimension of their body if they are placed directly on a slide or a petri dish. Therefore, to overcome this problem, we used the water absorbent filter paper to support the specimen, fix the morphological positions and measure part of specimen body. This tip facilitates measurement, observation and identification.

The soldiers in the samples were mainly used for identification. In addition, the workers can be used additionally. To determine the species name by basing on the external morphological features, it is necessary for us to implement the following steps: Observation of the external morphological characteristics of termites (head, antennae, postmentum, thorax, pronotum, alate...); separation of the body details that are the classification characteristics (if necessary); observationand measure of these details according to the instructions in the material of Roonwal (1970). Finally, based on the identification documents to determine the species name. The commonly used documents in the identification include: Termite identification according to the documents of Ahmad (1958, 1965); Nguyen Duc Kham et al. (2007); Huang et al (2000); Thapa (1981), Roonwal & Chhotani 1989, Tho 1982...

Results and discusstion

Species composition of Glyptotermes in Quang Nam province

Analysing nine termite samples belonging to Glyptotermes collected from Quang Nam province, Vietnam, we have recorded five species, of which three species have been identified scientific names (Glyptotermes fujianensis, Glyptotermes coorgensis, Glyptotermes dilatatus) (table 2).

Table 2. Species composition of Glyptotermes in Quang Nam, Vietnam

No	Specific name	Sign of sampling	Habitat	Altitude (m above sea level)
1	Glyptotermes coorgensis (Holmgren & Holmgren, 1917)	QN.050718.840	Rotten wood in natural forest	1,568
2	Glyptotermes fujianensis Ping 1983	QN.040819.14 QN.040819.45 QN.030819.34	Hard, dry wood in natural forest	351-525
3	Glyptotermes dilatatus (Bugnion and Popoff, 1910)	QN.050718.853 QN.020819.18	Hard, dry wood in natural forest	433 - 998
4	Glyptotermes sp.1	QN.040718.777 QN.010819.04	Hard, wet wood in natural forest	1,150 – 1,192
5	Glyptotermes sp.2	QN.010819.113	Hard, wet wood in natural forest	1,322

Table 2 shows that *Glyptotermes fujianensis* has the highest quantity of samples, with 3 samples (QN.040819.14, QN.040819.45, QN.030819.34), followed by two species *Glyptotermes dilatatus* and *Glyptotermes* sp.1, each has two samples. The remaining two species (*Glyptotermes coorgensis* and *Glyptotermes* sp.2), each has only one sample. All of these species are found in the natural forests,

however, *Glyptotermes coorgensis* is found in rotten wood at an altitude of nearly 1,600m, while *Glyptotermes fujianensis* and *Glyptotermes dilatatus* are found in hard, dry wood trunks at an altitude of below 1000m, both *Glyptotermes* sp.1 and *Glyptotermes* sp.2 are found in hard, wet wood trunks and at an altitude of above 1,000m.

Glyptotermes coorgensis has been recorded in Oriental Region — India: Karnataka, Kerala, Tamil Nadu; Glyptotermes dilatatus has been recorded in Oriental Region — Sri Lanka; Glyptotermes fujianensis: Oriental Region — China: Fujian, Guangdong, Hainan, Hubei, Sichuan, Yunnan; Taiwan. Palaearctic Region — Japan (Krishna et al., 2013). These three species were first recorded in Vietnam. Two unidentified species may be new species for the science. Below is a description of the external morphology of the two species in sp form.

External morphologic characteristics of two new termite species of Glyptotermes

Glyptotermes sp.1: The head is yellow-brown to reddish-brown, darker in the front from the prominent top. The sides of head are parallel and slightly narrower at the back, the widest at the posterior base of the antennae. In inclined viewing, the highest of head at 1/3 posterior part of the head. Head with some long, short scattered hairs. Postmentum has a clear waist, the widest part at about 1/3 anterior part, the narrowest (waist) at about 1/3 posterior part, the widest part is more than twice the narrowest part, anterior margin and posterior margin are all round gliding, sides of the widest are not round. Labrum is semicircular, broader than long, cover or not over 1/2 of the mandibles, with some long, short hairs at about 1/3 anterior half. Mandibles are rough, dark brown; the right mandible has 2 teeth; the left mandible has 3 distinct sharp teeth, the first tooth is smaller, the second tooth and the third tooth are similar, however, the distance between the top of tooth 1 and the tooth is only 1/2 of the distance between the top of tooth 2 and tooth 3. Antennae with 12 to 14 segments, segment 3 is very short, only 1/3 of segment 2; segment 2 is longer than segment 4 obviously. Pronotum is the same color as the back half of the head, the widest of the pronotum is slightly narrower than the widest of the head, the anterior margin is only slightly concave, the posterior margin is gradually narrowed at about 1/3 and slightly concave in the middle. Legs are short and rough; spur formula 3: 3: 3. The abdomen is enlongated.



Fig 1. Sodier of Glyptotermes sp.1

(A: Head viewed from above, B: head viewed from below, C: head viewed inclined)

Table 3. Maesurements (in milimetters) of sixteen solldier of *Gyptotermes* **sp.1** (including 2 soldiers of QN.040718.777 and 14 soldiers of QN.010819.04)

	Mean	Min	Max
Total body-length	8.46	7.50	10.00
Length of head with mandibles	3.13	2.80	3.30
Length of head to side base of mandibles	2.44	2.25	2.50
Maximum width of head	1.50	1.40	1.55
Head index (width/length without mandibles)	0.62	0.59	0.64

Height of head	1.39	1.25	1.50
Length of labrum	0.31	0.25	0.35
Width of labrum	0.39	0.32	0.45
Length of head with mandibles	0.98	0.92	1.05
Mandible-head index	0.40	0.38	0.43
Length of postmentum	1.78	1.50	1.95
Maximum width of postmentum	0.61	0.57	0.65
Minimum width of postmentum	0.26	0.25	0.30
Length of pronotum	0.69	0.60	0.75
Width of pronotum	1.42	1.30	1.50
Pronotum index (length/width)	0.18	0.17	0.21

Comments: This species is most similar to *G. taruni* Bose 1999. This species is similar to *G. taruni* in antennae and mandibles characteristics but there are some differences in head index (head width/length without mandibles) and labrum. The head length to the base of the mandibles and head height of this species are larger than *G. taruni*; labrum are semicircle, not nearly square as described in *G. taruni*. Comparing to *G. yui* Ping et Xu, this species is similar to the head sizes but differs in characteristics of posmentum, labrum and left mandible. In this species, postmentum of is not club, width of labrum broadens than length, the left mandible is shorter (0.92 to 1.05mm).

Glyptotermes sp2: Head is yellow to yellow-brown, darker gradually forward. Antennae, legs and abdomen are lighter color. The widest head at posterolateral ends of antennal carinae and narrows in the posterrior part. In lateral view, the highest is about 1/3 of the front of the head. Head with some long, short scattered hairs. Labrum are semicircular, width is larger than length, cover about half of mandibles, anterior margin is slightly concave, with some long, short hairs about one third of anterior part. Mandibles are thin, dark brown, sharply on top; right mandible with 2 distinctly sharp teeth; left mandible with 3 teeth, first tooth and second tooth sharply, third tooth unclearly, only tooth base. Antennae with 11 segments, segment 2, 3 and 4 the same length. The postmentum with clearly waist, the widest at about 1/4 of anterior part, the anterior part only slightly narrowed compared to the widest, the narrowest part (waist) at about 1/3 of posterior part. Pronotum is the same color as the posterior head, width is slightly larger than the widest of head and gradually narrows in the posterior; anterior margin is concave like moon; posterior margin is slightly concave in the middle. Spur formula 3: 3: 3. Abdomen is soft.

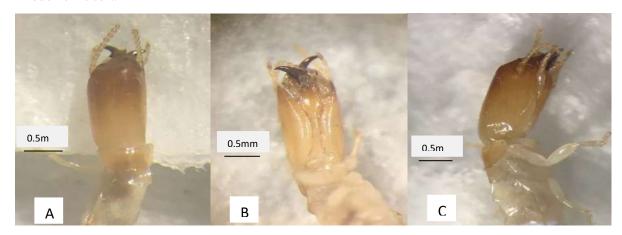


Fig 2. Soldier of *Glyptotermes* sp2. (A: Head viewed from above, B: head viewed from below, C: head viewed inclined)

Table 3. Maesurements (in milimetters) of one soldier of *Gyptotermes* sp2 (1 soldier of QN.010819.113)

Total body-length	5.10
Length of head to side base of mandibles	1.70
Maximum width of head	0.90
Height of head	0.87
Length of labrum	0.20
Width of labrum	0.30
Length of head with mandibles	0.85
Length of postmentum	1.30
Maximum width of postmentum	0.30
Minimum width of postmentum	0.15
Length of pronotum	0.47
Width of pronotum	0.95

Comments: This species is most similar to *G. shaanxiensis* Huang et Zhang. The head shape and postmentum characteristics are almost the same but different in left mandible. The left maldible of this species with three teeth and shorter.

Conclusions

Five species of the genus *Glyptotermes* have been identified in Quang Nam province, Vietnam. In which, three species were identified with scientific names and two species have not been identified yet. The study has added 3 new species to the termite fauna of Vietnam with detailed description of their external morphology of two species.

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Taxonomic Revision of *Coptotermes* spp. in East Malaysia Based on Morphometric and Genetic Analyses

by

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Abstract

The genus *Coptotermes* refers to the subterranean termites from the family Rhinotermitidae. Members of this genus are among one of the most destructive pests in Malaysia and the surrounding Southeast Asian nations as they may cause more than 90% damage in buildings and man-made structures. An accurate Coptotermes spp. identification is essential for proper pest control and management. However, this is hampered by difficulty in distinguishing the complex variations of the species under this genus. The relationship of the Coptotermes species was identified by using partial sequences of mitochondrial DNA sequences of cytochrome oxidase subunit II (COII) and was supported by morphometric measurement data. A total of 30 populations of C. curvignathus, C. sepangensis, and C. kalshoveni from selected areas in Sarawak were used in mtDNA analyses, along with other DNA sequences of C. gestroi, C. travians and other Coptotermes spp. from Peninsular Malaysia which were obtained from the GenBank. The morphometric assessment showed that many overlapping morphological characters between C. sepangensis and C. kalshoveni, and their morphological characters discriminated poorly from one another on Discriminant Function Analyses (DFA). They were found clustered together in both the DFA scatter plots and phylogenetic tree with a high bootstrap support of 99% on the neighborjoining tree. Based on the congruency from morphometric data and molecular phylogenetic analysis, we suggest that *C. sepangensis* is likely a junior synonym of *C. kalshoveni*.

Keywords: Coptotermes, morphometric, taxonomy, synonym, phylogenetics

Introduction

The termite genus of Coptotermes from the family of Rhinotermitidae is an invasive pest in urban and suburban areas. The genus is very destructive to wood and wooden materials in the world (Takematsu et al., 2000) and has a wide distribution throughout Asia, Australia, Africa and the New World. This pest has become a major concern worldwide and therefore collaboration efforts have been made between governmental and non-governmental partners on comprehensive approaches to pest suppression, containment and eradication. In Peninsular Malaysia, widespread infestation of the termite was reported on rubber trees (Hevea brasilliensis) before it was considered as a serious pest in the early 1900s. Today, the species is highly regarded as a destructive pest as it is highly destructive to buildings and its furnishings. It is also an agricultural nuisance in places such as oil palm plantations, garden landscapes and dwellings (Lee, 2002a) and brings damage to any cellulose materials such as books, papers, blankets, window frames and furniture (Chao et al. 1989). Termite taxonomy; especially the Coptotermes species is very challenging as the morphological identification alone cannot provide robust recognition of a single species of termites. The overdependence on the soldier morphology presents a major challenge to species identification because of the intraspecific variation in morphological characters in soldiers (Emerson, 1971; Husseneder & Grace, 2001) and lack of distinctive diagnostic features among the species of Coptotermes (Li et al., 2010) yet the identification based on soldier morphology is considered to be the best option thus far as compared to alates due to seasonal occurrences that prevent simultaneous collection and description of this caste (Jones et al., 2005). Therefore, the molecular techniques have been adopted for the termite classification, identification and phylogenetic analyses (Kambhampati et al., 1996; Miura et al., 1998; Clement et al., 2001; Austin et al., 2004; Szalanski et al., 2003, 2004) to overcome the limitations of morphological assessments.

Previous molecular studies of *Coptotermes* spp. have been carried out in West Malaysia (Yeap et al., 2009, Cheng et al., 2014) with limited representative *Coptotermes* samples from each region in Malaysia while no previous studies, to our knowledge, have included *Coptotermes* samples from East Malaysia that involves molecular phylogenetic studies. The goal of this project is to fill the gap in current knowledge on *Coptotermes* species particularly from East and West Malaysia in an attempt to: (1) provide a morphological variation of *Coptotermes* species from Sarawak, East Malaysia with clustering analyses based on discriminant function analyses (DFA) and (2) conduct a phylogenetic relationship inferred from mitochondrial gene of cytochrome oxidase II (COII) to complement morphological identifications.

Materials and methods

Termites were collected from 39 colonies from selected areas in Sarawak, East Malaysia (1.553⁰N, 110.359°E) from 2015 through 2016. Soldiers (one species found from one section was considered as one colony) were collected using a transect method by Jones et al. (2000) with modification of sampling transect (i.e., 100 m long and 2 m wide were divided into 5 small plots with 20 x 2 m each. Each plot was sampled by one person for 30 minutes). The samples were preserved in 100% alcohol and brought to the laboratory for identification. All specimens have been stored in the UNIMAS Insect Reference Collection, Department of Zoology, Universiti Malaysia Sarawak (UNIMAS). The identification of termites to the species level was done using taxonomic keys published by Thapa (1981), Tho (1992), and Ahmad (1965). Species comparison was traditionally made based on the diagnostic features such as head size and shape, mandible shape, pronotum width, and labrum shape for soldier caste (Hostettler et al., 1995). To establish the identity of Coptotermes spp., the soldier caste belonging to each colony was used for morphometric analyses. Soldiers' morphological measurement (n \geq 20) was recorded from 24 colonies of C. curvignathus, 10 colonies of C. sepangensis, two colonies of C. kalshoveni and three colonies of C. travians. The photographs of the soldiers were taken using a Motic SMZ-16B Series stereomicroscope attached to a Moticam 2000 camera. Calibrated measurements were done by using Motic Image Plus 2.0 software.

A minimum of 17 external characters was measured for each specimen; (a) total length (TL) (b) total length without head (TLH) (c) length of head at base of mandibles (TLM) (d) length to fontanelle (LF) (e) maximum width of head (WH) (f) width of head at base of mandibles (WHM) (g) length of labrum (LLb) (h) width of labrum (WLb) (i) length of antennae, segment 1 (AL1) (j) length of antennae, segment 2 (AL2) (k) width of antennae, segment 1 (WA1) (l) width of antennae, segment 2 (WA2) (m) length of pronotum (n) width of pronotum (WPr) (LPr) (o) length of postmentum (LPt) (p) maximum width of postmentum (MxWPt) (q) minimum width of postmentum (MnWPt). The indices for body index (BI), head index (HI), pronotum index (PI) and postmentum index (GI) were calculated as follows: (1) Body index (WH/TL) (2) head index (WH/WHM) (3) pronotum index (WPr/LPr) (4) postmentum index (MxWPt/MnWPt). These data were subjected to analysis of variance (ANOVA), and comparison of means was done based on Tukey's HSD (Honest Significant Difference) using STATISTIX Version 10.0. Next, morphometric data were $log_{10}x + 1$ transformed to reduce heterogeneity in variance and analysed using SPSS version 21 (SPSS Inc., 2006). Morphological affinities among species and individual specimens were evaluated by discriminant function analysis (DFA) using the transformed data set. This procedure maximizes between group variations, minimizes within group differences and identifies which variables contribute most to group separation (McGarigal et al., 2000).

For molecular analysis, the termite DNA was extracted using a DNeasy blood and tissue kit (QIAGEN, Valencia, CA) following the manufacturer's protocol. Polymerase chain reaction (PCR) of 900 bp region of partial mitochondrial COII (mtCOII) gene was conducted using the primers C2F2: 5'-

ATACCTCGACGWTATTCAGA -3' and TKN3785 reverse: 5'- GTTTAAGAGACCAGTACTTG-3' (Simon et al., 1994). The PCR reactions were conducted with 2µl of extracted DNA in a MyCycler thermal cycler (Bio-Rad Laboratories, Hercules, CA), with a profile consisting of 35 cycles of 94°C for 1 min, 47.9°C for 1 min and 72°C for 1 min in a final reaction volume of 25µl. The purified PCR products were sent to the 1st Base Laboratories (Selangor, Malaysia) for direct sequencing. The DNA sequences were aligned in Clustal X program version 2.0 (Thompson et al., 1997). Phylogenetic analyses were performed on 32 sequences obtained in this study together with 11 sequences obtained from GenBank. *Globitermes sulphureus* was used as outgroup in order to generate a rooted phylogenetic tree based on neighbor-joining method imcorporating Kimura-2-parameter distance with 1000 bootstrap replications.

Results and discussion

In this study, *C. curvignathus* and *C. sepangensis* have strongly curved, slender mandibles and the curvature begins at the middle of its length. The soldier's mandibles of *C. curvignathus* are distinctive as in being large compared to the other mandibles of *Coptotermes* species. Based on the mandible features, it was difficult to distinguish between *C. kalshoveni* and *C. travians*. Both species have less curved mandibles and the curvature begins at the anterior third of its length. The only reliable diagnostic feature to differentiate between these two species was that the head of *C. travians* is ovoid, much longer than wide compared to *C. kalshoveni* while *C. travians* was observed to be distinctively small in fontanelle size compared to *C. kalshoveni*. The ANOVA F-statistics indicated the presence of significant effect of character types on the *Coptotermes* species (P < 0.05; ANOVA test). Tukey-Kramer testing of individual characteristics provided groupings that indicated significant pairwise population differences.

The several measurements of the soldiers from the type, type localities and from the literature (Thapa, 1981, Tho, 1992) reflect this range of intraspecific measurements and the mean fall within the range of our measurements. Discriminant function analysis using morphometric identification formed four groups. *C. travians* and *C. curvignathus* were clearly distinguished from other groups; however, notable overlap of data points is evident between *C. sepangensis* and *C. kalshoveni*. In the DFA analysis, three significant functions were successful; Function 1, 2, and 3 explained 56.7%, 36.3% and 7.0% of the variance respectively. Function 1 has higher variability of characters in the analysis. The Wilk's lambda statistic for the tests of Function 1 through 3 functions (Wilk's lambda = 0.06), Function 2 through 3 functions (Wilk's lambda = 0.243) and Function 3 (Wilk's lambda = 0.724) has a probability of p = 0.000 respectively. It was suggested that MxPt (1.677) and WH (-2.261) played a prominent role in separating *Coptotermes* spp. through Function 1 (56.7% of trace). Meanwhile, in Function 2 (36.3% of trace), a separation of *Coptotermes* spp. was driven primarily by opposing contributions of WH (1.563) and TLH (-0.866).

Sequencing of the mtCOII partial gene generated 477 bp sequence fragment from all the individuals tested after a sequence trimming to align with sequence from GenBank. A pairwise sequence alignment of the partial COII gene sequences from the 34 specimens and 10 specimens of *Coptotermes* spp. from NCBI containing 91 variable sites (19.1% of total sites) and 87 characters (95.6% of the variable sites) were parsimoniously informative. Genetic diversity of COII gene showed intraspecies variation of *C. curvignathus* ranging from 0% to 0.7% while *C. gestroi* showed no genetic diversity. The genetic diversity of *C. sepangensis* ranged from 0% to 1.3% in COII while *C. kalshoveni* has a genetic diversity ranging from 0.4% to 2.3%. All sequences were separated by outgroup, *G. sulphureus* with a value range from 21.8% to 23.7%. The neighbour-joining (NJ) phylogenetic analyses conducted on COII partial gene sequences obtained from this study and from GeneBank allowed us to distinguish four major clades; Clade I (*C. curvignathus*), Clade II (*C. gestroi*), Clade III (*C. sepangensis* and *C. kalshoveni*) and Clade IV (*C. travians*). Clade I composed of all individuals that were identified morphologically as *C. curvignathus* in our study were clustered together and this cluster was consistent with the morphometric analyses conducted in the current study. A common Asian subterranean termite, *C. gestroi* in clade II formed a sister clade with the rubber tree termite, *C. curvignathus* in the NJ tree.

Clade III consists of combined individuals that were morphologically identified as *C. sepangensis* and *C. kalshoveni*, and the cluster was also consistent with the overlapping measurement characters in DFA analyses. *C. travians* in Clade IV formed a sister clade with Clade III in the NJ tree. Close relationship was observed between *C. sepangensis* and *C. kalshoveni* in Clade III with strong bootstrap values of 100% and 99% respectively. The genetic relationship observed between these two species is further supported by their morphometric similarity as observed in FDA analysis, whereby *C. sepangensis* and *C. kalshoveni* shared almost similar diagnostic features and close genetic distance. Interestingly, under Clade III, two subclades of *C. sepangensis* and *C. kalshoveni* were generated, in which individuals from Sarawak and Peninsular Malaysia was grouped separately according to their location.

Conclusion

The current effort to clarify the relationship of the *Coptotermes* species needs to be intensified by adding a description of alates along with soldiers and use quantitative morphometrics in the case of large samplings. Such tools will allow much greater certainty for the discovery of new cryptic species and clarify synonymous. The mtCOII genes implemented in the current study for phylogenetic relationship and molecular identifications are consistent with morphometric data and morphological identifications. Our survey provides new information on infestation patterns of *C. curvignathus* between East Malaysia and West Malaysia. The overlapping of morphological characteristics and close genetic relationship of *C. sepangensis* and *C. kalshoveni*, raised the likelihood of *C. sepangensis* to be a junior synonym of *C. kalshoveni*.

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Termite Taxonomy in Taiwan

by

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Abstract

Nine termite families contain ~3,000 species have been described worldwide. In Taiwan, five families, including Archotermopsidae, Kalotermitidae, Stylotermitidae, Rhinotermitidae, and Termitidae, were recorded. In this study, we examined > 5,700 sample collected across Taiwan. Based on both morphological measurement and genetic data, 22 termite species of 14 genera were confirmed. Three new or new record species of *Macrotermes*, *Neotermes*, and *Glyptotermes* were reported for the first time. Overall identification key of the 22 Taiwanese species based on both winged imago and soldier castes is provided. The checklist of termite species with Chinese common names were also provided for further fundamental and applied researches.

Keywords: Taiwan, Identification key, Termitoidae, Termite checklist.

Introduction

Termite taxonomic researches in Taiwan began at early 20th century, when Japanese researchers arrived Taiwan. Shiraki (1909) published 4 new termite species in Taiwan, Neotermes koshunensis (Shiraki), Coptotermes formosanus (Shiraki), Odontotermes formosanus (Shiraki), and Pericapritermes nitobei (Shiraki). Oshima published a Taiwan termite report annual, since 1911 to 1915. Oshima (1911) describe a new Reticulitermes species, R. flaviceps (Oshima). Next year, Oshima (1912) documented 2 new termite species, Glyptotermes fuscus Oshima and Incisitermes inamurai (Oshima), and 2 new recorded termite species, Cryptotermes domesticus (Haviland) and Glyptotermes satsumensis (Matsumura). Oshima and Maki (1919) describe Sinocapritermes mushae as a new species. Another researcher Nawa (1911) reported 2 Nasutitermes species in Taiwan, N. parvonasutus (Nawa), and N. takasagoensis (Nawa). Holmgren (1912) documented a new recorded species, Prorhinotermes flavus (Bugnion and Popoff), which only distribute at south of Taiwan. Hozawa (1915) described a new species, Nasutitermes kinoshitai (Hozawa) and documented the recorded of Hodotermopsis sjostedti Holmgren according to Holmgren 1912, in Taiwan. Morimoto (1968) survey the Reticulitermes of Taiwan and Japan, documented a new record Reticulitermes species, R. leptomandibularis (Hsia and Fan). Tsai (2003) examined all termite species in Taiwan, and confirmed the invasive termite species, Coptotermes gestroi (Wasmann), was a new record in Taiwan. Liang et al. (2017) describe a new species when they survey the pangolin habitat, and name the new species, Stylotermes halumicus Liang, Wu and Li. Wu (2019) documented the new record of R. kanmonensis Takematsu in Taiwan, which could be distinguished from R. flaviceps with enteric valves, the structure at hindgut.

According to Wu and Li (2010) generalize the termite records in Taiwan, and reported that Taiwan had 4 families, 12 genera, and 17 species. Tsai (2003) had published the mtDNA COII sequence of 8 termite species in Taiwan: *Cr. domesticus, I. inamurai, Co. formosanus, Co. gestroi, R. leptomandibularis, O. formosanus, Na. parvonasutus*, and *Na. takasagoensis*. About half of Taiwanese termites does not have molecular data for taxonomic research, especially the termites with limited specific morphological characters, such as *Glyptotermes* and *Neotermes*. In addition to shortage of molecular data, so far, an overall morphological key of Taiwanese termites is still unavailable. In the past five year, ~ 5,700 termite samples were collected across Taiwan and now deposited at the Termite

Collection of the National Chung Hsing University. In this study, we re-examine all available termite samples in Taiwan. The checklist of Taiwanese termite species is updated to 22 species. We also provide the morphological key of Taiwanese termite base on both wing imagoes and soldiers.

Materials and methods

Sample collection site and collecting method

A total of 5700 samples were collected across Taiwan. All samples were preserved in 95% ethanol and deposited in the NCHU Termite Collection, Department of Entomology, National Chung Hsing University (NCHU), Taichung, Taiwan. Samples could be distinguished to genera by the key in Krishna (2013).

DNA extraction, amplification, and sequencing

Genomic DNA was extracted from the muscle tissue of both head and thorax using Puregene DNA Isolation kit (Gentra Systems, Minnesota, USA) and QuickExtract DNA extraction kit (Epicentre Biotechnologies, Madison, WI) according to the manufacturer's protocol. Mitochondrial genes, 16S rDNA, COI rDNA, COII rDNA, and ITS genes, were amplified for the species identification.

Measurement and photography

A total of 5 and 7 quantitative characters of soldiers and winged imago, respectively, were selected for measuring and photography, including maximum head length, maximum head width, maximum pronotum length, maximum pronotum width, antenna articles for both castes, and maximum forewing and hindwing length for winged imago only. All sample measurements were record by using a Leica M205 C stereomicroscope and a Leica MC170 HD digital camera with LAS software (version 4.4.0, Leica Application Suite, Wetzlar, Germany). The measurements were taken according to Roonwal (1969). The color of samples was examined under the microscope and compared using the Munsell color system (Munsell Color Company 1975, Kelly and Judd 1976) to determine hue, value, and chroma of the described character. To prepare the key of Taiwanese Termitoidae, morphological data of previous taxonomic studies including Li et al. (2009), Wu and Li (2010), Li et al. (2011), Chiu et al. (2015), Liang and Li (2016), Liang et al. (2017), and Wu et al. (2019), were taken into consideration.

Results and discussion

Based on comparison of morphological data and genetic sequence of four genes (mt 16S rDNA, mt COI rDNA, mt COI rDNA, and ITS genes), 22 termite species were found in Taiwan. Three new or new record species, *Glyptotermes* sp., *Neotermes* sp., and *Macrotermes barneyi* Light. The 22 termites species were classified into 5 families, and 14 genera according to Krishna (2013) (Fig. 1.): Archotermopsidae: *Hodotermopsis* (1 species); Kalotermitidae: *Cryptotermes* (1 species), *Glyptotermes* (3 species), *Incisitermes* (1 species), *Neotermes* (2 species); Stylotermitidae: *Stylotermes* (1 species); Rhinotermitidae: *Coptotermes* (2 species), *Reticulitermes* (3 species), *Prorhinotermes* (1 species); Termitidae: *Macrotermes* (1 species), *Odontotermes* (1 species), *Nasutitermes* (3 species), *Pericapritermes* (1 species), *Sinocapritermes* (1 species). We provided the morphological key of Taiwanese termites based on winged imago caste (Table 1.) and soldier caste (Table 2.) with 5 families, 14 genera, and 22 species. A checklist of Taiwanese termite included 20 described species was provided (Table 3).

Table 1. Key to Taiwanese termites based on the characters of imago.

1. Forewing scale distinctly overlapping hind wing scales. (Lower 2 termites: Archotermopsidae, Kalotermitidae, Stylotermitidae, Rhinotermitidae)

Forewing scale not overlapping hind wing scale. (Higher termites: 15 Termitidae)

Anterior margin of forewing with 3 or more sclerotized veins.

2.	Anterior margin of forewing with 2 sclerotized veins.	10
3.	Ocellus absent; cercus 3 to 8 articles; Antenna more than 21 articles.	Hodotermopsis sjostedti
	Ocellus present; cercus 2 articles; Antenna less than 21 articles. (Kalotermitidae)	4
4.	Media not sclerotized and running midway between Radius sector and Cubitus.	5
	Media running close and parallel Radius sector to tip of wing.	6
5.	Media bending up and joining Radius sector in middle or beyond middle of wing.	Cryptotermes domesticus
	Media running midway between Radius sector and Cubitus to tip of wing.	Incisitermes inamurai
6.	Radius sector with branches.	7
	Radius sector without branches. (Glyptotermes)	8
7.	Wings transparent. Cubitus with 4-5 branches sclerotized.	Neotermes koshunensis
	Wings brownish. Cubitus with 6 or more branches sclerotized.	Neotermes sp.
8.	Head capsule and abdomen light orange yellow to dark orange yellow.	Glyptotermes satsumensis
	Head capsule and abdomen darker reddish brown to black.	9
9.	Body length with wing more than 10 mm, head width more than 1.2 mm.	Glyptotermes sp.
	Body length with wing less than 8 mm, head with less than 1 mm.	Glyptotermes fuscus
10.	3 tarsi articles.	Stylotermes halumicus
	4 tarsi articles. (Rhinotermitidae)	11
11.	Wing with dense setae. (Coptotermes)	12
12	Wing with few or without setae.	13
12.	Ocellus spots present; dorsal view of abdomen and head capsule reddish brown.	Coptotermes gestroi
10	Ocellus spots not obvious or absent, dorsal view of abdomen light orange yellow, head capsule reddish brown.	Coptotermes formosanus
13.	Head capsule orange yellow.	Prorhinotermes flavus
1.4	Head capsule dark reddish brown. (Reticulitermes)	14
14.	Pronotum with dark reddish brown.	Reticulitermes leptomandibularis
	Pronotum light yellow.	Reticulitermes flaviceps, Reticulitermes kanmonensis
15	Body length with wing more than 25 mm, forewing length more than	16
13.	20 mm.	
	Body length with wing less than 15 mm, forewing length less than 13 mm.	17
16.	Wings membrane brownish.	Odontotermes formosanus
	Wings membrane yellowish.	Macrotermes barneyi
17.	Media no sclerotized. (Nasutitermes)	18
4.0	Media sclerotized.	20
18.	Pronotum width less than 1 mm, the shallow V-shaped mark not reach the middle of pronotum.	Nasutitermes parvonasutus
	Pronotum width more than 1 mm, the shallow V-shaped mark reach the middle of pronotum.	19
19.	Fontanelle oval. V-shaped mark on pronotum with 6-10 setae on central area.	Nasutitermes kinoshitai
	Fontanelle slit-liked. V-shaped mark on pronotum with no setae on central area.	Nasutitermes takasagoensis
20.	Fontanelle with invert Y-shaped.	Pericapritermes nitobei
	Fontanelle not obvious.	Sinocapritermes mushae

	Table 2. Key to Taiwanese termites based on the characters of s	oldier.
1.	Cercus 3 to 8 articles. Antenna more than 21 articles. Body length	Hodotermopsis sjostedti
	more than 15 mm. Head width more than 4 mm.	
	Cercus 2 articles. Antenna less than 21 articles.	2
2.	Pronotum as wide as head.	3
	Pronotum narrower than head.	9
3.	Fontanelle absent. 4 tarsi articles. (Kalotermitidae)	4
	Fontanelle present. 3 tarsi articles.	Stylotermes halumicus
4.	Head in front vertical. Mandible not obvious.	Cryptotermes domesticus
	Head in front not vertical. Mandible obvious.	5
5.	Head not phragmotic. Mandible length equal or longer than half of	6
	head length.	
	Head phragmotic or slightly phragmotic. Mandible length shorter	7
	than half of head length. (Glyptotermes)	
6.	3 rd article of antenna with broadest at apex. Anterior margin of	Incisitermes inamurai
	pronotum with V-shaped caved.	
	3 rd article of antenna without broadest at apex. Anterior margin of	Neotermes koshunensis,
_	pronotum without caved.	Neotermes sp.
7.	Lateral margin of pronotum curved.	Glyptotermes satsumensis
0	Lateral margin of pronotum straight.	8
8.	Head width less than 1.1 mm. Body length less than 6 mm.	Glyptotermes fuscus
0	Head width more than 1.2 mm. Body length more than 8 mm.	Glyptotermes sp.
9.	Head without rostrum tube. Mandible obvious.	10
10	Head with rostrum tube. Mandible not obvious. (<i>Nasutitermes</i>)	16
10.	Mandible lateral symmetry.	11
11	Mandible without lateral symmetry.	18
11.	Pronotum flat.	12
12	Pronotum saddle shaped.	19 13
12.	Head with sides subparallel. Head with sides rounded.	15
13	Labrum with obvious transparent sharp tip.	Reticulitermes leptomandibularis
13.	Labrum without obvious transparent tip.	Reticulitermes flaviceps,
	Labrum without obvious transparent up.	Reticulitermes flaviceps, Reticulitermes kanmonensis
1/	Fontanelle at head front.	15
14.	Fontanelle at middle of head capsule.	Prorhinotermes flavus
15	Fontanelle with 1 seta at each side.	Coptotermes gestroi
15.	Fontanelle with 2 setae at each side.	Coptotermes formosanus
16.	Tip of rostrum surrounded with 2 to 4 setae. Head capsule dark	Nasutitermes takasagoensis
10.	brown.	rusumermes tunusugoensis
	Tip of rostrum surrounded with >20 setae. Head capsule orange	17
	yellow to yellowish brown.	-,
17	Rostrum with >100 setae covered from tip to base. Head width less	Nasutitermes parvonasutus
1/.	than 1.02 mm.	2. a.sumo mos pen ronasums
	Rostrum with dense setae limited at tip. Head width more than 1.02	Nasutitermes kinoshitai
	mm.	

Pericapritermes nitobei

Sinocapritermes mushae

Macrotermes barneyi

Odontotermes formosanus

18. Left mandible S-shaped curved.

Left mandible slightly twist.

minor soldier.

19. Left mandible with one marginal teeth.

Left mandible with marginal teeth serrated-like. With major and

Table. 3. Checklist of termites in Taiwan.

	Family, genus, and species names	Chinese common names
Arch	otermopsidae 原白蟻科	
1	Hodotermopsis sjostedti Holmgren, 1911	山林原白蟻
Kalote	ermitidae 木白蟻科	
2	Cryptotermes domesticus (Haviland, 1898)	截頭堆砂白蟻
3	Glyptotermes fuscus Oshima, 1912	黑樹白蟻
4	Glyptotermes satsumensis (Matsumura, 1907)	薩摩樹白蟻
5	Incisitermes inamurai (Oshima, 1912)	稻村楹白蟻
6	Neotermes koshunensis (Shiraki, 1909)	恆春新白蟻
Stylot	ermitidae 木鼻白蟻科	
7	Stylotermes halumicus Liang, Wu, and Li, 2017	穿山甲木鼻白蟻
Rhino	termitidae 鼻白蟻科	
8	Coptotermes formosanus Shiraki, 1909	台灣家白蟻
9	Coptotermes gestroi (Wasmann, 1896)	格斯特家白蟻
10	Prorhinotermes flavus (Bugnion and Popoff, 1910)	黄原鼻白蟻
11	Reticulitermes flaviceps (Oshima, 1911)	黄肢散白蟻
12	Reticulitermes leptomandibularis Hsia and Fan, 1965	細顎散白蟻
13	Reticulitermes kanmonensis Takematsu, 1999	關門散白蟻
Termi	tidae 白蟻科	
14	Macrotermes barneyi Light, 1924	黄翅大白蟻
15	Nasutitermes kinoshitai (Hozawa, 1915)	木下象白蟻
16	Nasutitermes parvonasutus (Nawa, 1911)	小象白蟻
17	Nasutitermes takasagoensis (Nawa, 1911)	高砂象白蟻
18	Odontotermes formosanus (Shiraki, 1909)	台灣土白蟻
19	Pericapritermes nitobei (Shiraki, 1909)	新渡户歪白蟻
20	Sinocapritermes mushae (Oshima and Maki, 1919)	霧社歪白蟻



Fig. 1. Taiwanese termites with alates, soldiers, and workers. Hodotermopsis sjostedti (1), Cryptotermes domesticus (2), Glyptotermes fuscus (3), Glyptotermes satsumensis (4), Incisitermes inamurai (5), Neotermes koshunensis (6), Stylotermes halumicus (7), Coptotermes formosanus (8), Coptotermes gestroi (9), Prorhinotermes flavus (10), Reticulitermes flaviceps (11), Reticulitermes

leptomandibularis (12), Reticulitermes kanmonensis (13), Macrotermes barneyi (14), Nasutitermes kinoshitai (15), Nasutitermes parvonasutus (16), Nasutitermes takasagoensis (17), Odontotermes formosanus (18), Pericapritermes nitobei (19), Sinocapritermes mushae (20), Glyptotermes sp. (triangle), Neotermes sp. (Circle)

Conclusion

A total of 22 good termite species classified into 5 families and 14genera were confirmed in this study. The identification key based on both winged imago and soldier castes are available for the first time. Taiwanese termite checklist with Chinese common names was also provided.

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O 09

Outbreak of Timber Pests in Philippines: As a Consequence of Frequent Typhoons

by

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Abstract

Evidence of climate change and its impact are now visible in many parts of the world. This report presents impact of natural disasters in particular typhoons on trees as it passes across the Philippines archipelago every year. Typhoons damage and destroy large number of trees which soon rot under moist and warm conditions. Wood beetles and termites find these sources very quickly, breaking down the dead trees. While doing so the pests establish new and healthy population in and around the devastated sites. Often events like these lead to pest build-up and lead to outbreak condition. This report presents a preliminary evaluation on pest density of two tree pests such as mound building termite Macrotermes gilvus and Rhinoceros beetle, Oryctus rhinoceros after Typhoon Hainan in 20013 and their control strategies.

Keyword: Climate change, pest outbreak, timber pest

Introduction

Evidence of climate change and its impact are now visible in many parts of the world. Examples of this can be had from changes in distribution, density or behavioural patterns of pests. Reports of new pest occurrence, such as reports of insect in previously unrecorded parts of the world are now attributed to climate change (Dhang, 2016).

Sims and Appel (2013) reported weather-influenced effects on timber pests from St. Louis area of USA. They reported termite swarming unusually early in the year, following the extremely mild winter of 2011-12. Quarles (2007) suggested temperature increases in the USA to favour warm weather pests such as ants, termite pests, clothes moths, flies, mosquitoes, fleas, stored product moths, wood boring beetles, and bed bugs. Evidence of termites in previously unrecorded latitudes are also coming to light now. Reports of tropical species, such as *Coptotermes gestroi*, becoming established in the subtropics (Grace, 2006); and subtropical species *C. formosanus* expanding their range northwards into more temperate areas (Jenkins et al., 2002) substantiate the role climate could play in timber pest distribution.

In colder areas of North America such as Wisconsin and southern Canada, populations of *Reticulitermes flavipes* appear to be gradually expanding their range and even swarming under natural outdoor conditions (Arango et al., 2014). However, colony growth and slow dispersal without the need for swarming has been associated with these northern colonies on the fringe of their range (Arango et al., 2014). Swarming events can occur in all months of the year. With increasing temperatures, both the total yearly number of swarms and frequency of swarms in cooler months can be expected to increase.

This paper reports a previously unreported aspect of climate change namely typhoon on tree pests. Though the observation is preliminary, the possibility of such an event is considered significant.

Materials and methods

The experimental site was the city of Tacloban Philippines, the epicenter if the Typhoon Hainan. The study method used were ocular surveys, published population monitoring methods and insect identification method. Dhang, (2014) method was used for monitoring population of mound building termite *Macrotermes gilvus* and Indriyanti (2018) method was used for Rhinoceros beetle, *Oryctus rhinoceros*.

The typhoon made a landfall in Nov 2013 and the population studies were conducted March 2014, Sept 2014 and March 2015. Fresh monitoring stations were installed during the months mentioned and each station was monitored for 60 days on a bi-monthly basis. The reason for discontinuous data collection was due to the fact that the site was not easily accessible throughout the year. A second site approximately 300 km south-west of Tacloban, namely Tagbilaran with similar geography and lesser impact was used as a control site. Control site had the same number of monitoring stations as the test site.

Results and discussion

Natural disaster and damaged trees

Natural disasters in particular typhoons leave destroyed and damaged trees as it passes across the Philippines archipelago every year. The dead trees soon rot under moist and warm conditions. Wood beetles and termites find these sources quickly, breaking down the dead stems to soil. While doing so the pests establish new and healthy population in and around the devastated sites. Often events like these lead to pest build-up to large sizes and outbreak are expected.

In addition to the dead trees which are often cut and salvaged for use, there is always a large quantity of dead tree debris left around, in particular the tree stumps which are hard to remove and process. These stumps often remain untouched or cut to size and buried. Large population of termites and wood infesting beetles have been observed in these stumps at times. At times these stumps are turned into termite mounds by *Macrotermes gilvus* species.

The magnitude of the above-mentioned issue can be judged by one single typhoon Haiyan which struck the island nation in Nov of 2013. Aftermath of which national and international agencies, including the Philippine government had to rush to salvage the wood from some 15 million coconut trees that were destroyed and damaged. The agencies were challenged by the scale of processing due to lack of adequate logistics. The numbers of chainsaws and handlers were not enough to timely harvest the timber.

A report published by UNDP (UNDP, 2014) had given an estimate how difficult was to salvage the timber from the downed trees in this single incident. The Philippines Coconut Authority is the premier institution which oversees these acts. The agency estimated that after the typhoon Haiyan, at the rate of 10 trees per chainsaw processed daily, it would mean some 21,000 trees can be turned into timber every day. But even at this rapid pace it would take nearly two years to just process the 15 million completely damaged coconut trees. This does not take into account the estimated 18 million less damaged ones that also require processing.

This act is further hampered by the fact that the Department of Environment and Natural Resources (DENR) in the Philippines, which controls chainsaw registration under legislation passed to prevent illegal logging, makes the work of salvage operation slower. Private chainsaws cannot be brought into the country without passing the regulatory authorities. Also finding and training chainsaw operators is another limitation due to an almost 20-year <u>nationwide ban on coconut-logging</u>. The law allows trees can be felled only if damaged by natural causes such as pests, typhoon or lightning.

In these circumstances it generally takes months and even years to clear the wood debris. Chances of incomplete clearing is often high particularly knowing the terrain and remoteness of some of the devastated localities affected by typhoons. Clearing the land of any rotting wood is critical because it attracts insect pests which build new population. These population can attack and kill newly planted seedlings, threatening the next generation of coconut trees. Re-establishing the coconut plantations, which can take more than a decade to cultivate, is vitally important for farmers, who lose their cash crop.

In addition to this the new houses being built initially for relief and then for resettlement of the displaced people in the territories use vast amount of coconut lumber. These lumbers coming from the downed trees hardly get the necessary time and resources for wood treatment. Thus, these lumbers stand susceptible to easy pest attacks and often are destroyed, needing periodic replacement over time.

Pests of coco lumber

In Philippines coconut wood is considered a "poor man's lumber" and it mostly sold and used in an untreated form. Occasionally a simple layer of paint of oil is used, which does not help to extend the service life to any great extent. Once moist or wet, these lumbers are estimated to last at most 3 years. Untreated coco lumber has limited natural durability, suggesting it cannot be used in weather-exposed conditions (Keating & Bolza, 1982). However high density, dry coco lumber is not susceptible to powder-post beetle (*Lyctus* species) and is suitable for use in fully protected applications. But untreated coco lumber is not resistant to termite attack, both from subterranean and drywood types.

Natural disaster and pest outbreak

One of the major predictions of climate change models is that extreme environmental events will become more frequent and will increase according to model predictions (IPCC, 2013). However, its potential impacts often focus on infectious and vectored diseases. Its influence on aspects such as building materials, quality of urban structures, non-vectors, nuisance pests are often marginalized and neglected (Dhang, 2016).

There is little or no history on wood related pest data available in the aftermath of natural disasters. But it is clearly predictable that left over wood, tree stumps, saw dusts after intensive sawing activities leave enough cellulosic residues in the soil for timber and tree related pests to be attracted and thrive. Ground surveys by the author in the areas have shown flourishing populations of Rhinoceros beetle, *Oryctus rhinoceros* in rotting woods and in live palm trees, and subterranean termites, *M. gilvus, Microtecrotermes losbanosensis, Nasutitermes lagunensis* on fallen and sawed tree stumps.

The activities were comparatively much higher in the aftermath of the typhoon (Table 1)

Population study	March 2014	Oct 2014	March 2015	
Number of In-ground stations				
intercepting M. gilvus population. A	25 (20)	202 (40)	242 (58)	
total of 250 stations were installed.				
Average number of <i>O. rhinoceros</i>				
caught in pheromone baited trap. A	4 (8)	18 (6)	16 (10)	
total of 25 traps were installed				
The number in parentheses indicate data in control site.				

Table 1: Population data of *M. gilvus* and *O. rhinoceros* in the aftermath of typhoon Hainan in Tacloban Philippines.

It is also imperative that all types of structures such as shelters, homes, commercial buildings in the surrounding areas will face the consequences of these pests. This was soon evident by a follow up survey done over 12 months on pest control operators in the region. Most reported an increase in damage reports over the period. It is also reasonable to accept that surging pest infestation and outbreaks would bring in use of more pesticide in the environment which adds to the overall concern.

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O 10

The Genome of *Prorhinotermes flavus*: A Termite Species with High Salinity Tolerance

by

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Abstract

Prorhinotermes flavus is a dampwood termite with unique habitats. They are found in coastal areas of islands or continents ranging over the Indian and the Pacific Ocean including Taiwan. Though a few genomes of termite species have been sequenced, that of a high salinity tolerant termite species has not been explored. Here, we performed de novo assembly of P. flavus by using a hybrid assembly pipeline. The high-quality genome was obtained by: 1. Building a genome draft by 10X Genomic Supernova assembly, 2. Scaffolding by SSPACE (Illumina mate-pair) and SSPACE-LongRead (PacBio longread), 3. Error correction using Illumina reads by Pilon, 4. Gap Filling and Inter Scaffolding by our developing tool Tinker. The final assembly of the P. flavus genome is about 969 Mb. The scaffold N50 length is 7 Mb, and 170 scaffolds can comprise 90 % of the genome. Accordingly, we established a P. flavus database that provides a user-friendly interface for visualization of the genomic structure together with the information of transcriptome profiling among different castes and sexes. While P. flavus can be easily cultured on Petri dishes and produce a substantial amount of progenies, our high-quality genome assembly could grant P. flavus a great potential to be an emerging model of eusocial insects for exploring basic biological questions related to caste development, developmental processes of hemimetabolous insect, and termite eusociality.

Keywords: genome, long-read sequencing, *Prorhinotermes flavus*, salinity resistance

Introduction

Termites are powerful wood degraders and can serve as an efficient biomass converter for producing bioenergy. Biologists, therefore, are highly interested in their genome content. *Prorhinotermes flavus* is a dampwood termite with unique habitats. They are found in coastal areas of islands or continents ranging over the Indian and the Pacific Ocean including Taiwan. Though a few genomes of termite species have been sequenced, that of a high salinity tolerant termite species has not been explored.

The first termite genome sequenced is the dampwood termite *Zootermopsis nevadensis*, which is a primitive lower termite (Terrapon et al., 2014). The genome of *Z. nevadensis* is estimated to be of 562 Mb. Intriguingly, in the genome of *Z. nevadensis*, the number of opsin orthologous genes are greatly reduced as two (Terrapon et al., 2014). The reduction of opsin family genes could reflect the physiological adaptation of their living in darkness during most of their lifetime. Moreover, the gene family expansion related to odorant reception is also evident, suggesting that chemical communication

could play a crucial role in eusociality (Terrapon et al., 2014). Almost in the same year, the 1.31 Gb draft genome of a fungus-growing termite, Macrotermes natalensis, was also published (Poulsen et al., 2014). In combing the metagenome analysis, the authors found that there is a functional complementarity between the domesticated fungus and gut microbiotas of worker and soldier for the enhancement of carbohydrate decomposition (Poulsen et al., 2014). Although these two published genomes represented opposite ends of the social complexity spectrum, the lower termite Z. nevadensis and the higher termite *M. natalensis*, a dramatic differences in gene composition is not found; except that the abundant presence of transposable elements could explain the larger genome of M. natalensis (Korb et al., 2015). The authors argue that the two termite genomes could only provide limited resolution unless more termite genomes are available. In 2018, the 2 Gb genome of the German cockroach, Blattella germanica, and the 1.3 Gb genome of the drywood termite Cryptotermes secundus were published (Harrison et al., 2018). The uncovering of the cockroach genome and the drywood termite, which evolutionarily positioned in between Z. nevadensis and M. natalensis, provides a good chance to perform comparative genomic studies. The results indicate that the higher termite M. natalensis presents a sophisticated evolution of genes linked to chemical communications, and this could be correlated to the highly eusocialization of this species (Harrison et al., 2018).

In this report, we provide the genomic information of the lower termite *P. flavus* with high quality by adopting third-generation sequencing techniques. This genome is not only the fourth termite genome that evolutionarily positioned in between the *M. natalensis* and *C. secundus*, it also provides a chance to discuss how the termite genome evolves and adapts the salty habitats.

Materials and methods

Genomic DNA isolation, total RNA extraction, and library preparation

For de novo genome assembly, 200 heads of male apterous neotenics were collected from a six-year Lab colony of P. flavus. The genomic DNA was extracted by using NTES (100 mM NaCl, 50 mM Tris pH 8.0, 50 mM EDTA, 1% SDS, and 0.25 mg/ml proteinase K) buffer and phenol/chloroform purified. The genomic DNA was used for the construction of a 20-Kb PacBio library, six mate-pair libraries (1-Kb, 3-Kb, 5-Kb, 8-Kb, 12-Kb, and 20-Kb), and a 10x Genomics library. SMRT sequencing was performed on the Sequel System with a total of eight SMRT Cells. For comparing the gene expression profiles among embryos, castes, and sexes in P. flavus, 14 RNA-sequencing (RNA-Seq) libraries were obtained from mixed-stage of embryos, ten pools of different castes and sexes including pseudergate, presoldier, soldier, male apterous neotenics, female apterous neotenics, male nymph stage 1, male nymph stage 3, female nymph stage 1, female nymph stage 3, male alate, female alate, male brachypterous neotenics, female brachypterous neotenics. Total RNA was extracted by using the Quick-RNA Miniprep kit (Zymo Research). The RNA samples were quantified and their qualities were examined by the BioAnalyzer RNA 6000 Nano Kit (Agilent). All libraries were constructed using TruSeq Stranded Poly-A RNA Library Prep Kit (Illumina). The paired-end 2 × 150 nt sequencing was conducted on the Illumina HiSeq 2000. All the sequencing was performed at the NGS High Throughput Genomics Core Facility at Academia Sinica, Taiwan.

Sequencing strategy & hybrid Genome Assembly

The *de novo* assembly of *P. flavus* was conducted by combing the current advanced technologies, including Illumina (both paired-end and mate-pair), PacBio long reads, and 10X Genomic linked reads with hybrid assembly pipelines (Draft assembly from 10x Genomics + Scaffolding + GapFilled + Error correction + InterScaffolding) by Supernova, SSPACE, SSPACE-LongRead, Pilon, and our developing tool Tinker. The established and constructed a *P. flavus* genome is 969 Mb with a scaffold N50 length over 7 Mb.

Gene prediction and annotation

The MAKER2 (https://www.yandell-lab.org/software/maker.html) pipeline was used to perform gene prediction. The input datasets include the 14 *P. flavus* transcriptomes of Illumina RNA-Seq and PacBio Iso-seq, protein sequences of *Zootermopsis*, *Cryptotermes*, and *Macrotermes*, and the pre-

existing *Drosophila* gene model. The predicted ORFs were annotated with homologs in the most updated nr database (April 2019) with GhostX (Suzuki et al., 2014). Protein sequence features, including signal peptides, transmembrane domains, and domains that described in Pfam database, were detected by SignalP (Petersen et al., 2011), TMHMM (Krogh et al., 2001), and HMMER (3.1b2), respectively (Mistry et al., 2013). Gene Ontology (GO) annotation was granted to genes with detectable Pfam domains, according to Pfam2GO. Furthermore, we mapped the protein-coding genes to the canonical pathway database KEGG pathway using KEGG Automatic Annotation Server (Moriya et al., 2007), setting the parameters for only mapping to related prokaryotic database in single-directional best hit (SBH) mode.

Estimation of Expression profiling

According to the assembly with GTF (Gene Transfer Format), raw reads generated from RNA-seq can be estimated by their expression profiling via an intuitive graphical interface in Docexpress (https://hub.docker.com/r/lsbnb/docexpress_fastqc).

Web framework construction

MOLAS is constructed using LAP system architecture (Ubuntu 14.04, Apache 2.04, PHP 5.1) with the Bootstrap 3 CSS framework (http://getbootstrap.com/), jQuery1.11.1, and jQuery Validation v1.17 to provide an intuitive user experience. The whole system runs in a virtual machine on the cloud infrastructure of the Institute of Information Science, Academia Sinica, Taiwan. The analysis process is implemented in scripts written in R (3.4.2).

Results and discussion

We performed de novo assembly of P. flavus by combing the advanced technologies including Illumina (both paired-end and mate-pair), PacBio long reads, and 10X Genomic linked reads. With hybrid assembly pipelines, we established and constructed a *P. flavus* genome with a size of 969 Mb. The genome size of *P. flavus* is smaller to the drywood termite *C. secundus* (1.3 Gb) and *M. natalensis* (1.31 Gb), but larger than that of Z. nevadensis (562 Mb). The high quality of P. flavus genome is shown by its scaffold N50 of the assembly, which is over 7 Mb by comparing to the N50 assembly of Z. nevadensis (0.76 Mb), C. secundus (1.18 Mb) and M. natalensis (2 Mb). Moreover, 170 scaffolds can comprise 90 % of the P. flavus genome. This indicates that 4-5 scaffolds could represent 90 % length of a chromosome. By using sequences of order Insecta as a database in BUSCO (Benchmarking Universal Single-Copy Orthologs), the score of genome assembly is up to 98.6 % with gaps less than 1.2 %. To evaluate the integrity of the *P. flavus* genome, we showed that all of the *Drosophila* Hox gene orthologues could perfectly found and aligned in a single scaffold. The P. flavus genome contains 19,886 protein-coding genes. The total number of protein-coding genes is more than that in other published termite genomes (Z. nevadensis: 15,459; C. secundus: 18,126; M. natalensis: 16,140) but less than that in cockroach genome (B. germanica: 29,216). According to the 14 transcriptome profiling of different castes and sexes obtained by either Illumina platform or PacBio IsoSeq, we annotated the P. flavus genome with transcript evidences and established a P. flavus genome database by MOLAS which provides a user-friendly interface with visual examination of the genomic structure together with the information of transcriptome profiling in various biological conditions. Our preliminary results showed that P. flavus could tolerate high salinity at levels that other termite species that die shortly upon saltwater feeding. Accordingly, we also executed a transcriptome analysis of a time-course experiment of the salinity tolerance in P. flavus for exploring the mechanism underlying the salinity tolerance of *P. flavus*.

Conclusion

In this report, we observed that the genome size of *P. flavus* is about 969 Mb with scaffold N50 over 7 Mb. Moreover, 170 scaffolds can comprise 90 % of the *P. flavus* genome. In addition to the high salinity tolerance, *P. flavus* can be easily cultured on Petri dishes and produce a substantial amount of

progenies. With the high-quality genome, *P. flavus* is granted a great potential to be an emerging model of eusocial insects for exploring basic biological questions related to caste development, developmental processes of hemimetabolous insects, and termite eusociality.

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O 11

Quantitative Analysis of "Termite Frass" and "Termite Droppings" Using Decayed and Undecayed Food

by

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Abstract

To reveal interaction between environmental microbes and termite and its gut microbes, we performed quantitative analysis of eating activity of termite against decayed and undecayed food. For this aim, we proposed "Pseudo round timber model" consist from paper or wood and we successfully decayed it as uniform food for termite. For quantitative analysis of termite feeding activity, we proposed "eating-excretion activity" as the number of droppings. Termites prefer decayed food to undecayed food. When termites eats decayed food, the number of droppings was kept about one dropping high level than they ate undecayed food and the termite body weight was increased and the number of protists in termite gut was also increased. But the amount of frass, we defined it as food shavings not include feces, was not proportional to the number of the droppings, that means frass did not reflect correct feeding amount. These results suggest that termite may use environmental microorganisms positively to decompose plant biomass. We could consider this "termite – gut microbes – environmental microbes" interaction as not strong but "gentle and moderate symbiosis" in the wide sense.

Keywords: damp-wood termite, Hodotermopsis sjostedti, feces, symbiosis

Introduction

It is well known that termite can decompose plant biomass with its gut microbes (Ohkuma 2008), but it's not the case that they can eat any type of wood. They are particular about their food (McMahan 1966, Bustamante and Martius 1998), especially important is the food decay level (Hendee, 1935, Cornelius 2012, Gazal 2012). For example, the famous house pest in Japan, *Coptotermes formosanus* and *Reticulitermes speratus* are often found at the rotting materials in the house. And dampwood termite, *Hodotermopsis sjostedti*, is also often found in the deeply rotten wood in the forest. These termites are not found in the fresh and unrotted wood. It seems that lower termite also uses environmental microbes to make their particular rotten food like the higher termite, *Odontotermes formosanus*, that positively use Termitomyces fungi living outside of the termite body.

To reveal the interaction between environmental microbes and termite and its gut microbes, it is important to prepare uniform food for quantitative analysis of the termite digestion system. But it is often difficult to make uniform rotted wood for termite food since fungus invasion and subsequent decaying of wood is dependent on wood structural complexity. Even thought we have a rotted piece of wood, it is difficult to cut it in same size with same decay condition for lab level experiment.

Therefore, the purpose of this study was (a) the quantitative analysis of termite eating activity compared to decayed and undecayed food. For this aim, we proposed that (b-1) the way how to prepare the decayed food for termite and (b-2) the way how to quantify the amount that termite ate.

Materials and methods

Preparation of damp wood termite

We collected damp wood termite *Hodotermopsis sjostedti*, from Yakushima at Kagoshima prefecture in Japan. We kept them in plastic containers with wood which the termite lived in, until we performed experiments.

Quantification of eating amount

We performed "eating-excretion activity" by counting the number of termite droppings. To confirm the validity of the "eating-excretion activity" method, we prepared nine containers. Each container had ten termites and wood from their nest as their food. We set three containers each at different degree of temperature (25, 15, and 7 °C), and we counted termite droppings everyday during three weeks.

Preparation of rotted food for termite

We prepared filter papers and thin wood as the food of termite. The half of them was used for the next step as undecayed food. The other half of them were bundled into hundred pieces each packed not to fall apart and we put it on the high termites activity area, which is at the Living Sphere Simulation Field, RISH, Kyoto University sited at Kagoshima prefecture in Japan. These woods and papers were allowed to decay by environmental microorganisms and we recovered them after two years. These samples were then dried in laboratory at room temperature.

Measurement of feeding activity against different food

We fed termite on the 4 types of food, which were two types of conditions, decayed/undecayed and two types of materials paper/wood. The experiment was performed triplicate, so we totally handled 12 containers. In each container ten termites and food were put and these were kept in 25 °C. Water was added to the food every day not to be dried the environment and within two or three days the food in the container were replaced on new food that was moistened with a fixed amount of water. The termite droppings were counted every day during three weeks. In addition the frass, which was food shavings not include feces, was collected and measured its weight. Termite body weight and death rate were also recorded.

Population dynamics of termite gut protists

We prepared another 16 containers (4 food types, 4 sampling times) for analysis of termite gut microbes. On the day-0, 7, 14, 21, we dissected the termite gut and collected gut contents. Two representative protists in the gut, *Trichonympha* sp. and *Eucomonympha* sp. were counted under the microscope.

Results and discussion

At first, we confirmed if we could estimate the "eating-excretion activity" by counting the number of termite droppings. We set up the termite container at different temperature with termite favorite wood as food that was also used as their house. Since it was known that termite activity is increased within this temperature range, it was expected that "eating-excretion activity" would be increased as the temperature goes up. In the results, the higher temperature led the much number of droppings. Therefore, we considered that the number of termite droppings would be a good index of "eating-excretion activity".

Here we proposed "Pseudo round timber model" consist form bundled papers or thin woods to prepare the uniform rotted food for termite. We decayed this model timbers by the environmental microorganisms in the forest that is known as the high termites activity field in Japan. Because each paper or wood sheet was decayed in the almost same manner, the model timber as a whole was almost uniformly decayed. Since we could easily peel off and broke the paper or the wood sheet on this model timber, it was easy to prepare uniform food for termite experiment in lab level. The "eating-excretion"

activity" showed the actual eating and excreting amount as the number of termite droppings, it is useful for quantitative analysis of termite digestion activity.

Then we fed termite the food, decayed/undecayed paper/wood, for quantitative analysis of "eating-excretion activity". Termites preferred wood to paper and they ate much decayed food than undecayed to a statistically significant level. Through out the experimental period, the number of droppings was kept about one dropping high level than they ate undecayed food. The termite body weight increased when they ate decayed food.

The absolute dry weight of paper/wood may be one of the indicators to quantitate how much termite eats the food (Uchima and Grace 2003, Hadi 2014), but the weight change would be really small in the case of laboratory small size experiment. And you may feel the amount of the frass was also usful as the indicator of termite activity, but it was not directly indicate termite digestion activity because in any case the frass was the food left over. In fact if we compared the relationship between the weight of frass and the number of droppings, there was no correlation. That was the reason why the frass weight was not suitable for estimating the termite "eating-excretion activity".

In addition, the number of protists in termite gut also increased if termite ate decayed food. This tendency is particularly noticeable with *Trichonympha* sp. more than *Eucomonympha* sp.

Conclusion

We proposed and validated "Pseudo round timber model" to prepare the decayed food for termite and we also proposed the usefulness of "eating-excretion activity" to quantify the amount that termite ate. We performed the quantitative analysis of termite eating activity compared decayed food and undecayed food. As the results, we indicated that termites ate and excreted decayed food more than undecayed food. It seems that the environmental microbes may help termite to decompose biomass.

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Distribution of *Odontotermes hainanensis* (Insecta: Isoptera) on Three River Dike Systems in the Northern Vietnam

by

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Abstract

Dike soil are usually taken from in-site along the river. Thus, soil is heterogenerous in different sites hence it can affect the distribution characteristics of termites in the dike. This study was carried out to seek the influence of environmental factors (humus content, pH index and salinity) of dike soil to the density of termite nests on three river dike systems in the Northern Vietnam. The results showed that humus content and pH index were not a direct factor deciding the distribution of *O. hainanensis* but the salinity of the dike soil had a role to the existence of termite nests.

Keywords: Termites, Isoptera, Distribution of termite, Vietnam river systems

Introduction

Red river, Thai Binh river and Ma river are three river systems with large basins (about 170,000 sq.km) in Vietnam. These three river systems have a river bed slope in the small delta (0.3 m/km on average), thus the river often causes major floods in the Northern Delta and the old Region IV. Because of the small slope of the river bed, the river meanders a lot and generates many tributaries making the dike system very long.

In the history of Vietnam, many dike-breaks have occurred, causing great losses like dike-breaks in 1945; some cases of dike- break of Thao river, Red river, Thai Binh river and Chu river in 1971 and 1975. In 1985 and 1986, happen the dike-break of Red river, Day river and Hoang Long river in Hanoi, Nam Dinh and Ninh Binh. Since then, small incidents occur every year such as leak, penetration, effervescent circuit, effervescent well etc., making the dikes weak and they are easy to break in the flood season. One of the most obvious reasons of causing dike damage and deterioration of the dike's safety is the infestation and nesting of termite in the dikes.

Previously, there were a number of studies relating to dike damage by termite in Vietnam, the most notably works are the works of Nguyen Duc Kham (1976), Nguyen Le (1976), Le Van Trien (1999), Bui Cong Hien et al (2000), Trinh Van Hanh (2008). The results of the studies above focused on termite composition and nest structure in certain dike section; how to find and excavate termite nests by manual methods. A series of issues such as composition of harmful termite species throughout the dike system of the main rivers; relationship between a number of specific environmental factors of different dike areas on different dike systems with species composition, density and distribution of termites have not been adequately studied. Understanding the behavioral changes of termite species under different dike environment conditions will relate to the organization, deployment, and supplement of more appropriate preventive measures.

Material and methods

Time and location of research

The research was conducted in 3 years from 2015 - 2018. The basic investigation work was carried out at the dike sections representing the midland, delta and coastal areas of all 3 systems of Red river, Thai Binh river and Ma river (Appendix 1).

The handling, storage and analysis of samples were implemented at the Laboratory of Institute of Ecology and Works Protection.

Methods of research

Method of sampling

Termite sampling: termite samples were collected along the route and according to the method of Nguyen Duc Kham (1971). The survey routes can have different length, ranging from 1 to 5 km long, each dike area may have many routes passing through the habitats near house, garden, field, etc. The sampling tools include hoe, shovel, screwdriver, trowel, soft clamps, plastic jar.

The termite samples include all individuals of different levels encountered in dead wood, dry twig, grass carpet, ground, in the nest... during the research.

Quantitative termite sample: On each dike area of each river system, collect samples on 5 dike sections, each dike section was 1km long, not consecutive. The termite samples were formed by 700 alcohol solution at the sampling location.

Method of sample identification

Determine spicies name by morphological method according to the documents of Ahmad (1958, 1965) Huang Fu Sheng (2000); Roonwal (1969); Thapa (1981).

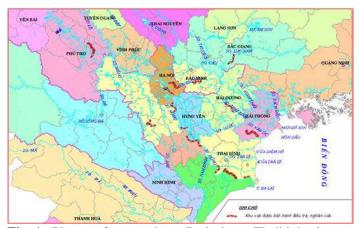


Fig 1. Places of research at Red river, Thaibinh river and Ma river systems "Map resourse: Government announcement 2014"

Method of researching soil samples

The soil samples on the dike were collected and analyzed to determine the physical and chemical properties (humus index, pH, salinity). Soil samples at the depth of about 10-15 cm from the ground were taken. The soil samples were collected at the edge of the dike roof about 0.5 meters, between the dike roof and about 0.5 meters away from the foot of dike roof. A sample was taken at 3 points on the same altitude with a distance of about 200 meters between the points. The soil samples were stored in 2 layers of plastic bags, labeled and had the document for describing the sampling location. pH target of soil was measured on Metrohm 620 glass electrode pH meter. Organic target (humus) was determined according to Tiurin method. The salinity index of soil was determined by the weight

method with the rate of extracting soil fluid by distilled water according to the rate: soil/distilled water = 1: 5.

Results and discussion

The distribution characteristics of *O. hainanensis* on the Red river dike system On the Red River dike system, the density of *O. hainanensis* termite nests fluctuates from 12.6 to 24.2 nests/km of dike; the highest one is in the dike of delta area, the second one is coastal dike and the lowest one is in midland dike. Through testing, it was confirmed that the density of termite nests in the dike of midland area was lower than that in the delta and coastal areas (table 1).

Table 1. Medium density of *O. hainanensis nest* according to area in dike systems

Dike systems	Area	Numnber of sections for survey (km)	Density (nest/km)	Reliability P
	Midland	5	12.6 ± 8.7	P(M-D) < 0.05
Red river	Delta	5	24.2 ± 2.0	P(D-C) > 0.05
	Coastal	5	16.8 ± 6.9	P(M-C) > 0.05
Theihinh	Midland	5	0	
Thaibinh river	Delta	5	5.4 ± 1.0	P(D-C) > 0.05
river	Coastal	5	8.4 ± 3.7	
Ma river	Midland	5	11.2 ± 5.1	P(M-D) < 0.001
	Delta	5	29.2 ± 8.4	P(D-C) < 0.001
	Coastal	11	9.6 ± 4.5	P(M-C) > 0.05

Note: M: Midland; D: Delta; C: Coastal

A total of 82 soil samples in 9 dike areas were analyzed some basic physical and chemical indicators namely humus, pH and salinity. The result values were tested by t-Test statistical function.

The analysis result of physio-chemical properties of the dike soil such as humus, pH (table 2) shows that the humus of the Red River midland is low (0.541%) and increases from the midland to the coastal area (1.558%). Based on the data of termite nest density and humus in the Red River dike, there is no positive correlation, that means it is not true that the higher the humus content in the soil is, the greater the termite nest density will be, it is clearly shown when the termite nest density in the Red River delta dike is the highest (24.2 nests/km) with the humus of 1.029%, higher than the termite nest density in midland dike and coastal dike with smaller humus in midland dike (0.541%) and even when the humus is greater in the coastal dike (1.558%). Like this, it does not mean that humus factor does not affect the distribution of *O. hainanensis* species, because humus was the main food of this species (Eggleton et al. (1997). But with this result, it can be seen that in the humus range of dike soil from 1.029% or more, the humus is not the main factor deciding the density of *O. hainanensis* termite nests on Red River dike.

Table 2. Humus content of soil in dike areas

Table 2. Humas content of son in dike areas				
Dike systems	Area	Number of soil samples tested	Humun content (%)	Reliability P
	Midland	9	0.541 ± 0.115	P(M-D) < 0.05
Red river	Delta	9	1.029 ± 0.173	P(D-C) < 0.05
	Coastal	8	1.558 ± 0.266	P(M-C) < 0.05
	Midland	9	0.810 ± 0.138	P(M-C) < 0.05
Thaibinh river	Delta	10	1.530 ± 0.269	P(D-C) > 0.05
	Coastal	8	1.273 ± 0.469	P(M-C) > 0.05

	Midland	9	1.195 ± 0.312	P(M-D) > 0.05
Ma river	Delta	9	1.380 ± 0.191	P(D-CB) > 0.05
	Coastal	11	1.420 ± 0.393	P(M-C) > 0.05

Note: M: Midland; D: Delta; C: Coastal

The comparison of pH of the Red River dike soil (table 3) shows that pH of the delta dike is significantly higher than that of the midland dike and the difference is not clear with pH of the coastal dike. In the midland dike, pH is more acidic (5.72), while in the delta dike and coastal dike, pH is nearly neutral (6.52 and 6.55). This allows us to assume that pH of the midland dike soil at the level of 5.72 has prevented the formation and development of *O. hainanensis* termite nest. However, the density of *O. hainanensis* termite nest is highest in delta dike, the density of *O. hainanensis* termite nest in coastal and midland dikes is not different. This shows that nearly neutral pH of the dike soil (6.52) is more suitable than the acidic pH at the midland dike. But when considering carefully, pH of the coastal dike is not different from pH of the delta dike, but the density of termite nest is different clearly. This shows that pH of the dike soil from 6.52 - 6.55 is not a decisive factor affecting the density of *O. hainanensis* termite nests on the Red River dike.

Table 3. pH of soil in dike areas

Dike systems	Area	Number of soil samples tested	pН	Reliability
	Midland	9	5.72 ± 0.69	P(M-D) > 0.05
Red river	Delta	9	6.52 ± 0.09	P(D-C) > 0.05
	Coastal	8	6.55 ± 0.06	P(M-C) < 0.05
	Midland	9	4.48 ± 0.44	P(M-P) < 0.001
Thaibinh river	Delta	10	6.25 ± 0.34	P(D-C) > 0.05
TIVEI	Coastal	8	6.03 ± 0.90	P(M-C) < 0.05
	Midland	9	6.53 ± 0.24	P(M-D) < 0.05
Ma river	Delta	9	6.03 ± 0.36	P(D-C) < 0.05
	Coastal	11	5.15 ± 0.61	P(M-C) < 0.001

Note: M: Midland; D: Delta; C: Coastal

The salinity data on the Red River dike shows clearly that the salinity increase is not clear from 0.054% to 0.076% when moving from the dike in the delta area to the dike in the coastal area but it is obvious that when the salinity does not change clearly, termite nest density also changes unclearly. Similarly, when considering the change in the salinity of the dikes in the midland area and the dikes in the delta area and the density of the termite nests of two dike areas, it shows that when the salinity of the midland dike is not different from that of the delta dike, the density of *O. hainanensis* termite nests on the dike is not significantly different. This proves that within the salinity limit of the dike soil of less than 0.076%, the salinity factor is not a determinant of the nest density of *O. hainanensis* species on the Red River dike.

Table 4. The mean salinity of soil in dike areas

Dike systems	Area	Number of soil samples tested	Salinity (%)	Reliability
	Midland	8	0.061 ± 0.003	P(M-D) < 0.05
Red river	Delta	9	0.054 ± 0.005	P(D-C) > 0.05
	Coastal	8	0.076 ± 0.012	P(M-C) < 0.05
TD1 '1' 1	Midland	9	0.067 ± 0.013	P(M-D) > 0.05
Thaibinh river	Delta	10	0.068 ± 0.008	P(D-C) < 0.05
	Coastal	8	0.147 ± 0.044	P(M-C) < 0.05

	Midland	9	0.057 ± 0.023	P(M-D) > 0.05
Ma river	Delta	9	0.071 ± 0.021	P(D-C) > 0.05
	Coastal	11	0.129 ± 0.066	P(M-C) < 0.05

Note: M: Midland; D: Delta; C: Coastal

Distribution characteristics of O. hainanensis termite species on Thai Binh river dike system On the dike system of Thai Binh river, O. hainanensis was found in the dikes of midland area, but in the dikes of delta area, it reached 5.4 nests/km and in the dikes of coastal area, there were 8.4 nests/km. The difference on nest density in delta dike and coastal dike was not clear.

It is noticeable that the low humus in the dike in the midland area of Thai Binh River (0.810%) is the cause of the phenomenon of almost no *O. hainanensis* nest. However, when compared with humus in the dike soil in the midland area of the Red River, at a lower humus (0.541%), the density of *O. hainanensis* nests remains relatively high (12.6 nests/km). This proves that the low humus factor in the midland dike of Thai Binh river is not the main cause of low density of termite nests. Tables 2 and 3 show that when the humus of delta dike soil (1.530%) and humus of coastal dikes (1.273%) are not significantly different, the density of *O. hainanensis* termite nests is not significantly different, this is consistent with normal inference. However, the humus of the delta dike soil of Thai Binh river (1,530%) is equivalent to the humus of the coastal dike of Red River (1.558%) but the density *O. hainanensis* nests on 2 dike areas is significantly different, respectively 5.4 nests/km and 16.8 nests/km and this rate of humus is relatively common on dike lines. This clearly proves that humus is not a main factor affecting the density of *O. hainanensis* termite nests at the dike.

Table 4, shows pH of dike land in the lowest midland area (4.48), it is different from pH of the dike soil in the delta and coastal areas, meanwhile, pH of soil of the delta dike (6.25) and coastal dike (6.03) is not significantly different. When considering the density of *O. hainanensis* termite nests, it shows that because pH is low in the midland dike of Thai Binh river (4.48), there is absence of termite nests of *O. hainanensis* species. However, Nguyen Duc Kham (1976) said that *O. hainanensis* could be distributed in soil environment with pH down to 3.2, therefore, it is possible that pH factor as low as 4.48 is not a direct factor causing this phenomenon. Clearly, low pH in the midland dike of Thai Binh river made the vegetational cover (grass) in the dike underdeveloped, resulting in *O. hainanensis* termites not develop. We think that pH is a factor relating to the vegetational cover at the dike, so it is related to the distribution of termites on the dike. This is a problem that needs to be further studied. For dikes in the delta area and dikes in the coastal area, the similarity of pH values between 2 areas above does not affect the density distribution of *O. hainanensis* in the dike of Thai Binh river.

The salinity at the dike of Thai Binh river varies insignificantly between midland dike and delta dike but there is a clear difference between delta dike and coastal dike and between midland dike and coastal dike (table 5). Although there is a clear difference in salinity, the density of the termite nests between coastal dike and delta dike is not significantly different, it proves that salinity of dike soil within the limit of 0.147% is not a determining factor to the density of *O. hainanensis* termite nests on Thai Binh river dike system. The absence of the nest of *O. hainanensis* in the midland dike area of Thai Binh river is not due to the salinity factor because the salinity in the midland dike of Thai Binh river is similar to that of the delta dike of Thai Binh river. Moreover, the salinity value is similar to the salinity in the midland dike of the Red River and Ma River, but in these places the density of termite nests is still relatively high (table 1).

As analyzed above, the factors of low pH, low humus, low salinity are not the direct factors causing the absence of *O. hainanensis* species in the midland dike of Thai Binh River. According to the general data in the overview part on research location, in the midland area of Thai Binh river system (Bac Giang), the meteorological data in 3 years (2016 - 2018) show that the average air humidity here is lower than that of other areas. On the other hand, according to our observation, the vegetational cover on the dike in this area is very poor, mainly stunted grass, many dike sections are bare, vacant land, it means that the food source for termites here is poor. Perhaps the loss of vegetational cover and the

factor of low air humidity that makes dike land dry is the main cause that makes *O. hainanensis* is almost not distributed on this dike area. It should be emphasized that, although the density of *O. hainanensis* termite nests on the midland dike was determined to be 0 nest/km, when re-investigating in the environment surrounding the dike in the midland area of Thai Binh river, the distribution of this termite species was still found. It proves that this species may make nest on the dike of Thai Binh river, but due to its low density, it may not have been detected in this study.

Nest distribution characteristics of O. hainanensis termite species on dike system of Ma river On dike system of Ma river, the density of O. hainanensis termite nest in plain dike was the highest (29.2 nests/km), different from midland dike (11.2 nets/km) and coastal dike (9.6 nests/km) with P < 0.001. The density of O. hainanensis nests in midland dike and coastal dike was not significantly different (table 1).

The change on density of *O. hainanensis* termite nests on Ma River dike cannot be explained by vegetational cover, because like Red River dike, the vegetational cover in Ma River dike is almost the same across the dike system from the midland area to the coastal area, the dike body is covered with grass layer. The grass layer is the source of food for termites. To better understand the distribution characteristics of *O. hainanensis* species in Ma river dike, the factors of humus, pH and salinity of dike soil are analyzed and evaluated.

The analysis of humus in Ma river dike (table 2) showed that humus in Ma river dike was relatively high, higher than the dike systems of Red river and Thai Binh river; changed little and was only in the range of 1.195-1.420%. The average humus was lowest in the midland dike (1.195%), increased gradually in the delta dike (1.380%) and the highest in the coastal dike (1.420%). However, when using the algorithm to check, the humus in the 3 different dike areas was not clear. When considering the correlation between the density of termite nests and the humus of dike soil, it was found that although the humus did not change significantly, the density of *O. hainanensis* termite nests changed greatly, particularly at delta dike, it was 29.2 nests/km, more than 2 times higher than the density of *O. hainanensis* nests in the midland dike (11.2 nests/km) and in the coastal dike (9.6 nests/km). Once again, it showed that humus in dike soil is not a main factor deciding the density of *O. hainanensis* termite nests.

In the consideration of pH (table 3), the soil environment of Ma river dike changed inversely with the changes in pH at the Red River dike and was different from Thai Binh dike; pH of soil of Ma river dike decreased gradually from midland dike to coastal dike. In contrast, pH of soil of the Red River dike increased gradually from the midland dike to coastal dike, while in Thai Binh River dike, highest pH was in the delta dike. Considering the correlation between the density of termite nests and pH between the dike areas of Ma River showed that when pH decreased gradually from midland area to coastal area, the density of termite nests changed and increased in the middle and decreased to 2 sides with high and low pH. That thing shows that pH factor is not a factor deciding the distribution of *O. hainanensis* species in Ma River dike.

When considering the correlation between the density of termite nests and pH of dike soil in the dike systems, it shows that pH of soil of Ma river midland dike (6.53) is equivalent to pH of soil of Red River delta dike (6.52) but the density of termite nests is less than 2 times (11.2 nests/km compared to 24.2 nests/km); pH of soil of Ma river delta dike (6.03) is equal to pH of soil of coastal dike of Thai Binh river (6.03) but the density of termite nests is more than 3 times (29.2 nests/km compared to 8.4 nests/km); pH of soil of coastal dike of Ma river (5.15) is not much different from that of soil of the midland dike of Thai Binh river (4.48), but the density of termite nests in coastal dike of Ma river is 9.6 nests/km, meanwhile, the density of termite nests in the midland dike of Thai Binh river is 0 nest/km. The analysis above shows that pH of the dike soil of Red river, Thai Binh river and Ma river varies from 4.48 to 6.55 and is not the main deciding factor to the density of *O. hainanensis* termite nests on the dike.

The analysis result on salinity of Ma river dike soil (table 4) showed that salinity of midland dike soil was not significantly different from that of delta dike soil, but much lower than that of coastal dike soil, respectively 0.057%; 0.071% and 0.129%. Through testing, the salinity value of delta dike soil (0.071%) was not different from that of coastal dike soil (0.129%). When considering the correlation of the density of termite nests on the dike with the salinity of the dike soil, it showed that, although the salinity of the midland dike soil of Ma river was much lower than the salinity of the coastal dike, the density of *O. hainanensis* termite nest in the midland dike was still higher than the nest density in the coastal dike with unclear level. This proved that salinity in the range of 0.057% to 0.112% was not a factor deciding the density of *O. hainanensis* termite nests on Ma river dike. This was further confirmed as analyzing the density of this termite nest in the delta dike and midland dike of Ma river, the density of *O. hainanensis* termite nests in Ma river delta (29.2 nests/km) was more than 2 times compared to the density of termite in midland dike, while salinity was not significantly different (tables 2 and 5).

It is easy to see that in all 3 dike systems, the rule of salinity change is shown clearly that the nearer the sea side, the higher the salinity of the dike soil and the density of *O. hainanensis* termite nests in the coastal dike is always lower than that in the delta dike. This shows that the high salinity of the dike soil may reduce the density of *O. hainanensis* termite nests. However, according to the analysis above, the influence of salinity to the distribution of termite nests is unclear.

According to the research result about the process of forming the Red river delta and Ma river delta, as reaching nearer the sea, the age of delta is younger. The process of expanding the delta happens gradually, so the time to escape from the influence of the seawater factor also changes slowly when going from the midland dike to the coastal dike. That means the salinity of the dike soil also changes slowly. On the other hand, when surveying the density of termite nests, we found that the dike lines near the estuary were unable to collect sample of *O. hainanensis* termite and other fungus termite species. That is, there cannot be fungus termite nest in a coastal dike near the sea passage. Therefore, it is sure that when the salinity increases to a certain level, there will be no fungus termite nest on the dike.

To see clearly the influence of salinity on the density of *O. hainanensis* termite nests on the dike, soil samples were collected representatively at the coastal dike at the section with termite and samples were collected in the coastal dike without termite nest of any fungus termite species, including *O. hainanensis*. The data is presented in table 5.

The data show that the average salinity value obtained from the soil samples collected at the dike near the estuary to the sea $(0.980 \pm 0.534\%)$ is statistical difference (P < 0.001) with all midland, delta and coastal areas a of all studied river systems. At the sections of the coastal dike b that have been sampled for this salinity range, through years of investigation, we could not find any *O. hainanensis* termite nest. Obviously, when the salinity value in the dike soil reaches as high as $0.980 \pm 0.534\%$, it is the cause of the absence of termite nests of the species causing damage to the dikes at the sections of coastal dike near the estuary, adjacent to the sea.

We have determined the dike sections without the termite nest namely: On the dike system of the Red River, it is the dike section from Km 9 of sea dike (called as sea dike but this is still the dike of the Red River) in Nam Hai commune, Tien Hai district, Thai Binh province to the sea. On the dike system of Thai Binh river, it is the dike section from Km 23 Ta Thai Binh in Tien Minh commune, Tien Lang district, Hai Phong. On the dike system of Ma river, it is the dike section from Km 13 Huu Lach Truong and from Km 51 Ta Ma dike toward the sea in Hoang Ha and Hoang Tan communes, Hoang Hoa district, Thanh Hoa province. The dike sections without termite above have a length of about 5 - 10 km, according to the direction from the estuary to the upstream. This result confirms that the dike in the coastal area within 5 km to the estuary is not required to prevent termites.

The research results presented above show that the humus and pH measured at the survey points in the midland, delta and coastal dike areas on the 3 river dike systems do not have decisive significance

affecting the density of *O. hainanensis* termite nests. However, the salinity of the dike soil environment has a role to the existence of termite nests.

Table 5. Distribution of *O. hainanensis* nest according to the salinity of dike soil

Dike systems	Area	Number of soil samples tested	Salinity (%)	Status of termites
	Midland	8	0.061 ± 0.003	+
Red river	Delta	9	0.054 ± 0.005	+
Red livel	Coastal A	8	0.076 ± 0.012	+
	Coastal B	7	0.980 ± 0.534	-
	Midland	9	0.067 ± 0.013	+
Thai Binh river	Delta	10	0.068 ± 0.008	+
Thai Billi fivei	Coastal A	8	0.147 ± 0.044	+
	Coastal B	7	0.980 ± 0.534	-
Ma river	Midland	9	0.057 ± 0.023	+
	Delta	9	0.071 ± 0.021	+
	Coastal A	11	0.129 ± 0.066	+
	Coastal B	7	0.980 ± 0.534	-

Note:

- + : dike with termites nest
- : dike without termite nest

The soil sample collected in dike coastal area, where was found out nest but it is 10 - 20 km far from water front.

Coastal B: the data of seven soil samples that collected from dike sections near water front (about 5 – 10km far from water front (without termites nest).

Conclusion

- The density of *O. hainanensis* termite nests is usually highest in plain dike, lower in midland dike and coastal dike.
- On three river dike systems in the Northern Vietnam, *O. hainanensis* nests only exist in the sections where the salinity of the dike soil is less than $0.98\% \pm 0.53\%$. The dike sections at estuary near the sea (about 5 10 km long) have no termite nest.
- Humus content and pH index is not a direct factor deciding the distribution of *O. hainanensis* on 3 river dike systems in the Northern Vietnam.

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Appendix 1. Location of research

Red river system	Location of research
Midland dike	Location of research
Thao river dike	Ha Thach, Xuan Huy communes - Lam Thao district - Phu Tho province
Thao river dike	Hien Quang, Thanh Uyen, Tam Cuong communes - Tam Nong district - Phu Tho province
Delta dike	
Red river dike	Kim Lan, Văn Đức communes - Gia Lam district - Ho Noi capital Thang Loi Commune - Van Giang district; Binh Minh, Da Trach, Tu Dan communes - Khoai Chau district - Hung Yen province
Duong river dike	Han Quang, Chi Lang, Mo Đao communes - Que Vo district - Bac Ninh province
Coastal dike	•
Red river dike	Nguyen Xa, Vu Tien, Vu Van communes - Vu Thu district; Vu Binh, Minh Tan communes - Kien Xuong district; Nam Hong, Nam Hung, Nam Phu communes - Tien Hai district - Thai Binh province
Red river dike	Tan Thinh, Nam Hong, Nam Thanh communes - Nam Truc district; Xuan Chau, Xuan Thanh, Xuan Tan communes - Xuan Truong district - Nam Đinh province
Thaibinh dike system	
Midland dike	
Luc Nam river dike	Tien Hung, Kham Lang, Bac Lung communes - Luc Nam district - Bac Giang province
Delta dike	
Thai Binh river dike	Ky Son, Dai Dong, Hung Dao, Binh Lang Communes - Tu Ky district - Hai Duong province
Khu vực đê ven biển	
Thai Binh river dike	Ly Hoc, Hoa Binh, Tran Duong communes - Vinh Bao district - Hai Phong province
Thai Binh river dike	Doan Lap, Tien Minh Communes - Tien Lang district - Hai Phong province
Ma dike system	
Khu vực đê trung du	
Chu river dike	Tho Dien, Tho Hai, Xuan Hoa communes - Tho Xuan district - Thanh Hoa province
Deltal dike	
Chu river dike	Xuan Khanh commune - Tho Xuan district; Thieu Toan, Thieu Minh, Thieu Do communes, - Thieu Hoa district - Thanh Hoa province
Khu vực đê ven biển	
Lach Truong river dike	Hoang Duc, Hoang Phuc, Hoang Dat, Hoang Ha communes - district Hoang Hoa - province Thanh Hóa
Ma river dike	Hoang Quang, Hoang Dai, Hoang Tan communes - Hoang Hoa district - province Thanh Hoa

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How to Confine Reproductives Using an Excluder for the Study of Subterranean Termites

by

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Abstract

Social insects, such as ants, bees, wasps, and termites, display division of labor - one of the traits of eusociality. Compared to social Hymenoptera, social behaviors of the subterranean termites are poorly understood because of their cryptic nature. A planar arena has been proposed to increase visibility and minimize disturbance for observation of subterranean termites. However, there is no means to designate specific areas, such as the central nest, because reproductives can move freely within the arenas. Therefore, there is a need to develop a reproductive excluder like that used in apiculture. We investigated the minimum width that different castes (workers, soldiers, and reproductives) of the Formosan subterranean termite, Coptotermes formosanus Shiraki, are able to pass through (passing size) to develop a reproductive excluder. We measured head widths and heights to correlate minimum passing sizes with head heights and widths. Our results showed that workers and soldiers of C. formosanus were able to pass through gaps greater than or equal to 0.7 mm, and that the passing size of reproductives was significantly larger than for workers and soldiers. Also, there were significant differences in head widths and heights among castes, and the correlation between minimum passing size and head height was stronger than the correlation with head width. Using the reproductive excluder in laboratory experiments enabled us to investigate the social behavior of subterranean termites with better visibility.

Keywords: social insect, division of labor, task allocation, queen excluder

Introduction

Subterranean termites are difficult to study because of their cryptic nature. The central nest of the termite colony is located either underground or within trees, and the foraging territory of subterranean termites can reach up to 100 m (King and Spink 1969, Su and Scheffrahn 1988).

Since some subterranean termites are important structural pests in urban environments, control of the subterranean termites has been widely investigated. However, the social behaviors of termites are poorly understood compared to social Hymenoptera, such as ants, bees and wasps. To study subterranean termites, the use of a 2D planar arena was proposed, and such arenas are widely used in the laboratory (Becker 1969, Chouvenc et al. 2011). The planar arena provides better visibility with less disturbance, and allows researchers to observe the colony for a prolonged time (Chouvenc et al. 2011).

The queen excluder in apiculture is widely applied to both beekeeping and research. This excluder takes advantage of differences in body sizes of different castes, such that worker bees are able to move freely, but the larger queens and drones cannot move freely. However, there is no such excluder for termites. In the present study, we developed a reproductive excluder for subterranean termites by testing the hypothesis that we may be able to exclude reproductives using caste-related head width or height differences.

Materials and methods

Workers and soldiers of *Coptotermes formosanus* Shiraki from three different colonies were collected from Broward County, Florida, USA, and brought back to the laboratory. Termites were kept in a plastic box $(30.5 \times 45.7 \times 15.2 \text{ cm}^3, \text{Carlisle}, \text{Oklahoma City}, \text{OK})$ containing pieces of *Picea* sp. and moistened organic soil.

Alates (winged reproductives) of *C. formosanus* were also collected during swarming season in April 2018 in Broward County, Florida, USA, using a light trap (Chouvenc et al. 2018). On the following day, the alates were sexed and each alate pair was introduced into a small vial containing moistened organic soil, a piece of wood and 3% agar solution. Both field populations and alate pairs were kept at $28 \pm 1^{\circ}$ C before testing.

An individual passing assay was carried out using each caste (i.e., workers, soldiers, queen and king). For the experiments, a planar area was made with a laser cutting machine (BOSS LS1416, Florida). The arena was designed to have an introduction chamber with 11 small chambers connected by a passageway. The passageway between the introduction chamber and the next chamber was 1.2 mm; then, the passageway progressively narrowed by 0.1 mm decrements (1.2 mm to 0.2 mm). An individual termite was introduced into the introduction chamber and recorded using a camcorder (4 HG10 AVCHD High Definition, Canon Inc., Tokyo, Japan) for 1 hour. After the experiments, the termites were collected and preserved in a vial with 95% ETOH for morphological measurements.

In addition to the individual passing assay, whole colonies were also tested for the practicality of the reproductive excluder. A planar arena with two chambers, the central nest (moistened sand) and foraging site (moistened sand and a piece of wood), were connected by a passage width of 0.7 mm (according to the result of the individual passing assay). A whole colony of *C. formosanus* (one-year-old) was introduced through an introduction hole. Whole colony experiments were conducted for one month to check whether reproductives were able to move freely. For the control, the chambers were connected with a 2 mm passage.

From the video file of each individual passing assay, the minimum passing size was measured. Using a microscope (Olympus SZX12, Tokyo, Japan), the head width and height of each termite was measured. A one-way ANOVA with caste as a factor was used, and Tukey's Honestly Significant Difference (HSD) test was used as a post-hoc analysis. Furthermore, Pearson's correlation coefficient (r) was analyzed between minimum passing size and head width, as well as minimum passing size and head height.

Results and discussion

Unlike social Hymenoptera, all members in a termite colony are juvenile, except reproductives and soldiers. These juvenile termites will undergo a series of molts, and their head size (i.e., width and height) will increase, whereas reproductives and soldiers have already reached a final molt, so their head widths and heights will not change over time.

In the individual passing assay, workers were able to pass through the gaps 0.7 mm wide, and soldiers and reproductives were able to pass through gaps 0.8 mm and 1.0 mm, respectively. We also found a significant difference in head width among castes. Workers and soldiers had significantly smaller head heights than reproductives. Correlations between minimum passing size and head width and between minimum passing size and head height revealed that head height was more positively correlated with minimum passing size.

When we tested the practicability of the reproductive excluder using one-year-old colonies of *C. formosanus*, we were able to exclude reproductives when we used 0.7 mm passageways; however, reproductives were able to freely move between both chambers with 1.0 mm passageways. Since we tested only *C. formosanus*, the reproductive excluder for other species should be determined based on head heights of caste members.

Most laboratory experiments are now using whole colonies of termites rather than only foraging populations. It allows us to advance our knowledge more deeply. Using the reproductive excluder, more topics on the social behaviors of subterranean termites can be addressed.

Conclusions

We developed a reproductive excluder for subterranean termites using caste-related head height differences. It can be applied to any studies investigating the biology of subterranean termites focused on the role of the reproductives in the colony with a need for high visibility of the central nest.

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Behavioral Patterns and Building Material of Termite Shelter Tube Construction

by

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Abstract

Termites construct various adaptive structures under natural environment. The nest-building materials has been proposed to predict feeding-habits of termites. When termites forage above ground, they build shelter tubes along their foraging trail. In the present study, we investigate the shelter tubes material and building behavior of two wood-feeding termites, *Nasutitermes takasagoensis* and *Coptotermes formosanus*, and one fungus-growing termites, *Odontotermes formosanus*. Organic material of shelter tubes was investigated by ignition and the building behaviors were observed by watching video recorded from the field. More organic materials was detected in the shelter tubes of *N. takasagoensis* and *C. formosanus* than that of *O. formosanus*. Although granule placement, fluid secretion and feces deposition are observed among all the three species, the building behavioral pattern and frequencies were different among species. We suggest that the difference in composition of shelter tubes and building behaviors among three species are related to feeding habits and foraging strategy.

Keywords: wood-feeding termite, fungus-growing termite, shelter tubes, building material, building behavior

Introduction

Termites build various adaptive architectures, such as nests and underground gallery systems, which provides the colony with regulated temperature, humidity and gas exchange under natural environment (Noirot and Darlington, 2000; Turner, 2001). The origin of all types of termite nest material not fully known; yet, feces and food resources are discovered to be incorporated in the nest of *Cubitermes*, *Coptotermes*, *Nasutitermes* and *Hospitalitermes* (Stoops, 1964; Lee and Wood, 1971; Thorne, et al. 1996; Miura and Matsumoto, 1997). Since feces and food resources are reservoir of organic matter, the organic matter lies in termite nest has been proposed to predict feeding-habits of termites (Amelung et al., 2002; Siebers et al., 2015).

During nest construction, termite agglomerate collected materials into particles (Zachariah et al., 2017) and cemented them with saliva and/or feces (Kadasami et al., 2016). The frequency of each building behavior has been further extended to feeding habits of termites. Saliva secretion is suggested to be more frequent in fungus-growing termites (Grassé, 1939; Noirot, 1970), while wood-feeding termites are suggested to frequently apply feces in the form of carton material (Lee and Wood, 1971). Building tasks and frequencies of building behavior may also correlate with labor division. By observing *Reticulitennes fukienensis* (Light), Crosland et al. (1998) discovered that workers with different instar exhibit different behavior during excavating tunnel.

When termites forage above ground, they build shelter tubes along their foraging trail. Shelter tubes are assumed to provide protection against predator (Kaiser et al., 2017) and desiccation (Jouquet, 2002). Thus, termites may develop building strategy in aspects of building material and behaviors in response to their foraging environment. In the present study, we investigate the building material and

building behavior of shelter tubes by wood-feeding and fungus-growing termites. Strategy of shelter tube construction and foraging activity are briefly discussed.

Materials and methods

Study sites and data collection

The study was conducted from June 2017 to July 2018 in Taiwan. *N. takasagoensis* were sampled in Lanyu Township, Taitung County (121.54°E, 22.04°N) and Siangjiao Bay Ecological Reserve Area, Kenting National Park, Hengchun Township, Pingtung County (120.82°E, 21.92°N). *C. formosanus* were sampled in South District, Taichung City (120.66°E, 24.11°N). *O. formosanus* were sampled in Donghai University, Situn District (120.60°E, 24.18°N) and National Chung Hsing University, South District, (120.67°E, 24.12°N) in Taichung City.

The shelter tubes on the surface of tree trunk was collected for the study. Only the shelter tubes presenting termite activity were used for the study. We scrubbed the shelter tubes by hands and placed them in a zipper bag. Termites were preserved in 95% ethanol with sites and dates recorded. Building behavior of workers was videotaped for 30 minutes. The equipment used for videotaping was ZenFone 3 (ZE552KL, Asustek Computer Inc., Suzhou, China) equipped with two pieces of macro lens (Scimaker macro lens, Scimaker, Taipei, Taiwan).

Shelter tube organic matter analysis

Proximate analysis was applied to investigate the component of shelter tubes. First, 1-g fresh shelter tube was dried at 105 °C for 24hrs and weighted (W1). Then, the dried samples were grinded into powder with pestle and ignited at 900 °C for 3hrs (CH-HTF, Chi-How Heating Co. LTD., Taoyuan, Taiwan), and weighted (W2). Finally, the samples were ignited again at 800°C for 8hrs and weighted (W3). The formula is below:

Water content (%) = (1 - W1)/1*100%

Volatile organic compound (%) = (W1-W2)/1*100%

Fixed carbon (%) = (W2-W3)/1*100%

Ash (%) = W3/1*100%

Only organic matter and ash were used in the study, since the relative humidity in study sites might be different and affect the water content of shelter tubes. Thus, we modified the formula to explicit water content. The modified formula is below:

Organic matter (%) = [(1-W1-W2)+(W2-W3)]/(1-W1)*100%Ash (%) = W3/(1-W1)*100%

Building behavior

Building behaviors of 50 workers from each colony were observed per video. The building behaviors include granule placement, fluid secretion and feces deposition. The frequency of each building behavior is determined by summing up the total of each behavior performed by the same colony and then divided it by all observed behaviors. For labor division, *N. takasagoensis* have three workers from three castes, including major worker, medium workers and minor workers (Liang and Li 2016). *C. formosanus* only have one worker caste, while *O. formosanus* have minor workers and major workers. We distinguish the instar of each worker and record its building behavior.

We estimated the granule placement speed by observing each video for 20 minutes. The number of granule placement on the repairing site was counted, which was divided by 20 minutes to obtain the number of granule placement per minute.

Statistics

The organic matter was tested by utilizing Kruskal-Wallis test with Mann-Whitney pairwise (HSD). The frequencies of each building behavior were tested by Kruskal-Wallis test with Mann-Whitney test. Building behavior by each instar was tested by Kruskal-Wallis test with Mann-Whitney pairwise

(HSD). The pattern of building behavior of each colony was analyzed by Markov chain, and each behavior was considered as independence event. Individual was treated as a unit source.

Transition probabilities of building behaviors were calculated by counting the frequencies between two events and was further divided by the total occurrence of first event (Haccou & Meelis 1992). Speed of granule placement was tested with ANOVA with Tukey's pairwise (HSD). All statistics were carried out by Past v3.14 (Øyvind Hammer 2016). Treatments were performed with significant difference at α <0.05 level.

Results and discussion

Shelter tube organic matter and feeding type

The organic matter in shelter tubes was significantly different among N. takasagoensis (95.32 \pm 0.80%), C. formosanus (51.57 \pm 31.52%) and O. formosanus (5.91 \pm 0.63%) (Kruskal-Wallis, $X^2 = 13.24$, p < 0.001). Chewed wood and feces were observed to be utilized by N. takasagoensis and C. formosanus, which could result in high proportion of organic matter in shelter tubes. Additionally, mineral particles were also used by C. formosanus, and the organic matter content differed among colonies, suggesting that C. formosanus may construct shelter tubes with different proportion of organic materials to meet their environmental need.

Fungus-growing termites cultivated *Termitomyces* spp. by making fungus combs with plant litter and feces (Darlington 1994; Hydo et al. 2002). Fungus degraded the lignin and make more cellulose available for fungus-growing termites. (Hydo et al. 2002). Due to different purpose of construction, we suggested that *O. formosanus* mainly allocate organic matter in fungus comb instead of shelter tubes to cultivate fungus colony.

Building behavior pattern and labor division

Building patterns of *N. takasagoensis*, *C. formosanus* and *O. formosanus* were summarized in Fig.2, composed of building behavioral sequence and the transition probabilities. After arrival, *N. takasagoensis* placed granules (0.52) and deposited feces (0.48), while *C. formosanus* and *O. formosanus* tend to only placed granules (0.94 and 0.96, respectively). After placing granules, fluid secretion was performed by all species and followed by feces deposition in *N. takasagoensis* and *C. formosanus* (0.53 and 0.30 respectively), while *O. formosanus* left without feces deposition. The result imply that termites shared similar building behavior, but the building pattern could be diverse among *N. takasagoensis*, *C. formosanus* and *O. formosanus*.

The frequencies of building behavior in granules placement, fluid secretion and feces deposition were significantly different among species. (Fig. 3, Kruskal-wallis test: $X^2 = 12.72$, p < 0.01, $X^2 = 11.46$ and p < 0.01, $X^2 = 14.82$, p < 0.001, respectively). N. takasagoensis had lowest frequency of granule placement (p < 0.001 in all cases), which may result from about half of N. takasagoensis individuals deposited feces without placing granules. The frequency of feces deposition was significantly higher in N. takasagoensis than C. formosanus, implying that N. takasagoensis utilized more feces than C. formosanus. Although fungus-growing termites had been suggested to have higher frequency in fluid secretion (Grassé 1939; Noirot 1970), we found that there was no significant difference between N. takasagoensis and O. formosanus, however C. formosanus had highest frequency in fluid secretion.

All of *N. takasagoensis*'s cates were devote in the building activity. Since it is sometime hard to distinguish medium and minor workers, they were recorded as a group. Major workers has significant higher number than medium and minor workers in the combination of granule placement and fluid secretion (Mann-Whitney test: U = 4.0, p < 0.01) and the combination of granule placement, fluid secretion, and feces deposition combination (Mann-Whitney test: U = 9.0, p < 0.05). The result indicated that major workers conducted more building tasks than medium and minor workers. Inversly, though having major and minor workers, only major workers participated in the building activity in

O. formosanus. Such labor division may result from foraging mainly done by major workers, while fungus combs were primarily built by minor workers (Li et al., 2015).

Table. 1 Number of building behavioral combination by major workers, medium workers and minor workers in N. takasagoensis (n=8, mean \pm SD) by observing 50 individuals from each colony.

	W	Worker caste ^a		
Behavior ^b	Major worker	Medium & minor worker	U value	p value
Feces deposition	8.75 ± 4.27	9.87 ± 6.56	29.5	0.83
Granule placement and fluid secretion	7.75 ± 3.40	1.75 ± 1.75	4.0	< 0.01
Granule placement and feces deposition	0.25 ± 0.46	0 ± 0		
Granule placement, fluid secretion and feces deposition	14.00 ± 5.35	7.63 ± 3.74	9.0	< 0.05

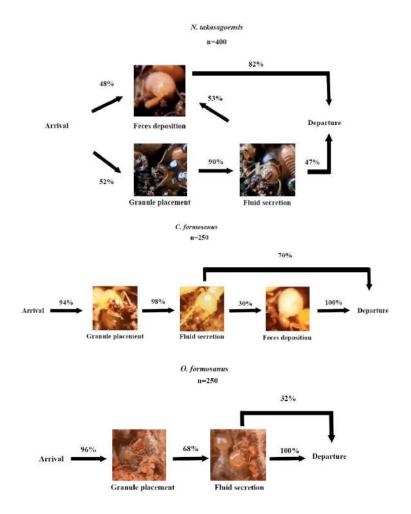
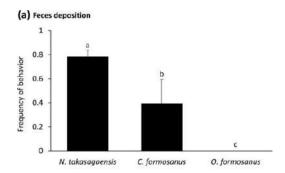
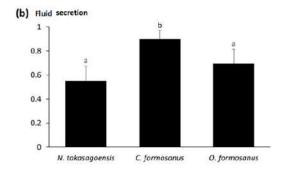


Fig. 1 Building behavioral sequence of *N. takasagoensis*, *C. formosanus* and *O. formosanus*. Each value represents the probability out of the given state.





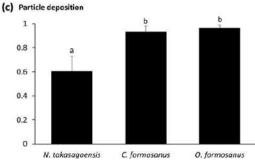


Fig. 2 The frequencies of building behavior of *N. takasagoensis*, *C. formosanus* and *O. formosanus*. The frequency of feces deposition (a), Fluid secretion (b) and particle deposition (c). Different letter above histogram means significantly different at p < 0.05. Vertical lines above chart represent standard deviation.

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O 15

Exploring the Cryptic Life-History of the Rove Beetles Associated with Formosan Subterranean Termite

by

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Abstract

Termitophiles are the organisms living inside the termite nest or having some obligatory relationship with termites. Most of their life-history and relationship with termite were unknown. Two staphylinid genera, *Japanophilus* Maruyama & Iwata and *Sinophilus* Kistner, had been found in the nest of *C. formosanus*, and used for interpreting the native range of *C. formosanus*. However, the complete life-history of these two staphylinid genera is still in mystery. By using the wooden bait as a monitoring tool for termitophiles, the seasoning occurrence of termitophile could be accessible. We also speculate the dispersal stages of the termitophile based on their functional anatomies and symbiotic relationships. The internal organs of both species were examined by the Micro-CT, and reveal a similar reproductive strategy between two species.

Keywords: social insect symbiont, Staphylinidae, immature stage, dispersal stage

Introduction

Termitophiles are the organisms living inside the termite nest or having some obligatory relationship with termites (Kistner 1969). That is, an organism has some stages of its life-cycle completely depend on termite society is regarded as a termitophile. Over the years, many organisms, mainly arthropods, have been found from the termite nest (Kistner 1982). Most of those species perform a variety of specialized morphology, presumed for adapting into the termite nest environment and therefore generally regard as termitophile, even though their life-history and relationship with termite are mostly unknown.

Beyond being like the pests stealing food and resources from the termite, termitophiles exhibit diverse relationships with their host termite and could play a crucial role in the framework of termite ecology and evolutionary biology. For example, the termitophiles using chemical mimicry to integrate into termite society have been reported in staphylinid beetles (Howard et al. 1980). The termitophile could act as nematode carrier that was found in the fungus-growing termite (Kanzaki et al. 2019).

As some of the termitophiles are species-specific and not likely transported by human activities or cross the natural barrier. Their presence is thought to indicate the native range of host termite (Kistner 1985, Maruyama et al. 2012). Two staphylinid genera, *Japanophilus* Maruyama & Iwata and *Sinophilus* Kistner, had been found in the nest of *C. formosanus*, and used for interpreting the native range of *C. formosanus* in China and Japan (Kistner 1985, Maruyama and Iwata 2002, Maruyama et al. 2012). The high degree of host specificity and dependence of *Japanophilus* and *Sinophilus* was reported from Taiwan and it supports those termitophiles could be a good indicator for interpreting the native range of host termite (Liang and Li 2018). However, until now, the complete life-history of these two staphylinid genera is still in mystery. In present study, we use *Japanophilus* sp. and *Sinophilus* sp. associated with *C. formosanus* in Taiwan as our studying materials and aim to investigate their life-history by synthesizing the data of their functional anatomy, symbiotic relationship, and host termite biology.

Materials and methods

The termitophiles examined in the present study were collected by dissecting the deadwood materials infested by *Coptotermes formosanus* or by the wooden bait, which is originally used as termite population monitoring technics for pest control. The wooden bait is a bundle of radiata pine slates (*Pinus radiate* D. Don; 18 pieces, 8.5 by 13 by 0.2 cm) using in the modified underground monitoring station of Chiu et al. (2016), which is originally designed by Su et al. (1993). The wooden baits were set up under two rotten fallen logs infested by *C. formosanus*. The wooden baits were renewed and took back the old baits every 1–2 months in the year 2015 and 2018. The old wooden baits infested by *C. formosanus* were dissected in the laboratory to look for the termitophilous beetles. We carefully examined the anatomy of the termitophilous beetles collected from the termite nest. Several specimens were fixed in the Bouin's fixative for examing internal organs by micro computed tomography (Micro-CT).

Results and discussion

1. Termite wooden bait as collecting and monitoring method for termitophiles
Based on the wooden baits set up at the field, we were able to examine the annual occurrence of termitophiles. The results show the adult of *Japanophilus* sp. and *Sinophilus* sp. occurred in the termite colony in the whole year. Additionally, the 1st instar larvae of *Japanophilus* sp. (the larvae were identified by barcoding) were found in the wooden baits for the first time and those larvae only occurred from March to July.

In general, termitophile collecting methods are usually destructive to the termite nest due to the scarcity of termitophiles in the termite colony. To maximize the outcomes of the time-consumed field survey, the substrates contained termites, e.g. termite feeding sites (deadwood trunk, branches) or termite-constructed structures (mud sheet, tunnel, and nest) are opened and broke into pieces for searching the termitophile. In our investigation on termitophiles of *C. formosanus*, the spaces between the wood pieces of wooden baits could generate the greatest number of target termites to forage inside in a short time period. Therefore, it maximizes the probability of accompanying termitophiles into the baits. Based on this application of using the wooden bait as a monitoring tool for termitophiles, the very important information of life-history, like the seasoning occurrence of termitophile could be accessible.

2. Functional anatomy reveals dispersal stages of termitophile. The termitophile, especially the symphile, which is obligatorily ass

The termitophile, especially the symphile, which is obligatorily associated with certain termite host, must have dispersal stages to another termite colony in its lifetime to avoid the population walking into the dead-end when the host termite colony die. Based on this assumption, we speculate the dispersal stages of the termitophile based on their functional anatomies and symbiotic relationships, like flight ability and host dependence.

The larvae of *Japanophilus* sp. are abundant at the soil/litter nearby the termite-infested wood, not the area with high termite activities. These larvae are moving quickly without evident interaction with their host termites. Furthermore, adult *Japanophilus* sp. is found to be wingless, which is presumed to be the evolutionary consequence of that adults are not the major stage for the dispersal. Furthermore, the adult *Japanophilus* sp. highly depends on living termite and can not live without termite for a long time (Liang and Li 2018). This suggests that the larva of *J. hojoi*, at least first instar, is the potential dispersal stage of its lifetime. In contrast, the genus *Sinophilus*, at least two species, *S. yukoae* Maruyama & Iwata and *S. xiai* Kistner, are fully winged. The third species, *S. rougemonti* Pace, had been collected from the flight-interception trap (Pace 1998), suggesting the adult of *Sinophilus* will leave the host nest and probably disperse by flight.

3. Micro-CT is an ideal tool for internal organ examination

The internal organs of both species were examined by the Micro-CT. We focus on the female adult because of the reproductive organs are crucial for interpreting the life-history of an organism. The results show *Japanophilus* sp. could bear up to 13 mature eggs in its coelom. Those eggs arrange in its abdomen and could reach to the metathorax. Another species, *Sinophilus* sp., is found to have a similar egg size with *Japanophilus* sp. Although the larva of *Sinophilus* sp. is not discovered yet, we can speculate that it larva should have a similar size with the larva of *Japanophilus* sp. we found. The study herein shows the number and size of eggs are easily observed in these termitophilous beetles. Due to the scarcity of the specimens available in most of the termitophiles, we suggest the non-destructive examination of internal organs by micro-CT is an ideal method for applying to investigate the life-history of other termitophiles.

Conclusion

The life-history of termitophilous beetles associated with the Formosan subterranean termite is investigated through three aspects, the seasoning occurrence, dispersal stages, and reproductive strategies. We are not yet to completely realized the whole life-history of these two species, however, the continued data accumulation will shed light on it. The ideas presented in this study are intended to help with exploring the life-history of the other social insect symbiontic arthropods.

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Fungal Biotrophic Parasites of Taiwanese Termite: The First Record of *Termitaria*, *Mattirolella*, *Antennopsis*, *Laboulbeniopsis* in Taiwan

by

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Abstract

Several entomopathogenic fungi were found on termites and were considered as biotrophic parasites. In this study, we examined twenty termite species in Taiwan and new recorded four ectoparasite fungal genera. Eleven termite species are the hosts of *Termitaria* and the infection of soil-feeding termite is the first record. Our survey shows the high diversity of the parasitic ectoparasitic fungi of termite in Taiwan. Further study could focus on the host-parasite specificity between entomopathogenic fungi and termite by genetic information.

Keywords: Termitaria, entomopathogenic fungi, termite, Mattirolella, Antennopsis

Introduction

Termites generally forage in soil and litters, which is a habitat that accommodates various entomopathogenic fungi (Mäkipää et al. 2017). The entomopathogenic fungi may enter, grow, and spread in the nest of termites, which is a closed and humid environment (Rosengaus et al. 2011). To date, more than 8 ectoparasite fungal genera were reported growing on the exoskeleton of termites, include *Laboulbenia*, *Dimeromyces*, *Coremycetopsis*, *Hormiscioideus*, *Termitaria*, *Antennopsis*, *Laboulbeniopsis* and *Mattirolella* (Blackwell and Rossi 1986). Most of the ectoparasitic fungi were considered as biotrophic rather than necrotrophic. For example, *Antennopsis* and *Laboulbeniopsis* grow only on the cuticle of termites (Myles et al. 1998, Henk et al. 2003, Guswenrivo et al. 2018).

Termitaria, a common ectoparasite fungal genus, in which its large sporodochi was visually observed on legs, antenna, and abdomen of termites, without using microscopes (Vega and Blackwell 2005). In 1920, Thaxter primarily discovered Termitaria on Reticulitermes virginicus, Reticulitermes flavipes, Nasutitermes costalis, with the description of two species. Four additional Termitaria species were later described in 1923 and 1982 (Reichensperger 1923, Kimbrough and Lenz 1982). The morphological identification of Termitaria usually depend on the size of sporodochia, phialides, phialoconidia and haustoria. Hojo et al. created a new way to classify the Termitaria into white, black and intermediate type by the shape and surface color of the sporodochia (Hojo et al. 2001). In 1986, Blackwell and Rossi revised the genus Termitaria and their termite hosts, and concluded that Termitaria has low host-specificity (e.g. T. cornata occurred in >20 termite species). However, the species diversity of Termitaria could be underestimated without a survey of genetic diversity (Hojo et al. 2002), and the conclusion of low host-specificity could be misleading. In this study, we aimed to survey the morphological diversity of ectoparasitic fungi growing on the cuticle of termites in Taiwan.

Materials and methods

A total of 954 termite colonies of 20 termite species (Table 1) were examined to survey the occurrence of ectoparasitic fungi. For each colony, 10–12 workers were randomly selected and examined under a Leica[®] M205 C stereomicroscope (Leica Microsystems, Wetzlar, Germany). Photographs of termites and fungi were taken by Leica[®] MC170 HD digital camera (Leica Microsystems, Wetzlar, Germany) with the software LAS (LeicaVR Application Suite V4.4.0, Leica Microsystems, Wetzlar, Germany). The termites were collected from various habitats, including soil, woody litters, and the dead part of living trees in Taiwan, from 2006 to 2019. All termite samples are kept in 95 % alcohol and preserved in National Chung Hsing University, Taiwan. Fungal genera were identified based on the taxonomic key of Blackwell (Blackwell and Kimbrough 1978).

Results and discussion

A total of >10,000 individuals from 20 Taiwanese termite species were examined. The results showed that 11 termite species are hosts of *Termitaria* (8 termite species are new hosts record), 4 termite species are hosts of *Mattirolella* (4 termite species are new hosts record), 7 termite species are hosts of *Antennopsis* (7 termite species are new hosts record), and 4 termite species are hosts of *Laboulbeniopsis* (4 termite species are new hosts record) (Table 1).

The *Termitaria* is the most common fungi which can be found on termite host from Archotermopsidae, Rhinotermitidae and Termitidae. But we didn't find any biotrophic fungus in Kalotermitidae or Stylotermitidae after checking 348 colonies. It seems related to the habitat of Kalotermitidae, a group is well-known living in wood and seldom encountering the soil. In the other hand, 75% termite species (9/12) which are living around the soil and wood can found the *Termitaria*. And we first recorded the *Termitaria* infected soil-feeding termite. Both soil-feeding termite species recorded in Taiwan, *Pericapritermes nitobei* and *Sinocapritermes mushae*, were infected by *Termitaria* and show different appearance. All *Termitaria* be found previously have black excipulum but the fungi we found on soil-feeding termite are totally white (Fig 1, I and J).

Conclusion

We conducted a survey of parasitic ectoparasitic fungi of 20 termite species in Taiwan and found that there are 13 species infected by ectoparasitic fungi. *Termitaria* is most frequently encountered which infected 11 termite species. The high morphological diversity of *Termitaria* were observed, and further taxonomic study will rely on genetic information. In addition to taxonomy, studies of host-parasite specificity between termite and *Termitaria* could be also tested by using genetic data of both termite and fungi.

Table 1. Fungal biotrophic parasites of Taiwanese termite

	Termite species	Number of colonies be	Number of colonies infected by fungal biotrophic parasites			rasites
	·	examined	Termitaria	Mattirolella	Antennopsis	Laboulbeniopsis
Archotermopsidae	Hodotermopsis sjostedti Holmgren, 1911	7	4	-	-	
	Cryptotermes domesticus (Haviland, 1898)	84	-	-	-	
	Glyptotermes fuscus Oshima, 1912	18	-	-	-	
Kalotermitidae	Glyptotermes satsumensis (Matsumura, 1907)	6	-	-	-	
	Incisitermes inamurai (Oshima, 1912)	138	-	-		
	Neotermes koshunensis (Shiraki, 1909)	99	-	-	-	
Stylotermitidae	Stylotermes halumicus Liang, Wu, and Li, 2017	3	-	-	-	
	Coptotermes formosanus Shiraki, 1909	89	1	-	8	
Rhinotermitidae	Coptotermes gestroi (Wasmann, 1896)	21	-	-	3	
	Prorhinotermes flavus (Bugnion and Popoff, 1910)	50	-	-	1	
	Reticulitermes flaviceps (Oshima, 1911)	18	4	-	5	
	Reticulitermes leptomandibularis Hsia and Fan, 1965	3	3	-	2	
	Reticulitermes kanmonensis Takematsu, 1999	21	2	-	2	:
Termitidae	Macrotermes barneyi Light, 1924	9	-	-	-	
	Nasutitermes kinoshitai (Hozawa, 1915)	5	2	-	-	
	Nasutitermes parvonasutus (Nawa, 1911)	64	4	10	-	
	Nasutitermes takasagoensis (Nawa, 1911)	72	4	-	1	
	Odontotermes formosanus (Shiraki, 1909)	167	24	2	-	
	Pericapritermes nitobei (Shiraki, 1909)	52	1	9	-	
	Sinocapritermes mushae (Oshima and Maki, 1919)	28	1	1	-	

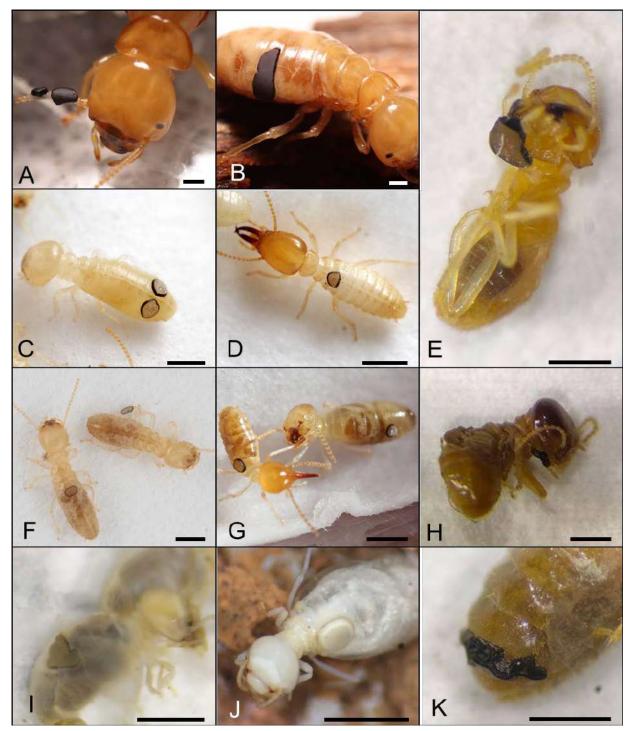


Figure 1. The *Termitaria* on Taiwanese termite species. *Hodotermopsis sjostedti* (A) and (B), *Coptotermes formosanus* (C) and (D); *Odontotermes formosanus* (E); *Reticulitermes leptomandibularis* (F); *Nasutitermes parvonasutus* (G); *Sinocapritermes mushae* (I); *Pericapritermes nitobei* (J); *Nasutitermes takasagoensis* (H) and (K). Scale bar is 1 mm.

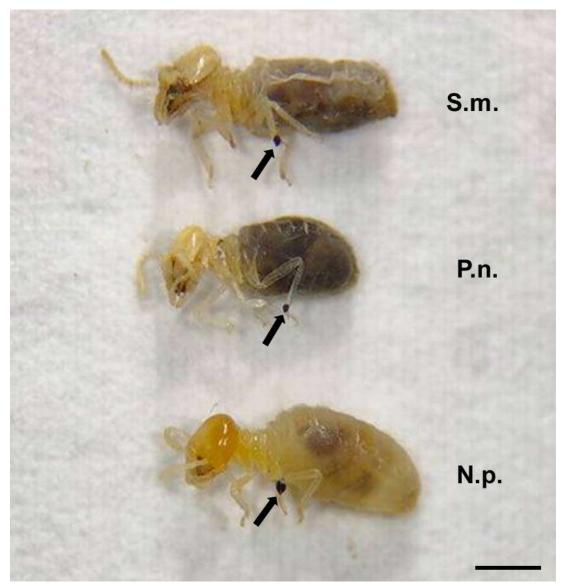


Figure 2. The *Mattirolella* on Taiwanese termite species, *Sinocapritermes mushae* (S.m), *Pericapritermes nitobei* (P.n.), and *Nasutitermes parvonasutus* (N.p). Black arrows mark the thallus of *Mattirolella*. Scale bar is 1 mm.

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Seasonal Feeding Ecology of an Obligate Myrmecophagous Mammal, Chinese Pangolin

by

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Abstract

Ant and termite colonies are so-called superorganism and have great biomass in most tropical and subtropical ecosystems. Ant- and termite-eating, or myrmecophage, is a foraging behavior for many mammalian species. Seasonal climatic factors strongly affect the life cycles of ants and termites, including colony development and dispersal flight. Chinese pangolin is an obligate myrmecophagous mammal. Previous field observation indicated that the dietary content of Chinese pangolin is seasonal, however, the sample size and number of checking time points of the previous studies were very limited. In this study, we analyzed Chinese pangolin's fecal samples and estimate the approximate dietary biomass intake of ants and termites across all seasons. The results revealed that the dietary biomass and the prey caste composition of Chinese pangolin were seasonal. Pangolins consumed more ants in summer than in winter, while the quantity of termite intake significantly decreased from July to September. Termite alates consisted the majority of termite biomass intake from April to June, which presents seasonality of food availability associated with termite colony development. Our results indicated that the food composition of Chinese pangolin changes seasonally. The species and castes compositions of ant and termite provide us further information of feeding ecology of Chinese Pangolins.

Keywords: termites, ants, dietary content, feces, seasonality

Introduction

Myrmecophagy, or ant- and termite-eating, is a foraging behavior among over 200 mammal species, and of these, about 12% are obligate myrmecophagous mammals (Redford, 1987). Knowledge of dietary component in myrmecophagous mammals is insightful to understand the impact on ants and termite populations as well as the prey-predators' role in the ecosystem (Klare et al., 2011).

The life history features of ants and termites are strictly associated with seasonal variations of climate factors (Chiu, et al., 2018; Lopatina, 2018; Mullins, et al., 2015; Nutting, 1969; Tschinkel, 1987). For most ant species, the colony development process strictly proceeds in annual cycle (Hölldobler and Wilson, 1990; Wilson, 1985). High temperature and humidity stimulate ant's foraging activity (Kaspari et al., 1999; Levings and Windsor, 1984), and ants generally rear larva in warm and wet seasons to produce the greatest individuals of various castes and thus to maximize the fitness of survival rate and colony growth (Herbers, 1985; Lopatina, 2018). For termites, especially termite

families, Rhinotermitidae and Termitidae, their foraging activity, alate development, and dispersal flight occurred at specific timing of a year, which associated with climate factors (Chiu, et al., 2010; Chiu, et al., 2018; Hu, et al., 2007; Mullins, et al., 2015; Nutting 1969; Neoh and Lee 2009; Nalepa et al. 2001; Sangamma and Chimkod 2012).

Chinese pangolin (*Manis pentadactyla*) is an obligate myrmecophagous mammal with a widespread historical distribution in southern and eastern Asia (Corlett, 2007; Challender et al., 2014). When feeding on termites, Chinese pangolins can excavate foraging burrows extending to 5 meters and more (Lin, 2011; Trageser, et al., 2017). Wu et al. (2005) reported the presence of feeding burrow increased in winter than in summer. In addition, based on gut content analysis of 12 wild pangolins, ants were the major diet content in summer and termite was the majority of diet in winter, which indicating feeding behaviors of Chinese pangolin is seasonal. However, the sample size and number of checking time points of the previous studies were very limited, based on which we cannot draw a solid conclusion. Hence, in this study, we investigated the dietary content of Chinese pangolin through analyzing 132 fecal samples across all seasons to assess the prey composition and biomass. Three hypotheses were tested in this study: (1) the composition of prey species is seasonal (2) the dietary biomass is seasonal; (3) the composition of prey castes is seasonal.

Materials and methods

A total of 132 pangolin fecal samples were collected from Luanshan, Taitung County, Taiwan between 2009 and 2016. Ten grams of dried homogenized sample of each feces were analyzed. Fecal analysis techniques (Sun et al. 2019) were used to identify and calculate the prey species. The ant head capsule and termite left mandible were used for species identification and calculation of number of ants and termites in each fecal sample. Climate factors including temperature and rainfall from 1985 to 2015 were obtained from Taitung weather station. Four seasons were pooled, such as Jan to Mar (winter), April to Jun (spring), Jul to Sep (summer), and Oct to Dec (autumn). Temperature and rainfall were higher in spring and summer.

In order to estimate the number of termite and ant fed by pangolin based on fecal remains, feeding trials were conducted by feeding captive pangolins counted ants and termites. The ant head capsule and termite mandible recovery rates from the fecal sample were 0.65 and 0.35, respectively (Sun et al. unpublished data), which was used to estimate the number of ants and termites in this study. The biomass estimation of consumed ant was conducted by estimating according to head length index of each caste reported by Kapari and Weiser, 1999. For termites, biomass of each caste of *Odontotermes formosanus* was measured based on field-collected termite samples. For other termite species, the biomass estimation was assessed according to head width index of each caste introduced by Dahlsjö et al. 2015. The differences of prey dietary biomass and caste were compared with four seasons using a Kruskal-Wallis test and the level of significance was set to $\alpha = 0.05$.

Results and discussion

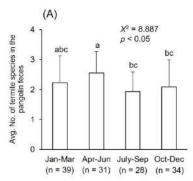
From the pangolin fecal samples, four termite species (2 families and 4 genera) and >70 ant species (5 families, 25 genera) were identified. Seven ant species composed of 80.2% ant dietary dry mass (i.e. *Pheidologeton yanoi* 23.4%; *Pheidole nodus* 15.6%; *Camponotus monju* 15.1%; *Anoplolepis gracilipes* 12.4%; *Pheidole fervens* 6.9%; *Crematogaster nawai* 3.5%; *Pseudolasius binghami* 3.3%). Fungus-growing termite, *O. formosanus*, consisted of 84.8% termite dietary dry mass. The average number of prey species in each feces was 2.2 for termite and 12.5 for ant. The number of termite species fed by pangolin was significantly higher ($X^2 = 8.887$, p < 0.05) in spring than that of summer and autumn, while the number of ant species was the same ($X^2 = 4.571$, P < 0.2036) among the four seasons (Fig. 1). In addition, both ants and termite dietary biomass showed clear seasonality. The dietary biomass of termite was significantly lower ($X^2 = 43$, P < 0.001) in summer. In contrast, ant dietary biomass was significantly higher ($X^2 = 16.5$, P < 0.001) in summer (Fig. 2 and Fig. 3, A-1 and B-1).

The dietary biomass of termite worker and soldier were seasonal and both significantly lower ($X^2 = 41.76$, p < 0.001; $X^2 = 42.8$, p < 0.001, respectively) in summer than other three seasons (Fig. 3. A-2 and A-3). The dietary biomass of termite alate was clearly higher ($X^2 = 32.05$, p < 0.001) from April to June than other seasons. The dietary biomass of ant worker was not seasonal ($X^2 = 4.571$, P = 0.1314) (Fig. 3. B-2). The dietary biomass of ant soldier peaked in autumn ($X^2 = 28.16$, P < 0.001) (Fig. 3, B-3). Lastly, the ant alates presented in every season and significantly higher ($X^2 = 28.38$, P < 0.001) in autumn as well (Fig. 3, B-4).

The number of ant species in the Chinese pangolin's diet was higher than termite, while the total dietary biomass of termite and ant was similar. Ants and termite alates were found in every season indicating Chinese pangolin exhibit nest-raiding behavior (Redford, 1986). The results confirmed the biomass intake as well as the presence of termite alates was seasonal which coordinated the dispersal flight season of *O. formosanus* (Chiu et al., 2010). The termite alate biomass fed by pangolin were much higher (67.8% of total termite biomass) compared to ant alate (0.15% of total ant biomass), indicating termite alates were an important food resources for pangolin especially before termite dispersal flight season. The seasonal pattern of ant biomass and caste composition in the pangolin's diet was similar to the previous studies of ant activity in the field (Kaspari et al., 1999; Levings and Windsor, 1984). From the nutrition aspect, alate or nymphs of ants and termites was contained substantially higher percentages of fat and thus providing more calories than workers and soldiers (Redford and Dorea, 1984; Oyarzun et al., 1996). The nutritional value of various prey castes in the diet of myrmecophagous mammals have seldom been quantified and analyzed. To obtain a comprehensive understanding of feeding ecology of Chinese pangolin, analyzing correlation between pangolin's body weight and prey nutrition composition is required.

Conclusion

Our results revealed that the prey biomass and caste composition of Chinese pangolin were seasonal. Pangolins consumed more ants in hot and wet seasons than in cold and dry seasons. Pangolins eat similar amount of ant biomass among four seasons but eat more termite in spring and less termite in summer. The termite alates appear in the pangolins' diet seasonally and most found in spring, which synchronized the termite dispersal flight season. The dietary biomass of termite alate were much higher than that of ant. Further studies are required to investigate the effects of prey nutritional value among various prey species and castes on the body weight of pangolin.



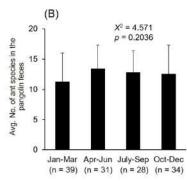


Figure 1. The number of prey species of Chinese pangolin (*Manis pentadactyla*) revealed from 132 fecal samples collected in different seasons.

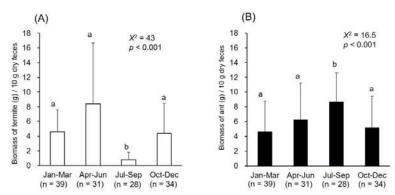


Figure 2. Dietary biomass of termite and ant consumed by Chinese pangolin (*Manis pentadactyla*) estimated from 132 fecal samples collected in different seasons.

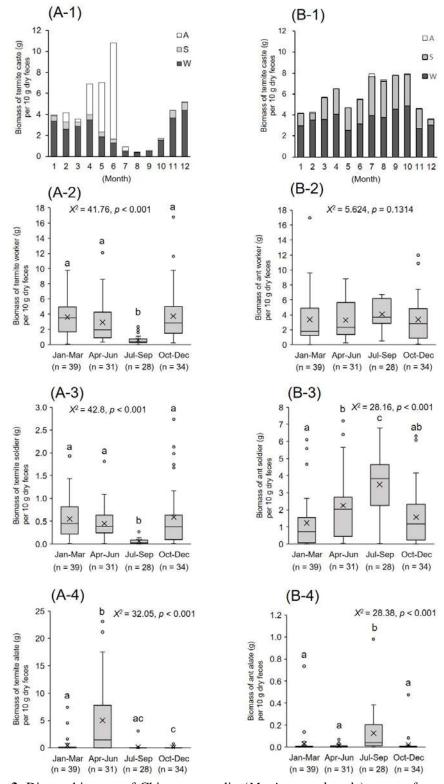


Figure 3. Dietary biomass of Chinese pangolin (*Manis pentadactyla*) among four seasons. Overall biomass of termite and ant castes fed by pangolins, and biomass of worker (W), soldier (S), and alate (A) castes were estimated by using 132 feces samples.

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The predator of ants and Termites: pangolins

by

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Abstract

The predator of ants and termites: pangolins is facing a huge crisis. Severe poaching continues to occur throughout the world, accelerating the extinction of this species.. More and more confiscated pangolins are brought into rescue centers to wait for the wild. Do we have the ability to save these animals? For ex-situ conservation, efficient captive technologies are important, and urgent to be developed. Taipei Zoo focused on the development of diet, husbandry, medical science, and research of basic biology, behavioral and reproductive physiology, and has been accumulating results and experiences over the years in pangolin captive management. The most important and key of captive pangolin is to know what they eat and what nutrition we have to provide. We combine nutrition and dietary ecology to try to understand the food diversity and digestion status of pangolins in the wild, and the nutritional composition of ants and termites. Food to meet the nutritional needs of captive pangolins at different stages of their life. In the future, the pangolin's ex-situ conservation husbandry technology will be further developed to assist in the rescue, rehabilitation to develop Ex-situ populations to save this animal that is about to become extinct.

Keywords: pangolin, dietary ecology, termites, ants, artificial diet

Introduction

Pangolin is the only species under the Pangolinidae family, there are 8 species in the world, distributed in tropical and subtropical regions of Africa and Asia. In Asia, pangolin's meat is regarded as a wild game, and scale is considered to have the effect of promoting blood circulation and swelling, which is why it has always faced huge hunting pressure in the Chinese market. From 2010 to 2015, an estimated 120 tones pangolin was arrested for illegal smuggling trade. The International Union for Conservation of Nature (IUCN) has formally announced on October 23, 2014 that the Chinese pangolins have been upgraded to "Critically Endangered" (CE). In addition, the eight species of pangolins have been officially upgraded from Appendix II to Appendix I on September 28, 2016 in the CITES (Convention on Endangered Species of Wild Fauna and Flora). Taiwan's Wildlife Conservation Law lists them as precious and rare conservation wildlife of conservation level II.

Taipei Zoo assists domestic conservation authorities in hosting pangolins through wildlife rescue work. During the containment period, we were given rescue treatment and accumulated pangolin medical specialty. We continued to collect basic pangolin biological data, and gradually established the ecological habits and behaviors of the pangolin in captivity in Taiwan. Fill a vacancy for the difficulties and deficiencies in the field pangolin research.

Pangolins are the important conservation focus species captive in Taipei Zoo. Over the past three decades, the Taipei Zoo has dedicated to the development of the husbandry and breeding techniques for pangolins. In physiological research, blood samples are regularly collected to analyze kinship data and cooperate with hormone detection to make the most suitable pair for reproduction; analyze the nutritional composition of termites and ants in the pangolin field staple food to develop artificial diets that are more suitable for the nutritional requirements of pangolins. In this topic, I will focus on the diet of pangolin in zoo and the development of artificial diet.

Pangolins are myrmecophagous mammals and the predator of ants and termites whose biology and ecology remain poorly studied. They face a huge crisis in the world whose are the critically endangered species. More and more confiscated pangolins enter the rescue center to wait for the wild. For ex-situ conservation, the efficient captive technologies are importance and urgency to development. Taipei Zoo focuses on the development of diet of pangolin, husbandry, medical technology, and research of basic biology, behavioral and reproductive physiology, has accumulated results and experiments over the years in captive management. The most important and key of captive is to know what they eat and what nutrition we have to provide. Many myrmecophagous mammals like giant anteater, armadillo or aardvark have developed fairly complete husbandry to know how to captive. But those successful experiences cannot be applied to this particular species of pangolin.

The development of pangolin diet in Taipei Zoo.

The diet is one of the key to improve the survivability of captive pangolin. But captive ants and termites to feed pangolin is really a challenge. Research and development of artificial diet becomes another resort. From the field study of pangolin diets, we found that invertebrates are especially important for pangolins, if we further look into the captive diets of pangolins, that different zoo had experience in keeping different pangolin species, while, the captive diets of pangolins varied from institutions. The food items used in zoo or rescue center could be divided into 7 categories, which were invertebrates (like meal worms or nee larvae), vertebrates (for example: beef), plant matter (coconut powder), concentrates (cat or dog food), dairy (milk powder), supplements (vitamins or minerals), water. Most of the institution failed on keeping pangolins, they died in a short time, in most cases, and these were because of poor acceptance of captive diets and digestive problems. So in the beginning of keep pangolins, we had to solve how to maintain them longer. Not only did we have to improve the environment but also the captive diets.

The first diets of Chinese pangolin was composed of cooked unpolished rice, cooked potato, bread, egg yolk, bee larvae, milk powder, honey, feline food, vitamins and minerals supplements. Continuous improvement over 15 years base on the health problem of pangolins. After 2010, we already have a good recipe. As the diet improve, the number of days of survival under captivity increases significantly. But that is not enough. Animal welfare is more than just survive.

Build a healthy and sustainable captive population is our goal. The natural food resources are difficult to obtain. Proper diet formulas are important for captive and rescued pangolins. From an ecological point of view, we must develop artificial diets that are more suitable for the nutritional requirements of pangolins, so that the captive environment can be more closely combined with the wild pangolin diet. We hope that the captive pangolin can also be providing as much nutrition as it needs, satisfying all the life history of the pangolin and the nutrition required at different stages. This must be closely combined with ecology and pangolin food habits in the wild.

Termite mandibles and ant head capsules are the two primary remains found in pangolin feces collected in the field. Determining the retention time of insect cuticles is important for understanding the digestive physiology of pangolins, while determining the recovery rate of termites and ants in feces is required to estimate the number of these preys that are consumed by pangolins.

Clarify the digestibility and digestion status of pangolins to ants and termites, thereby helping to estimate the ants and termite content in wild pangolin feces, and help to more accurately estimate the

dietary composition of wild pangolins. The improvement of artificial diets has substantial help and reference value to elevate the animal welfare in captive.

To achieve this goal, we have the following challenges in the development of diet. The challenges are (1) What is the compositions of wild diet. (2) Knowledge gap of nutritional requirement in each life stages of pangolins.(3) How to increase palatability of diet for pangolin.

Digesta retention time and recovery rates of ants and termites in Formosan pangolins (Manis pentadactyla pejtadactyla)

In this study, we conducted feeding trials with captive Formosan pangolins (*Manis pentadactyla pentadactyla*). Sixty grams of the fungus-growing termite *Odontotermes formosanus* (18,816 insects) and 15–20 grams of the yellow crazy ant *Anoplolepis gracilipes*(14,400–19,200 individuals) were fed to each pangolin in two feeding trials. After feeding, pangolin feces were collected daily for 1 week. We also assessed the accuracy of using chromium (III) oxide power (Cr_2O_3) as a proxy for determining gut passage time in pangolins, as has been done in previous studies. The results showed that remaining termite mandibles and ant head capsules in feces peaked at 66 and 90 hours after feeding, respectively. The recovery rates of termite mandible sand ant head capsuleswere0.35 \pm 0.10 and 0.65 \pm 0.04, respectively. In both feeding trials, the retention time of Cr_2O_3 was much shorter than that of the termite mandibles and ant head capsules, indicating that Cr_2O_3 is not an appropriate indicator for estimating food retention time of myrmecophagous animals. Our results revealed that the ant head capsules were preserved better in feces compared with the termite mandibles, suggesting that termites may be considerably underestimated in the feces of wild pangolins.

Nutrition analysis of pangolin diet and termite symbiosis fungus nursery.

The analysis of nutrition of 3 years artificial diets showed that there was no significant difference of total calories among different years, but were different significantly on amino and fatty acid. It indicated that the current diets couldn't supply stable nutrition for captive pangolins. The symbiosis fungus nursery of termites and artificial diet of Taipei Zoo both contained high concentration Arginine, Glutamine, Aspartic, Linoleic acid (9,12-cis-Linoleic acid) and Palmitic acid.

Analysis of the amino acid content in the old and new termite symbiosis nursery showed that there was no significant difference in the amino acid content of the new and old nursery (p = 0.1208, Wilcoxon Signed Rank Test). Overall, the glutamic acid content of the termite symbiosis nursery was the highest, followed by aspartic acid and pyridonate, but the glutamic acid content of the old termite symbiosis fungus nursery was 10 times than the new. The reproduction-related formamide and amidine contents of the new and old symbiosis fungus nursery were 111.1 and 108.2 mg / 100g, and 291.9 and 298.2 mg / 100g, respectively (Figure 2).

Comparing the amino acid content of the artificial diet and termite symbiosis nursery, the results show that both have high contents of amidine, glutamic acid, and aspartic acid. All are lower than the artificial food in the symbiosis nursery. Analysis of the fatty acids in the old and new termite symbiosis nursery showed that there was no significant difference (p = 0.4237, Wilcoxon Signed Rank Test). Overall, the percentage of fatty acids in termite symbiosis nursery was the highest with linoleic acid (9,12-cis-Linoleic acid), the content of which was higher than 30%, followed by Palmitic acid, which contained 18% (Figure 3).

Comparing the percentage of fatty acids in the artificial diet and termite, the results shows that both have a high proportion of linoleic acid and palmitic acid. Not detected in the termite symbiosis nursery. The preliminary analysis shows that the amino acid and fatty acid composition of the termite symbiosis nursery is similar to that of the termite, and there is no significant difference in the amino acid and fatty acid composition between new and old termite symbiosis nursery. Depends on this, the termite symbiosis nursery may can be developed as an ingredients of artificial diets, or as a food directly used to feed pangolin. The collection of termite symbiosis nursery is relatively easier than termites. Therefore, it is more feasible as a main material for artificial diets or directly as food for pangolins than for collecting or breeding termites or ants, and it is expected that pangolins can obtain proper

nutrition. In the process of excavating termite nests and feeding termites in the wild, pangolins should also feed on termite symbiosis nursery. Therefore, termite symbiosis nursery may be able to be one of the important food sources for pangolins.

The results also show that both the termite symbiosis nursery and the artificial diet have a high proportion of fatty acids such as linoleic acid and palmitic acid. In general, the main amino and fatty acid components of artificial diets and termite symbiosis nurseries are similar, but the two reproduction-related amino acids (methionine and lysine) in termite symbiotic nurseries are lower than artificial diet.

Research on pangolin foraging ecology relies heavily on the cooperation of experts in different fields. Through cooperation with pangolin ecologists, ant experts, termite experts and Taipei zoo, Taiwan 's understanding of pangolin foraging ecology has made significant progress. The in-depth study on the ecology of pangolins and ants and termites is expected to contribute to the conservation work of this critically endangered animal.

Conclusion

The pangolins of Taiwan adopt anteaters (including ants of the Hymenoptera family and termites of the genus Termitidae) as the main food (Lin Zongqi et al., 2017; Li et al, 2011). In order to make artificial diets closer to the nutrition of wild foods and to meet the needs of various life history stages of pangolins, the park has invested in artificial diet nutrition research in recent years, hoping to develop a diet composition that better meets the nutritional requirements of pangolins. The team continued to cooperate with the National Institute of Changhua Normal University's Biology-Social Insect Research Department and the National ZTE University's Department of Entomology to understand the feeding habits of wild pangolins and find ant species suitable for captive pangolin feed based on ant / termite characteristics. The artificial pangolin diets developed by this garden have good feeding conditions and stable animal health. However, there seems to be a breakthrough in reproductive capacity. The limiting factors have yet to be clarified. This study hopes to provide pangolin breeding facilities by adjusting the nutrition of the diet. The required nutrients are targeted for testing and improvement of the diet. In addition, in recent years, some individuals have discovered problems such as poor scale growth and dry skin. They also hope to improve the nutrition of the diet.

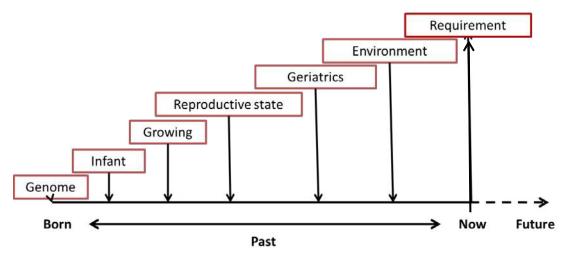


Figure 1. The artificial feed of pangolin should meet the nutritional needs of each stage in life history.

Old termite symbiosis nurseriesNew termite symbiosis nurseries

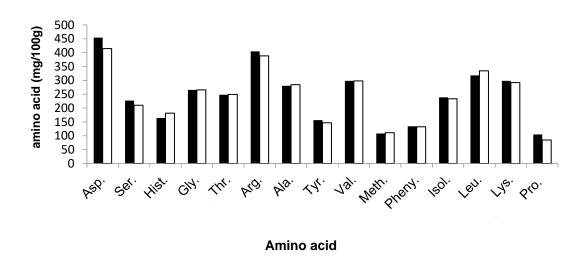


Figure2. Comparison of amino Acid composition in new and old termite symbiotic nursery (Formamidine and Asp Acid are related to reproduction) °

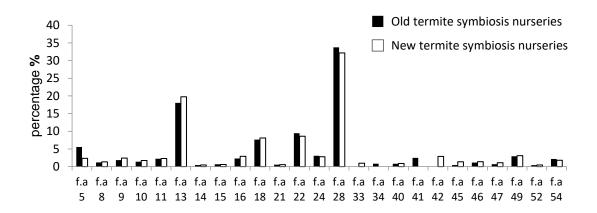


Figure3. Fatty acid composition in old and new termite symbiosis nurseries (* indicates fatty acids related to reproduction). Each fatty acid code is as follows: f.a5:Undecanoic acid; f.a 8:Myristic acid; f.a 9: 9-trans-Tetradecenoic acid; f.a 10:Myristoleic acid; f.a 11:Pentadecanoic acid; f.a 13:Palmitic acid; f.a 14: 9-trans-Hexadecenoic acid; f.a 15:Palmtoleic acid; f.a 16:Heptadecanoic acid; f.a18:Stearic acid; f.a21:1-trans-Octadecenoic acid; f.a 22: 6-cis-Octadecenoic acid; f.a24:11-cis-Vaccenic acid; f.a 26: 9-cis,12-trans-Octadecadienoic acid; f.a28:9,12-cis-Linoleic acid; f.a33:Arachidic acid*; f.a 34: 9-cis, 12, 15-trans- Octadecatrienoic acid+9,12-; f.a40:11,14-cis-Eicosadienoic acid; f.a41: 8,11,14-cis-Eicosatrienoic acid; f.a 42:Behenic acid; f.a45:(13-cis-Erucic

acid); f.a 46: Tricosanoic acid; f.a47: 13,16-cis-Docosadienoic acid; f.a 49:Lignoceric acid; f.a 52, 4,7,10,13,16-cis-Docosapentaenoic acid; f.a 54:4,7, 10,13,16,19 -cis-Docosahexaenoic acid, DHA_o

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O 19

Replacing Some Myths by Truths about Termites

by

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Abstract

Older termite myths are replaced. Termites are no longer confined to be tropical insects, their presence are not limited by latitude, altitude or country boundaries. Alates swarming cannot take place throughout the year. There is no fixed swarming schedule due to global warming. Termite infestation suggests bad luck and churns out many ghost stories, but in fact it is a result and not a cause. Termites are said to destroy homes, but only if the owners have an indifferent attitude to home care. There is an optimistic conclusion that termite prevention is still possible to save from termite damage. Steel structures cannot avoid termite infestation. The pain suffered by a female termite refuted the myth that mother insects couldn't care less about their babies. The outlook appearance of worker, soldier and queen look so different but they come from the same parents. They integrate to form one composite organism. The soul power or instinct is transmitted from the queen to all organs, workers, soldiers, alates and the digestive fungus gardens.

Keywords: termite, instinct, myths, swarmers, termite-proof structure, man-made organs

Introduction

The knowledge about termite is fascinating but is changing in its terrain. It is important for both the expert and layman to know the fact rather than the fiction of termites. When it comes to paying for the cost of pest control against termite, knowing more about the myths can avoid paying for a costly termite control problem. The study of termite in a scientific way is relatively young as compared with the existence of termite itself on Earth. The only systematic study began with Marias (2010) who has carried out tedious and trustworthy observation less than a century ago. The work has not surfaced to the public knowledge domain until after three decades. However, reading through his study we can derive many correct fact about termites. Therefore, in my study below, I have relied on his work as a basis for listing the truths to replace the myths. The reason I trust his work is because I found his observation supported by minute details which I have had previous experience with or previously seen. Therefore, I have conviction on his work and believe that they are true. The current advancement of man-made organs and artificial intelligence has coincided with the study by Marais (2010) which again has added credence to his theories and observations. The different members of the caste system in a termite nest has a footprint of a human body now being used in artificial intelligence to produce organs in treating incurable human diseases, basing on genetic, nano- and robotic technologies. One final aim of this research is for a consolidation of some research papers in the previous PRTRG conferences to be used and applied as much as is possible.

Materials and methods

The research is literature review based. It is a qualitative research all done by deskwork. According to Kelly's personal construct theory (1963), "all theories are hypotheses created by people, which may be valid at any particular time, may suddenly become invalid in some unforeseeable respect and replaced by a better theory". From this perspective the previous experience and understanding about termites as a fiction or a myth are of the same type. Myths came by from superstitious and primitive

minds. They are personal construct of the reality by the people. They fall into the cycle of the Kelly's theory.

Results and discussion

Some myths and truths are disclosed as follows. They have been arranged according to their scale and size:

- 1. Termites are tropical insects Termites (Hill, Hore and Thornton, 1982) are truly tropical insects and thus are not abundant in Hong Kong for the climate here is not hot enough for their liking. Another consequence of local temperatures is that locally the mound-building termites in the family Termitidae do not produce a mound but keep the entire colony underground. Chen (2003) suggests that termites originated in tropical forests. After years of evolution, they chose warm and humid climate. Therefore, nowadays, termites found their habitat in both tropical as well as sub-tropical climate conditions. Globally speaking, termites spread in lands between 45⁰ north and south to the equator. The above are all myths. The truth is: Deng, et al. (2016) collect Reticulitermes species at altitude 2356 m above sea level. There were two species in Tibet, R. tibetanus and R. assamensis. It was proven that irrespective of altitude, the two Reticulitermes species in Tibet have a close relationship with other Chinese continental Reticulitermes, including those from neighbouring provinces of Yunan, Sichuan, Jianxin, Jiangsu and Fujian. They are not relic species confined to Tibet. Singham (2016) writes that Macrotermes gilvus is an indigenous subterranean and fungusgrowing termite species of Southeast Asia. They are found in areas from Indochina to Malayan Peninsula, Sumatra, Java, Borneo and the Philippines. These places were full of environmental forces including tectonic movements, glacial cycles, climatic changes and volcanic activities. He opines that M. gilvus is well suited to investigate the relative impact by these forces. Singham (2016) suggests that the phylogeographic pattern of M. gilvus is likely relics from Pleistocene (2,580,000 – 11,700 years ago) environmental changes. Most genetic differentiation was associated with Pleistocene geographical division. The Sunda Shelf was recorded to be 120 meters lower than the current sea level 11,700 years ago during the last Ice Age. He notices that the gene flow between geographical groups is rare, an obvious exception to this is a strong genetic propinquity between Malayan Peninsula and North
- 2. **Any time** Termite can swarm throughout the year. The truth is: termites only swarm one time a year (in the spring). The temperature needs to reach 21⁰ C. The swarm usually happens the first sunny day after a heavy rain. If you see "swarm-like" insect in other times of the year, it is probably an ant swarm, which looks very similar.

boundaries as previously envisaged.

Sumatra populations despite the presence of a formidable physiographic barrier – the straits of Malacca separating the two regions. It was detected that during the early Pleistocene, there was a spreading out of *M. gilvus* from Indochina towards Java and the Philippines reaching to across Southeast Asia. So the demarcation is not confined to latitude, altitude or country

3. **Fixed swarming roster** Chen (2003) observes a fixed time schedule of alate swarming in Guangzhou China: that *Coptotermes formosanus* swarms from early May to late June; *Odontotermes formosanus* swarms from early April to mid-June; *Macrotermes barneyi* swarms from mid-April to mid-May and *Reticulitermes guangzhouensis* swarms as early as late March. The traditional thinking is Formosan and Asian termites swarmed at different times which would make it impossible for them to interbreed. However, the abovementioned regular dates are now disrupted probably by global warming. SCMP (2015) reports Professor N Y Su's findings that two of the most destructive termite species in the world are mating in South Florida, producing hybrid colonies that are growing at twice the normal rate of other termites. The types of Asian and Formosan termites used to live in separate territories and swarm at different times.

- 4. **Termites are vicious** Property having termites is a sign of bad "fung shui" or vicious spirit, therefore during quiet moments in the night, the "tik-tik" noise is made by ghosts. The truth is Marais (2010) writes that the noise comes from the soldier termites. The termite nest must be kept intact at all times. In case the nest is damaged, the pin-pointed looking creatures will first appear at the spot, they will examine the damage and the new openings, and will investigate from all angles. In case there are no worker termites nearby, or if the number of workers are not enough, the soldiers will "shout" by moving their shoulder bone, the pronotum, producing a series of sudden actions of "tik-tik-tik". If there are termites at our home, at night it is possible to hear the "tik-tik" noise from all directions. Soldiers use this noise to call workers to help, this is the same sound when food is found. It is recognizable for assembly, no matter what duties workers are engaging, like carrying water, transporting nymph, cultivating a fungi garden, feeding the king or feeding the queen, they will all quit and come to the sound source for an assembly. Cho et al. (2008) write that the parts of a building like timber rafters near wall, the corner of the pedestal, the bottom of staircase, the connections with staircase and the ground floor, the built-in wood cupboard, footing of column, the skirting near the entrance door, etc. can attract termites. At night when there are sounds of "tik-tik-tik" near the front door, it might not be about a ghost story, neither should this be interpreted as a bad "fung shui" or bad spirit. This is a matter of a cause and result. Many Fung Shui masters treat termites in a built environment as vicious. This is proven by the fact that vacant buildings were infested by termites. Termite infestations are found in buildings where they are found quieter or when they are empty. The result that they are vacant is not caused by the termites but by a lowering of business or other activities. So, the termite infestation should not be considered as a cause rather an outcome of other factors.
- 5. **Termites eat your home** Termite swarmers are damaging our properties. The truth is: termite swarmers are not eating your home. They have one purpose in life and that is to mate and create new colonies. While it is true that termites cause over US\$2 billion in all sorts of damage each year, it takes most colonies a while to do any significant damage. It is when the evidence of termites is ignored that serious damage can occur.
- 6. Termite prevention is impossible There are things you can do to help prevent termites. Most professional pest control companies will provide you with a free inspection. The report will list any conducive conditions for termites, such as moisture areas, high soil condition along the structure or wood that is in contact with the ground. Correcting these types of things will reduce the chance that termites will invade the structure.
- 7. **Steel house is termite-proof** Steel frame house is immune from termite attacks is a myth. Termites don't just attack timber houses. The truth is termites can get into your home through a small crack and eat away at the window frames, skirting boards, floor boards and wall linings. The overall risk of major structural damage to steel or masonry house is low, but all types of houses are at risk. Metal can be bitten through though. Cheung et al. (2014) say that, termites are not capable of damaging hard building materials such as steel, glazing and porcelain, concrete and stone. Nevertheless, owing to their small stature, termites can penetrate these materials through small cracks. In a building lacking maintenance, it is not uncommon to find cracks in concrete beams, walls, etc. As termites penetrate these cracks they will leave traces, such as mud that can lead people to think termites made the cracks. In fact, this is not the case.
- 8. Giving birth is painless Termites lay eggs leisurely. The nest is like those of other homes, which is full of food. Most insects lay eggs leisurely and casually, like having food, drinking water, shrugging the feathers and cleaning the tentacles. After the honeymoon, the male insect duty will end, the female insect will produce eggs after the nest is prepared and then her duty

will be over. Sometimes, the female insect will never come across the produced babies. This also applies to termites but this is merely a myth. The truth is Marais (2010) observes that the female termite is very attentive whilst laying eggs, has not been slightly distracted in the presence of human's lighting. Her preparation work is very impressive. She spends a long time standing in where she will lay eggs, and then starts to produce eggs. Her antenna curls and lays about in all directions. Her mouth opens and shuts constantly, at times she raises her lower body high in the air. Before laying eggs, she turns around two or three times and look at the ground, like checking what is on it. When the actual laying takes place, her body will seriously shrink. When the termite gives birth to the first batch of eggs, she will turn around, spend a long time and examine the eggs carefully, use her mouth part and front legs to gently touch them. The next moment will see her lying motionless beside the eggs for a period of time. The series of actions suggest that what we see is the evidence of pain of production in nature.

9. Worker, soldier and queen have different parents Worker, soldier and queen look so different, they must have been born from different parents. The truth is they are from the same. Then how does it happen that the worker and soldier not only inherit instinct which the father and mother did not possess, but they do not inherit the specialized instinct that the father and mother do possess? Marais (2010) writes that the individual worker or soldier possess no individual instincts. It forms part of a separate and composite organism, of which the queen is the psychological centre. The queen has the power - call it instinct - of influencing the workers and soldiers in a certain way, which enable them to perform collective duties. The power or instinct is transmitted to all queens born from her. As soon as the queen is destroyed, all the instincts of workers and soldiers immediately cease. She transmits this psychological power to the future queens, just as she transmits to them the power of producing three infinitely different form of insects: the queen, the worker and the soldier. The powers are transmitted differently to these three forms but certainly they still come from the same parents. That is the most spectacular observation I have come across. Marais's (2010) years of unceasing work on the veld led him to formulate a theory that the individual nest of the termites is similar in every respect to the organism of an animal. He observed that the workers and soldiers resemble red and white blood corpuscles, while gardens with fungus are the digestive organ. The queen functions as the brain and the sexual flight of swarmer is similar in every respect to the escape of spermatozoa and ova. The current advancement of artificial intelligence has coincided with the study by Marais (2010) which again has added credence to his theories and observations. The different members of the caste system in a termite nest has a footprint of a human body, which is being used in artificial intelligence to produce organs in treating incurable human diseases, basing on genetic, nano- and robotic technologies. In the book *The Singularity is Near*, Raymond Kurzweil (2005) postulates that we will have millions of blood-cell sized devices, known as nanobots, inside our bodies fighting against diseases, improving our memory, and cognitive abilities. With a new revelation of the instinct of termites, the law of accelerating returns can push forward the frontier of knowledge, and to see the number of inventions exponentially increased.

Conclusion

From the literature reviewed and the information gathered, the myths have been arranged from a macroscopic to a microscopic perspective. The results in the preceding paragraphs have seen some replacement of myths by truths. Termites are no longer confined to be tropical insects, their presence are not limited by latitude, altitude or country boundaries. Alates swarming cannot take place

throughout the year. There is no fixed swarming schedule due to global warming effects. Termite infestation suggests bad luck and churns out many ghost stories, but in fact it is a result and not a cause. Termites are said to destroy homes, but only if the owners have an indifferent attitude to home care. There is an optimistic conclusion that termite prevention is still possible to save from termite damage. Steel structures cannot avoid termite infestation. Cracks between the heavy steel members will allow the passage of termites to forage for food. The pain suffered by a female termite refuted the myth that mother insects couldn't care less about their babies. The outlook appearance of worker, soldier and queen look so different. In fact, the three forms of insects come from the same parents. They integrate to form one composite organism. The soul power or instinct is transmitted from the queen to all organs, workers, soldiers, alates and the digestive fungus gardens. This reminds me of the current advancement of artificial intelligence to repair humans. By storing the brain memories and experience in the "cloud", the rest of the human bodies are all artificially manufactured by genetic, nano- and robotic technologies. Decayed or decaying body parts will be replaced, like those done by worker and soldiers, by manmade organs. In this way, humans can be immortal, the same as following the foot print of the termites.

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O 20

Control of Drywood Termites (*Cryptotermes dudleyi* Banks) Using Carbon Dioxide

by

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Abstract

The project aimed to evaluate the performance of carbon dioxide (CO₂) in controlling drywood termites; identify the recommended concentration and exposure period that will eliminate drywood termite infestation as remedial treatment; and to determine the cost of controlling drywood termites using CO₂.

The performance of CO₂ as treatment to drywood termites, *Cryptotermes dudleyi* Banks was determined inside 5.0 cm x 10 cm x 10 cm plastic trays as enclosed termite chambers. Each termite chamber was provided with a conditioned wood block of 1.0 cm x 2.0 cm x 6.0 cm of Moluccan sau [*Paraserianthes falcataria* (L.) Nielsen] and 100 termite workers were introduced in the set-up. Different levels of CO₂ treatment application at loading times of 30, 45, 60, 75 and 90 secs per termite chamber at a constant pressure of 10 kg/cm² was used. The efficacy of CO₂ treatment was based on the mortality of test insects after treatment application which was observed at intervals of 0, 1, 3, 5 and 7 days.

The effective dosages of 60 secs, 75 secs and 90 secs loading times that were obtained in the above test was also applied on 1.25 cm x 30 cm x 30 cm drywood termite-infested wood blocks of *P. falcataria* and untreated marine plywood as remedial treatment to control the infestation. The efficacy of the treatment application was likewise based on insect mortality at 3, 5 and 7 days-treatment period.

Results showed that CO₂ was highly toxic and caused 92.7% to 98.0% kill to *C. dudleyi* regardless of exposure periods. In contrast, the lower loading times of 30 secs to 60 secs of CO₂ were moderately toxic to drywood termites with mortalities ranging from 40.7% to 73.3% in 3 days to 7 days of exposure period.

The performance of CO₂ using infested termite lumber of Moluccan sau, *P. falcataria* and marine plywood conformed with the results in the toxicity of the fumigant in the preliminary screening of the toxicity of CO₂ to *C. dudleyi*. The CO₂ at loading times of 75 secs and 90 secs were also highly toxic and extremely reduced 95.1% to 99.5% of *C. dudleyi* in infested lumber and marine plywood. However, the CO₂ even at the longest loading time of 90 secs and exposure period of 7 days was not able to completely eliminate termite problem compared to the 100% kill provided by the standard chemical aluminum phosphide (AlP). The CO₂ can be used as alternative compound for AlP and should be combined with chemical treatment to entirely control drywood termite infestation.

The cost of treatment using aluminum phosphide is PHP 44.00 much less compared to PHP 67.00 cost of CO_2 per treatment of 1 m² or m³ of termite-infested area. However, aluminum phosphide is hazardous to human and to the environment compared to the application of relatively safe CO_2 .

Keywords: Fumigation, carbon dioxide, drywood termites, *C. dudleyi*

Introduction

Drywood termites (*Cryptotermes dudleyi* Banks and *C. cyanocephalus* Light) are the major destructive species of termites of houses and building in the Philippines. Occurrence of drywood termites in wood is difficult to detect since they live deep inside the wood and will become noticeable only upon the appearance of fecal pellets. Drywood termite infestations are usually confined to a small area and may be found in structural wood, trim, hardwood floors, furniture, or other wood items (Galloway and Lay 1993). Lewis (2002) cited that because of the difficulty in detecting drywood termites and determining the extent of the damage done, do-it-yourself treatment are not recommended.

Drywood termite control methods can be categorized as either whole structure or localized. Whole structure treatments for existing infestations include the use of fumigants and application of heat to eliminate the existing population of drywood termites. Localized and spot treatments involved chemical methods using chemical liquid and dusts, foams and liquid nitrogen (Lewis 2002). Non-chemical control measures are also available in the form of application of biological control, electrocution, heat and microwaves.

Fumigation is one of the effective methods of controlling pests or insect infestations. Its advantage over localized treatment is that it eliminates infestations that are not visible to the naked eye. Major issues to consider with the use of fumigants include the difficulty of installing tarpaulins, difficulty in determining the proper dosage, the need to protect bag food items, and lack of residual control (Lewis 2002). Fumigation ("tenting") has been the only method used for over forty years which insures complete eradication of all drywood termites from a structure (Scheffrahn and Su 1997). The most commonly used fumigants for insect control include chloropicrin, 1, 3-dichloropropene, dichlorvos (DDVP), ethylene oxide, hydrocyanic acid gas, methyl bromide, methyl isothiocyanate, phosphine and sulfuryl fluoride (Rodriguez undated). Methyl bromide as fumigant was proven effective to control different kinds of pests. However, according to the US Environmental Protection Agency, it depletes the stratospheric ozone layer, hence the amount of methyl bromide produced and imported in the U.S. was reduced incrementally until it was phased out in January 1, 2005, pursuant to its obligations under the Montreal Protocol on Substances that Deplete the Ozone Layer (Protocol) and the Clean Air Act (CAA) (http://www.epa.gov/ozone/mbr/). Because of this, alternative treatments that are more environmentally friendly are being pursued (ISPM No. 15 2009). The application of inert fumigants such as carbon dioxide (CO₂) and nitrogen in structural pest control has gained interest due to environmental issues associated with the use of methyl bromide. CO2 is a fumigant that produces no harmful residues and is relatively safe to use (Food & Fertilizer Technology Center 2004).

On the other hand, several studies have already been conducted on the efficacy of CO_2 in controlling stored-product pests. Page *et al.* (2002) examined the use of CO_2 as a method for controlling thrips on onions for export. Six CO_2 treatments – an air control and 15%, 30%, 45%, 60% and 100% CO_2 , (all in balance air) were applied for 6, 12, 24, 48 and 72 hrs. Results of the study revealed that mortality was 100% at CO_2 concentrations of 30% or more after at least 24 h. Keever 1989 conducted fumigation of a 12,706 m³ warehouse containing cases (1.2 m X 0.76 m X 0.76 m) and hogsheads of flue-cured tobacco using CO_2 in order to kill the cigarette beetle, *Lasioderma serricorne* (F.). Results confirmed that all insects to include 500 eggs, 288 larvae, 270 pupae and 494 adults placed in the free space (with 35-60% CO_2 concentrations) and inside tobacco containers (with 38-45% CO_2 concentrations) were killed during the 5-day and 7-day treatments.

With the success of using CO₂ to manage pests in stored-products, it can also be a promising control method to address the serious problem on drywood termites. The technology will benefit the wood industry, developers, home and building owners, pest control operators and the general public as well.

This project aimed to 1) evaluate the performance of CO_2 in controlling drywood termites; 2) identify the recommended concentration and exposure period that will eliminate drywood termites; and 3) determine the cost of controlling drywood termites using CO_2 .

Review of Literature

Termites belong to the insect Order Isoptera. In nature, termites are considered beneficial because they break down dead and dying plant material (Cress 1997). However, they are also regarded as pests because they feed on wood components of houses and buildings.

Several methods of termite control are being practiced worldwide. Termite bait technology, conventional barrier treatments (liquid termiticides) and the use of borate wood treatments are approaches that are proven effective in protecting structures from subterranean termites provided they are properly applied (Clemson University 2008). For drywood termites, control measures can either be spot-treatment by injecting insecticides into the nest or fumigation for large infestations where colonies are in the same structure (Shelton et al. 2000). Fumigation is also an important tool for the control of termite and wood-boring beetle infestations (Delate et al. 1995). One of the most commonly used fumigants is the methyl bromide (MB). It has a relatively quick killing effect on insects, but because of its contribution to stratospheric ozone depletion has been phased out in developed countries since 2005, and in developing countries phase out will take place by 2015 (Navarro undated).

In the Philippines, the Bio-tech Environmental Services, Inc. started to use non-flammable pre-mixed cylinderized mixture of phosphine and CO₂, which provides highly effective fumigation in both sealed and unsealed storage facilities. In addition, the Horn Diluphos System, is a unique and revolutionary fumigation application technology that safely and effectively blends pure cylinderized phosphine directly with air to safe and effective levels of phosphine gas (10,000 ppm), well below the lower flammability limit (LFL) of 18,000 ppm (Neri 2012).

Other methods such as electro gun, microwave, and freezing with liquid nitrogen are local or spot treatments designed to eradicate termites in a specific area (California Structural Pest Control Board 2005). Studies have also been conducted on the use of carbon dioxide to control stored-product and wood destroying insects. Carbon dioxide, being a natural component of the atmosphere, is a safe chemical and has been permitted for use as an additive to many types of drinks and foods (Nakakita and Kawashima 1994). The use of CO₂ for grain fumigation has been proven effective but requires lengthy exposure intervals, typically 10 days or longer (Caliboso et al. 1994). Results of their study revealed that exposure of relevant development stages of major pests of stored grain to high concentrations of CO₂ (98%) for short intervals (5-20 minutes) produced complete mortality.

The study of Riudavets *et al.* 2010 aimed to establish the efficacy of CO_2 at high pressure against different stages of several insect and mite pests that affect stored-food products. Results confirmed that a high level of control was achieved for most species and development stages when they were treated with CO_2 at 20 bar for 60 min. Nakakita and Kawashima 1994 suggests that with CO_2 at 20 kg/cm², an exposure time of 5 minutes was sufficient to kill all adults of tested species of maize weevil, red flour beetle, lesser grain borer and cigarette beetle. Delate et al. found out that a 24-hr exposure to \geq 95% CO_2 caused significant termite mortality, but 60 h were required for complete mortality. The study further revealed that exposure to 50% CO_2 for 60 h resulted in approximately 70% termite mortality, while complete mortality was recorded after 120 h. In addition, when termites were sealed in wooden blocks (90 x 90 x 152 mm), 72-96 h exposure to \geq 95% CO_2 was necessary for complete control.

With the encouraging results of researches on the use of CO₂ gas against stored pests, there is a possibility that this can be applied to drywood termites or "unos", where damage occur in patches in a piece of wood or plywood. In this regard, there is a need to generate information of the effective dosages, treatment time and exposure period to effectively eliminate structural pests. This alternative measure will not only control drywood termites which is a serious problem of homeowners but will also discourage the use of more hazardous fumigants.

Material and methods

Test Insects

Drywood termites, *Cryptotermes* dudleyi Banks served as test insects and were collected from the cultured colony at the FPRDI Entomology Laboratory. Collection of test insects was done by gently splitting infested lumber or plywood from the rearing chamber. Termites collected were placed into a tray lined up with pieces of white papers. Only workers and soldiers that cling to the paper after few minutes were collected and transferred to another tray provided with pieces of wood veneers. The test insects were conditioned for 24 hours prior to introduction to respective termite chambers.

Test Wood Blocks

Wood blocks with dimensions of 1.0 cm x 2.0 cm x 6.0 cm were prepared from Moluccan sau [Paraserianthes falcataria (L.) Nielsen] and 1.27 cm x 2.0 x 6.0 cm commercial marine plywood. Wood blocks of P. falcataria of 5.0 cm x 10.0 cm x 30 cm were also prepared for further evaluation of the toxicity of CO_2 . Test wood blocks were sanded on all sides, properly labeled and gradually oven-dried to about 14.0% moisture content (MC). The percent MC of test wood blocks was determined by conventional oven-dry method calculated using the formula:

MC (%) =
$$\frac{\text{W1 - W2}}{\text{W2}}$$
 X 100 where: W!=Green weight (g) and W2 = OD weight (g)

Fabrication and Calibration of Carbon Dioxide Cylinder

The dispensing apparatus of carbon dioxide (CO_2) made of cylindrical chamber of 5-mm thickness steel was fabricated. This was provided with control valves (inlet and outlet) and a pressure gauge. The inlet valve was used for refilling CO_2 while the outlet valve was intended to discharge CO_2 . The outlet valve was equipped with a nozzle to serve as channel of CO_2 into the termite infested wood or termite chamber. The volume of CO_2 to be released for each test was 10 kg/cm^2 and determined by the pressure gauge provided.

Toxicity Test

Toxicity of carbon dioxide to drywood termites using termite chamber

A plastic container with a dimension of $5.0~cm \times 10.0~cm \times 10.0~cm$ served as termite chambers for the toxicity test of CO_2 . Each termite chamber was provided with a single $1.0~cm \times 2.0~cm \times 6.0~cm$ wood block at the center. The chamber was tightly sealed after introducing 100~workers and 2~soldiers of drywood termites. A small hole was made on the cover of each chamber that served as opening to release CO^2 .

The volume of CO_2 treatment was based on the length of time it was released at 30, 45, 60, 75 and 90 secs per termite chamber using a pressure of 10 kg/cm². Each treatment was replicated 10 times. The set-up was kept at room temperature in the dark room and mortalities of test insects were determined after 0-day, 1-day, 2-days, 3-days, 5 days and 7 days of exposure period. A standard chemical of aluminum phosphide and untreated set-up were provided for comparison.

Remedial treatment of termites infested wood blocks and marine plywood using CO₂

Wood blocks of *P. falcataria* with dimensions of 5.0 cm x 10.0 cm x 30 cm and marine plywood samples measuring 1.25 cm x 30 cm x 30 cm were prepared as substrate for drywood termites. Two holes were drilled at the center of wood block at a depth of 2.5 cm and 0.75 cm in wood and plywood samples, respectively. Each hole was introduced with 100 workers and 2 soldiers of *C. dudleyi* and plugged with wood dowel. The population was allowed to establish inside the substrates for four (4) weeks prior to toxicity test.

The performance of CO₂ was further evaluated as remedial treatment to control drywood termites. Based on results of the toxicity test, the treatment time of CO₂ used were 60, 75 and 90 secs with pressure of 10 kg/cm² and exposure periods of 5 days and 7 days. Treated and untreated materials were retrieved after the exposure period and cut or split gently with a sharp knife to determine the percent mortality of test insects.

Evaluation of the Efficacy of Treatment

Wood blocks containing active termite population were examined after exposure to CO₂. Woodblocks were gently split and the live and dead termites were counted. Percent mortality of termites was recorded and computed using the following formula:

% Mortality =
$$\frac{\text{Number of dead test insects}}{\text{Total number of termites}} \times 100$$

The efficacy of treatment of CO₂ was rated as follows:

% Mortality	Classification of the Efficacy of Treatment
76 - 100	Highly toxic (HT)
51 - 75	Toxic (T)
26 - 50	Moderately Toxic (MT)
1 - 25	Slightly Toxic (ST)
0	Not Toxic (NT)

Statistical Design

The experiment was laid-out following the Completely Randomized Design (CRD). The toxicity of CO_2 was classified based on the arbitrary rating cited in the methodology.

Cost of Treatment

The estimated cost of treatment of CO_2 was determined based on the cost of needed materials and the cost of CO_2 . The cost of treatment of CO_2 was comparted with the cost of treatment application of standard furnigant aluminum phosphide.

Results and discussion

A. Toxicity of Carbon Dioxide to Drywood Termites in Termite Chamber

The results of the toxicity of CO₂ at 3 days fumigation period showed that the mortalities of drywood termites *C. dudleyi* Banks increases remarkably with the increasing time of release of CO₂ and observation period (Table 1). At 30 seconds and 45 seconds of releasing time, the CO₂ was moderately toxic and caused 34.7% to 43.3% kill to test at 0-day after released of fumigant. The mortality of *C. dudleyi* Banks rose to 60.7% when the releasing time of CO₂ was set at 60 secs. The releasing time of CO₂ for 75 secs and 90 secs provided the highest mortalities of drywood termites that ranged from 88.0% to 94.7%, respectively.

The mortalities of *C. dudleyi* slightly increased in 1 day to 3 days after application of CO₂. However, the level of the efficacy of treatment remained the same regardless of releasing period of CO₂. The toxicity of fumigation at 30 secs loading time remained moderately toxic and gave 36.0% to 40.7% kill to *C. dudleyi* in 1-day to 3-days of observation period after release of CO₂. At 45 secs and 60 secs loading time, the CO₂ was toxic and provided 55.3% to 61.3% kill to test insects. The highest kill of 90.0% to 98.0% of drywood termites was recorded in 75 secs and 90 secs releasing time of CO₂. The results indicate that the longer releasing time of 75 secs and 90 secs of CO₂ into the chamber was more fatal than the releasing time of 30 secs to 60 secs to *C. dudleyi* Banks. Mortalities of test insects in the control group were 0% to 4.0%.

The toxicity of CO₂ to drywood termites at 5-days treatment period (Table 2) was similar with the performance of CO₂ when *C. dudleyi* was fumigated for 3-days. There were steady slight increases in termite mortalities from the 30 secs to 90 secs loading time period of CO₂ but the level of efficacy of treatment to drywood termites remained the same. The lowest loadings times of 30 secs and 45 seconds remained moderately toxic and the CO₂ affected 35.3% and 48.0% of test insects, respectively at 0-day after released of fumigant (Table 2). The loading period of 60 secs provided 60.0% mortality and the treatment was toxic drywood termites. Highest mortality rates of 90.0% to 94.0% were observed in loading times of 75 secs and 90 secs at 0 day after released of CO₂.

Table 2. Toxicity of carbon dioxide against drywood termites (<i>C. dudleyi</i>) at 5 days of treatment					
period.	_	,			
	Loading % Mortality After Release of Carbon Dioxide			Dioxide	
Treatment	Time	0 day	1 day	3 days	5 days
1. Carbon dioxide	30 secs	35.3 MT	40.7 MT	42.7 MT	48.7 MT
2. Carbon dioxide	45 secs	48.0 MT	50.7 T	52.7 T	58.0 T
3. Carbon dioxide	60 secs	60.0 T	60.7 T	64.7 T	66.0 T
4. Carbon dioxide	75 secs	90.0 HT	92.0 HT	92.0 HT	92.7 HT
5. Carbon doxide	90 secs	94.0 HT	94.0 HT	94.0 HT	98.0 HT
6. Control	-	0	0	6	6

Legend:

% Insect Mortality (Efficacy of Treatment): 76-100% (Highly toxic, HT); 51-75% (Toxic, T); 26-50 (Moderately toxic' MT); 1-25% (Slightly toxic, ST); and 0% (Not toxic, NT).

The 30 secs loading time of CO_2 remained moderately toxic to *C. dudleyi* at 1 day to 3 days after fumigation. It caused 40.7% to 48.7% kill within the period. The 45 secs and 60 secs loading time remained toxic and affected 50.7% to 66.0% of the test insects. The 75 secs and 90 secs loading time remained highly toxic and gave a mortality high of 92.0% to 98.0% within the period.

The results on the efficacy of CO_2 at 7 days exposure period against drywood termites are presented in Table 3. The efficacy of treatment to *C. dudleyi* at 7 days exposure period demonstrated the same level of efficacy at 5 days exposure period. Similarly, there were minimal increased in mortalities but the level of efficacy of treatment remained the same per loading time. The 30 secs loading time affected 40.7% of *C. dudleyi* and remained moderately toxic at 0 day to 3 days after 7-days of fumigation period. The 45 secs and 60 secs loading times remained. toxic to drywood termites at 0 day to 3 days.

Table 3. Toxicity of carbon dioxide against drywood termites (<i>C. dudleyi</i>) after 7 days of							
treatment period.	treatment period.						
	Fumigation	Fumigation % Mortality After Release of Carbon Dioxide					
Treatment	Period	0 day	1 day	3 days	7 days		
1. Carbon dioxide	30 secs	40.7 MT	46.0 MT	46.0 MT	48.0 MT		
2. Carbon dioxide	45 secs	64.7 T	68.0 T	70.0 T	70.7 T		
3. Carbon dioxide	60 secs	64.7 T	66.7 T	69.3 T	73.3 T		
4. Carbon dioxide	75 secs	89.3 HT	92.7 HT	93.3 HT	93.3 HT		
5. Carbon dioxide	90 secs	94.0 HT	97.3 HT	97.3 HT	97.3 HT		
6. Control	-	0	0	8	12		
I accord.	-	•	•		•		

Legend:

% Insect Mortality (Efficacy of Treatment): 76-100% (Highly toxic, HT); 51-75% (Toxic, T); 26-50 (Moderately toxic' MT); 1-25% (Slightly toxic, ST); and 0% (Not toxic, NT).

The fumigant caused 64.6% to 73.3% within the period of observation. The highest loading times of 75 secs and 90 secs displayed the highest rates of mortality of 89.3% to 94.% at 0 day. The treatment remained highly toxic and offered 92.7% to 97.3% kill after 1 to 3 days of exposure period.

Based on the results, the application of CO_2 can markedly reduce about 92.0% to 98% of the population of drywood termites by using 75 secs or 90 secs loading times and fumigation periods of 3 days to 5 days. However, it was unlikely to eliminate 100% of drywood termites even using the longest loading time of 90 secs and longer fumigation period of 7 days.

Remedial Treatment Using Carbon Dioxide in Infested Wood Blocks and Marine Plywood by Drywood Termites

Fumigation of CO₂ remarkably affected active termite populations of *C. dudleyi* using 60 secs loading time at 5 days fumigation period. The treatment was toxic to drywood termites and caused 75.0% kill of test insects in infested Moluccan sau (Table 4). It was noted however, that it was highly toxic to *C. dudleyi* in infested marine plywood and affected 82.9% of test insects using the same loading time and 5 days fumigation period. Higher mortalities were noted in the latter indicating that the penetration of CO₂ was easier than in penetration of voids in solid wood or lumber of Moluccan sau. Dead termites and pellet-like materials were noted inside the lumber Moluccan sau and composite marine plywood. The longer loading times of 75 secs and 90 secs of CO₂ provided higher mortalities of drywood termites in both Moluccan sau and marine plywood materials. Both treatments were highly toxic to *C. dudleyi* with mortalities ranging from 95.6% to 96.2% in 75 loading time and 95.1% to 99.5% in 90 secs loading time in infested Moluccan sau and marine plywood. The standard fumigant Aluminum Phosphide provided 100% kill of termites in both infested Moluccan sau lumber and plywood specimens. The results indicate that destruction of Moluccan sau and marine plywood might continue

Table 4. Toxicity of carbon dioxide as remedial treatment to Moluccan sau infested with drywood
termites, C. dudlevi at 5 days treatment period.

termites, et attate je at e aujs treatment person				
	Loading	% Mortality		
Treatment	Time	Moluccan Sau	Marine Plywood	
T1 - Carbon Dioxide.	60 secs	75.0 T	82.9 HT	
T2 - Carbon Dioxide.	75 secs	96.2 HT	95.6 HT	
T3 - Carbon Dioxide.	90 secs	99.5 HT	95.1 HT	
T4 - Standard, AlP	3 tablets/cu m	100 HT	100 HT	
T5 – Control	-	4.3	5.3	

Legend:

as the remaining active population might have survived from CO₂ application. In contrast, complete elimination of the termite problem can be provided by the standard fumigant, Aluminum phosphide.

The effect of longer fumigation period of CO_2 for 7 days to *C. dudleyi* is presented in Table 5. The CO_2 was highly toxic to *C. dudleyi* at fumigation period of 7 days regardless of loading times (60 secs, 75 secs and 90 secs) and termite infested materials (Moluccan sau and marine plywood). The mortalities of *C. dudleyi* ranged from 82.9% to 98.9% and all treatments of CO_2 were highly toxic as the standard fumigant AlP. The mortalities of *C. dudleyi* ranged from 82.9% to 98.9% and all treatments of CO_2 were highly toxic as the standard fumigant AlP. The only downside of CO_2 was that it cannot eliminate 100% of *C. dudleyi* in both infested lumber and marine plywood even if the fumigation period was extended to 7 days.

Table 5. Toxicity of carbon dioxide as remedial treatment to drywood termites, *C. dudleyi* infested materials at 7.0 days of treatment period

_	Loading	% Mortality	
Treatment	Time	Moluccan Sau	Marine Plywood
T1 - Carbon Dioxide.	60 secs	87.1 HT	82.9 HT
T2 - Carbon Dioxide.	75 secs	98.9 HT	98.2 HT
T3 - Carbon Dioxide.	90 secs	98.3 HT	98.3 HT
T4 - Standard AlP	3 tablets/cu m	100 HT	100 HT
T5 - Control	-	1.7	5.3

Legend:

[%] Insect Mortality (Efficacy of Treatment): 76-100% (Highly toxic, HT); 51-75% (Toxic, T); 26-50 (Moderately toxic' MT); 1-25% (Slightly toxic, ST); and 0% (Not toxic, NT).

[%] Insect Mortality (Efficacy of Treatment): 76-100% (Highly toxic, HT); 51-75% (Toxic, T); 26-50 (Moderately toxic' MT); 1-25% (Slightly toxic, ST); and 0% (Not toxic, NT).

Cost of Treatment

The total capital investment for CO_2 dispenser assembly and CO_2 container is PHP 6,000. The CO_2 dispenser assembly and CO_2 container are re-usable and could last for at least a minimum of 5 years. The full load of CO_2 in a 1 kg. cap canister cost PHP 200 to PHP 250.00 which can treat a 3 m x 3 m infested area. A minimal cost of P 10.00 for plastic and masking tape for sealing the infested area per square meter. The cost of treatment is estimated to be PHP 77.00 to 93.00 per square meter including the depreciation cost of CO_2 assembly and cost of plastic and masking tape. In contrast, the aluminum phosphide cost PHP 44.00 much less compared to PHP 67.00 cost of CO_2 per treatment of 1 m² or m³ of termite infested area. The aluminum phosphide is quite hazardous to human and environment compared to CO_2 .

Conclusion

The toxicity of CO₂ to *C. dudleyi* by termite chamber method showed that the toxicity increases with increasing loading times of CO₂. The higher loading times of 75 secs and 90 secs of CO₂ were highly toxic and caused 92.7% to 98.0% kill to *C. dudleyi regardless* of exposure periods. In contrast, the lower loading times of 30 secs to 60 secs of CO₂ were moderately toxic to toxic to drywood termites with mortalities ranging from 40.7% to 73.3% in 3 days to 7 days of exposure period. It is presumed that higher volume of CO₂ was dispensed with longer loading periods which provided higher mortalities of *C. dudleyi* than in the shorter loading periods.

The efficacy of CO₂ as remedial treatment of infested termite lumber of Moluccan sau and marine plywood conformed with the results of the toxicity test of CO₂ to *C. dudleyi* at higher loading times of 75 secs and 90 secs and treatment periods of 5 days and 7 days, respectively. The CO₂ at loading times of 75 secs and 90 secs were also highly toxic and reduced population by 95.1% to 99.5% of *C. dudleyi* in infested lumber and marine plywood. However, the CO₂ even at the longest loading time of 90 secs and exposure period of 7 days was not able to completely eliminate termite problem compared to the 100% kill provided by the standard chemical aluminum phosphide. The CO₂ can be used as an alternative compound for aluminum phosphide and should be combined with chemical treatment to entirely control drywood termite infestation.

Based on the results of the study, the cost of remedial treatment of infested area by drywood termites using CO₂ was P 67.00 per square meter compared to P 44.00 of aluminum phosphide cost. The aluminum phosphide is quite hazardous to human and environment compared to CO₂.

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O 21

Unique, Effective, Bio-Based Termiticide Microemulsion Solutions for Aboveground Protection of Malaysian Native Hardwoods Against Subterranean Termite Threats in Buildings and Outdoors

by

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Abstract

The bio-based termiticide solutions presented in this paper are water-based wood preservatives approved for dipping treatment providing 25 years of termite protection for solid wood and woodbased products in Europe and for more than 10 years in Indonesia. These bio-based solutions are patented formulations based on concentrated microemulsions (ME) diluted with water as a dipping treatment, but also for vacuum pressure treatment. Field trials conducted in Malaysia by UNIMAS confirmed the efficacy of such bio-based solutions at three product concentrations on short dip-treated kempas (Koompassia malaccensis) heartwood, a major hardwood species in the Malaysian wood construction market, against the Southeastern Asian subterranean termite Coptotermes curvignathus exposed to aboveground H2 (indoor, non-wetting conditions) hazard class targeting termites compared to CCA-treated kempas and radiata pine (*Pinus radiata*) sapwood. Prior to the H2 hazard class termite field test exposure, treated wood blocks were conditioned to either a non-leaching volatilization (H2 hazard class weathered wood blocks) or to a leaching followed by volatilization (H3 hazard class weathered blocks) as well as non-leaching/non-volatilization and leaching/non-volatilization reference treatments. After 6 months field exposure, untreated kempas was severely (termite ratings: 0, mean mass loss: 97.4%) or moderately attacked (mean ratings: 7.7, mean mass loss: 17.5%), while none of the leached-volatilized (H3 hazard class) or non-leached-volatilized (H2 hazard class) test blocks treated with those bio-based products at both target retentions were regarded as attacked (mean ratings: 9.7-10, negligible mean mass loss) regardless of biocide retention, leached or non-leached wood, volatilized or non-volatilized wood treatments. Excellent performance also prevailed with the remaining treatment combinations of treated wood. Due to their unique compositions, the bio-based termiticide solutions presented here showed excellent performance against Coptotermes curvignathus with a low biocide retention where conventional agro-insecticides do not work. In conclusion, these solutions are effective for long-term wood protection in buildings and aboveground outdoors against Southeast Asian Coptotermes subterranean termites.

Keywords: subterranean termites, *Coptotermes curvignathus*, kempas, cypermethrin, permethrin, diptreatment, microemulsion, H2 and H3 hazard classes.

Introduction

Various laboratory and field termite tests have reported termite resistance of solid wood or wood composite dip-treated, brushed-on or sprayed-on (collectively termed envelope treatments) with traditional emulsifiable concentrates, suspension concentrates or light organic solvent formulations often using pyrethroid and other organic termiticides at high dosages and/or without prior artificial

weathering of freshly treated wood before termite testing (Sornnuwat et al 1994, Peters and Creffield 2003, Donath et a 2008, Sukartana et al 2009, Fadillah et al 2014, Tawi and Wong 2016). These termiticides were also evaluated as pressure-treated wood when increased termiticide penetration into the wood was desired (Creffield et al 2013, Scown and Creffield 2009). A new generation of bio-based termiticidal formulations has now emerged which are well-known technologies in Europe for dip- and pressure-treatment of wood and wood-based products. These solutions have been recently considered in Indonesia using the lowest emission costs (eco-costs) Life Cycle Assessment (LCA) methodology (Siswanti Zuraida et al 2016). The laboratory of Adkalis - Berkem Group - has the expertise and knowledge in biocidal formulations, especially for wood preservation for over 50 years. The first ME-Microemulsion technology for dipping treatments was patented in Europe by this laboratory. Waterborne products can, with practical dipping or aspersion treatments and adequate dipping times and effective concentrations, also enhance durability performance of wood against termites under Malaysian/Australasian H2-H3 biological hazard class conditions (Wong 2004) in Southeast Asian Regions such as Malaysia. A summary of the key results of field trials conducted in Malaysia is provided in this document. The aim was to evaluate the performance of microemulsion technology of bio-based solutions on treated wood against Coptotermes curvignathus.

Materials and methods

The field trials were undertaken by The Wood Biodeterioration and Protection Laboratory, University Malaysia Sarawak (UNIMAS), Sarawak/Malaysia, using the H2 hazard class aboveground termite field test protocol Wong (2005), also described by Xuan et al (2017), meant to target termite pests (and exclude fungal decay) on a sheltered (shielded from sunlight and water) and 1-m high stack of termite-infested keruing (Dipterocarpus sp.) wood at a well-drained suburban open-spaced field site where Coptotermes curvignathus are prevalent. Hence, the test against Coptotermes curvignathus, is representative of the aggressive subterranean termites found attacking construction wood in Malaysia and much of Southeast Asia.

Table 1: Bio-based solutions (over 60% bio-based content according to E.U norm NF 16640, 2017

and International norm ASTM D 6866) - Properties and concentrations used

Products (ME-Microemulsion)	Nominal concentrations of active ingredient (%w/w)	Diluted product concentration (%)	Concentrations of a.i after dilution (%)
		5 (5:95)	0.08
Product 1	Cypermethrin: 1.6% w/w	10 (10:90)	0.16
		20 (20:80)	0.32
		5 (5:95)	0.05
Product 2	Permethrin: 1.0% w/w	10 (10:90)	0.10
		20 (20:80)	0.20

n=24.

The Malaysian wood selected for the preservative treatment and testing was kempas heartwood wellknown to be termite-susceptible (Wong 2000, Cirad 2012) and with wide commercial structural use in Malaysia and Indonesia. Air dry sawn Kempas (Koompassia malaccensis) heartwood samples were converted into replicated test blocks (2 x 2 x 5 cm). Kempas blocks were dipped, for three minutes, into one of three dilutions (product concentrations or concentrations of termiticides cypermethrin or permethrin) of Product 1 and Product 2 respectively as listed in Table 1. Freshly dip-treated blocks were air dried for 2 weeks. The treated and untreated blocks were next subjected to artificial weathering: a 10 day full vacuum treatment at 40°C known as volatilization (blocks weathered aboveground indoor exposure = H2 hazard class) or EN84 (EN 1997) leaching regime followed by volatilization (blocks weathered aboveground outdoor exposure = H3 hazard class). Other artificial

weathering regimes (ie. no leaching + no volatilization, leaching + no volatilization) were also included for comparison. Untreated control blocks and referenced volatized and/ or leached blocks already pressure-treated to about 5.6 kg/m³ CCA retention (radiata pine sapwood, kempas heartwood) meant for Malaysian H2 hazard class were also included. These untreated and treated and variously weathered wood blocks were exposed to the H2 hazard class field test of Wong (2005) for up to 6 months to expose wood to target subterranean termites, *Coptotermes curvignathus*.

After six-month field exposure to termites, blocks were retrieved, carefully cleaned of adhering termite carton matter, bait wood and bait cardboard, and oven-dried. All wood blocks were visually rated for degree of termite attack on the AWPA E7-07 scale (10: sound with surface nibbles permitted to 0: test block destroyed) (**Table 2**), and percent mass loss of wood blocks also determined (AWPA 2008). Data were analysed using ANOVA and MINITAB-14, with multiple comparison t-tests of mean values by Least Significant Difference (LSD, P<0.05) to examine the influence of leaching, volatilization and combinations of weathering effects, and termiticide concentrations/retention for protection from termites.

Table 2: AWPA E7-07 termite rating scheme (AWPA 2008)

Rating	Description
10	Sound
9.5	Trace, surface nibbles permitted
9	Slight attack, ≤3% of cross sectional area affected
8	Moderate attack, 3-10% cross sectional area affected
7	Moderately severe attack and penetration, 10-30% of cross sectional area affected
6	Severe attack, 30-50% of cross sectional area affected
4	Very severe attack, 50-75% of cross sectional area affected
0	Failure (destroyed)

Results and discussion

Results (Table 3) of the pooled data from all weathered regimes shows that untreated kempas blocks sustained severe attack with mean wood mass loss of 51.7% (mean termite rating 4.2) which were significantly (P<0.05) different from that of kempas treated with the 2 bio-based solutions and both radiata pine and kempas treated with CCA.. All treatments sustained complete protection against termites regardless of wood preservative (CCA versus ME formulations) and ME product concentrations (hence termiticide concentrations).

Both solutions even at their lowest termiticide concentrations among volatilized and/or leached kempas blocks, conferred complete termite control with typical short duration dip-treatments (3 minutes) (Table 3).

Separately, the percentage mass loss and termite ratings at each concentration for the both products were equivalent to those of CCA-treated blocks with and without leaching and volatizing procedures (Tables 4 & 5). This same high termite resistance against *Coptotermes curvignathus* confirmed the strong fixation of cypermethrin and permethrin to the wood, due to the unique bio-based formulations. Comparisons between cypermethrin and permethrin would not be valid due to the different formulations. In all cases, percentage mass loss was negligible with termite ratings higher than 9.6.

Table 3: Pooled mean percentage mass loss (%) and termite rating of treated wood of different products concentrations of ME formulations pooling across weathering regimes

Treatments		Percent mass loss (%)	Termite rating
Untreated kempas		51.7 (40.4) b	4.2 (3.8) <i>a</i>
CCA treated kempas		0.2 (0.7) a	10 (0) b
CCA treated radiata pine		0.5 (0.8) a	9.8 (0.3) <i>b</i>
	20%	1.1 (0.5) a	9.9 (0.2) <i>b</i>
Product 1 Treated kempas	10%	0.8 (1.3) a	9.9 (0.2) <i>b</i>
Trouved Hempus	5%	0.7 (0.8) a	9.9 (0.2) <i>b</i>
	20%	0.7 (0.3) a	9.8 (0.3) <i>b</i>
Product 2 Treated kempas	10%	0.6 (0.3) a	9.8 (0.3) <i>b</i>
	5%	0.4 (0.3) a	9.7 (0.3) <i>b</i>

^(...) = Standard deviation, n=24. Within-column values sharing same italicized letters denote not significant (P<0.05) by LSD; LSD values 7.6% (% mass loss) and 0.7 (termite rating)

Table 4: Mean percentage mass loss (%) of volatilized and non-volatilized, leached and nonleached, CCA treated pine. CCA treated kempas and kempas treated with bio-based solutions

CCA treated pine, CCA treated kempas and kempas treated with bio-based solutions					
T _{**}	aatmant		Cond	<u>itions</u>	
116	<u>eatment</u>		<u>Volatilized</u>	Non-volatilized	
YY		(L)	19.6 (21.5) b	97.4 (6.3) d	
Untreated kempas		(NL)	17.5 (27.5) <i>b</i>	72.2 (24.6) <i>c</i>	
CCA tracted redicts nine		(L)	0.8 (1.5) a	0.5 (0.3) a	
CCA treated radiata pine		(NL)	0.3 (0.1) a	0.5 (0.3) a	
CCA treated kempas		(L)	0.4 (0.8) a	0 (0.1) a	
		(NL)	0.6 (1.2) a	-0.2 (0.2) a	
	5 0/	(L)	0.9 (0.1) a	1.4 (0.6) a	
	5%	(NL)	0.9 (0.2) a	1.4 (0.7) a	
Product 1	10%	(L)	0.6 (0.2) a	1.6 (2.6) a	
Treated kempas		(NL)	0.5 (0.2) a	0.2 (0.1) a	
	20%	(L)	0.6 (0.2) a	0.8 (0.4) a	
		(NL)	0.5 (0.4) a	0.9 (1.6) a	
	5 0/	(L)	0.9 (0.3) a	0.8 (0.5) a	
	5%	(NL)	0.7 (0.2) a	0.5 (0.2) a	
Product 2	100/	(L)	0.8 (0.2) a	0.6 (0.2) a	
Treated kempas	10%	(NL)	0.7 (0.4) a	0.3 (0.1) a	
	200/	(L)	0.5 (0.2) a	0.4 (0.2) a	
	20%	(NL)	0.4 (0.3) a	0.2 (0.3) a	

L= leached, NL= non-leached, (...) = standard deviation, n=6. Values sharing same italicized letters denote no significant difference (P<0.05) by LSD. LSD values (% mass loss) = 8.4%

These results revealed that those test concentrations were effective and comparable to those generally confirmed efficient against *Reticulitermes* (Adkalis 2018). Interestingly and of academic interest, judging by the relative percent mass loss and termite ratings, weathering enhanced termite resistance of kempas; the untreated volatilized-leached and untreated volatilized-nonleached kempas provided a rating of moderate termite resistance (termite ratings: 7.0, 7.7) while untreated nonvolatilized-leached and untreated nonvolatilized-nonleached counterparts remained nonresistant (termite ratings: 0, 2.2) to termites (Tables 4 & 5). Perhaps volatile extractives of kempas heartwood were nontoxic and stimulated termite feeding, and their depletion from heartwood after 10 days weathering made the wood less palatable to termites. Alternatively volatilization may have caused movement of volatile kempas heartwood compounds from the core to the wood block surface, thus concentrating volatiles at the enveloped surface. Nevertheless such "natural wood protection" possibly accorded by such

likely concentrated volatile deposition on the wood surface is perceivably only transitory, and may not confer longer term wood protection for the untreated kempas.

Table 5: Mean termite rating and standard deviation of volatilized and non-volatilized, treated pine (reference species) and kempas using biobased solutions at different concentrations and leaching conditions

Treatment			Cond	<u>itions</u>
<u>11</u>	<u>Treatment</u>			Non-volatilized
Untreated kempas		(L)	7.0 (1.5) <i>c</i>	0 (0) a
		(NL)	7.7 (2.0) c	2.2 (3.4) <i>b</i>
CCA treated radiata pine		(L)	9.8 (0.3) <i>d</i>	9.7 (0.3) d
		(NL)	10 (0) d	9.5 (0) d
CCA treated kempas		(L)	10 (0) d	10 (0) d
·		(NL)	10 (0) d	10 (0) d
	50/		9.9 (0.2) d	9.9 (0.2) d
	5%	(NL)	9.8 (0.3) d	9.8 (0.3) d
Product 1	10%	(L)	9.9 (0.2) d	9.7 (0.3) d
Treated kempas		(NL)	10 (0) d	9.8 (0.3) d
	20%	(L)	10 (0) d	9.8 (0.3) d
		(NL)	9.9 (0.2) d	9.8 (0.3) d
	5%	(L)	9.8 (0.3) <i>d</i>	9.8 (0.3) d
	3%	(NL)	9.8 (0.3) d	9.7 (0.3) d
Product 2 Treated kempas	10%	(L)	9.8 (0.3) d	9.8 (0.3) d
	10%	(NL)	9.8 (0.3) d	9.8 (0.3) d
	2004	(L)	9.8 (0.3) d	9.6 (0.2) d
	20%	(NL)	9.8 (0.3) d	9.8 (0.3) d

L= leached, NL= non-leached, (...) = standard deviation, n=6. Values sharing same italicized letters denote no significant difference (P<0.05) by LSD. LSD values (termite rating) = 0.8

Conclusion

The termite test results confirm the excellent performance using dip-treatments of the two ME termiticides to protect kempas wood from termites. The bio-based water-based solutions, presented in this paper, have unique and original compositions that ensure effective H2 and H3 hazard class termiticidal treatment of Malaysian woods. This is comparable to CCA-treated vacuum-pressured kempas and pine. It is recognised that for complete H3 hazard class wood protection which requires prevention of decay by Basidiomycetes, these novel formulations have been already supplemented with a suitable fungicidal biocide which control decay threats under exposed aboveground (H3) outdoors. The excellent performances of both these bio-based termiticidal solutions for long-term wood protection, was confirmed again, in tropical regions.

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Laboratory Evaluation and Bioavailability of Termiticides in Tropical Soils to Subterranean Termites at Different Temperatures

by

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Abstract

Soil termiticide treatment is a fundamental method to control termite population and infestation. This method involves creating a continuous barrier surrounding the structures. However, termiticides may dissipate, depending on a half-life, degradation rate, leaching activity and storage method. In this study, the degradation rate and half-life of three commercially available termiticides, with active ingredient such as bifenthrin, fipronil and imidacloprid were determined under the field and laboratory conditions. The objective of this study was to determine the bioavailability of termiticides towards subterranean termites, *Coptotermes gestroi* under the laboratory conditions at two different temperature 30°C and 40°C using two types of soils i.e. sandy loam and loamy sand. No-choice bioassay was done to determine the mortality rate for each termiticide tested. Types of termiticides showed a significant difference toward termite mortality (F= 82.744; df= 2; P= 0.00). Bifenthrin revealed a higher termite mortality compared to fipronil and imidacloprid. Thus, the bifenthrin LT₅₀ and LT₉₅ values were lower compared to fipronil and imidacloprid. Termiticide concentration did affect the termite mortality (F= 9.407; df= 2; P= 0.00) where the higher the concentration, the higher the termite mortality. The laboratory study on termiticide degradation indicated that bifenthrin was more persistent in the soils compared to fipronil and imidacloprid.

Keywords: termiticides, bioavailability, laboratory condition, temperature

Introduction

Termites infestation are enormous, ranging from US\$ 22 billion to US\$ 40 billion worldwide (Rust & Su, 2012; Su, 2002). Damages from subterranean termite alone are approximately US\$ 400 million per year (Lee, 2007). Chemical-based termiticides mainly soil termiticides represent almost 90% of the subterranean control products with bifenthrin and imidacloprid make up 65% of the products (Koehler et al., 2011). Properly applied soil-termiticide treatment should be able to provide a minimum of 5 years effective protection (Richman et al., 2006). Soil termiticide treatment effectiveness may vary according to locations (Ramakrishnan et al., 2000) while susceptibility to termiticide may be different according to species (Dhang, 2011). Toxicity and mode of action of termiticides are two crucial factors in determining the effectiveness of termiticide-treated soil.

The objective of this study was to determine the bioavailability of termiticides towards subterranean termites (*Coptotermes gestroi*) termiticides containing active ingredients of fipronil, bifenthrin and imidacloprid under the laboratory conditions at two different temperature 30°C and 40°C using two types of soils i.e. sandy loam and loamy sand. This was done using a no-choice bioassay to determine the mortality rate for each termiticide tested

Materials and methods

Laboratory degradation study

Soil sampling

Soil samples were collected from two sites i.e. Durian valley, Universiti Sains Malaysia (USM) (5°21.35'N; 100°18.16'E) for sandy loam and Teluk Bahang, Penang (5°26.47'N; 100°13.04'E) for loamy sand. This two types of soil selected according to Ab Majid & Ahmad, (2013), which stated that termites in Penang accounted 44% to loamy sand and 56% to sandy loam soil. Termite was recorded found only on two types of soil namely, sandy loam and loamy sand. The soils were taken approximately 10 cm from the top layer (A-horizon). The remaining debris such as stones, vegetation and macro faunas were removed. The soils were air-dried at a room temperature (20-25°C). Then, the soils were sieved through 2 mm siever, stored at an ambient temperature and were kept air-dried. The soils were subsequently analysed for particle size, pH and organic matter content. Then, the soil pH was determined using a pH meter (HANNA HI 8424, Romania). The soils were mixed with distilled water at a ratio of 1:2 and were left overnight to obtain the pH value (Chan, 2010).

Termiticides

Termiticides used represented three chemical classes: chloronicotinyl (imidacloprid), phenyl pyrazole (fipronil) and pyrethroid (bifenthrin). Formulated products i.e. Imidacloprid 200 SC (Ensystex, MALAYSIA Sdn. Bhd., Kuala Lumpur), Bifenthrin 100 SC (Ensystex, MALAYSIA Sdn. Bhd., Kuala Lumpur) and Fipronil 5.0 SC (Hextar Chemicals Sdn. Bhd., Selangor, Malaysia) were purchased from a local distributor.

Soil treatment

A stock solution for each termiticide was prepared in 1000 ml deionised distilled water. Three termiticide concentrations were prepared; high (2000 ppm), medium (1000 ppm) and low (500 ppm). Then, 100 ml of each termiticide from the stock solution were applied on 1000 g of soil (sandy loam and loamy sand) and were filled into two plastic containers (1 L). Each plastic container was placed in the oven with temperatures of 30°C and 40°C. Three replications were used for each concentration. For control, 1000 g of the soil samples were treated with 100 ml of deionised water. The soil samples were collected during the 1st month, 3rd month, and 9th month and 12th month for analysis.

Bioavailability of termiticides

In the no-choice bioassay, the data were analysed using log-probit analysis to obtain LT50 and LT95 with 95% confidence limit. A factorial analysis of variance (ANOVA) was also performed on termiticide, concentration, temperature and soil types as independent variables to find the significant differences between the independent variable tested with termite mortality (dependent variable). A two-way ANOVA was performed which termite mortality as dependent variable and the Tukey's test at P<0.01 was assessed to determine significant differences between the groups. The control groups exceeded 10% mortality were repeated and all data were corrected using Abbott's formula (Abbott, 1925).

 $P = (Po-Pc)/(100-Pc) \times 100$

Where.

P = corrected mortality (%).

Po = observed mortality (%).

Pc = control mortality (%).

Results and discussion

Lethal time values (LT50 and LT95) of bifenthrin, fipronil and imidacloprid obtained by counting dead termites in the 1st, 3rd, 6th, 12th and 20th month. Generally, the result indicated that LT50 and LT95 values increased over time, implying that the efficacy of termiticides decreased over time. Bifenthrin showed the lowest LT50 and LT95 values compared to fipronil and imidacloprid for all months tested. While, imidacloprid indicated the highest LT50 and LT95 values for all months tested compared to fipronil and bifenthrin.

Statistical analysis indicated that there was a significant difference on month toward termite mortality (F=11.794; df=3; P=0.00). The LT50 and LT95 were lower in the first month compared to in the 12th month (Table 3.11), implying that time taken to kill 50% and 95% termites in the 1st month was faster compared to in the 12th month. Types of termiticides showed a significant difference toward termite mortality (F=82.744; df=2; P=0.00). Bifenthrin revealed a higher termite mortality compared to fipronil and imidacloprid. Thus, the bifenthrin LT50 and LT95 values were lower compared to fipronil and imidacloprid. Termiticide concentration did affect the termite mortality (F=9.407; f=2; f=0.00) where the higher the concentration, the higher the termite mortality (Table 1).

Generally, bifenthrin caused the highest termite mortality compared to fipronil and imidacloprid. A similar result was obtained by Smith & Rust (1990) of which the termite mortality in soil treated with bifenthrin was high although being exposed to low concentration level. Imidacloprid, on the other hand, caused the lowest termite mortality. This result corroborated the ideas of Manzoor & Pervez, (2017), who suggested that bifenthrin provided the highest mortality among termiticides tested (bifenthrin and fipronil). The results were also supported by Saran & Kamble (2008), who revealed that LT50 and LT90 for bifenthrin were shorter, followed by fipronil and imidacloprid.

The degradation of termiticides is highly influenced by temperature, the higher the temperature, the higher the degradation process (Ying & Kookana, 2002; Zhu et al., 2004). In this study, there was no effect of temperature towards termite mortality. A study by Gautam & Henderson (2012) on exploratory activities of C. formosanus Shiraki at different temperatures has found that this termite species tunnelled significantly greater in sands with the temperature of 22°C compared to in sands at 28°C. The result of bioavailability in the laboratory for imidacloprid was similar to the results in the field. A study by Ramakrishnan et al., (2000) on termite mortality using imidacloprid at different concentrations (25, 35 and 50 ppm) on four types of soils has revealed that the termite mortality was insignificant for imidacloprid applied in sandy loam and loam soils. While, Manzoor & Pervez, (2017) indicated that bifenthrin and fipronil were more effective in bioavailability experiment at sandy loam compared to sandy clay loam. Results obtained by Ramakrishnan et al., (2000) further supported this present study where no significant difference of termite mortality in soils treated with imidacloprid in sandy loam and loamy sand. However, contrary to this study, Manzoor & Pervez (2017) found that there was a significant difference between types of soils (sandy loam and sandy clay loam) and termite mortality. In conclusion, the bioavailability laboratory studies showed that bifenthrin had the lowest LT₅₀ and LT₉₅ values among termiticide tested. Types of soils and temperature had no significant effects on the degradation and bioavailability of termiticides tested in the laboratory experiment.

Table 1: Effects of soil, month, termiticide, concentration and temperature on termite mortality for laboratory degradation study

Source	df	Mean Square	F	Sig.
Soil	1	188.5	0.399	0.528
Month	3	5578.502	11.794	0
Termiticide	2	39137.72	82.744	0
Concentration	2	4449.48	9.407	0
Temperature	1	421.508	0.891	0.345
Soil * Month	3	386.655	0.817	0.484
Soil * Termiticide	2	831.488	1.758	0.173
Soil * Concentration	2	31.322	0.066	0.936
Soil * Temperature	1	32.812	0.069	0.792
Month * Termiticide	6	553.94	1.171	0.319
Month * Concentration	6	40.027	0.085	0.998

Month * Temperature	3	584.451	1.236	0.295
Termiticide *	4	225.931	0.478	0.752
Concentration	4	223.931	0.478	0.732
Termiticide *	2	524.961	1.11	0.33
Temperature	2	324.901	1.11	0.55
Concentration *	2	13.822	0.029	0.971
Temperature	2	13.822	0.029	0.971
Soil * Month *	6	135.174	0.286	0.944
Termiticide	O	133.174	0.280	0.744
Soil * Month *	6	38.423	0.081	0.998
Concentration	O O	36.423	0.001	0.776
Soil * Month *	3	110.334	0.233	0.873
Temperature	3	110.334	0.233	0.675
Soil * Termiticide *	4	22.792	0.048	0.996
Concentration	4	22.192	0.048	0.550
Soil * Termiticide *	2	76.36	0.161	0.851
Temperature	2	70.30	0.101	0.031
Soil * Concentration *	2	9.626	0.02	0.98
Temperature	2	9.020	0.02	0.76
Month * Termiticide *	12	48.925	0.103	1
Concentration	12	40.923	0.103	1
Month * Termiticide *	6	281.345	0.595	0.735
Temperature	O O	201.343	0.393	0.755
Month * Concentration	6	22.087	0.047	1
* Temperature	O	22.007	0.047	1
Termiticide *				
Concentration *	4	4.982	0.011	1
Temperature				
Soil * Month *				
Termiticide *	12	36.356	0.077	1
Concentration				
Soil * Month *				
Termiticide *	6	149.548	0.316	0.929
Temperature				
Soil * Month *				
Concentration *	6	14.42	0.03	1
Temperature				

Soil * Termiticide *				
Concentration *	4	16.078	0.034	0.998
Temperature				
Month * Termiticide *				
Concentration *	12	7.803	0.016	1
Temperature				
Soil * Month *				
Termiticide *	12	12.505	0.026	1
Concentration *	12	12.303	0.020	1
Temperature				

df: degree of freedom; bold: significant value

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Reliable Laboratory Trial for Testing Innovative Anti-Termite Barriers

by

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Abstract

Subterranean termites are a real threat for wooden commodities and they do generate considerable economical losses. The increasing use of wood in the building sector, under a gradual termite territorial expansion calls for proactive approaches in termite management. In this scope, anti-termite barriers are a technological step forward since insecticides are coming under stricter scrutiny. Therefore, anti-termite barriers need to be evaluated before being released into market. This paper presents a reliable laboratory test method to assess the efficacy of innovative physical anti termite barriers.

Keywords: Reticulitermes, physical barrier, laboratory methodology, reliability

Introduction

Termites are ubiquitous social insects present in most parts of the world. Being an essential member of the soil ecosystem, subterranean termites have a high ecological importance providing many benefits in terms of breakdown and recycling of organic matters, soil fertility, etc. In return, these cellulose feeders are pests of forestry having a significant impact on both plantation and urban forestry, as well as a huge threat for wooden commodities (Govorushko, 2019). Due to climate change and environmental degradation, together with global trade, a significant global expansion of termites is predicted along with the economic losses due to their activities (Buczkowski and Bertelsmeier, 2017). On the other hand, wood, as a renewable bio-based material, sees its use increasing in the building sector due to its ability to stock carbon as well as its high-level technological characteristics. However, the wood preservation sector has faced many changes since the ban of organochloride insecticides and chromated copper arsenate (Grace, 2006). Together with different alternatives development in the field of wood protection, anti-termite barriers for buildings became serious candidates for termite management. Since the early development of particle barriers, different strategies have been envisaged, such as physical (particle, metal, shield) and physico-chemical (a matrix associated with a termicidal ingredient) barriers (Ahmed and French, 2008; Lewis, 1997). Anti-termite barriers present the advantages to be non-invasive systems, excluding termites from the buildings, protecting the structure, fittings and contents. During development phases and before being on a market, barriers must be evaluated for their abilities and efficacy. The aim of this research is to present a reliable laboratory methodology to evaluate the performances of different candidate physical barriers.

Materials and methods

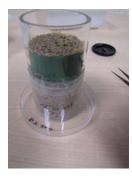
Following four physical barriers were tested, none of them contain insecticidal active ingredients:

- 1. (A) a 100% bio-based polyamide film,
- 2. (B) an ultra-high molecular weight polyethylene film,

- 3. (C) a polyethylene film, which is the matrix without insecticide for the TERMIFILM® (Messaoudi et al., 2016).
- 4. (D) a mesh made of bi-component multi-filament polyester yarn which has sheath end core part each filament. The grid pattern allows holes of 0.63 mm^2 .

Each barrier was cut into 6 cm² samples with no preferential side. The barriers were tested: (i) as they were (undamaged), or (ii) with 4 small holes of 0.5 or 0.7 mm, drilled using 25G and 22G needles respectively. Barrier D, being already a mesh, was used only in its original form. The holes are bored at the corners of a 1cm² square, at the center of each sample (Fig. 1). The barriers are then placed in between glass tubes (5 cm height and diameter), as presented in Fig 2 and 3. In the control devices, the barrier was replaced by a thermoplastic film (Parafilm®, Sigma). A pine (*Pinus sylvestris*, L.) sapwood bait of dimensions 15 x 25 x 25 mm³ (L,R,T), covered by wet Fontainebleau sand (4 vol sand /1 vol deionized water), is placed in the lower tube. On the upper tube, 600 termite workers (*Reticulitermes flavipes* ex. *santonensis*), 6 nymphs and 6 soldiers were introduced on the humid floral foam. The termites are harvested once a year in Saint Trojan forest, Oleron Island (France), and are kept in the dark, in large breeding boxes at 27°C, Relative Humidity (RH) > 75%, and fed by poplar wood. Within the 2 breeding boxes used for this trial, the noticed percentage of nymphs and soldiers was about 1% of the population. This proportion between the casts was kept for the test.





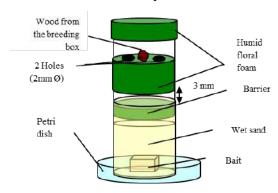


Figure 1.Film drilled

Figure 2. Test device

Figure 3. Scheme of the test device

Each modality (barrier/intact or drilled), as well as controls, were tested in four replicates. The test devices were kept 8 weeks at 27°C, 75% RH, and observed twice a week. At the end of the test, the devices are disentangled, the survival rate of the termites is calculated, a visual rating is given to each Pine bait (based on the visual assessment of the EN117) (EN117, 2013). The barriers, or the Parafilm®, are left to condition at 20°C, 65% RH prior to their observation.

Results and discussion

The results for all barriers are presented in table 1. For all the controls, the Parafilm® was damaged in many places, the bait was reached and presented a severe attack, and the survival of the termites was above 50%. These criteria validate our test in terms of sufficient termite feeding pressure and termite virulence, the required level of termite activity being achieved (Fig 4) (Ewart and Rawlinson, 2000).



Figure 4. Controls film and baits

The 4 films did present different results. The film C, which is a matrix remained intact when used undamaged. On half of the drilled film, the film was penetrated several times and the bait reached. The termites' survival rate was between 30.7 and 62.8%. The holes act as primer aperture but are not initially large enough to let the termite go through, being smaller that the head and the mandibule width (Zaremski and Fouquet, 2009). The termites use this failure in the film integrity as an opportunity for further degradation (Fig 5).



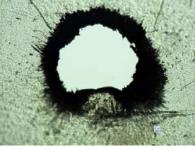




Figure 5. Hole (0.7 mm)

Figure 6. Hole (0.7 mm) enlarged by the termites (same scale as fig 5)

Figure 7. Aspect of the barrier C and the bait

The barrier A did perform better as the termites did no go through the film, even when drilled. However some nibbling was noticed when the films were observed (Fig.8). The barrier B did effectively exclude the termite from crossing, and no sign of attempted degradation was noticed (Fig 9).

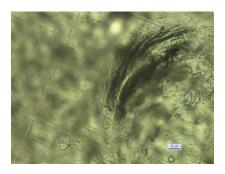




Figure 8. Termite nibbling on barrier A

Figure 9. Untouched hole (0.5mm) on barrier B

Table 1. Performances of the physical barriers (*No attack, **Severe attack)

Barrier	Treatment	Barrier observation	Pine bait Visual	Termite survival rate
			rating	(%)
A	-	No degradation, no material removal from the film, nibbling signs on few samples	0*	0
	4 holes, 0.5 mm Ø	No degradation even around the holes, no	0	0
	4 holes, 0.7 mm Ø	material removal from the film, nibbling signs on few samples	0	0
В	-	No degradation, no material removal from the film	0	0
	4 holes,		0	0
	0.5 mm Ø	No degradation even around the holes, no		
	4 holes, 0.7 mm Ø	material removal from the film	0	0

С	-	No degradation, no material removal from the film	0	0
		2 samples : No degradation even around the holes, no material removal from the film	0	0
	4 holes, 0.5 mm Ø	1 sample : material removal, attempt of tunneling through the barrier	0	0
		1 sample: one of the 4 holes enlarged, crossing of the barrier	4**	30.7
		1 sample: one of the 4 holes enlarged, but no crossing of the barrier	0	0
	4 holes, 0.7 mm Ø	3 samples: 1 or 2 holes enlarged, crossing of the barrier	4	62.8, 31.2 and 38.2 for the 3 samples respectively
D	-	No degradation, no material removal from the film	0	0
Controls	16 samples	Degradation of the Parafilm® used as a control barrier, crossing of the film	4	Higher than 50%

The barrier D, a grid, did also perform well: the pattern holes are too small to let the termite go through, and the termites did not degrade the mesh (Fig.10).

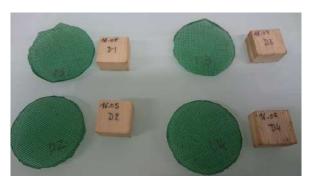


Figure 10. Aspect of the grid D and the bait

The test method set up to test the performance of different barriers was shown to be an adequate and discriminant method. The number of termites used seems an adequate group size (Thorne *et al.*, 2010) and brought sufficient biological pressure to make the difference between the controls and tested barriers. The survival rate (above 50%) and the severe attack needed on the bait have been largely experienced in European standardized methods where a non-choice test is applied (such as EN117, 2013). Here, these validity criteria were easily reached for the controls, meaning that the termite population chosen could feed and survive on the volume of wood available (bait) for the test period defined (8 weeks).

Concerning the different barriers tested, it appears that they were sufficiently challenged by the method. The holes made on the barrier are not intended to be degradations but only primer failures, and their size does not allow the termite to go through (Zaremski and Fouquet, 2009). The termites do still have to degrade the barrier around those holes or anywhere else to go through. Indeed, in the field/on site, some "pioneers" *Reticulitermes* workers explore systematically in every direction in search for food. These workers initiate a foraging process by covering each direction of the foraging territory (Reinhard *et al.*, 1997). Thus, the drilled holes are a privileged foraging point, a weak part of the barrier. Once the pioneer workers have started to degrade the barriers, far more workers will be attracted by the recruitment trail along with the volatiles from the wood. In this case, if the barrier is not performant enough, the termites can go through the barrier down to the bait.

Conclusions

The method presented here is easy to perform in a laboratory, reliable and discriminant between different physical barriers. From the different barriers tested in this study, barriers B and grid D performed the best as no nibbling, and no termite passed through the barriers. The method is also reproducible, and has also been used and proven for physico-chemical barriers. As a consequence, this method has been validated and accredited during a COFRAC (French Accreditation Committee) audit (accreditation n°1-1686, scope available at https://www.cofrac.fr/).

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O 24

Household Termite Infestation Survey and Swarming Season

by

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Abstract

Termites are considered as major urban pests in Taiwan for over one hundred years. However, the quantitative studies of termite economic damage, pest species composition in urban construction, and their dispersal flight seasons are not yet conducted. In this study, we launched a citizen scientific project to invite homeowners and pest control operators to collect termite samples. In addition, a questionnaire survey was conducted at the main train stations of each prefecture. A total of 1,736 valid questionnaires showed termite building infestation rate is 9.6%. Over 3,000 termite samples collected in past three years indicated 95% termite infestation in Taiwan was caused by *C. gestroi* (Wasmann) and *Coptotermes formosanus* Shiraki. *C. formosanus* was mostly found in northern and eastern Taiwan and *C. gestroi* were mostly found in southern Taiwan. The two *Coptotermes* spp. are sympatric at central Taiwan. The dispersal flight season of *C. gestroi* and *C. formosanus* peak at late April to late May, respectively. Three minor pests, drywood termite, *Cryptotermes domesticus* (Haviland), fungusgrowing termite, *Odontotermes formosanus* (Shiraki), and subterranean termite, *Reticulitermes flaviceps* (Oshima) were also reported in this study.

Keywords: Coptotermes, swarming, urban pest, citizen science project

Introduction

Among over 3,000 species of termites all over the world, 80 species are regarded as serious pests, including subterranean termites, fungus-growing termites, and drywood termites. Estimated worldwide economic loss from control and repair cost is over \$40 billion annually (Rust and Su 2012). In Taiwan, three subterranean termites, *Coptotermes formosanus*, *C. gestroi*, *Reticulitermes flaviceps*, one fungus-growing termite, *Odontotermes formosanus*, and one drywood termite, *Cryptotermes domesticus* are considered common in urban area (Yang and Li 2012).

Although serve termite damage occurred in Taiwan have been documented since early 20th century (Li et al. 2011), quantification of termite damage remains unavailable. The building termite infestation is difficult to be recognized by homeowners and researchers had limited access of private buildings. Hence, no enough household infested termites were available to researchers. Identification of termite species is another challenge which resulted in misunderstandings among homeowners, pest control operators, and scientists. Sample acquiring for species confirming to scientists is indispensable. In addition to survey of household infested termites, prediction of dispersal flight (swarming) season is critical to prevent termite infestation. However, observing termite swarming is also difficult due to complicated flight phenology (Chouvenc et al. 2017), and duration of swarming is usually less than 30 minutes (Bess 1970). Both unpredictability and temporariness of termite swarming resulted in challenges of studying termite swarming behavior.

The objectives of this study are to estimate household termite infestation rates, species composition, and their dispersal flight season. Three methods including questionnaire survey, Facebook advertisement, and setting up a webpage for obtaining termite collection data and samples were used.

The fundamental information of urban termite infestation and swarming is important to termite control industry and homeowners.

Materials and methods

In this study, we quantify household infestation rate through questionnaire and analyze termite species composition and their dispersal flight seasons by identifying termite samples provided by homeowners and pest control operators.

Questionnaire design

Questionnaire was designed for surveying the household termite infestation rate. In 2015, we randomly picked up homeowners at the major train stations of each prefecture in Taiwan. The question is: Has your house/apartment ever been infested by termite? Personal background information of resident is also documented.

Termite sample collection

Samples including soldiers, workers, and alates are collected by homeowners, pest control operators and from 2015, and sent to our lab, Laboratory of Urban Entomology, National Chung Hsing University, Taichung, Taiwan (LUE-NCHU).

a. Advertisement through Facebook

A Facebook group, Termite and Alate Club (https://www.facebook.com/groups/398182670532605/), is established in 2017 to promote a citizen science project and to recruit more people to collect and send their samples to our laboratory. General information of termite biology and control is also offered and posted in the Facebook page.

b. Providing termite identification service through webpage

When homeowners or pest control operators have a termite sample, they could log in the website Taiwan Termite Identification Service (http://www.termite.nchu.edu.tw) and add new collection data including collection location (address, or longitude and latitude), collection date, collector name, types of collecting environment (inside buildings, on the trees, on the walls or plants near buildings, in natural environment, or others). After providing required data, one unique sample code is given automatically to each sample. We encouraged collectors sending their samples to our lab. After we receive and identify the sample, we key in the termite species name in the website system. The data register will receive a notice through e-mail. Each data register has their own account, and all termite collection data they input is saved in the website system. Pest control companies could use this system as their termite control business data logger. Termite sample distribution map powered by Google Map is provided to each register.

Preservation and Identification of vouchers

All vouchers are preserved in 95% alcohol in glass vials and stored in NCHU Termite Collection. Species identification for all samples are followed by the descriptions from Yang and Li (2012). Samples collected inside buildings with soldiers or workers are referred to building infestation samples, and alate sample, without other castes, are considered as samples collected during dispersal flight seasons.

Results and discussion

Building infestation rate

The building infestation rate in each county is consistently near to 10%, no significant difference was found among the 19 counties. Of the 1,736 valid questionnaires, household termite infestation rate is 9.6%. In addition to infestation, 18.7% of homeowners encountered dispersal alates in their house, which indicates termite infestation in their neighborhood. Over 61.5% homeowners have not seen any termite in their houses, and the rest, 10.2%, had no idea of termites or termite infestation.

Termite species composition of building infestation

A total of 1,296 termite samples collected from infested building during 2015-2019 indicate *C. formosanus* and *C. gestroi* are two major termite pests (~95%) (Figure 1.). At northern Taiwan, such as Taipei-Keelung metropolitan area, Taoyuan, and Miaoli City, over 90% household infestation caused by *C. formosanus*. However, at southern Taiwan, such as Tainan, Kaohsiung, and Pingtung City, *C. gestroi* is responsible for over 90% building termite damage. The transition zone is central Taiwan with 85% and 15% *C. formosanus* and *C. gestroi* in Taichung City, respectively, and 50% both species in Chiayi and Yunlin City.

Dispersal flight seasons of C. formosanus and C. gestroi

The 363 alate samples collected during 2017-2019 showed the peak flight seasons of *C. gestroi* and *C. formosanus* are in early-mid April and mid-late May, respectively. The overlapped period of their dispersal flight season is late April to late May (Figure 2.).

With synergy of citizen science project, over 3,000 termite samples were sent to our lab in the past three years. These data provided crucial information of termite infestation in urban environment. In Taiwan, *C. gestroi* is an invasive species, and previous recorded restricted in central and southern Taiwan. In the past three years, four *C. gestroi* infested building in New Taipei City and Taoyuan City have been confirmed. In addition, on April 6th in 2019, *C. gestroi* alates were firstly discovered in Eastern Taiwan (Hu and Ho 2019), which also suggests the potential extension and increasing economic impact of *C. gestroi* in Taiwan.

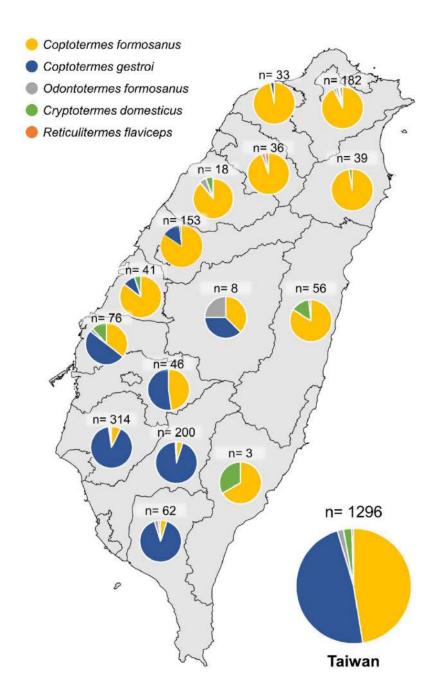


Figure 1. Termite species composition of building infestation in Taiwan. A total of 1,296 samples are collected during 2015- 2019 July by pest control operators and homeowners.

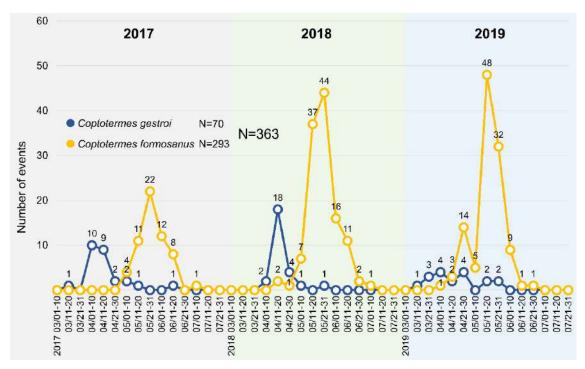


Figure 2. Swarming seasons of *C. formosanus* and *C. gestroi*. 363 Samples are collected during 2017-2019.

Conclusion

Overall termite building infestation rate is ~10%, and 95% of termite infestation were caused by *C. gestroi* and *C. formosanus*. The dispersal flight season of *C. gestroi* and *C. formosanus*, occurs between March to June and peaks early-mid April and mid-late May, respectively.

Cooperation between homeowners, pest control operators, and researchers is a constructive strategy to study urban termite infestation. Correct termite identification, detailed distribution maps, and dispersal flight seasons are crucial information for pest control industry.

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O 25

Termite infestation survey and baiting Formosan subterranean termite (Blattodea: Rhinotermitidae) of protected old trees in urban areas

by

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Abstract

We investigated 228 protected old trees among four major tree species in Taichung, Taiwan. Of the 94.7% trees were older than 50 years, and termite infestation rate was 52.2%. Seven termite species were identified, and Coptotermes formosanus Shiraki was the dominant species on protected old trees that accounted for 18.9%. Two above-ground and eight in-ground monitoring stations were installed around nine C. formosanus-infested Mangifera indica L. Of the nine trees, four trees were infested by one C. formosanus colony respectively; three trees were infested by one C. formosanus colony at the same time; and the other two trees were suffered from multiple termite colonies infestation simultaneously: one was infested by two colonies of C. formosanus, and the other one was infested by C. formosanus and Reticulitermes flaviceps Oshima. Termite bait with 0.5% hexaflumuron was applied in the in-ground monitoring station that stained termites appeared for the seven C. formosanus colonies among nine trees. Each C. formosanus colony were eliminated in 6-26 weeks after consumed the bait. R. flaviceps and Odontotermes formosanus (Shiraki) invaded in-ground monitoring stations and consumed bait after elimination of C. formosanus were observed in this study. We could not confirm that whether the active ingredient of bait had effects on R. flaviceps and O. formosanus colony, but we should concern that the additional bait consumption by untargeted termite species would increase the cost of baiting.

Keywords: Coptotermes formosanus, termite control, underground baiting system, tree protection, termite reinvasion

Introduction

Termites are known as major decomposers of dead plant materials (Ulyshen and Wagner 2013). Approximate 80 termite species are considered serious pests of woodwork and wooden products (Edward and Mill 1986, Su and Scheffrahn 1990, 2000, Rust and Su 2012), some of them can also infest living trees (Harris 1969, Sands 1974, Chang and Hung 1986, Mitchell 1989, Wood 1996).

Coptotermes spp. are notorious for causing serious damage on living trees (Tho 1974, Apolinário and Martius 2004, Jasmi and Ahmad 2011), which can infest heartwood and hollow out tree trunks. Osbrink et al. (1999) noticed that collapse risks of urban trees were related to termite infestations, 21 of the 28 hurricane-damaged trees were infested by *C. formosanus* Shiraki. Termites are generally regarded as the most important pest on old trees compared to other insect pests, and *Coptotermes* spp. are probably dominant termite species on urban trees (Jasmi and Ahmad 2011, Zorzenon and Campos 2015). *C. formosanus* and *C. gestroi* (Wasmann) are the two *Coptotermes* spp. recorded in Taiwan (Tsai and Chen 2011), both of them were confirmed to infest living trees by Li et al. (2011) in the tropical botanical garden.

Termite bait contains chitin synthesis inhibitor (CSI) has highly specific effects on *Coptotermes* spp. and *Reticulitermes* spp. which can result in elimination of 90-100% individuals in a mature *C. formosanus* colony with only few grams of active ingredient (Su 1994). In this study, we conducted a

termite infestation survey to find out termite infestation rate and species composition on urban old trees; and carried out a baiting protocol for subterranean termite control to verify the effectiveness of the underground baiting system on termite-infested trees.

Materials and methods

This study investigated termite infestations on 228 protected old trees in Taichung, Taiwan. We chose four major tree species of the protected old trees, and of the 94.7% trees were older than 50 years. 66 Chinese banyans (Ficus microcarpa L. f.), 56 Camphor trees (Cinnamomum camphora (L.) Presl.), 54 Mangoes (Mangifera indica L.), and 52 Bishop woods (Bischofia javanica Bl.) were inspected, and the inspection method was modified from Li et al. (2011). Each person carried out a 10-minute inspection on the trunk within two-meter height above the ground, and recorded the inspection result of termite infestation on each tree. Termite were collected and identified by termite classification key (Li 2010, Yang and Li 2012), and specimens were deposited in the National Chung Hsing University (NCHU) Termite Collection. 11 M. indica that collected living C. formosanus were chose for termite monitoring and control. We installed two above-ground monitoring stations on bark and eight inground monitoring stations in the soil to monitor termite activities. Termite activities in all stations were checked every two weeks, and wood resources were replaced with new ones when the consumption was more than 50%. To know if termites found in the above-ground and in-ground monitoring station were the same colony, we stained termites in one above-ground monitoring station to observe the spread of stained termites. The termite-staining method was modified from Chiu et al. (2016). Termite bait with 0.5% hexaflumuron was applied in the in-ground monitoring station that stained termites appeared for termite control. Consumption of termite bait was checked once a week when termites still feeding; and checked every two weeks when termites were absent.

Results and discussion

A total of 228 protected old trees were investigated in this study, and 118 (51.8%) trees were infested by seven termite species including C. formosanus (18.9%), Odontotermes formosanus (Shiraki) (14.0%), Cryptotermes domesticus (Haviland) (4.8%), R. flaviceps Oshima (0.9%), Neotermes koshunensis (Shiraki) (0.4%), Glyptotermes satsumensis (Matsumura) (0.4%), C. gestroi (0.4%). Compared with the studies conducted in Taiwan (Li et al. 2011, Chiu 2013 (unpublished), Kuo 2016) (Table 1), termite infestation rate of protected old trees was higher than that of younger trees in urban areas and nature environments. Li et al. (2011) inspected 3,253 trees with a similar method in the tropical botanical garden (149-151 m) that was established more than 70 years, and 280 (8.6%) trees were infested by five termite species including O. formosanus (7.5%), N. koshunensis (0.8%), C. gestroi (0.3%), C. formosanus (<0.1%), and Cr. domesticus (<0.1%). Chiu inspected 594 trees with a similar method that modified from Li et al. (2011) on 2013 December in the NCHU campus. All of trees were less than 50 years old, and 36 (6.1%) trees were infested by O. formosanus (4.4%) and C. formosanus (1.7%) (unpublished). Kuo (2016) surveyed termites and ants' succession on 30-40 yearold Japanese cedar (Cryptomeria japonica D. Don) in the experimental forest (1,070-1,093 m), and 16 living trees were cut into segments by 3.6 m. Each person inspected the segment with an axe for five minutes, three trees (18.8%) were infested by G. satsumensis and one tree (6.3%) was infested by R. flaviceps.

Nine of the 228 surveyed trees were infested by two kinds of termite species simultaneously in this study, indicating that old trees can provide diverse niches for different termite species at the same time. *C. formosanus* is the dominant termite species on protected old trees. To evaluate effectiveness of the underground baiting system on *C. formosanus*-infested trees, we confirmed that nine of the 11 *M. indica* were infested by the same *C. formosanus* colony in the above-ground and in-ground monitoring station with termite-staining. Of the nine trees, four trees were infested by one *C. formosanus* colony respectively; three trees were infested by one *C. formosanus* colony at the same time; and the other

two trees were suffered from multiple termite colonies infestation simultaneously: one was infested by two colonies of *C. formosanus*, and the other one was infested by *C. formosanus* and *R. flaviceps*.

Termite bait was applied in the in-ground monitoring station that stained termites appeared for the seven *C. formosanus* colonies among nine trees. Each *C. formosanus* colony were eliminated in 6-26 weeks after consumed the bait. Several studies pointed out that after the dominant species, usually are *Coptotermes* spp., was eliminated, other termite colonies would invade the in-ground monitoring station and consume the bait (Messenger et al. 2005, Mullins et al. 2011, Chiu et al. 2016, Lai et al. 2018). *R. flaviceps* and *O. formosanus* invaded in-ground monitoring stations and consumed bait after elimination of *C. formosanus* were observed in this study. We could not confirm that whether the active ingredient of bait had effects on *R. flaviceps* and *O. formosanus* colony, but the additional bait consumption by untargeted termite species would increase the cost of baiting.

Table 1. Termite species composition among four species of protected old trees

	Ficus $microcarpa$ $(n = 66)^1$	Cinnamomum camphora $(n = 56)^2$	Mangifera indica $(n = 54)^3$	Bischofia $javanica$ $(n = 52)^4$	Total trees (n = 228)
Cryptotermes domesticus	6	0	1	4	11 (4.8%)
Neotermes koshunensis	1	0	0	0	1 (0.4%)
Glyptotermes satsumensis	0	1	0	0	1 (0.4%)
Coptotermes formosanus	6	14	16	7	43 (18.9%)
Coptotermes gestroi	0	0	1	0	1 (0.4%)
Reticulitermes flaviceps	0	1	0	1	2 (0.9%)
Odontotermes formosanus	2	22	4	4	32 (14.0%)
Total termite occurrences	14 (21.2%)	32 (57.1%)	21 (38.9%)	15 (28.8%)	82 (36.0%)

Number in parenthesis represents percentage of termite infestation rate.

Conclusion

Termite infestation of urban trees is common, and *C. formosanus* is the dominant termite species that threatens protected old trees. Considering the inspection method was relatively preliminary in this study, the actual termite infestation rate of urban old trees might be higher. To prevent collapse risks of old trees from serious termite damage, monitoring and control for *C. formosanus* are necessary. This study confirmed the underground baiting system could eliminate the *C. formosanus* colony that infested old trees, but untargeted termite species, such as *R. flaviceps* and *O. formosanus*, would invade in-ground monitoring stations and consume the bait after elimination of *C. formosanus*. In order to limit termite infestations on old trees, it is important to detect and control termite reinvasion.

¹ One tree was infested by both *O. formosanus* and *Cr. domesticus*.

² One tree was infested by both *O. formosanus* and *G. satsumensis*; four trees were infested by both *O. formosanus* and *C. formosanus*; one tree was infested by both *O. formosanus* and *R. flaviceps*.

³ One tree was infested by both *O. formosanus* and *C. formosanus*.

⁴ One tree was infested by both *C. formosanus* and *R. flaviceps*.

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Smartphone Image-Based Identification of Four Major Termite Pest Species by Using Deep Learning

by

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Abstract

Termites are important urban pests, which cause economic and social impact on urban communities. Termite identification is crucial for effective control strategies. However, the termite identification requires expertise and could be labor-intensive. Despite availability of in-depth learning technologies in pest recognition, the termite recognition has still not been well studied. In this study, we collected four major termite species in various locations in Taiwan and acquired large number of termite images using a smartphone. Then, we implement a termite classification system by using a deep learning model, MobileNetV2. Finally, our prediction results achieved the mean accuracy of 0.930, 0.936, and 0.859 for soldier, worker, and both castes, respectively. The proposed method has provided a new approach for termite recognition automatically.

Keywords: deep learning, termite, artificial intelligence, pest control, pest identification

Introduction

Termites are important urban structural pests, which causes more than 40 billion USD economic loss worldwide annually (Rust and Su, 2012). Invasive termite pests including subterranean termites and drywood termites continue expanding their territories (Evans et al., 2013), hence increasing damage and cost of termite control is expected.

Various control methods have been invented and applied on different species, such as creating underground soil barrier, control wood infested colony, or eliminating subterranean colony. So far, no universal method is available for controlling all termite species. Hence, in order to control specific termite damage, correct identification of termite species is crucial. However, the termite identification requires expertise and could be labor-intensive. The requirement for automatic method for termite species identification is indeed needed. Despite great achievements on pest recognition in recent years, the termite recognition has still not been studied.

The rapid development of image processing and deep learning technologies leads to an increased opportunity for pest recognition. Compared with conventional image-based method, deep learning techniques do not require feature extraction by domain experts in a long term and subjective handcrafted process (Wäldchen and Mäder, 2018). Instead, the deep learning approaches can automatically extract the essential features from images for solving classification problems. A convolutional neural networks (CNN) as a deep learning approach has achieved good results for agricultural pest identification. Xie et al. (2015)applied multiple-task sparse representation for enhancing recognition performance on 24 common pest species of field crop. Cheng et al. (2017) recognized ten pest species with an accuracy of 98.67% using deep residual learning method. With application of transfer learning, a pretrained version of AlexNet also obtained a recognition rate of 93.84% for ten types of pest species (Dawei et al., 2019). A recent study developed the sensor device to automatically monitor the mosquito species using deep learning (Huang et al., 2018).

In this work, we collected four major termite species from various locations in Taiwan. We took advantage of well-known deep learning architecture of MobileNet (Howard et al., 2017), which is designed to image recognition and particularly suitable for mobile designs, for classification of termite species in Taiwan. To train deep learning models, large quantity of images with labelled information is needed to deliver accurate results. We thus used continuous model of smart phone camera to acquire large number of images. In order to demonstrate the robustness of deep learning models, we applied the cross-validation method by shuffling the images acquired from different locations. Our prediction results achieved the mean accuracy of 0.930, 0.936, and 0.859 for soldier, worker, and both castes, respectively. To our best knowledge, our study is the first study to demonstrate the applicability of deep learning in the termite recognition.

Materials and methods

Termite collection

Four termite species were collected in Taiwan from June 2018 to September 2018, including *Odontotermes formosanus* (Shiraki), *Coptotermes formosanus* (Shiraki), *Reticulitermes flaviceps* (Oshima) and *Cryptotermes domesticus* (Haviland). *O. formosanus* nests were collected in Donghai University, Situn District (120.60°E, 24.18°N) and National Chung Hsin University, South District, (120.67°E, 24.12°N) in Taichung City. *C. formosanus* nests were collected in Dakeshan, Zhuolan Township, Miaoli county (120.89°E, 24.31°N), National Chung Hsin University, South District, Taichung City (120.67°E, 24.12°N) and Taichung Port, Qingshui District, Taichung City (120.53°E, 24.29°N). *R. flaviceps* nests were collected in Fushan, Yuanshan Township, Yilan County (121.58°E, 24.76°N). *Cr. domesticus* nests were collected in Siangjiao Bay Ecological Reserve Area, Kenting National Park, Hengchun Township, Pingtung County (120.82°E, 21.92°N).

Image acquisition

The types of images were categorized into three termite caste groups: worker group, soldier group and worker-and-soldier group. Worker group and soldier group contained five individuals, while worker-and-soldier group contained five workers and three or five soldiers. Since only few soldiers were found in *Cr. domesticus* colonies, three individuals of soldiers were used in each case. Three colonies each of *O. formosanus*, *C. formosanus* and *R. flaviceps* were used in the experiment, while five colonies of *Cr. domesticus* were used to compensate the shortage of soldier caste. Except of *Cr. domesticus*, each caste group of each colony was conducted for three replicates without reused any individual (3 colonies x 3 caste groups x 3 replicates). For *Cr. domesticus*, a total of 27 replicates were conducted with 5 termite colonies. 1,000 photos were continuous shot for each replicate by using a cellular phone (ZenFone 3, ZE552KL, Asustek Computer Inc., Taiwan). During photographing, the cellular phone was placed on an acrylic device and the termites were place in an acrylic ring to restrict the activity range of termites (Fig.1). Termites were placed on a black panel to make the shape of termite can be clearly distinguished.



Figure 1. Photography devices. Termites were placed in the ring. Cellular phone was placed on an acrylic stage with its lens aiming at the termite through the gap.

Individual termite segmentation

The image of termites comprised several individuals in the plate (Fig. 2a). We employed image processing algorithms for capturing individual termite from the original images. First, a RGB image was converted into a binary image using Otsu thresholding (Otsu, 1979). Since the black background and pale-colored termites in the images of our dataset represent high contrast in the histogram of Otsuthreshold segmentation, we can obtain well segmented binary images with more precise outline of termites (Fig. 2b). Second, we reversed the binary images by subtracting from 1 for the use of connected component filtering (Haralock and Shapiro, 1991). We labeled every connected component as 1, 2, 3,...etc, using D8 connecting mode, to make it able to be calculated for the area and be separated one by one. For termites in the images, the area is usually between approximately 13,000 and 50,000 pixels. In the third step, we filtered the areas by the pixel sizes for termites to form individual-termite mask with 0 as background and 1 as termite (Fig. 2c). Due to the different angles of the termites increasing the difficulty to crop individual termites, we applied skeleton method (Ju et al., 2007) to create the one-pixel bones to represent the termite area (Fig. 2d). Next, we used several times of opening to form a thicker skeleton and used Hough transform (Duda and Hart, 1972) to rotate the whole images to adjust the given termite straight (Fig. 2e). Later, we applied bounding box to frame the area of the termite using a rectangle as an image mask, and then cropped the original image to acquire individual termite areas (Fig. 2f).

Because the side length the rectangles were varied, an individual termite image was padded into a squared image with an equal value for width and height using zero padding. For practicality, we downsized each images of individual termite to a size of 130×130 pixels. This reduced the image size from around 3 MB to 23 KB, making processing much more practical and faster during model development and run-time execution.

Termite classification using MobileNetV2 with pretrained weights

We adapted the MobileNetV2 models (Sandler et al., 2018) with pretrained weights (or parameters) as classifier for termite identification. MobileNetV2 is a successor to MobileNets (Howard et al., 2017) designed by Google. In addition to traditional convolution layers and fully connected layers of MobielNets architecture, the depthwise convolution and pointwise convolution layers is designed to be tuned easier and fast. The light weight of model makes it eligible for applications on mobile devices, such as cell phones, with lower hardware requirements and almost the same speed. Therefore, we used MobileNetV2 in this study for possible future applications.

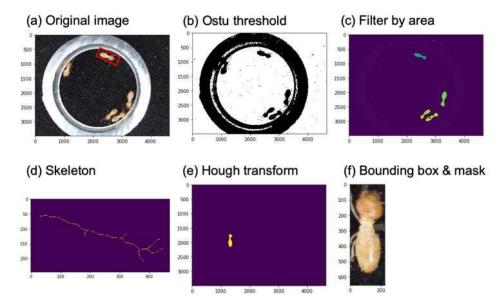


Figure 2. Pipeline for image segmentation of individual termites. (a) The original full image; (b) a binary image by Ostu threshold; (c) filtering termites by certain area size; (d) skeleton of individual termite object; (e) rotating image straight by Hough transform; (f) bounding box of single termite and mask.



Figure 3. A leave-one-location-out cross-validation (LOLO CV) procedure. The datasets were divided according to the collection locations and the testing datasets were independent from training datasets.

Training and testing

We implemented recognition tasks with MobileNetV2 in python 3.7, PyTorch 1.3.0, torchvision 0.4.1 and CUDA 10. We used adaptive moment estimation (Kingma and Ba, 2014) as the optimizer and categorical cross-entropy (De Boer et al., 2005) as the loss function for training classifiers. Each classifier was trained for 100 epochs with an initial learning rate of 0.0001 and the batch size of 64. The dataset contained 400 images of individual termites of each caste from three different locations. Models were evaluated using a leave-one-location-out cross-validation (LOLO CV) procedure that models are repeatedly trained by leaving the images from one of the 3 locations out (Fig. 3). Because our dataset are spatially and temporally dependent, LOLO CV has been suggested to avoid optimistic bias of k-fold cross-validation (Roberts et al., 2017).

Results and discussion

The datasets of termites from different locations are displayed in Fig. 4a. Applying our segmentation pipeline, the individual termite images contain minimum background and considerable variations in termite posture and morphology are observed among images (Fig. 4b).

Our dataset consists of 9600 termite images for both soldier and worker castes collected from different locations in Taiwan. We conducted experiments for three different classifiers, using different castes, *i.e.* soldier, worker, and both castes, as the input. The performance of three classifiers achieved the mean accuracy of 0.930, 0.936, and 0.859 for soldier, worker, and both castes respectively using LOLO CV (Table 1). For the classifiers using soldier caste images as the input, the soldier termites of *O. formosanus* and *Cr. domesticus* were more easily confused with each other (Fig. 5a-c). For the classifiers using worker caste images as the input, the worker termites of *C. formosanus* and *R. flaviceps* were more easily confused with each other (Fig. 5d-f).

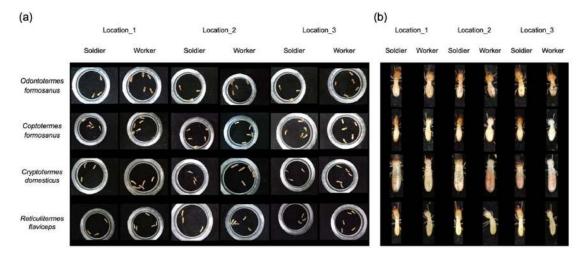


Figure 4. Termite images. (a) original full images and (b) individual termite images.

Table 1. Performance of different classifiers using the MobileNetV2 model. Experiments were conducted by LOLO CV procedure.

Classifiers	Experiments	Accuracy	Mean Accuracy
	1	0.968	
Soldier caste	2	0.920	0.930
	3	0.902	
	1	0.949	
Worker caste	2	0.894	0.936
	3	0.966	
	1	0.879	
Both castes	2	0.832	0.859
	3	0.866	

These observations were similar to the human experts' experience since the morphology between *C. formosanus* and *R. flaviceps* workers are much similar (Fig. 4b). In contrast, the classifiers of both castes obtained relatively lower performance than the classifiers of single caste. The failures in the classifiers of both castes could be largely accounted for the confusion between workers and soldiers within same species (Fig. 5g-i). There is a smaller intra-species variance in the color of body in some species. For example, the head of *O. formosanus* workers is yellowish, which look much similar to the soldier caste. For *Cr. domesticus*, the abdomen color in both workers and soldiers is pinkish in contrast to other classes. In sum, the classifiers of single castes obtained a promising accuracy while the

classifiers of both castes remained a space for improvement. This limitation will be overcome by increasing the number and quality of termite images.

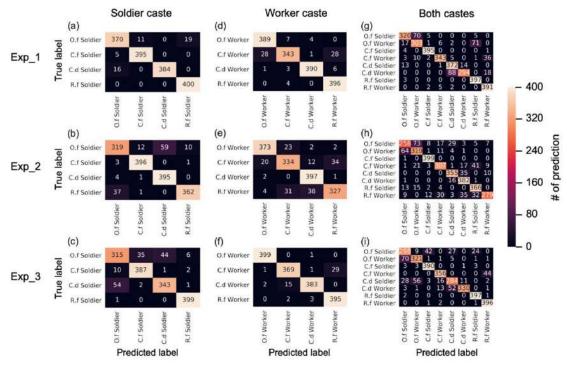


Figure 5. Confusion matrix of different classifiers. The number of testing data in each class were 400. O.f.: *O. formosanus*, C.f.: *C. formosanus*, C.d.: *Cr. domesticus*, R.f.: *R. flaviceps*.

Conclusion

In conclusion, it is worth noting that this is the first report of using a deep learning approach for image-based identification of termites. An image-based identification system can facilitate precise termite control by providing immediate support to control agents, including ordinary citizens. Further research will be conducted to evaluate how deep learning model works using other computer vision techniques in order to improve the performance of recognition outcome. In addition, more termite species will be considered to examine in the future. Our current results indeed warrant further investigation.

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Furfurylation Treatment on Mangium and Pine Woods Extremely Reduces Subterranean Termite Feeding Activities

by

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Abstract

Mangium (*Acacia mangium*) and pine (*Pinus merkusii*) trees are commonly planted for timber production, and these trees could be cut at young age, consequently the wood containing a lot of sapand juvenile-wood is susceptible to attack by subterranean termite. Impregnation of furfuryl alcohol (FA) into the wood is one method reducing termites attack the wood. The purpose of the study was to reduce subterranean termite feeding activity through furfurylation treatment on wood via laboratory test. Wood specimens of mangium and pine were treated with FA, and then tested to subterranean termite in the laboratory according to Indonesian standard SNI 7207-2014. The results showed that weight percent gain of mangium was 18% and pine 43%. Furfurylation on wood could reduce termite feeding activity which was indicated by increasing termite mortality by 500%, decreasing feeding rate by 300% and decreasing wood weight percent loss by 96%, compared with the untreated wood. Following Indonesian standard, untreated mangium was classified into resistance class IV or poorly resistant and pine class V or very poorly resistant, but both furfurylated woods were classified into resistance class I or very resistant.

Keywords: furfurylated wood, subterranean termite, resistance class, termite feeding activity.

Introduction

Supply logs for Indonesian wood industry in 2017 reached 43 million m³, and 87% of them were from plantation forests which were cut at young age (Ministry of Environment and Forestry 2018). The timbers were mostly dominated by sapwood and containing juvenile wood which were susceptible attacked by subterranean termite attack (Fajriani et al. 2013). Furfurylation on wood could enhance wood quality and to reduce termite feeding activity especially in no choice test in laboratory.

Forest plantation is being developed to substitute natural forest especially for timber supply purposes. Mostly the planted trees are fast growing species because the demand on wood is increasing year by year, and the stand could be exploited after 6-10 years old. The timber is dominated with sapwood and containing juvenile wood, hence it is inferior in terms of physical-mechanical properties and susceptible attacked by bio-deterioration organisms, especially subterranean termite. Some favorite species for industrial plantation forest are mangium (*Acacia mangium*), sengon (*Falcataria muloccana*), jabon (*Anthocephalus cadamba*), and pine (*Pinus merkisii*).

Hadi et al. (2005) investigated Scots pine (*Pinus sylvestris*), agathis (*Agathis dammara*) and sengon (*Falcataria muloccana*) for furfurylation treatment, and the results showed that weight percent gain of 40% or more could enhance wood resistant to dry wood termite and subterranean termite attacks, but the wood color became darker, and a higher weight percent gain resulted in more dark intensity. The other researcher Lande et al. (2004) mentioned that furfurylated wood improved resistance to decay and insect attacks, and Esteves et al. (2011) mentioned that with the furfurylation process there was a clear decrease in mass loss due to fungal attacks. Mass loss due to *Postia placenta* and

Coniophora puteana decreased from 28.23% to 1.11% and from 5.69% to 0.78%, respectively, corresponding to a 96% and 86% decrease in relation to untreated wood.

The purpose of this study was to determine effect of furfurylation treatment of wood on termite feeding activity and wood resistance to subterranean termite attack conducted in no choice laboratory test according to Indonesian standard.

Materials and methods

Wood preparation

Mangium and pine woods were from Bogor area, West Java, Indonesia. The wood was cut into specimens with size of 0.5 cm by 2.5 cm in cross section by 2.5 cm in the longitudinal direction according to Indonesian standard SNI 7207-2014 (SNI 2014). Impregnation process was done to fill cell voids with furfuryl alcohol (FA) solution. To accelerate polymerization process, the FA solution was added with tartaric acid at amount of 5/100 (w/w). The wood specimens were placed in the chamber and then vacuumed at 600 mmHg for 30 minutes. During released period of vacuum process, the FA solution allowed to flow into the chamber and subsequently pressured at 10 kg/cm² for 30 minutes. After vacuum-pressure process was finished, the wood specimens were wrapped with aluminum foil and then heated in an oven at 100 °C for 24 hours. Afterward, the aluminum sheet was removed and the wood specimens were weighed for weight percent gain (WPG) calculation. For comparison purposes untreated wood was included as the control, and six samples as replication were prepared for each treatment combination. The specimens were conditioned in a room condition for three weeks prior to be exposed for subterranean termite attack in laboratory test.

Subterranean termite test

The laboratory subterranean termite attack test was based on Indonesian standard SNI 7207-2014. Each wood test specimen was placed in a glass container with 200 g of sterilized sand, water to reach moisture content 7% under water holding capacity, and 200 healthy and active workers of *Coptotermes curvignathus* Holmgren subterranean termites from a laboratory colony. The containers were put in a dark room at a temperature of 25 °C to 30 °C and 80% to 90% relative humidity during four weeks and weighed weekly. If the moisture content of the sand decreased by 2% or more, water was added to achieve moisture content standard. Test method diagram according to SNI 7207-2014 was following Arinana et al. (2012). At the end of the test, the wood specimens were clean up and then put in the oven at 67 °C to reach oven dry weight.

Data analysis

To analyze the effect of treatments upon all responses, i.e. wood density, wood weight loss, termite mortality, a 2×2 factorial in completely randomized design was used for data analysis. The first factor was wood species (mangium and pine), and the second factor was treatment (untreated and furfurylated woods). Duncan's multiple range tests were used for further analysis if interaction factor was significantly different at $p \le 0.05$. The data was analyzed with Microsoft Excel and Statistical Product and Service Solution version 22.

Results and discussion

Mangium is a broadleaf species, while pine is conifer. The density of untreated mangium was 0.57 g/cm³ and pine 0.66 g/cm³, and the durability class was IV for mangium and V for pine. The durability class according to Martawijaya et al. (2014) ranged from I to V with class I being very resistant and V very poorly resistant. The average values of wood density, termite mortality, termite feeding rate, wood weight loss, and wood resistant class are shown in Table 1, and analysis of variance (Anova) is resumed in Table 2.

Table 1. Density, weight loss, resistant class and mortality for each wood species and treatment.

Wood species	Treatment	Wood density (g/cm ³)	Termite mortality (%)	Feeding rate (µg/termite/ day)	Wood weight loss (%)	Resista nt class
Mangiu m	Untreated	0.57 (0.08) a	21.1 (2.0) b	47.5 (13.4)	12.26 (3.27) b	IV
	Furfurylated	0.67 (0.05) b	100(0) c	4.1 (3.3)	0.55 (0.46) a	I
Pine	Untreated	0.66 (0.04) b	12.3 (1.3) a	90.3 (11.1)	21.27 (2.78) c	V
	Furfurylated	0.88 (0.07) c	100(0) c	8.4 (3.8)	0.75 (0.31) a	I

Remarks: Values in parentheses are standard deviation. Values followed by the same letter within the columns in each respond are not statistically different regarding to Duncan's multi range test.

Table 2. Resume of Anova

Parameter	Wood species	Treatment	Interaction
Wood density (g/cm ³)	**	**	*
Termite mortality (%)	**	**	**
Feeding rate			
(µg/termite/day)			
Wood weight loss (%)	**	**	**

Remarks: ** Highly significantly different ($P \le 0.01$); * Significantly different ($P \le 0.05$).

According to Table 2, wood density was affected by wood species, treatment and interaction of both factors, further data analysis explained that wood density of furfurylated wood was higher than untreated one, and the increment was linear with WPG while mangium reached 18.1% and pine 43.0%. Pine had higher WPG than mangium despite its higher density. Pine belongs to the conifer class which has more simple anatomical characteristics compared to mangium which belongs to the broadleaf class. Softwood is mostly arranged by tracheid cells while hardwood more complex which is composed of at least five basic cells namely vessel, fibers, parenchyma, tracheid and epithelial cells. This result was in line with Ding et al. (2008), who reported that the enhanced density due to polymer impregnation depended on the wood species. During impregnation process, FA solution entered the void in wood with a pressure and then it hardened because of heat and catalyst presences to develop polymer among furan rings, and some of them could graft to the cell wood (Gerardin 2015).

Termite mortality of untreated mangium (21.1%) was higher than pine (12.3%), and these results were similar with Arinana et al. (2012) mentioned termite mortality of mangium reached 27% and pine 9%. Both furfurylated woods had 100% termite mortality or increased 500% compared with untreated wood. The assumption regarding to the chemical changed of furfurylated wood resulting higher density and more hydrophobic, consequently the termite could not feed anymore, and finally all termites died even though the test period has not yet finished.

Termite feeding rate was affected by wood species, treatment and interaction of both factors. Further data analysis explained that termite feeding rate of untreated mangium (47.5 μ g/termite/day) was significantly lower than pine (90.3 μ g/termite/day), and these values were similar with Arinana et al. (2012) who had reported 43 and 79 μ g/termite/day, respectively. Furthermore pine was recommended for inclusion in the test in laboratory regarding to Indonesian standard. In terms of treatment, termite feeding rate of furfurylated wood (6.3 μ g/termite/day) was much lower than untreated one (68.9 μ g/termite/day) or it was 91% compared with untreated wood and these values was incorporate with weight loss linearly, i.e. higher mortality had lower feeding rate, in other words furfurylation on wood could extremely reduce termite feeding activity.

After exposed to subterranean termite, weight percent loss of furfurylated wood was much lower than untreated wood, as it was reduced to 96% compared to the control. This observation indicated that termite totally could not feed in the furfurylated wood test, as the wood had already changed physically and chemically. Regarding Indonesian standard SNI 7207-2014 (SNI 2014), based on weight loss values, the untreated mangium is classified to resistance class IV or poorly resistant and pine is class V or very poorly resistant, but after furfurylation treatment the class resistances of both wood species were changed, both were now classified as class I or very resistant.

Conclusion

It can be concluded that furfurylation on wood generated higher wood density and chemically changed wood component compared with untreated wood. Furfurylation treatment could extremely reduce termite feeding activity which was indicated by higher termite mortality, lower termite feeding rate, lower wood weight percent loss, and consequently it could enhance wood resistance to subterranean termite attack. Untreated mangium wood was classified into resistant class IV or poorly resistant and pine class V or very poorly resistant, but furfurylated woods were classified into resistant class I or very resistant.

Acknowledgement

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Poster Presentations



Evolution of Termite Symbiosis Informed by Transcriptome-Based Phylogenies

by

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Abstract

Termites are the oldest social insect lineage, one of the few animal groups able to feed on lignocellulose, and amongst the most abundant animal groups in tropical ecosystems. To understand the key evolutionary transitions that shaped modern termites, including the evolution of sociality and the loss of gut protozoa, a robust molecular phylogenetic tree is required. Here, we sequenced 53 termite transcriptomes and two genomes which we used to reconstruct time-calibrated phylogenetic tree of 68 termite species. Our analyses, based on up to 4065 protein-coding genes, confirm the branching pattern of major termite taxa, as well as the polyphyly of Rhinotermitidae and Termitinae, and the paraphyly of Heterotermes in respect to Coptotermes. Our analyses challenge previous molecular phylogenies by recovering sister relationships between the comb-builders Sphaerotermitinae and Macrotermitinae, which changes our understanding of the chronology of the loss of protozoa and the origin of fungus-growing. The termite phylogeny indicates that comb building is a derived trait within Termitidae and that externalization of some of the digestion to comb-like structures involving bacteria or fungi may not have driven the loss of protozoa from ancestral termitids, as previously hypothesized. Instead, dietary shifts from wood to other plant-based substrates and recruitment of new gut prokaryotic symbionts may have played the major role in this symbiotic transition.

Keywords: termites, transcriptomes, phylogeny, symbiosis, Sphaerotermitinae

Characterizing the feeding behaviour of a generalist inquiline termite

by

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Abstract

Among the various lifestyles adopted by termites, inquilines live in a nest built by other termite species. Some of these do not forage outside the nest, but rather feed on food stored by the host or on the nest material itself. *Cavitermes tuberosus* (Termitidae: Termitinae) is a broad-spectrum inquiline of arboreal termites, which does not seem to forage outside the nest. Therefore, our aim was to define the actual feeding behaviour of this species. We used an integrative framework combining ecological measures (physico-chemical parameters, stable isotopic ratios of N and C) and Illumina MiSeq sequencing of 16S rRNA gene to identify bacterial communities and to analyse termites as well as the material from nests constructed by different termite hosts. Our results indicate that: (i) the physico-chemical composition of nests shifts in secondarily colonized nests; (ii) stable isotopic ratios suggest that *C. tuberosus* feeds on the nest organic matter; (iii) its gut microbiome is highly diverse and most similar to the one of its main host, *Labiotermes labralis*; and (iv) nitrogen depletion in the nest material might be prevented by the substantial presence of N-fixing bacteria. These results highlight the generalist character of this inquiline and potential for ectosymbioses with nest bacteria, features which may in turn explain its great ecological success.

keywords: Ecology, Humivory, Microbiota, Neotropical, Nest

Intestinal Protists of the Enigmatic Termite Family Stylotermitidae (Insecta: Blattodea) and its Phylogenetic Position

by

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Abstract

The termite family Stylotermitidae, which phylogenetically locates between Rhinotermitidae and Kalotermitidae, is consisted of 2 fossil genera and 1 extant genus Stylotermes (45 species). Due to their cryptic biology, no molecular data is subjected until recently (Wu et al. 2018). All lower termites harbor intestinal protists, which play an irreplaceable role in wood digestion of termites, including Stylotermitidae. Mutualism between termites and protists can traces back to early Cretaceous (Poinar Jr 2009). Though co-evolution exists between termites and protists, the phylogenetic tree of protists is not always consistent with its hosts (Boscaro et al. 2017). Many protists have been reported, however, few is described and fewer is given molecular data. Intestinal protists are extremely difficult to cultivate, which making the traditional methods of sequencing nearly impossible. In this study, Stylotermes halumicus, as well as its intestinal protists, is sequenced with next generation sequencing (NGS). SSU rRNA is identified and compared with reported sequences in GenBank. Hypermastigote genus Pseudotrichonympha is identified. The most similar species of Pseudotrichonympha sp. in S. halumicus is P. pearti in the termite, Prorhinotermes simplex, with the similarity of 95.34%. Protists are observed under optical microscope, Pseudotrichonympha sp. composes the majority of protists. This suggests that Pseudotrichonympha sp. likely play a central role in the digestion of cellulous in Stylotermitidae and its enigmatic biology of feeding on living trees. Pseudotrichonympha is reported in Rhinotermitidae and Archotermopsidae, but not in Kalotermitidae. We suggest that the difference of protists fauna may be the key to the habitat shift from Kalotermitidae which feed on woods only, to Rhinotermitidae, which feed on both soil and woods.

Keywords: Pseudotrichonympha, Stylotermitidae, phylogeny, intestinal protists

The Assembly of Fungal Communities in Termite Guts is Affected by Diet and Taxonomy

by

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Abstract

Termites are one of the most abundant insects in tropical and subtropical regions and play crucial role as ecosystem engineers feeding and degrading different types of substrates. Although it is known that gut symbionts are necessary for termites in order to effectively use their feeding substrate, such as wood, there is a little knowledge about role of fungal community in termite gut. To understand this further we tried to fill this gap of knowledge and have sequenced two fungal markers - ITS and LSU, in more than 50 termite species for each marker. We have covered different feeding groups as well as different locations and taxonomical groups.

There was an obvious trend in low diversity and abundance of fungal species in the guts of lower termites. On the other hand, highest diversity and richness of fungal species was recorded for soil feeders. Termitomyces fungus was present in all fungal-growers (Macrotermitinae) as expected. Interestingly, fungal community of fungal-grower Macrotermes was unexpectedly rich and abundant in fungal orders Hypocreales and Pleosporales. Lichen-feeders, fungus-growers and lower termites were significantly different in the composition of their fungal microbiota from other groups. Occurrence of some fungal orders was influenced by the location as well. For example, ubiquitous order Eurotiales were significantly more often found in the guts of termites from Africa.

Overall, to our knowledge, this is the first comprehensive study on fungal communities in termite guts.

Keywords: fungi, termites, diet, ITS, LSU

Genus *Coptotermes* (Rhinotermitidae) in Sumatra and West Java: Morphological and Phylogenetic Studies

by

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Abstract

Coptotermes Wasmann is considered an economically important group of termite. However, until now, the distribution of Coptotermes group in Indonesia is not fully known due to the lack of surveys. The genus Coptotermes, is well-known as urban pest all over South East Asian Countries, but information with regards to its range merely existed in Indonesia. This study focused on collection of samples from 8 locations in Batam, Simeulue, Karimun Islands, and around West Java Province by using transect and casual sampling method. As much as 5 species of Coptotermes group were collected and identified. Morphological differences among C. gestroi antennae segmentation and head measurements were observed. Two pairs of mitochondrial primers were used for targeting the 12S and 16S genes. Amplification of the 12S gene was performed by 12SF and 12SR primers. While LR-N-13398 and LR-J-12887 primers were used to amplify the 16S gene. Sequence results were manually edited and the genetic distance was analyzed according to the Kimura 2-parameter model of sequence evolution in Mega 7. Several head character measurements significantly splitted Cipinang and Serpong samples into different group based on their localty. However, the result of 12S and 16S phylogenetic tree put both of them into the same clade. Consequently, morphological variation and measurement complex in both specimens underlined the perplexity of species determination on the field. The results do not rule out the possibility of C. gestroi to be considered as a potential major termite pest in Indonesian urban areas. The study gave information on Coptotermes Wasmann existence in Sundaic Region and their phylogenetic relationship.

Keywords: Coptotermes, termite pest, Rhinotermitidae, morphological variation, phylogeny

Termites Investigation on Forest Plantation Area in Riau, Sumatera Indonesia

by

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Abstract

Termites infestation may become inevitable incidence in simplified vegetation such as forest plantation. The occurrence of the termites was investigated on both *Eucalyptus pellita* and *Acacia crassicarpa* in forest plantation as well as in its housing facilities in Riau Province, Sumatera, Indonesia. Species diversity of the termites and their impact on the trees were studied. Efficacy of several insecticides were tested against the most dectructive species.

In the plantation area, eight termites species were found belonging to two families: Capritermes sp. (Family: Termitidae), Coptotermes curvignathus (Family: Rhinotermitidae), Longipeditermes longipes (Family: Termitidae), Macrotermes sp. (Family: Termitidae), Microcerotermes duplex. (Family: Termitidae), Microtermes sp. (Family: Termitidae), Procapritermes sp. (Family: Termitidae) and Schedorhinotermes sp. (Family: Rhinotermitidae). In the housing area, one species was found: Cryptotermes domesticus (Family: Kalotermitidae). This C. domesticus was found colonizing dry wood of abandoned housing facilities, but it was not found in the plantation. C. curvignathus was the most species found in A. crassicarpa plantation, while M. duplex was the most destructive species attacking E. pellita plantation.

Insecticides with active ingredient Fipronil 2 ml per L and Chlorfenapyr + indoxacarb 3 ml per L were effective in controlling *M. duplex* up to 100% and 93.8%, respectively; where no new infestation was found in all observation plots up to two months after application.

Keywords: Termite, *Coptotermes*, *Microcerotermes*, *Cryptotermes*, *Acacia crassicarpa*, *Eucalyptus pellita*, fipronil.

De novo Development and Polymorphism Analysis of Microsatellite Markers in Prorhinotermes flavus (Blattodea: Rhinotermitidae)

by

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Abstract

Prorhinotermes can be found in the coastal areas of tropical islands. According to previous studies of invasive termite biology, it has been hypothesized that the transoceanic colonies dispersal was achieved by converting sterile workers into reproductive caste. To test the dispersal hypothesis, developing a genetic tool is required. Microsatellites are 2~6 bases of tandem nucleotide repeat motifs found in the genome and usually display a high degree of length polymorphism due to a higher mutation rate and considered as a powerful genetic tool for evolutionary, ecology and population genetic studies. Here we utilized a Prorhinotermes draft genome sequence for the de novo development of di-nucleotide (CA) or tri-nucleotide (AAT) repeats-containing genetic markers. PCR primers were designed using Primer3 with default settings. To detect the degree of allelic diversities, PCR was carried out with a pool of DNA extracted from an outbred-and-lab-reared colony. The forward primer of 7 highly polymorphisms loci were labeled with various fluorescent dyes, including 6-FAM, VIC, NED, and PET, for multiplexing PCR products for the subsequent fragment analyses. To test the panel, termite samples come from a colony collected at Kenting Taiwan were used. There are from 1 to 4 alleles per locus in the tested colony, with 85.71% of loci were polymorphic. Comparing the observed (Ho) and expected (He) heterozygosity, we conclude that the allele of Kenting population is in Hardy-Weinberg equilibration, and the fixation index indicating that the colony is a panmictic population.

Keywords: Prorhinotermes, Microsatellites, DNA fragment analyses.

Worldwide Historical Biogeography of Termites (Blattodea: Isoptera)

by

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Abstract

Termites are well-known for their importance in organic matter decomposition and soil fertilization in tropical and subtropical ecosystems. Termites diverged from other cockroaches ~170 Ma, and all modern termite species share a common ancestor dated at 149 Ma. Given the antiquity of termites, both vicariance, through plate tectonics, or dispersal, through oceanic rafting or across land bridges, can explain the current distribution of termites. The phylogenetic relationships among termite families and subfamilies are well-studied. However, the relationships among lineages that diverged more recently have been unknown until recently. In our project, we used complete mitochondrial genomes to build time-calibrated phylogeny of termites, with the final aim of resolving the global historical biogeography of termites. We selected a representative set of samples, including species from all lineages, and from across the geographic locations where termites are known to occur. This dataset allowed us to reconstruct the precise origins and dispersal patterns of termites. Finally, we will sequence mitochondrial genomes of around 2000 samples including almost all termite species of the Neotropical regions.

Keywords: termites, historical biogeography, mitochondrial genomes, Neotropical regions

Plasticity of Reproductive Caste Development of *Prorhinotermes flavus* (Blattodea: Rhinotermitidea)

by

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Abstract

The caste development of the termite genus, *Prorhinotermes*, is considered more plastic than other genera in the termite family Rhinotermitidae when analyzed morphometrically and morphologically. Their distinctive distribution around tropical oceanic coast and islands worldwide might be attribute to the high composition of secondary reproductive caste, neotenics, which make up to 2-12.9% of individuals in the colony. We hypothesized that neotenics play a major role in increasing the adaptive fitness and dispersal strategy. However, the development of neotenics remains unclear.

In this study, we focus on the neotenics development of *Prorhinotermes flavus* by measuring the maximum width of head, pronotum and the number of antennal segments. As the result, pluripotent immatures (pseudergates), retaining the ability to differentiate into the apterous neotenics at 3-7 larval instar stage. And the nymph may undergo the regressive molt become brachypterous neotenic with wing pads. By censusing caste composition of 25 *P. flavus* colonies, we did not find any primary reproductive. Reproductive castes, winged imago (alate) and neotenics were found in a colony almost exclusively. The caste composition of the 25 colonies indicates that *P. flavus* is likely to disperse through two ways: alate-dispersal strategy (ADS) and neotenic-dispersal strategy (NDS). Neotenics are barely found in ADS type colony; in contrast, in the NDS type colony, neotenics are the mainly reproductive caste but alates and nymphs are rare.

The phylogenic position of *Prorhinotermes* is between the two worldwide invaded termite groups, Kalotermitidae and Rhinotermitidae. Knowing the caste development pathway and the dispersal strategy of *P. flavus* would contribute to the future study of termite invasion.

Keywords: oceanic termite, neotenics, caste development pathway, termite dispersal strategy

Does Body Size Variation of Formosan Fungus-Growing Termite Fit Bergmann's Rule?

by

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Abstract

Bergmann's rule describes that organism body size increases with decreasing environmental temperature for thermoregulation. Formosan fungus-growing termite, Odontotermes formosanus (Shiraki), is the most common and widely distributed termite species in Taiwan. We checked 1,194 colony samples of O. formosanus preserved in the National Chung Hsing University Termite Collection. For sample selection, we divided Taiwan into three regions from north to south by 23- and 24-degrees north latitude. From sea level up to 1,500m elevation, every 300 meters is defined as one section. A total of 15 divisions divided by latitude and altitude were used in this study. We use stratified random sampling in the sample distribution range. In each division, more than 5 termite colony samples were randomly selected. Of each selected colony, at least 5 workers were submitted to maximum head width measurement. We analyzed the correlations of termite's head width and latitude, longitude, elevation and annual mean temperature, precipitation, maximum and minimum additional 19 bioclimatic variables by using simple linear regression analysis. The bioclimatic variables were available from worldclim dataset. A total of 460 workers from 76 colonies were measured. The range of maximum head width of worker was 1.03-1.67mm, with an average of 1.35±0.01mm. Significant correlations between twelve environmental factors and head width were found. Latitude, elevation, mean diurnal range and temperature annual range show positive correlation, and longitude, annual mean temperature, maximum and minimum temperature of warmest and coldest month or season are negative correlation with head width. The temperature of coldest month was the most relevant factor. The worker size of O. formosanus tend to decrease with increase in environmental temperature, which is consistent with Bergmann's pattern. However, the mechanism behind required further studies. In addition to increasing individual body size, social insects are selected as groups, so called superorganism. Whether variation of termite colony biomass fit with Bergmann's pattern remains unknown.

Keywords: Odontotermes formosanus, body size, Bergmann's rule, superorganism



Transport of Microplastics from Surface into Soil by Termites

by

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Abstract

Even though plastics have enormous benefits to society, it causes a serious environmental problem. In particular <5mm size of plastics, which is called microplastics (MP), have been intensively studied in aquatic environments. However, information about terrestrial environments is lacking. There is a report saying that soil probably receives much more plastic waste than oceans. Thus, it is necessary to conduct research on this problem of soil being polluted by plastics. Our experiment verified the effect of soil-organism activity to the movement of MP in terrestrial environments, using termites as a representative of soil-organisms, in experiment using laboratory pots. The size of pots was 5.8 cm in diameter and 15 cm in height. We used two subterranean termites, Formosan subterranean termite (FST: Coptotermes formosanus) and Asian subterranean termite (AST: C. gestroi). We used a combination of plastics namely polyethylene (PE) and polypropylene (PP) microplastics varying in three different sizes (2-5 mm, 1-2 mm, 0.5-1 mm), and polystyrene (PS) blocks (2×2×1 cm). Three weeks after introducing the termites to pots with soil with plastics, we observed that microplastics were not only buried in the piled-up soil but also transported up to >3 cm depth. In the cases of PE and PP, smaller particles tended to be transported deeper, and particles of <0.5mm size were also detected. In the case of PS, termites heavily attacked PS blocks and broke them into small particles. MP were seen in a deeper layer than those of PE and PP. Our study showed that termites were associated with the movement of MP from surface into the soil and were likely to break plastics into smaller particles. These effects depend on the size and type of plastics. When termites break plastics into smaller parts and those are retained in soil where environment is relatively stable, it would be possible that those in soil can give negative impacts on soil biota, soil organisms, and plants in some ways for a long period. We need to conduct further researches to understand the fate of MP in soil.

Keywords: microplastic, termite, polyethylene (PE), polypropylene (PP), polystyrene (PS)

Monitoring the Hybridization of Two Notorious Termite Pests in Taiwan

by

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Abstract

Formosan subterranean termite, Coptotermes formosanus Shiraki, and Asian subterranean termite, C. gestroi (Wasmann), are considered two of the most destructive termite pests worldwide. They are invasive and both present in Florida, Hawaii, and Taiwan. Previous studies in Florida showed that hybrid colonies of these two species can be established, exhibiting hybrid vigor in fertility under laboratory conditions. If the hybrid colonies can robustly survive in the wild, it might pose immense problem to human society. C. formosanus was found throughout Taiwan, while C. gestroi most found in southern and central Taiwan with a major overlapped region at the central Taiwan. Considering over 100 years of sympatry history, we speculated that natural hybrids occur among the wild populations. To investigate the hybrid potential of C. formosanus and C. gestroi in the field, alates of C. formosanus and C. gestroi were caught in three cities in the central part of Taiwan including Taichung, Yunlin and Chiayi. Dispersal flight events were inspected every night from March 20th to June 20th, 2019. Among the 92 days of our daily monitoring, we observed dispersal flight in 27 days in Taichung, and both species flew together in 9 nights (9/27); in Yunlin, five dispersal flight events were recorded, and no overlapped flight was found (0/5); in Chiayi, termite flew in 38 nights and 3 of them with both species (3/38). To verify whether the hybrid colonies can be established, we set up reciprocal crosses between C. formosanus and C. gestroi using collected alates. Six months after laboratory rearing, the survival rate of \mathcal{C} . formosanus× \mathcal{C} . gestroi and \mathcal{C} . formosanus× \mathcal{C} . gestroi were 19.4% (14/72) and 48.1% (38/79). The survival rates of conspecies colonies of C. formosanus and C. gestroi were 33.3% (42/126) and 40.4% (40/99) respectively. Our results indicated that in Taiwan, C. formosanus and C. gestroi have an overlapped swarming seasons and the mating of the two species has ability to form an initial colony in the laboratory. We further employed a panel of microsatellite genetic markers established by Chouvenc et al. (2017) to investigate the genetic polymorphism of alates between cities, and tested whether the hybrid colonies do exist and survive in wild populations.

Keywords: Coptotermes, hybrid, microsatellite marker, dispersal flight

P 13

Identification of Termite – Ectoparasitic fungi Relationship

by

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Abstract

Ectoparasitic fungi attach to and grow on the body surface of their hosts. Twenty-two species of termite ectoparasitic fungi have been reported, with the two most common species being Laboulbeniopsis termitarius Thaxt and Antennopsis gallica Buchli and Heim. They might affect on behaviour, reproductive and survival of host termites. Since the size and the existing of ectoparasitic fungi were to small, visual observation under a dissecting microscope has been a common method to screen the presence of fungi on termites, a high number of termites are required. The detection of the ectoparasitic fungi in a robust yet economic manner, a multiplex nested PCR assay, using speciesspecific primers, was then developed. However, the infection process and the effect of the fungi on termite behavior remind unclear. In this study, the observation of penetration and growing of ectoparasitic fungi on termite cutile was revealed. The infected termite by ectoparasitic fungi were separated and preserved inside glutaraldehyde (GA) solution. The fixation of infected termite body was carried out. Observation of infection part was carried out by using transmission electron microscope (TEM) The result of this study might help to understand and answer the question of how the fungi infect the termite, how the fungi-infected termites are impaired, why the fungi-infected termites have a shorter lifespan, the effects on the termite of fungi infection, the processes and conditions of fungal infection of termites how the fungi infected termite.

Keywords: ectoparasite fungi, termite, penetration, TEM

P_14

A Nematode Species Associated with Termitophilic beetle, Ziaelas formosanus – A Study of its Highly Derived Morphological/Structural Characters

by

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Abstract

The nest of an agricultural termite, *Odontotermes formosanus* harbors many symbiotic arthropods, e.g., beetles, flies and millipedes. In the previous studies on the termite and symbionts-associated nematodes we revealed that there are two different sets of nematode fauna in the nest, i.e., both termite and symbionts have their associated nematodes, but no nematode species overlap between termite and symbionts.

In the present study, we examined a species of nematode associated with one of the termitophilic tenebrionid beetle, *Ziaelas formosanus*. The nematode was isolated from the adult beetle as the dauer (dormant) juvenile, but it was not found from termite body or nest materials (fungus comb). The dauer juveniles developed to the adults via fourth-stage propagative juveniles on the dissected body of the beetle, and can be cultured using nematode growth media (NGM) plate previously seeded with *E. coli* strain OP50, i.e., the nematode is a bacteria-feeding species.

Based upon a molecular phylogenetic analysis using two ribosomal RNA loci (near-full-length of 18S and D2-D3 expansion segments of 28S), the nematode was identified to belong to the genus *Acrostichus*, which contain the entomophilic (phoretic) species associated with soil-dwelling bees, mushroom beetles, bark and ambrosia beetles and weevils. However, its typological characters, especially feeding (stomatal) structures, did not fit the generic characters of *Acrostichus*, but fit much closer to *Rhabditolaimus*, the entomophilic genus associated with various groups of insects, and *Mehdinema*, the parasite of mole crickets, suggesting that the species is highly adapted to feed on bacteria (or liquid nutritional source) in highly humid condition. This highly derived structural characters may represent the plasticity of the nematodes, and further biological examination on carrier *Ziaelas* beetles may provide environmental factors affecting the adaptation of nematodes and other meiofaunal symbionts.

Keywords: morphology, nematode, *Odontotermes formosanus*, phylogeny, symbiont, *Ziaelas formosanus*

P_15

Detection of Potential Compound from Wood-Decay Fungi for Bioattractant of Termite

by

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Abstract

Basidiomycete fungi was studied to evaluate for their potential volatile compound as bio-attractant for controlling a wood-attacking insect, such as termite. Termite is an economically important pest of wood industry which attack lignocellulosic materials. Ten species of basidiomycete fungi classified as wood-decay fungi (SC, PC, COR, Gano, M3, M4, M7, H2A, TH2 and TP) were grown in jars containing a mixture of rubber wood-sawdust and JIS (Japanese Industrial Standards) media. The fungi were incubated at ambient temperature for 14 days until the entire surface of the media was covered by fungal mycelium. Then, the volatile compounds of fungi were trapped with head space sampling method. Hexane washed monotrap discs were exposed to workers of Subterranean termite (*Coptotermes* sp.) for 24 hours. Aggregation index (AI) was calculated to determine the attractance behaviour. Subsequently, GC–MS was performed to identify the specific chemical compounds. The termites showed significant preferences on crude volatile extract of M7, COR, Gano and TH2 after 2 hours observation. Based on GC-MS analysis, methyl 2-furoate was suspected as specific compound which induced attractance behavior of the termite.

Keywords: bio-attractant, termite, wood-attacking insect, wood-decay fungi

Patented Biobased Microemulsion-Based Pyrethroid Solutions provide Permanent Wood Protection against *Coptotermes* termites

by

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Abstract

Biobased microemulsion-based pyrethroid solutions are approved for dipping treatment providing 25 years of termite protection for solid wood and wood-based products in Europe and for more than 10 years in Indonesia. Those water-based wood preservatives are patented formulations based on concentrated microemulsions (ME) diluted with water as a dipping treatment but also for vacuum pressure treatment. Field trials conducted in Malaysia by UNIMAS confirmed the efficacy of those biobased solutions at three product concentrations (nominal cypermethrin concentrations used: 0.08, 0.16 & 0.32% w/w; nominal permethrin concentrations used: 0.05, 0.10, 0.20 % w/w) on 3-minutes dip-treated kempas (Koompassia malaccensis) heartwood, a major hardwood species in the Malaysian wood construction market, against the Southeastern Asian subterranean termite Coptotermes curvignathus. Such treated wood blocks were exposed to aboveground H2 (indoor, non-wetting conditions) hazard class termite field test and compared with CCA-treated kempas heartwood and radiata pine (Pinus radiata) sapwood. Prior to the H2 hazard class termite field test exposure, treated wood blocks were conditioned to either a non-leaching volatilization (H2 hazard class weathered wood blocks) or to a leaching followed by volatilization (H3 hazard class weathered blocks) as well as nonleaching/non-volatilization and leaching/non-volatilization reference treatments. After 6 months field exposure, untreated kempas was severely (termite ratings: 0, mean mass loss: 97.4%) or moderately attacked (mean ratings: 7.7, mean mass loss: 17.5%), while none of the leached-volatilized (H3 hazard class) or non-leached-volatilized (H2 hazard class) test blocks treated with the products at both target retentions were regarded as attacked (mean ratings: 9.7-10, negligible mean mass loss) regardless of applied termiticide concentration, leached or non-leached wood, volatilized or non-volatilized wood treatments. Excellent performance also prevailed with the remaining treatment combinations of treated wood. Due to their unique compositions, those solutions showed excellent performance against Coptotermes curvignathus with remarkably low termiticide concentrations where conventional agroinsecticides fail to perform. In conclusion, those biobased microemulsion-based pyrethroid are effective for wood protection in buildings and outdoors aboveground against Southeast Asian Coptotermes subterranean termites.

Keywords: subterranean termites, *Coptotermes curvignathus*, kempas, dip-treatment, biobased microemulsion, H2 and H3 hazard classes.

Monitoring Uptake and Penetration of Pesticide during Impregnation of an easily Impregnable (*Pinus sylvestris*) and a Refractory (*Picea abies*) Wood Species with Bio-Based Emulsion Gel Industrial Formulations

by

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Abstract

Wood samples from spruce (Picea abies) and pine (Pinus sylvestris) even in dry state are susceptible Preservation against biological decay in exposed conditions is to biological degradation. conventionally achieved by impregnating the wood with pesticides applied in professional and industrial processes. Solution penetration in wood is a complex and largely unknown process which involves numerous parameters. Here we focus on the relation between the penetration of wood preservatives, the wood macro- and microstructure, and the physical characteristics of emulsion gel formulations. Due to distinct anatomical characteristics, pine wood is considered as easily impregnable contrary to the more recalcitrant spruce. In the present study, specimens from the two species were impregnated with three types of commercial bio-based emulsion formulations, of insecticide, and of fungicide plus insecticide preservatives. The degrees of penetration and uptake of active agents were evaluated over the thickness of the specimens. The impact of different modes of application of the pesticide formulations such as dipping, surface spraying, and vacuum-impregnation, on the penetration and of retention of the active agents was analyzed. The levels of impregnation were visually assessed, and qualitative and quantitative analyses of the active agents, cypermethrin, permethrin and propiconazole were analyzed by gas liquid chromatography coupled to mass spectroscopy. Due to the suitable combinations of solvents and surfactants used in the preparation of the bio-based emulsions of pesticide agents, the present aqueous gel formulations of low-toxicity exhibited rapid wood penetration and enabled high yields retention, particularly when vacuumimpregnation was implemented. The respective penetration and distribution of the active agents, permethrin and propiconazole, were quantified as a function of depth in the specimen. The difference of susceptibility of pine wood versus spruce wood to the pesticide treatment is discussed in relation to the permeability of the two species, and to the connectivity of their conducting cells network, in terms of their anatomical and ultrastructural characteristics. The present data will be useful for estimating the capacity of penetration and retention of low-toxicity degradable bio-based preservative formulations with minimal environmental impact, and their potential on wood species preservation.

Keywords: wood preservatives, emulsion gels formulations, permethrin, cypermethrin, propiconazole, impregnation, *Pinus sylvestris, Picea abies*, wood microstructure

Developing Evaluation Methods for Termiticide used in Taiwan

by

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Abstract

Coptotermes spp. has been recorded as a severe urban pest to construction across Taiwan. In northwestern Taiwan, buildings damaged is primarily caused by *C. formosanus*, while *C. gestroi* is primarily buildings pest in southwestern Taiwan. It is estimated that both termites cause one billion NTD loss annually (~33 million USD) in Taiwan. So far, no standard evaluation method for testing termiticide efficacy has been legislated in Taiwan. Hence, the Environmental Protection Administration (EPA), Taiwan, would like to set up a protocol for screening major termiticide used and available in the market.

In Taiwan, pest control operators (PCOs) primarily offer remedial termite control. Pretreatment is not enforced by law. In order to provide a proper and useful methods to test termiticide efficacy, we conducted a questionnaire survey to licensed PCOs first. We randomly selected registered PCOs to conduct telephone interview, with a goal to obtain 56 valid questionnaires (5% of 1107 companies). Except of background information of the company, the two questions asked were: 1) What kind of termite control methods do your company offer? What is the frequency of conducting each method? And 2} What kind of termiticide do you apply? What is the application frequency of each chemical? The result obtained show, four termite control methods were used in Taiwan, including liquid termiticide injection into wood (89%), drill and injection liquid pesticide into soil (75%), indoor spraying on wood (50%), baiting (27%). Five active ingredients were most used in Taiwan, include fipronil (96%), hexaflumuron (25%), imidacloprid (12%), chlorpyrifos (5%), sodium metaborate tetrahydrate (1%).

According to the questionnaire survey, we suggested EPA to design and accept three termiticide evaluation methods, including applying liquid termiticide on wood and in soil, and feeding termite with bait. Since both *C. gestroi* and *C. formosanus* are major pests, all termiticide evaluation should be conducted with both species. At least 36 tests should be conducted for screening a termiticide, which are: 2 termite species x 3 termite colonies per species x 3 replicates per colony x 2 groups (experimental group and control group).

Keywords: Environmental Protection Administration, liquid termiticide, termite bait, questionnaire survey, pesticide efficacy evaluation

Termite Infestations in Important Wooden Cultural Properties of Korea

by

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Abstract

In the Korean peninsula, 2 species of subterranean termites are reported, *Reticulitermes speratus kyushuensis* (Morimoto) and *R. kanmonensis* Takematsu. In the past, termite damages were reported mostly in the southern region of Korea such as Busan, Jinju, Yeosu, etc. However, new damage has been detected in the northern part. Activities of termites were also noticed to have become much longer and swarming was found to start much earlier than past. This could be due to global climate change. This change could be a reason for serious damages in wooden cultural properties (WCPs) by termites. According to a survey done by the National Research Institute of Cultural Heritage, 70% of WCPs were infested by termites. Among the WCPs, some of the buildings were built in the year 1500 and are very important as national treasures. Despite such importance, protection against termites had been neglected., could be because the damages were not severe in the past. However, at present, it is not difficult to find termites damages in the important structures such as pillars which could cause instability to the structures. In Korea, liquid termiticides, fumigations, and baiting are currently used to control the subterranean termites. In this research paper, we would like to share current termite problems and discuss IPM methodologies in certain area-wide projects in Korea

Keywords: Subterranean termites, area-wide project, wooden cultural properties

Features of Semiautomatic and Fully Automatic Systems for Monitoring Termite Activity using Electromagnetic Induction with Non-Looping Method

bv

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Abstract

Monitoring termite activity plays an important role in termite management. Here we described wireless systems for monitoring of termite activities based on DEKAN electromagnetic induction with non-looping (short as DEMINL) method. We have developed semiautomatic and fully automatic systems. Both of them hold independent intellectual property rights. Semiautomatic termites intelligent monitoring system consists of a termite monitoring station and a handheld receiver, while a fully automatic system consists of a termite monitoring station, an information collection station and a PC-end management software. The signals in semiautomatic system are sent from the termite monitoring station to signal receiver by radio frequency identification (RFID). Lora technology was used for signal transmission between termite monitoring station and information collection station at fully automatic system. The device uses special anti-aging materials to delay degradation especially in humid and acidic environments. System uses non-contact and non-wire structure and the electronic components are completely encapsulated in water resistance environment. Field tests showed that the accuracy rate in detecting termites was more than 97%. The device based on DEMINL has shown its accuracy and durable characteristics in detecting of termites.

Keywords: termites, automatic, DEMINL, non-looping, electromagnetic

Phylogeny of the Subfamily Rhinotermitinae (Blattodea: Rhinotermitidae) Based on Mitochondrial COII and 16SrRNA Sequences

by

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Abstract

It is verified that Rhinotermitidae is not monophyletic according to the studies on the phylogenetic relationships among higher taxa of Blattodea. Within Rhinotermitidae, however, the relationships among some subfamilies and genera remain confusing. Taxonomic status of the subfamily Rhinotermitinae may be questionable due to particular morphological characters of soldiers. Species in 4 subfamilies of Rhinotermitidae were chosen, the sequence composition and genetic distance within the family and between the two families Rhinotermitidae and Termitidae were analyzed; molecular phylogenetic trees were reconstructed by maximum likelihood (ML) and Bayesian inference (BI) methods based on DNA sequences of the partial mitochondrial COII and 16SrRNA genes. The results indicated that the genetic distances of both genes between Rhinotermitinae and the other 3 subfamilies were larger than those between the 3 subfamilies and the subfamilies of Termitidae. In the phylogenetic trees reconstructed based on 16SrRNA gene, 4 genera of Rhinotermitinae composed a monophyletic clade which was the sister group of the clade including Termitidae and the other 3 subfamilies of Rhinotermitidae. The ML tree and BI tree based on COII gene were different in the relationships between subfamilies of Rhinotermitidae. The ML tree showed that Rhinotermitinae composed a monophyletic clade with Prorhinotermitinae which was the sister group to the clade containing Coptotermitinae, Heterotermitinae and Termitidae, while Rhinotermitinae clustered into one clade with other 3 subfamilies in the BI tree. The combined trees based on the tandem COII and 16 SrRNA genes showed the same phylogenetic relationships as in the ML tree of COII gene. In consideration of the primitiveness of the morphological characters existing in the soldiers of Rhinotermitinae, it was supposed that the relationship of Rhinotermitinae with other subfamilies of Rhinotermitidae was not as closer as that indicated in the current taxonomy system. It was suggested that the subfamily Rhinoterminae should be separated and be upgraded to the family rank.

Keywords: Rhinotermitinae, COII, 16SrRNA, phylogeny

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Molecular Mechanism of Soldier Differentiation in the Formosan Subterranean Termite

by

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Abstract

Termites have a distinct polyphenism controlled by concise hormonal and molecular mechanisms. Workers undergo double molts to transform into soldiers (worker-presoldier-soldier). Juvenile hormone analog, such as methoprene, can induce workers to transform into presoldiers. However, the molecular mechanism underlying the worker-to-presoldier transformation in *Coptotermes formosanus* Shiraki is still not clear. We sequenced the transcriptome of workers four days after they had fed on methoprene-treated filter paper and control group workers which fed on acetone-treated filter paper. The transcriptome of *C. formosanus* was assembled using the *de novo* assembly method. Expression levels of unigenes in the methoprene-treated group and the control group were compared. The differentially-expressed genes were further analyzed by Gene Ontology term enrichment analysis and Kyoto Encyclopedia of Genes and Genomes pathway enrichment analysis. Tetrapyrrole binding, oxidoreductase activity, and metal ion binding are the only three enriched GO terms. Juvenile hormone synthesis was the first ranked enriched pathway. Carbohydrate, amino acid, and lipid metabolism pathways were also enriched. These three pathways may be related to fat body development which is critical for presoldier formation. Our results have indicated the significance of JH synthesis pathways, and pathways related to fat body development in the artificial induction of presoliders.

Keywords: caste regulation, juvenile hormone, methoprene bioassay

Hybridization Between the Two Sympatric Termite *Reticulitermes*Species Under Laboratory Condition

by

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Abstract

Closely related species have similar reproductive behaviors and recognition systems, which contributes to interspecific interactions and introgression. Few studies, however, have explored the relationship between the preference for species in reproduction choice and interspecific mating in termites. Here, we combined field observations with laboratory experiments to investigate the impact of the preference for species in reproduction choice between the two termites *Reticulitermes flaviceps* and *R. chinensis* on their reproductive strategy. We found that there were no any significant differences in the frequencies of tandem and mating behaviors between intraspecific and interspecific partners, but the allogrooming frequencies of interspecific partners were significantly higher than intraspecific partners. There were no significant differences in the duration of tandem allogrooming or mating behavior at each time between conspecific partners and heterospecific partners. Moreover, genetic analysis showed that both intraspecific and interspecific mating was able to produce offspring. Our results indicated that the two termites belonging to *Reticulitermes* species did not display marked intraspecific preference in tandem and mating behaviors, which facilitates the interspecific hybridization and would likely increase the genetic diversity.

Keywords: Termites, Hybridization, Reproductive behavior, Introgression, Genetic diversity

Proteomic Quantification of Lysine Acetylation in the Termite Reticulitermes chinensis

by

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Abstract

Protein lysine acetylation is a reversible posttranslational modification and plays a pivotal role in a broad array of physiological functions. In our study, a strategy combining immunoaffinity enrichment of acetylated peptides based on anti-acetyl-lysine antibody with high-resolution tandem mass spectrometry was employed for a systemic survey of acetylation sites in *Reticulitermes chinensis*. 901 acetylated proteins containing 1589 lysine acetylation sites were identified in *Reticulitermes chinensis*. 62 proteins were up-regulated and 18 proteins were down-regulated, After Normalizing by proteome proteins, 6 lysine acetylation proteins were up-regulated and 43 lysine acetylation proteins were down-regulated when compared to the control sample. Functional enrichment analysis showed that proteins up-regulated were implicated in the regulation of diverse KEGG pathways, including ribose phosphate metabolic process, amino sugar metabolism and oxidative phosphorylation. In particular, a large fraction of metabolic enzymes, including multiple rate-limiting enzymes, were also found to be acetylated. Overall, our study provides a comprehensive view of acetylation sites, facilitating an in-depth investigation of functional roles of acetylation in *Reticulitermes chinensis* in the future.

Keywords: lysine acetylation, *Reticulitermes chinensis*, protein

GC-MS Analysis of Metabolites Produced by *Coptotermes formosanus*Bred on Bagasse

by

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Abstract

Bagasse is the main waste of the sugar industry, with a large annual output. However, due to the high content of cellulose and hemicellulose in bagasse, the degree of lignification is high, resulting in low conversion and utilization. Therefore, the comprehensive utilization of bagasse has become one of the research hotspots. Termites are important decomposers on earth as they efficiently produce energy from lignocellulose, which may be used to treat bagasse. In this study, gas chromatography time-of-flight mass spectrometry (GC-TOFMS) was applied to detect the metabolic effects of termites feeding on bagasse. Samples collected from termites fed for 10 days on bagasse diet were analyzed and compared with the pine wood diet. Principal component analysis (PCA) of metabolite profiles demonstrated a separation of different clusters corresponding to different diets. A total of 55 differentially expressed metabolites were found in the bagasse diet, including 34 up-regulated metabolites, e.g. sorbitol, and 21 down-regulated metabolites, e.g. maltose. Searching against the KEGG database, 13 pathways were identified, suggesting that metabolites were abundant in amino acid and sugar metabolism. These results demonstrate the potential to investigate bagasse conversion and utilization with termite's own cellulase system. Further studies on the mechanism of the termite digestion are proposed.

Keywords: termite, bagasse, GC-TOFMS, metabolites

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Differential Response of Cellulose and Hemicellulose Hydrolysis System to Laccase Inhibition of *Coptotermes formosanus*

by

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Abstract

The dual lignocellulolytic system, along with hindgut symbionts are important for the survival of lower termites. Laccase (LAC) belongs to phenol oxidase, which is one of the important endogenous oxidases in termites which decompose lignin. For providing theoretical basis for termite control, in this study, the effects of laccase activity on cellulases and hemi-cellulase activity and corresponding gene expressions from endogenous tissue and protozoan, as well as the variation characteristics of abundance and structure of symbiotic flagellates and bacterial communities in the hindgut were analyzed.

The results showed that: (1) kojic acid could significantly inhibit the phenoloxidase activity of Coptotermes formosanus under the condition of normal feeding. Although the activity of endogenous and microbial cellulases and hemi-cellulase all decreased at varied degrees, hydrolase activity from hindgut were inhabited much more significantly. The order of influence of phenol oxidase activity on different hydrolases was β - 1,4-endoxylanase (EX) > cellobiohydrolase (CBH) > β -glucosidases $(BG) > \beta$ - 1,4-endoglucanases (EG). The salivary gland *lac* gene expression was significantly inhibited with the phenoloxidase activity, while cellulase and hemi-cellulase genes mostly showed significant up regulation. The up-regulation of these hydrolase gene expressions is in direct proportion to the corresponding inhibition of enzyme activity: Pex (protozoan) > Peg (protozoan) > bg (salivary gland) > eg (salivary gland). (2) In accordance with the variation trend of cellulase activities in the hindgut, the number of Pseudonympha grassi, Holomastigotoides hartmanni and their symbiotic bacteria Bacteroides and Spirochete (dominant phyum) all decreased but without significant (p > 0.05). Inversely, the number of Spirotrichonympha leidyi increased significantly. Meanwhile, kojic acid promoted the abundance of a large number of rare bacteria in phyla Firmicutes, Proteobacteria and Actinobacteria. We suppose that these phyla are responsible for the detoxify metabolism of termites. Therefore, this study suggests a high correlation between the endogenous laccase and the hemicellulase in the hindgut. The degradation of lignin, cellulose and hemicellulose may have different priority levels in optimal hydrolysis regulation order. And the non-flagellate symbiotic bacteria are likely important in survival adaptation mechanism of termites.

Keywords: kojic, laccase, lignocellulase, bacterial community, termite

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