

(Cover)

**The 15<sup>th</sup> Conference of**



**PRTRG 15**

**Philippines**

**March 12 – 13, 2024**

**Program Book and Proceedings**

The 15<sup>th</sup> Conference of  
PRTRG | Pacific Rim Termite Research Group  
March 12 – 13, 2024  
Philippines International Convention Center (PICC)  
Manila, Philippines

Organizer:



Supported by



ZM Crop Protection Corporation



G Chemtrading Corporation



Groupe Berkem, France



EnsysTex



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LEADS Environmental Health Products Corp.

# Foreword of the President



Dear participants,

I am very pleased to welcome you all to the 15<sup>th</sup> International Conference of Pacific Rim Termite Research Group (PRTRG) in Manila, Philippines, on March 12 – 13, 2024.

The PRTRG Conference is a well-established biannual event at the PRTRG, devoted to gathering eminent speakers and scholars engaged in termite research, young researchers and students, as well as professionals from pest control industries. With PRTRG Conference, we continue providing the participants with a forum for the transfer of knowledge and future research collaboration through scientific and technical presentations.

The 15<sup>th</sup> PRTRG Conference Philippines is a historic and important event in PRTRG history. It is our first conference held in Philippines and it marks our return

to biannual cycle of the PRTRG conference since we overcome the challenges of Covid-19 global outbreak. With the help and participation of our society members, friends, and colleagues, we manage to hold it in March 2024, a year after the postponed PRTRG 14 Conference in Bangkok, Thailand in March 2023.

We also consistently support young scientists/students to take participation in the PRTRG conference by providing 6 travel fund awards for the current event. We hope by giving them chance to join our conference, they will challenge themselves to learn and gain invaluable scientific experience.

Last but not least, I would like to thank the local organizers, Dr. Partho Dhang (Chairman of the Organizing Committee) & his team, for their dedicated work to preparing the conference; to Dr. S Khoirul Himmi (Secretary General of PRTRG) for preparing the program and proceedings; to all partners for their enormous support and participation.

I wish you all a successful and fruitful conference.

*Su-Chart LEE*

**Su-Chart LEE**  
**President of PRTRG 2020 – 2024**

# PRTRG Committee

## Pro-Team Executive Committee

President	:	Mr. Su-Chart Leelayouthytin (King Service Center, Thailand)
Secretary General	:	Dr. S Khoirul Himmi (National Research and Innovation Agency/BRIN, Indonesia)
Honorary Treasurer	:	Mr. Steph Chua Yang Peng (Bentz Jaz, Singapore)
Executive Committee Members	:	Prof. Dr. Chow-Yang Lee (University of California, Riverside, USA) Prof. Dr. Sulaeman Yusuf (National Research and Innovation Agency/BRIN, Indonesia) Prof. Dr. Dayu Zhang (Zhejiang A&F University, China) Prof. Dr. Hou-Feng Li (National Chung Hsing University, Taiwan) Dr. Eric Cheng Shing Kwong (Ridgid Plumbing Limited, Hong Kong) Dr. Veera Singham (Universiti Sains Malaysia, Malaysia) Dr. Partho Dhang (Urban Entomologist, Philippines)
Honorary Auditor	:	Dr. Chun-I Chiu (Chiang Mai University, Thailand) Dr. Rungarun Tisgratog (Kasetsart University, Thailand)

## Proceedings Editor

Dr. S Khoirul Himmi (National Research and Innovation Agency/BRIN, Indonesia)  
Dr. Partho Dhang (Independent Consultant of Urban Pest Management, Philippines)

## The Organizing Committee of the 15<sup>th</sup> PRTRG Conference

Dr. Partho Dhang (Chairman)  
Jess M. Asistin

# Office

## PRTRG Main Office

48 Toh Guan Road East, #06-139 Enterprise Hub Singapore 608586

Website: [www.prtrg.org](http://www.prtrg.org) | Email: [enquiry@prtrg.org](mailto:enquiry@prtrg.org)

## Country Representative

- China : Prof. Dr. Dayu Zhang (Zhejiang A&F University)
- Hong Kong : Dr. Cheng Shing Kwong (Ridgid Plumbing Limited)
- Indonesia : Prof. Dr. Sulaeman Yusuf (National Research and Innovation Agency/BRIN)
- Malaysia : Dr. Veera Singham (Universiti Sains Malaysia)
- Philippines : Dr. Partho Dhang (Urban Entomologist)
- Singapore : Mr. Steph Chua Yang Peng (Bentz Jaz Pte. Ltd.)
- Taiwan : Prof. Dr. Hou-Feng Li (National Chung Hsing University)
- Thailand : Mr. Khwanchai Charoenkrung (Royal Forest Department)
- Vietnam : Ms. Tô Thị Mai Duyên (Sumitomo Chemical Asia)

## The 15<sup>th</sup> Conference of



Philippines is an archipelagic country in Southeast Asia, known for its diverse culture and traditions. More than 7,000 islands make up the Philippines, but the bulk of its fast-growing population lives on just 11 of them. The country has a total area of approximately 300,000 square kilometers and a population of more than 100 million people.

Manila is the capital and second-most populous city of the Philippines. Located on the eastern shore of Manila Bay on the island of Luzon, it is classified as a highly urbanized city. *Maynilà*, the Filipino name for the city, comes from the phrase *may-nilà*, meaning "where indigo is found". *Nilà* is derived from the Sanskrit word *nīla* (नील) or "nilad" plant (*Scyphiphora hydrophylacea*), which refers to indigo dye and, by extension, to several plant species from which this natural dye can be extracted.

The name "Manila" is commonly used to refer to the whole metropolitan area, the greater metropolitan area, and the city proper. Metro Manila, the officially defined metropolitan area, is the capital region of the Philippines, and includes the much-larger Quezon City and the Makati Central Business District. It is the most-populous region in the country, one of the most-populous urban areas in the world, and one of the wealthiest regions in Southeast Asia. The people are known for their hospitality and friendliness, making it a great place to visit and experience the local culture. Whether you're exploring the bustling streets of Manila or relaxing on a tropical beach, Philippines offers a truly unforgettable experience.

**Please ask the art team to put iconic landmark/icon of Manila / Philippines  
on this page**

# Venue & Transportation

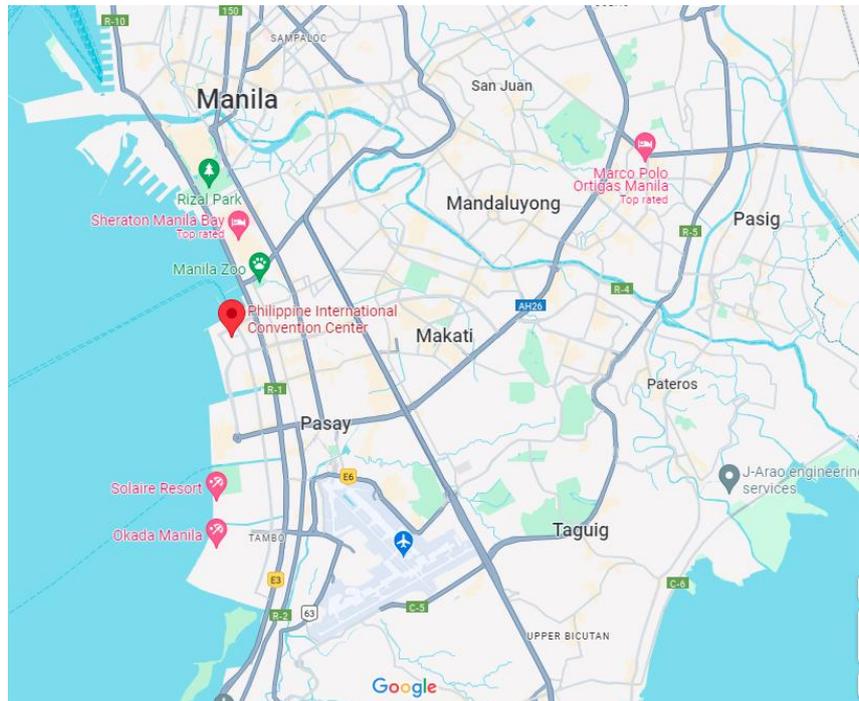
## Philippine International Convention Center (PICC)

xxx-xxxx-xxxx (Please put info of room number/ballroom, and floor)

Address: PICC Complex, 1307 Pasay City, Metro Manila, Philippines.

Email: [info@picc.gov.ph](mailto:info@picc.gov.ph)

Trunkline: (+63) 8 789 4789



Going to the PICC quite easy even though it is not accessible to the usual utility jeep. Simply follow the guidance from this web to reach the venue: <https://mattscradle.com/get-to-picc-pasay/>

# Program at a Glance

## Tuesday | 12 March 2024

- 08:00 – 08:45 **Registration**
- 08:45 – 09:00 **Opening Ceremony**  
Welcome Speech – President of PRTRG, Mr. Su-Chart Lee
- 09:00 – 09:45 **Keynote Lecture**  
Hou-Feng Li, Ph.D – National Chung Hsing University, Taiwan
- 09:45 – 10:00 **Photography Session**
- 10:00 – 10:15 **Tea Break**
- 10:15 – 11:45 **Kunio Tsunoda Memorial Lecture**  
Dr. Chow-Yang Lee – University of California, Riverside, USA  
Edward L. Vargo, Ph.D – Texas A&M University, USA  
Dr. Menandro Acda – University of the Philippines Los Banos, Philippines
- 11:45 – 12:00 **The PRTRG Travel Fund Award Ceremony**
- 12:00 – 13:00 **Lunch Break**
- 13:00 – 14:00 **Special Talk “Current Termite Research and Global Challenges”**  
Vernard Lewis, Ph.D – University of California, Berkeley, USA  
Don Ewart, Ph.D – Pest Management Consultant, Australia
- 14:00 – 14:45 **Poster Presentation – Tea Break**
- 14:45 – 16:30 **Session for Oral Presentation**
- 16:30 – 17:30 **Biennial General Meeting (PRTRG Member Only)**
- 17:30 – 17:45 **Closing of Day 1**
- 18:00 – 20:00 **Welcome Dinner**  
Philippines International Convention Center (PICC)

## Wednesday | 13 March 2024

- 08:00 – 09:00 **Registration**
- 09:00 – 10:45 **Session for Oral Presentation**
- 10:45 – 11:00 **Tea Break**
- 11:00 – 12:00 **Special Forum: Innovation in Termite Management**  
Sasaki Motoyuki – ZMCP Corporation  
Daouia Messaoudi – Groupe Berkem  
Steve Broadbent – Ensystem  
Ann Margaret L. Castilan – Leads Environmental Health Products Corporation
- 12:00 – 12:15 **The next PRTRG 16 Conference Announcement**
- 12:15 – 13:30 **Lunch and Preparation for the Excursion**
- 13:30 – 18:00 **Excursion Program**  
Visiting the walled city of Intramuros
- 18:00 – 21:00 **Gala Dinner and Closing Ceremony**  
Century Park Hotel, Manila

# Excursion

Participants will be treated to a short excursion trip to the walled city of Intramuros. Intramuros, or the *'Walled City'*, is one of the oldest districts of Manila, built on the south bank of the Pasig River around 1571. It was built by the Spaniards. It is bound on all sides by moats and thick, high walls, with some over 6 meters high.

While the district was originally home to an Indianized-Malayan-Islamic settlement, it became the center of religious, political, and military power in the 16th century, when the country was under the control of Spain. Only the elite Spaniards and Mestizos were allowed accommodation in Intramuros. That's obviously not the case now, but the historic buildings and features remain as testament to the Philippines' challenging past.

Manila Intramuros is one of the highlights of 10 Best Things to Do in Manila.

## Excursion Program

13:30 – 14:00 **Bus pick-up and preparation for the excursion**

14:00 – 14:30 **Heading to Manila INTRAMUROS**

14:30 – 16:00 **Guided tour to the Old City**

16:00 – 17:30 **Visit to the beach arena for shopping and sunset viewing**

17:30 – 18:00 **Bus pick-up and heading to the Century Park Hotel for Gala Dinner**



Walk past the iconic  
Manila Cathedral



Explore the old city  
of Intramuros



Have a look at the  
City skyline



Enjoy a sunset by the  
Manila bay



# Speakers

## Keynote Lecture

Tuesday | March 12, 2024 | Time: 09:00 – 09:45

### **Data goldmine: Revolutionizing termite research through collective insight of citizens and industries**



**Hou-Feng Li, Ph.D**

**National Chung Hsing University, Taiwan**

Hou-Feng Li is a distinguished professor at the Department of Entomology, National Chung Hsing University, Taiwan. He received his Ph.D. and M.S. degree from the University of Florida, USA and his B.A. degree from the National Taiwan University, Taiwan. His research focuses on termite biology and management. He has published over 80 peer-reviewed papers and book chapters and given over 160 presentations. Hou-Feng received many awards at national and international levels, including the Ta-You Wu Memorial Award (2019) from the Ministry of Science and Technology, Taiwan, and William L. and Ruth D. Nutting Award for outstanding achievements in basic termite biology (2008) from the International Union for the Study of Social Insects. He served as the President (2018-2020) and Secretary-General (2016-2018) of the Taiwan Entomological Society, and the Secretary-General (2016-2020) of the Pacific Rim Termite Research Group.

## Kunio Tsunoda Memorial Lecture

Tuesday | March 12, 2024 | Time: 10:15 – 10:45

### Management of subterranean termites using liquid termiticides



**Dr. Chow-Yang Lee**

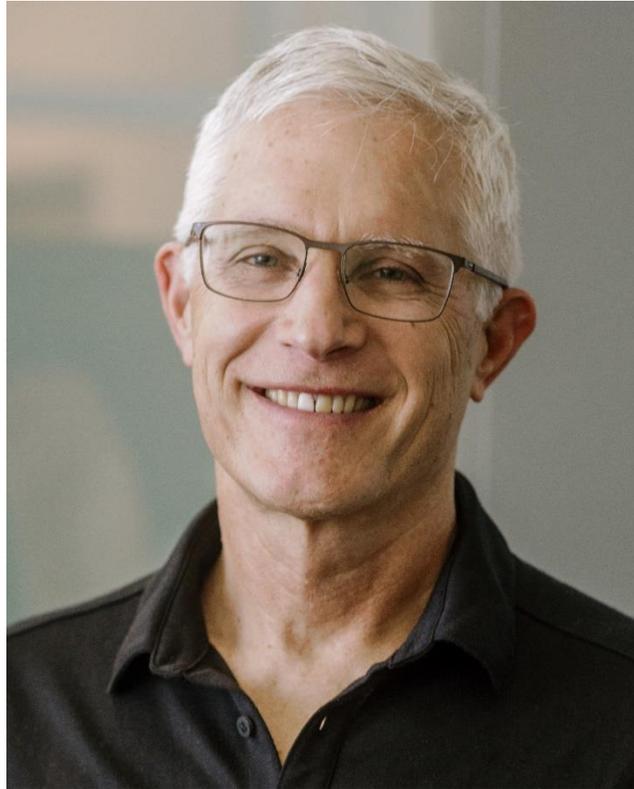
**University of California, Riverside, USA**

Chow-Yang Lee is the Professor and Endowed Presidential Chair in Urban Entomology at the University of California, Riverside, USA. His research involves understanding behavioral, ecological, and physiological adaptations of insect pests in the urban environment. He has published over 280 peer-reviewed papers and books and mentored 17 Ph.D. and 32 M.S students. Chow-Yang received many awards at international and national levels, including the *Recognition Award in Medical, Urban and Veterinary Entomology (2022)* from the Entomological Society of America, *Arnold Mallis Distinguished Achievement Award in Urban Entomology (2022)* from the National Conference on Urban Entomology (USA). He co-edited a new book *Biology and Management of the Formosan Subterranean Termite and Related Species* with Nan-Yao Su, and was recently published.

## **Kunio Tsunoda Memorial Lecture**

Tuesday | March 12, 2024 | Time: 10:45 – 11:15

**Termite invaders: Where do they come from and how do they spread?**



**Edward L. Vargo, Ph.D.**

**Texas A&M University, USA**

Ed Vargo holds the Endowed Chair in Urban and Structural Entomology at Texas A&M. He received his Ph.D. in Entomology from the University of Georgia. Before joining Texas A&M, Dr. Vargo was Professor at North Carolina State University. He is well known in the field of urban entomology for his contributions to the biology and management of termites, ants, and bed bugs. Dr. Vargo has published more than 170 scientific articles on urban pests and has given more than 200 talks at professional meetings and scientific conferences.

## **Kunio Tsunoda Memorial Lecture**

Tuesday | March 12, 2024 | Time: 11:15 – 11:45

### **Performance of soil termiticides in open field and under roof overhang**



**Dr. Menandro N. Acda**

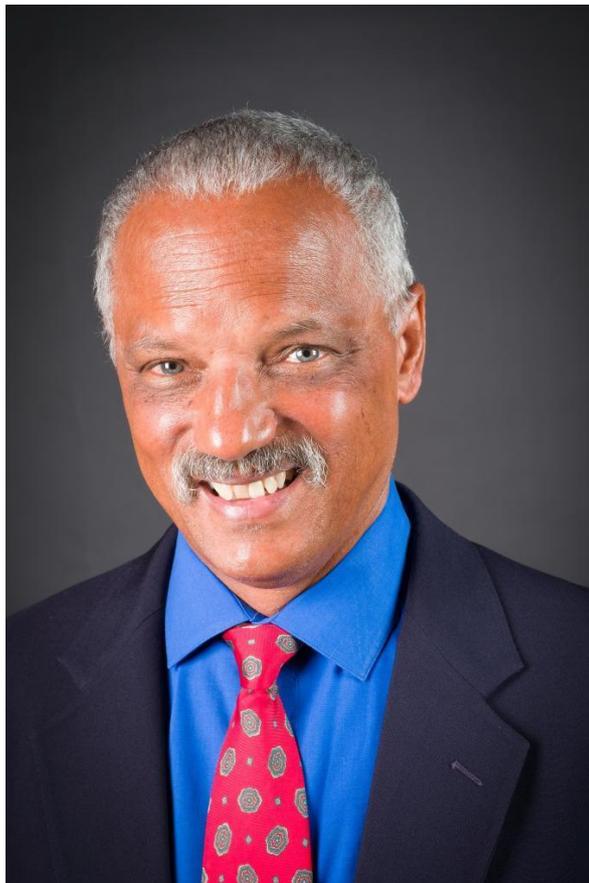
**University of the Philippines Los Banos, Philippines**

Dr. Menandro N. Acda is Professor of Wood Science and Technology at the Department of Forest Products and Paper Science, University of the Philippines Los Banos. He completed his Bachelor of Science and Master of Science degrees from the University of the Philippines Los Banos both in the area of Wood Science and Technology. He obtained his Ph.D. and Post-Doctoral training in Forest Products from Oregon State University, USA in Wood Preservation and Biodeterioration. Dr. Acda's research interests include wood materials science and tropical termite biology and control. Results of his research projects are published in international journals and proceedings of conferences from which he received various awards and recognitions. Dr. Acda is an elected Fellow of the International Academy of Wood Science (IAWS) and the Philippine-American Academy of Science and Engineering (PAASE). Recently, he was included in the Stanford's 2023 World's Top 2% Scientists across all disciplines. In addition to his research and extension works, Dr. Acda is also actively involved in teaching classes in wood science and technology and academic adviser to both graduate and undergraduate students.

**Special Talk:**  
**Current Termite Research and Global Challenges**

Tuesday | March 12, 2024 | Time: 13:00 – 13:30

**Drywood termites: an increasing global concern**



**Vernard Lewis, Ph.D**

**University of California, Berkeley, USA**

Vernard Lewis is Emeritus Professor Coop. Extension at Rasser College of Natural Resource, University of California Berkeley, USA. He received his B.S., M.S., and Ph.D. degrees in Entomology at the University of California Berkeley (UCB). He joined the faculty in 1991 and specialized in Urban Entomology and retired from UCB in 2017. During his career, he authored or co-authored more than 160 publications and gave hundreds of lectures and presentations. He also have consulted with the United Nations and had two structural pest control licenses for the State of California. His research involved developing methods of detection and nonchemical control for termites and other structural pests. He was inducted into the National Pest Management Hall of Fame in 2016. In retirement, his focus has been mentorship of students and membership on advisory boards for the UCB Botanical Garden and Rasser College of Natural Resources. He also enjoy facilitating partnerships between the University and community groups and to increase underrepresented groups participation in STEM careers and activities.

**Special Talk:**  
**Current Termite Research and Global Challenges**

Tuesday | March 12, 2024 | Time: 13:00 – 14:00

**Rigorous testing with Australian *Coptotermes*:**  
**Collection and test methods to provide maximum challenge**



**Don Ewart, Ph.D**

**Consulting Entomologist**  
**The Institute of Pest Risk Management, Australia**

Dr. Don has been working with, and talking about, termites and timber pests for over forty years. He began with a baiting study of the ecology of semi-arid subterranean termites before moving to *Coptotermes* in a mountain eucalypt forest. A post-doc at the University of Hawaii drove his interest in the economic aspects of termites. Following work has centered on least toxic termite management with barriers and control with baits.

Don studied and taught at La Trobe University, taught forest entomology for the University of Melbourne, and general entomology for Monash University before becoming School Chairperson & Head, Human Development Centre, University of Melbourne School of Early Childhood Studies, where he taught widely in science and administrative computing. A return to termites came with a particle barrier project at CSIRO Forest Products, and 15 years with Granitgard Pty Ltd, which resulted in a mature non-toxic management system and 4 patents. Don's work includes development of Australian termite standards and codes, contract research, expert witness assessments, consulting and, training pest managers for The Institute of Pest Risk Management & others. Don can be found through [drdons.net](http://drdons.net) and [tiprm.com](http://tiprm.com)



# Program Agenda

# Program Agenda

Tuesday | March 12, 2024

08:00-08:45

Registration

08:45-09:00

Opening Ceremony

Welcome Speech – President of PRTRG, Mr. Su-Chart Lee

## Keynote Lecture

Moderator: Jim Creffield

09:00-09:45

**K\_01 Data goldmine: Revolutionizing termite research through collective insight of citizens and industries**

Hou-Feng Li

09:45-10:00

Photography Session

10:00-10:15

*Tea Break*

## Kunio Tsunoda Memorial Lecture

Moderator: S Khoirul Himmi

10:15-10:45

**K\_02 Management of subterranean termites using liquid termiticides**

Chow-Yang Lee

10:45-11:15

**K\_03 Termite invaders: Where do they come from and how do they spread?**

Edward L. Vargo

11:15-11:45

**K\_04 Performance of soil termiticides in open field and under roof overhang**

Menandro N. Acda

11:45-12:00

The PRTRG Travel Fund Award Ceremony

12:00-13:00

*Lunch*

## Special Talk:

Moderator: Eric Cheng

### Current Termite Research and Global Challenges

13:00-13:30

**K\_05 Drywood termites: An increasing global concern**

Vernard Lewis

13:30-14:00

**K\_06 Rigorous testing with Australian *Coptotermes*: Collection and test methods to provide maximum challenge**

Don Ewart

14:00-14:45

Poster Presentation – *Tea Break*

## Oral Presentation

### Section 1: Biodiversity, Ecology, and Behavior

Moderator: Bramantyo Wikantyooso

14:45-15:00

**O\_01 Task list for the revision of *Coptotermes* in asia**

Chia-Chien Wu, Hou-Feng Li

- 15:00-15:15 **O\_02 Species composition and infestation status of termites in ancient Shan tea trees in northern Vietnam**  
 Nguyen Thi My, Nguyen Quoc Huy, Nguyen Minh Duc, Nguyen Thuy Hien, Nguyen Manh Cuong, Dang Ngoc Bich, Ha Tra My, Nguyen Thi Tuyen
- 15:15-15:30 **O\_03 Commensal bacteria enhance organic acids release from the dead termites and elicit corpse-burying behavior of *Coptotermes formosanus***  
 Bosheng Chen, Weisong Zhao, Chenxin Wu, Lulu Wang, Dayu Zhang
- 15:30-15:45 **O\_04 Advance and future prospects in phototaxis of termite: What can we learn from decades of research?**  
 Qi Long, Qiu-Ying Huang
- 15:45-16:00 **O\_05 The influence of desaturase on fatty acid synthesis and trophallaxis in *Coptotermes formosanus***  
 Lulu Wang, Danni Xu, Yuxin Tong, Weisong Zhao, Bosheng Chen, Dayu Zhang

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## Oral Presentation

### Section 2: Physiology, Morphology, and Structure

Moderator: Dayu Zhang

- 16:00-16:15 **O\_06 Analysing the changes in chemical profiles of ageing *Cryptotermes brevis* frass for use in termite management: Preliminary analysis**  
 William Haigh, R Andrew Hayes
- 16:15-16:30 **O\_07 Gross analysis of the hair-type sensory receptors distribution on the antennae of an open-column termite *Hospitalitermes sp.* (Nasutitermitinae, Blattodea)**  
 Bramantyo Wikantyo, S. Khoirul Himmi, Didi Tarmadi, Sulaeman Yusuf

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16:30-17:30 Biennial General Meeting (Member Only)

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17:30-17:45 Closing of Day 1

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18:00-20:00 *Welcome Dinner* – Philippines International Convention Center (PICC)

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# Program Agenda

Wednesday | March 13, 2024

08:00-09:00 Registration

## Oral Presentation

### Section 2: Physiology, Morphology, and Structure

Moderator: Sulaeman Yusuf

09:00-09:15 **O\_08** Diets rich in nitrogen enhance termite reproductive performance on both primary and secondary reproductive castes

Wan-Jen Li, Takanori Tomita, Mu-Xuan Chen, Hou-Feng Li

09:15-09:30 **O\_09** Comparative analysis of softwood and hardwood lignocellulose degradation by drywood termites, *Cryptotermes dudleyi*

Ni Putu Ratna Ayu Krishanti, Didi Tarmadi, S. Khoirul Himmi, Yuki tobimatsu

## Oral Presentation

### Section 3: Economic Impact and Innovative Management

Moderator: Chun-I Chu

09:30-09:45 **O\_10** Advantage and challenge of employing micro-CT technology for termite study

Hauchuan Liao and Hou-Feng Li

09:45-10:00 **O\_11** Actual state of termites (Isoptera) that damage some dam works with big capacity in Vietnam

Nguyen Minh Duc, Le Quang Thinh, Nguyen Manh Cuong

10:00-10:15 **O\_12** Efficacy performance of bio-based termiticide containing plant polyphenolic extracts from Berkem Biosolutions® against subterranean termites: Laboratory and field evaluation

Daouia Messaoudi, S. Khoirul Himmi, Didi Tarmadi, Ikhsan Guswenrivo, Sulaeman Yusuf

10:15-10:30 **O\_13** Evaluation of different sea sand particle's mixtures as an effective physical control barrier against *Heterotermes indicola* (Blattodea: Rhinotermitidae) in the laboratory conditions

Maid Zaman, Imtiaz Ali Khan, Abid Farid, Misbahullah, Muhammad Izhar Shafi

10:30-10:45 **O\_14** Termite management by entomopathogenic fungi: Recent advances and future prospects

Ali Hassan, Zhiqiang Li, Xuguo Zhou, Jianchu Mo, Qiuying Huang

10:45-11:00 Tea Break

## Special Forum

### Innovation in Termite Management

Moderator: Partho Dhang

11:00-11:15 **F\_01** Introducing a novel termiticide Metamisalt® 20SC (Dicloromezotiaz 20 SC)

Sasaki Motoyuki

- 11:15-11:30 **F\_02 Laboratory and field evaluations of selected plant polyphenolic extracts from Berkem Biosolutions® as bait toxicants for termite control in tropical zones**  
Florent Chopinet, Stéphane Savriama, Daouia Messaoudi
- 11:30-11:45 **F\_03 Hydrotrail – A termite guiding strategy creating a termite defence zone**  
Steve Broadbent
- 11:45-12:00 **F\_04 Navigating termite management innovations in the Philippines**  
Ann Margaret L. Castilan

12:00-12:15	The next PRTRG 16 Conference Announcement
12:15-13:30	<i>Lunch</i> and Preparation for the Excursion
13:30-18:00	Excursion Program Visiting the walled city of Intramuros
18:00-21:00	<i>Gala Dinner and Closing Ceremony</i> Century Park Hotel, Manila

## List of Poster Presentations

14:00 – 14:45 | Tuesday | March 12, 2024

- 
- P\_01 **Mud-tube repairing behavior and capacity in *Macrotermes gilvus* (Termitidae, Blattodea)**  
Bramantyo Wikantyo, S. Khoirul Himmi, Didi Tarmadi Ikhsan Guswenrivo, Sulaeman Yusuf
- 
- P\_02 **Are borate solutions effective in wood protection against drywood termites?**  
Andrew H.H Wong, Carlson A.D. Tawi
- 
- P\_03 **Wood protection against termites with selected new generation permethrin-azole based preservatives for The Philippines**  
Malcolm Farmer, Andrew H.H. Wong
- 
- P\_04 **High termite resistance of kempas (*Koompassia malaccensis*) hardwood protected with a novel vegetal extracts-cypermethrin wood preservative under outdoor aboveground tropical environment**  
Daouia Messaoudi, Andrew H.H. Wong
- 
- P\_05 **Twisting tale of termite's elastic mandible form and function**  
Tzu-Chia Liu, Kuan-Chih Kuan, Chun-I Chiu, Hou-Feng Li, Kai-Jung Chi
- 
- P\_06 **Applications of Computational Systems for Monitoring Termite Activity in China**  
Junfeng Shen, Zheng Fang, Hongyue Li, Junhong Zhong, Lingli Wu, Bosheng Chen, Dayu Zhang
- 
- P\_07 **Termiticidal activity of *Lantana camara* and *Dioscorea hipsida* extracts against subterranean termite, *Coptotermes gestroi***  
Didi Tarmadi, Ardiansyah, Canra Aguslan Siregar, Riana Anggraini, S. Khoirul Himmi, Bramantyo Wikantyo, Ni Putu Ratna Ayu Krishanti, Sulaeman Yusuf
- 
- P\_08 **Evaluating the effectiveness of *litsea angulata* leaf extract as an organic wood preservative against subterranean termite, *Coptotermes curvignatus***  
Muhammad Akmal Rizqullah, Harlinda Kuspradini, Erwin, Titik Kartika, Ikhsan Guswenrivo
- 
- P\_09 **Behavioral changes in termites triggered by fungal volatile compounds**  
Titik Kartika, Deni Zulfiana, Anis Sri Lestari, Ni Putu Ratna Ayu Krishanti, Sulaeman Yusuf
- 
- P\_10 **Efficacy determination of an entomopathogenic fungi-based cellulose casings bait in termite control**  
Xiao-Nan Zhang, Qiu-Ying Huang
- 
- P\_11 **The role of the olfactory system in burial behavior triggered by oleic acid in *Coptotermes formosanus***  
Hongyue Li, Jiahan Liu, Qian Wang, Yuanfei Ma, Weisong Zhao, Bosheng Chen, Jennifer Hackney Price, Dayu Zhang
-



# Proceedings



# **Abstract Book**

## **Keynote and Invited Lectures**

Tuesday | March 12, 2024 | 09:00-09:45

**K\_01** Keynote Lecture

**Data goldmine: Revolutionizing termite research through collective insight of citizens and industries**

by

Hou-Feng Li

Department of Entomology, National Chung Hsing University, Taiwan

Email: houfeng@nchu.edu.tw

**Abstract**

Assessing termite pests in urban environments is challenging, particularly in the context of global climatic change and rapid urbanization. Understanding the composition of termite species and their threat to wooden structures is essential for developing effective control tools and management strategies. However, limited access to private buildings hampers research on urban termite pests. In Taiwan, we addressed this challenge through a citizen science project, enlisting the public and pest management professionals to collect over 3,000 termite samples between 2015 and 2020. This initiative revealed the composition of termite pests in each prefecture and their dispersal flight seasons, identifying three potential invasive pests.

In addition to sample collection, we conducted questionnaire interviews with citizens at main train stations, randomly selecting >100 passengers in each prefecture. Insights into customers' attitudes and behaviors regarding termite pests and control were obtained, including information on termite infestations, problem resolution, and background details of accommodations and homeowners. This data allowed us to estimate the nationwide termite infestation rate, identify common damage items, assess popular control tools, and understand the costs of termite management and structure repairs.

The rise of social media, freight services, logistic advancements, and AI-assisted pest identification facilitated the collection of extensive urban pest information. In the context of fast climate change, urban development, and international shipments, timely access to urban termite pest status is crucial. The success of our citizen science project in Taiwan demonstrates a viable approach for studying urban termite pests, suggesting its potential applicability across the Pacific Rim.

**Tuesday | March 12, 2024 | 10:15-10:45**

**K\_02 Kunio Tsunoda Memorial Lecture**

## **Management of subterranean termites using liquid termiticides**

by

Chow-Yang Lee

Department of Entomology, University of California, Riverside, USA

Email: chowyanl@ucr.edu

### **Abstract**

For over 120 years, liquid termiticides have been employed to address subterranean termite issues in the soil. Changes in environmental concerns, new chemical formulations, progress in insect toxicology, and regulatory modifications have significantly influenced the selection and application of termiticides. Over the past seven decades, no less than ten classes of insecticides have served as termiticides. Despite these changes, liquid termiticides continue to be the preferred choice for controlling subterranean termites on a global scale. In this presentation, I will discuss the historical and current perspectives surrounding using liquid termiticides. Specifically, I will explore the various types of termiticides (Type I, II, III, IV), distinctions between repellent and nonrepellent termiticides, factors impacting their effectiveness (including termiticide physical properties and soil characteristics), and a comparison with termite baits.

Tuesday | March 12, 2024 | 10:45-11:15

**K\_03 Kunio Tsunoda Memorial Lecture**

**Termite invaders: Where do they come from and how do they spread?**

by

Edward Vargo

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**Abstract**

There are some 28 species of invasive termites in the world, and many are highly destructive pests of human built structures. The Pacific Rim Region is the source of many species of invasive termites and has also served as the source of many invasive termites in other parts of the world. All invasive termites share the following three traits: they are wood eaters, they nest in wood, and they readily produce neotenic. The vast majority of species come from the families Kalotermitidae and Heterotermitidae. Retracing the invasion history of species requires both historical information and genetic data. I will review some of the studies examining the origin and spread of invasive termites, focusing on species important to the Pacific Rim Region. Consistent with studies of other invasive insects, especially ants, the introduction of termites often occurs secondarily from bridgeheads, that is, from other areas where the species has become invasive. The best studied species with regard to its invasion history is the Formosan subterranean termite *Coptotermes formosanus*, which is native to eastern Asia and is one of the most destructive invasive pests in the world. It has established populations in Japan, Hawaii and the southeastern US. In a recent study using a panel of single nucleotide polymorphisms (SNPs), we retraced the invasion history of this species through approximate Bayesian computation. We found a complex invasion history, with initial introductions to Hawaii which then served as the source for an introduction to the southeastern US. A separate introduction event from southcentral China subsequently occurred in Florida. We also examined the breeding structure of colonies in various native and introduced populations. We found population varied in the proportions of colonies forming simple families (a single queen and king present) and those headed by extended families (multiple inbreeding queens and kings), but no consistent difference between native and invasive populations. Overall, these findings reinforce the pivotal role of bridgeheads in the spread of invasive species and illustrate that the global distribution of *C. formosanus* has been shaped by multiple introductions out of China, which may have prevented and possibly reversed the loss of genetic diversity within its invasive range, and that changes in colony breeding structure cannot account for the invasion success of this species.

**Tuesday | March 12, 2024 | 11:15-11:45**

**K\_04 Kunio Tsunoda Memorial Lecture**

## **Performance of soil termiticides in open field and under roof overhang**

by

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### **Abstract**

The performance of soil termiticides using concrete slab test installed in open field and under roof overhang in the Philippines was investigated over a 5-year study period. Plots treated with cypermethrin and chlorpyrifos in open field showed higher proportion of termite penetration compared to that installed under roof overhang. The high proportion of plots attacked by termites in open field could be attributed to environmental factors such as presence of a wider diversity of termite species, moisture, temperature, soil properties, microbial communities common in tropical climates, etc. The results of these two methods of installation could affect protocol used for field trial and the granting of registration or performance warranty to candidate termiticides in Philippines and other tropical countries.

Tuesday | March 12, 2024 | 13:00-13:30

**K\_05 Special Talk: Current Termite Research and Global Challenges**

## **Drywood termites: An increasing global concern**

by

Vernard Lewis

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### **Abstract**

The family Kalotermitidae contains the third highest species abundance among termites. They also are the second most invasive and economically importance. Their unique physiological ability to conserve water, nest in small pieces of wood, and flexible reproductive capacity contribute to their continued global spread. Academia and industry have proposed and developed novel means of detecting drywood termite infestations, albeit these methods have had mixed results. The drawbacks in detection performance include the challenges of detecting infestations through wall coverings and volume of wood to be inspected. Methods of drywood termite control have had a long history of reporting in the literature that goes back 100 years. Local treatments with chemicals historically have been important and still predominate today. However, the current consumer climate includes wanting alternatives to pesticides that achieve high levels of efficacy, non-intrusive to the environment, and economically affordable. I conclude my talk with a review of a recent paper that proposes an economic model for determining the consequences of control strategy used.

### **Outline:**

- Biodiversity and invasive species
- Biological and physiological survival skills
- Damage potential and detection
- Management
- Economic consequences of management choices

Tuesday | March 12, 2024 | 13:30-14:00

**K\_06** Special Talk: Current Termite Research and Global Challenges

**Rigorous testing with Australian *Coptotermes*:  
Collection and test methods to provide maximum challenge**

by

Don Ewart

Consulting Entomologist  
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**Abstract**

Subterranean termite attack on structures is sometimes unpredictable. A product or system intended to deter termites may fail unexpectedly. Installers, suppliers, manufacturers, regulators, and sometimes consumers, all have an interest in the predicted failure rates. Early in my career, I settled on a maximum call back rate of fewer than one in a thousand jobs with a target of fewer than one in two thousand. To achieve this level of certainty requires quality testing covering all the intended applications. Termites, unlike their cockroach relatives, are not robust creatures. When testing products or systems, the results can be skewed by small injuries. Collection, handling, and presentation all provide the potential for reduced vigour. In the worst case, the termite vigour is reduced so that the challenge is insufficient and a Type II error results, where an unsuitable product is passed as fit for purpose. In this paper we explore the common means by which vitality is lost and the means to avoid such losses.



# Oral Presentation

Tuesday | March 12, 2024 | 14:45-15:00

## Section 1: Biodiversity, Ecology, and Behavior

**O\_01**

### Task list for the revision of *Coptotermes* in Asia

by

Chia-Chien Wu, and Hou-Feng Li\*

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#### Abstract

The genus *Coptotermes* Wasmann, 1896, comprising 59 living species distributed widely across various regions, holds economic significance due to its invasive nature. Despite extensive studies for population management, the taxonomy of *Coptotermes*, particularly in Asia, remains unsettled, with potential synonymies. This article conducts a comprehensive review of all *Coptotermes* species, examining and listing insufficient information regarding uncertain species in Asia. A total of 35 *Coptotermes* species have their type locality in Asia. Twenty of them need the description of winged imago castes, and one species requires the description of soldier castes. Thirty species need molecular data from the type localities. The steps to solve the taxonomy issues in the Asian area are proposed. The collection of specimens from the type localities of the uncertain species is urgently needed. Detailed morphological descriptions and whole mitochondrial genome sequencing are suggested to be conducted after collection. The citizen science project would help speed up the collection of *Coptotermes* species.

**Keywords:** *Coptoterems*, Asian region, citizen science, uncertain species, species list

#### Introduction

The genus *Coptotermes* Wasmann, 1896 (Blattodea: Rhinotermitidae) is widely distributed across the Australian, Ethiopian, Nearctic, Neotropical, Oriental, Palaearctic, and Papuan regions (Krishna et al., 2013). As a pest genus, a total of 18 species of *Coptotermes* are recognized as pests of significant economic importance worldwide (Rust and Su, 2012). *Coptotermes* is a popular research subject due to its invasive ability and economic significance. Despite its wide distribution and the extensive scientific literature associated with population management worldwide, the taxonomy of *Coptotermes* remains unsettled, and many species names may be synonyms of other species, especially in the Asian region.

Termites (Blattodea: Termitoidae) are eusocial insects. Within a termite colony, individuals can be broadly categorized into three castes: reproductive castes (king, queen, and winged imago), soldier castes, and worker castes. In termite taxonomy, species descriptions typically depend on the morphological characters of the soldier and winged imago castes. A comprehensive species description ideally includes both soldier and winged imago castes. However, the description of the winged imago castes is occasionally omitted due to the seasonal occurrences of these castes. Consequently, some original species descriptions are solely based on the soldier castes (Li, 2000). In the case of *Coptotermes*, challenges arise from the similarity in soldier morphology among species and the variation observed intraspecifically (Li et al., 2010; Emerson, 1971). Only a few morphological characters of soldier castes can be reliably used for species identification.

In recent years, molecular technology has been recognized as an efficient tool for solving taxonomic problems, such as validating species and synonymization. Molecular data appear to be a growing trend and essential for species description. However, there is still a lack of molecular data for many *Coptotermes* species.

There are 59 *Coptotermes* species listed in the revision roadmap proposed by Li and Wu (2023) (Table 1). According to the species list, the 59 *Coptotermes* species are categorized as 23 valid species and 36 uncertain species. Valid species include those with detailed morphological descriptions (winged imago and soldier castes) and genetic information. Uncertain species have been less studied in comparison to other species and lack reliable molecular data to support their validity. Regarding the type locality of uncertain species, 30 out of 36 species are documented to be from the Asian region (Table 1).

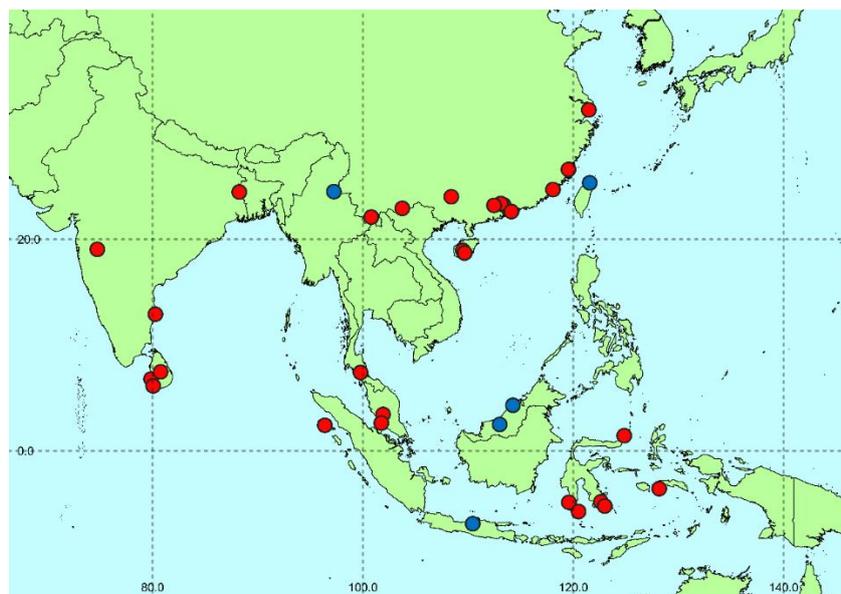
In this article, we conduct a comprehensive review of all *Coptotermes* species from the Asian region. We examine and list the insufficient information regarding uncertain species in Asia, including the morphological descriptions of winged imago and soldier castes, as well as genetic information. We scrutinize the taxonomy status of *Coptotermes* in the Asian area and discuss research directions for the future.

## Materials and methods

To ascertain the taxonomic status of *Coptotermes* in the Asian region, we conducted a thorough literature review, encompassing original species descriptions and references obtained from Google Scholar. Relevant literature was identified using keywords like "*Coptotermes* taxonomy," "*Coptotermes* new species," and "*Coptotermes* synonyms." We systematically documented the type localities of each species and compiled a list highlighting the insufficient information available for each species from Asia. Subsequently, we discuss potential strategies for improving and guiding future research in Asian region.

## Results and discussion

According to Table 1, 35 out of 59 *Coptotermes* species have their type locality in the Asian region. However, only five valid species have their type localities in Asia (Fig. 1). These include *C. kalshoveni* Kemner, 1934 from Indonesia, *C. travians* (Haviland, 1898) and *C. curvignathus* Holmgren, 1913 from Malaysia, *C. gestroi* (Wasmann, 1896) from Myanmar, and *C. formosanus* Shiraki, 1909 from Taiwan. Regarding the type locality of uncertain species, 30 out of 36 species are documented to be from the Asian region (Fig. 1). Specifically, 14 species recorded their type localities in China, with all 14 identified as uncertain species. Three species have type localities in India, and all of them are categorized as uncertain species. Eight species noted their type localities in Indonesia, and 7 out of 8 are uncertain species. Four species listed their type localities in Malaysia, with 2 of them being uncertain species. One species recorded its type locality in Thailand and is also classified as an uncertain species. Three species have type localities in Sri Lanka, and all of them are uncertain species.



**Fig 1.** Type localities of *Coptotermes* in Asian region. Blue dots are valid species. Red dots are uncertain species.

Concerning the 30 uncertain species in Asia, only 10 species have a description of the winged imago, and one species requires a description of the soldier castes. In China, 10 out of 14 species need a description of the winged imago castes. In India, 2 out of 3 species require a description of the winged imago castes. In Indonesia, 5 out of 7 species need a description of the winged imago, and one species requires the description of the soldier castes. In Malaysia, both of the two species need a description of the winged imago castes. In Sri Lanka, 2 out of 3 species need the description of the winged imago castes. None of the 30 uncertain species have molecular data available from their type localities.

To address the taxonomy issues related to these uncertain species, the following steps are proposed:

Step 1: Collect specimens from the type localities of uncertain species (species marked as Bold in Table 1). Ensure the collection for each species, at least 10 colonies are collected within each 100 km<sup>2</sup> area. For instance, if the administrative regions is 300 km<sup>2</sup>, then a total of 30 colonies of *Coptotermes* termites should be collected. Each colony should comprise 100 individuals, including more than 10 soldiers for the ensuing research. Maintain a minimum distance of 100 meters between each collection site to ensure individuals are from different colonies.

Step 2: Collect specimens from the type localities of species that require a detailed description of winged imago castes. The use of Malaise traps and light traps can be effective in collecting these castes (Chiu et al., 2016). Citizen science projects can play a crucial role in collecting both winged imago and soldier castes of *Coptotermes* in urban environments. Inviting the public and pest management professionals to participate in termite collection efforts proves to be a practical approach for gathering a large number of samples within a limited time (Huang et al., 2022).

Step 3: Conduct mitochondrial gene sequencing (such as 16S, COI, and COII sequences) or whole mitochondrial genome sequencing. Detailed morphological descriptions are also necessary and should be conducted after collection.

If the type specimens are missing or not selected, the selection of a neotype or lectotype is necessary. Colonies collected with soldier, winged imago castes, and molecular data make excellent samples to represent the species and should be considered for selection as type specimens.

## Conclusion

A total of 30 uncertain species of *Coptotermes* have their type localities in Asia. Twenty species require detailed descriptions of winged imago castes, and one species needs the description of soldier castes. All of the 30 species need molecular data from the type localities. Proposed steps to solve taxonomy issues in the Asian area include the necessary collection of soldier and winged imago castes from the type localities, as well as conducting the morphological description and molecular data of the missing castes from the specimen collection from the type localities. Citizen science project, involving the public and pest management professionals, will provide a practical avenue for swiftly amassing termite samples.

## References

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**Table 1.** List of *Coptotermes* species.

	<b>Species name</b>	<b>imago</b>	<b>soldier</b>	<b>molecular</b>	<b>Type locality</b>	<b>LATDD</b>	<b>LONDD</b>
1	<i>C. testaceus</i> (Linnaeus, 1758)	Y	Y	Y	America	3.919305	-56.027783
2	<i>C. cooloola</i> Lee et al., 2017	X	Y	Y	Australia: Brisbane city	-27.4704528	153.026034
3	<i>C. acinaciformis</i> (Froggatt, 1898)	Y	Y	Y	Australia: Halls Creek	-18.2229842	127.67014
4	<i>C. nanus</i> Lee et al., 2017	X	Y	Y	Australia: Kununurra	-15.7783483	128.7439
5	<i>C. frenchi</i> Hill, 1932	Y	Y	Y	Australia: Melbourne	-37.8226005	145.03537
6	<i>C. michaelseni</i> Silvestri, 1909	Y	Y	Y	Australia: Mundijong	-32.2972426	115.984966
7	<b><i>C. fumipennis</i> (Walker, 1853)</b>	<b>Y</b>	<b>X</b>	<b>X</b>	<b>Australia: New Holland</b>	<b>-38.1106687</b>	<b>145.293876</b>
8	<i>C. lacteus</i> (Froggatt, 1898)	Y	Y	Y	Australia: Shoalhaven	-35.0810677	150.489229
9	<i>C. acinaciformis raffrayi</i> Wasmann, 1900	Y	Y	Y	Australia: Swan River	-31.8962877	115.957502
10	<i>C. brunneus</i> Gay, 1955	Y	Y	Y	Australia: Western Australia	-27.8273573	114.687824
11	<i>C. dreghorni</i> Hill, 1942	Y	Y	Y	Australia: Timber Reserve	-25.4924414	152.655929
12	<i>C. sjostedti</i> Holmgren, 1911	Y	Y	Y	Cameroon: Bonge	7.369722	12.354722
13	<b><i>C. chaoxianensis</i> Huang and Li, 1985</b>	<b>Y</b>	<b>Y</b>	<b>X</b>	<b>China: Chaoxian County</b>	<b>23.814084</b>	<b>108.400169</b>
14	<b><i>C. hainanensis</i> Li and Tsai, 1985</b>	<b>X</b>	<b>Y</b>	<b>X</b>	<b>China: Danxiaan County</b>	<b>18.759448</b>	<b>109.653022</b>
15	<b><i>C. guangdongensis</i> Ping, 1985</b>	<b>X</b>	<b>Y</b>	<b>X</b>	<b>China: Guangzhou City.</b>	<b>23.095201</b>	<b>113.36764</b>
16	<b><i>C. gulangyuensis</i> Li and Huang, 1986</b>	<b>X</b>	<b>Y</b>	<b>X</b>	<b>China: Gulangyu Island</b>	<b>24.446318</b>	<b>118.066232</b>
17	<b><i>C. varicapitatus</i> Tsai and Li, 1985</b>	<b>X</b>	<b>Y</b>	<b>X</b>	<b>China: Hainan Island</b>	<b>19</b>	<b>109.5</b>
18	<b><i>C. dimorphus</i> Xia and He, 1986</b>	<b>Y</b>	<b>Y</b>	<b>X</b>	<b>China: Hekou County</b>	<b>22.78527</b>	<b>103.74128</b>
19	<b><i>C. longignathus</i> Xia and He, 1986</b>	<b>X</b>	<b>Y</b>	<b>X</b>	<b>China: Hekou County</b>	<b>22.7854332</b>	<b>103.741124</b>
20	<b><i>C. bannaensis</i> Xia and He, 1986</b>	<b>X</b>	<b>Y</b>	<b>X</b>	<b>China: Jinghong County</b>	<b>22.0088096</b>	<b>100.794409</b>
21	<b><i>C. grandis</i> Li and Huang, 1985</b>	<b>X</b>	<b>Y</b>	<b>X</b>	<b>China: Lianjiang County</b>	<b>26.1975299</b>	<b>119.53957</b>
22	<b><i>C. longistriatus</i> Li and Huang, 1985</b>	<b>X</b>	<b>Y</b>	<b>X</b>	<b>China: Nanhai County</b>	<b>23.213796</b>	<b>113.101938</b>
23	<b><i>C. melanoistriatus</i> Gao, Lau and He, 1995</b>	<b>X</b>	<b>Y</b>	<b>X</b>	<b>China: New Territories</b>	<b>22.498919</b>	<b>114.11283</b>
24	<b><i>C. shanghaiensis</i> Xia and He, 1986</b>	<b>Y</b>	<b>Y</b>	<b>X</b>	<b>China: Shanghai</b>	<b>31.230416</b>	<b>121.473701</b>
25	<b><i>C. monosetosus</i> Tsai and Li, 1985</b>	<b>Y</b>	<b>Y</b>	<b>X</b>	<b>China: Yaxian County</b>	<b>18.76725</b>	<b>109.682548</b>
26	<b><i>C. cyclocoryphus</i> Zhu, Li and Ma, 1984</b>	<b>Y</b>	<b>Y</b>	<b>X</b>	<b>China: Zhaoqing</b>	<b>23.0468999</b>	<b>112.46528</b>
27	<b><i>C. silvaticus</i> Harris, 1968</b>	<b>X</b>	<b>Y</b>	<b>X</b>	<b>Gabon: Belinga</b>	<b>1.145035</b>	<b>13.1999909</b>

28	<i>C. intermedius</i> Silvestri, 1912	Y	Y	Y	Guinea-Bissau: Rio Cassine	11.803749	-15.180413
29	<i>C. kishori</i> Roonwal and Chhotani, 1962	X	Y	X	<b>India: Berhampur</b>	<b>24.2290007</b>	<b>88.2461183</b>
30	<i>C. beckeri</i> Mathur and Chhotani, 1969	X	Y	X	<b>India: Chennai</b>	<b>13.0826802</b>	<b>80.2707184</b>
31	<i>C. heimi</i> (Wasmann, 1902)	Y	Y	X	<b>India: Wallon</b>	<b>19.0948287</b>	<b>74.7479789</b>
32	<i>C. amboinensis</i> Kemner, 1931	Y	Y	X	<b>Indonesia: Ambon</b>	<b>-3.6386665</b>	<b>128.168856</b>
33	<i>C. minutissimus</i> Kemner, 1934	X	Y	X	<b>Indonesia: Berra Massidi</b>	<b>-4.901629</b>	<b>122.627746</b>
34	<i>C. boetonensis</i> Kemner, 1934	X	Y	X	<b>Indonesia: Boeton Island</b>	<b>-5.3096355</b>	<b>122.988832</b>
35	<i>C. menadoe</i> Oshima, 1914	Y	X	X	<b>Indonesia: Manado</b>	<b>1.4748305</b>	<b>124.842079</b>
36	<i>C. oshimai</i> Light and Davis, 1929	X	Y	X	<b>Indonesia: Maros</b>	<b>-4.9519888</b>	<b>119.577607</b>
37	<i>C. peregrinator</i> Kemner, 1934	X	Y	X	<b>Indonesia: Pamatata</b>	<b>-5.836344</b>	<b>120.499121</b>
38	<i>C. kalshoveni</i> Kemner, 1934	X	Y	Y	Indonesia: Semarang	-7.0051453	110.438125
39	<i>C. sinabangensis</i> Oshima, 1923	X	Y	X	<b>Indonesia: Sumatra</b>	<b>2.4765338</b>	<b>96.3804554</b>
40	<i>C. truncates</i> (Wasmann, 1897)	X	Y	X	Madagascar: Nossi Bé	-13.3150186	48.2592606
41	<i>C. bentongensis</i> Krishna, 1956	X	Y	X	<b>Malaysia: Bentong</b>	<b>3.522168</b>	<b>101.910353</b>
42	<i>C. travians</i> (Haviland, 1898)	Y	Y	Y	Malaysia: Marudi	4.4063366	114.262825
43	<i>C. curvignathus</i> Holmgren, 1913	Y	Y	Y	Malaysia: Sarawak	2.5574285	113.001199
44	<i>C. sepangensis</i> Krishna, 1956	X	Y	X	<b>Malaysia: Sepang</b>	<b>2.691369</b>	<b>101.750527</b>
45	<i>C. mauricianus</i> (Rambur, 1842)	Y	X	X	<b>Mauritius</b>	<b>-20.3319806</b>	<b>57.5497207</b>
46	<i>C. gestroi</i> (Wasmann, 1896)	Y	Y	Y	Myanmar: Bhamo	24.2629574	97.2383178
47	<i>C. gambrinus</i> Bourguignon and Roisin, 2011	X	Y	Y	Papua New Guinea: Bulolo.	-7.2011074	146.644666
48	<i>C. dobonicus</i> Oshima, 1914	X	Y	X	<b>Papua New Guinea: Dobo</b>	<b>-8.7558031</b>	<b>148.366666</b>
49	<i>C. remotus</i> Hill, 1927	Y	Y	Y	<b>Papua New Guinea: Kaewieng</b>	<b>-2.5781167</b>	<b>150.808608</b>
50	<i>C. elisae</i> (Desneux, 1905)	Y	Y	Y	Papua New Guinea: Simbang	-6.724345	146.993629
51	<i>C. pamuae</i> Snyder, 1925	Y	Y	Y	Solomon Islands: Pamua	-10.5737447	161.809694
52	<i>C. grandiceps</i> Snyder, 1925	Y	Y	X	Solomon Islands: Tulagi Island	-9.1015826	160.147069
53	<i>C. emersoni</i> Ahmad, 1953	X	Y	X	<b>Sri Lanka: Colombo.</b>	<b>6.9270786</b>	<b>79.861243</b>
54	<i>C. gaurii</i> Roonwal and Krishna, 1955	X	Y	X	<b>Sri Lanka: Marambekana</b>	<b>7.616667</b>	<b>80.766667</b>
55	<i>C. ceylonicus</i> Holmgren, 1911	Y	Y	X	<b>Sri Lanka: Peradeniya</b>	<b>6.2664541</b>	<b>80.0604774</b>
56	<i>C. formosanus</i> Shiraki, 1909	Y	Y	Y	Taiwan: Taipei	25.0329636	121.565427
57	<i>C. amanii</i> (Sjöstedt, 1911)	Y	Y	Y	Tanzania: Amani	-4.75	38.5

<b>58</b>	<b><i>C. premrasmii</i> Ahmad, 1965</b>	<b>Y</b>	<b>Y</b>	<b>X</b>	<b>Thailand: Ka-Chong</b>	<b>7.5548287</b>	<b>99.7746812</b>
<b>59</b>	<b><i>C. paradoxus</i> (Sjöstedt, 1911)</b>	<b>Y</b>	<b>X</b>	<b>X</b>	<b>Togo: Bismarckburg</b>	<b>8.18333333</b>	<b>0.7</b>

Bold species are the uncertain species.

Tuesday | March 12, 2024 | 15:00-15:15

## Section 1: Biodiversity, Ecology, and Behavior

O\_02

### Species composition and infestation status of termites in ancient Shan tea trees in northern Vietnam

by

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#### Abstract

The study was conducted at 10 ancient tea areas in 3 provinces in the North of Vietnam (Yen Bai, Tuyen Quang, and Ha Giang provinces). Total of 17 species belonging to 11 genera, 6 subfamilies and 3 families were recorded in ancient tea farms, in which 7 species were pests including *Glyptotermes satsumensis*, *Glyptotermes tsaii*, *Reticulitermes assamensis*, *Reticulitermes guangzhouensis*, *Macrotermes barneyi*, *Odontotermes formosanus*, and *Odontotermes pujiangensis*. Infestation status of these termite pests was also recorded.

**Keywords:** Species composition, damage, ancient Shan tea, termites

#### Introduction

Shan (*Camellia sinensis*) tea is a kind of specialty tea in quality and high value. In Vietnam, this type of tea has been cultured for a long time in some localities in mountainous areas from 800-1,800 m above sea level in some provinces in the north of Vietnam such as Tuyen Quang, Ha Giang, Yen Bai, Son La, Dien Bien, etc. The Shan tea area is about 24% of the total tea plantation of Viet Nam (Do Van Ngoc et al., 2006). However, it was found to be infested by some of organisms including termites (Le Van Bao, 2014, 2016); Nguyen Thi Tuyen et al., 2019).

In many tropical and subtropical ecosystems, termites play a key role, as soil engineers and decomposers (Bignell, 2006; Jouquet et al., 2011). However, some of species are pests. About 371 (12.4%) of the total 3,000 recorded species have been reported as destructive pests; in which, 30 species infest to coffees and teas (Krishna et al., 2013). In Vietnam, 8 species belonging to 5 genera (*Reticulitermes*, *Macrotermes*, *Odontotermes*, *Pericapritermes*, *Pseudocapritermes*) were founded in the Ancient Shan tea in Yen Bai province (Le Van Bao, 2014), however, there is limited data on its infestation status. In this study, the composition of termites in the ancient Shan tea and its infestation status were investigated. The result is a science basis to contribute to the further investigation of control method for these pests.

#### Materials and methods

##### Study sites

In 2023, the survey was conducted in 10 ancient tea areas in 8 communes in 3 provinces in the North of Vietnam (Yen Bai, Tuyen Quang and Ha Giang provinces) (Table 1). Termites were collected and brought to the laboratory of the Institute of Ecology and Works Protection in Hanoi, Vietnam for further works of identification and storage.

**Table 1.** The studied sites

No	Site (commune, district, province level)	Location coordinates	Altitude (m) a.s.l
1	Suoi Giang, Van Chan, Yen Bai	21°37'21"N 104°35'37"E	1,020
2	Phinh Ho, Tram Tau, Yen Bai	21°31'34"N 104°32'19"E	970
3	Son Phu, Na Hang, Tuyen Quang	22°20'43"N 105°27'42"E	800
4	Sinh Long, Na Hang, Tuyen Quang	22°31'45"N 105°23'36"E	870
5	Tho Binh, Lam Binh, Tuyen Quang	22°22'09"N 105°13'23"E	1,050
		22°46'44"N 104°51'05"E	800
6	Cao Bo, Vi Xuyen, Ha Giang	22°46'30"N 104°52'16"E	880
		22°45'44"N 104°50'55"E	920
7	Nam Ty, Hoang Shu Phi, Ha Giang	22°36'55"N 104°45'55"E	1,200
8	Thong Nguyen, Hoang Shu Phi, Ha Giang	22°35'49"N 104°42'42"E	960

***Termite collection and identification***

Termite samples were collected by both quantitative and qualitative method.

***Quantitative termite collection:***

At each area (tea farm), termites signs were searched along a route in middle of the farm such as nests (in the tree or on the ground), swarming holes, the tunnel on tree trunks, branches up to a height of 2m, rotten wood or leaf litter on the ground.

***Qualitative termite collection:***

Based on the method of Jones and Eggleton (2000), a transect (100m long and 2m wide) was created at each area (tea farm). The transect was divided into 20 sections; each section was a 5m × 2m area. For each designated section, two experienced collectors searched the entire section from 10cm beneath the ground and up to 2 m height of trees above the ground in the period of 30 minutes. All encountered termites were collected and especially, soldiers were paid special attention during the collection for further work of identification.

Termites collected were preserved in plastic tubes containing 75% alcohol and were brought to the laboratory of the Institute of Ecology and Works Protection for identification and preserve.

During the investigation, researchers recorded the number of Shan tea trees and its health status. The parts of these trees infested by termites were also recorded. The health status of the trees were then categorized into 3 groups: (1): the normally growing trees; (2): the weak trees (yellow leaves, wilting, excessive leaf loss, etc.); (3): the dead trees. Termites encountered locations were classified into 4 groups: (1) in the tunnel on living tree; (2) in the living tissues of the tree (with the more detail as termites penetrate the outer bark of branches, trunks, roots or into the wood); (3) only in the dead tissue of the tree; (4) under humus layer.

***Termite identification:*** Termites were observed under the Leca MZ6 binocular magnifying glasses. The parameters of soldiers and alates were measured followed the instructions of Roonwal (1970). The identification keys were used such as Ahmad (1958, 1965), Nguyen Duc Kham et al., (2007), Huang et al. (2000), Roonwal & Chhotani (1989), Thapa (1982), and Chhotani (1997).

***Termites as pests in Ancient Shan tea***

The consideration as a pest for termite species was based on the feeding group and its encountered location in the tea farms. The species was considered as pest if they were found to infest the living trees and belonged to the wood feed group (Group I, II).

- The feeding group was based on the document of Donovan et al. (2001), and Jones and Eggleton (2011), in which group I contains lower termite dead wood and grass-feeders; group II contains Termitidae with a range of feeding habits including dead wood, grass, leaf litter, and micro-epiphytes; group III contains Termitidae feeding in the organic-rich upper layers of the soil; group IV contains the true soil-feeders)
- Encountered locations: (0) no finding on the tea tree; (1) attacking into living tea trees (build the tunnel on branches, nest inside the trunk or under the tree, etc.).

## Results and discussion

### Composition of termites

17 species belonging to 11 genera of 3 families (Kalotermitidae, Rhinotermitidae and Termitidae) (Table 2) were recorded in this study. In which two species (*Odontotermes pujiangensis* Fan, 1987 and *Euhamitermes yuntaishanensis* Zhu & Huang, 1987) have first time recorded for Vietnam termite fauna.

Table 2 showed that seven species (*Glyptotermes satsumensis*, *Glyptotermes tsaii* *Reticulitermes assamensis*, *Reticulitermes guangzhouensis*, *Macrotermes barneyi*, *Odontotermes formosanus* and *Odontotermes pujiangensis*) were pests to ancient tea tree in northern Vietnam. These species were found to infest the living trees and belonged to the feeding group I or II. The remaining species were not pest. Five species (*Reticulitermes chinensis*, *Euhamitermes yuntaishanensis*, *Ahmaditermes perisinuosus*, *Peribulbitermes dinghuensis*, *Pilitermes jiangxiensis*) were collected from rotten branches in the ground to dry died stumps. Although *Pericapritermes dunensis* and *Pericapritermes latignathus* were found on the living tree, they did not damage trees. *Pericapritermes* spp. feed on organically rich upper soil layers (humus feeders – feeding group III) (Donovan et al., 2001; Jones and Eggleton, 2011) and build nests under the humus layer in the rotten wood or under the ground. They may penetrate the mulch layer inside hollow cavities of the tea trunk after the part has rotted.

**Table 2.** Composition of termites and pest status in ancient Shan tea areas in northern Vietnam

No	Scientific name	Collection site	Feeding group	Pest
<b>KALOTERMITIDAE Ederlein, 1909</b>				
KALOTERMITINAE, Froggatt, 1897				
1	<i>Glyptotermes satsumensis</i> (Matsumura, 1904)	1	I	x
2	<i>Glyptotermes tsaii</i> Huang & Zhu, 1986	1	I	x
3	<i>Glyptotermes</i> sp.	0	I	
<b>RHINOTERMITIDAE Light, 1896</b>				
HETEROTERMITINAE Froggatt, 1896				
4	<i>Reticulitermes assamensis</i> Gardner, 1943	1	I	x
5	<i>Reticulitermes chinensis</i> Snyder, 1923	0	I	
6	<i>Reticulitermes guangzhouensis</i> Ping, 1985	1	I	x
<b>TERMITIDAE Westwood, 1840</b>				
MACROTERTERMITINAE Kemner, 1934				
7	<i>Macrotermes barneyi</i> Light, 1924	1	II	x
8	<i>Odontotermes formosanus</i> (Shiraki, 1909)	1	II	x
9	<i>Odontotermes pujiangensis</i> Fan, 1987*	1	II	x
APICOTERMITINAE GRASSE' & NOIROT, 1955				
10	<i>Euhamitermes yuntaishanensis</i> Zhu & Huang, 1987*	0	III	
TERMITINAE SJOSTEDT, 1926				
11	<i>Pericaptitermes dunensis</i> (Roonwal & Sen-Sarma, 1960)	1	III	
12	<i>Pericaptitermes latignathus</i> (Holmgren, 1914)	1	III	
13	<i>Pseudocapritermes albipennis</i> Tsai & Chen, 1963	0	III	
14	<i>Procapritermes</i> sp.	0	III	
NASUTITERMITINAE HARE, 1937				
15	<i>Ahmaditermes perisinuosus</i> Li & Xiao, 1989	0	II	
16	<i>Peribulbitermes dinghuensis</i> Li, 1985	0	(II)	
17	<i>Pilitermes jiangxiensis</i> He, 1987	0	(III)	

*Noted:* (\*): New species recorded for termite fauna in Vietnam; x: the pest species

### Termites as pest damaging the ancient Shan tea trees

Seven pest species belonging to 3 subfamilies including Kalotermitinae, Heterotermitinae and Macrotermitinae were identified as pest for the ancient Shan tea trees. In which, 2 species belonged to the

subfamily Kalotermitinae were *Glyptotermes satsumensis* and *Glyptotermes tsaii*. While *Glyptotermes satsumensis* was found in Thong Nguyen commune, Hoang Shu Phi district and Cao Bo commune, Vi Xuyen district, Ha Giang province, the *Glyptotermes tsaii* was only found in Cao Bo commune, Vi Xuyen district, Ha Giang province. These species were found to build nest inside the trunk of living ancient tea trees. They penetrated into the living tissues to the heartwood of the tree. Their nests consisted of long galleries with a nearly circular horizontal section, diameter ranging from 2 - 2.5mm. The galleries were connected to each other diagonally, horizontally, vertically, and irregularly (Fig. 1A and 1B).



**Fig 1.** The ancient Shan tea was infested by *Glyptotermes*. A: by *G. satsumensis*; B: by *G. Tsaii* (Photos by: Nguyen Thi My, 2023)

All of these nests were related to the rotten point on the tea tree. The same phenomenon was found in *Glyptotermes dilatatus* that their incipient colony initiated by a pair of alates at the wound of the tree (by cutting, branch breaking or other reasons) where was rotted by wood decay fungus for a long time. As the individual number of colonies increase, they need food and therefore move to the heartwood by building the feeding galleries and expanding their territory (Senanayak et al., 1915; Nawarathna, 2016). Besides, they disperse wood rot fungi while feeding on heart wood of tea bush (Senanayak et al., 1915). Because of termite activities, the supply of nutrients to the tree is affected that gradually cause the trees weakening and dying.

In subfamily Heterotermitinae, *Reticulitermes assamensis* was found in Sinh Long commune, Na Hang district, Tuyen Quang province, Cao Bo commune, Vi Xuyen district, Ha Giang province and Suoi Giang commune, Van Chan district, Yen Bai province, while *Reticulitermes guangzhouensis* was found Cao Bo commune, Vi Xuyen district, Ha Giang province and Phin Ho, commune, Tram Tau district, Yen Bai province. 2 species were found on the dead part of living tea trees. They usually build nest in hard core of the trunk or stump. The cavities of nest are not certain shape. The gnaw traces were clearly visible on the cavity surfaces and without tunnels outside compared to that of species belonging to genus *Coptotermes* and *Schedorhinotermes* (Fig. 2A and Fig. 2B). Data on the harmful characteristics of the *Reticulitermes* genus and its species are limited. The Kapur's observations (1962) showed that *Reticulitermes chinensis* were found under the soil and in damaged roots of the pine trees (*Pinus longifolia*). The bark of the roots, in each case, was found to be quite intact while the hard cores were mostly tunneled through. When severely damaged, the roots showed galleries which were rather irregular and ran almost parallel to the length of the root were occasionally filled with soil and excreted wood. This characteristic was found in *Reticulitermes assamensis* in our study (Fig. 2C and Fig. 2D)



**Fig 2.** The ancient Shan tea was infested by *Reticulitermes*  
(Photos by Nguyen Thi My, 2023)

Three species belonging to subfamily Macrotermitinae (*Macrotermes barneyi*, *Odontotermes formosanus*, and *Odontotermes pujiangensis*) were record as pests to ancient tea tree in the northern Vietnam. In which, *Odontotermes formosanus* was found all study areas with the highest of samples. These species nests are difficult to locate as its nest are totally underground with completed nest system. The common damage characteristics of these species tunnel on the tree trunk and gradually corrode part of the bark, then they find a way to penetrate into the trunk and branches through wounds (Fig.3).



**Fig 3.** The ancient Shan tea was infested by *Macrotermitinae*.  
A: by *Macrotermes barneyi*; B: by *Odontotermes formosanus*; C: *Odontotermes pujiangensis*  
(Photos by: Nguyen Thi My, 2023)

Das (1962) called subfamily Macrotermitinae with “scavenging termites” name because they usually clean up dead and dying tissues though it is possible. Continued removal of dead and dying tissue prevents the healing of the wounds, resulting in the formation of cavities and hollows in the stems, which are progressively enlarged.

This is further aggravated during the wet season, particularly when rainwater is lodged, providing further material for the termite to act upon. Unlike genera *Glyptotermes* and *Reticulitermes*, species belonging to the Macrotermitinae subfamily which their food are quite diverse (wood, tree roots, grass roots, dry leaves, etc.) and they have a complex food processing process through growing fungus gardens. By mean of the symbiotic relationship with the *Termitomyces* fungus, termites go out to forage and chew food materials to build the fungus garden substrate through primary fecal pellets, while the *Termitomyces* fungus appears to be largely to break down lignin complexes in the food residues, freeing more cellulose which the termites can use (Sand, 1973) and creating nutritious food sources for termites through white asexual spores (noduli) (Nobre & Aanen, 2012). They mostly feed on dead plants, but sometimes also eat live plants, causing widespread and dangerous damage, especially in semi-arid Africa and India (Logan et al., 1990). Besides, “fungal grower” termites often have a large number of individuals in the colony and they can make foraging tunnels in tree trunks or roots underground. In this study, we also recorded some evidence of *Odontotermes formosanus* damage the roots of ancient Shan tea in Suoi Giang commune, Van Chan district, Yen Bai province (Fig. 4). They built external paths adjacent to the root bark, along the base of tree to the cap of root, attacked a part of the root system (Fig. 4A), gnawed even the tip of the fresh root (Fig. 4B).



**Fig 4.** The root system of ancient Shan tea was damage by *Odontotermes formosanus*  
(Photos by: Nguyen Thi My, 2023)

In Vietnam, Macrotermitinae and Heterotermitinae infested the trunks and roots of trees were also

reported in previous studies including Le Van Bao (2014) [15], Nguyen Thị Tuyen et al. (2019) [21], Nguyen Van Quang et al. (2007) [20] and Bui Thi Thuy (2015) [4]. The infested evidence of *Glyptotermes* to trees, especially the ancient tea was first recorded.

## Conclusion

In this study, 17 species belonging to 11 genera and 3 families were recorded in ancient tea tree areas in the North of Vietnam. In which, 7 species were found as pests, and the genus *Glyptotermes* was first recorded as pest to ancient tea trees. The study added two new species to the termite fauna of Vietnam.

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Tuesday | March 12, 2024 | 15:15-15:30

## Section 1: Biodiversity, Ecology, and Behavior

O\_03

### Commensal bacteria enhance organic acids release from the dead termites and elicit corpse-burying behavior of *Coptotermes formosanus*

by

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#### Abstract

Living in social communities, termites have developed a vast capability to prevent the spread of pathogens and various hazardous components within their colony. Burial behavior is essential for termites to remove dead corpses of infected individuals that evolve by "death cues" such as oleic acid. However, it remains less known how these burial behavior-related signals arose in the corpse and whether they played distinct roles during the corpse-burial process. We tested the burial efficacy of *Coptotermes formosanus* workers and found that termite corpses with long post-death periods were buried earlier than those freshly killed individuals. Our spatial metabolomics and metagenomic analysis of the corpse showed that oleic acid, the major signal that guides burial behavior, was released jointly by lipolytic enzymes produced by host dead cells and the commensal bacteria *Bacillus cereus*. We further show that organic acids produced by commensal bacteria enriched in corpses could attract *C. formosanus* workers to the location of corpses. Our result highlights the cooperation of commensal bacteria after the host died in the colony and the corpse burial mechanism that bacteria metabolites of corpses can cooperate together and serve as the signal molecule to attract workers, hence eliciting the burial behavior of eusocial termites.

**Keywords:** Termites, corpse management, burial behavior, commensal bacteria, organic acids, signal molecules

#### Introduction

Commensal bacteria colonized in the gut enable termites to digest their food substrates (wood, soil, grass, etc.) more efficiently (Brune and Dietrich 2015). In fact, wood-feeding termites harbor a large amount of gut bacteria that release digestive enzymes such as cellulase, lipases, and proteases (König et al. 2013). These microbes could hydrolysis the hard-to-degrade substances in food and provide nutrients like vitamins, and free-amino acids to their host. Moreover, commensal bacteria could produce various secondary metabolites during their biodegradation process in the host tissue, including short-chain fatty acids, terpenoids, and organic acids like short chain fatty acids and  $\gamma$ -aminobutyric acid (Bown et al. 2006). These small molecules could serve as signal molecules and participate in the host metabolism process, impacting the host metabolism and physiological processes. Additionally, it is also very important that even though the host is dead, its commensal bacteria could still keep living in the host tissue. Bacteria in insect corpses could accelerate the corruption and also release a large number of volatile compounds (Martin et al. 2019). For solitary insect species, these corpse-released compounds might only serve as odorant signals for saprophagous species. However, in a eusocial insect colony, the dead corpse of an individual could still affect the physiology and behavior of other individuals. Therefore, the activity of commensal bacteria obtained in termite corpses should be considered and their potential functions still need further investigation.

In this study, we compared the burial efficiency of termite corpses with different post-death periods. We applied distinct strategies to suppress the proliferation of bacteria in corpses and estimated the influence

of corpse bacteria loading on termite burial behavior. To further confirm the function of the burial behavior-related bacteria, we performed 16S rRNA gene-based amplicon sequencing, shotgun metagenomic analysis, and spatial metabolomic analysis. We showed that oleic acid released by termite corpses was generated by the degradation of lipids. Both the host's endogenous enzymes and bacterial-produced enzymes could catalyze this process. Moreover, bacterial-produced compounds accumulated in corpses showed an attractive effect on *C. formosanus* workers. Our result indicated that commensal bacteria of termites participate in the corpse management process, which might be finished by multiple steps (the worker was attracted by corpses initially and then started burial corpses).

## Materials and methods

### ***Termite rearing and sampling***

*Coptotermes formosanus* individuals used in this study were collected from the lab-maintained colony. Workers used for the corpse management, metabolomic analysis, high throughput sequencing, behavior test, and electroantennogram studies were transferred into a plastic box (20 × 30 × 15 cm) and fed on wood blocks and water-rinsed filter papers. All collected termite individuals were maintained in a totally dark and 27°C, 75% RH condition.

### ***Burial behavior test***

Termite corpses were obtained by freeze-killed active *C. formosanus* workers (- 20°C for 15 min). Obtained corpses were transferred into a 9 cm petri dish embedded with rinsed filter paper, then incubated at 27°C, 75% RH condition for further use. The concentration of NaClO and antibiotic solution used for treatment were 2.5% and 100 µg/mL respectively.

### ***High throughput sequencing and metabolomic analysis***

Pooled samples were used for 16S rDNA amplicon sequencing. A total of 50 individuals were pooled for each sample. Four replications were used for each treatment. V3-V4 region of 16S rRNA was sequenced and the prepared samples were introduced to Illumina NovaSeq 6000 platform for high throughput sequencing.

Untargeted Metabolomics were performed by termite corpses incubated by 0 h, 6 h, 12 h, and 24 h. Each sample was pooled by 50 corpses and four replicates were prepared for each group. Metabolites of termite corpses were analyzed by Agilent 1290 Infinity LC system equipped with ACQUITY UPLC BEH Amide column (1.7 µm, 2.1 mm × 100 mm). TripleTOF 6600 System was applied for mass accuracy analysis.

## Results and Discussion

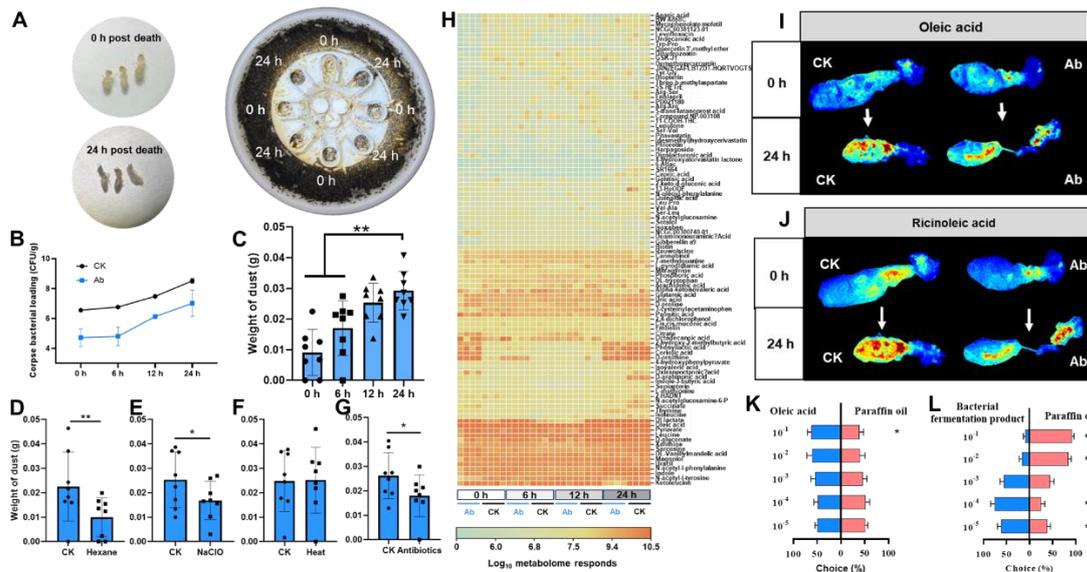
### ***Commensal bacteria involved in corpse oleic acid accumulation***

Our previous study depicted that oleic acid released by the dead corpse of *Coptotermes formosanus* could drive workers in the same colony to move soil (dust) to the corpses. Our previous GC analysis of the corpse 24 h post-death showed that long-chain fatty acids and long-chain hydrocarbons were dominant in the cuticle extraction of *C. formosanus* corpse. To test whether termite corpses could accumulate death cues after death, we estimated the burial efficiency of corpses at different post-death periods. After a 12 h burial test, the dust weight in 24 h post-death corpses were significantly higher than 0 h and 6 h post-death corpses (Figure 1A, 1C). This confirmed that chemical compounds accumulated in the corpse promote the burial behavior of *C. formosanus* workers.

Next, we tried to figure out whether commensal bacteria of termites participate in the accumulation of "death cues." Similarly, the weight of dust in each cell containing termites' corpses was used to assess the efficiency of burial behavior. Totally four distinct strategies were used to treat the termite corpses (hexane, NaClO, heat, and antibiotics). We observed that the 24 h post-death corpse treated with hexane had lower dust weight than the control group, indicating that hexane treatment might remove the "death cues" accumulated in the corpses (Figure 1D). Apart from the heat treatment, which might break the lipid structure and release free fatty acids (Figure 1F), both NaClO and antibiotic-treated corpses showed significantly lower dust weight than the control group (Figure 1E and 1G). This can be explained by the reduced bacteria loading of corpses (Figure B).

To find out the potential chemicals that correlated with termite corpse management behavior. We performed an untargeted metabolomic analysis by comparing the chemical composition of the corpses between normal corpses (CK) and antibiotic-treated corpses (Ab). The result showed that bacteria enriched

in termite corpses provide a large amount of oleic acid, DI-lactate, Indole, Leucine and its derivatives, and other fatty acids in corpses (Figure 1H). Among these compounds, we selected the most abundant and significantly enriched (24 h post-death normal corpses vs. 24 h post-death antibiotic-treated corpses) chemicals for further analysis. The result showed that the oleic acid and DI-lactate acid were initially accumulated in the gut tract of termite corpses. During the putrefaction process, the gut membrane collapsed and these compounds were further released to the whole body, even outside the body. The spread of these compounds was faster, and the amount was higher in the corpse of the control group, which contained more bacteria than the antibiotic-treated corpse (Figure 1I and 1J). Among all the selected compounds, we found that termite workers were attracted by low concentrations ( $< 10^{-2}$ ) of DI-lactate. This result indicated that DI-lactate released by corpse-derived bacteria might attract workers and show them the location of corpses (Figure 1L).



**Fig 1.** Corpse-derived bacteria contribute to the corpse burial behavior of *Coptotermes formosanus* workers. A. Termite corpse incubated for different times and their burial efficiency. B. Bacteria loading of normal (CK) and antibiotic-treated (Ab) corpses. C. Phenotype of termite corpses and bury condition (6 h) of corpses incubated for different times (0 h vs 24 h). D-G, Burial efficiency of corpse treated by hexane (D), NaClO (E), Heat (F), and antibiotics (G). H, Metabolome responds to termite corpse treated by antibiotics (Ab) (incubated for different times). Chemicals with top 100 fold-change rate (CK vs. Ab) and top 100 abundance were visualized. I and J, spatial metabolomic analysis of accumulated fatty acids including oleic acid (I) and Ricinoleic acid (J) in termite corpses. K and L, preference test of oleic acid (K) and the Bacterial fermentation product (L) among *C. formosanus* workers.

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Tuesday | March 12, 2024 | 15:30-15:45

Section 1: Biodiversity, Ecology, and Behavior

O\_04

**Advance and future prospects in phototaxis of termite:  
What can we learn from decades of research?**

by

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**Abstract**

Phototaxis is the behavioral characteristic of creatures to chase or escape under the stimulation of light source. The phototropic behavior of insects is closely related to important physiological behaviors such as swarming, foraging and circadian rhythms, Termites are the main pests that harm water conservancy facilities and wooden buildings, and have received much attention from researchers because of their high reproductive ability, mass swarming, and hidden nesting characteristics, even though the vision of the worker and soldier castes of termites is extremely underdeveloped, while the light source is still as an important abiotic factor that affects the swarming behavior of alates. However, it remains unclear how termites perceive different light sources and how light sources influence their swarming process. This review summarizes physiological and behavioral and genomic studies on termite vision, hoping to deepen the understanding of termite phototaxis, with a view to better research on the mechanisms of termite phototaxis and to provide new strategies for termite control.

**Keywords:** Termites, social insects, vision, light-wavelength, pest management

**Introduction**

Phototropism is a behavioral that causes organisms to chase light sources (positive phototropism) and avoid them (negative phototropism) when stimulated by external light sources (Randel and Jékely 2016), most creature, including mammals, birds and insects (Spudich et al 2000; Paulk et al 2013; Buschbeck & Bok 2023) have been recognized to possess phototropism. Termites is one of the most widespread and abundant animals in terrestrial ecosystems (Mertl et al. 2012). The polymorphism of vision is particularly evident in termites, where most termites have extremely underdeveloped eyes in workers and soldiers, while alate have relatively well-developed compound eyes, for which visual perception and phototropism play an important role in the process of swarming. Based on the characteristics of social insects, we can intuitively interpret this phenomenon as termite caste differentiation, however, the reason behind this polymorphism of different visual development is still unclear, and the molecular mechanism behind it still needs to be revealed.

In this study, we integrated the studies on termite phototaxis from the reported behavioral studies to the light wavelength sensitivity experiments, in order to provide new insights into the study of termite phototaxis.

**Differentiation of spectral sensitivity in termites**

Compared to the social insects of *Hymenoptera*, visual studies on termites have lagged behind.

Although the preference for different photosensitive wavelengths and the influence of phototropic behavior of the western drywood termites were initially reported as early as last century, the progress is still limited (Cabrera et al. 1996). Compared to other insect species, only a few termites have reported spectral sensitivity studies. Ferreira et al. reported that *Cryptotermes brevis*, a wood-feeding termite with an urban distribution, was detected by the researchers with settings of 395 nm (UV), 460-555 nm (white light), 470 nm (blue light), 525 nm (Green light), 590 nm (yellow light), and 625 nm (red light) to test the sensitivity of *C. brevis* alates to different light-wavelengths. The results showed that white light, blue light and green light (460-550 nm) were the most effective for the trapping of winged ants (Ferreira and Borges, 2012). Chang et al. (2001) first reported the photosensitive wavelengths of the *Coptotermes formosanus*, and through the comparison of the trapping effect of trapping lamps with three different wavelengths, the sensitivities of the blue (367~583 nm) and green (525~648 nm) wavelengths were preliminarily determined, then Wakako et al. (2014) conducted further experiments with *Incisitermes minor* and *C. formosanus* under laboratory conditions.

Based on electroretinogram (ERG) technique, the photosensitive wavelengths of worker, soldier, and alate of those two termites were measured, and the results showed that *I. minor* and *C. formosanus* were sensitive to UV and violet (350-400 nm) light. *Odontotermes formosanus* is a termite widely distributed in Asia that feeds exclusively on wood fibers, and has received extensive attention from the water conservancy departments of China, Japan, South Korea, and other Asian countries because of the great damage it can cause to dams and other water conservancy facilities. (Hu 2006; Chiu et al., 2018; ) Hu (2006) used ERG to determine the spectral sensitivity of the compound eyes of the *O. formosanus* alates, and the results showed that after adaption to different light conditions, the alates were more sensitive to the 523 nm green light (after adaption to dark conditions) and 388 nm(violet light) (after adaptation to violet and white light), and found that no difference in the sensitive wavelengths of alates between sexes. The results indicate that *O. formosanus* has at least these two types of visual receptors.

In addition, although the color discrimination to light wavelength was not addressed, Mizumoto and Bourguignon (2022) conducted a study on the light stimuli on the tandem coordination of *Reticulitermes speratus* and *C. formosanus*, the results indicated both *R. speratus* and *C. formosanus* could perceive light and showed positive phototaxis, *R. speratus* showed higher activity under light conditions, while the nocturnal *C. formosanus* were relatively insensitive to light. The above studies show that the photosensitive wavelengths of termites are consistent with those of other insects, which are more sensitive to short-wave light. However, there is no molecular study on the optic of termite to address the underlying mechanism at the present time.

### **Genomics of termites involving opsin**

The expression of opsin is usually regulated by circadian rhythms and environmental factors. The evolution of opsin genes is often associated with spectral adaptation of insects to specific environments, a phenomenon that highlights the selective pressure of specific light conditions on the evolution of photoreceptor and the divergence of opsin regulation from circadian rhythms. (Warrant and Dacke 2011; Futahashi et al 2015). Insect photoreceptor for sensitivity to ultraviolet, blue, and short-wavelength light has been longstanding and highly conserved (van der Kooi et al 2021). Although the results of previous wavelength-sensitive studies on termite suggest that the differentiation of termite opsin may be similar to that of ants. Unfortunately, in termites there is a lack of sufficient evidence, especially at the genomic level and molecular level, to support this evidence. Currently, there are only two researches of termite opsin have been reported. Terrapon et al have assembled and annotated the genome of *Zootermopsis nevadensis*, and reported four opsin genes ( ZooNev1.0, GCF\_000696155.1), which is the first deciphered termite genome. Harrison et al assembled the genome of the *Cryptotermes secundus* and characterized six opsin genes (Csec\_1.0, GCF\_002891405.2).

Studies on insects have shown an association between environmental demands (e.g., light, temperature, nutrients, competitors and predators) and physiological adaptations to vision (Yilmaz et al 2022). Yilmaz et al. found higher expression levels of LW1 and UV optic than BL optic in *Camponotus rufipes*, which supports the prominent function of these two opsin in visual orientation (Yilmaz et al 2016). For some termites, such as the *Reticulitermes* and *Coptotermes*, produce a large number of alates during the swarming season, while vision plays an important role in the navigation of alates. Neither the differential expression of opsin in the alates during this period nor the underlying mechanisms behind the "activation" of vision are well addressed. At present, the genomic information of only six termite species have been registered in NCBI, in addition to the two species mentioned above, *R. speratus* (TAMU-RSper-2021, GCA\_021186555.1), *Reticulitermes lucifugus* (UNIBO \_RLUCI\_2021, GCA\_026260175.1) and *C. formosanus* (CopFor1.0,

GCA\_013340265.1), as well as an incompletely assembled genome of the *Nasutitermes exitiosus* (GCA\_001404035.1). We urgently require more evidence at molecular levels to reveal the underlying mechanisms behind the diversity of opsin in termite. On the other hand, the genomes of social insects can generate and regulate very distinct phenotypes, and sociality itself can shape the genomes of different populations in unique ways (Mikhailova et al 2023). Thus, resolving visual differentiation could provide more evidence to analyze the differentiation of social insect genome.

## Conclusion

The study of insect visual system is a research field that has received wide attention. In this study, we integrate the experimental reports on termite vision and discuss the problems and directions of development of termite vision research, as well as emphasize the necessity of the research on termite vision, and call for more researchers to explore termite vision more deeply in the genomic and experimental levels, in order to provide new insights for the prevention and control of this widely distributed species in the world.

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Tuesday | March 12, 2024 | 15:45-16:00

## Section 1: Biodiversity, Ecology, and Behavior

O\_05

### The influence of desaturase on fatty acid synthesis and trophallaxis in *Coptotermes formosanus*

by

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#### Abstract

Fatty acid desaturase plays a key role in the lipid synthesis pathway of unsaturated lipid synthesis. In this study, the transcriptome data of *C. formosanus* were annotated and analyzed. Seven first desaturase family genes were screened, and 7 desaturase genes were named *Cfor-desatsA1*, *Cfor-desatA2-a*, *Cfor-desatA2-b*, *Cfor-desatB-a*, *Cfor-desatB-b*, *Cfor-desatD*, *Cfor-desatE*. The full-length of these 7 *Cfor-desats* genes was amplified, and the dsRNA corresponding to the 7 *Cfor-desats* genes was synthesized. We observed and recorded the survival rate of workers within 10 days after interference. Except for the ds*Cfor-desatE* treatment group, the survival rate of the remaining 6 desaturase genes was significantly reduced after dsRNA interference. However, in the ds*Cfor-desatA2-b* treatment group, the normal soldiers reared with the disturbed workers also showed higher mortality. Because soldiers lack feeding ability, they can only achieve their required nutrition through workers, revealing that this gene affects the trophallaxis of *C. formosanus*. We also observed that the amount of filter paper consumed by workers decreased significantly after 24 h in the ds*Cfor-desatA2-b* treatment group. However, soldiers rearing with RNAi treated workers kept the same amount of dye at 24 h, 48 h and 72 h. It shows that the composition may change during trophallaxis. In summary, *Cfor-desatA2-b* can affect fatty acid synthesis and trophallaxis in termite population.

**Keywords:** *Coptotermes formosanus*, desaturase, fatty acid, trophallaxis, RNAi

#### Introduction

Unsaturated fatty acids (UFAs) play crucial roles in the physiology and ecology of insects, participating in various fundamental biological processes such as lipid metabolism, cell signalling and maintenance of membrane fluidity. These molecules serve as precursors of insect hormones, pheromones and various cuticular hydrocarbons (CHCs) that govern insect development and behaviours. Although evidence for the importance of UFA is accumulating, how these compounds are synthesised and their impacts on the phenotype and metabolism of insects still require investigation.

Fatty acid desaturase (FAD) is a crucial enzyme in UFA biosynthesis that localises in the endoplasmic reticulum of the cell and utilises saturated fatty acids (e.g., C16:0, C18:0) synthesised by fatty acid synthase (FAS) to produce UFA. Desaturation reactions catalysed by desaturases produce different unsaturated products depending on the chain length of their substrate. FADs have also been classified into eight distinct subfamilies in termites. However, their specific functions, regulation mechanism and influences on the development and social behaviour of termites are still unclear.

Trophallaxis is a special behavior of termites and other social insects to maintain group stability. It is a phenomenon that social insects exchange substances in the digestive tract among group members. Trophallaxis is of great significance to the transmission of nutrients and symbionts, the promotion of insect growth and development, as well as grade regulation and behavioral differentiation.

In this study, we characterised the desaturase gene family in a termite *Coptotermes formosanus* and

investigated the function of FADs belonging to the first desaturase family (*DesatA1*, *DesatA2*, *DesatB*, *DesatC*, *DesatD* and *DesatE*). Our previous studies have shown that the survival rate of workers injected with ds*Cfor-desatA2-b* was lower than that of workers injected with ds*GFP* when the RNA interference desaturase gene *Cfor-desatA2-b* was fed with soldiers without any treatment. Unexpectedly, the survival rate of the soldiers injected with ds*Cfor-desatA2-b* was significantly lower than that of the soldiers injected with ds*GFP*. The soldiers had no ability to feed independently, they could only obtain the nutrition they needed through the worker's feeding, it is speculated that the worker's trophallaxis has an impact on the survival rate of the soldiers.

## Materials and methods

### **RNA preparation and qRT-PCR**

Total RNA was extracted from (1) the whole body of *C. formosanus* workers; and (2) the head, gut, fat body and cuticle of workers. After removing the head, the gut tissue and fat body, the remaining part was washed again in the ice-cold PBS solution. After all fat and muscle tissues were removed completely, the tissue-free cuticles were used for further RNA extraction. The reaction was performed as follows: 95 °C for 30 s, followed by 39 cycles at 95 °C for 10 and 60 °C for 30 s. Three independent biological replicates with three technical repetitions were prepared for each gene, and the relative expression was calculated using the  $2^{-\Delta\Delta C_t}$  method.

### **Preparation of dsRNA and RNA interference**

The DNA templates of *Cfor-desats* were amplified by PCR with genespecific primer sets containing T7 RNA polymerase recognition sequences and then purified. The dsRNAs were orally injected into workers with a FemtoJet 4i microinjection device by force-feeding method. Each individual received an injection of 200 ng of *Cfor-desats* dsRNA. Workers injected with GFP (ds*GFP*) and water were used as the control group. RNAi-treated worker and the control groups were reared together (worker: soldier = 50: 10) in a 90-mm Petri dish containing moist sands and filter paper. The expression level of each *Cfor-desats* was measured by RT-qPCR after injection. The survival rates of each group (three replicates per group) were monitored for 10 days after injection.

### **Fatty acid analysis in *C. formosanus***

Thirty RNAi-treated workers from eight groups (ds*Cfor-desatA1*, ds*Cfor-desatA2-a*, ds*Cfor-desatA2-b*, ds*Cfor-desatB-a*, ds*Cfor-desatB-b*, ds*Cfor-desatD*, ds*Cfor-desatE* and a control group treated with ds*GFP*) were selected and maintained in the dark ( $28 \pm 2$  °C, RH >80%) for five days. After the treatment period, the termites were collected and homogenised in liquid nitrogen for fatty acid extraction.

### **Determination of trophallaxis**

0.1 g of Nile Blue A was dissolved in 10 mL of ddH<sub>2</sub>O, and the filter paper was immersed in the solution. It was then used for termite feed. After RNAi, the termite samples were collected in a 1.5 mL centrifuge tube, and liquid nitrogen was added. The samples were fully grounded. The centrifuge tube was placed in a pre-cooled 4 °C centrifuge, centrifuged for 5 min, and the supernatant was transferred to a new centrifuge tube with a pipette. The liquid in the centrifuge tube was absorbed with a 1 mL disposable syringe, and the membrane was filtered and transferred to a new centrifuge tube. High performance liquid chromatography Waters 600 high performance liquid chromatography was used to detect the amount of Nile Blue A.

## Results and discussion

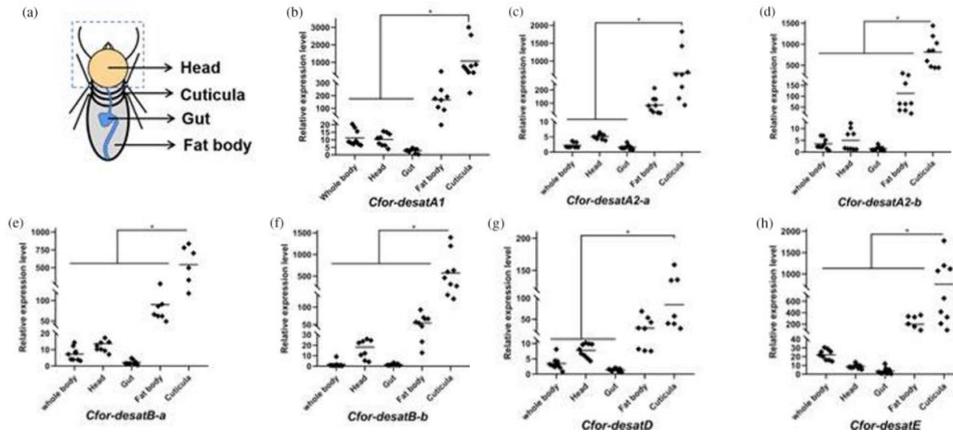
### **Comparison of *C. formosanus* desaturases**

We examined the expression levels of the seven desaturase genes in the head, gut, fat body and cuticle of workers (Figure 1a). Our analysis revealed that seven desaturase genes identified in *C. formosanus* were highly expressed in the cuticle and fat body (Figure 1 b- h). This result suggests that these desaturases may play a role in the biosynthesis of cuticular hydrocarbons. Overall, the expression pattern of *C. formosanus* desaturases indicated that these enzymes are mainly distributed in the cuticle and fat body of worker termites.

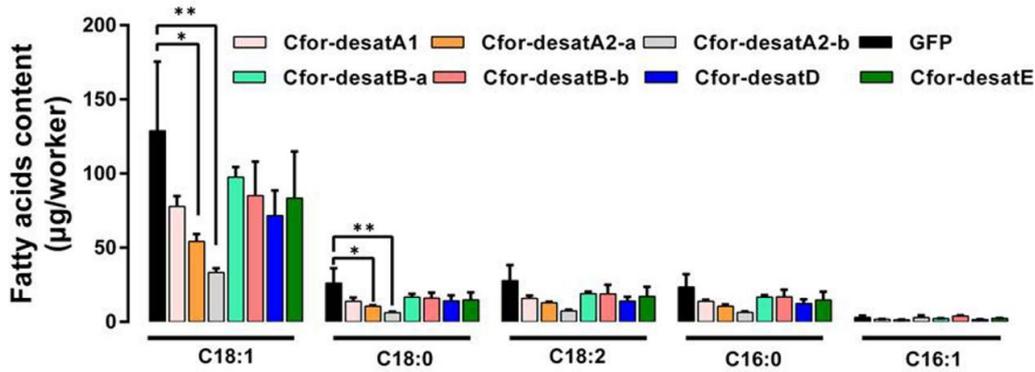
### **Influence of *Cfor-desats* suppression on fatty acid contents**

We further analysed the fatty acid content in ds*Cfor-desat* treated groups. Our result revealed that termites treated with ds*Cfor-desatB-b*, ds*Cfor-desatA2-b* and ds*Cfor-desatA2-a* exhibited the largest difference in fatty

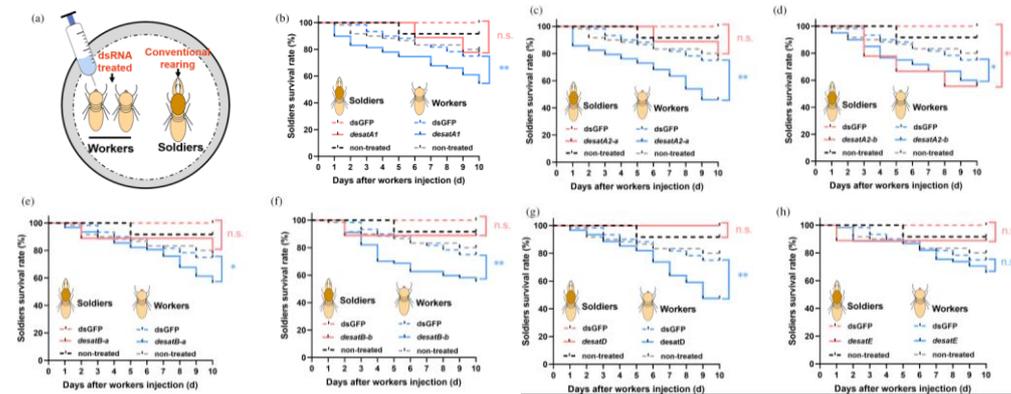
acid content when compared with those in the GFP-treated group (Figure 2). Other desaturases such as *Cfor-desatE*, *Cfor-desatB-a*, *Cfor-desatA1* and *Cfor-desatD* may have minor function in the fatty acid synthesis of termites.



**Fig 1.** The transcript levels of seven desaturase genes in different tissues of *C. formosanus* workers.



**Fig 2** The composition of fatty acids in *C. formosanus*.



**Fig 3.** Impacts of *Cfor-desats* on the survival rates of *C. formosanus* workers and soldiers. (a) Experimental design of this experiment. The worker injected with *Cfor-desatA1* (b), *Cfor-desatA2-a* (c), *Cfor-desatA2-b* (d), *Cfor-desatB-a* (e), *Cfor-desatB-b* (f), *Cfor-desatD* (g) and *Cfor-desatE* (h) respectively. *GFP* (ds*GFP*) and non-injection workers were used as control.

### *Influence of Cfor-desats suppression on the survival rate of C. formosanus*

The study analysed the survival rate of *C. formosanus* workers and non-treated soldiers 10 days after RNAi treatment (Figure 3a). The results demonstrated a significant decrease in the survival rate of workers in the ds*Cfor-desatA2*, ds*Cfor-desatB* and ds*Cfor-desatD* groups compared with the ds*GFP* group (Figure

3b- g). Interestingly, the survival rate of larvae treated with ds*Cfor-desatE* showed no significant difference from the control group (Figure 3h). Regarding the survival of soldiers, the majority of treatment groups (ds*Cfor-desatA1*, ds*Cfor-desatA2-a*, ds*Cfor-desatB-a*, ds*Cfor-desatB-b*, ds*Cfor-desatD* and ds*Cfor-desatE*) did not display a significant difference from the control group. However, soldiers that were reared together with workers that were treated with RNAi of *Cfor-desatA2-b* showed a significant higher death rate (Figure 3d).

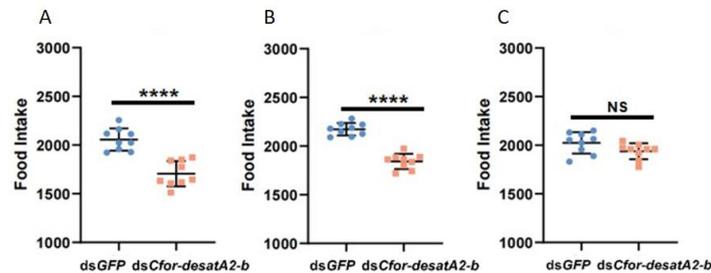


Fig.4 Nile Blue A amount in workers after RNAi. 24 h (A), 48 h (B), 72 h (C).

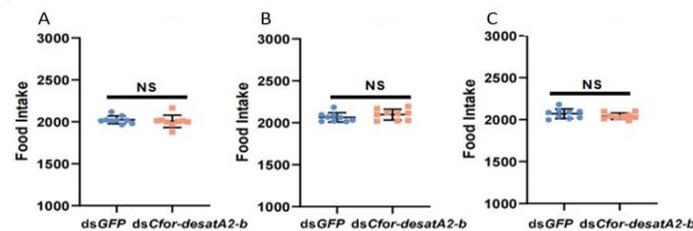


Fig. 5. Nile Blue A amount in soldiers. 24 h (A), 48 h (B), 72 h (C).

### Determination of trophallaxis

The filter paper was stained with Nile Blue A and used as feed for *C. formosanus* workers. The untreated soldiers and workers with *Cfor-desatA2-b* interference were reared together for 24 h, 48 h and 72 h. The amount of Nile Blue A was detected by HPLC. The amount of Nile Blue A decreased significantly after 24 h of interference (Figure 4 A - C ). However, there was no significant change in the amount of Nile Blue A consumed between soldiers rearing with RNAi treated workers and soldiers rearing with GFP treated workers at 24 h, 48 h and 72 h (Figure 5 A - C ). It showed that the composition of the feeding fluid changed.

### Conclusion

In this experiment, the transcriptome data of *C. formosanus* were screened, and 7 First desaturase family genes were screened. The dsRNA corresponding to 7 *Cfor-desats* genes was synthesized for microfeeding injection, and dsRNA interfered with the expression of desaturase in workers. It was confirmed by fluorescence quantitative PCR that the interference efficiency was still significant on the 3rd and 5th days. In the presence of normal soldiers, the survival rate of workers decreased more slowly than that of workers alone, but in the ds*Cfor-desatA2-a*, ds*Cfor-desatA2-b*, ds*Cfor-desatB-a*, ds*Cfor-desatB-b* treatment groups, the survival of all workers was significantly lower than that of the control group. The survival rate of untreated soldiers in the ds*Cfor-desatA2-b* treatment group also decreased significantly, and this phenomenon was supplemented and confirmed. The effect of desaturase gene *Cfor-desatA2-b* on the trophallaxis of *C. formosanus* was determined by dsRNA interference. The amount of filter paper consumed by workers decreased significantly after 24 h in the ds*Cfor-desatA2-b* treatment group. However, soldiers rearing with RNAi treated workers kept the same amount of dye at 24 h, 48 h and 72 h compared with control. It shows that the composition may change during trophallaxis.

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Tuesday | March 12, 2024 | 16:00-16:15

## Section 2: Physiology, Morphology, and Structure

**O\_06**

### **Analysing the changes in chemical profiles of ageing *Cryptotermes brevis* frass for use in termite management: Preliminary analysis**

by

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#### **Abstract**

Drywood termites are problematic timber pests worldwide and none more so than *Cryptotermes brevis*, the West Indian drywood termite. This is in part due to the cryptic nature of the insect making it hard to detect in structures and thereby easy to transport. Primary detections are commonly made by observations of the frass deposits they leave behind, often leading to fumigation of the building to effectively eradicate the pest. However, this presents a problem after treatment as the frass is often later “kicked out” by other insect species. Therefore, when frass is seen after treatment, it is challenging to determine if further action is needed as the building is reinfested or if it is simply old frass being removed from the nests. This leads to a number of wasted fumigations and associated costs or non-treatment of buildings, leading to increases in the termite population. The work described here tests the idea of using the chemical profiles of frass as a method to reliably age a sample and distinguish the old from the new. Using hexane extraction and gas chromatography-mass spectrometry on West Indian drywood termite frass samples that were left to age for various times (between 0-22 months), the resulting profiles of the age groups were compared. Differences in these profiles were seen between fresh frass (under 6 months) and those aged over 12 months, with frass in between undistinguishable from either group. The eight compounds responsible for over 70 % of these differences were identified as targets for any technique developed to age the frass samples in the field. Overall, the use of chemical profiles in *C. brevis* frass has merit in the development of a technique to age newly discovered frass deposits and is likely a useful tool in the management of this timber pest to reduce the occurrence of unneeded fumigations in the future.

**Keywords:** Pest management, chemical analysis, fumigation

#### **Introduction**

Drywood termites cause massive damage to timber in service globally, none more so than *Cryptotermes brevis* (Walker), the West Indian drywood termite, commonly recognised as the most destructive drywood termite in the world (Scheffrahn et al., 2009). The species’ capacity to resist desiccation and their cryptic lifestyle creates a remarkable ability to be transported as a hitchhiker pest (Horwood, 2008). The invasive introduction of the termite species has brought huge costs in repair and control measures. Although global estimates are challenging, drywood termites are thought to be responsible for around 20 % of the \$40 billion USD spent annually on termite control (Rust & Su, 2012), with many countries reporting millions of dollars annually on *C. brevis* management alone. The most affected countries are the US, Australia, Spain, South Africa and numerous Pacific and Atlantic islands (Najjari et al., 2023).

A large part of the impacts of *C. brevis* and other drywood termites come from the management costs in attempts to eradicate the infestations and limit losses. The most effective method of extermination is fumigation of the structure, commonly using sulfuryl fluoride due to high mortality rates (Osbrink et al., 1987). While this solution is effective at killing termites, it runs into several issues when applied in

management plans. In particular, the cost of treatment can be substantial, especially in countries with few established contractors, where prices will run into the tens of thousands of dollars (Horwood, 2008). There is also a disturbance problem, as families will have to move out of their homes during treatment. These two factors alone may prevent individuals from proceeding with fumigation, enabling the termites to propagate within and beyond the original structure. Other management solutions are available and are currently being developed in Australia to address this issue, such as the use of spot treatments (e.g., Hassan & Fitzgerald, 2023) utilising lethal heat or gas/liquid-propelled termiticides to individually remove colonies of *C. brevis*. The essential factor to consider with all current treatment plans is they do nothing to prevent reinfestation of the same structure by nearby colonies. This results in buildings being reinfested by termites in subsequent years due to migration from adjacent infestations that were not identified. The issue of multiple infestations and thus treatments on the same structures exacerbates the impact that *C. brevis* has on a location.

The cryptic nature of this species means the termite is rarely seen even in extensive infestations. Instead, the colonies are normally identified due to the presence of frass deposits (Oi et al., 2008). This frass is characteristic to a species level with *C. brevis* (Bobadilla et al., 2020), making it an effective and non-invasive detection method, however, reliance on this method at the early stage of infestation and post-treatment may cause an issue (Haigh et al., 2022). It is common for *C. brevis* colonies to store large amounts of frass within the nest architecture and after eradication of the termites, it is also common for other insect species, such as ants, to inhabit the empty nests. This leads to old frass deposits being pushed out of the nests and creating the distinctive piles that were first used to detect the termite. It is thus difficult post-fumigation to distinguish whether a newly seen pile of frass is created due to other insect movement or ongoing termite feeding, signifying treatment failure or re-infestation. The ability to evaluate a frass deposit for age would reveal if it was created before or after treatment was applied and, consequently, if further action is needed.

One potential method that could be utilised to achieve this is the analysis of the compounds that are present on the frass itself. Chemical profiles of frass of different ages can be compared to assess for significant differences. Similar work has been done on other termite species, Lewis et al. (2010) investigated the changes in hydrocarbons over time on the frass of *Incisitermes minor*, a closely related drywood termite. They concluded that the chemical profiles did alter significantly as it ages. If this time-related change holds true for the frass of *C. brevis*, this knowledge could be used to assess the age of a discovered frass sample. The work presented here investigates the use of this idea, building on preliminary investigations done at the Ecosciences Precinct, Brisbane, Australia (Hayes et al., 2021). It is hoped that the data gained from this trial will lead to a reduced incidence of unnecessary fumigations, a lower requirement for localised treatments and a decreased economic burden of *C. brevis* where the termite is established.

## **Materials and methods**

### ***Termite collection***

West Indian drywood termites were collected from a well-established infestation in Maryborough, Queensland, Australia. The species was confirmed by frass examination and infested materials, such as floorboards, doors and panels, were transported back to the laboratory at the Ecosciences Precinct, Brisbane using protocols approved by Biosecurity Queensland. Here, they were kept in controlled environmental conditions (25 °C, 75 % R.H.) before termites were manually extracted from the wooden material using a chisel. Collected colony members were maintained on veneers of hoop pine (*Araucaria cunninghamii*) for a minimum of four weeks to ensure any other material in their guts had passed through.

### ***Frass collection and ageing***

Acclimated termites were then moved to fresh veneers of hoop pine within plastic containers and sorted into colonies consisting of 100 members (one male and one female reproductive and 98 pseudergates). Three replicate colonies were formed with hoop pine of different thicknesses to allow natural variety to the colony construction between groups. Colonies were left undisturbed in the dark. Approximately once a month, the containers were opened and all frass produced by the termites was collected manually using a fine paintbrush. Samples were weighed, labelled in open plastic trays, and maintained under the same conditions as the termites from collection until analysis to imitate natural ageing. Collection of frass occurred in this way for over 22 months, with 19 sampling events for each colony.

### ***Chemical extraction and sampling***

After ageing samples, the chemicals on the frass were extracted with a solvent. Samples were placed into hexane (1 mL) and mixed for 24 hours on a shaker table. The solvent was then filtered off, evaporated

to dryness at ambient temperature under nitrogen gas and then redissolved in hexane (100  $\mu$ L). These extracts (3  $\mu$ L) were analysed by gas chromatography-mass spectrometry (GC-MS) (Agilent 6890 & Agilent 5975 respectively). The GC-MS conditions were optimised based on Haverty et al. (2005) and were as follows: inlet temperature 250 °C, carrier gas helium at 15 cm s<sup>-1</sup>, split ratio 13:1, transfer-line temperature 280 °C, initial temperature 40 °C, rate 1: 40 °C min<sup>-1</sup> to 200 °C, rate 2: 3 °C min<sup>-1</sup> to 300 °C, final time 11 min. The MS was held at 280 °C in the ion source, with a scan rate of 2.40 scans s<sup>-1</sup>. Resulting peaks were assessed and identities were tentatively assigned using the National Institute of Standards and Technology (NIST) mass spectral library.

### Analysis

Chemicals found in the samples were recorded and compared between treatments, with age classes (old, medium and fresh (see Table 1.)) being assigned to aid comparisons. The GC-MS data were square root transformed and compared using a Bray-Curtis similarity matrix. Samples were compared using non-metric multidimensional scaling ordination (nMDS) and Analysis of Similarity (ANOSIM). Similarity Percentages (SIMPER) analyses were also used to highlight the contribution of each compound to the difference seen between age classes. All analyses were conducted using PRIMER software (V 7.0.13).

**Table 1.** Time of *C. brevis* frass ageing prior to analysis and mass of frass collected (mg).

Age class	Age range (days)	Colony 1 mass	Colony 2 mass	Colony 3 mass
Fresh	0 – 39	28.8	48.2	28.1
	40 – 66	17.8	54.5	25.3
	67 – 105	16.7	36.2	2.00
	106 – 133	23.3	0.20	2.60
	134 – 165	11.0	3.90	5.60
	166 – 196	41.1	47.9	2.10
Medium	197 – 228	54.1	19.9	21.9
	229 – 259	30.7	4.60	19.1
	260 – 288	110.3	12.3	11.9
	289 – 312	10.9	36.0	5.80
	313 – 353	31.2	239.3	70.1
	354 – 381	213.3	3.90	85.0
Old	382 – 416	68.4	3.20	25.2
	417 – 442	50.8	2.90	5.40
	443 – 499	35.6	14.4	228.2
	500 – 533	53.7	42.4	48.1
	534 – 567	56.8	62.9	139.7
	568 – 599	30.6	31.8	119.9
	600 – 690	255.5	183.3	453.3

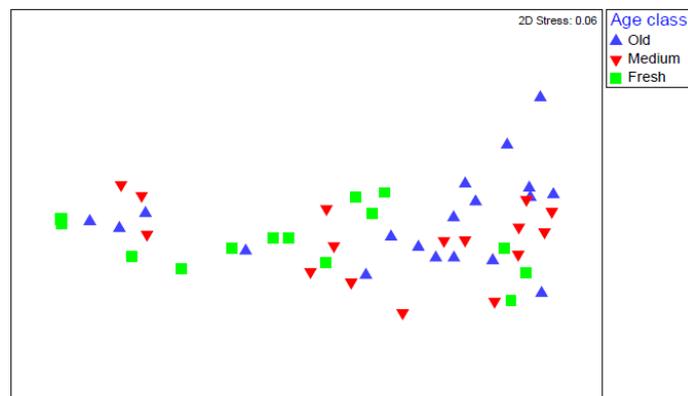
## Results and discussion

Compounds identified by GC-MS analysis of the frass extracts are shown (Table 2). The compounds shown were detected in many samples and therefore provided the best targets for comparisons between age groups.

A nMDS plot (Figure 1) shows that some differences exist between age classes, though a fair amount of overlap in the data can be seen. The Analysis of Similarity confirmed significant differences between age groups (Global R = 0.067, P = 0.047). Pairwise tests showed only fresh frass differed significantly from old frass, with no difference between old and medium frass, and also not between fresh and medium frass. This finding agrees with previous work (Haverty et al., 2005), that suggested changes can only be measured after an extended ageing time. This result means that the technique has limits, only being able to distinguish frass older than 12 months from fresh frass. Management strategies using this technique must account for this when determining when to sample the frass.

**Table 2.** Mean ( $\pm$  SEM) relative percentage area of compounds tentatively identified in *C. brevis* frass (and the percentage of samples of that age class containing the compound) across three age classes: old 12+ months, medium 6-12 months and fresh 0-6 months.

Ret time (min)	Compound	Old (12+ months) 19 results	Middle (6-12 months) 16 results	Fresh (0-6 months) 16 results
6.55	tetradecanal (C14-al)	0.16 $\pm$ 0.06 (32)	0.33 $\pm$ 0.12 (47)	0.04 $\pm$ 0.04 (5)
10.05	heptadecanal (C16-al)	0.04 $\pm$ 0.03 (16)	0.05 $\pm$ 0.03 (16)	0.02 $\pm$ 0.02 (5)
17.13	nonadecane / eicosane	0.35 $\pm$ 0.11 (42)	0.18 $\pm$ 0.10 (32)	0.02 $\pm$ 0.02 (5)
17.79	pentadecanal (C15-al)	0.07 $\pm$ 0.03 (26)	0.04 $\pm$ 0.03 (5)	0 (0)
18.48	7-hexyldocosane	2.73 $\pm$ 0.63 (74)	1.26 $\pm$ 0.50 (47)	0.61 $\pm$ 0.21 (32)
19.32	pentacosane (C25)	19.17 $\pm$ 2.69 (79)	19.35 $\pm$ 2.92 (68)	14.25 $\pm$ 3.06 (58)
20.92	3-ethyltetracosane	5.73 $\pm$ 0.88 (79)	3.33 $\pm$ 0.74 (68)	2.16 $\pm$ 0.65 (47)
21.50	hexacosane (C26)	2.31 $\pm$ 0.35 (79)	1.84 $\pm$ 0.34 (63)	0.58 $\pm$ 0.31 (21)
22.27	oxygenated hydrocarbon	0.41 $\pm$ 0.24 (42)	0.23 $\pm$ 0.09 (37)	0 (0)
23.73	heptacosane (C27)	29.74 $\pm$ 2.93 (100)	29.61 $\pm$ 3.10 (84)	21.59 $\pm$ 4.40 (63)
25.31	9-octylhexacosane	0.31 $\pm$ 0.07 (53)	0.35 $\pm$ 0.10 (42)	0.12 $\pm$ 0.07 (16)
28.06	octacosane (C28)	1.71 $\pm$ 0.28 (74)	1.40 $\pm$ 0.26 (63)	0.46 $\pm$ 0.24 (21)
32.37	cholesterol	0.11 $\pm$ 0.09 (16)	0.20 $\pm$ 0.09 (37)	0.20 $\pm$ 0.11 (16)
32.54	cholestanol	0.26 $\pm$ 0.11 (42)	0.25 $\pm$ 0.13 (37)	0.13 $\pm$ 0.10 (11)
33.77	steroid 1	14.61 $\pm$ 2.98 (95)	17.76 $\pm$ 3.84 (84)	22.91 $\pm$ 3.11 (84)
36.56	stigmastanol	1.00 $\pm$ 0.31 (79)	0.43 $\pm$ 0.28 (42)	0.27 $\pm$ 0.13 (21)
37.55	steroid 2	14.24 $\pm$ 2.64 (100)	14.75 $\pm$ 2.44 (84)	25.66 $\pm$ 3.89 (84)
42.07	steroid 3	6.68 $\pm$ 1.18 (100)	8.37 $\pm$ 1.50 (84)	10.90 $\pm$ 1.87 (84)



**Fig 1.** Non-metric multidimensional scaling ordination showing distribution of samples from *Cryptotermes brevis* frass. Each point in the ordination represents an individual sample, with age classes demonstrating significant difference with overlap.

Eighteen compounds were identified in frass extracts (Table 2), however the changes in relative area of these compounds between groups varied. A Similarity Percentages (SIMPER) analysis determined the seven compounds that account for at least 70 % of these differences (Table 3)

**Table 3.** The seven compounds identified from *Cryptotermes brevis* frass that account for at least 70% of the differences between age class samples.

Compound name	Contribution to dissimilarity (%)	Cumulative (%)
heptacosane (C27)	14.24	14.24
pentacosane (C25)	13.79	28.03
steroid 2	11.40	39.43
steroid 1	11.21	50.64
3-ethyltetracosane	8.49	59.13
steroid 3	7.83	66.97
hexacosane (C26)	6.34	73.31

While these seven compounds contributed to at least 70 % of the differences between frass chemical profiles of varied ages, it is worth noting that just four compounds (heptacosane, pentacosane, steroid 2 and steroid 1) contributed more than 50 % of the difference alone. For a real-world application of these results, this is the most important finding as it is these chemicals that would be the target for any simplified method of sampling the compounds in frass deposits since the analytical technique used here is generally inaccessible to most homeowners and pest inspectors.

## Conclusion

The results described here provide a promising basis for the idea of using the chemical profiles of *Cryptotermes brevis* frass as a method to age the samples. The pattern of chemical compounds extracted from the frass changes significantly as it ages, specifically with older frass (12+ months) having noticeable differences to fresh frass. Further work to develop a simple assay that can be deployed in the field to measure the seven (or perhaps just four) key compounds highlighted in this work and produce a resulting age class for the sample is warranted. Rigorous field testing of any such tool to confirm its efficacy would provide those working in this field an easy way to age a discovered frass sample.

The work completed here affords evidence that this method of determining the approximate age of a termite frass sample serves as a suitable solution to the specific problem of newly found frass deposits post-treatment of a building. It is hoped that the development of these ideas will lead to an eventual solution that prevents unnecessary fumigations where *C. brevis* is no longer present and can save afflicted individuals cost and disturbance from this devastating timber pest.

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Tuesday | March 12, 2024 | 16:15-16:30

## Section 2: Physiology, Morphology, and Structure

O\_07

### Gross analysis of the hair-type sensory receptors distribution on the antennae of an open-column termite *Hospitalitermes* sp. (Nasutitermitinae, Blattodea)

by

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#### Abstract

Foraging tunnel is necessarily built by subterranean termites to travel towards the available resources. However, some termite groups have different mechanisms as they do not rely on the foraging tunnel. Open-column foraging termites have a particular mechanism to build invisible tunnels based on chemical signals and physical barriers. The soldier caste has a particular role in the open column foraging behavior by establishing soldier walls on both lateral sides of the marching workers. Hence, open-column termite soldiers may encounter various threats in an open environment. Apart from sensing the chemical signals from the trail pheromone by using the antennae as sensory organs, various airborne vibrational stimuli may also circle around their foraging line. Until now, the study of termite soldier sensitivity to airborne vibration is limited. In this study, the observation of sensory receptors on the antennae of open-column termite (*Hospitalitermes* sp.) was conducted. Roughly, three types of hair-type sensory receptors (chaeticum, trichodeum, basiconicum) were classified into two functionalities based on the structure of the sensory receptors (mechano-chemotactile and olfactory). By comparing to subterranean soldiers in other references, open-column foraging termite soldiers had more abundant mechano-chemotactile receptors on their antennae. The distribution of the mechano-chemotactile receptors was also more scattered. The structure of each sensory receptor was also discussed in detail. The high number of mechanoreceptors on the antennae of open-column termite soldiers might reflect the importance of antennae in detecting various airborne vibrational inputs from the open environment.

**Keywords:** Defensive caste, functional morphology, electron microscopy, sensilla, sensory system

#### Introduction

The existence of biotic and abiotic threats may underly morphological adaptation in insects (Patterson 1984, Bernays et al. 1991, Scheffrahn et al. 1998). The need to overcome predator and competitor threats affected the jaw and head size and shape alteration in some social insects (Grüter et al. 2012, Wills et al. 2014). Morphological adaptation in termites is also observed in their defensive caste across genera. Some termite soldier's defensive organs are specialized to have phragmotic and projecting (nasute) head, twisted mandibles, and sensory system modification reflected by a variety distribution of body hair (setae) (Lubin and Montgomery 1981, Scheffrahn et al. 1998, Matsuura 2002, Kuan et al. 2020, Wikantyo et al. 2022).

The profiling of sensory receptors on the antennae of some closed-column foraging termites (CCS) has been well studied. In *Coptotermes formosanus*, Several types of sensory receptors on the antennae belong to sensilla chaeticum, trichodeum, basiconicum, capitulum, which are included in the hair-type sensory receptor, and campaniforma which is included in the plate-type sensory receptor (Deng et al. 2006, Yanagawa et al. 2009, Fu et al. 2020). In an open-column foraging termite species (OCS), however, there is still limited

information regarding the sensory receptor's information on the antennae. The differences of habitat and foraging behavior might also affect the distribution of sensory receptors on the antennae as the defensive apparatus (peripheral sensory system) along with the head and mandibles (Scheffrahn et al. 1998, Koshikawa et al. 2002, Matsuura 2002, Ishikawa et al. 2007, Seid et al. 2008, Kaji et al. 2016, Kuan et al. 2020).

In this study, we performed the gross analysis of hair-type sensory receptors distribution on the antennae of OCS *Hospitalitermes* sp. The analysis was conducted by utilizing electron microscopy analysis. We classified the putative function of each type of sensory receptor by observing the structure of the cuticle.

## Materials and methods

### *Open-column termite specimens*

Specimens of the OCS was collected in 2017. The specimens belong to the genus *Hospitalitermes*. It was collected in Botanical Garden of Kuningan, West Java. The specimens were kept in 70% alcohol for morphological identification and analysis. Three specimens were used in this study (3 pair of antennae). The remaining collections were stored in National Research and Innovation Agency (BRIN), Cibinong, Indonesia.

### *Electron microscopy analysis*

Specimens were cleaned up by using ultrasonic cleaner in cacodylate buffer in 5 min. Solution of 2,5% glutaraldehyde were used to further fix the specimens overnight. After the prefixation, the specimens were soaked in 2% tannic acid overnight. Specimens were prepared to go through serial dehydration protocol (50%, 70%, 85%, 95%, and absolute alcohol) in 4°C. Platinum sputtered coating was applied to the specimens before analysis with JSM-IT200 scanning electron microscope (JEOL, Tokyo, Japan) at a 5kv accelerating voltage.

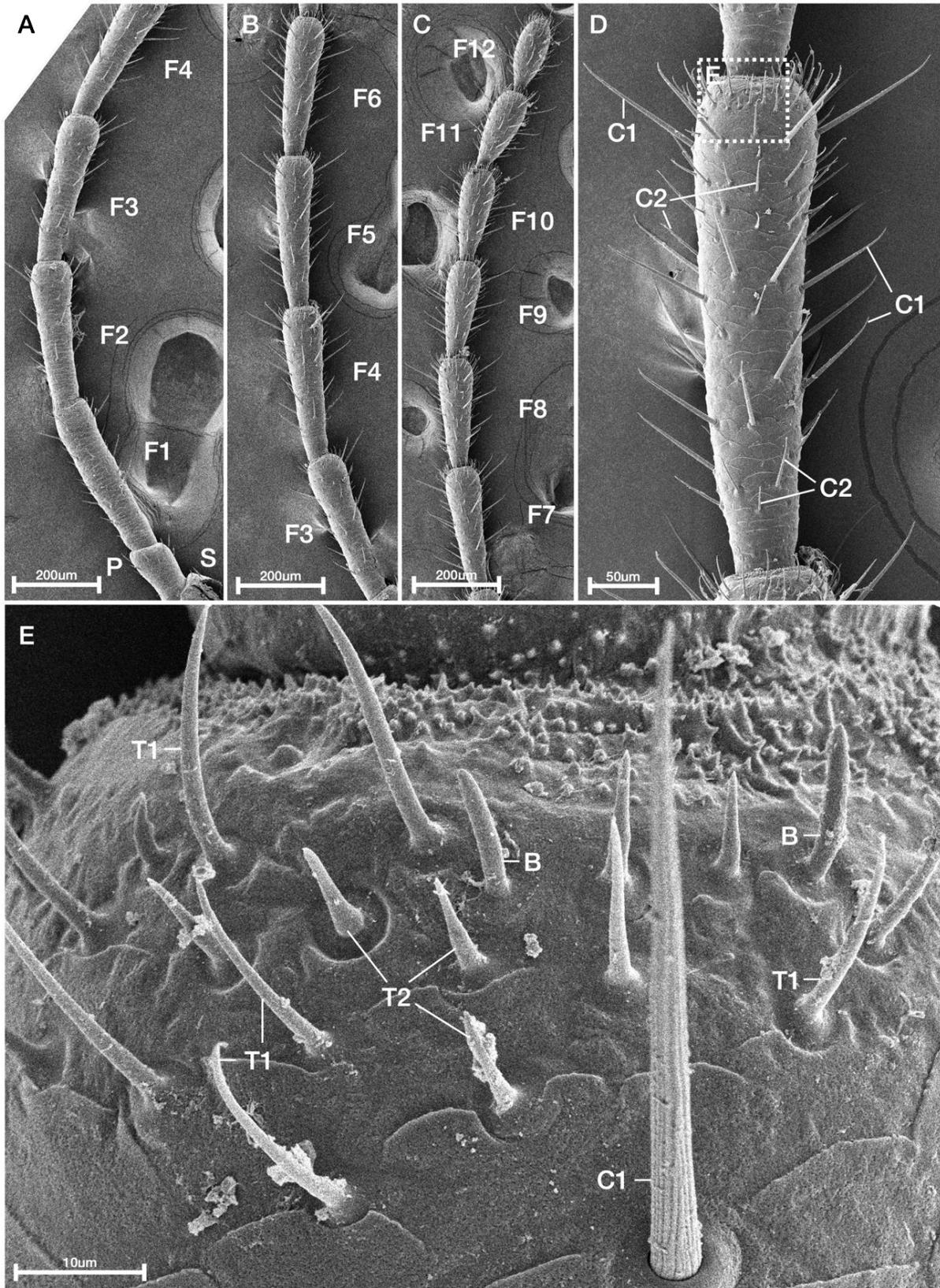
### *Sensory receptors classification*

The sensory receptors were classified according to the respective references (Altner et al. 1978, Altner and Prillinger 1980, Altner et al. 1981, Altner and Loftus 1985, Yanagawa et al. 2009, Fu et al. 2020).

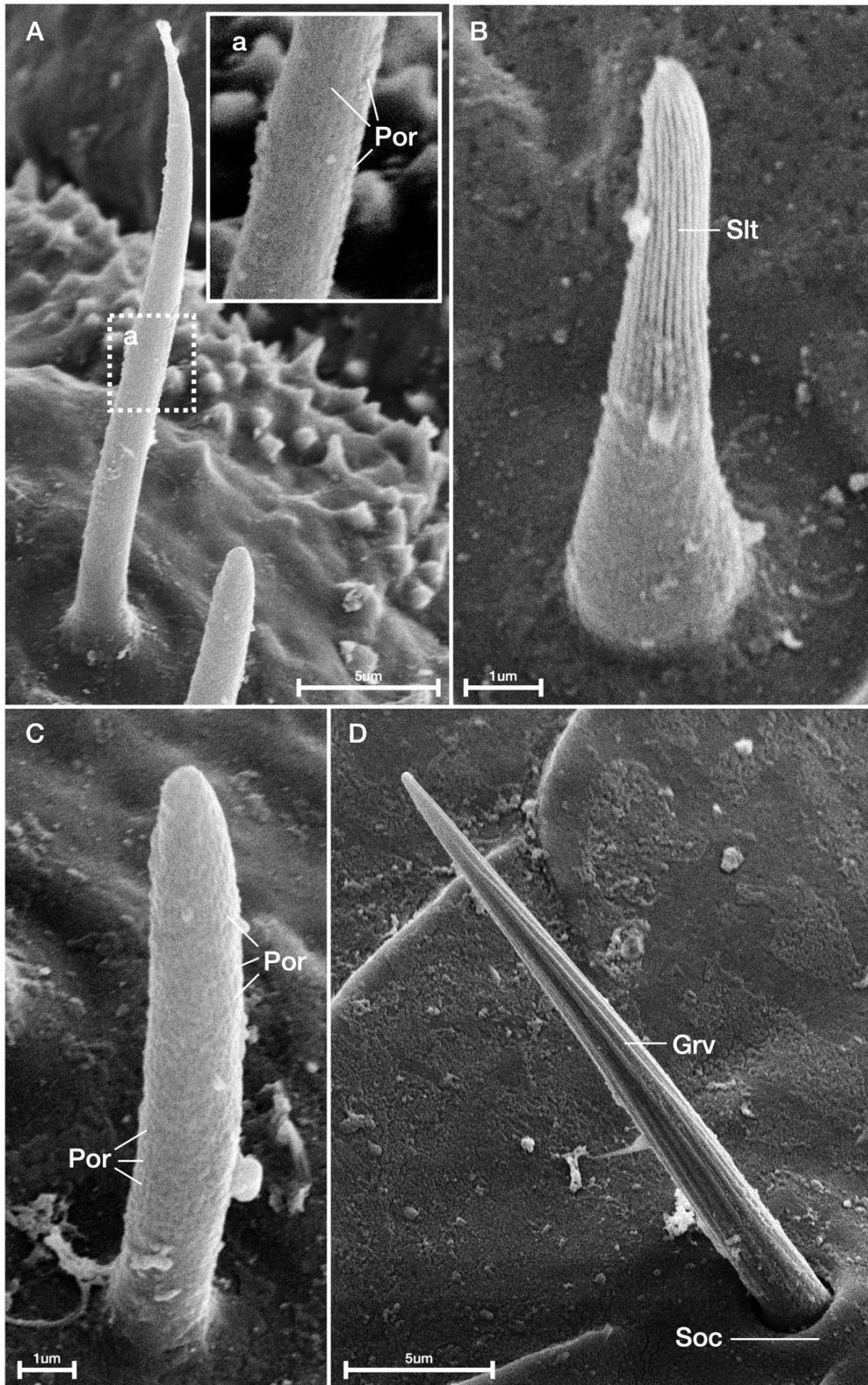
## Results and discussion

The results showed the antennae of the OCS has 12 flagellar segments with several types of sensory receptors, such as chaeticum, trichodeum, and basiconicum as hair-type sensory receptors (Fig. 1). Campaniforma was also observed as a plate-type sensory receptor. In this study, two types of chaeticum was differentiated by the length of the sensory receptors. Chaeticum 1 as the longest sensory receptor (>70µm) and chaeticum 2 as the shorter one (<40µm), filling the space among chaeticum 1. Chaeticum sensory receptor has structures that support the mechano-chemotactile (MCT) reception (Yanagawa et al. 2009, Fu et al. 2020, Wikantyoso et al. 2022). The availability of sockets circling the receptor peg explained the flexibility potential of the sensory receptor. Although it was not obvious in this study, this type of sensory receptors has terminal pore at the tip of the peg (Altner and Prillinger 1980, Yanagawa et al. 2009). The length of the chaeticum 1 showed the longest touching range among the other sensory receptors, explained the higher chance for chaeticum 1 to touch substrates or other medium. The chaeticum 2 has shorter length with also flexibility potential may detect other mechanical signal that does not need long touching range but has bigger energy, such as airborbe vibrational stimulus. Short mechanoreceptor in Crickets needs stronger input to be able to sensed rather than the longer mechanoreceptor (Kumagai et al. 1998).

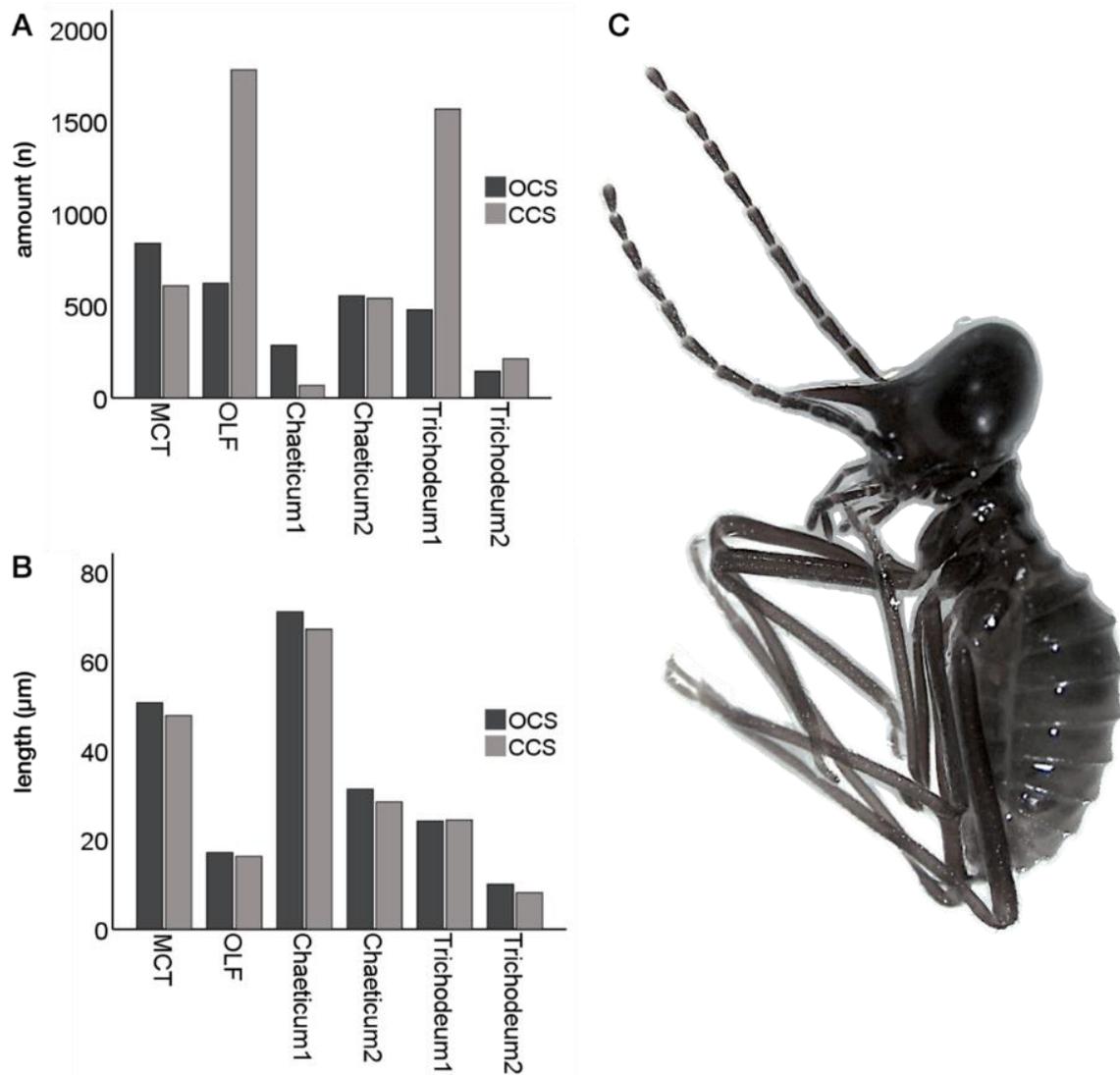
Trichodeum sensory receptor was also observed on the antennae of OCS. Two types of trichodeum were observed. Both of them have structures that support the olfactory (OLF) reception. The cuticle has multiple pores or slits as the potential structure of OLF functionality (Fig. 2). The base of the peg was not flexible. Trichodeum 1 had longer peg (around 25µm) and multiple pores scattered along the cuticle wall. Trichodeum 2 had shorter peg (aroung 15µm) and slits structure on the two third of distal peg. The pores and the slits have similar function by letting any airborne molecules in particular size to penetrate the cuticle and were sensed by the dendritic elongation inside the peg lumen (Altner et al. 1977, Altner and Prillinger 1980, Zacharuk 1980).



**Fig 1.** The antennae of OCS *Hospitalitermes* sp. A–C: antenna with 12 flagellar segments, D: The 5<sup>th</sup> flagellar segment, E: distal part of the flagellar segment. B: basiconicum, C: chaeticum, F: flagellar segment, P: pedicel, S: scape, T: Trichodeum



**Fig 2.** The profile of sensory receptors. A: Trichodeum 1 with multiple pores, B: Trichodeum 2 with slits structure, C: Basiconicum with multiple pores, D: Chaeticum 1 or 2 with a socket. Grv: longitudinal groove, Por: pore, Slit: slit, Soc: socket.



**Fig 3.** The distribution and length of the sensory receptors. Data comparison was taken from Yanagawa (2009). A: the number of each sensory receptor, B: the length of each sensory receptor, C: representation of *Hospitalitermes* sp.

If the sensory receptors number in this study was compared to the results of Yanagawa (2009), the obvious differences between the OCS (*Hospitalitermes* sp.) and the CCS (*C. formosanus*) are OCS possessed higher number of MCT receptors and lower number of OLF receptors, while CCS in the other hand possessed higher number of OLF receptors and lower number of MCT receptors (Fig. 3). In insects, chemosensation is mostly utilized by using volatile pheromone that could be sensed by olfactory system over long distance. The small body size might limit the ability to apprehend visual and auditory signal (Wertheim et al. 2006, McKinney et al. 2015). In termites, several pheromones in *Trinervitermes bettonianus* and *Nasutitermes costalis* have been suggested to be volatile and provide a long-lasting orientation cue (Oloo and Leuthold 1979, Traniello 1982). However, the depletion of the OLF receptors on OCS soldier's antennae might help the localization of the detection of their own volatile pheromone. Thus, soldiers could focus on the possible closest threats and may have elevate the boldness to guide the worker caste march out of the nest and collect food. While the higher MCT receptors might be needed by the soldiers of OCS to evaluate the airborne vibrational signals from their surroundings. As soldier's antennae could sense airflow (Wikantyo et al. 2023), wind velocity was evaluated by subterranean soldiers before they give the green light to the alate to swarm out (Leong et al. 1983, Watson and Gay 1991, Sugio and Miyaguni 2019). Further detail analyses are needed to confirm the function of each sensory receptor and provide deeper distribution understanding.

## Conclusion

Roughly, three types of hair-type sensory receptors (chaeticum, trichodeum, basiconicum) were classified into two functionalities based on the structure of the sensory receptors (mechano-chemotactile and olfactory). By comparing to subterranean soldiers in other references, open-column foraging termite soldiers had more abundant mechano-chemotactile receptors but lower olfactory receptors on their antennae. The distribution of the mechano-chemotactile receptors was also more scattered on each segment of the flagellomere. The high number of the mechanoreceptors on the antennae of open-column termite soldiers might reflect the importance of antennae to detect various airborne vibrational inputs from the open environment.

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Wednesday | March 13, 2024 | 09:00-09:15

## Section 2: Physiology, Morphology, and Structure

O\_08

### Diets Rich in Nitrogen Enhance Termite Reproductive Performance on Both Primary and Secondary Reproductive Castes

by

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#### Abstract

Nitrogen is a crucial nutrient for termite growth and reproduction, but nitrogen is limited in their primary food resource. In this study, we investigated the impact of a high-nitrogen food source on reproductive ability. We fed termites with a high nitrogen food source, spent mushroom substrates (SMS), to replace a comparatively low-nitrogen food source, peat moss soil for six months. Two types of reproductive castes were studied: primary reproductive, king and queen, of *Coptotermes formosanus* Shiraki and *Coptotermes gestroi* (Wasmann) and secondary reproductive, neotenic, of *Prorhinotermes flavus* Bugnion and Popoff. The assessment of reproductive ability involves metrics such as the increase in the total number of individuals, larvae, eggs, and neotenics, as well as the weight gain of the king and queen. Furthermore, we estimated the weight loss of nitrogen and carbon content in peat moss soil, SMS, and wood through the 6-month experimental period. The results demonstrated a positive impact of the high-nitrogen food on the numbers of total individuals and larvae in *C. gestroi*, and the numbers of larvae and eggs in *P. flavus*. Additionally, the weight of the queen significantly increase in both *C. formosanus* and *C. gestroi*. This study proves that high-nitrogen diets stimulate termite reproductive ability.

**Keywords:** High-nitrogen food source, spent mushroom substrates, *Coptotermes formosanus*, *Coptotermes gestroi*, *Prorhinotermes flavus*

#### Introduction

Nitrogen plays a crucial role in the growth, reproduction, and survival of termites. The nitrogen ratio in the termite body is around 8% to 13% (Higashi et al., 1992). However, the primary food resource for wood-feeding termites is nitrogen-limited, ranging from approximately 0.03% to 0.15% (Cowling and Merrill, 1966). Kakkar et al. (2017) suggested that workers of *Coptotermes formosanus* Shiraki tend to return to the central nest, where reproductive castes and larvae are located, to molt and recycle nitrogen from the exuviae. According to the study by Brent and Traniello (2002), enhanced dietary nitrogen has a significant effect on the reproductive maturation of the termite *Zootermopsis angusticollis*. This effect manifests in significant increases in the number of ovarioles and fecundity for neotenic females. These studies highlight the importance of nitrogen to termites, but the influence of nitrogen on an incipient colony remains unknown.

The reproductive castes of termites can be classified into primary reproductive castes (king and queen) and secondary reproductive castes (male and female neotenics) (Thorne, 1996). Typically, termite colonies are established by a single pair of alates following their dispersal flight. In the case of the Formosan subterranean termite, *C. formosanus*, and the Asian subterranean termite, *Coptotermes gestroi* Wasmann, both incipient colonies are initiated by a pair of dealates, with queens serving as the primary reproductive castes. Mature colonies of these species can reach sizes of several million individuals. As queens age or die, some workers or nymphs may undergo molting to become secondary reproductive castes, neotenics, to assume the reproductive role of the queen.

The oceanic termite genus, *Prorhinotermes*, is commonly found in tropical islands (Krishna et al., 2013), with documented instances of numerous neotenic individuals (Bordereau et al., 2002). The number of neotenic in *Prorhinotermes flavus* Bugnion and Popoff can be as high as 12.9%, as observed in Lanyu Island, Taiwan (Li et al., 2011). The colony size of *P. flavus* ranges from 40 to 6343 individuals (Chiu, 2022).

In this study, our objective is to investigate whether reproductive ability increases when termites consume a high-nitrogen food. The assessment of reproductive ability is conducted through various metrics, including the increase in the total number of individuals, larvae, eggs, and neotenic. Additionally, the weight gain of the king and queen is also measured. We test whether the nitrogen content of the food source affects reproduction of both primary and secondary reproductive castes in the same way. *C. formosanus* and *C. gestroi* are employed to represent the alate reproductive condition, and *P. flavus* is representative of the neotenic reproductive condition.

## Materials and methods

### Termite

To observe the king and queen, whole colonies of both *C. formosanus* and *C. gestroi* were used in the study. These colonies contain 165–542 individuals, which were raised from one pair of alates in the laboratory for 15 months before testing. To observe neotenic, three colonies of *P. flavus* (TW4672, TW7247, and TW7956) were collected from tree trunks and wood debris in the Siangjiao Bay Ecological Reserve Areas, Kenting, Pintung, Taiwan, in 2017 and 2018, and then raised in the laboratory. For each trial of *P. flavus*, 100 individuals (10 neotenic, 20 soldiers, and 70 pseudergates) were used.

### Food Source

Termites fed on wood and soil (low-nitrogen group) or wood and spent mushroom substrates (SMS, high-nitrogen group) for 6 months. The wood are slabs (8.5 by 13 by 0.3 cm) of radiata pine (*Pinus radiata* D. Don). The soil is peat moss soil (T053B200, Klasmann, Germany). SMS is the leftover material or medium used for cultivating *Lentinula* mushrooms. SMS consists of a mixture of organic materials such as agricultural residues, straw, and other plant-based components. Nitrogen and carbon content of the wood, soil, and SMS were analyzed by elemental analysis (elementar vario EL cube, Germany). Before the trials, the nitrogen content of the wood is 0.09%, that of peat moss soil is 0.91–0.95%, and that of SMS is 1.22–2.24%. For the trials of *C. formosanus* and *C. gestroi*, each setup contained 15 wood slabs and 150 g of soil (low-nitrogen group) or SMS (high-nitrogen group) in dry weight. For the trials of *P. flavus*, each setup contained 3 slabs of wood and 50 g of soil (low-nitrogen group) or SMS (high-nitrogen group) in dry weight.

### Experimental design and data collection

A total of 12 trials of *C. formosanus* were tested (2 nitrogen-concentration food x 6 colonies). A total of 12 trials of *C. gestroi* were tested (2 nitrogen-concentration food x 6 colonies). A total of 12 trials of *P. flavus* were conducted (2 nitrogen-concentration food x 3 colonies x 2 replications). To calculate the population growth, the total number of individuals in each trials were counted before and after testing. To calculate reproductive rates, the total number of larvae in all trials and that of *P. flavus* eggs were counted before and after trials. The number of neotenic in *P. flavus* were counted before and after the trials. The body weight of the king and queen of *C. formosanus* and *C. gestroi* was measured before and after the trials. After the 6-month experimental period, the remaining food source, wood and soil or SMS were separated from termites and underwent a drying process using a constant temperature oven (DKN402C, Yamato Scientific Co., Ltd., Japan) set at 105 degrees Celsius for 3 days. Following the drying process, we separated the wood from the soil or SMS and recorded their respective weights. The soil and SMS were then sent to the Instrument Center of National Chung Hsing University for nitrogen and carbon content analysis using elemental analysis (Elementar Vario EL cube, Germany).

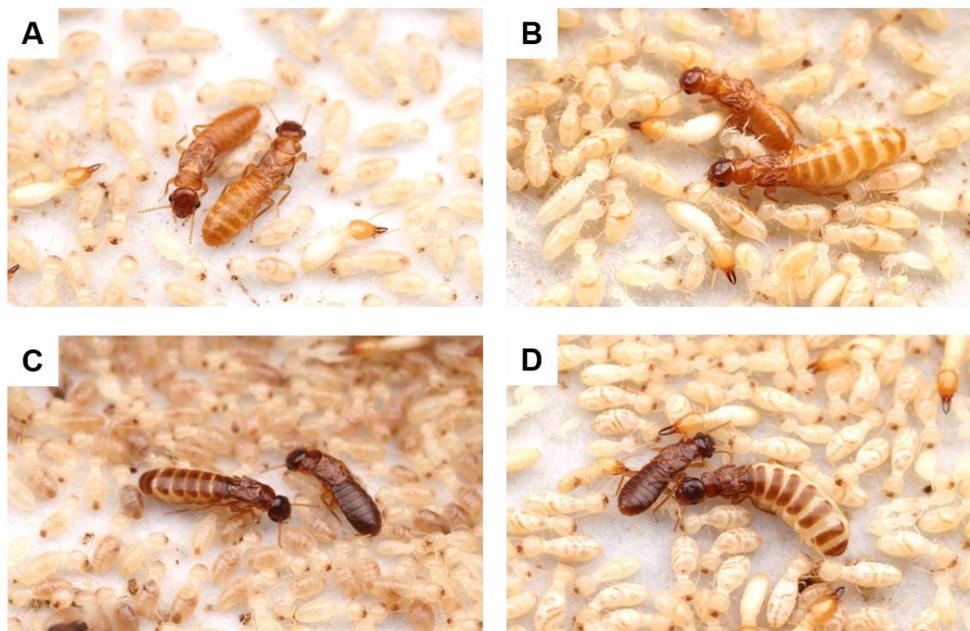
The means of total individuals and larval numbers in the three species, the increased weight of the king and queen in the case of *C. formosanus* and *C. gestroi*, and the number of neotenic, eggs and pseudergates in *P. flavus* between low- and high nitrogen food groups were analyzed using t-test, with a significance level ( $\alpha$ ) set at 0.05.

## Results and discussion

### Reproductive caste

Most termite colonies survived the 6-month experimental period. Only two *C. gestroi* colonies in the

low-nitrogen group and two trials of *P. flavus* in the high-nitrogen group died. The body weight increase rate of *C. formosanus* queens is  $89 \pm 41\%$  (low-nitrogen food,  $n = 6$ ) and  $215 \pm 91\%$  (high-nitrogen food,  $n = 6$ ,  $p < 0.05$ ). The body weight increase rate of *C. gestroi* queens is  $64 \pm 39\%$  (low-nitrogen food,  $n = 4$ ) and  $384 \pm 174\%$  (high-nitrogen food,  $n = 6$ ,  $p < 0.05$ ). The body weight of *Coptotermes* queens significantly increases by feeding on high-nitrogen food compared to that in low-nitrogen food groups. However, the weight of kings of both *Coptotermes* species is not significantly different before and after feeding on low- and high-nitrogen food for 6 months. For *C. formosanus*, the increased weight of kings is  $30 \pm 6\%$  ( $n = 6$ ) and  $33 \pm 12\%$  ( $n = 6$ ) in low- and high-nitrogen groups, respectively ( $p > 0.05$ ). For *C. gestroi*, the increased weight of kings is  $18 \pm 11\%$  ( $n = 4$ ) and  $17 \pm 5\%$  ( $n = 6$ ) in low- and high-nitrogen groups, respectively ( $p > 0.05$ ). For *P. flavus*, after feeding on high nitrogen food for 6 months, the number of neotenics is 10, 12, 40, and 42, on average increased by  $16.0 \pm 17.4$  ( $n = 4$ ). Compared to high-nitrogen food group, after the 6-month experimental period, the number of neotenics is 8 to 14 in the low-nitrogen food group, only increased by  $0.3 \pm 2$  ( $n = 6$ ,  $p = 0.053$ ).



**Fig 1.** Morphological comparison of termites fed with low- or high-nitrogen food sources for 6 months. The queen of the *C. formosanus* colony feeding on low-nitrogen food (A) is significantly smaller than that feeding on high-nitrogen food (B). The queen of the *C. gestroi* colony feeding on low-nitrogen food (C) is also significantly smaller than that feeding on high-nitrogen food (D).

**Table 1.** Increased number of larvae and all colony individuals of *Coptotermes formosanus* and *Coptotermes gestroi* after feeding on low-nitrogen food (wood and soil) or high-nitrogen food (wood and SMS) for 6 months.

Species	Treatments	n	Larvae	All individuals
<i>C. formosanus</i>	Low-nitrogen food	6	$-63.3 \pm 45.1$	$603.7 \pm 174.4$
	Hing-nitrogen food	6	$114.2 \pm 230.6$	$960.0 \pm 601.0$
	T-test (p-value)		0.09	0.19
<i>C. gestroi</i>	Low-nitrogen food	4	$-64.0 \pm 26.7$	$330.2 \pm 640.0$
	Hing-nitrogen food	6	$973.0 \pm 253.8$	$3885.8 \pm 956.4$
	T-test (p-value)		$< 0.01$	$< 0.01$

### Non-reproductive caste

Large variation in population growth was observed in *C. formosanus* and *C. gestroi* colonies (Table 1). The increased numbers of larvae and all colony individuals in *C. formosanus* is not significantly different between the low- and high-nitrogen food group (larvae,  $p = 0.09$ ; all individuals,  $p = 0.19$ ). For *C. gestroi*, the number of larvae and all castes significantly increased in the high-nitrogen food group compared to that

in the low-nitrogen food group (larvae,  $p < 0.01$ ; all individuals,  $p < 0.01$ ). The average increased population in the high-nitrogen group ( $3885.8 \pm 956.4$ ) is ten times more than that in the low-nitrogen group ( $330.2 \pm 640.0$ ).

In *P. flavus*, the numbers of larvae and eggs were significantly higher in the high-nitrogen food group than in the low-nitrogen food group (larvae:  $p < 0.01$ ; egg:  $p < 0.01$ , Table 2). However, the number of pseudergates and the overall population growth were not significantly different between the two groups.

**Table 2.** Increased number of neotenic, larvae, eggs and all castes of *Prorhinotermes flavus* after feeding on low-nitrogen food (wood and soil) or high-nitrogen food (wood and SMS) for 6 months.

Treatments	n	Neotenic	Larva	Egg	Pseudergate	All individuals
Low-nitrogen food	6	$0.3 \pm 2$	$17.3 \pm 11.8$	$9.2 \pm 2.9$	$175.3 \pm 46.2$	$129.2 \pm 49.6$
High-nitrogen food	4	$16.0 \pm 17.4$	$38.0 \pm 16.0$	$25.0 \pm 8.1$	$137.3 \pm 48.4$	$122.5 \pm 49.1$
T-test (p-value)		0.0538	$< 0.01$	$< 0.01$	0.24	0.84

### Nitrogen and Carbon Depletion

The results clearly demonstrate that feeding on the high-nitrogen food source enhances termite's reproductive ability. To validate the impact of nitrogen, it is crucial to know the total nitrogen and carbon depletion under each treatment. Based on the weight loss and the nitrogen and carbon content of wood, soil, and SMS before and after the experiments, we are able to calculate nitrogen and carbon depletion. The findings reveal that total nitrogen and carbon depletion are significantly higher in the high-nitrogen food group than in the low-nitrogen food group among three termite species (Table 3). It is interesting to note that total nitrogen increased in the low-nitrogen food group (wood and peat moss soil) after being fed on by *P. flavus* for 6 months. Even though the mechanism behind nitrogen increase remains unknown, nitrogen fixation by termites and their associated microbes would be a topic for further investigation (Breznak et al., 1973).

### Conclusion

This study provides evidence to support that high-nitrogen food source has a positive impact on termite reproductive ability, including the body weight increase of queens of *C. formosanus* and *C. gestroi*, and an increasing number of secondary reproductive caste members of *P. flavus*. Furthermore, a high-nitrogen diet stimulates queens and neotenic to lay more eggs, and subsequently, more larvae were observed. The increased depletion of nitrogen and carbon in the high-nitrogen food substantiates the positive correlation between nitrogen content in food and termite's reproductive success.

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**Table 3.** Nitrogen and carbon depletion of soil, SMS, and wood after being fed on by three termite species for 6 months.

Species	Food source	Soil/SMS		Wood		Soil/SMS + Wood	
		Nitrogen Depletion (g)	Carbon Depletion (g)	Nitrogen Depletion (g)	Carbon Depletion (g)	Nitrogen Depletion (g)	Carbon Depletion (g)
<i>C. formosanus</i>	Low-nitrogen food	0.54 ± 0.05	29.60 ± 2.74	0.02 ± 0.00	8.94 ± 1.77	0.56 ± 0.05	38.53 ± 3.76
	High-nitrogen food	1.70 ± 0.07	27.32 ± 1.49	0.05 ± 0.01	28.11 ± 7.48	1.75 ± 0.07	55.43 ± 6.40
	P-value	< 0.01	0.10	< 0.01	< 0.01	< 0.01	< 0.01
<i>C. gestroi</i>	Low-nitrogen food	0.43 ± 0.01	22.07 ± 1.11	0.01 ± 0.00	7.23 ± 1.28	0.45 ± 0.01	29.30 ± 0.51
	High-nitrogen food	1.66 ± 0.08	21.44 ± 2.09	0.08 ± 0.02	46.62 ± 13.90	1.74 ± 0.07	68.06 ± 13.37
	P-value	< 0.01	0.60	< 0.01	< 0.01	< 0.01	< 0.01
<i>P. flavus</i>	Low-nitrogen food	-0.18 ± < 0.02	-1.30 ± 0.43	< 0.01	1.95 ± 0.43	-0.18 ± 0.00	0.64 ± 0.48
	High-nitrogen food	0.07 ± 0.02	10.01 ± 0.25	< 0.01	1.93 ± 0.34	0.07 ± 0.02	11.94 ± 0.54
	P-value	< 0.01	< 0.01	0.93	0.93	< 0.01	< 0.01

Wednesday | March 13, 2024 | 09:15-09:30

## Section 2: Physiology, Morphology, and Structure

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### Comparative analysis of softwood and hardwood lignocellulose degradation by drywood termites, *Cryptotermes dudleyi*

by

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#### Abstract

Wood-destroying insects, primarily classified into two major orders of Insecta, namely Coleoptera (beetles) and Blattodea (termites), pose a considerable economic threat to the wood industry. Termites, in particular, are recognized as important wood consumers, playing a crucial role in wood decomposition and carbon recycling within terrestrial ecosystems. Conversely, wood-feeding beetles exploit a range of wood sources, from live and dead trees to new and old timbers with varying degrees of decay. Subterranean termites, especially species like *Coptotermes formosanus*, have been studied for their efficient hydrolysis and assimilation of polysaccharides (cellulose and hemicelluloses) and their ability to overcome recalcitrant lignin barriers. The current study focuses on elucidating the mechanisms underlying lignocellulose digestion by drywood termites (*Cryptotermes dudleyi*), comparing them with subterranean termites and wood-attacking beetles. Understanding these processes is crucial not only for advancing our knowledge of the physiology and evolution of herbivorous insects but also for implications in the development of biotechnological strategies for wood pest management and the refinement of biochemical production methods from lignocellulosic biomass.

**Keywords:** Lignocellulose digestion, drywood termite, *Cryptotermes dudleyi*

#### Introduction

In nature, the intricate breakdown of lignocellulose involves a collaborative effort among various symbiotic microorganisms. These microorganisms contribute enzymes that collectively dismantle the complex structure of biomass. The adaptability of lignocellulose transformation becomes evident under diverse conditions, encompassing aerobic and anaerobic environments, a wide range of temperatures, various pH levels, and different redox potentials (Cragg et al., 2015). This inherent diversity significantly influences and shapes the mechanisms responsible for lignocellulose degradation.

Xylophagous insects, found primarily in the three major orders of Insecta Coleoptera (beetles), Blattodea (cockroaches and termites), and Hymenoptera (ants, bees, and wasps) (Pournou, 2020) emerge as vital wood consumers, impacting wood decay and carbon recycling in terrestrial ecosystems (Tokuda, 2019). Many are recognized as wood pests due to their ability to cause severe damage to economically important forest trees and drywood materials (Pournou, 2020). These wood-feeding insects have evolved unique mandibles to crush lignocellulose and diverse digestive systems often associated with intestinal microbial symbionts, producing enzymes essential for lignocellulose decomposition the resistant biocomposite of cellulose, hemicelluloses, and lignin constituting woody plant cell walls (Watanabe & Tokuda, 2010; Ni & Tokuda, 2013; Brune, 2014; Douglas, 2015; Tokuda, 2019). Subsequently, in the hindgut, the ground and predigested biomass undergo anaerobic fermentation orchestrated by microbial consortia residing in the termite gut (Brune, 2014).

The endosymbiotic behavior extends beyond termites to include wood-borer insects, establishing symbiotic relationships, notably with  $\gamma$ -Proteobacteria, facilitating the digestion of scraped wood particles (Geib et al., 2008). In addition to prokaryotes, eukaryotes also contribute to lignocellulose degradation. For instance, fungus-growing termites engage in a process where lignocellulose is predigested by fungi before being assimilated by termites (Johnson et al., 1981). Similarly, lower termites rely on flagellated protists for the majority of lignocellulose digestion (Hongoh et al., 2003).

Recently, the deconstruction processes of lignocellulose in insect gut digestive systems have been revisited through structural analyses of digested lignocellulose residues in feces using advanced analytical techniques. Notably, studies employing pyrolysis-gas chromatography/mass spectrometry (Py-GC/MS) (Geib et al., 2008; Ke et al., 2011; Ke et al., 2013; Dumond et al., 2021) and/or two-dimensional (2D) nuclear magnetic resonance (NMR) spectroscopy (Li et al., 2017; Tarmadi et al., 2018; Dumond et al., 2021; Krishanti et al., 2021) have revealed differential degradation patterns of cell wall polysaccharides, such as cellulose and hemicelluloses. However, information about lignocellulose degradation in higher termites, such as dry-wood termites, remains unclear and has not yet been conducted. Therefore, in this study, we aim to analyze the process of lignocellulose decomposition within the gut of drywood termites and compare it with the mechanisms observed in subterranean termites and wood-attacking beetles.

Additionally, these studies have provided evidence of previously overlooked chemical alterations of lignin polymers during their passage through the insect digestive systems. Through a comprehensive exploration of chemical analysis and insights derived from NMR, our objective is to bridge the gap between entomology, biomaterial science, and environmental sustainability. This exploration opens doors to innovative solutions in both biological and industrial contexts.

## Materials and methods

### *Insects feeding and feces collection*

The termite used in this study was drywood termite *Cryptotermes dudleyi* which collected from Bogor, Indonesia. Insects feeding and feces collection was conducted according to Tarmadi et al., (2018). Two hundred termite workers of *Cryptotermes dudleyi* were starved for 2 days and then allowed to fed on sapwood blocks [2 (L) x 2 (W) x 1 (H) cm] of Rubber wood (*hardwood*) and Pine wood (*Softwood*). The cylindrical fecal pellets were collected once a week for 6 months to obtain sufficient material for lignocellulose analysis. The feces immediately frozen and kept at  $-25^{\circ}\text{C}$  to prevent further degradation. The obtained feces (digested) and original (undigested) diet samples were pulverized, washed successively with water and 80% ethanol, and then lyophilized to obtain cell wall residue (CWR) samples (Tarmadi et al., 2018; Krishanti et al., 2021) for wet chemistry and NMR analyses.

### *Chemical compositional analysis of lignocelluloses digested by C. dudleyi*

Digested lignocellulose samples (feces) from *C. dudleyi* workers were initially characterized through lignocellulose compositional analysis using wet chemical methods. The starch content was determined by measuring the amount of glucose released after treatment with thermostable  $\alpha$ -amylase and amyloglucosidase, following the method outlined by Hattori et al. (2012). Crystalline cellulose and non-crystalline glycan contents were determined using the two-step acid hydrolysis method, employing trifluoroacetic acid and sulfuric acid, as described by Lam et al. (2017). In brief, matrix polysaccharides in CWR samples were first hydrolyzed with 2M trifluoroacetic acid ( $100^{\circ}\text{C}$ , 5 hours). The resulting monomeric sugars were then derivatized using the alditol-acetate method and quantified by GC/MS, with myo-inositol as an internal standard.

The trifluoroacetic acid-insoluble pellets containing crystalline cellulose were washed with Updegraff reagent and then completely hydrolyzed with 72% sulfuric acid. Glucose concentrations in the obtained solutions were measured using Glucose C-II Test Wako reagent (Wako Pure Chemical) following the kit protocol. For the thioglycolic acid lignin assay to determine lignin content (Suzuki et al., 2009), milled wood lignins prepared from each original lignocellulose sample were utilized to construct the standard curves. Analytical thioacidolysis was performed to determine lignin composition, following the method outlined by Yamamura et al. (2012). The released lignin monomers were derivatized with N,O-bis(trimethylsilyl)acetamide and quantified by gas chromatography/mass spectrometry (GC/MS), using 4,4'-ethylidenebisphenol as an internal standard (Yue et al., 2012).

### *Statistical analysis*

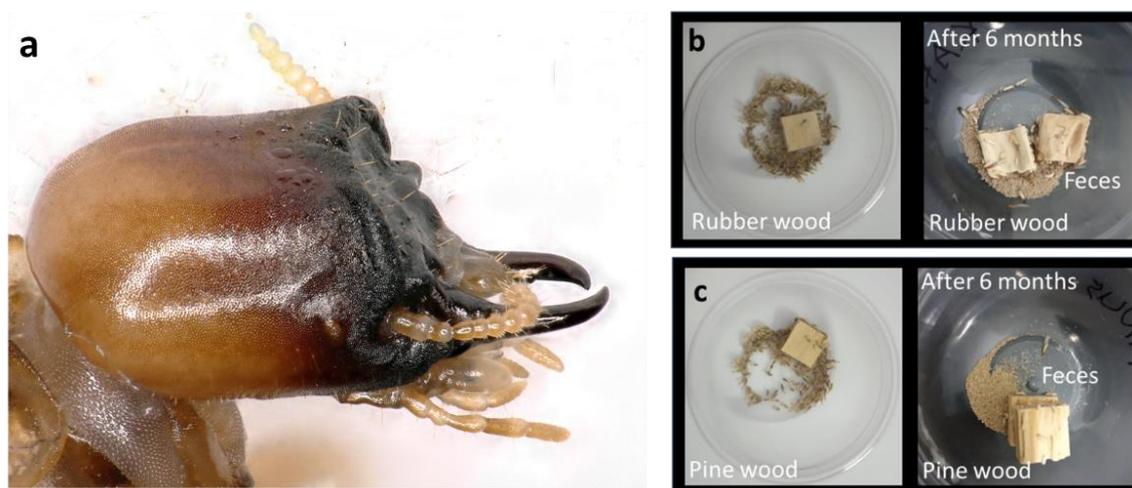
Student's *t*-test ( $p < 0.05$ ) and one way analysis of variance (ANOVA) followed by Tukey's honestly

significant difference (HSD) test ( $p < 0.05$ ) were performed using IBM SPSS Statistics version 27 (IBM Corporation, Armonk, NY, USA).

## Results and discussion

### *Drywood termite, Cryptotermes dudleyi*

The drywood termite is recognized for its tendency to reside and forage within a single piece of drywood, exhibiting a behavior known as a one-piece nesting habit. This characteristic places the colony in a sheltered environment (Himmi et al., 2016). Notably, this protected habitat often extends into human living spaces, rendering drywood termites as pests. Their capacity to target a variety of wooden objects within human spaces, including furniture, flooring, and even the structural components of houses, underscores their potential for causing damage (Rust et al., 2012; Lewis & Forschler, 2014). Given this scenario, monitoring drywood infestations becomes crucial, particularly in prevalent environments like wooden buildings or various materials composed of cellulose (Lewis & Forschler, 2014). We focused on a native drywood termite of Java Island, *Cryptotermes dudleyi*, a well-known dry-wood termite wood for our study (Fig. 1a).



**Fig 1.** Feeding experiment of *Cryptotermes dudleyi* with two types of wood. (a) Morphology of *C. dudleyi*, (b) *C. dudleyi* fed with rubber wood block and (c) *C. dudleyi* fed with pine wood block.

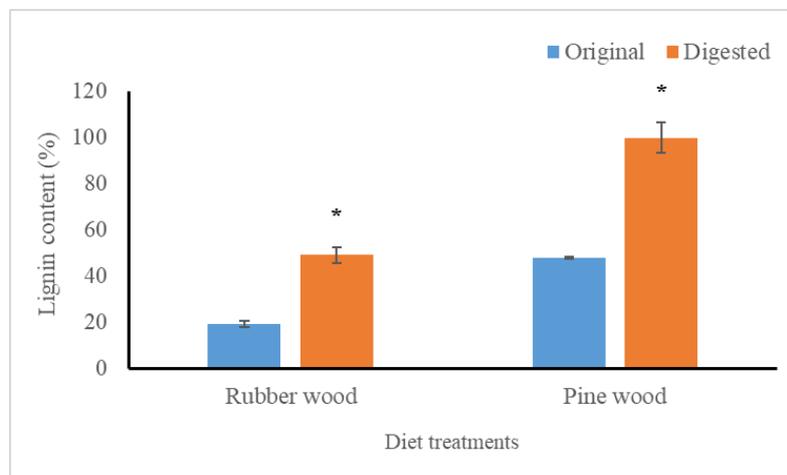
Another observation indicated a discernible impact of different feeding materials on termites, specifically those fed rubber wood (Hardwood) compared to pine wood (Softwood). While the weight loss of each diet treatment was not measured, findings from the orphaned colony revealed variations in the size of fecal pellets (Fig. 1b and 1c). This aligns with the results reported by Zega et al. (2021), where fecal pellets of *C. dudleyi* collected from infested pine wood were smaller compared to those produced when feeding on hardwood. This suggests that different feeding materials may lead to variations in the characteristics and preferences, with feeding on hardwood diets exhibiting a broader range of values.

The survival rates and dietary mass consumed by *C. dudleyi* workers fed lignocellulose diets were observed. The survival rates of workers fed pine wood (Softwood) and rubber wood (Hardwood) diets after 6 months of feeding were similar (approximately 90%). Additionally, it was noted that *C. dudleyi* workers consumed both softwood and hardwood diets.

### *Lignocellulose decomposition*

Sugar content analyses determined an approximately ~72% proportional decrease in cellulosic crystalline glucan for pinewood diets (Table 1). While mannan was moderately (48%) depleted, the other major hemicellulosic sugars, i.e., xylan, arabinan, and galactan, were significantly enriched in the digested pinewood lignocellulose. The result suggests the selective digestion of cellulose over hemicelluloses in the *C. dudleyi* digestive system. Meanwhile, in the digestion of rubberwood, our result found that an approximately 31% of glucan (Amorphous) was proportional decrease, lower than termite fed with pinewood. Among the hemicellulosic sugars, about 137% and 113.7% proportional increase in hemicellulose Arabinan and Galactan, respectively, were likewise recorded (Table 1). Similar in the digestion of pinewood, however,

this study observed significant decreases in crystalline glucan (~73%), xylan (~40%), and mannan (~40%) in the digestion system of *C. dudleyi*. Overall, the data are in line with previous studies in lower termites, *C. formosanus* (Tarmadi et al., 2018) reporting preferential conversions of cell-wall polysaccharides in the termite digestive system. It is most likely that polysaccharide conversions in the *C. formosanus* and *C. dudleyi* digestive system are highly selective for cellulose degradation, especially in softwood lignocellulose, but expand to include hemicellulosic sugar conversions in hardwood.



**Fig 2.** Total lignin content determined by thioglycolic acid lignin analysis. Value are means  $\pm$  SD (n=3) and asterisks indicate significant difference between original and digested lignocellulose diets ( $P < 0.05$ ).

### Enrichment of residual lignin

The lignin content analyses were analyzed by using thioglycolic acid lignin and thioacidolysis analysis. Our results found that lignin content (%) augmented in fecal pellet rather than the original diet (Fig. 2 and Table 2), indicated that lignin is hardly utilized as a nutrient source to *C. dudleyi*. This result shows that, as reported for other wood-feeding insects such as *Coptotermes formosanus* (Tarmadi et al., 2018) and wood-beetle *Nicobium hirtum* (Krishanti et al., 2021), *C. dudleyi* also digest primarily polysaccharides, leaving a majority of lignin polymers in fecal digestive residues. Total of lignin content was ~90% proportional increase in in the digested softwood pinewood and rubber wood (Fig. 2).

In addition, a complementary lignin compositional analysis using thioacidolysis was conducted on *C. dudleyi* fed with a hardwood diet (Table 2), which quantifies lignin-derived monomeric compounds released from non-oxidized  $\beta$ -O-4 units in the lignin polymer (Lapierre et al., 1985). The results of thioacidolysis analyses showed that the ratio of non-oxidized S and G lignin units (S/G ratio) was slightly reduced in the digested fecal pellet compared with the original diet. Notably, a similar result was also found in lower termites, *C. formosanus* when fed with a hardwood diet. Since thioacidolysis measures only the S/G unit ratio in the monomers released by cleaving  $\beta$ -O-4 bonds, the results also revealed that the Guaiacyl lignin unit in the fecal pellet was augmented over the Syringyl lignin unit (Table 2). These results indicate that lignin can be structurally modified during its passage through the *C. dudleyi* digestive system, as further demonstrated below. To further examine the lignocellulose decomposition structures by the drywood termite, *C. dudleyi*, a comprehensive analysis by 2D-NMR is currently needed and will be conducted in future studies.

## Conclusion

In this study, softwood and hardwood diets were provided to *C. dudleyi* workers, and structural differences between the original lignocellulose diets and the resulting feces were examined through wet-chemical analysis. Overall, the data support the perspective that lignin polymers undergo at least partial modification/decomposition during their passage through the termite gut digestive system. However, it is evident that polysaccharide decomposition predominates the overall lignocellulose deconstruction process, and the majority of lignin polymers remain intact in the digestive residues. This result strongly aligns with findings from a previous study in lower termites, specifically *C. formosanus*, suggesting that drywood termites and/or their gut symbionts may exhibit a preference for the degradation of cellulose over lignin. The fundamental insights gained from this study contribute to the growing understanding of lignocellulose

decomposition in drywood termites. Further investigations, particularly through comprehensive analyses such as 2D-NMR, are crucial for a more detailed exploration of the lignocellulose decomposition structures by *C. dudleyi*. This will enable a deeper comprehension of the intricate interplay between termites and their gut microorganisms in the context of lignocellulose degradation. Such knowledge holds significant implications not only for advancing our understanding of termite physiology but also for its potential applications in biotechnological strategies for wood pest management and the development of sustainable biochemical production methods from lignocellulosic biomass.

**Acknowledgments** The authors thank the Japan-ASEAN Science Technology Innovation Platform (JASTIP) Program for their support, a collaborative project of Kyoto University, Japan and National Research and Innovation Agency (BRIN), Indonesia under Work-Package (WP) 3: Bioresources and Biodiversity.

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**Table 1.** Chemical composition of lignocellulose samples digested by the termite *C. dudleyi*

Chemical components	Pine wood (Softwood)			Rubber wood (Hardwood)		
	Original	Digested	Change (%)	Original	Digested	Change (%)
Carbohydrate content (mg/g CWR)						
Glucan (Crystalline)	348.5 ± 25.0	95.2 ± 16.6	<b>-72.7</b>	459.5 ± 136.5	123.4 ± 12.6	<b>-73.1</b>
Glucan (Amorphous)	33.0 ± 1.9	15.6 ± 1.1	<b>-52.6</b>	15.5 ± 1.9	10.6 ± 0.4	<b>-31.7</b>
Arabinan	8.2 ± 0.3	12.5 ± 1.2	<b>51.7</b>	3.8 ± 0.3	9.1 ± 0.3	<b>137.7</b>
Xylan	29.0 ± 1.0	32.4 ± 5.5	<b>12.0</b>	136.4 ± 14.2	82.5 ± 4.2	<b>-39.5</b>
Mannan	87.0 ± 3.7	44.5 ± 4.4	<b>-48.9</b>	12.8 ± 1.5	7.7 ± 0.2	<b>-39.9</b>
Galactan	18.1 ± 0.4	28.3 ± 2.2	<b>56.2</b>	7.5 ± 0.5	16.0 ± 0.5	<b>113.7</b>

Values are means ± standard deviation (n = 3) and asterisks indicate significant difference between original and digested samples (Student t-test, \*P < 0.05; \*\*P < 0.01).

**Table 2.** Lignin composition of lignocellulose hardwood digested by the termite *C. dudleyi*

Lignin components	<i>C. dudleyi</i>			<i>C. formosanus</i> (Tarmadi et al., 2018)		
	Original	Digested	Change (%)	Original	Digested	Change (%)
Thioglycolic acid lignin (% CWR)						
Total lignin	19.3 ± 1.2	49.2 ± 3.3	<b>154.6</b>	19.6 ± 0.4	33.5 ± 0.3	<b>70.9</b>
Thioacidolysis (% CWR)						
H	0.0 ± 0.0	0.0 ± 0.0	<b>0.0</b>	0.0 ± 0.0	0.0 ± 0.0	<b>0.0</b>
G	36.3 ± 2.6	38.7 ± 0.8	<b>6.8</b>	24.8 ± 0.1	29.7 ± 1.3	<b>19.7</b>
S	63.7 ± 2.6	61.3 ± 0.8	<b>-3.9</b>	75.2 ± 0.1	70.2 ± 1.3	<b>-6.6</b>
S/G	1.8 ± 0.2	1.6 ± 0.1	<b>-10.4</b>	3.1 ± 0.0	2.4 ± 0.1	<b>-22.3</b>

Values are means ± standard deviation (n = 3) and asterisks indicate significant difference between original and digested samples (Student t-test, \*P < 0.05; \*\*P < 0.01).

Wednesday | March 13, 2024 | 09:30-09:45

### Section 3: Economic Impact and Innovative Management

O\_10

## Advantage and Challenge of Employing Micro-CT Technology for Termite Study

by

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### Abstract

Micro-computed tomography (micro-CT) is a specialized imaging technology extensively used for observing small objects, particularly in the field of entomology. However, termites present a challenge due to their soft bodies and tissues, making direct examination using micro-CT difficult. In this article, we have explored various preparation methods for fixation and staining to improve the quality of images. These efforts aim to facilitate the clarification of termite behavior, physiology, or evolution by utilizing non-destructive 3D models through micro-CT.

**Keywords:** Micro-computed tomography, termite, external structure, radiocontrast agents

### Introduction

Micro-computed tomography (micro-CT) is an imaging technology. Noninvasive X-rays can penetrate the surface and confirm the presence of the target within. Recently, computed tomography has increased the precision of localization and volume calculation by three-dimensional images (Schwass et al., 2009). The imaging involves exposing the target to X-rays from multiple angles, and a detector receives the X-rays with varying degrees of attenuation to produce the final three-dimensional imaging. As advance of imaging analyses, the resolution of computed tomography has become even higher to micro-level. Micro-CT offers the for accurate observation of extremely minute samples, even in biology, particularly focus on entomology, where the study involves organisms with tiny body sizes (Ritman, 2004, Kypke and Solodovnikov, 2020).

Insects, as highly diverse and complex organisms, have applications in various fields such as agriculture (Eteraf-Oskouei and Najafi, 2013), industry (Franceschini et al., 2007), and disease prevention (Gan et al., 2021). Among of them, termites are famous pests in both forestry and urban areas with approximately 3000 recorded species worldwide. They are distributed globally excluding Antarctica, with a concentration in tropical. The relationship between human activities and termites is very close due to the food resources and habitat preferences of termites. Consequently, humans need to spend substantial funds annually to control termite damage. Despite this, termite as scavenger in the ecosystem, break down wood cellulose through symbiotic microorganisms. Additionally, termite eusociality show interactive communications and diverse castes in one or more colonies, it further is shown their complexity and significance in the ecosystem.

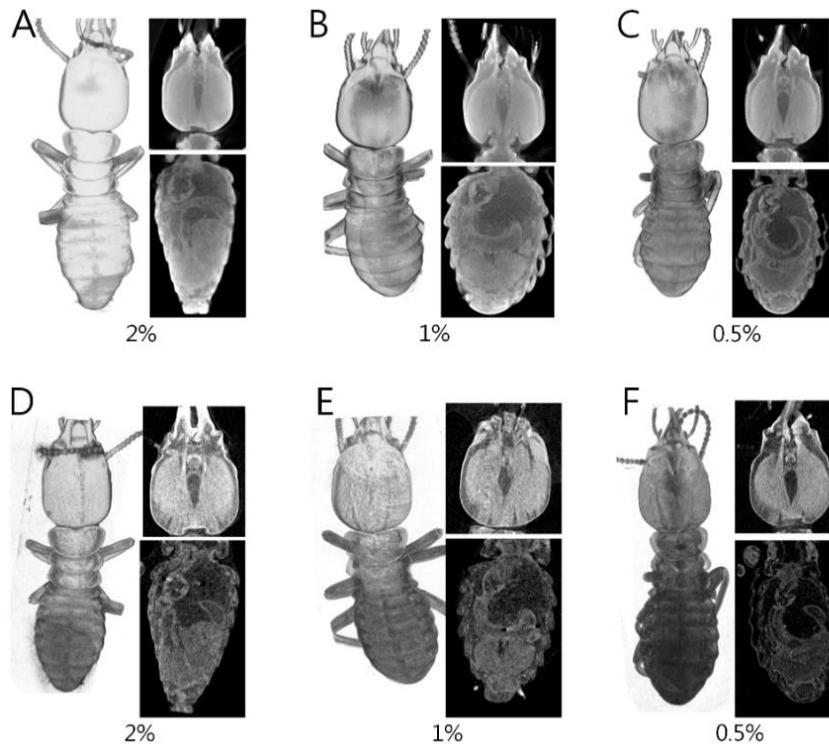
To contribute to the understanding of studies on behavior, physiology, or evolution of termite, examinations of external structures and internal tissues are comprehensively conducted. However, termites have extremely soft bodies and internal tissues that are prone to deformation during processing, such as paraffin sectioning or dissection (Ho and Hutmacher, 2006). Therefore, we expect that termites can be examined more precisely through the non-destructive approach and three-dimensional imaging.

## Materials and methods

For preserving and stabilize the whole structures, termites were immersed the termite in 70% alcohol and gradually increased the concentration every hour until it reached a 99% alcohol solution. For staining methods, we compared the difference between 0.5%, 1% and 2% iodine solution with 99% alcohol in *Prorhinotermes flavus* soldier processing 24 hours and 48hours. To determine the location and size of the internal structures, *Coptotermes formosanus* alate were stain in 1% iodine solution with 99% alcohol processing 4 days. All samples were washed excess agent by 99% alcohol before scanning. The images were captured using the Delta DELab  $\mu$ CT-100X (Delta Electronics, Taiwan), composed by DELInsect 3D Recon (version 2.0.0.9981, Delta Electronics, Taiwan), and examination by DELCTomo (version 2.1.0.647, Delta Electronics, Taiwan).

## Results and discussion

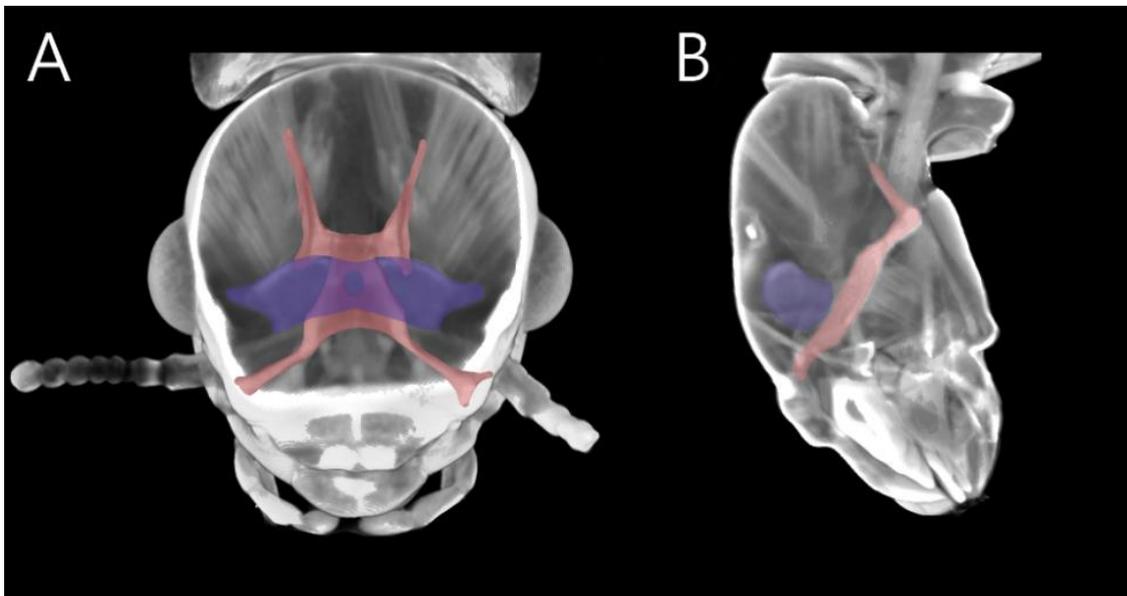
Radiopaque contrast agents can enhance the contrast and provide a clear examination for muscle and soft tissue with lower density. In our results, increasing the concentration of iodine solution could enhance the contrast of external structures of termite, but it was not shown in internal tissue significantly (Fig.1). Thus, high concentration of the agent could not accelerate the staining time of internal soft tissues. Instead, the contrast of surface density is significantly enhanced after staining, it may cause beam hardening effect and decrease the imaging quality of internal soft tissues (Van de Castele et al., 2004, Jung et al., 2005). Although iodine solution presents quick and convenient staining methods, prolonged staining can lead to overstain, leading to reduced contrast between soft tissues and causing unexpected deformation (Li et al., 2015, Callahan et al., 2021). Phosphotungstic acid solution (PTA) is a useful alternative agent that provides excellent contrast for soft tissue without causing overstaining for termite staining (Liao et al., 2023). The staining process requires a duration of over 1 or 2 months (Lesciotto et al., 2020). Therefore, depending on the target and require, the samples may process various treatment methods.



**Fig 1.** Different concentrations by iodine staining in *Prorhinotermes flavus* soldier. A, B, C, processing 24 hours; D, E, F, processing 48 hours; the concentration present at the bottom of each figure. The images were captures using 3  $\mu$ m resolution, 50 mV, and 512 projection number. The contrast of external structures (left) was significantly enhanced in 2% iodine solution processing 24 hours and 48 hours, but no difference was observed in tissues and muscles (upper right and bottom right).

The convenience of examining samples has been enhanced by 3D imaging through micro-CT compared to the limitations of shooting angles in 2D images (Kundrata et al., 2020). However, the mesosoma of termite imago still experienced deformation during the dehydration process. To address this, it is necessary to extend the immersion time (more than 12 hours) in alcohol solutions of different concentrations to ensure the complete removal of moisture. Alternatively, immersing in Bouin solution before staining can help fixate and stabilize the sample.

The study of taxonomy has yielded significant breakthroughs in understanding the functionality, ontogeny, and physics of various biological parts through micro-CT imaging (Schoborg et al., 2019, Adachi et al., 2020). Micro-CT has been successful in examining the internal structures of the head and brain development, allowing for precise location determination and volume calculations (Fig. 2). Previous research on brain development revealed that reproductive castes (ergatoid, nymphoid, and alate) exhibit larger brain volumes than soldiers and workers (Ishibashi et al., 2023). Furthermore, micro-CT has been employed to study the specific developments in the optic lobe of the brain in alates as an adaptation to environmental changes (Niven and Laughlin 2008, Ishibashi et al., 2023). In our studies of reproductive gland development, both imago and neotenic castes in the genus *Prorhinotermes* present reproductive capabilities. However, there is currently insufficient morphological evidence to identify genders for the other castes (nymph, worker). Therefore, examining reproductive gland through micro-CT, is useful to determining the gender of termites (Liao et al., 2023), and estimating the maturity time of the reproductive gland without dissection.



**Fig 2.** 3D image of brain in *Coptotermes formosanus* imago (female). The images were captured using 3  $\mu\text{m}$  resolution, 60 mV, and 720 projection number. The location and size of brain (blue) and tentorium (red) is also shown significantly. The volume of brain were calculated approximately  $2.4 \times 10^7 \mu\text{m}^3$ .

The current limitations of micro-CT in analyzing soft tissues indicate that it has not reached the level of detail provided by established anatomical or optical observation methods like scanning electron microscopy (SEM). In recent years, advancements in X-ray technology, such as 4D-CT and synchrotron CT, have offered higher levels of resolution and the ability to observe living organisms. Combining with other medical image methods such as magnetic resonance imaging or positron emission tomography could provide more detailed information on termites in future research.

## Conclusion

Micro-CT provides the non-destructive approach and 3D imaging for termites. The termite sample presents good contrast of soft tissues in the image through staining processes using iodine and PTA solution. The imaging technology is helpful for measuring and locating the external structures and internal tissues without dissection, advancing our knowledge of termite biology.

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Wednesday | March 13, 2024 | 09:45-10:00

### Section 3: Economic Impact and Innovative Management

**O\_11**

## **Actual state of termites (isoptera) that damage some dam works with big capacity in Vietnam**

by

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### **Abstract**

The survey was carried out at 30 large dams in Vietnam. 23 species belonging to 20 genera, 5 subfamilies and two families were identified. In which, 13 species were severely harm to the dam; 2 species were moderate harmful pests; 2 species were harmful to structures on the dams; and 7 species were unharmed to the dams. There were 96,7% of surveyed dams occupied by termites. Among them, 50% of the surveyed dams recorded 4-6 species, 43% of the dams with 1-3 species, and 1 dam (Cau Moi dam in Dong Nai province) with 8 species. The results also revealed that 8 dams were at high risk of unsafety caused by termites that need an immediate treatment plan including Dai Lai dam (Vinh Phuc), Ray River dam, Thuong Ea Sup dam, Krong BukHa dam, Phuoc Hoa dam, Cau Moi dam, Sao River dam (Nghe An) and Yen My dam.

**Keywords:** Dam, species composition, termites, dam safety

### **Introduction**

The report of the American Dam Safety Association states that the activities of some animal species are the main cause or a major contribution to the unsafe operation or complete failure of a dam works, especially for homogeneous earth dams. Some animal species build nests, dig caves, create entrances into the dam for shelters, hunting, etc. These all activities cause harmful effects to the safety and performance of the dam works. Some of these effects can be easily identified, such as landslide, surface erosion, other problems such as porosity, crack, seepage, etc. inside may not be visible until the dam works is in danger. Among the organisms that can damage the dam works, termite (Isoptera) is one of primary concern, especially in tropical countries.

Vietnam has invested to build 6,648 irrigation reservoirs, in which 702 reservoirs are classified as large and special (Report of the Conference on safety management of dams and irrigation reservoirs 2018). Although designed and constructed with strict standards, the majority of dams creating irrigation reservoirs are earthen dams, built in the 70s and 80s of the last century. Many reservoirs over 50 years are degraded that are in risk of incidents. The first publication on termites damaging dams in Vietnam were Vu Van Tuyen (1982, 1989). The composition of termite species consisted of 52 species, in which 2 dangerous group i.e. *Odontotermes* and *Macrotermes*. More than 10 years later, the research on dams of Le Van Trien et al. (2000) in the North Central region recorded 9 species belonging to 5 genera, in which *Odontotermes* genus consisted of the highest number of species with 4 species. In 2001, Le Van Trien et al. continued to investigate the termite fauna that damages dam works in Lam Dong and reported 30 species belonging to 14 genera in 3 families. One year later, Le Van Trien et al. published a list of composition of termite species on dams in the Central Highlands including 17 species belonging to 7 genera and 2 families. The investigation result of the species composition and distribution of termites that damages dams in the Southeast region by Nguyen Quoc Huy et al. (2007) recorded 29 species, in which 18 species were pests that damages dams. Nguyen Quoc

Huy (2011) found 23 species belonging to 11 genera of 2 families on 15 dams and reservoirs in the Central Highlands. Among these, 10 species were pest to reservoir dams, in which *Macrotermes gilvus*, *M. annadalei* and *Odontotermes ceylonicus* were the main pest caused damages. Investigation on termites cause dams damage had received a lot of attention from researchers, and certain results had also been achieved. Researches on termites that damage dams were mainly carried out in certain regions, which were areas with many built reservoir and dam works such as the Central Highlands and the Southeast, etc. Up to now, it has been more than 10 years since the publish of Nguyen Quoc Huy in 2011, we want to investigate and re-survey the current state of termites that damage the dam, thereby making assessments on the change in composition of harmful termite species over time to have appropriate adjustments in managing termites that damage this important works system. The scope of the research was focused on big reservoir dams managed by the Ministry of Agriculture and Rural Development and reservoirs operated according to the inter-reservoir operation Procedure (mainly reservoirs with capacity  $\geq 3$  million m<sup>3</sup>).

## Materials and methods

The research was conducted between March 2020 and December 2021. The survey and investigation works were conducted at 30 dam works showed in Table 4.

*Methods of investigating and assessing the level of damage of termites:* The data on composition and level of damaging of termites is collected directly at the field complied with Vietnamese national standards: TCVN: 8479; TCVN: 8480; TCVN: 8227. Locating termite nests on the works and surrounding environment by visual observations and GPS devices.

*Scope of survey at the works:* For dams formed according to Article 5.3.1 of the standard TCVN: 8480-2010, this work includes the entire area of the downstream slope of the dam body and the surrounding environment at downstream of the dam body. The surrounding environment is determined with a range of 100m from the edge of the dam base and the two dam shoulders to the height of 0.1H but not exceeding 100m (except for flooded areas, overflows and culverts, ponds, etc.).

*Methods of collecting and identification of termite species:* At each sampling location, we used simple tools such as hoes, screwdrivers, etc. and specialized soft forceps to collect termites (at the roof line, at termite nests, places of searching for food or flying termite in herds,...). We tried to collect all castes (soldiers, workers, nymphs and alates) for each sample. Termites were preserved in small tubes containing 75% alcohol and brought to the laboratory of the Institute of Ecology and Works Protection for filtering, changing alcohol, identification and storage.

The external morphological characteristics of termites were observed using a magnifying glass or stereo microscope with some supporting tools such as small needles, soft forceps, etc. Size parameters of termites were measured according to instructions of Roonwal (1970). Termite samples were identified based on the keys of Nguyen Duc Kham et al. (2007), Ahmad (1958, 1965), Huang et al. (2000), Roonwal & Chhotani (1989) and Thapa (1981).

## Results and discussion

### *Composition of termite species composition discovered at investigated dam works*

Twenty three species belonging to 2 families, 5 subfamilies and 10 genera were identified from total of 2,716 collected samples at 30 dam works (Table 1). The number of termite species found in this research was only accounted for 50% of that published by Trinh Van Hanh et al., 2019 (46 species, 16 genera, 8 subfamilies).

Table 1 showed that the majority termite species at the dams belonged to the Termitidae family with 21 species, 4 subfamilies and 9 genera. This species number accounted for 91.3% of the total species found during the investigation (21 out of 23 species); 58% (21 out of 36 species) of total species belonging to the Termitidae that caused damage to dams and dikes in Vietnam (Trinh Van Hanh et al., 2019); and 10.6% (21/198 species) of total species belonging to the Termitidae family recorded in Vietnam (Nguyen Quoc Huy et al., 2021).

In Rhinotermitidae family, there was only two species belonging to a single genus namely *Coptotermes* accounted for 25% number of species reported in Trinh et al. 2019; and 4.1% of the species number recorded in Vietnam (Nguyen Quoc Huy et al., 2021).

### *Termite species that cause damage to dam works*

Among 23 termite species founded at investigated dam works, not all 23 species caused harm to the

safety of the dam. Some species nests were simple structures, small, shallow chambers with the small number of individuals that were unlikely to have impacts on the structure of the dams. Therefore, they were categorized as non-harmful species. Based on Vietnam standard (TCVN), termite species are classified and ranked according to the level of impact on dam works showed in Table 2.

**Table 1.** Composition of termite species collected at investigated dams

No.	Taxon	No.	Taxon
	<b>Rhinotermitidae Light, 1869</b>	14	<i>Macrotermes serrulatus</i> Snyder, 1934
	<b>Coptotermitinae Holmgren, 1910</b>	15	<i>Macrotermes beaufortensis</i> Thapa, 1981
	<b>Coptotermes Wasmann, 1896</b>	16	<i>Macrotermes barneyi</i> Light, 1924
1	<i>Coptotermes gestroi</i> (Wasmann, 1896)		<b>Hypotermes Holmgren, 1813</b>
2	<i>Coptotermes formosanus</i> Shiraki, 1909	17	<i>Hypotermes obscuriceps</i> (Wasmann, 1902)
	<b>Termitidae Westwood, 1840</b>		<b>Microtermes Wasmann, 1902</b>
	<b>Macrotermitinae Kemner, 1934</b>	18	<i>Microtermes pakistanicus</i> Ahmad, 1955
	<b>Odontotermes Holmgren, 1912</b>		<b>Amitermitina Kemner, 1934</b>
3	<i>Odontotermes hainanensis</i> (Light, 1924)		<b>Microcerotermes Silvestri, 1901</b>
4	<i>Odontotermes ceylonicus</i> Wasmann, 1902	19	<i>Microcerotermes bugnioni</i> Holmgren, 1911
5	<i>Odontotermes feae</i> (Wasmann, 1896)		<b>Globitermes Holmgren, 1912</b>
6	<i>Odontotermes measodensis</i> Ahmad, 1965	20	<i>Globitermes sulphureus</i> (Havilandi, 1898)
7	<i>Odontotermes angustignathus</i> Tsai et chen, 1963		<b>Termitinae Sjostedt, 1926</b>
8	<i>Odontotermes longignathus</i> Holmgren, 1914		<b>Termes Linnaeus, 1912</b>
9	<i>Odontotermes formosanus</i> (Shiraki, 1909)	21	<i>Termes propinquus</i> (Holmgren, 1914)
10	<i>Odontotermes yunnanensis</i> Tsai et Chen, 1963		<b>Pericapritermes Silvestri, 1915</b>
	<b>Macrotermes Holmgren, 1909</b>	22	<i>Pericapritermes nitobei</i> (Shitaky, 1909)
11	<i>Macrotermes gilvus</i> Hagen, 1858		<b>Nasutitermitinae Hare, 1937</b>
12	<i>Macrotermes carbonarius</i> (Hagen, 1858)		<b>Bulbitermes Emerson, 1949</b>
13	<i>Macrotermes annandalei</i> (silvestri, 1914)	23	<i>Bulbitermes prahae</i> Krishna, 1965

**Table 2.** Ranking of termite species according to the levels of impact on dam works

No.	Serious damage to dam	Damage to structures on dam	Moderate damage	Not damage to dam
1	<i>Odontotermes hainanensis</i>	(O.) <i>Coptotermes gestroi</i>	<i>M. serrulatus</i>	<i>Microtermes pakistanicus</i>
	<i>O. ceylonicus</i>	<i>C. formosanus</i>	<i>M. beaufortensis</i>	<i>Microcerotermes bugnioni</i>
3	<i>O. feae</i>			<i>Termes propinquus</i>
4	<i>O. measodensis</i>			<i>Globitermes sulphureus</i>
5	<i>O. angustignathus</i>			<i>Pericapritermes nitobei</i>
6	<i>O. longignathus</i>			<i>Bulbitermes prahae</i>
7	<i>O. formosanus</i>			
8	<i>O. yunnanensis</i>			
9	<i>Macrotermes gilvus</i>	(M.)		
10	<i>M. carbonarius</i>			
11	<i>M. annandalei</i>			
12	<i>M. barneyi</i>			
13	<i>Hypotermes obscuriceps</i>			

Table 2 showed 13 species that caused severe damage to dams, in which, 2 species caused moderate damage, and 2 species caused damage to architectural works built within the scope of the dam (such as operating house, valve tower, etc.) that were required prevention and control action. The remaining species were not required to be treated as categorized as non-harmful species to the dams.

**Damage level of termites to investigated dams**

Among the 30 investigated dam works, 29 out of 30 dam works were found to be damaged by termites at different levels, accounting for the rate of 96.7% of the investigated works (Table 4), while the remaining dam, Yen Lap dam in Quang Ninh province, was not located termites nests. The distribution level of termite species at each works is shown in Table 3.

**Table 3.** Actual status of distribution of harmful termite species at the investigated works

No.	Dam	Province	Number of species	Serious damage to dam
<b>Northern</b>				
1	Cam Son	Bac Giang	5	3
2	Trang Vinh	Quang Ninh	3	0
3	Yen Lap	Quang Ninh	0	0
4	Đai Lai	Vinh phuc	4	2
5	Đong Mo	Ha Noi	4	3
<b>North Central</b>				
6	Song Muc	Thanh Hoa	5	3
7	Yen My	Thanh Hoa	3	2
8	Vuc Mau	Nghe An	2	1
9	Song Sao	Nghe An	4	2
10	Song Rac	Ha Tinh	1	0
11	Kim Son	Ha Tinh	1	0
12	Ke Go	Ha Tinh	1	0
13	Rao Da	Quang Binh	6	3
14	An Ma	Quang Binh	5	3
15	Vuc Tron	Quang Binh	2	1
16	Bau Nhum	Quang Tri	2	1
17	Ho Truoi	Thua Thien Hue	4	2
<b>South Central</b>				
18	Phu Ninh	Quang Nam	5	3
19	Khe Tan	Quang Nam	4	2
20	Nuoc Trong	Quang Ngai	1	0
21	Đinh Binh	Binh Đinh	3	2
22	Thuan Ninh	Binh Đinh	3	2
23	Da Ban	Khanh Hoa	3	1
<b>Central Highland</b>				
24	Ia Mlah	Gia Lai	3	3
25	Ea Sup Thuong	Dac Lak	5	3
26	Krong Bukha	Dac Lak	6	4
<b>South East</b>				
27	Song Ray	Vung Tau	6	4
28	Suoi Giai	Binh Phuoc	6	2
29	Phuoc Hoa	Binh Phuoc	6	3
30	Cau Moi	Đong Nai	8	6

The results of Table 3 showed that 50% of the investigated dams were found 4-6 species, 43% of the dams found 1-3 termite species. The dam that found the largest number of termite species (8 species) was Cau Moi dam (Dong Nai province). It can be seen that the diversity on composition of termite species distributed on the dam was relatively low. This is understandable because the dam environment is inherently a homogeneous habitat, mainly convenient for subterranean termites to grow and develop, and less suitable for other termite groups such as groups of damp wood termites, dry wood termites or humus eating group.

In terms of damage of termites to dams, there were 6 works that were not found serious damage species namely: Trang Vinh, Yen Lap, Song Rac, Kim Son, Ke Go and Nuoc Trong dams. These were termite-safe dams during the investigation.

There were 4 works that termite species was found causing serious damage namely Vuc Mau, Vuc Tron, Bau Nhum and Da Ban.

The number of works that encountered 2-3 species causing serious damage accounted for a fairly big rate of 56.7%. There were 8 works that encountered 2 species causing serious damage including Dai Lai, Yen My, Song Sao, Ho Truoi, Khe Tan, Dinh Binh, Thuan Ninh and Suoi Giai dams, and 9 dams that were encountered 3 species including Cam Son, Dong Mo, Song Muc Rao Da, An Ma, Phu Ninh, Ia Mlah, Ea Sup Thuong and Phuoc Hoa dams.

There were 3 dams were serious damage by high number of termite species, i.e. Krong Bukha dam and Song Ray dam with 4 species; and Cau Moi dam with the highest number of 6 species.

In result, there were 8 dam works categorized as high risk of safety loss caused by termite that need to be treated immediately i.e. Dai Lai dam (Vinh Phuc province), Song Ray dam (Vung Tau province), EaSup Thuong dam, Krong BukHa dam (Dak Lak province), Phuoc Hoa dam (Binh Phuoc province), Cau Moi dam (Dong Nai province), Song Sao dam (Nghe An province) and Yen My dam (Thanh Hoa province).

## Conclusion

Twenty three termite species belonging to 2 families, 5 subfamilies and 10 termite genera were identified. In which, 13 species severely damaged the dams, 2 species damaged moderately, and 2 species damaged the architectural works on the dams, and 7 species that did not damage the dams.

Twenty nine of 30 investigated dams were found termite distribution at different levels. Among them, 50% of investigated dams were founded 4-6 termite species, 43% of dams found 1-3 termite species and Cau Moi dam (Dong Nai province) found 8 termite species.

Six dams were not recorded termites that damaged seriously on the dams, while 4 dams found 1 species, 8 dams found 2-3 species and 3 dams found 4-6 serious species that damaged seriously on the dam body.

Among 30 investigated works, 8 works were assessed to be at a high risk of safety loss due to the damage of termites and required immediate treatment plans namely: Dai Lai dam (Vinh Phuc), Song Ray dam (Vung Tau), EaSup Thuong dam, Krong BukHa dam (Dak Lak), Phuoc Hoa dam (Binh Phuoc), Cau Moi dam (Dong Nai), Song Sao dam (Nghe An) and Yen My dam (Thanh Hoa).

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Wednesday | March 13, 2024 | 10:00-10:15

### Section 3: Economic Impact and Innovative Management

O\_12

## Efficacy performance of bio-based termiticide containing Arnica extract from Berkem Biosolutions® against subterranean termites: Laboratory and field evaluation

by

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### Abstract

The efficacy performance of a bio-based termiticide product containing Arnica extract from Berkem Biosolutions® in soil treatment tests against subterranean termites had been evaluated in both laboratory and field conditions. The laboratory assessment was carried out using a Bottle H test unit (Fig. 1) following the Japan Wood Preservation Association (JWPA) standard, JWPAS-TS-(1) 2018 using *Coptotermes gestroi*. The field assessment was conducted using a simulation test unit, in which the treated soil surface was covered with a concrete slab (dimension of 63 x 63 x 10 cm) and a PVC pipe (10 cm in diameter and height) with a cover was put in the middle of it. A wood bait (rubberwood, dimension 5 x 5 x 10 cm) is placed inside the PVC pipe as the indicator of termite presence/attack to pass the treated soil barrier. Various subterranean termites were found surrounding the testing area, such as *Coptotermes sp.*, *Macrotermes gilvus*, *Microtermes sp.*, and *Odontotermes sp.*. The results of both laboratory and field assessments suggested that the product showed high resistance against subterranean termites.

**Keywords:** Soil treatment, bio-based termiticide, Arnica extract, subterranean termites

### Introduction

Subterranean termite management is essential to prevent structural damage, economic loss, and maintain environmental balance. The effective management approach includes wood treatments, soil barrier treatments, and population control using bait systems. The annual damage potential of subterranean termite attacks in southeast Asia and the United States alone is estimated to exceed US \$4 million and US\$5 billion, respectively (Lee et al. 2007; Peterson 2010), and the latest report on the global damage was estimated at US \$22 billion to US \$40 billion (Kalleswaraswamy et al. 2022). Preconstruction treatment of sub-slab soil using liquid termiticide is an effective method for preventing subterranean termite infestations (Su and Scheffrahn 1998) to establish a chemical barrier between the soil, where subterranean termites live, and the building.

The current trend in sustainable termite management is to make a transition from termiticides that use chemicals identified as Persistent Organic Pollutants (POPs) to a new environmentally safer control measure (Verma et al. 2018) as encouraged by the United Nations Environmental Programme (UNEP). POPs are a set of toxic chemicals that are persistent in the environment and able to last for several years before breaking down, They negatively affect humans, plant and animal species, and natural ecosystems both in close proximity and at significant distances away from the source of discharge. Research on bio-based pesticides using plant extract and natural resources has gained attention (Himmi et al. 2013; Mishra et al. 2021; Souto et al. 2021; Tarmadi et al. 2014).

The present study evaluated the efficacy performance of a bio-based termiticide product containing Arnica extract from Berkem Biosolutions® in soil treatment tests against subterranean termites in both laboratory and field conditions. Laboratory tests and field tests are both essential in research and have different strengths. Laboratory tests are conducted in a controlled environment that makes it easier to establish cause-and-effect relationships. However, the artificial setting may not accurately reflect real-world conditions and therefore, laboratory tests need to be complemented by field tests.

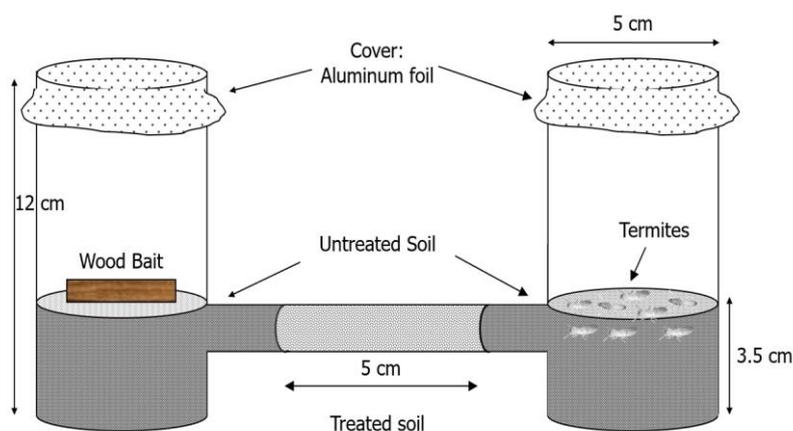
## Materials and methods

### *Bio-based termiticide product (Arnica Biosolutions)*

The bio-based termiticide tested is a water-based liquid product containing one specific plant polyphenolic extract from Berkem Biosolution®: Arnica extract, rich in bioactive flavonoids. Its formulation includes the patented Groupe Berkem advanced gel technology that provides right-dose application while ensuring optimal flavonoid stability.

### *Laboratory assessment*

The laboratory assessment was carried out using a Bottle H test unit (Figure 1) following the Japan Wood Preservation Association (JWPA) standard, JWPAS-TS-(1) 2018. The soil (20 mesh) was treated with the bio-based termiticide product with a concentration of 5 L/m<sup>2</sup> and air-dried for 1 week. The treated soil (7 g) was put into a glass tube tunnel (5 cm in length; 1.5 cm in diameter), while the untreated soil was added at the edge of the glass tube. For the control unit, untreated soil was put into the 5 ml glass tube tunnel as well as the glass container.



**Fig 1.** The Bottle H test unit

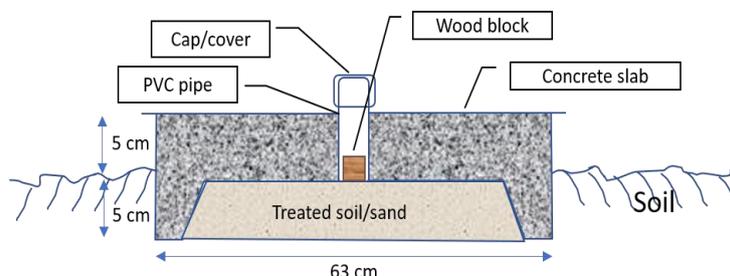
A set number of *Coptotermes gestroi* (150 workers and 15 soldiers) were introduced into one side of a glass container (12 cm in height; 5 cm in diameter), while a rubberwood with the dimension of 20 (R) x 20 (T) x 10 (L) was placed in the opposite side to lure termite (Figure 1). The length of tunnel penetration (mm) of treated and untreated soil (control unit) by termites was observed every day for 21 days to determine the efficacy score and criteria (Table 1). Termite mortality rate was recorded at the end of the test period. The test is deemed valid if the mortality rate in the control units does not exceed 20%.

**Table 1.** Efficacy criteria of soil treatment test, based on the length of penetration by termites in the glass tube tunnel.

Length of tunnel penetration (cm)	Score	Efficacy Criteria
0 – 1.0	1	High resistant
1.1 – 2.0	2	Moderate resistant
2.1 – 3.0	3	Low resistant
> 3.0	4	Non- resistant

### Field assessment

The field assessment was conducted using a simulation test unit as presented in Figure 2. The bio-based termiticide product was mixed evenly with soil (20 mesh) with a concentration of 5L/m<sup>2</sup> and air-dried for 1 hour. The treated soil surface is covered with a plastic layer with a 10 cm hole in the middle, and a concrete slab (dimension of 63 x 63 x 10 cm) is put on top of the area (Figure 1). A PVC pipe (10 cm in diameter and height) with a pipe cover is put in the middle of the concrete slab. A wood bait (rubberwood, dimension 5 x 5 x 10 cm) is placed inside the PVC pipe as the indicator of termite presence/attack to pass the treated soil barrier. The wood bait was replaced regularly or when the wood was deteriorated by termites or fungi.



**Fig 2.** Schematic test unit for field assessment on the efficacy performance of the bio-based termiticide product against subterranean termites.

The unit tests were installed in January 2021 and the monitoring was conducted twice per month, with the assessment data collected for three years running (ongoing observation). The efficacy criteria were measured based on the absence or the presence of termites during the monitoring period inside the PVC pipe (Table 2).

**Table 2.** The efficacy criteria of the bio-based termiticide product in the soil treatment test (field evaluation) against subterranean termites

Test results	Efficacy Criteria
No termite presence/termite attack on wood bait during the monitoring period	Resistant against subterranean termites
Termite presence/termite attack on wood bait is observed during the monitoring period	Non-resistant against subterranean termites

## Results and discussion

### Laboratory assessment

The laboratory assessment offers tests with a standardized testing method in a controlled environment (Hassan and Morrell 2021) and is cost-effective. The laboratory efficacy results of the bio-based termiticide product against subterranean termite *C. gestroi* in the soil-treatment test are presented in Table 3.



**Fig 3.** Record of tunnel penetration by *C. gestroi*: *Left* The bottle H experimental set-up; and *right* Control (untreated soil) unit, in which termites were observed to fully penetrate the tunnel (length 5 cm) during the observation period.

The results suggested that the product showed high resistance (efficacy score = 1) against *C. Gestroi* (Table 3). There was no termite penetration in all treated soil test units, indicating that the product provided good soil-barrier protection. The product also showed a high lethal effect on *C. gestroi*, as indicated by a 100% termite mortality rate in all treated-soil test units.

**Table 3.** Laboratory efficacy test of the the bio-based termiticide product against subterranean termite, *C. gestroi* in the soil-treatment test.

Sample code	Replication unit	Length of tunnel penetration (cm)	Score	Efficacy Criteria	Termite Mortality rate (%)
Bio-based termiticide product from Berkem Biosolutions®	R1	0	1	High resistant	100
	R2	0	1	High resistant	100
	R3	0	1	High resistant	100
	R4	0	1	High resistant	100
	R5	0	1	High resistant	100
Control Unit	R1	5.0	4	Non-resistant	18.0
	R2	5.0	4	Non-resistant	17.3
	R3	5.0	4	Non-resistant	16.7
	R4	5.0	4	Non-resistant	15.3
	R5	5.0	4	Non-resistant	18.7

### Field assessment

The lifespan of soil barriers chemical products against termites can vary depending on the quality of the product and the specific conditions of the application (Borchers and Pieler 2010). In her review, Faith Oi stated that a good termiticide would be (1) toxic or repellent; (2) applied in a continuous barrier; and (3) last at least 5 years (Oi 2022) as long as the soil barrier is not disrupted. In some sources of professional termite management, high-quality chemical termite barriers are designed to last up to a maximum of 8 to 10 years.

**Table 4.** Field efficacy test of the bio-based termiticide product against subterranean termites in the soil-treatment test.

Sample code	Replication unit	Observation*			Efficacy Criteria against subterranean termites
		2021	2022	2023	
Bio-based termiticide product from Berkem Biosolutions®	R1	(-)	(-)	(-)	resistant
	R2	(-)	(-)	(-)	resistant
	R3	(-)	(-)	(-)	resistant
	R4	(-)	(-)	(-)	resistant
	R5	(-)	(-)	(-)	resistant
Control Unit (untreated soil)	R1	(+)	(+)	(+)	Non-resistant
	R2	(+)	(+)	(+)	Non-resistant
	R3	(+)	(+)	(+)	Non-resistant
	R4	(+)	(+)	(+)	Non-resistant
	R5	(+)	(+)	(+)	Non-resistant

\*The monitoring is conducted twice per month from January 2021 to January 2024, and the monitoring in 2024 is still ongoing

Table information:

(-) = No termite is found inside the PVC pipe

(+) = termite present inside the PVC pipe/attack the wood bait

The field evaluation efficacy results of the bio-based termiticide product against subterranean in the soil-treatment test of observation are presented in Table 4. The results suggested that the product showed high resistance and protection against subterranean termites (Table 4), as there was no termite penetration in all treated soil test units during three years of observation, indicating that the product provided good soil-barrier protection. In the control units, termites were observed to fully penetrate the untreated soil and attack

the wood bait in the first month of observation and the wood bait was regularly replaced during the observation period. Various subterranean termites were found surrounding the testing area, such as *Coptotermes sp.*, *Macrotermes gilvus*, *Microtermes sp.*, and *Odontotermes sp.*...

## Conclusion

In conclusion, the laboratory efficacy results of the bio-based termiticide product containing Arnica extract from Berkem Biosolutions® not only showed high resistance (efficacy score = 1) but also a high lethal effect against subterranean termite *C. gestroi* in the soil-treatment test. The ongoing observation on the field showed that during the three-year assessment, the bio-based termiticide product had high resistance and protection against subterranean termites in the soil barrier test, indicated by no termite penetration found in all treated test units.

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Wednesday | March 13, 2024 | 10:15-10:30

### Section 3: Economic Impact and Innovative Management

O\_13

## Evaluation of different sea sand particle's mixtures as an effective physical control barrier against *Heterotermes indicola* (Blattodea: Rhinotermitidae) in the laboratory conditions

by

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### Abstract

Termites including *Heterotermes indicola* are economically important pests causing damages to the urban settlements. It makes underground nests and satellite galleries for access to the food source and thus controlling and eradication is not always an easy task. For a sustainable control, various mesh size of sea sand particles was assessed as a physical barrier in a hollow glass tube (5cm) for evaluating tunneling in equal (03 subgroups) and unequal (14 subgroups sand mixtures; based on A+C and A+BC pre-sand categories made) combinations of different ratios. C-20 (0.85 mm) and C-80 (0.25-0.18 mm) sand particles were used as a control standard. After the release of 50 workers + 02 soldiers in each setup, cumulative tunneling data was recorded for 03 days and mortality with blotting paper consumption data after 07 days. Overall, the pest covered significantly longer mean distance and made significantly higher mean number of tunnels in 5cm control barrier (C-80) as compared to equal sand combination (subgroup I-to-III) mixtures. In unequal combination it travelled significantly longer mean distance in 5cm control (C-80) and zero mean distance in sand mixture combination of subgroup VIII, IX, X and control (C-20) with significantly more mean number of tunnels in control (C-80) and zero mean number of tunnels in sand mixture combinations of group VIII, IX, X and control C-20. Similarly, significantly longer mean distance was observed in C-80 and zero mean distance in unequal sand mixtures combination (A+BC) subgroup XVI, XVII and C-20 with significantly more mean number of tunnels in C-80 and zero mean tunnels in sand mixture combinations of subgroup XVI, XVII and control (C-20). Overall, coarse sand particles alone (0.85-0.30 mm) and in combination with fine particles (0.25+mm) of equal and unequal ratios have successfully stopped the tunneling and caused maximum mortality with overall zero blotting paper consumption. While increasing the ratio of fine particles (0.42+mm) have increased the chances of tunneling with the reduction in the mortality.

**Keywords:** Sand mixtures, *Heterotermes indicola*, tunneling, sustainable management

### Introduction

Termites are eusocial insects living in colony and caste system and one of the most important damage causing agents to human belongings. They consist of reproductive, soldiers and workers caste (80-90% ) in which only workers are responsible for cleaning, foraging, mud tubes construction (Hickey, 2006) and all the economic damages caused by attacking on both soft and hard wood (Ahmed and French, 2008). Damages to structures and wooden houses are caused by the various species belonging to the family Kalotermitidae, Hodotermitidae, Termitidae and Rhinotermitidae (Ahmed and Qasim, 2011). Various genera of Termitidae

also causing severe losses to the agricultural crops, commercial forests, and structures (Batista-Pereira et al., 2004). In July 2007, Archicentre Institute of Australia calculated that annual cost of damage and replacement caused by termites was about 910 million dollars in which 100 million dollars' losses were caused by subterranean termites, alone (Ahmed and French, 2008). In Pakistan termites are causing enormous damages but no well documented information is available about the damages caused by the pest species. *Heterotermes indicola* is a devastating subterranean termites' specie, found across the country. They make satellite nests or mud galleries for reaching the food source. Termites were destructive pests, when left untreated, causing damages to apricot (5.68%), pear (7%), plum, peach, oranges, and leman at Tarnab, Lala kally and The University of Agriculture, Peshawar, Pakistan. In buildings severe damages are caused by *H. indicola*, *Coptotermes heimi* and *Odontotermes obesis*, *Anacantotermes vagas* and *Microtermes* species (Saljoqi et al., 2012).

Although chemicals are the main stay of termite's control but there are also alternative control methods (Hickey, 2006) including physical control methods that could be ecofriendly, sustainable, and cheap for easy and successful management. Sea sand barriers could be a potential physical control barrier if utilized scientifically but less emphasis has been given to this area of control method because of the lack of scientific knowledge and easily available termiticides. Although these termiticides are causing several environmental issues and responsible for the losses of biodiversity and ill effects on the ecosystem (Akhter, 2008). To provide a sustainable, eco-friendly, and cheap solution for the control of termites, this study was carried out at Termite lab, Nuclear Institute for Food and Agriculture (NIFA) Peshawar, Pakistan against *H. indicola*. for the construction of a successful physical barrier by evaluating various mesh size sand mixtures under laboratory conditions.

## Materials and methods

### Culture collection

Termite's culture was collected by installing NIFA termaps followed by collection after 14 days. Collected traps were brought to laboratory and gently jerked into plastic tub. Wet blotting paper pieces were put on the surface of the culture in the tub. Sticked termites to blotting paper were again gently jerked into a separate clean tub and stored in petri dishes by following Salihah et al. (1990).

### Preparation of various sand particles combinations

Two groups were made of sea sand graded particles from mesh size 20, 30, 40, 50 and 60-90+ in equal and unequal combinations. Equal combination group contained subgroup I, II, and III while unequal combinations contained subgroup IV to XVII. Subgroup I was made of mesh size 20 and 30, subgroup II of mesh size 20, 30 and 40, and subgroup III of mesh size 20, 30, 40 and 50 mesh in the ratio of 1:1, 1:1 and 1:1:1:1, respectively (Table 1).



**Fig 1.** Evaluating setup of different mesh size mixtures against *Heterotermes indicola* under the laboratory conditions. (A= Sieving mesh machine; B= sterilized sand of different mesh sizes; C= Termite culture; E-D= Glass tubes filled with sand mixtures and sealed; F= Termites tunneling in the barrier)

Prior to making mixtures of unequal combinations three main mixture categories were made containing

20 and 30 (1:1) mesh size in category A, 40 and 50 (1:1) in category B and a mixture of 60-90+ mesh size sand particle in category C (Table 2). Sub group IV to X were made by mixing of category A and C main mixtures in the ratio of 1:1; 1:2; 1:3; 1:4; 1:0.5; 1:0.25, 1:0.1 respectively and sub group XI to XVII were made by mixing of main category mixture A and BC (while B+C were used in 1:1) in the same ratio of 1:1; 1:2; 1:3; 1:4; 1:0.5; 1:0.25, 1:0.1, respectively (Table 1).

**Table 1.** Sand mixtures in various mesh size combinations for evaluation of a physical barrier against *Heterotermes indicola* under laboratory conditions

Subgroup	Mixture number	Number of sieve sizes mixed	Ratio
I	20_30	20+30	1:1
II	20_30_40	20+30+40	1:1:1
III	20_30_40_50	20+30+40+50	1:1:1:1
IV	A+C-1:1	(20+30)+(60+70+80+80+)	1:1
V	A+C-1:2	(20+30)+(60+70+80+80+)	1:2
VI	A+C-1:3	(20+30)+(60+70+80+80+)	1:3
VII	A+C-1:4	(20+30)+(60+70+80+80+)	1:4
VIII	A+C-1:0.5	(20+30)+(60+70+80+80+)	1:0.5
IX	A+C-1:0.25	(20+30)+(60+70+80+80+)	1:0.25
X	A+C-1:0.1	(20+30)+(60+70+80+80+)	1:0.1
XI	A+BC-1:1	(20+30)+{(40+50)+(50+60+70+80+)}	1:1
XII	A+BC-1:2	(20+30)+{(40+50)+(50+60+70+80+)}	1:2
XIII	A+BC-1:3	(20+30)+{(40+50)+(50+60+70+80+)}	1:3
XIV	A+BC-1:4	(20+30)+{(40+50)+(50+60+70+80+)}	1:4
XV	A+BC-1:0.5	(20+30)+{(40+50)+(50+60+70+80+)}	1:0.5
XVI	A+BC-1:0.25	(20+30)+{(40+50)+(50+60+70+80+)}	1:0.25
XVII	A+BC-1:0.1	(20+30)+{(40+50)+(50+60+70+80+)}	1:0.1
C-20	Control-I	20	-
C-80	Control-II	60+70+80+pan	1:1:1:1

An 18% distilled water was mixed to each subgroup, followed by filling the marked glass tubes with the moist sand of 5cm barrier for equal and unequal combinations subgroup. Then termites (50 workers + 2 soldiers) were released at one end and a single weighed and sterilized blotting paper strip (3 x 5cm) at other end before sealing each glass tube with aluminum foil and rubber band as shown in the Figure 01. Cumulative tunneling distance data was recorded for 3 days after 24hr along with % mortality and amount of blotting paper consumed data on day 7th after disassembling the setup. C-80 was used as a control treatment for equal proportion (subgroup I-III) while C-20 and C-80 was used as a control treatment for unequal proportion (subgroup IV-XVII). All the ratios were taken in grams and each treatment was replicated four times.

**Table 2.** Main mixture categories made prior to making of unequal combinations of physical barriers against *Heterotermes indicola* under laboratory conditions.

Mixture Category	Mixture of mesh sieve sizes	Ratio
A	20+30	1:1
B	40+50	1:1
C	60+70+80+90	1:1:1:1

### Data analysis

Collected data was analyzed by using SYSTAT Statistical Package Software, SPSS. Means were calculated by using descriptive statistics and Tukey's HSD was calculated by using one-way ANOVA (SPSS, 2008).

## Results and discussion

### Equal combination group

Equal combination sand barrier group (I, II, III), results in Table 3 revealed that *H. indicola* travelled significantly higher mean distance of 1.61, 1.61 and 4.42cm in control of 5cm sand barrier on day 1, 2 and 3, respectively. In sand mixture combination of subgroup-I (20\_30) the pest travelled zero distance while in subgroup-II (20\_30\_40) and subgroup-III (20\_30\_40\_50) only 0.19cm (nonsignificant) distance was recorded on day 1, 2 and 3, respectively. The mean number of tunnels made by *H. indicola* were significantly more of 01 in control while 0.52 and zero number of tunnels in subgroup III and I; and II on day 1, 2 and 3 respectively.

**Table 3.** Mean distance travelled, and mean number of tunnels made by *Heterotermes indicola* in 5cm barrier of equal combination of sand mixtures under laboratory conditions

Mixture group/size	Mean distance travelled in 5cm barrier on			Mean numbers of tunnel made in 5 cm on		
	Day1±SE	Day2±SE	Day3±SE	Day1±SE	Day2±SE	Day3±SE
subgroup I (20_30)	0 a	0 a	0 a	0 a	0 a	0 a
subgroup II (20_30_40)	0 a	0 a	0 a	0 a	0 a	0 a
subgroup III (20_30_40_50)	0.19±0 a	0.19±0.078 a	0.19±0.078 a	0.52±0.19 b	0.52±0.18 b	0.52±0.18 b
Control-I C-80	1.61±0.27 b	1.61±0.27 b	4.42±0.11 b	1±0 b	1±0 b	1±0 b

### Unequal composition group

Mixtures of Subgroup IV to X (A+C)

Investigation of subgroup IV to X (Table 4) reveals that *H. indicola* travelled significantly longer distance of 7.87, 8.52 and 8.52cm in control (C-80) while zero distance for subgroup VIII to X (A+C-1:0.5, A+C-1:0.25, A+C-1:0.1) and control (C-20) on day 1, 2 and 3. Similarly mean number of tunnels made by the pest were significantly more in control (C-80) of 2.83, 3.12 and 3.12 with no tunnels in the sand mixture combinations of subgroup VIII to X (A+C-1:0.5, A+C-1:0.25, A+C-1:0.1) and C-20 on day 1, 2 and 3. Subgroup IV to VII were tunneled but successfully resisted to the penetration/breaching of the barrier with non-significant mean number of tunnels as shown in the Table 4.

Mixture of subgroup XI to XVII (A+BC)

Investigation of unequal composition of subgroup XI to XVII (A+BC) showed that *H. indicola* travelled significantly longer mean distance of 8.96, 9.05 and 9.05cm in control-II (C-80) with zero distance in subgroup XV, XVI, XVII (A+BC-1:0.5, A+BC-1:0.25, A+BC-1:0.1) and Control-I (C-20) on day 1, 2 and 3 (Table 5). The number of tunnels made were significantly more in control-II (C-80) of 6.84, 7.03 and 7.03 tunnels on day 1, 2 and 3 but zero tunnels were made in sand mixture combinations of subgroup XVI and XVII (A+BC-1:0.25, A+BC-1:0.1) and control-I (C-20). Subgroup XI to XV were tunneled but successfully resisted to the penetration/breaching of the barrier with non-significant mean number of tunnels as shown Table 5.

Literature survey reveals that the particle size plays a significant role in the tunneling and a similar combination of mesh size 20 and 30 (0.84-0.59mm) and 20-to-50 (0.84-0.30mm) were assessed for the tunneling behavior of *Coptotermes formosonus* by Carnelius (2005) and reported that tunneling was significantly affected by the particle size. Similarly, Acda and Ong (2005a) studied uniform mixture of mesh size 16-8 (1.18-2.36mm) and found effective against *C. vastator* penetration (tunneling). Acda and Ong (2005b) concluded that uniform mixture of mesh size 16-8 (1.18-2.36mm) or mesh size 12-8 (1.70-2.36mm) was effective against penetration (tunneling) of *Nasutitermes luzonicus* or *Microcerotermes losbanosensis* and *Macrotermes gilvus*, respectively. Su et al., (1991) found mesh size 16-7 (1.18-2.80mm) effective as a barrier made of uniform mixtures against *C. formosonus* and *Reticulitermes flavipes*. French et al, (2003) reported that maximum mortality leading to no tunneling could be caused either the sticking of termites or

cut off and rupturing of lightly sclerotize body with the rough/angular particles in all groups of both equal and unequal proportions with no blotting paper consumption.

**Table 4.** Mean distance travelled, and mean number of tunnels made by *Heterotermes indicola* in the various sand mixtures of subgroup IV to X (A+C) under laboratory conditions.

Sand mixture group	Mean distance travelled in 5cm barrier on			Mean number of tunnels made on		
	Day1±SE	Day2±SE	Day3±SE	Day1±SE	Day2±SE	Day3±SE
Subgroup IV A+C-1:1	0.51±0.15 b	0.75±0.11 b	0.75±0.11 b	0.86±0.26 ab	1.45±0.12 bc	1.45±0.12 bc
Subgroup V A+C-1:2	1±0 b	2.00±0 c	2.98±0.05 c	1.45±0.12 bc	1.45±0.12 bc	1.69±0.11 bc
Subgroup VI A+C-1:3	0.88±0.14 b	2.24±0.05 c	3.42±0.01c	0.68±0.19 ab	0.68±0.19 ab	0.68±0.19 ab
Subgroup VII A+C-1:4	1.12±0.06 b	2.34±0.04c	3.50±0.01 c	0.86±0.26ab	0.86±0.26 b	0.86±0.26 b
Subgroup VIII A+C-1:0.5	0 a	0 a	0 a	0 a	0 a	0 a
Subgroup IX A+C-1:0.25	0 a	0 a	0 a	0 a	0 a	0 a
Subgroup X A+C-1:0.1	0 a	0 a	0 a	0 a	0 a	0 a
Control-I C-20	0 a	0 a	0 a	0 a	0 a	0 a
Control-II C-80	7.87±0.1 c	8.52±0.07 d	8.52±0.07 d	2.83±0.18 c	3.12±0.15 c	3.12±0.15 c

**Table 5.** Mean distance travelled, and mean number of tunnels made by *Heterotermes indicola* in various sand mixtures of subgroup XI to XVII (A+BC) under the laboratory conditions.

Sand mixture group	Mean Distance travelled in 5cm barrier on			Mean numbers of Tunnels made on		
	Day1±SE	Day2±SE	Day3±SE	Day1±SE	Day2±SE	Day3±SE
Subgroup XI A+BC-1:1	1.10±0.17 b	1.17±0.18 b	1.26±0.21b	1.91±0.15 c	2.13±0.18 c	2.31±0.22b
Subgroup XII A+BC-1:2	1.60±0.18 bc	1.91±0.19 bc	2.21±0.21 bc	2.22±0.08 c	3.23±0.06 c	3.64±0.08b
Subgroup XIII A+BC-1:3	1.81±0.06 bc	1.89±0.06 bc	1.95±0.05 bc	2.46±0.09 c	2.46±0.09 c	2.95±0.09 b
Subgroup XIV A+BC-1:4	2.92±0.07 c	3.37±0.07 c	3.49±0.04 c	3.16±0.13 c	3.16±0.13 c	3.36±0.16 b
Subgroup XV A+BC-1:0.5	0.17±0.06 a	0.19±0.07 a	0.19±0.07 a	0.68±0.19 b	0.68±0.19 b	0.68±0.19 a
Subgroup XVI A+BC-1:0.25	0 a	0 a	0 a	0 a	0 a	0 a
Subgroup XVII A+BC-1:0.1	0 a	0 a	0 a	0 a	0 a	0 a
Control-I C-20	0 a	0 a	0 a	0 a	0 a	0 a
Control-II C-80	8.96±0.13 d	9.05±0.13 d	9.05±0.13 d	6.84±0.12d	7.03±0.14 d	7.03±0.14 c

## Conclusion

Overall, coarse sand particles alone (0.85-0.30 mm) and in combination with fine particles (0.25+mm) of equal and unequal ratios have successfully stopped the tunneling and caused maximum mortality. Increasing the ratio of fine particles (0.42+mm) have increased the chances of tunneling thus proving to be a potential control agent against *H. indicola* under laboratory condition but similar study shall be carried out according to the field conditions for further evaluation of the effectivity of the particles. These particles sizes are specific to *H. indicola* only under lab condition and shall not be used without the recommendation of local specialist.

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Wednesday | March 13, 2024 | 10:30-10:45

### Section 3: Economic Impact and Innovative Management

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## Termite management by entomopathogenic fungi: Recent advances and future prospects

by

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### Abstract

Termites are global pest for man-made structures, and in some parts of the world, they infest agricultural crops as well. Termite control relies predominately on chemical insecticides, but the negative impacts of synthetic termiticides on the environment limit their applications. Entomopathogenic fungi (EPF) are considered an environmentally friendly alternative. Nevertheless, termites have developed various defensive behavioral strategies to circumvent EPF. In this review, we summarize 1) the use of EPF as a biological control agent and strategies/defenses of termites that minimize the effectiveness of EPF, 2) the potential of integrating entomopathogenic fungi with RNA interference (RNAi) as an effective termite control strategy, 3) discussed nanoparticles (silver, chitosan, titanium, etc) which are biosynthesized from different EPF. Nanoparticles have the ability to deliver RNAi triggers (dsRNA/siRNA), so we proposed the synthesis of EPF-based nanoparticles and dsRNA/siRNA complex as potential strategy to control termite pests.

**Keywords:** Termites, biological control, entomopathogenic fungi, RNA interference, nanoparticles

### Introduction

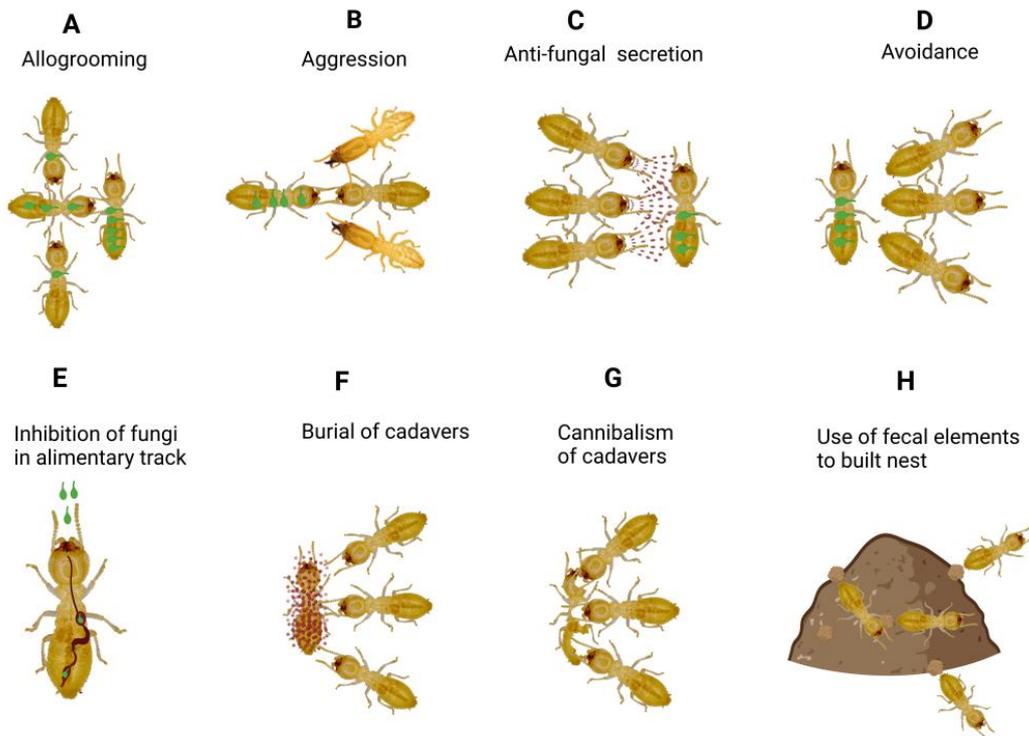
Termites eat all plant remains, including dying wood materials, which make them wonderful decomposers (Mugerwa, 2015). Annually one-third dead wood is recycled and broken down by termites in subtropical and tropical environments (Verma et al., 2009). Agricultural crops, including legumes (chickpea, beans, pigeon pea and cowpea), vegetables (tomato, pepper, eggplant and okra), root crops (yam, potato and cassava), fruits (mulberry, mango, coffee, guava, almond, plum and litchi), sugarcane, cotton, tea and tobacco are damaged by termites (Qasim et al., 2015). Termites not only damage crops but also trees (Rao et al., 2012). Approximately 300 species of trees from 76 families have suffered from termite attack. About 40%-60% of trees die due to termite attack in southern hilly areas of China. Termites also damage structures, including houses, dams, bridges, decks (Li et al., 2010), roads, poles, walls, underground pipes, cables (Lenz et al., 2013), historical materials, archives, and works of art with high historical value (Liotta et al. 1999). Damage and repair costs due to termite infestations exceed 40 billion dollars per year around the world (Rust

and Su, 2012).

Different strategies are used to effectively manage termite population. However, various factors like information about termite biology and ecology, infestation level, soil characteristics, cropping systems, and cost of control can influence termite management. Chemical control depends upon the effective use of termiticides and information about resistance against insecticides. Non-chemical control strategies used to control termites include physical and mechanical control (heating, freezing, digging nests, non-toxic barriers), cultural control (crop rotation, mulching, and intercropping), biological control (predators, parasitoids, and entomopathogens), and emerging biotechnological tools (Ahmad et al., 2021). Biological control has excellent potential for controlling termites commercially (Chouvenc et al., 2011). In this review, we will discuss the biological control of termites, focusing on entomopathogenic fungi (EPF) and strategies of termites to minimize EPF effectiveness. The use of EPF and RNA interference (RNAi) combination to weaken termite's defenses against EPF then proposed the production of EPF-based nanoparticles and siRNA/dsRNA complex as a potential strategy to control termite pests.

### Termite's defense against fungal infections

Termites have the ability to detect the odor of conidia, which leads to the start of allogrooming behavior (Mburu et al., 2009). Allogrooming behavior is an important part of the defense behavior of termites, during which termites can externally remove pathogens from cuticles and clean the cuticles to prevent the growth of pathogens during the early infection stage (Liu et al., 2019). Pathogens are attached to the surface of infected termites loosely during early infection stage, so it's easy for nestmates to clean these pathogens through allogrooming (Yanagawa and Shimizu, 2007). Meanwhile, soldiers are unable to provide grooming activities, but they produce volatiles or chemicals to contribute in sanitary care (Mitaka et al., 2017; He et al., 2018). Termites eat infected individuals or bury dead ones (Yanagawa et al., 2011). Besides external defense, termites also use the immune system to serve colony-level defenses. Termites infected with the fungus *Metarhizium* can transmit low doses of pathogens to caregivers through allogrooming, and after acquiring a low level of infection, caregivers can trigger active immunization against fungal growth (Liu et al., 2015). The mechanism of different defense strategies of termites is also depicted in Figure 1.

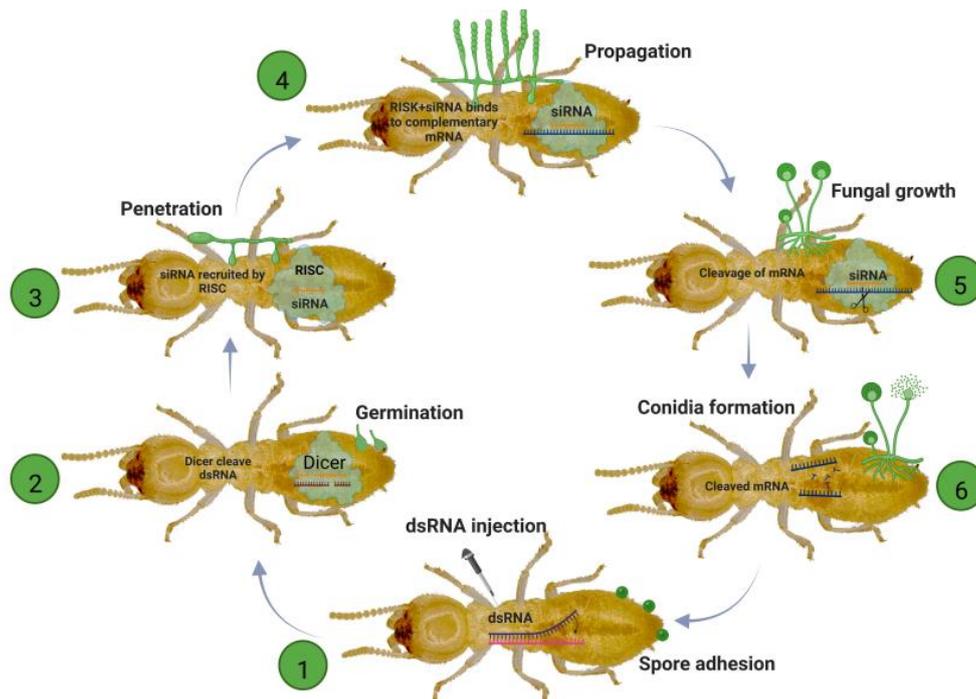


**Fig 1.** Different defense strategies of termites against EPF. (A) Allogrooming, (B) showing aggression towards an infected individual, (C) secreting anti-fungal chemicals towards an infected individual, (D) avoiding a fungus-exposed individual, (E) inhibition of fungi in the alimentary canal, (F) burial of infected cadavers, (G) cannibalism of infected cadavers, (H) use of fecal elements in construction of nest

## Combination of RNAi and EPF as a potential strategy to weaken termite's defenses

Although EPF are effective against termites in laboratory experiments, however efficiency of EPF could be better when used in the field against termites. Therefore, the combination of EPF with some other environmentally friendly technologies like RNAi can enhance the effectiveness of EPF. One of the main reasons in the low efficiency of EPF against termites is the social and individual immunity of termites against pathogens. RNAi can break these immune responses of termites and can make EPF more effective, as shown by Hamilton and Bulmer (2012), when knockdown of important immune genes GNBPs and termicin increased mortality of the termite *R. flavipes* following exposure to the *M. anisopliae*. Silencing of selenoprotein T gene decreased anti-fungal activity and impaired active immunization in the termite *R. chinensis* against *M. anisopliae* and increased mortality (Zhao et al., 2020). Zhou et al., (2021) also showed a close relationship between selenium-binding protein (SeBP) and active immunization in termites. Silencing SeBP gene in nestmates of fungus-exposed termites reduced the survival of their nestmates in the termite *R. chinensis*, which suggested that dysfunctional immune response through RNAi can be used to make EPF more effective.

The effectiveness of RNAi is not limited to genes related to immunity. Silencing of IDH gene involved in the metabolism (tricarboxylic acid cycle) resulted in the reduced immunity of termites after the fungus *M. anisopliae* infection along with increased apoptotic lesion, which ultimately improved the mortality of termites (Liu et al., 2020). Similarly, the combination of phosphofructokinase gene silencing and *M. anisopliae* infections impaired the glycolytic process, altered locomotion and allogrooming, decreased immunity, and reduced survival in *R. chinensis* (Hassan et al., 2022). RNAi of essential genes related with immunity or metabolism makes termites vulnerable against EPF, which can be used as a potential biological control strategy to control termites. The suggested mechanism of the synergistic effect of combining RNAi with EPF against termites is depicted in Figure 2.

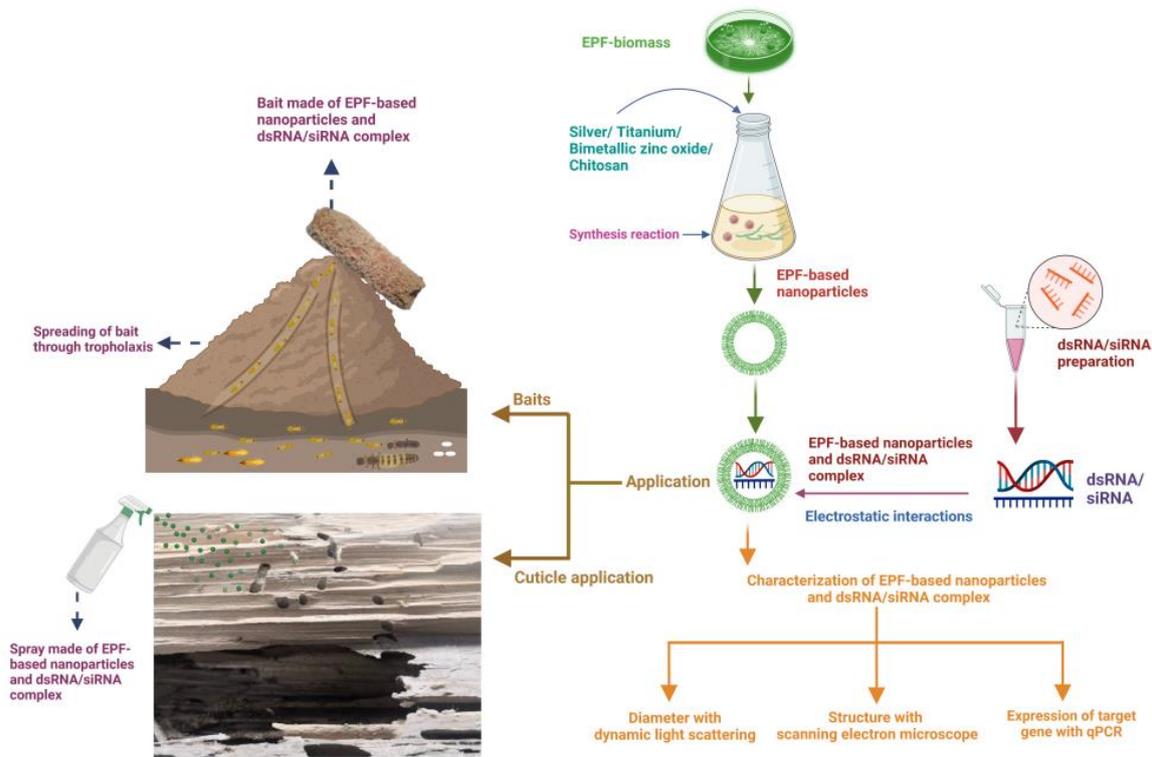


**Fig 2.** The suggested mechanism of the synergistic effect of integrating RNAi with EPF against termite pests.

### EPF-based nanoparticles and RNAi triggers as potential termite control strategy

RNAi is used not only as a tool for gene functional analysis but also as a potential alternative approach to chemical pesticides for controlling insect pests, leading toward sustainable agriculture (Cai et al., 2018). Main challenge in the RNAi based pest management of insects is delivery of dsRNA/siRNA because traditional delivery systems like injection, oral feeding, soaking and transgenic plants are not considered as much effective. Nanoparticles such as dendrimers, chitosan, cationic and liposomes can deliver dsRNA/siRNA effectively. These nanoparticles have the ability to protect dsRNA/siRNA from enzymatic

degradation and help in translocation across cell membranes (Ahmadzada et al., 2018; Chalbatani et al., 2019). Nanoparticle-mediated RNAi also proved effective by oral feeding in *Aedes aegypti*, *Blattella germanica*, and *Agrotis ypsilon* (Xu et al., 2014; Zhang et al., 2015; Lin et al., 2017; Chen et al., 2019). Chitosan nanoparticles made from EPF *M. anisopliae* proved very effective against *Plutella xylostella* (Wu et al., 2022).



**Fig 3.** Proposed system for the complex formation, characterization, and application of EPF-based nanoparticles, and dsRNA/siRNA to potentially control termite pests

Similarly, various other nanoparticles like silver, titanium and bimetallic zinc oxide can be made from EPF (Yosri et al., 2018; Srivastava et al., 2019; Kumaravel et al., 2021). Nanoparticles have ability to attach with dsRNA through electrostatic forces (Yan et al. 2021; Ma et al. 2022;). Chitosan nanoparticles can be made from EPF (Wu et al., 2022), and also have ability to deliver dsRNA (Zhang et al., 2015). Similarly, other nanoparticles (silver, titanium, bimetallic zinc oxide) may also have ability to deliver dsRNA/siRNA too like chitosan nanoparticles, which can be applied in the field in the form of spray or baits. Forager workers share baits with their nestmates through trophallaxis (Li et al., 2010; Chouvenec and Su, 2017). Thus, we proposed EPF-based nanoparticles and dsRNA/siRNA complex system for the control of termite pests (Figure 3). Although there is a lack of research work on dsRNA/siRNA delivery with the help of EPF-based nanoparticles against termites, but this will be an interesting research area to be explored in the future.

## Conclusion

Termites have evolved various defense strategies against EPF, which makes the biocontrol of termites difficult. Previously, EPF have been used to control termites, but there is a need to make EPF more effective against termites.

Recent studies reported that the combination of RNAi and EPF is effective in disrupting the immunity of termites and improving mortality in the laboratory. EPF and RNAi together have the potential to break termite defense mechanisms.

Nanoparticles (silver, chitosan, titanium, etc) have the ability to deliver RNAi triggers (dsRNA/siRNA), and these nanoparticles can be made from EPF. Future studies must focus on the selection of important genes and the synthesis of EPF-based nanoparticles. Then, combining those nanoparticles with RNAi triggers of selected genes could potentially control the termites in field conditions.

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**Special Forum:  
Innovation in Termite Management**

Wednesday | March 13, 2024 | 11:00-11:15

Special Forum: Innovation in Termite Management

F\_01

## Introducing a Novel Termiticide Metamisalt®20SC (Dicloromezotiaz 20 SC)

by

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### Abstract

ZMCP, a Japanese joint venture company of Zen-noh and Mitsubishi Corporation, will present the technical profile of a novel new termiticide, Metamisalt®20SC, in cooperation with its partnering company ENSYSTEEX. Metamisalt® has a unique insecticidal mode of action, classified under IRAC sub-group 4E as mesoionic, a competitive modulator of the Achr binding site in nAChR in the neuro-system. The new termiticide Metamisalt® 20SC has been launched in the Japanese market successfully in 2022. Metamisalt® is highly effective in controlling subterranean termites, such as *Coptotermes formosanus*, with soil application. Metamisalt® is a non-repellent and slow-killing termiticide with excellent transfer effect through the grooming behavior of the termite population. With this excellent transfer effect, Metamisalt® demonstrated colony elimination performance in a trial conducted in actual infested house structures. The active ingredient, dicloromezotiaz is odorless with very low water-solubility, low vapor pressure, and highly resistant to alkali at elevated temperature conditions. Metamisalt® is highly safe for mammals both in acute and chronic toxicity with a high ADI of 1.2 mg/kg body weight/day and it is also environmentally safe. These physicochemical and toxicological properties make the product a suitable termiticide for urban use. In the near future, ZMCP and ENSYSTEEX aim to bring this new product into the Asian market as an innovative new solution for termite control.

Wednesday | March 13, 2024 | 11:15-11:30

Special Forum: Innovation in Termite Management

F\_02

## Laboratory and field evaluations of selected plant polyphenolic extracts from Berkem Biosolutions® as bait toxicants for termite control in tropical zones

by

Florent Chopinet<sup>1</sup>, Stéphane Savriama<sup>1</sup>, Daouia Messaoudi<sup>2\*</sup>

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### Abstract

Baiting technology for managing termite populations has shown great potential for almost three decades. Studies have shown successful use of chitin synthesis inhibitors from the benzoylphenyl urea chemical group in commercially available baiting systems. Recent efforts in Europe and other economically developed regions are targeting on sustainable nature-friendly technologies to replace these conventional synthetic agents with negative impacts on the environment and human health.

The plant polyphenolic extracts from Berkem Biosolutions® are 100% plant-based ingredients, rich in active molecules, particularly polyphenols, specially adapted to the needs and challenges of termite pest management. The efficacy performance of the polyphenolic extracts was evaluated against two species of subterranean termites, *Coptotermes gestroi* and *Prorhinotermes canalifrons*, currently present in Reunion Island. The laboratory assessment was carried out against *Coptotermes gestroi* using choice and no-choice protocols. Bait consumption, termite behavior, and termite mortality were recorded with cellulose paper and compressed cellulose tablets both treated with the polyphenolic extracts. The field assessment was conducted with input of pest control organizations at various sites in Reunion Island where the current commercial bait systems had failed. The period to eliminate the colony for both species of termites was recorded from 3 months up to 12 months with the polyphenolic extracts impregnated in wooden stakes installed in the ground all around the residential houses.

This work reported the excellent potential of the plant polyphenolic extracts from Berkem Biosolutions® as biobased bait toxicant for the control of subterranean termite populations *Coptotermes gestroi* and *Prorhinotermes canalifrons* in tropical zones.

**Keywords:** *Coptotermes gestroi*, *Prorhinotermes canalifrons*, polyphenolic plant extracts, biobased bait toxicant, overseas department

Wednesday | March 13, 2024 | 11:30-11:45

**Special Forum: Innovation in Termite Management**

**F\_03**

**Hydrotrail-a termite guiding strategy creating a termite defence zone**

by

Steve Broadbent

Technical Director, Ensystem

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**Abstract**

Increased societal mobility during the last 100 years, climate changes, and resistance to pesticides have had an enormous impact on the distribution of pestiferous insects around the globe. Termites have always been moved by human travel and commerce, but the frequency of such movements (that is, the rate of invasion) has dramatically increased. Consumers are also requesting more environmentally sustainable pest management strategies.

HydroTrail is a new termite “Connect the stations” guiding technology. Thanks to the hydration-based physical mechanism of action, it aggregates water forming a consolidated water trail after application to soil. Thus, it directs termites to detection stations resulting in substantial reduction of the detection time to improve increased efficacy of termiticide treatments creating a trail to lure termites to the treatment zone or bait stations. It helps to implement control measures lowering the possible damage, whilst also helping to determine the effectiveness of the *control methods* implemented and *adjust* treatments, as required.

It is a relatively cheap, biodegradable, and easy-to-apply formulation (with no digging nor trenching required) with a 90-days persistence in soil.

In field evaluations, termites only tunneled to pop-up detection stations that had Hydrotrail treatment lines. Control detection stations, which were surrounded by untreated soil, were not discovered by termites. Once the termites found a detection station, they only stayed active in the station for a couple of months due to the limited food source (wood) present in the pop-up detection stations. These findings suggest that, in field conditions, termites could locate and follow a Hydrotrail to a detection station.

**Wednesday | March 13, 2024 | 11:45-12:00**

**Special Forum: Innovation in Termite Management**

**F\_04**

**Navigating termite management innovations in the Philippines**

by

Ann Margaret L. Castilan

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**Abstract**

Pest management professionals (PMPs) grapple with the ever-evolving challenges in termite management. As technologies rapidly advance and pesticide resistance looms as a threat, the pest management industry must adapt and innovate to maintain effective termite control strategies from detection and monitoring tools to well-designed termite control programs.

A key aspect of termite control revolves around the species identification and detection of infestations. While traditional methods like visual inspections and bait stations have been staples, technological advancements have introduced more efficient strategies. Acoustic emission technology (i.e. Termatrac's iTraker) has proven highly effective in pinpointing hidden termite colonies using radar, moisture and temperature as parameters of detection. The integration of this innovative detection method into pest management practices highlights the importance of data-driven decision-making which enhances the confidence, reliability, and professionalism of PMPs. Most importantly, promoting precision and productivity while reducing reliance on conventional, time-consuming approaches.

The evolving nature of termiticides, and the selection of suitable active ingredients (a.i.) integral to termite control, also demands attention. While there have been several termiticide products of various a.i. that are out on the market today, bifenthrin (IRAC Group 3A) has been traditionally used as an effective termite prevention with its long stability and residual (repellency) properties. Alongside is another novel compound, broflanilide (IRAC Group 30), which was recently introduced as a termiticide and has been recognized to be a game-changer in breaking pesticide resistance to the commonly used neuro-insecticides. Although either is effective, the coexistence of both traditional and novel technologies provides an opportunity of choice, competitive advantage, innovation and market evolution.

Either and/or the combination of approaches is effective with in-depth knowledge and professional experience in using appropriate application strategies. Aside from comprehending the biology and behavior of termites, understanding the mode of action of termiticides is paramount to optimizing its use (i.e. dilution rate, timing and circumstance) and ensuring more successful outcomes. It is imperative for PMPs to continuously elevate their pest knowledge and pioneer innovations in termite control, positioning them as leaders in the field. PMPs' thrive for innovation fosters a culture of continuous learning, adapting to cutting-edge technologies, and addressing the dynamic challenges posed by termites.



# Poster Presentation

Tuesday | March 12, 2024 | 14:00-14:45

**P\_01** Poster Presentation

## **Mud-tube repairing behavior and capacity in *Macrotermes gilvus* (Termitidae, Blattodea)**

by

Bramantyo Wikantyo\*, S. Khoirul Himmi, Didi Tarmadi, Ikhsan Guswenrivo, Sulaeman Yusuf

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Bogor, Indonesia

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### **Abstract**

Termites spend most of their lives searching for food resources. Unlike other insects accustomed to open habitats, subterranean termite groups scavenge under mud tubes that connect nests and food resources either underground or aboveground. Several studies on mud-tube repairing behavior have been conducted in *Macrotermes* spp. and *Odontotermes* spp. However, many details of this repairing behavior are yet to be observed. In this study, the mud-tube repairing rate and capacity in *Macrotermes gilvus* were analyzed. The mud-tube was purposively broken down to a particular length, and the behavior of the mud-tube repairing was observed and recorded. The capacity of the mud-tube was analyzed using a Computer Vision based analysis. Machine learning model evaluation was established, and further mud-tube capacity prediction was conducted. The results showed that mud-tube repair was performed in a 'zipping pattern'. The process started from the lowest area and edge of the broken mud-tube, and gradually moved upward, followed by the repairment of the middle part of the mud-tube. In total, the mud-tube repairing rate of *M. gilvus* was able to cover a 5.62 cm length broken mud-tube in 780 s. Each worker spent 7.37 s attaching new material or blocking onto the broken mud-tube, excluding travel time. After the model prediction, as many as 60 workers passed through the broken mud tube under 25 s of observation. The utilization of computer vision to track and count foraging termites in real time may open new possibilities for monitoring systems using artificial intelligent technology.

**Keywords:** Termite monitoring, tunneling behavior, fungus-growing termite, urban termite pest, artificial intelligent

Tuesday | March 12, 2024 | 14:00-14:45

**P\_02** Poster Presentation

## **Are borate solutions effective in wood protection against drywood termites?**

by

Andrew H.H Wong<sup>1,2</sup> and Carlson A.D. Tawi<sup>1</sup>

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### **Abstract**

Drywood termites can be very destructive to indoor wooden structures but their cryptic activity and slow attack rates often make wood damage undetectable until their faecal pellets are found on infested wood much later. It is hence crucial to prescribe proven pre-construction wood protection strategies against such termites. The ability of borates to protect wood against termites has been questioned occasionally due to sporadic observations of poor performance of borates as wood protecting termiticides. This question is also relevant to termite control professionals who conduct topical application of borates (eg. DOT) indoors. This study evaluated the efficacy of rubberwood (*Hevea brasiliensis*) blocks dip-treated with different concentrations of a glycol-borate product (containing nominally 40% Disodium Octaborate Tetrahydrate = DOT) and dipping times against drywood termites. Rubberwood blocks were dip-treated for 5 or 60 seconds using 3.64%, 5%, 6.67%, 13.33% and 20% DOT. Replicated air-dried treated rubberwood blocks were randomly sandwiched between drywood termite (*Cryptotermes cynocephalus*) infested sawn hardwood Binuang (*Octomeles sumatrana*) materials for 7 months, after which the level of termite resistance of the wood blocks were assessed by a 10-point termite rating scale and percentage mass losses recorded. Despite the slow rate of attacks typical of drywood termites, it was shown that untreated controls generally sustained superficial attack (mean mass loss: 1.3%, mean termite rating: 9.4) especially one block which had the lowest rating 8. While attacks on untreated blocks were broadly similar to many treated counterparts (mass loss: 0.1 – 1.3%, rating: 9.4 – 10), there were however notable exceptions observed on treated rubberwood: (i) blocks treated with 13.33% sustained moderate to severe attacks (mean mass loss: 7.1%, rating: 8.3); (ii) there were treated blocks rated 8 [3.64% (60s), 5% (5s), 6.64% (60s)]; (iii) one treated block was rated 6 [13.33% (5s)] and rated 7 [13.33% (60s)]; and blocks treated at 20% sustained nibbling to slight attacks. Increased dipping times and concentrations did not appear to confer higher wood protection. Therefore termite attacks can still occur on rubberwood treated even at considerably high surface DOT concentrations (13.33 & 20%), implying that other wood protection chemicals are preferred against drywood (and subterranean) termites. This finding is also useful to the relevant termite control professionals.

**Keywords:** Drywood termites, *Cryptotermes cynocephalus*, envelope treatment, rubberwood, Disodium Octaborate Tetrahydrate, wood protection

Tuesday | March 12, 2024 | 14:00-14:45

**P\_03** Poster Presentation

## **Wood protection against termites with selected new generation permethrin-azole based preservatives for The Philippines**

by

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### **Abstract**

Subterranean and drywood termites continue to ravage wood in construction throughout much of the tropics, incurring considerable costs in property damages as well as post-construction remedial treatment costs to the wood materials, and The Philippines is no exception to this nuisance. Wood protection offers a proven strategy to protect both home-grown hardwoods and softwoods against termite threats in The Philippines, and wood-protecting chemicals have been made available in the marketplace for immediate pre- and post-construction wood protection needs. Hardwood (*Pterocarpus indicus* = Narra) and softwood (*Pinus insularis*) Two novel “Solignum” trade named permethrin-azole based microemulsion wood preservatives (permethrin: 0.2% m/m, tebuconazole: 0.15% m/m, propiconazole: 0.15% m/m), compared with conventional permethrin-triazoles based LOSP (of similar concentrations) used in The Philippines, have been subjected to an H2-Hazard Class (indoor aboveground exposure) termite test for 24 months at a Los Banos, Laguna. Replicated wood blocks of these woods were envelope-treated with 125 and 250 ml/m<sup>2</sup> solution retentions. At the end of testing, all three variants of the permethrin-azole based formulations completely prevented subterranean termite attack on both hardwood and softwood at both applied solution retentions unlike all the untreated counterpart wood blocks that sustained attacks [mean termite attack severity: 2.1 (hardwood), 3.2 (softwood)] according to a 5-point termite rating scale. Clearly, such waterborne formulations are suitable for wood protection against termites when incorporated in any wooden construction in this region.

**Keywords:** Solignum, wood protection, envelope treatment, permethrin-triazoles, termite control

Tuesday | March 12, 2024 | 14:00-14:45

**P\_04** Poster Presentation

## **High termite resistance of kempas (*Koompassia malaccensis*) hardwood protected with a novel vegetal extracts-cypermethrin wood preservative under outdoor aboveground tropical environment**

by

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### **Abstract**

An environmentally-conscious society in Europe and other economically developed regions requires that wood-protecting chemicals contain lesser or lower levels of synthetic actives and be supplemented instead with bio-based ingredients to enhance the bioefficacy of the wood treatment formulations. Particular reference to the novel proprietary Xilix 7000K<sup>®</sup> water-borne formulation developed by Groupe Berkem (France), containing vegetal extracts-cypermethrin mixtures (SYNERKEM<sup>®</sup> technology), was made to report the remarkable ability of such formulation to protect enveloped-treated tropical hardwood kempas (*Koompassia malaccensis*) against *Coptotermes curvignathus* subterranean termites under aboveground outdoor conditions. Replicated air-dried kempas test heartwood blocks brushed-on with this formulation and with the reference permethrin-based LOSP biocide, were subjected to the laboratory EN84 leaching regime and then to laboratory volatilization regime (simulating long-term aboveground outdoor weathering exposure of treated wood) and then subjected to an innovative severe Malaysian/Australian H2-hazard class termite field test at a humid tropical test site in Sarawak, Malaysia. After 6 months, untreated kempas blocks were severely attacked (high wood mass losses, low-to-zero visual termite ratings) by *C. curvignathus*, contrasting with those treated with the novel SYNERKEM technology and with the hazardous reference LOSP that were conferred high termite resistance (negligible wood mass losses, maximum termite rating). These findings demonstrate the remarkable ability of such novel formulation in establishing permanent protection against termites (and obviously against wood boring insects) of enveloped-treated wood structure under aboveground outdoor (and obviously indoor) conditions.

**Keywords:** Non-biocidal additive, termite test, tropical hardwood, envelope treatment, pyrethroid, kempas, *Coptotermes curvignathus*

Tuesday | March 12, 2024 | 14:00-14:45

**P\_05** Poster Presentation

## Twisting tale of termite's elastic mandible form and function

by

Tzu-Chia Liu<sup>1</sup>, Kuan-Chih Kuan<sup>2</sup>, Chun-I Chiu<sup>3</sup>, Hou-Feng Li<sup>2\*</sup>, Kai-Jung Chi<sup>1,4,5\*</sup>

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### Abstract

Elastic mandible snapping, found in ants and termites, is the fastest animal movement known to date, among which the asymmetric elastic mandibles of *Pericapritermes nitobei* termite soldiers rank top with snapping speed up to 132 m/s. Snap-jaws present symmetric and asymmetric types, with the latter only reported in termites. Our previous study showed that termite's elastic mandibles can be categorized into three types, symmetric hook-like (SHO), asymmetric hook-like (AHO), and asymmetric hammer-like (AHA), and suggested that asymmetric form might have evolved prior to existence of hammer-like tip. To compare the snapping performance of AHO and AHA elastic mandibles, we applied previously developed 2D models to simulate the elastic energy stored in mandibles with different geometries, and compared the geometric features of models with optimized energy storage to the morphology of 40 extant termite species with asymmetric elastic mandibles. Our results show that the AHA mandibles of extant species are close to predicted mandible geometries for optimal energy storage at the two deformable "joints" at middle and base of the twisted left mandible. However, the results from simulating AHO distributed widely: some are close to the predictions for AHA mandibles, but the others may function more like the symmetric ones (SHO). Such results from mechanical analyses further supported that AHO might be the intermediate form of elastic mandible evolution, from SHO to AHA, in termites, and show functioning with broad mechanical performance spectrum.

**Keywords:** Elastic mandible termites, biomechanics, geometric modeling, morphospace, mechanospace

Tuesday | March 12, 2024 | 14:00-14:45

**P\_06** Poster Presentation

## **Applications of computational systems for monitoring termite activity in China**

by

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### **Abstract**

Termites cause severe economic damages in households, reservoirs, agriculture, and forests. Monitoring termite activity plays an important role in integrated termite management. A few computational systems have been developed and widely used in China. In some termite-management projects, automatic termite monitoring system become a typical termite monitoring approach. Among these devices, and a wireless system using Dekan electromagnetic induction with non-looping (DEMINL) method for the monitoring of termite activities is especially widely applied. The system comprises of a termite bait station and a handheld reader or LoRa gateway. While the wood pieces in the bait station were consumed by termites, the device could generate alarm signals and displayed. Field tests over a two-year period showed an accurate detection rate of more than 95%. The DEMINL-based device demonstrated high accuracy and durability in detecting termites, making it suitable for termite monitoring in households, crop fields, forests, and reservoirs. This device has been applied in more than two hundred locations including some very famous historical sites.

**Keywords:** Termites; automatic; monitoring; DEMINL; integrated termite management

Tuesday | March 12, 2024 | 14:00-14:45

**P\_07** Poster Presentation

## **Termiticidal activity of *Lantana camara* and *Dioscorea hipsida* extracts against subterranean termite, *Coptotermes gestroi***

by

Didi Tarmadi<sup>1\*</sup>, Ardiansyah<sup>2</sup>, Canra Aguslan Siregar<sup>2</sup>, Riana Anggraini<sup>2</sup>, S. Khoirul Himmi<sup>1</sup>,  
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### **Abstract**

Subterranean termite, *Coptotermes*, is one of the most economically important termite groups in Southeast Asia. Until now, synthetic chemical pesticides have generally been used to efficiently control subterranean termite infestation in urban areas. However, the overuse of chemical pesticides may also harm the environment. Therefore, it is important to develop environmentally safe materials as alternative controlling agents, such as developing environmentally friendly control agents from plants. This study was conducted to evaluate the termiticidal activity of *Lantana camara* and *Dioscorea hipsida* crude extracts against subterranean termite, *Coptotermes gestroi*. The termite mortality was observed at 2, 4, 6, 8, 10, 12, and 14 d at concentrations of 1, 2, 3, 4, and 5%. The results showed that the crude extract of *L. camara* and *D. hipsida* had low termiticidal activity at concentrations of 1, 2, and 3%. However, at concentrations of 4 and 5%, the crude extracts of *L. camara* and *D. hipsida* showed moderate and high termiticidal activities, respectively. Overall, *L. camara* and *D. hipsida* have the potential to be developed as alternative sources of termite control agents.

**Keywords:** Plant extracts, termite control, termite mortality, *Coptotermes gestroi*

Tuesday | March 12, 2024 | 14:00-14:45

**P\_08** Poster Presentation

**Evaluating the effectiveness of *Litsea angulata* leaf extract  
as an organic wood preservative against subterranean termites,  
*Coptotermes curvignatus***

by

Muhammad Akmal Rizqullah<sup>1\*</sup>, Harlinda Kuspradini<sup>1</sup>, Erwin<sup>1</sup>, Titik Kartika<sup>2</sup>, Ikhsan Guswenrivo<sup>2</sup>

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**Abstract**

*Litsea angulata*, belonging to the Lauraceae family, is a fragrant botanical specimen indigenous to the wooded regions of East Kalimantan, Indonesia, capable of synthesizing essential oils. Aromatic plants possess insecticidal properties, rendering them potentially toxic to various insect pests, including termites. Consequently, these plants serve as promising eco-friendly alternatives for termite control. This investigative study endeavors to assess the efficacy of leaf extracts derived from *L. angulata* as a natural preservative for wood against subterranean termites, specifically *Coptotermes curvignatus*. The extraction of *L. angulata* leaf extract was achieved using the maceration method with ethanol. Various physio-chemical attributes of the extract, such as yield, organoleptic characteristics, and chemical composition, were scrutinized employing GC-MS analysis. Durable class IV-V sengon wood underwent preservation through cold soaking for 72 hours at diverse concentrations, encompassing negative control, 1%, 2%, 4%, and positive control. The wood resistance assessment against termites adhered to the specifications outlined in SNI 7207:2014. The extraction process yielded a 5.436% output, with organoleptic properties manifesting as a semisolid substance exhibiting a blackish-brown hue and possessing a distinctive aroma of medium intensity. The extract was found to be comprised entirely of  $\alpha$ -Phellandrene, a monoterpene. The outcomes of the wood durability examination unveiled that the optimal concentration exerted a tangible impact, mitigating the percentage weight loss of the test samples and elevating the wood's durability classification from class IV-V to class II.

**Keywords:** *Litsea angulata* extract, Natural preservatives wood, *Coptotermes curvignatus*, Biotermicide

Tuesday | March 12, 2024 | 14:00-14:45

**P\_09** Poster Presentation

## **Behavioral changes in termites triggered by fungal volatile compounds**

by

Titik Kartika<sup>1\*</sup>, Deni Zulfiana<sup>2</sup>, Anis Sri Lestari<sup>3</sup>, Ni Putu Ratna Ayu Krishanti<sup>1</sup>, Sulaeman Yusuf<sup>1</sup>

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### **Abstract**

In natural ecosystems, it is widely acknowledged that the foraging behavior of subterranean termites can be influenced by wood-decaying fungi, with specific fungal species being actively avoided by termites. Furthermore, chemical constituents derived from wood-decaying fungi have been recognized for their potential in managing termite populations. In this study, we investigated the response of termites to volatile compounds emitted by selected wood-decaying fungi. Ten species of wood-decaying fungi were collected from botanical gardens, designated as SC, M4, TH2, PC, TP, H2A, M3, COR, M7, and Gano. These fungi were cultured on rubber wood flakes supplemented with JIS media for one month. Volatile compounds were then extracted via headspace sampling using activated charcoal followed by hexane solvent washing. The resulting compounds were applied to paper discs and presented to worker termites in a choice test. Termite response was quantified using an aggregation index ranging from -1 (indicating a repellent response) to 1 (indicating an attractive response). Initially, termites predominantly avoided volatile compounds during the first hour of observation, although exceptions were noted for extracts from M7, COR, and, to some extent, Gano. However, beyond the initial hour, termites exhibited attraction towards most paper discs before reverting to avoidance after four hours. Several chemical compounds, including dodecane, hexadecane, tetradecane, and methyl-2-furoate, were frequently identified in the extracts via GC-MS analysis, along with other compounds. Subsequently, the synthetic counterparts of these compounds were evaluated for their effects on termite behavior. Bioassay results indicated that termites were attracted to all compounds except dodecane, which induced repellent behavior in approximately 60% of exposed termites after two hours. We propose that excluding dodecane from the compound blend could potentially enhance the synergistic effects of volatile compounds, thereby facilitating their development for use in bait systems.

**Keywords:** wood-rotting fungi, termite, volatile compounds

Tuesday | March 12, 2024 | 14:00-14:45

**P\_10** Poster Presentation

## **Efficacy determination of an entomopathogenic fungi-based cellulose casings bait in termite control**

by

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### **Abstract**

Termites are eusocial insects that exclusively feed on wood fibers, which threatened housing construction, water conservancy projects, cultural relics, historical sites, landscaping. We estimate the invasion of termites in Hubei Province of China and the test an environmentally friendly control approach of termite in this study. Our results showed that several main species of termites that harmed building were *Reticulitermes chinensis* Snyder, *Reticulitermes flaviceps* and *Coptotermes formosanus*, as well as *Odontotermes formosanus*, which harmed the greening facilities around houses. The main damage site caused by termite were wooden door frames and window frames. The percentages of termite hazard classes in the 47 assessment sites investigated were 7.84% for Class I, 49.02% for Class II, and 43.13% for Class III, respectively. In addition, termite monitoring trap was put in assessment sites and the inspection after one year revealed that the mold rate of the trapped wood was up to 30%. Furthermore, in order to test the effectiveness of an fungi-based cellulose casings baits in termite control, we developed an *Metaria anisopliae* mixed casings baits to carry out indoor termite toxicity test and field termite control experiments. Our results showed that the mortality rate of *O. formosanus* reached 76% after 7 days of *M. anisopliae* mixed casings baits treatment, and the casings baits had no obvious repellent effect on termites. An area of 100 square meters in Lion Mountain, Wuhan, China was selected as bait experimental site, after 20 days of placing the casing baits containing *M. anisopliae* in the field, the casing baits were taken clean and there were no signs of active termites around the experimental site. Our results suggest that fungi-based cellulose casings baits could achieve effective control of termites, which provides approaches to economical, efficient and safe termite control.

**Keywords:** Termites, monitoring technology, integrated control, pest management

Tuesday | March 12, 2024 | 14:00-14:45

**P\_11** Poster Presentation

## **The role of the olfactory system in burial behavior triggered by oleic acid in *Coptotermes formosanus***

by

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### **Abstract**

Social insects maintain hygienic conditions through their social immunity behaviors such as burial behavior, which is central for protecting healthy individuals from corpses. It is generally believed that chemicals released from corpses, such as oleic acid, are the most important cues for triggering burial behavior in termites. However, how does the oleic acid trigger burial behavior in a termite population remains unclear. Here we report the contribution of the olfactory system to burial behavior in *Coptotermes formosanus* Shiraki. We demonstrated that one of OBPs, *CforOBP7* is highly expressed in the antennae of workers. Fluorescent competition binding experiments exhibited that *CforOBP7* has a strong affinity for oleic acid. Furthermore, *CforOBP7*-silenced termites showed the antennal response to oleic acid was significantly reduced and oleic acid triggered burial behavior was also inhibited compared with the control. Therefore, we concluded that *CforOBP7* governs the burial behavior of *C. formosanus* triggered by oleic acid. The other elements of olfactory system involved in this burial behavior are under investigation.



# Notes

