



STUDY OF ULTRASTRUCTURE, GENETICS, AND MOLLUSCICIDAL
ACTIVITY OF *STEINERNEMA* AND *HETERORHABDITIS*



A Thesis Submitted to the Graduate School of Naresuan University
in Partial Fulfillment of the Requirements
for the Doctor of Philosophy in Parasitology
2025

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Thesis entitled " STUDY OF ULTRASTRUCTURE, GENETICS, AND
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has been approved by the Graduate School as partial fulfillment of the requirements
for the Doctor of Philosophy in Parasitology of Naresuan University

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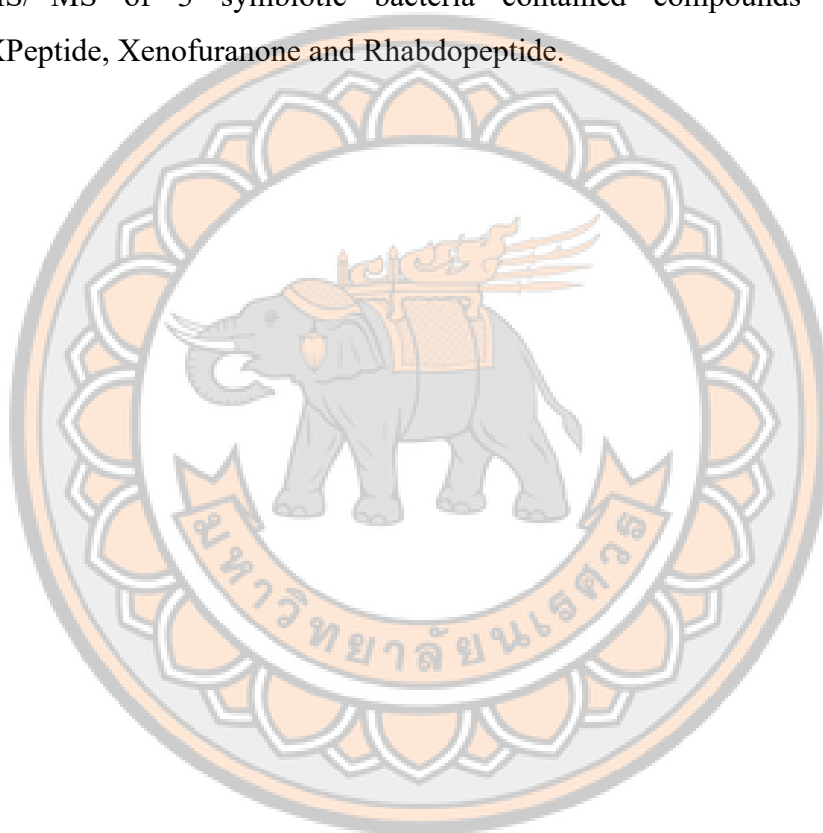
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ABSTRACT

Biomphalaria glabrata acts as the intermediate host of schistosomes that causes human schistosomiasis. Entomopathogenic nematodes (EPNs) of the genera *Steinernema* and *Heterorhabditis* and their symbiotic bacteria, *Xenorhabdus* and *Photorhabdus*, respectively. They have been used as an effective biological control agent. The Symbiotic bacteria produce secondary metabolites with several biological activities. Controlling *B. glabrata* is a potential strategy to limit the transmission of schistosomiasis. In this study we isolated and identified EPNs and their symbiotic bacteria from soil samples in agricultural areas of northern Thailand and evaluate their molluscicidal activity against *B. glabrata* snail. A total of 51 of 1,000 soil samples were positive (5.1% prevalence) for EPNs, which were identified through sequencing of the rDNA and ITS to 37 *Steinernema* isolates (3.7%) and 14 *Heterorhabditis* isolates (1.4%). Thirty-one bacterial isolates belong to *Xenorhabdus* (n=19) and *Photorhabdus* (n=12). *Xenorhabdus ehlersii*, *X. stockiae*, *X. indica*, *X. griffinae*, *P. luminescens*, *P. akhurstii* and *P. laumondii* subsp. *laumondii* were molecularly identified based on *recA* sequencing. Five isolates of bacterial extracts showed potential molluscicide with 100% mortality of snail. *Photorhabdus laumondii* subsp. *laumondii* (bALN19.5_TH) showed highest effectiveness with the lethal

concentration (LC) values at 54.52 $\mu\text{g/mL}$ and 89.58 $\mu\text{g/mL}$ for LC50 and LC90, respectively. Histopathological changes of the snail were observed in the head-foot region, which showed ruptures of the epithelium covering the foot and deformation of the muscle fiber. A hemocyte of the treated snails was observed in the digestive tubules of the digestive glands. The hermaphrodite glands of treated snails showed a reduction in the number of spermatozoa, degeneration of oocytes, and deformation and destruction in the hermaphrodite gland. In addition, LC- MS/ MS of 3 symbiotic bacteria contained compounds such as GameXPeptide, Xenofuranone and Rhabdopeptide.



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CHAPTER I

INTRODUCTION

Background and significance of the study

Entomopathogenic nematodes (EPNs) are microscopic, soil-dwelling roundworms that have been broadly utilized as biocontrol agents due to their ability to infect and kill a wide range of insect pests (Georgis et al., 2006). EPNs in the genera *Steinernema* and *Heterorhabditis* are the most extensively studied in biological control and have the ability to kill insect pests quickly. These nematodes are symbiotically associated with *Xenorhabdus* and *Photorhabdus* bacteria, respectively (Boemare et al., 1993). These bacteria produce various secondary metabolites or toxins including antibacterial, antifungal, antiparasitic, acaricidal, cytotoxic, insecticidal, molluscicidal, and nematocidal activities (Grundmann et al., 2014; Bode, 2009; Bi & Yu, 2018; Abd-Elgawad, 2022; Dumidae et al., 2024).

The infective juvenile stages (IJs) of EPNs contain symbiotic bacteria in their intestines and enter insect host through natural orifice such as mouth, anus, or spiracles, or by penetrating directly through the cuticle. The EPNs release their bacterial symbionts from intestine into the blood system of insect. The symbiotic bacteria produce several toxins which cause septicemia in insects. Finally, the insect was killed within 24 to 48 hours (Park & Kim, 2000; Forst et al., 1997; Kaya & Gaugler, 1993).

For many years, *Steinernema* and *Heterorhabditis* have been used to manage many insect pests. Species of the genus *Steinernema* have been employed as biocontrol agents to effectively manage populations of the sugarcane froghopper (*Aeneolaimia varia*), a major pest that causes significant damage to sugarcane crops, Spittlebug (*Mahanarva spectabilis*), and Ground pearl scale (*Eurhizococcus colombianus*) while *Heterorhabditis* have been used to control Coffee root mealybug (*Dysmicoccus texensis*), Fall armyworm (*Spodoptera frugiperda*), and Mexican bean beetle (*Copturus aguacatae*) (Andaló et al., 2006; 2012; Aristizábal et al., 2015; Batista et al., 2014). Furthermore, prior studies have demonstrated the pathogenicity of EPNs toward gastropod snails. For instance, exposure to *Heterorhabditis indica* LPP1 resulted in 55% mortality in *Bradybaena similaris*, along with a significant reduction ($P < 0.05$)

in the glucose content of the hemolymph in infected snails. These findings indicate that infection by *H. indica* can disrupt the host's physiological functions, contributing to its pathogenic effect (Tunholi et al., 2014; 2017b). Subsequently, in 2017 they evaluated susceptibility of *Lymnaea columella* to *H. baujardi*, with mortality rate of 66% and demonstrated the strong pathogenic potential of *H. baujardi* suggesting the use of EPNs as a potential alternative for biological control of these snail (Tunholi et al., 2017a). Recently, the susceptibility of *B. glabrata* to the *H. bacteriophora* isolate HP88 was reported. The infection induces breakdown of carbohydrate homeostasis in *B. glabrata* but did not show lethality in the population of infected snails (Santos-Amaral et al., 2022).

Biomphalaria glabrata is an aquatic pulmonate gastropod in the family Planorbidae. This snail is a significant intermediate host of *Schistosoma mansoni*, a trematode parasite, which is the most significant cause of schistosomiasis in humans. Currently, over 250 million people in 78 countries are affected by this infection. Moreover, due to the COVID-19 pandemic since 2021, work to control this schistosomiasis has been reduced (World Health Organization, 2023). Chronic schistosomiasis can cause significant disability and, in some cases, can result in death (Evan Secor, 2014). The World Health Organization (WHO) recommends the use of mass drug administration (MDA) with the anthelmintic praziquantel to control schistosomiasis. However, MDA is not 100% successful. Furthermore, no licensed or commercial schistosomiasis vaccine is currently available in the market against any strain or species of schistosome infecting humans (Faust et al., 2020; Siddiqui et al., 2023). Other available control approaches include environmental management, health education, and intermediate host snail control, which is the most practical approach to interrupt the schistosome life cycle (Cioli et al., 2014; Evan Secor, 2014). The use of synthetic molluscicide niclosamide to control the snail populations was recommended by World Health Organization Pesticide Evaluation Scheme (WHOPES) (World Health Organization, 2017). There are certain limitations for applying niclosamide, including limitations to the usage of potable water immediately after application and temporary high toxicity to non-target organisms including fish, amphibians, and humans ((USEPA), 1999). Also, the high cost and appearance of snails resistant to niclosamide has limited its usefulness in controlling the snails (Coelho & Caldeira, 2016; Dai et al.,

2014). The development of novel molluscicides of synthetic or biological origin is an important approach. The use of plant-based molluscicides (extracts and compounds isolated from plants) is one of the most effective methods to reduce snail populations (Araújo et al., 2018; Matos et al., 2020; Mello-Silva et al., 2010).

Among the biological agents, EPNs have shown great successful to control many insect pests as well as the intermediate host snail (Larissa Bitencourt Vidal et al., 2021; Santos Amaral et al., 2022; Schurkman & Dillman, 2021). However, to our knowledge, no previous study has been conducted with the symbiotic bacteria, *Xenorhabdus* and *Photorhabdus*, to control the snails. In addition, EPNs are globally distributed in several ecologies except Antarctica (Vitta et al., 2017; Půža et al., 2025). In 2025, *Steinernema* and *Heterorhabditis* have been reported for 114 and 21 species, respectively (Půža et al., 2025). In Thailand, a diverse range of EPNs and their bacterial symbionts have been documented throughout the country. Six species of *Steinernema*—*S. abbasi*, *S. khoisanae*, *S. kushidai*, *S. scarabaei*, *S. seungi*, and *S. websteri*—as well as seven species of *Heterorhabditis*—*H. bacteriophora*, *H. baujardi*, *H. gerrardi*, *H. indica*, *H. zealandica*, *Heterorhabditis* sp. SGgj, and *Heterorhabditis* sp. SGmg3—have been reported. Additionally, three species of the symbiotic bacterium *Xenorhabdus* have been identified: *X. stockiae*, *X. japonica*, and *X. miraniensis*. Moreover, three species comprising six subspecies of *Photorhabdus* have been recorded, including *P. luminescens* subsp. *akhurstii*, *P. luminescens* subsp. *hainanensis*, *P. luminescens* subsp. *laumondii*, *P. asymbiotica* subsp. *australis*, *P. luminescens* subsp. *namnaonensis*, and *P. temperata* subsp. *temperata*. Several surveys on EPNs in Thailand have shown that most EPNs were isolated from soil samples collected along roadsides or in national parks. Only a few studies have reported the isolation of EPNs from agricultural areas, such as those in Phitsanulok Province in central Thailand (Ardpairin et al., 2023). Moreover, native EPNs hold potential for future applications in local environments due to their inherent adaptability to regional ecological conditions (de Brida et al., 2017b). Therefore, the overall goal of this study was to isolate and identify EPNs and their symbiotic bacteria from agricultural areas and evaluate their efficacies on *B. grabrata*.

Purposes of the study

1. To isolate and identify entomopathogenic nematodes (EPNs) from soil samples in agricultural areas of northern Thailand
2. To isolate and identify *Xenorhabdus* and *Photorhabdus* bacteria associated with EPNs
3. To analyze the phylogenetic relationships of EPNs and their bacterial symbionts
4. To examine the ultrastructure of selected EPN isolates using a scanning electron microscope
5. To evaluate the effectiveness of EPNs and their bacterial symbionts against the snail *Biomphalaria glabrata*, an intermediate host of *Schistosoma mansoni*

Scope of the study

In this study, one thousand soil samples were randomly collected from 200 agricultural sites across 10 provinces in northern Thailand. EPNs were isolated from all soil samples in the laboratory using *Galleria mellonella* larvae as bait. A modified White trap technique was used for isolating infective juvenile stages (IJs) of EPNs from *G. mellonella* cadavers. Molecular techniques based on polymerase chain reaction (PCR) and sequencing of a partial region of the 28S rDNA for *Steinernema* and an internal transcribed spacer (ITS) for *Heterorhabditis* were performed to classify the isolated EPNs. Furthermore, the ultrastructural study of the IJs was performed using scanning electron microscope (SEM). Species identification of their symbiotic bacteria was performed based on sequencing of a partial sequence of the *recA* gene. Phylogenetic tree based on maximum likelihood method was used to study their evolutionary relationships. Molluscicidal activity of symbiotic bacteria against *B. glabrata* and histopathological change of the tested snail were evaluated in the laboratory by hematoxylin and eosin (H & E) staining. In addition, liquid chromatography–tandem mass spectrometry (LC–MS/MS) analysis together with molecular networking of symbiosis bacteria extracts was performed to elucidate the secondary metabolites produced by the symbiotic bacteria.

CHAPTER II

Related works and studies

Entomopathogenic nematodes

Entomopathogenic nematodes (EPNs), the insect parasite of the families Steinernematidae (genus *Steinernema*) and Heterorhabditidae (genus *Heterorhabditis*) are symbiotically associated with bacteria in the genera *Xenorhabdus* and *Photorhabdus*, respectively (Boemare & Gaugler, 2002). Entomopathogenic nematodes (EPNs) have been commonly used as biocontrol agents due to their effective biocontrol activity against a variety of significant insect pests (Andaló et al., 2012; Lacey & Georgis, 2012). These nematodes naturally inhabit the soil and are commonly present in areas where vegetation or plants are found. They are also commercially available to farmers and are used in various cropping systems. The United States Environmental Protection Agency (EPA) has confirmed that entomopathogenic nematodes pose no harm to humans, vertebrates, or plants (Kaya & Gaugler, 1993).

Genus *Steinernema*

The first EPN species of the genus *Steinernema* was initially described as *Aplectana kraussei* by Gotthold Steiner in 1932. Later, in 1927, Travassos reclassified it as *Steinernema kraussei*. Genus *Steinernema* classifies to the phylum Nematoda, class Chromadorea, order Rhabditida, and family Steinernematidae (Travassos, 1927). These nematodes form a symbiotic relationship with bacteria from the genus *Xenorhabdus* (Boemare & Gaugler, 2002). The bacteria located in the anterior part of the intestine (Figure 1) (Ciche et al., 2006). To date, approximately 114 species of *Steinernema* have been described (Table 1) (Půža et al., 2025).

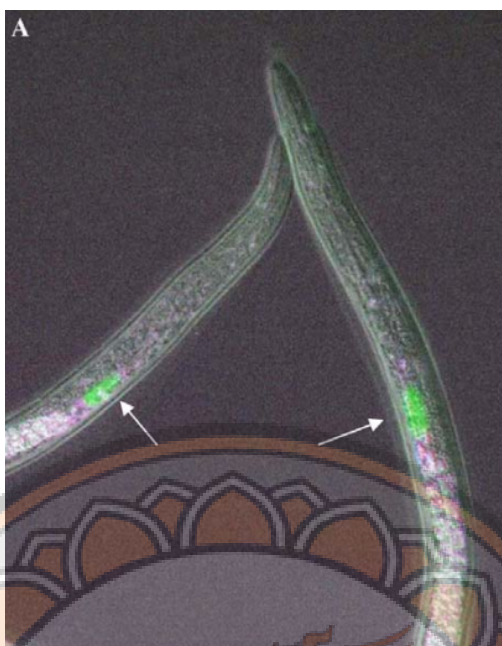


Figure 1 *Xenorhabdus nematophila* cells located in the intestinal vesicle (arrow) of *Steinernema carpocapsae* infective juveniles.

Source: Ciche et al. (2006)

Table 1 Entomopathogenic nematode in genus *Steinernema*

Genus	Species	References
<i>Steinernema</i>	<i>abbasi</i>	(Elawad et al., 1997)
	<i>aciari</i>	(Qiu et al., 2005a)
	<i>adamsi</i>	(Baniya et al., 2024)
	<i>affine</i>	(Wouts et al., 1982)
	<i>africanum</i>	(Machado et al., 2022)
	<i>akhursti</i>	(Qiu et al., 2005b)
	<i>ananthnagense</i>	(Bhat et al., 2023a)
	<i>anatoliense</i>	(Hazir et al., 2003)
	<i>(carpocapsae)</i>	
	<i>apuliae</i>	(Triggiani et al., 2004)
	<i>arasbaranense</i>	(Nikdel et al., 2011)

Table 1 (Cont.)

Genus	Species	References
<i>Steinernema</i>	<i>arenarium</i>	(Wouts et al., 1982)
	<i>ashiuense</i>	(Phan et al., 2006a)
	<i>asiaticum</i>	(Anis et al., 2002)
	<i>australe</i>	(Edgington et al., 2009a)
	<i>backanense</i>	(Phan et al., 2006b)
	<i>bakwenae</i>	(Malan et al., 2023)
	<i>balochiense</i>	(Fayyaz et al., 2015)
	<i>batswanae</i>	(Didiza et al., 2021)
	<i>beddingi</i>	(Lihong Qiu et al., 2005)
	<i>beitlechemi</i>	(Çimen et al., 2016a)
	<i>bertusi</i>	(Katumanyane et al., 2020)
	<i>bicornutum</i>	(Tallósi et al., 1995)
	<i>biddulphi</i>	(Cimen et al., 2016b)
	<i>bifurcatum</i>	(Fayyaz et al., 2014)
	<i>boemarei</i>	(Lee et al., 2008)
	<i>borjomiense</i>	(Gorgadze et al., 2018)
	<i>brazilense</i>	(Nguyen et al., 2010)
	<i>cameroonense</i>	(Kanga et al., 2012)
	<i>carpocapsae</i>	(Wouts et al., 1982)
	<i>caudatum</i>	(Xu et al., 1991)
	<i>ceratophorum</i>	(Jian et al., 1997)
	<i>changbaiense</i>	(Ma et al., 2002)
	<i>cholashanense</i>	(Nguyen et al., 2008)
	<i>citrae</i>	(Stokwe et al., 2011)
	<i>colombiense</i>	(López-Núñez et al., 2008)
	<i>costaricense</i>	(Uribe-Lorío et al., 2007)
	<i>cubanum</i>	(Mráček et al., 1994)
	<i>cumgareense</i>	(Phan et al., 2006b)

Table 1 (Cont.)

Genus	Species	References
<i>Steinernema</i>	<i>diaprepesi</i>	(Nguyen & Duncan, 2002)
	<i>eapokense</i>	(Phan et al., 2006b)
	<i>ethiopiense</i>	(Tamiru et al., 2012)
	<i>everestense</i>	(Khatri-Chhetri et al., 2011a)
	<i>fabii</i>	(Abate et al., 2016)
	<i>feltiae</i>	(Wouts et al., 1982)
	<i>glaseri</i>	(Wouts et al., 1982)
	<i>goweni</i>	(San-Blas et al., 2016)
	<i>guangdongense</i>	(Qiu et al., 2004)
	<i>hebeiense</i>	(Chen et al., 2006)
	<i>hermaphroditum</i>	(Stock et al., 2004)
	<i>huense</i>	(Phan et al., 2014)
	<i>ichnusae</i>	(Tarasco et al., 2008)
	<i>indicum</i>	(Patil et al., 2023)
	<i>innovationi</i>	(Çimen et al., 2014a)
	<i>intermedium</i>	(Poinar, 1985)
	<i>jeffreyense</i>	(Malan et al., 2015)
	<i>jollieti</i>	(Spiridonov et al., 2004)
	<i>kandii</i>	(Godjo et al., 2019)
	<i>karii</i>	(Waturu et al., 1997)
	<i>khoisanæ</i>	(Nguyen et al., 2006a)
	<i>khuongi</i>	(Stock et al., 2018)
	<i>kraussei</i>	(Travassos, 1927)
	<i>kushidai</i>	(Mamiya, 1988)
	<i>lamjungense</i>	(Khatri-Chhetri et al., 2011b)
	<i>leizhouense</i>	(Nguyen et al., 2006b)
	<i>litchii</i>	(Steyn et al., 2017)

Table 1 (Cont.)

Genus	Species	References
<i>Steinernema</i>	<i>litorale</i>	(Yoshida, 2004)
	<i>loci</i>	(Phan et al., 2001a)
	<i>longicaudum</i>	(Shen & Wang, 1991)
	<i>meghalayensis</i>	(Ganguly et al., 2011)
	<i>(carpocapsae)</i>	
	<i>mictlani</i>	(Haidel et al., 2023)
	<i>minutum</i>	(Maneesakorn et al., 2010)
	<i>monticolum</i>	(Stock et al., 1997)
	<i>neocurtillae</i>	(Nguyen & Smart, 1992)
	<i>nepalense</i>	(Khatri-Chhetri et al., 2011c)
	<i>nguyeni</i>	(Malan et al., 2016)
	<i>nyetense</i>	(Kanga et al., 2012)
	<i>oregonense</i>	(Liu & Berry, 1996b)
	<i>pakistanense</i>	(Shahina et al., 2001)
	<i>papillatum</i>	(San-Blas et al., 2015)
	<i>phyllophagae</i>	(Nguyen & Buss, 2011)
	<i>poinari</i>	(Mráček & Nermut, 2014)
	<i>populi</i>	(Tian et al., 2022)
	<i>puertoricense</i>	(Román & Figueroa, 1994)
	<i>pui</i>	(Qiu et al., 2011)
	<i>puntauvense</i>	(Uribe-Lorío et al., 2007)
	<i>pwaniensis</i>	(Půža et al., 2016)
	<i>ralatorein</i>	(Grifaldo-Alcántara et al., 2017)
	<i>rarum</i>	(Doucet, 1986)
	<i>riobrave</i>	(Cabanillas et al., 1994)
	<i>riojaense</i>	(Půža et al., 2020)
	<i>ritteri</i>	(Doucet & Doucet, 1990)
	<i>robustispiculum</i>	(Phan et al., 2005)
	<i>sacchari</i>	(Nthenga et al., 2014)

Table 1 (Cont.)

Genus	Species	References
<i>Steinernema</i>	<i>sandneri</i>	(Lis et al., 2021)
	<i>sangi</i>	(Phan et al., 2001b)
	<i>sasonense</i>	(Phan et al., 2006b)
	<i>scapterisci</i>	(Nguyen & Smart, 1990)
	<i>scarabaei</i>	(Stock & Koppenhöfer, 2003)
	<i>schliemanni</i>	(Spiridonov et al., 2010)
	<i>serratum (longidaudum)</i>	(Liu, 1992)
	<i>shori</i>	(Soni et al., 2023)
	<i>siamkayai</i>	(Stock et al., 1998)
	<i>sichuanense</i>	(Mráček et al., 2006)
	<i>silvaticum</i>	(Sturhan et al., 2005)
	<i>surkhetense</i>	(Khatri-Chhetri et al., 2011c)
	<i>taiwanensis</i>	(Tseng et al., 2018)
	<i>tami</i>	(Luc et al., 2000)
	<i>texanum</i>	(Nguyen et al., 2007)
	<i>thailandensis</i>	(Tangchitsomkid, 1998)
	<i>thesami</i>	(Gorgadze et al., 2016)
	<i>thanhi</i>	(Phan et al., 2001a)
	<i>thermophilum (abbasi)</i>	(Ganguly & Singh, 2000)
	<i>tielingense</i>	(Ma et al., 2012a)
	<i>tophus</i>	(Çimen et al., 2014b)
	<i>unicornum</i>	(Edgington et al., 2009b)
	<i>vulcanicum</i>	(Clausi et al., 2011)
	<i>websteri (carpocapsae)</i>	(Cutler & Stock, 2003)
	<i>weiseri</i>	(Mráček et al., 2003)
	<i>xinbinense</i>	(Ma et al., 2012b)
	<i>xueshanense</i>	(Mráček et al., 2009)
	<i>yirgalemense</i>	(Nguyen et al., 2004)

Genus *Heterorhabditis*

Genus *Heterorhabditis* was first described in Australia in 1976 as *Heterorhabditis bacteriophora* (Poinar, 1975). Poinar suggested that *Heterorhabditis* evolved from a Pellioiditis-like ancestor in a sandy marine habitat. It is classified under the phylum Nematoda, class Chromadorea, order Rhabditida, and family Heterorhabditidae (Poinar, 1975). The nematode forms a symbiotic association with bacteria from the genus *Photorhabdus* (Boemare & Gaugler, 2002) which reside in the anterior and midgut regions of the infective juvenile stage (Figure 2) (Ciche et al., 2006). The number of *Heterorhabditis* species is fewer than *Steinernema*, currently EPNs in genus *Heterorhabditis* have been describe 21 species (Table 2) (Půža et al., 2025).



Figure 2 *Photorhabdus luminescens* cells located in the anteriors and midintestine (arrow) of *Heterorhabditis bacteriophora*.

Source: Ciche et al. (2006)

Table 2 Entomopathogenic nematode in genus *Heterorhabditis*

Genus	Species	References
<i>Heterorhabditis</i>	<i>amazonensis</i>	(Andaló et al., 2006)
	<i>atacamensis</i>	(Edgington et al., 2010)
	<i>bacteriophora</i>	(Poinar, 1975)
	<i>baujardi</i>	(Phan et al., 2003)
	<i>beicherriana</i>	(Li et al., 2012)
	<i>casmirica</i>	(Bhat et al., 2023b)
	<i>downesi</i>	(Stock et al., 2002)
	<i>egyptii</i>	(Abd-Elgawad, 2021)
	<i>floridensis</i>	(Nguyen et al., 2006)
	<i>georgiana</i>	(Plichta et al., 2009)
	<i>hambletoni</i>	(Poinar, 1975)
	<i>indica</i>	(Hominick, 2002)
	<i>marelatus</i>	(Liu & Berry, 1996a)
	<i>megidis</i>	(Poinar et al., 1987)
	<i>mexicana</i>	(Nguyen et al., 2004)
	<i>noenieputensis</i>	(Malan et al., 2008)
	<i>ruandica</i>	(Machado, Bhat, et al., 2021)
	<i>safricana</i>	(Malan et al., 2008)
	<i>taysearae</i>	(Abou-El-Sooud et al., 1996)
	<i>zacatecana</i>	(Machado, et al., 2021)
	<i>zealandica</i>	(Poinar, 1990)

Life cycle of entomopathogenic nematodes (EPNs)

The nematode exists in the environment as a non-feeding infective juvenile (IJ), which is relatively resistant to environmental stresses and is commonly found in soil (Poinar, 2018). *Steinernema* IJs carry their symbiotic bacteria, *Xenorhabdus*, in a specialized intestinal vesicle, while *Heterorhabditis* IJs carry *Photorhabdus* in the midgut region of the intestine. The infective juveniles of *Steinernema* typically enter insect hosts through natural openings such as respiratory spiracles, the mouth, or the

anus, before migrating to the haemocoel. In contrast, *Heterorhabditis* possess a tooth-like structure near the mouth that enables them to directly penetrate the insect cuticle and access the haemocoel. *Steinernema* spp. induce a toxicogenesis and produce an immune depressive factor active against antimicrobial peptides from the insects (Boemare, 2002). The IJs release the symbiotic bacteria into the haemolymph of insect which is a rich source of nutrients and then the bacteria can grow and multiply to a high cell density within the cadaver, release toxins and exoenzymes and rapidly kill the insect, usually in less than 24 hours (Forst et al., 1997; Hu et al., 2006). The IJ grows and develops to the fourth stage larvae and become adult within 2-3 days. After mating, the females lay eggs that hatch into first-stage juveniles, which then undergo successive molts to reach the second, third, and fourth developmental stages. The nematodes continue to reproduce for 2–3 generations until nutrients become scarce. At that point, they transform into infective juveniles. These infective juveniles (IJs), which do not feed, emerge from the insect cadaver and move into the surrounding soil in search of new insect hosts. IJs are capable of surviving without a host for 2 to 3 months (Figure 3).

In *Steinernema*, reproduction is amphimictic, involving the fusion of male and female gametes. The infective juvenile (IJ) of *Steinernema* develops into either a male or female adult. In contrast, the IJ of *Heterorhabditis* first develops into a hermaphroditic female, which then produces a second generation consisting of amphimictic males and females. *Steinernema carpocapsae* lays more eggs during both the first and second generations compared to *Heterorhabditis bacteriophora*. In the third generation, eggs from *S. carpocapsae* develop through a process known as endotokia matricida. The IJs produced via endotokia matricida do not fully develop into the infective stage until they have exited the mother nematode's body (Figure 3) (Burnell & Stock, 2000).

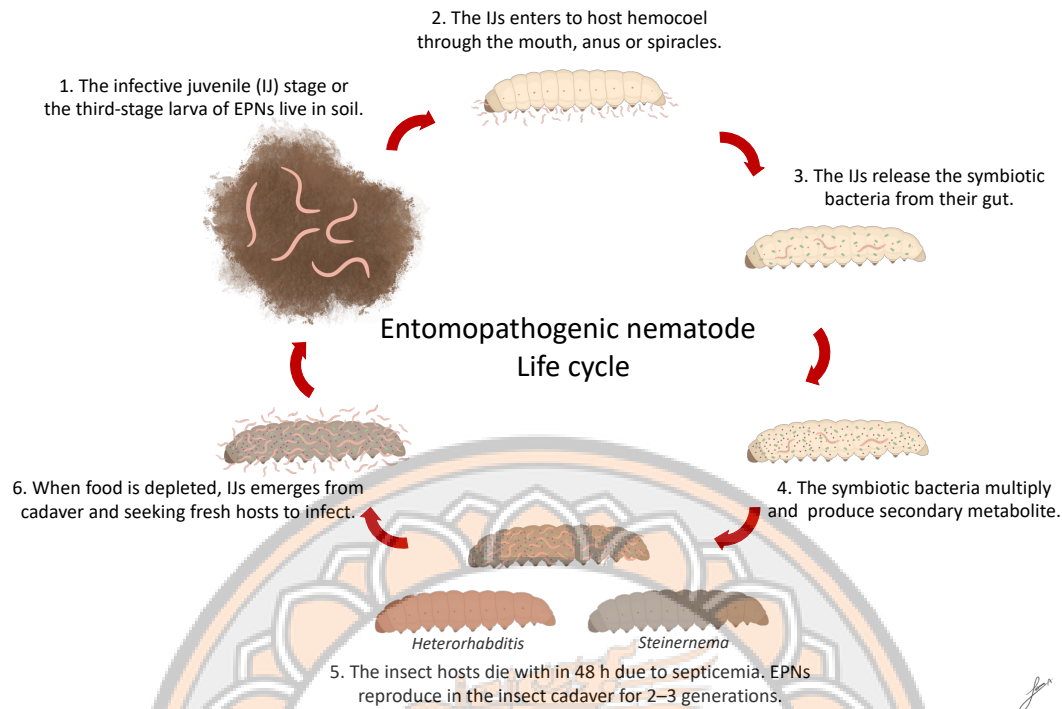


Figure 3 Entomopathogenic nematode Life Cycle

Identification of entomopathogenic nematodes (EPNs)

Morphological identification of EPNs can be performed by examining structural features using a scanning electron microscope (SEM), allowing differentiation of the external characteristics of infective juveniles, males, and females. However, limitation of this method is ability to distinguish in genera only because EPNs have similar morphology in species.

A polytomous key can be used to identify the species of *Steinernema* and *Heterorhabditis* following these steps; 1) For consistency, the nematodes should be cultured in *Galleria mellonella*; 2) Since variation occurs among individuals within the same species, examine at least ten infective juveniles and males of the target nematode; 3) Compare the body length of the infective juveniles with those listed in the key that have similar measurements; 4) Cross-reference your data with the bolded measurements and distinguishing features of selected nematode species; 5) Identify which nematode species in the key closely resemble your specimens; 6) Refer to the original species descriptions for additional details to validate your identification (Byron, 2002).

Morphology of *Steinernema* on SEM observations, for male: head is swollen or not. There are 4 cephalic papillae which often larger than 6 labial papillae. Posterior region has one testis and one pair of spicules with long gubernaculum but without bursa and normally have 6 - 10 pairs of genital papillae. The spicule is large. Tail tip has rounded, with or without mucron (Figure 4). Females are larger in size than males and possess either a smooth or annulated cuticle. The head is rounded or ringed and features six lips. Females have paired, oppositely positioned ovaries. The vagina is short and muscular, and the vulva is located near the midpoint of the body, with or without protruding lips. An epiptygma may be present or absent. Tail tip round, with mucron, bluntly pointed (Figure 5). The IJ has slender shape with or without a sheath. Head with 4 cephalic papillae and 6 labial papillae or with only 4 cephalic papillae without labial papillae. Esophagus and intestine appear to narrow tube. Digestive system is not active. "The lateral field exhibits a pattern of ridges extending from the head to the tail. The tail is either conoid or filiform in shape (Figure 6), with phasmids located at the mid-tail region (Byron, 2002). Additionally, species within the genus *Steinernema* can be distinguished based on morphological characteristics, as presented in Table 3 (Nguyen, 2010).

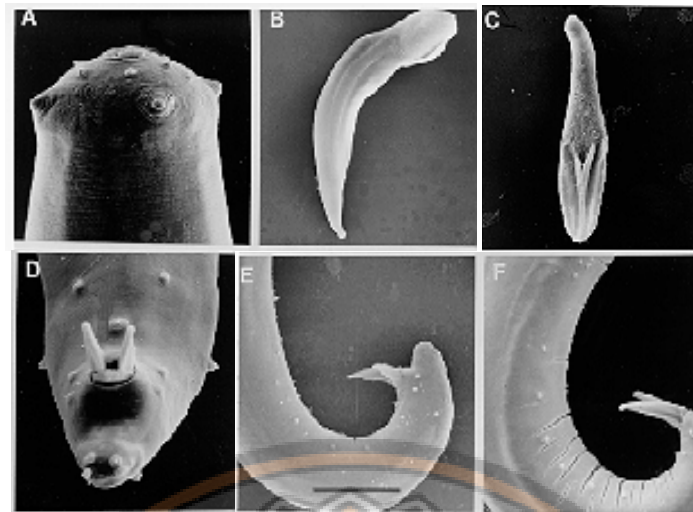


Figure 4 Structures of *Steinernema* males; A) head with labial and cephalic papillae, B - C) spicules, D – F) posterior part showing spicules and pre-anal genital papillae

Source: Adams and Nguyen (2002)

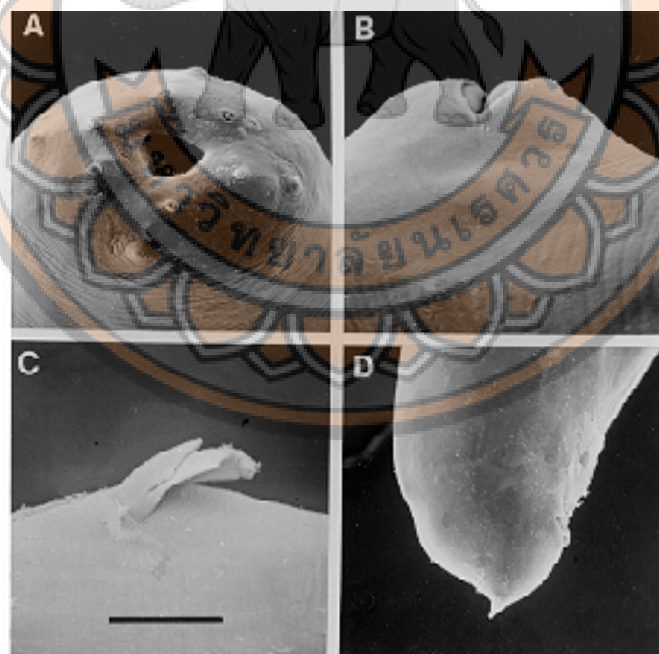


Figure 5 Structures of *Steinernema* female; A) Face view, B) Vulva, C) Epiptygma and D) Female tail

Source: Adams and Nguyen (2002)

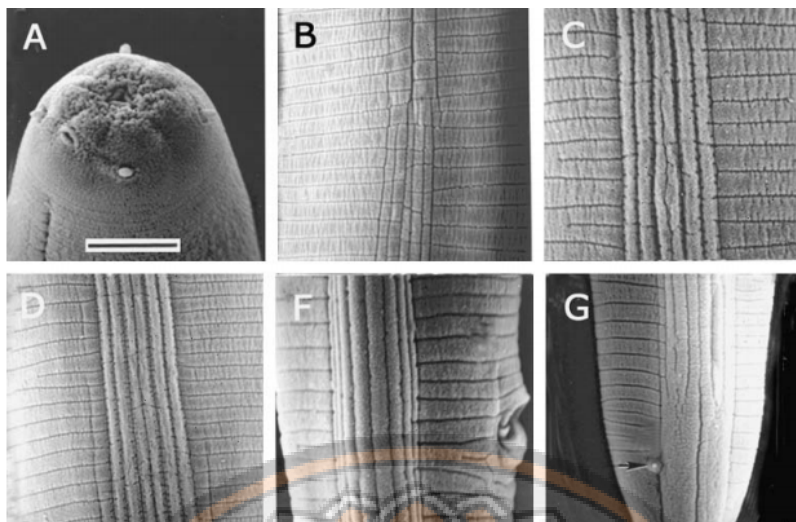


Figure 6 Morphological structures of the infective juvenile stage of *Steinernema glaseri*

Source: Adams and Nguyen (2002)

Table 3 Classification of *Steinernema* group based on morphology of IJ stage

Group	Morphology	<i>Steinernema</i> species
<i>glaseri</i>	Length of IJ more than 1,000 μm	<i>S. aciari</i>
		<i>S. apuliae</i>
		<i>S. arenarium</i>
		<i>S. australe</i>
		<i>S. boemarei</i>
		<i>S. brazilense</i>
		<i>S. caudatum</i>
		<i>S. cubana</i>
		<i>S. diaprepesi</i>
		<i>S. ethiopiense</i>
		<i>S. glaseri</i>
		<i>S. guangdongense</i>
		<i>S. hermaphroditum</i>

Table 3 (Cont.)

Group	Morphology	<i>Steinernema</i> species
<i>glaseri</i>		<i>S. innovation</i>
		<i>S. khoisanae</i>
		<i>S. khuongi</i>
		<i>S. lamjungense</i>
		<i>S. leizhouense</i>
		<i>S. longicaudum</i>
		<i>S. phyllophagae</i>
		<i>S. puertoricense</i>
		<i>S. pui</i>
		<i>S. tophus</i>
		<i>S. vulcanicum</i>
<i>feltiae</i>	Length of IJ between 700 to 1,000 μm	<i>S. akhursti</i>
		<i>S. ashiuense</i>
		<i>S. beitlechemi</i>
		<i>S. jeffreyense</i>
		<i>S. changbaiense</i>
		<i>S. cholashanense</i>
		<i>S. citrae</i>
		<i>S. ethiopense</i>
		<i>S. everestense</i>
		<i>S. fabii</i>
		<i>S. feltiae</i>
		<i>S. hebeiense</i>
		<i>S. ichnusae</i>
		<i>S. jollieti</i>
		<i>S. kari</i>
		<i>S. kraussei</i>
		<i>S. litorale</i>
		<i>S. loci</i>

Table 3 (Cont.)

Group	Morphology	<i>Steinernema</i> species
<i>feltiae</i>		<i>S. monticolum</i>
		<i>S. neocurtillae</i>
<i>feltiae</i>	Length of IJ between 700 to 1,000 μm	<i>S. oregonense</i>
		<i>S. puntauvense</i>
		<i>S. robustispiculum</i>
		<i>S. sacchari</i>
		<i>S. sangi</i>
		<i>S. scarabaei</i>
		<i>S. schliemanni</i>
		<i>S. silvaticum</i>
		<i>S. texanum</i>
		<i>S. thanhi</i>
		<i>S. tielingense</i>
		<i>S. unicornum</i>
		<i>S. weiseri</i>
		<i>S. xinbinense</i>
		<i>S. xueshanense</i>
<i>intermedium</i>	Length of IJ between 600 to 700 μm	<i>S. affine</i>
		<i>S. anatoliense</i>
		<i>S. arasbaranense</i>
		<i>S. asiaticum</i>
		<i>S. backanense</i>
		<i>S. balochiense</i>
		<i>S. beddingi</i>
		<i>S. cameroonense</i>
<i>bicornutum</i>	IJ has 2 horn-like structures	<i>S. abbasi</i>
		<i>S. bicornutum</i>
		<i>S. biddulphi</i>
		<i>S. bifurcatum</i>

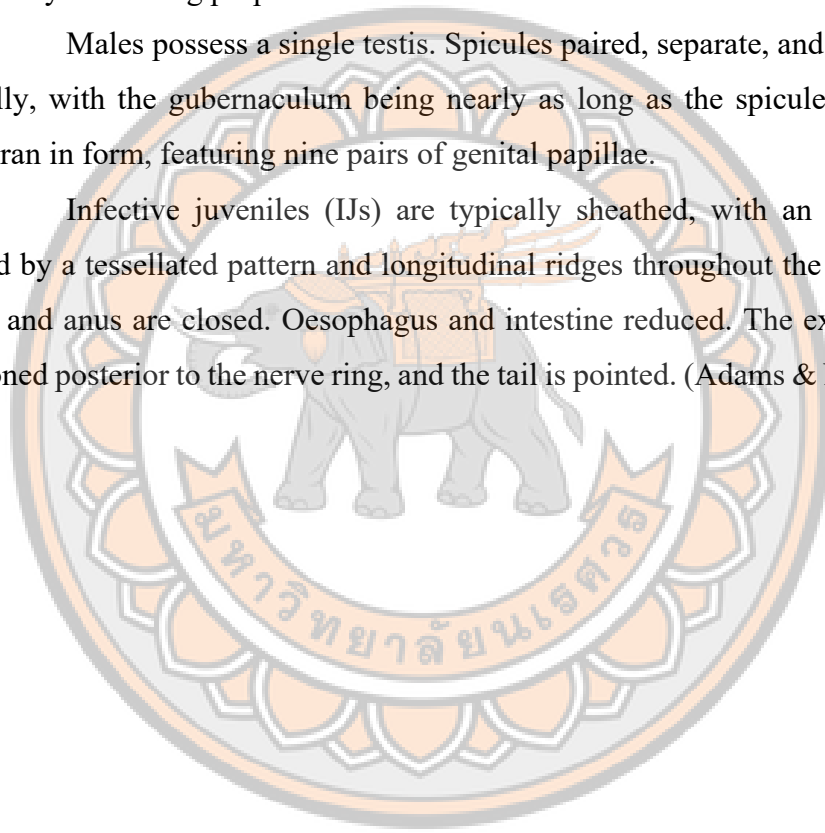
Table 3 (Cont.)

Group	Morphology	<i>Steinernema</i> species
<i>bicornutum</i>		<i>S. ceratophorum</i>
		<i>S. costaricense</i>
		<i>S. goweni</i>
		<i>S. pakistanense</i>
		<i>S. riobrave</i>
		<i>S. yirgalomense</i>
<i>carpocapsae</i>	Length of IJ less than 600 µm	<i>S. carpocapsae</i>
		<i>S. colombiense</i>
		<i>S. cumgareense</i>
		<i>S. eapokense</i>
		<i>S. huense</i>
		<i>S. intermedium</i>
		<i>S. kushidai</i>
		<i>S. meghaleyense</i>
		<i>S. minutum</i>
		<i>S. nepalense</i>
		<i>S. nyetense</i>
		<i>S. pwaniensis</i>
<i>carpocapsae</i>	Length of IJ less than 600 µm	<i>S. rarum</i>
		<i>S. ritteri</i>
		<i>S. sasonense</i>
		<i>S. scapterisci</i>
		<i>S. siamkayai</i>
		<i>S. sichuanense</i>
		<i>S. surkhentense</i>
		<i>S. tami</i>
		<i>S. thermophilum</i>
		<i>S. websteri</i>

Morphology of *Heterorhabditis* on SEM shows in Figure 7. Females are categorized into two types: hermaphroditic and amphimictic. Hermaphroditic females have a truncate to slightly rounded head with six well-developed, distinct conical lips, each bearing a terminal papilla. The stoma is broad and shallow. The vulva is slit-like and located slightly anterior to the mid-body. The tail is pointed and longer than the anal body width, usually with a postanal swelling. Amphimictic females are similar in morphology to hermaphroditic females but are generally smaller. Their vulva serves exclusively for mating purposes.

Males possess a single testis. Spicules paired, separate, and slightly curved ventrally, with the gubernaculum being nearly as long as the spicules. The bursa is peloderan in form, featuring nine pairs of genital papillae.

Infective juveniles (IJs) are typically sheathed, with an anterior region marked by a tessellated pattern and longitudinal ridges throughout the body. Both the mouth and anus are closed. Oesophagus and intestine reduced. The excretory pore is positioned posterior to the nerve ring, and the tail is pointed. (Adams & Nguyen, 2002).



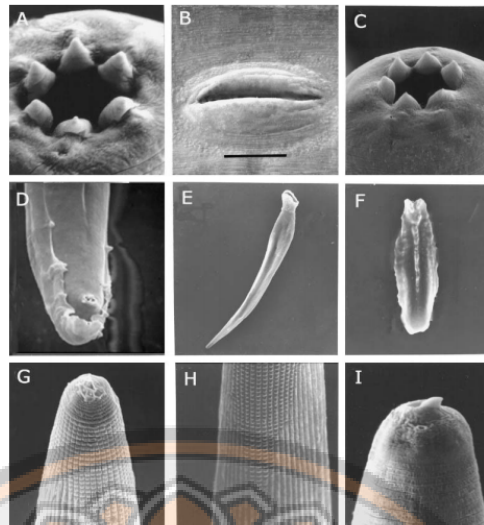


Figure 7 Morphological structures of *Heterorhabditis* spp.: A) Facial view of a hermaphroditic female, B) Vulva of the hermaphroditic female, C) Facial view of an amphimictic female, D) Male tail showing peloderan bursa, E) Spicule, F) Gubernaculum, G–I) Anterior region of the infective juvenile (IJ).

Source: Adams and Nguyen (2002)

However, limitation of this method is ability to distinguish in genera only because different species of *Steinernema* and especially *Heterorhabditis*, look similar. Therefore, the new techniques have been developed for study about EPNs.

Molecular techniques have been widely used for classifying EPNs within the genera *Steinernema* and *Heterorhabditis*. Several molecular methods are used for EPN identification. Among these, the most commonly used genetic marker—due to its extensive representation in genetic databases—is the DNA sequence of the 18S or small subunit (SSU) ribosomal RNA gene (Dorris et al., 1999; Blaxter et al., 1998). Restriction fragment length polymorphism (RFLP) analysis, which utilizes restriction enzymes targeting the 26S rDNA gene and the internal transcribed spacer (ITS) regions, has also been applied for species-level classification of EPNs, although it requires the use of multiple enzymes (Nasmith et al., 1996). While 18S rDNA sequencing can distinguish between the genera *Steinernema* and *Heterorhabditis*, its low variability limits its effectiveness at the species level (Blaxter et al., 1998). In addition, the 28S

rDNA sequence provides sufficient variation to differentiate species within the genus *Steinernema* (Stock et al., 2001; Nguyen et al., 2010; Thanwisai, 2012). Similarly, the ITS region, known for its high sequence variability, enables species-level identification within *Heterorhabditis* (Hominick et al., 1997; Thanwisai, 2012). Furthermore, phylogenetic analyses based on specific nucleotide sequences are valuable for studying evolutionary relationships among EPN species. (Nguyen et al., 2004; Adams et al., 1998; Nguyen et al., 2001; Stock et al., 2001; Thanwisai, 2012)

Phylogenetic relationship of entomopathogenic nematodes (EPNs)

For phylogenetic analyses at the species level of EPNs, the researchers have commonly analyzed the internal transcribed spacer (ITS) region of rDNA for *Heterorhabditis* and the 28S rRNA gene for *Steinernema* (Fukruksa, 2014; Hominick et al., 1997; Nguyen et al., 2010; Stock et al., 2001; Suwannaroj, 2014; Thanwisai, 2012). Subkrasae (2022) constructed the phylogenetic tree based on the neighbor-joining (NJ), maximum likelihood (ML) to examine the diversity and phylogenetic relationships of EPNs from 12 provinces that covered four regions in Thailand. According to 28S rDNA region, most of *Steinernema* isolates were closely related to *S. surkhetense* while only one isolate was close to *S. kushidai*. For ITS region, the *Steinernema* were group with *S. huense*, *S. guangdongense*, *S. siamkayai*, *Steinernema* sp. YNd80 and *Steinernema* sp. YNc215. *Heterorhabditis* isolates were mainly affiliated with *H. indica* while some isolates were closely related to *H. baujardi* and *Heterorhabditis* sp. SGmg3. In 2012, Thanwisai also mostly found *H. indica*. However, *S. websteri* were the most species found for *Steinernema*. In Mae Wong National Park, Muangpat et al. (2017) found EPN isolates related to *H. zealandica*, *H. indica*, *H. baujardi*, *S. kushidai* and *S. websteri*. Similarly, Yooyangket et al. (2018) reported isolates of *S. websteri* and *H. baujard* from Nam Nao National Park.

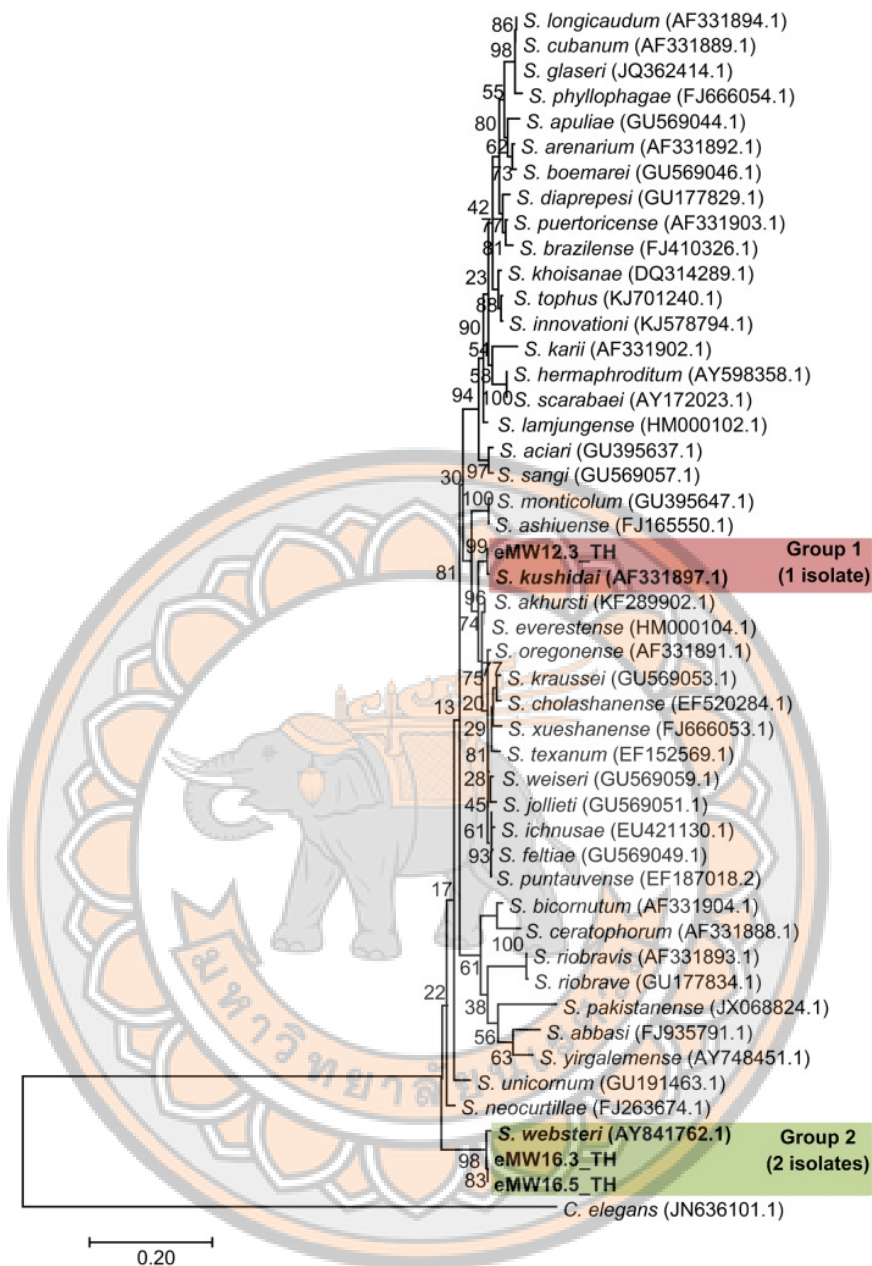


Figure 8 Maximum likelihood phylogenetic tree of *Steinerema* species in Mae Wong National Park, Thailand.

Source: Muangpat et al., 2017

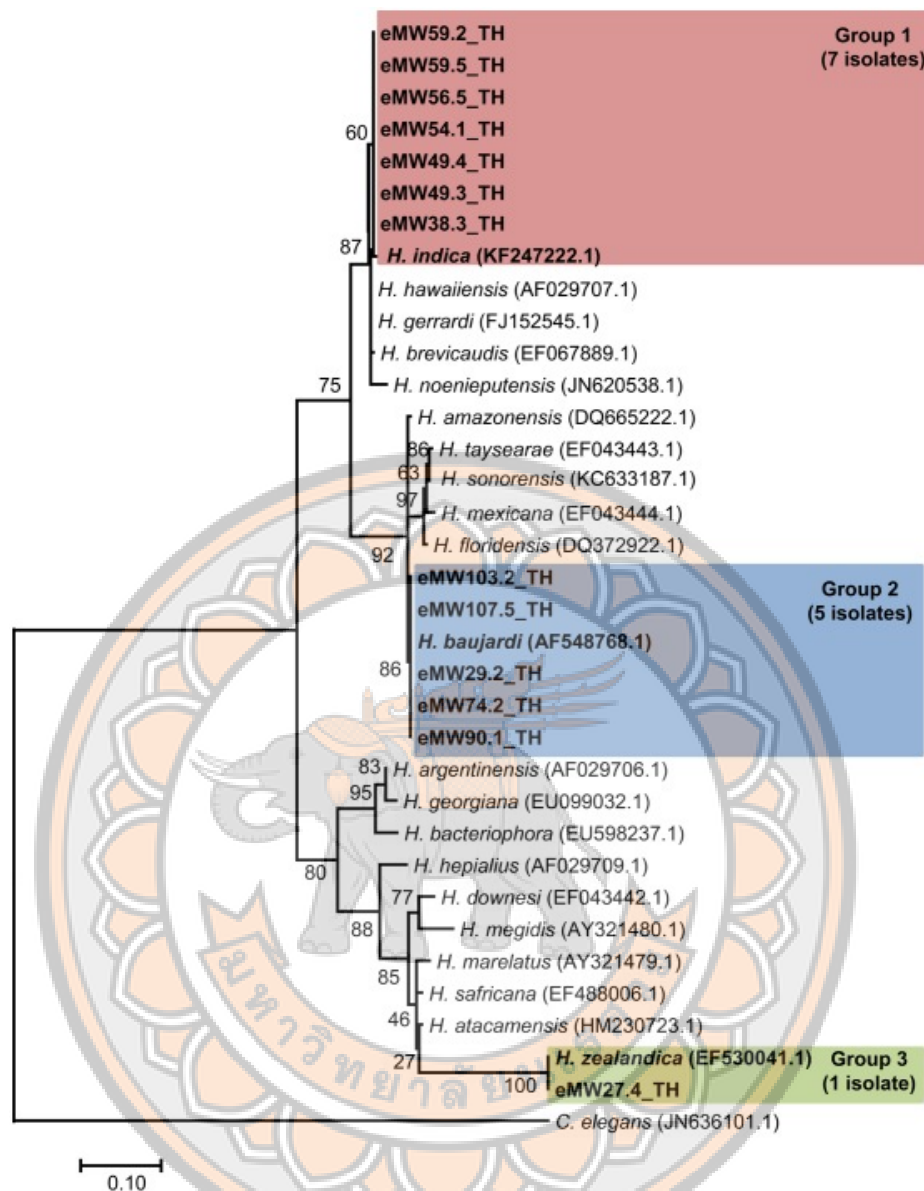


Figure 9 Maximum likelihood phylogenetic tree of *Heterorhabditis* species in Mae Wong National Park, Thailand.

Source: Muangpat et al., 2017

Distribution of entomopathogenic nematodes (EPNs)

The EPNs are globally found in several habitats except Antarctica (Griffin et al., 1991). Currently, there are 36 references from European countries, eight from North America and the Caribbean, four from South America, 12 from Asia, one from Africa, and two from the Australia–Pacific region. The extensive research on entomopathogenic nematodes in Europe is largely attributed to strong support from the European Union for collaborative studies. As a result, the biogeography and biodiversity of these organisms are more thoroughly documented in Europe than in any other region of the world. However, much of the global distribution of entomopathogenic nematodes remains largely unexplored.

Habitat and factor of distribution of entomopathogenic nematodes (EPNs)

The distribution of entomopathogenic nematodes (EPNs) is influenced by factors such as habitat type, soil texture, pH, altitude, and the availability of suitable insect hosts (de Brida et al., 2017a). Surveys conducted in Germany identified habitat preferences among *Steinernema* species (Sturhan, 1999). *Steinernema affine* was commonly found in cultivated fields and grasslands, and also occurred in woodlands, whereas *S. intermedium* and *S. kraussei* were primarily associated with forest habitats. *S. affine* and *S. feltiae* were found exclusively in arable soils. Similarly, Hominick et al. (1995) reported that *S. feltiae* and *S. affine* were predominantly located in fields and roadside verges across the UK and the Netherlands. In the Slovak Republic, *S. affine* was mostly observed in arable soils, while *S. intermedium* occurred in forested areas. *S. feltiae*, on the other hand, showed no strong habitat preference (Sturhan, 1999). In Belgium, *S. kraussei* was frequently found in woodlands, particularly under coniferous trees, while *S. feltiae* appeared both within and along the edges of forests. In California, sampling of natural habitats by Stock et al. (1999) revealed that coniferous forests supported the greatest diversity of EPNs, with oak woodlands showing the highest prevalence. Among the species, *S. kraussei* emerged as the most abundant and widely distributed, typically inhabiting forested areas. Overall, *Steinernema* species are most prevalent in woodland ecosystems, which appear to support the richest diversity of this genus (Stock et al., 1999; Sturhan, 1999).

Entomopathogenic nematodes (EPNs) are distributed throughout Thailand. In 2012, Thanwisai et al. reported the presence of *Steinernema websteri* and *S. khoisaniae* across several provinces. Specifically, *S. websteri* was detected in Khon Kaen, Phetchabun, Suphan Buri, Nakhon Nayok, and Kanchanaburi. Additionally, several *Heterorhabditis* species, including *H. indica*, *H. bacteriophora*, *H. baujardi*, and two unidentified species, *Heterorhabditis* sp. SGmg3 and *Heterorhabditis* sp. SGgi, were also reported (Thanwisai, 2012). *S. websteri*, *S. scarabaei*, *H. indica*, *H. gerrardi* and *Heterorhabditis* sp. SGmg3 were also found in the upper northern. According to Vitta et al (2017) suggests that *H. indica* and *S. websteri*, is nation-wide in Thailand. *S. saimkayi* was isolated in Phetchabun Province and was identified as a novel species (Stock et al, 1998). *S. minutum* is a second novel specie in Thailand which was found in Chumphon Province (Maneesakorn et al., 2010). Moreover, in 2017 and 2018 there were reported of *H. zealandica*, *S. websteri* and *S. kushidai* from Mae Wong Nation Park, Kamphaeng Phet Province and *H. baujardi* and *S. websteri* from Nam Nao National Park, Phetchabun province (Muangpat et al., 2017; Yooyangket et al., 2018). In addition, the recently surveying of EPNs in Phitsanulok Province, lower northern Thailand. EPN isolates were identified as *S. surkhetense*, *H. indica* and *Heterorhabditis* sp. (Suwannaroj et al., 2020).

Entomopathogenic nematodes (EPNs) were predominantly found in loamy soils, within a temperature range of 26°C to 33°C, pH levels between 5.0 and 7.0, and soil moisture content ranging from 0.5% to 6.8% (Vitta et al., 2017; Vitta et al., 2015). In general, nematodes are more commonly found in light soils such as sandy or silty types, rather than in heavier soils with either low or high organic content. While soil texture may not significantly influence nematode prevalence, it plays a crucial role in the survival and movement of infective juveniles. Most species, including *Steinernema feltiae*, *S. affine*, *S. arenarium*, *S. carpocapsae*, *S. weiseri*, *S. intermedium*, *S. bicornutum*, and *S. silvaticum*, show a strong preference for light, sandy or silty soils. An exception is *S. kraussei*, which is tolerant of both silt/clay and silt/clay organic soils. (Mráček et al., 2005).

Entomopathogenic nematodes (EPNs) were detected in soil samples throughout all seasons. While most samples were collected between April and September, nematodes were also found in early spring (March) and early winter (November and December). These organisms can survive the repeated freeze-thaw cycles that occur in the upper layers of soil during winter. Overall, seasonal variation did not have a significant impact on nematode prevalence (Mráček et al., 2005).

Entomopathogenic nematodes (EPNs) were found across various elevations, including lowlands (below 300 m), hilly regions (300–500 m), highlands (500–800 m), and mountainous areas (above 800 m). *Steinernema kraussei* was most identified in the hilly and highland zones, while *S. feltiae* appeared more frequently in lowland and hilly regions. *S. affine* was predominantly found in the hilly countryside and highlands. Overall, there were no significant differences in EPN prevalence among these elevation zones. Notably, the highest recorded occurrence of *S. kraussei* was at 1100 m in the Kumava Mountains (Mráček et al., 2005).

Application of EPNs for the control of insects

EPNs in the genera *Heterorhabditis* and *Steinernema* are obligate parasites of several insects (Kaya & Gaugler, 1993). They have been commonly reported that different stages of an insect species respond differently to EPN species. *Steinernema glaseri* was firstly used for controlling scarabs beetles (Gaugler et al., 1992). EPNs that exhibited good field control of *Popillia japonica* included *S. scarabaei* (100%), *H. bacteriophora* (strain GPS11) (34–97%), *H. bacteriophora* (strain TF) (65–92%), and *H. zealandica* (strain X1) (73–98%) (Grewal et al., 2005; Koppenhöfer et al., 2006). The two species, *H. bacteriophora* and *H. zealandica*, are commercially available for grub control (Grewal et al., 2005). The nematode species *Steinernema kraussei* and *S. carpocapsae* demonstrated highly effective control, achieving 100% mortality of *Aethina tumida* larvae. Additionally, sequential applications of these nematodes after the larvae entered the sand to pupate maintained excellent control for up to three weeks (Andrews et al., 1982).

Application of EPNs for the control of snails

There are few studies of the pathogenicity of *Steinernema* and *Heterorhabditis* on snails. Since 1986, Li et al started testing *Steinernema glaseri* to infect the snail of species *Oncomelania hupensis*. This EPN can infect, kill and develop in the semi-aquatic snail *O. hupensis*. In 1994, Jaworska described the susceptibility of *Deroceras agreste* and *Deroceras reticulatum* exposed to 3 EPN species (Wilson et al., 1994). Moreover, the effect of exposure of *Bradybaena similaris* to *H. indica* LPP1 were investigated. The exposure to *H. indica* LPP1 caused 55% mortality in *B. similaris*, with the highest rate observed in the first week. Similar to report in 2017 they suggest that the use of *H. indica* LPP1 is a potential alternative for biological control of *B. similaris* (Tunholi et al., 2017b). Other species of snail had been studied with EPNs is *Lymnaea columella*, an aquatic snail. This study demonstrated the susceptibility of *Lymnaea columella* to infective juveniles of *Heterorhabditis baujardi* LPP7, resulting in an average mortality rate of 66.66% among the snail population following exposure (Tunholi et al., 2017a).

Symbiotic bacteria

Xenorhabdus and *Photorhabdus* bacteria form symbiotic partnerships with entomopathogenic nematodes; *Xenorhabdus* with steinernematids and *Photorhabdus* with heterorhabditids. Together, the nematode-bacterium complex can infect and kill the larval stages of many insect species. These gram-negative, motile bacteria from the family Morganellaceae are carried inside the gut of the infective juvenile stage of the nematode. In the soil, infective juveniles actively locate insect larvae and penetrate their haemocoel. Once inside, the nematodes release their symbiotic bacteria into the haemolymph. The bacteria rapidly multiply and secrete a cocktail of toxins and hydrolytic enzymes that kill the host and break down its body into a nutrient-rich medium, supporting nematode development and reproduction. When resources are depleted, a new generation of infective juveniles is formed and recolonized by the bacteria, completing this finely tuned and highly specialized life cycle.

The symbiotic bacteria exist in two distinct physiological states, each adapted to a different ecological niche. The first is the phoretic state, in which the bacteria are carried by the nematode during its infective juvenile stage. In this state, *Xenorhabdus*

resides in a specialized intestinal vesicle of *Steinernema* infective juveniles, while *Photorhabdus* is primarily found in the anterior gut of *Heterorhabditis* infective juveniles. The second is the vegetative state, during which the bacteria actively reproduce inside an insect host after being released into the insect's haemolymph. Thus, these symbionts alternate between environments with low and high nutrient availability. Unlike many other animal-associated symbiotic bacteria, their life cycle is unique—they are mutualistic with nematodes but pathogenic to insects (Boemare, 2002).

***Xenorhabdus* bacteria**

In 1965, George and Thomas reported the discovery of the first *Xenorhabdus* species located in the anterior intestine of *Steinernema* nematodes. *Xenorhabdus* cells are rod-shaped, non-spore-forming (asporogenous) bacteria, measuring approximately $0.3\text{--}2 \times 2\text{--}10\text{ }\mu\text{m}$. They are Gram-negative and facultatively anaerobic, capable of both respiratory and fermentative metabolism. Additionally, they test negative for both catalase activity and nitrate reduction (Yamanaka et al., 2004).

Bacterial colonies on nutrient-bromothymol blue-triphenyl tetrazolium chloride agar (NBTA), which contains bromothymol blue and TTC, colonies incubated at $25\text{--}28\text{ }^{\circ}\text{C}$ typically appear convex, circular, and have undulated edges. They exhibit a blue or brown coloration due to the absorption of bromothymol blue (Thanwisai et al., 2012). At present, 26 (39) species of *Xenorhabdus* were reported (Table 4).

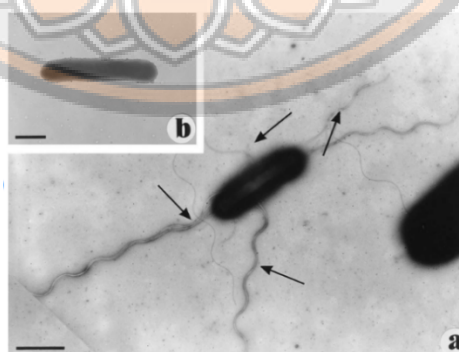


Figure 10 Morphology of *X. nematophilus*; a) showing numerous flagella, b) without flagella.

Source: Givaudan et al. (1995)

Table 4 Symbiotic bacteria in genus *Xenorhabdus*

Genus	Species	References
<i>Xenorhabdus</i>	<i>aichiensis</i>	(Machado, Bhat, Castaneda-Alvarez, Askary, et al., 2023)
	<i>anantnagensis</i>	(Machado, Bhat, Castaneda-Alvarez, Askary, et al., 2023)
	<i>beddingii</i>	(Akhurst & Boemare, 1988)
	<i>bharatensis</i>	(Thanwisai et al., 2024)
	<i>bovienii</i>	(Akhurst & Boemare, 1988)
	<i>budapestensis</i>	(Lengyel et al., 2005)
	<i>cabanillasii</i>	(Tailliez et al., 2006)
	<i>doucetiae</i>	(Tailliez et al., 2006)
	<i>eapokensis</i>	(Kämpfer et al., 2017)
	<i>ehlersii</i>	(Lengyel et al., 2005)
	<i>entomophaga</i>	(Thanwisai et al., 2024)
	<i>griffiniiae</i>	(Tailliez et al., 2006)
	<i>hominickii</i>	(Tailliez et al., 2006)
	<i>indica</i>	(Somvanshi et al., 2006)
	<i>innexi</i>	(Lengyel et al., 2005)
	<i>ishibashii</i>	(Kuwata et al., 2013)
	<i>japonica</i>	(Nishimura et al., 1994)
	<i>khoisanae</i>	(Tiarin Ferreira et al., 2013)
	<i>koppenhoeferi</i>	(Tailliez et al., 2006)
	<i>kozodoii</i>	(Tailliez et al., 2006)
	<i>lircayensis</i>	(Castaneda-Alvarez et al., 2021)
	<i>littoralis</i>	(Palma et al., 2024)
	<i>luminescens</i>	(Thomas & Poinar, 1979)
	<i>magdalenensis</i>	(Tailliez et al., 2006)
	<i>mauleonii</i>	(Tailliez et al., 2006)
	<i>miraniensis</i>	(Tailliez et al., 2006)
	<i>nematophila</i>	(George & Thomas, 1979)

Table 4 (Cont.)

Genus	Species	References
<i>Xenorhabdus</i>	<i>poinarii</i>	(Akhurst & Boemare, 1988)
	<i>romanii</i>	(Tailliez et al., 2006)
	<i>santafensis</i>	(Palma et al., 2024)
	<i>siamensis</i>	(Thanwisai et al., 2024)
	<i>stockiae</i>	(Tailliez et al., 2006)
	<i>szentirmaii</i>	(Lengyel et al., 2005)
	<i>thailandensis</i>	(Thanwisai et al., 2024)
	<i>thuongxuanensis</i>	(Kämpfer et al., 2017)
	<i>vietnamensis</i>	(Tailliez et al., 2006)
	<i>yunnanensis</i>	(Machado et al., 2023)

***Photorhabdus* bacteria**

Photorhabdus are Gram-negative bacilli which have asporogenous, rod-shaped ($0.5\text{--}2 \times 1\text{--}10 \mu\text{m}$), motile via peritrichous flagella. These bacteria are facultatively anaerobic, capable of both respiration and fermentation. They typically grow best at 28°C, though some strains can grow at 37–38°C. They differ from many other members of the Morganellaceae family by lacking several characteristic traits. *Photorhabdus* exist in primary and secondary forms, with the secondary form known for producing bioluminescence and all strains are catalase positive but do not reduce nitrate (Leisman et al., 1995). The colony characteristics on NBTA medium are described as convex, circular, with entire margins, and displaying a green coloration (Thanwisai et al., 2012). *Photorhabdus* is associated with *Heterorhabditis* and is carried in the intestine. At present, 25 species of *Photorhabdus* were reported worldwide (Table 5).

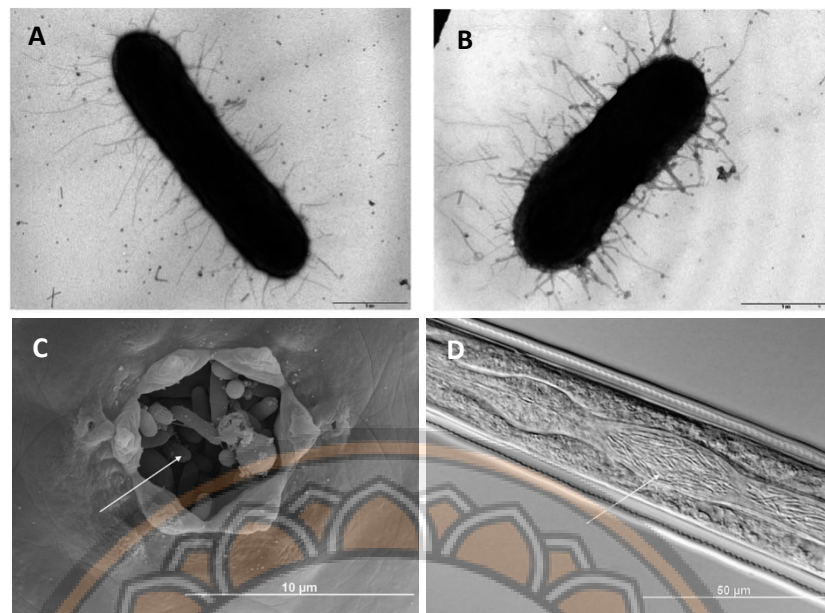


Figure 11 A) Morphology of *P. luminescens* by electron microscopy (Evelyne et al., 2006). B) *Photobacterium temperata* rods visible (arrow) inside the nematode mouth.

Source: Sajnaga and Kazimierczak (2020)

Table 5 Symbiotic bacteria in genus *Photobacterium*

Species	Previous described Species
<i>aballayi</i> (Machado et al., 2023)	-
<i>aegyptia</i> (Machado et al., 2021)	-
<i>africana</i> (Machado et al., 2024)	-
<i>akhurstii</i> (Machado et al., 2018)	<i>luminescens</i> subsp. <i>akhurstii</i> (Fischer-Le Saux et al., 1999)
<i>antumapuensis</i> (Castaneda-Alvarez et al., 2022)	-
<i>asymbiotica</i> (Fischer-Le Saux et al., 1999)	-
<i>australis</i> (Machado et al., 2018)	<i>asymbiotica</i> subsp. <i>australis</i> (Akhurst et al., 2004)
<i>bodei</i> (Machado et al., 2018)	-

Table 5 (Cont.)

Species	Previous described Species
<i>caribbeanensis</i> (Machado et al., 2018)	<i>luminescens</i> subsp. <i>caribbeanensis</i> (Tailliez et al., 2010)
<i>cinerea</i> (Machado et al., 2018)	<i>temperata</i> subsp. <i>cinerea</i> (Tóth & Lakatos, 2008)
<i>hainanensis</i> (Machado et al., 2018)	<i>luminescens</i> subsp. <i>hainanensis</i> (Tailliez et al., 2010)
<i>heterorhabditis</i> (Ferreira et al., 2014)	-
<i>hindustanensis</i> (Machado et al., 2021)	-
<i>kayaii</i> (Machado et al., 2018)	<i>luminescens</i> subsp. <i>kayaii</i> (Hazir et al., 2004)
<i>khanii</i> (Machado et al., 2018)	<i>temperata</i> subsp. <i>khanii</i> (Tailliez et al., 2010)
<i>kleinii</i> (Machado et al., 2018)	<i>luminescens</i> subsp. <i>kleinii</i> (An & Grewal, 2011)
<i>laumondii</i> (Machado et al., 2018)	<i>luminescens</i> subsp. <i>laumondii</i> (Fischer-Le Saux et al., 1999)
<i>luminescens</i> (Boemare et al., 1993)	-
<i>namnaonensis</i> (Machado et al., 2018)	<i>luminescens</i> subsp. <i>namnaonensis</i> (Glaeser et al., 2016)
<i>noenieputensis</i> (Machado et al., 2018)	<i>luminescens</i> subsp. <i>noenieputensis</i> (Ferreira et al., 2013)
<i>stackebrandtii</i> (Machado et al., 2018)	<i>temperata</i> subsp. <i>stackebrandtii</i> (An & Grewal, 2010)
<i>tasmaniensis</i> (Machado et al., 2018)	<i>temperata</i> subsp. <i>tasmaniensis</i> (Tailliez et al., 2010)
<i>temperate</i> (Fischer-Le Saux et al., 1999)	-
<i>thracensis</i> (Machado et al., 2018)	<i>luminescens</i> subsp. <i>thracensis</i> (Hazir et al., 2004)
<i>viridis</i>	(Machado et al., 2024)

Pathogenicity

Xenorhabdus and released into the host's hemolymph within five hours of nematode invasion, typically resulting in the host larva's death within 24 to 48 hours (Akhurst, 1982). The effectiveness of this nematode-bacterium complex in killing insect larvae varies depending on the insect species, as well as its immune and physiological condition, and the specific bacterial strain involved (Akhurst, 1982). *Photorhabdus* rapidly multiply in haemolymph and release metalloprotease to degrade midgut epithelium of intestine and immune system (Silva et al., 2002). On the other hand, *Xenorhabdus*, release endotoxins such as lipopolysaccharides (LPS) from their outer membranes, which target and damage the insect host's hemocytes (Brillard et al., 2001; Dunphy & Thurston, 1990). To successfully proliferate within the insect hemolymph, *Xenorhabdus* and *Photorhabdus* must overcome the host's immune defenses—either by resisting recognition, tolerating the response, or actively suppressing it. As part of their natural life cycle, these bacteria are well-adapted to survive in the hemolymph, where they produce secondary metabolites with antimicrobial properties that can target bacteria, fungi, and yeast (Akhurst, 1982). Insects killed by these pathogens often decompose slowly and exhibit distinctive dark red or black coloration (Emelianoff et al., 2008). Due to their high pathogenicity, the nematode-bacterium complex has proven valuable as a biological control agent against insect pest.

Identification of symbiotic bacteria

Photorhabdus and *Xenorhabdus* bacteria can be identified using several techniques. They are Gram-negative, rod-shaped, motile, facultative anaerobes that lack the ability to form spores. Molecular analysis of 16S rDNA has shown that *Xenorhabdus* is closely related to *Photorhabdus*, and both form a distinct sister group within the Morganellaceae family (Forst et al., 1997).

1. Biochemical test

Biochemical tests are useful for distinguishing *Xenorhabdus* and *Photorhabdus* from other members of the Morganellaceae family, though they often fail to differentiate between the two genera due to shared negative results across many tests. However, *Photorhabdus* can be distinguished by its ability to produce catalase,

yielding a positive catalase test, and its unique trait of bioluminescence which is a feature not found in *Xenorhabdus* (Akhurst & Boemare, 1988).

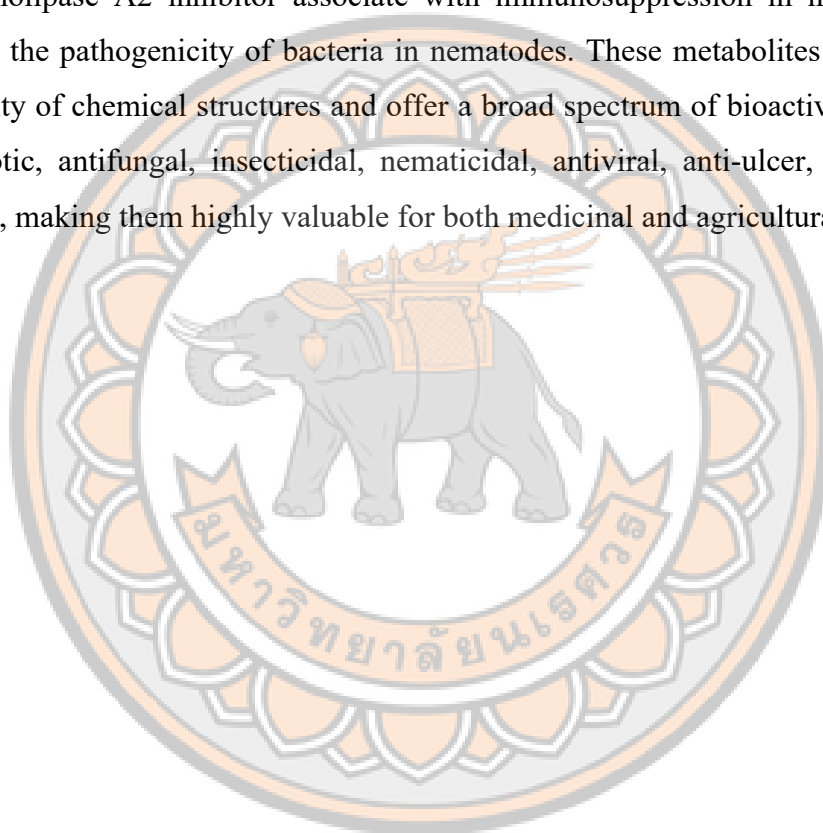
2. Molecular techniques

Molecular characteristics can be employed to identify, diagnose, and differentiate species, much like morphological traits. The appeal of molecular data lies in their well-defined genetic foundation, which is a significant advantage considering the considerable environmental and host-related morphological variation observed in entomopathogenic nematodes. Differentiating between *Xenorhabdus* and *Photorhabdus* species through nucleotide sequencing primarily involves analyzing the DNA sequences of housekeeping genes. Complete 16S rDNA sequencing was used to distinguish *P. asymbiotica*, *P. temperata*, and *P. luminescens* in 1991 (Fischer-Le Saux et al., 1999). However, 16S rDNA proved unreliable for differentiation at the subspecies level (Akhurst et al., 2004). Subsequent studies included sequencing of genes such as recombinase A (*recA*), DNA gyrase subunit B (*gyrB*), 50S ribosomal protein L2 (*rplB*), glutamyl-tRNA synthetase (*gltX*) and DNA polymerase III subunit beta (*dnaN*). Among these, *recA*, *gyrB*, *dnaN*, and *gltX* exhibited greater sequence variability than *rplB* and 16S rDNA. Despite this, *gyrB* and *rplB* were unable to differentiate all *Photorhabdus* and *Xenorhabdus* species. Additionally, the *gltX* gene may have been acquired via lateral gene transfer, making it unsuitable for species-level differentiation.

Phylogenetic analyses based on *recA* and *dnaN* provided more accurate species clustering. Therefore, using individual gene sequences such as *recA* or *dnaN* offers a more effective approach for distinguishing between *Photorhabdus* and *Xenorhabdus* species (Tailliez et al., 2010).

Secondary metabolites

The symbiotic bacteria produce several secondary metabolites both small and large molecule (Figure 12). The majority of *Xenorhabdus* and *Photorhabdus* species synthesize multiple groups of bioactive secondary metabolites. Secondary metabolites produced by *Xenorhabdus* bacteria, including xenorhabdins and xenocoumacins, as well as compounds from *Photorhabdus* such as hydroxystilbenes and anthraquinones, exhibit strong activity against Gram-positive bacteria (McInerney et al., 1991). Phospholipase A2 inhibitor associate with immunosuppression in insect larva and induce the pathogenicity of bacteria in nematodes. These metabolites display a wide diversity of chemical structures and offer a broad spectrum of bioactivities, including antibiotic, antifungal, insecticidal, nematocidal, antiviral, anti-ulcer, and anticancer effects, making them highly valuable for both medicinal and agricultural applications.



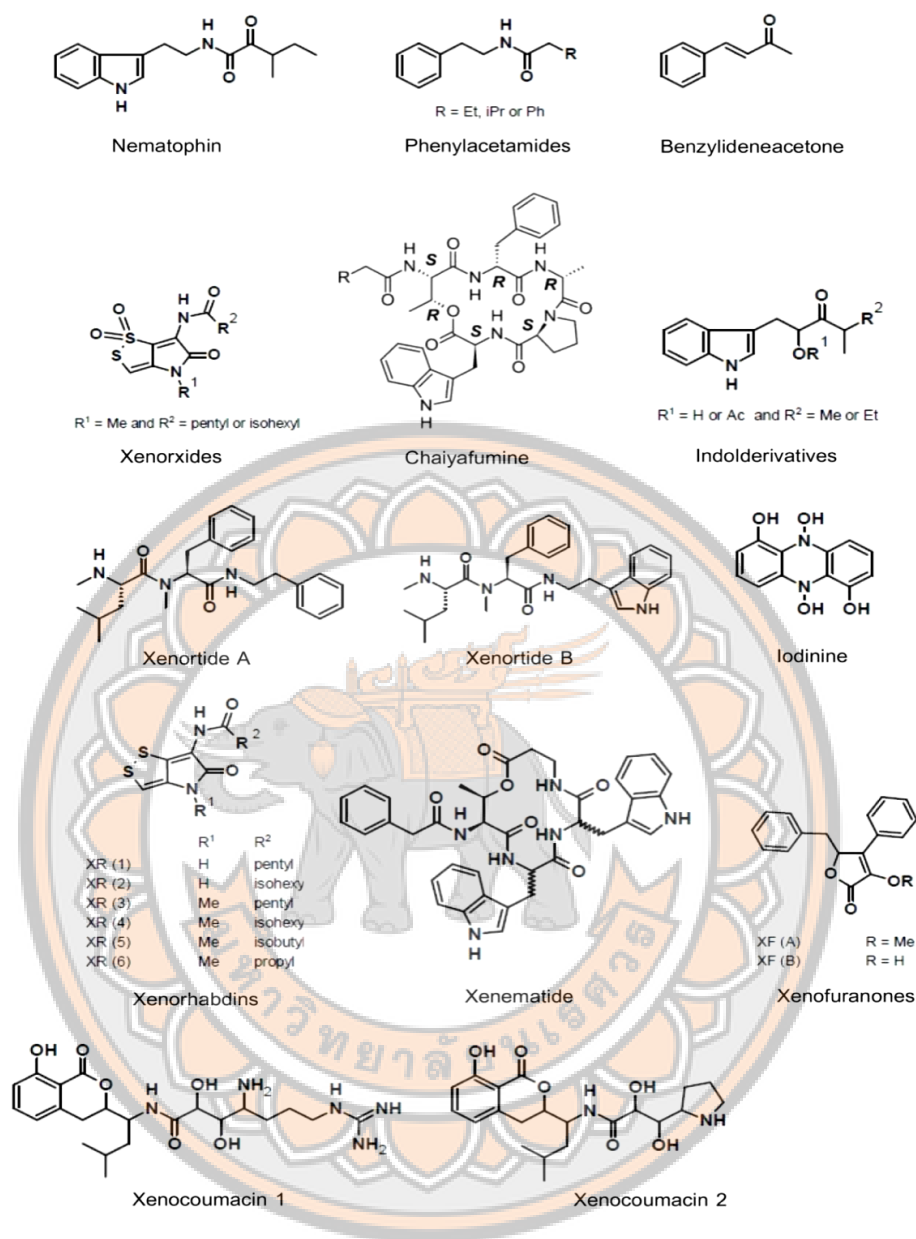


Figure 12 Secondary metabolites and derivatives structure of *Xenorhabdus* species.

Source: Brachmann (2009)

Phylogenetic relationship of *Photorhabdus* and *Xenorhabdus*

The phylogenetic relationships between *Photorhabdus* and *Xenorhabdus* species were analyzed in 2010 using neighbor-joining methods based on sequences of *recA*, *gyrB*, *dnaN*, *gltX*, and *16S* rDNA. Results showed that *recA* and *dnaN* provided more accurate clustering (Tailliez et al., 2010). *Xenorhabdus* strains are distinctly distinguished from strains of *Photorhabdus*. Later, in 2012, Thanwisai et al investigated the symbiotic bacteria found in Thailand using *recA* gene sequences analyzed via maximum-likelihood methods. Their study revealed that *Xenorhabdus* species in Thailand formed two main clusters—one closely related to *X. stockiae* strain TH01 and the other to *X. miraniensis* strain Q1. *Photorhabdus* species were divided into three groups: one linked to *P. luminescens* subsp. *akhurstii* strain FRG04 and *P. luminescens* subsp. *hainanensis* strain C8404; another to *P. luminescens* subsp. *laumondii* strain E21; and the third to *P. asymbiotica* subsp. *australis* strain 9802892 (Thanwisai et al., 2012). In 2022, Subkrasae reported the presence of symbiotic bacteria from four regions of Thailand. Based on analysis of the *recA* gene region, *Xenorhabdus* strains were classified into five groups, most of which were closely related to *X. stockiae*, followed by *X. indica*, *X. griffiniae*, *X. thuongxuanensis* and *X. japonica*. While one isolate of *X. eapokensis* was found using 16s rRNA. *Photorhabdus* isolates were clustered with *P. luminescens* subsp. *akhurstii*, *P. asymbiotica* subsp. *australis* and *P. luminescens* subsp. *hainanensis*. Moreover, the evolutionary relationship of symbiotic bacteria from 2 national park in Thailand were analyzed. In 2017, Muangpat reported that symbiotic bacteria isolated from entomopathogenic nematodes in Mae Wong National Park, Kamphaeng Phet Province, were closely related to *X. stockiae*, *X. japonica*, *P. temperata* subsp. *temperata*, and *P. luminescens* subsp. *akhurstii*. Later, Yooyangket found that symbiotic bacteria from Nam Nao National Park, Phetchabun Province, were associated with *X. vietnamensis*, *X. japonica*, *X. stockiae* TH01, and *P. luminescens* subsp. *akhurstii* FRG04 (Yooyangket et al., 2018).



Figure 13 Phylogenys of *Xenorhabdus* from upper northern Thailand together with *Xenorhabdus* spp. worldwide based on maximum-likelihood analysis of 508 bp of the *recA* region.

Source: Fukruksa et al. (2017)

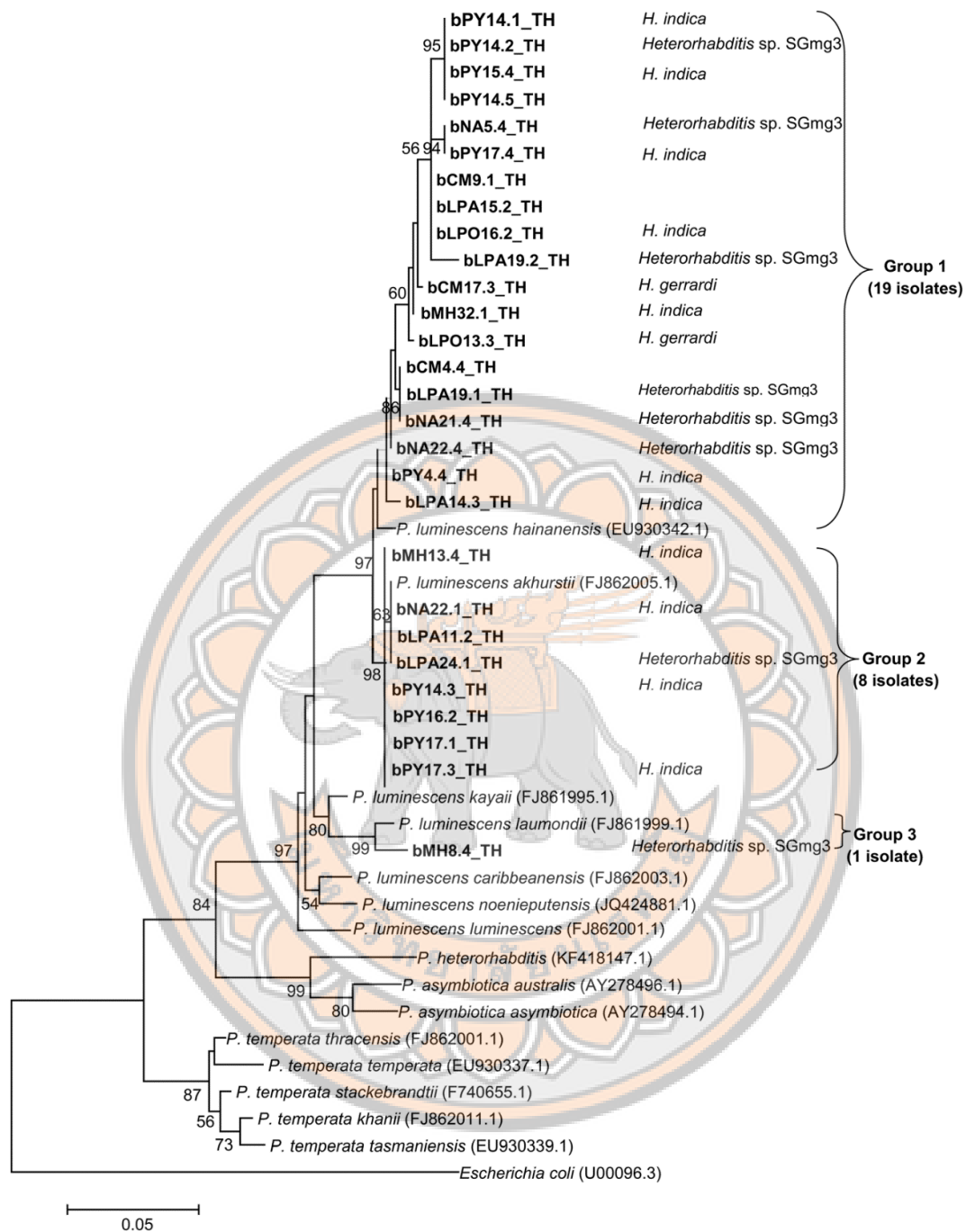


Figure 14 Phylogenetic relationships of *Photorhabdus* from EPNs of upper northern Thailand and *Photorhabdus* spp. worldwide based on maximum-likelihood analysis of 508 bp of the *recA* region.

Source: Fukruksa et al. (2017)

Relationship of entomopathogenic nematodes and their symbiotic bacteria

It is generally believed that each species within the genus *Steinernema* forms a symbiotic relationship with only a single species of *Xenorhabdus*. However, *Xenorhabdus* species can associate with multiple nematode species. In contrast, the symbiotic relationships between *Heterorhabditis* and *Photorhabdus* are more flexible, with both bacteria and nematodes capable of establishing partnerships with multiple species of their respective symbiotic counterparts (Koppenhöfer et al., 2006; Koppenhöfer & Gaugler, 2009). The association between EPNs and their symbiotic bacteria is in Table 6.

Table 6 List of entomopathogenic nematodes and their symbiotic bacteria

Symbiotic bacteria	Entomopathogenic nematode
<i>P. asymbiotica</i>	<i>H. indica</i>
<i>P. australis</i>	<i>H. gerrardi</i>
	<i>H. indica</i>
<i>P. heterorhabditis</i>	<i>H. zealandica</i>
<i>P. akhurstii</i>	<i>H. baujardi</i>
	<i>H. indica</i>
<i>P. caribbeanensis</i>	<i>H. bacteriophora</i>
<i>P. hainanensis</i>	<i>H. bacteriophora</i>
<i>P. kayaii</i>	<i>H. bacteriophora</i>
<i>P. kleinii</i>	<i>H. heliothidis</i>
	<i>H. megidis</i>
<i>P. laumondii</i>	<i>H. bacteriophora</i>
<i>P. luminescens</i>	<i>H. bacteriophora</i>
<i>P. sonorensis</i>	<i>H. sonorensis</i>
<i>P. thracensis</i>	<i>H. bacteriophora</i>
<i>P. cinerea</i>	<i>H. downesi</i>
	<i>H. megidis</i>
<i>P. khanii</i>	<i>H. heliothidis</i>
	<i>H. megidis</i>

Table 6 (Cont.)

Symbiotic bacteria	Entomopathogenic nematode
<i>P. stackebrandtii</i>	<i>H. bacteriophora</i>
<i>P. tasmaniensis</i>	<i>H. marelatum</i>
	<i>H. zealandica</i>
<i>P. temperata</i>	<i>H. megidis</i>
<i>P. zealandica</i>	<i>H. zealandica</i>
<i>X. beddingii</i>	<i>S. longicaudum</i>
<i>X. bovienii</i>	<i>S. affine</i>
	<i>S. ceratophorum</i>
	<i>S. feltiae</i>
	<i>S. intermedium</i>
	<i>S. jolietii</i>
	<i>S. kraussei</i>
	<i>S. litorale</i>
	<i>S. oregonense</i>
<i>X. bovienii</i>	<i>S. puntauense</i>
	<i>S. sichuanense</i>
	<i>S. weiseri</i>
<i>X. budapestensis</i>	<i>S. bicornutum</i>
	<i>S. ceratophorum</i>
<i>X. cabanillasii</i>	<i>S. riobrave</i>
<i>X. doucetiae</i>	<i>S. diaprepesi</i>
<i>X. ehlersii</i>	<i>S. longicaudum</i>
	<i>S. serratum</i>
<i>X. hominickii</i>	<i>S. arenarium</i>
	<i>S. karrii</i>
	<i>S. monticolum</i>

Table 6 (Cont.)

Symbiotic bacteria	Entomopathogenic nematode
<i>X. griffithiae</i>	<i>S. hermaphroditum</i>
<i>X. indica</i>	<i>S. thermophilum</i>
	<i>S. abbasi</i>
<i>X. innexi</i>	<i>S. scapterisci</i>
<i>X. Ishibashii</i>	<i>S. aciari</i>
<i>X. japonica</i>	<i>S. kushidai</i>
<i>X. khoisanae</i>	<i>S. khoisanae</i>
<i>X. koppenhoeferi</i>	<i>S. scarabaei</i>
<i>X. kozodoii</i>	<i>S. aquiliae</i>
	<i>S. arenarium</i>
<i>X. magdalenensis</i>	<i>S. austral</i>
<i>X. miraniensis</i>	<i>S. khoisanae</i>
<i>X. namatophila</i>	<i>S. carpocapsae</i>
	<i>S. feltiae</i>
	<i>S. websteri</i>
<i>X. poinarii</i>	<i>S. cubanum</i>
	<i>S. glaseri</i>
<i>X. romanii</i>	<i>S. puertoricense</i>
<i>X. stockiae</i>	<i>S. siamkayai</i>
	<i>S. websteri</i>
<i>X. szentirmaii</i>	<i>S. rarum</i>
<i>X. vietnamensis</i>	<i>S. sangi</i>

Biology of *Biomphalaria glabrata*

Biomphalaria glabrata is a freshwater snail classified in phylum Mollusca, class Gastropoda, family Planorbinae, and genus *Biomphalaria*. It is the major intermediate snail host for the *Schistosoma mansoni* trematode, which causes schistosomiasis, a chronic disease that affects 240 million people every year (Gryseels et al., 2006).

Biomphalaria glabrata shell have a disc-like shape in size $5-8 \times 20-27$ mm. Shell aperture is rounded. The umbilicus is wide, funnel-shaped depression on one side. The shell is planispiral coiling and has a sinistral (left-handed) coiling pattern. However, the shell coiled upside down, so it appears to be dextral (pseudodextral discoid). It typically has 4 to 5 rounded whorls that gradually increase in size. The shell is thin and fragile (Lotfy and Lotfy, 2015; Palasio et al, 2017) (Figure 15).

Biomphalaria glabrata has a wide geographic distribution that is found in streams, lakes, and wetlands in South and Central Americas as well as in Africa. Snails are commonly found in surface waters near the edges of water bodies (Jurberg et al., 1997; Appleton, 1978). However, they can also survive at depths of up to 10 meters, either when placed there or by sinking on their own (Deschiens & Jadin, 1954; Jurberg et al., 1987). Laboratory studies have shown that they can tolerate pressures equivalent to a depth of 50 meters without any noticeable harm (Jurberg et al., 1988). Additionally, *Biomphalaria glabrata* can survive out of water by aestivating, and have been found buried up to 3 meters from the water's edge (Olivier & Barbosa, 1956; Paraense et al., 1955). When unable to reach the surface, they are capable of switching from aerobic to anaerobic respiration, allowing them to survive up to 16 hours without dissolved oxygen; however, they eventually die from asphyxiation if deprived for longer (Von Brand et al., 1950). In addition, statistical analysis failed to uncover any correlation between distribution and water quality. It is this very ability to function as a generalist that contributes to their success in marginal environments. The data indicate that this organism is neither fastidious in its diet nor narrow in its tolerances. On the contrary, it is monoecious, reproduces rapidly, has pulmonary respiration, tolerates physical-chemical variations (Melo et al., 2014).



Figure 15 Morphology of adult stage *Biomphalaria glabrata*

Control of *B. glabrata*

Snail control is a crucial part of the global schistosomiasis elimination strategy. In the past, molluscicides particularly niclosamide has been used for the elimination of this snail. However, there are limitations with the use of niclosamide due to temporary toxicity to non-target aquatic organisms including fish, amphibians and invertebrates, together with the high cost of synthetic molluscicides. Based on the WHO recommendation, the new snail control products have been explored from chemical, plants and microorganism (World Health Organization, 2020).

The use of chemical products has proven to be one of the most effective strategies for mollusk control. It is estimated that over 7,000 chemicals have been evaluated, including copper sulfate, gramoxone, calcium hydroxide, N-tritylmorpholine, and niclosamide. Among these, niclosamide stands out for its high efficacy, effectively eliminating 100% of both adult mollusks and embryos at concentrations below 1.5 µg/ml within just 2 hours of exposure (Ribeiro et al., 2009).

The natural products from plants have a lot of benefit from their secondary metabolites. There is various plant have been used against *B. glabrata* snail. For example, the extract from *Moringa oleifera* flowers, which contains flavones, flavonols, saponins, tannins, xanthones, and trypsin inhibitor activity. This flower extract with LC50: 2.37±0.5mg/mL showed significant ($P<0.05$) delay of the development of *B. glabrata* embryos and promoted mortality of adult snails.

Furthermore, the development of embryos generated by snails were disrupted by this extract, with most of them remaining in the blastula stage while control embryos were already in the gastrula stage (Rocha-Filho et al., 2015). The natural substances derived from lichen is one of the most studied. Secondary metabolites from lichen, the usnic acid isolated from *Cladonia substellata* have been evaluated their molluscicidal activity on embryos adult mollusks. The result show LC90 for 24 h of exposure were 1.62, 4.45, 5.36, and 4.49 $\mu\text{g/ml}$ for blastula, gastrula, trocophore, and veliger embryonic stages, respectively, and 3.45 $\mu\text{g/ml}$ for adult snails (Araújo et al., 2018). Moreover, the potassium salt of usnic acid from *C. substellata* was tested with this mollusk. The lowest lethal concentration for the embryos and adult snails was 10 and 1 $\mu\text{g/ml}$, respectively. The lichen of the species *Cladia aggregate* produces barbatic acid (BAR). The greatest molluscicidal activity of BAR was observed at 25 $\mu\text{g/mL}$, which showed 100% lethality, while the LC50 value for BAR was 11.9 $\mu\text{g/mL}$ (Martins et al., 2017).

Microbial pathogens have been explored as biocontrol agents for managing snail populations. *Bacillus thuringiensis*, a Gram-positive, spore-forming bacterium that produces various toxins, has demonstrated broad-spectrum activity and has been tested against *Biomphalaria alexandrina*. Additionally, *Brevibacillus laterosporus* has been shown to be pathogenic to juvenile stages of *B. glabrata* (de Oliveira et al., 2004). Exposure of healthy snails to *Paenibacillus* caused massive mortality in *B. glabrata*. Moreover, eggs laid by infected snails were also infected and decrease in hatching (Duval et al., 2015).

CHAPTER III

Research Procedures of the Study

Collection of soil sample

In this study, 1,000 soil samples from 200 soil sites were randomly collected from agriculture areas in northern Thailand (Chiang Mai, Chiang Rai, Kamphaeng Phet, Lamphun, Nan, Phitsanulok, Phrae, Sukhothai and Uttaradit). From each sample, approximately 500-1,000 grams of soil samples were collected using hand shovel with 10-centimeter depth. Soil samples were kept in plastic bag to prevent losing moisture. The extrinsic factor soil temperature was recorded with soil survey instrument AMT-300 (Model: KC-300B, Yanchang Kecheng Optoelectronic Technology Co., Ltd, China). In addition, soil pH and moisture were recorded by using the soil pH and moisture tester (Modal DM-15, Takemura electric works, Ltd, Japan). Soil textures were observed and noted. The latitude, altitude, and meters above mean sea level of each soil site were determined using Garmin nuvi 1250 GPS navigator (Garmin, Taiwan). All soil samples were transferred to the department of Microbiology and Parasitology, faculty of Medical Science, Naresuan University, Phitsanulok province, Thailand for further isolation of the EPNs.

Isolation of entomopathogenic nematodes from soil sample

Baiting technique was performed for isolating the infective juvenile stage of EPNs from the soil samples using the *Galleria mellonella* (Bedding & Akhurst, 1975). Each soil sample was placed in a plastic container along with five *G. mellonella* larvae. The container was sealed with a lid and kept at room temperature (approximately 25-28 °C) for five days. The dead larvae were observed and collected for further isolation of the IJs of EPNs. The baiting technique was repeated with 5 new *G. mellonella* larvae to maximize the EPNs.

Isolation of entomopathogenic nematodes from *G. mellonella* larvae

The IJs were isolated from deceased *G. mellonella* larvae using the White trap method. This setup involved two plastic petri dishes: a smaller dish (60 × 15 mm) was

placed upside down inside a larger dish (100 × 15 mm). A piece of filter paper was laid across the smaller dish to act as a bridge. Sterile distilled water was poured into the bottom of the larger dish, and infected *G. mellonella* larvae were placed on the filter paper. The setup was incubated in the dark at room temperature for 15–20 days. Infective juvenile stages of the EPNs that emerged from the cadavers were examined under a stereomicroscope and collected into a tissue culture flask. The nematodes were then cleaned using sedimentation and repeated washes with sterile distilled water. Finally, the IJs were stored in the flask at 13°C for up to 2–3 months. In addition, five microcentrifuge tubes containing EPNs were kept at -20°C for DNA extraction.

Propagation and storage of entomopathogenic nematodes

The propagation of entomopathogenic nematodes (EPNs) was carried out to increase their population for use as biocontrol agents. Five *Galleria mellonella* larvae were placed in a plastic petri dish (30 × 15 mm), and a few drops of infective juvenile (IJ) suspension were applied directly onto the larvae. The dish was sealed with paraffin film and incubated at room temperature for 2 to 4 days. Larval mortality was monitored daily. Once the larvae had died, they were transferred to a White trap to allow the EPNs to emerge. The emerging nematodes were collected and transferred into a 50 mL culture flask. The nematodes were cleaned by repeatedly rinsing with sterile distilled water to remove any debris or contaminants. The cleaned EPNs, suspended in approximately 5–10 mL of sterile water, were stored in the culture flask at 13°C.

Identification of entomopathogenic nematodes

DNA extraction of EPNs

Genomic DNA of entomopathogenic nematodes (EPNs) was isolated using the Phire Tissue Direct PCR Master Mix Kit (Thermo Scientific, USA), in accordance with the manufacturer's protocol. About 200–500 infective juveniles (IJs) were transferred into a microcentrifuge tube and kept at -20°C until further processing. The nematodes were then mixed with 20 µL of dilution buffer, followed by the addition of 0.5 µL of DNA release solution. The sample was thoroughly homogenized using a sterile 1 mL pipette tip. After homogenization, the tube was briefly spun down and incubated in a water bath at 95°C for 5 minutes. It was then centrifuged at 12,000 × g for 5 minutes.

The resulting supernatant, containing the genomic DNA, was used as a template for polymerase chain reaction (PCR).

Polymerase chain reaction for EPNs

Polymerase chain reaction or PCR was carried out using the Phire Tissue Direct PCR Master Mix kit (Thermo Scientific, USA) in a thermal cycler (Applied Biosystems, Pittsburgh, PA, USA). Partial fragments of the ITS region (1,000–1,100 bp), 28S rDNA (870 bp), and D2/D3 regions were amplified for both *Heterorhabditis* and *Steinernema* species. The primers used for amplifying 28S rDNA in this study were 539_F 5'-GGATTCCTTAGTAACTGCGAGTG-3' and 535_R 5'TAGTCTTTCGCCCCCTATACCCTT- 3', for ITS were TW81_F 5'-GTTTCCGTAGGTGAACCTGC-3' and AB28_R 5'-ATATGCTTAAGTTCAGCGGGT-3' and D2/D3 were D2_F 5'-CCTTAGTAACGGCGAGTGAAA-3' and 536_R 5'-CAGCTATCCTGAGGAAAC-3'. The nucleotide primers used are listed in Table 7, and the PCR conditions and thermal cycling parameters are provided in Tables 8 and 9, respectively. The amplified products were analyzed by electrophoresis on a 1.2% agarose gel run at 100 volts for 30 minutes. The gel was stained with ethidium bromide (EtBr) for 10 minutes, rinsed with distilled water for 15 minutes, and DNA bands were visualized under the UV light.

Table 7 Primer for identification of *Steinernema* and *Heterorhabditis*

Genus of Entomopathogenic nematode	Gene	Primer
<i>Steinernema</i>	28S	539_F 5'-GGATTCCTTAGTAACTGCGAGTG-3' 535_R 5'TAGTCTTTCGCCCCCTATACCCTT- 3'
<i>Heterorhabditis</i> , <i>Steinernema</i>	ITS	TW81_F 5'-GTTTCCGTAGGTGAACCTGC-3' AB28_R 5'-ATATGCTTAAGTTCAGCGGGT-3'
<i>Steinernema</i>	D2/D3	D2_F 5'-CCTTAGTAACGGCGAGTGAAA-3' 536_R 5'-CAGCTATCCTGAGGAAAC-3'

Table 8 PCR reagent components used for amplifying *Steinernema* and *Heterorhabditis*

Reagent (concentration)	Volume (μL)
Phire Tissue MasterMix (2X)	15
Forward primer (5 μM)	1.2
Reward primer (5 μM)	1.2
DNA (20-200 ng)	1.8
Distilled water	10.8
Total Amount	30

Table 9 Thermal cycling conditions for amplifying entomopathogenic nematodes

Parameter	Temperature	Time	
Initial denature	98°C	5 min	
Denature	98°C	5 sec	
Annealing	<i>Steinernema</i> : 55°C <i>Heterorhabditis</i> : 50°C	5 sec	30 cycles
Extension	72°C	30 sec	
Final extension	72°C	1 min	

DNA purification and sequencing for Entomopathogenic nematodes

Purification of the amplified PCR products was carried out using the NucleoSpin® Gel and PCR Clean-up kit, following the manufacturer's instructions. A total of 29 μl of PCR product was mixed with 58 μl of NTI buffer. The mixture was then applied to a NucleoSpin® Gel and PCR Clean-up column placed in a 2 ml collection tube and centrifuged at $11,000 \times g$ for 30 seconds. The flow-through was discarded, and the column was returned to the same collection tube. Next, 700 μl of NT3 buffer was added to the column and centrifuged again at $11,000 \times g$ for 30 seconds. After discarding the flow-through, a final centrifugation at $11,000 \times g$ for 1 minute was performed to remove any residual NT3 buffer. The column was then transferred to a

new 1.5 ml microcentrifuge tube, and 20 µl of sterile water was added. Following a 1-minute incubation at room temperature, the tube was centrifuged at $11,000 \times g$ for 1 minute to elute the purified DNA. To verify the purified PCR products, 1 µl was loaded into a 1.2% agarose gel and subjected to electrophoresis at 100 volts for 30 minutes. The gel was stained with ethidium bromide, rinsed with distilled water, and examined under UV light to detect the DNA fragments. The confirmed purified products were then submitted to Macrogen, Inc. in South Korea for sequencing.

Sequence analysis of entomopathogenic nematodes

To identify EPN species, the sequences of ITS, 28S rDNA and D2/D3 were checked and edited with SeqManII (DNASTAR inc., Wisconsin, USA). The edited sequences were compared with known sequences in NCBI database using a BLASTN search (<http://www.ncbi.nlm.nih.gov/blast/Blast.cgi>). A cut-off of $\geq 97\%$ identity were considered for the same species.

Phylogenetic analysis

Alignment of the nucleotide sequences in this study with known sequences was analysed by ClustalW. Phylogenetic tree of EPNs were constructed based on maximum likelihood methods using the Tamura-Nei-parameter model, 1,000 Bootstrap replicates in MEGA Version 7.0 (Kumar et al., 2016).

Ultrastructural study of EPNs by scanning electron microscope

For scanning electron microscopy (SEM), the IJs of *Steinernema* and *Heterorhabditis* were washed five times in sterile PBS for 3 minutes (Lacey, 1997) to get rid of impurities. The nematodes were then fixed in 4% glutaraldehyde for 24h at 20°C. And they were then sent to Faculty of Science, Burapha University to continue process for SEM. After fixation, the nematodes were washed three times in PBS for 15 minutes. Dehydration was used a series of alcohols for 10 min (ethyl alcohol: 30, 50, 70, 90, and 100%). After that the alcohol was changed to amyl acetate (ethyl alcohol: amyl acetate, 25: 75, 50: 50, 75: 25, 100% amyl acetate), 10 minutes each turn. The samples were dried at the critical point and coated with a 30 nm layer of gold palled. The samples were observed in scanning electron microscope at the voltage of 15 kV (Skrzypek et al., 2004).

Isolation of *Photorhabdus* and *Xenorhabdus* from entomopathogenic nematodes

Photorhabdus and *Xenorhabdus* bacteria were isolated from the hemolymph of dead *G. mellonella* larvae infected with the IJs of EPNs. The dead *G. mellonella* larvae were washed with sterilized water and they were surface sterilized by dipping into absolute ethanol for 1 minute. The larvae were dried on a sterile petri dish. The third segment from the head of *G. mellonella* larvae were teared by sterile forceps. The hemolymph on the teared area of dead larvae were picked up by sterile loop and streaked on nutrient agar supplemented with bromothymol blue and triphenyl-2,3,5-tetrazolium chloride (NBTA). The NBTA plates were sealed with parafilm and incubated at dark in room temperature (25-28 °C) for 4 days. Preliminary identification based on colony morphology was conducted by observing colony color: *Photorhabdus* displayed green colonies, while *Xenorhabdus* formed blue colonies (Thanwisai, 2012).

Identification of *Photorhabdus* and *Xenorhabdus*

DNA extraction of symbiotic bacteria

A single colony from each symbiotic bacterial isolate was transferred into a 15 ml centrifuge tube containing 5 ml of Luria-Bertani (LB) broth. The cultures were incubated at room temperature with shaking at 150 rpm for 18–24 hours. After incubation, the cultures were spun at $12,000 \times g$ for 1 minute to harvest the bacterial pellets. Genomic DNA was then extracted from the pellets using the Genomic DNA Mini Kit (Blood/Cultured Cell) provided by Geneaid Biotech Ltd., Taiwan. The pellets were mixed with 200 μ l of GB buffer, vortexed thoroughly, and incubated at room temperature for 5 minutes. Next, 20 μ l of Proteinase K was added, and the mixture was incubated at 60°C for 8 minutes. Afterward, 200 μ l of GB buffer was added and incubated at 70°C for 10 minutes. During this time, 50 μ l of elution buffer per sample was pre-heated to 70°C. Following incubation, 200 μ l of absolute ethanol was added to the lysate and the entire mixture was transferred to a GD column. The column was centrifuged at $12,000 \times g$ for 2 minutes, and the flow-through was discarded. The column was placed into a new collection tube, and 400 μ l of W1 buffer was added. After centrifugation at $12,000 \times g$ for 30 seconds, the flow-through was discarded. Next, 600 μ l of Wash Buffer containing ethanol was added to the column, followed by centrifugation at $12,000 \times g$ for 30 seconds. The flow-through was discarded, and the

column was spun again at $12,000 \times g$ for 3 minutes to completely dry the matrix. The dried column was then transferred to a clean 1.5 ml microcentrifuge tube, and 50 μ l of pre-heated elution buffer was added. The setup was allowed to stand for at least 3 minutes to facilitate complete absorption. Finally, the column was centrifuged at $12,000 \times g$ for 30 seconds to elute the purified genomic DNA, which was stored at -20°C for subsequent use as PCR template.

Polymerase chain reaction for symbiotic bacteria

Polymerase chain reaction (PCR) was conducted using the EconoTaq® PLUS 2X Master Mix (Lucigen, USA) in a thermal cycler (Applied Biosystems, Pittsburgh, PA, USA). A partial fragment of the *recA* gene (approximately 890 bp) from *Photorhabdus* and *Xenorhabdus* species was amplified using the primer pair: *recA_F* (5'-GCTATTGATGAAAATAAACA-3') and *recA_R* (5'-RATTTTTRTCWCCRTTTRTAGCT-3'). The composition of the PCR reaction mixture and the thermal cycling conditions are detailed in Tables 10 and 11, respectively. The resulting PCR products were analyzed by electrophoresis on a 1.2% agarose gel at 100 volts for 30 minutes. The gel was stained with ethidium bromide (EtBr), rinsed with distilled water, and the DNA bands were visualized under ultraviolet (UV) light.

Table 10 Components of PCR reagent for amplification of symbiotic bacteria

Reagent (concentration)	Volume (μ L)
EconoTaq PLUS Master Mix (2X)	15
Forward primer (1 μ M)	1.5
Reward primer (1 μ M)	1.5
DNA (10 ng/ μ l)	1.5
Distilled water	10.5
Total Amount	30

Table 11 Thermal cycling for amplification of symbiotic bacteria

Parameter	Temperature	Time
Initial denature	94°C	5 min
Denature	94°C	1 min
Annealing	45°C	45 sec
Extension	72°C	2 min
Final extension	72°C	7 min

DNA purification and sequencing of symbiotic bacteria

Purification of the PCR products from symbiotic bacteria was carried out using the same procedure as that used for EPNs, except for using different primers for sequencing.

Sequence analysis of symbiotic bacteria

PCR products were sequenced by Macrogen Inc. (South Korea). A partially nucleotide sequences of *recA* were edited using SeqManII (DNASTAR Inc., Madison, WI, USA) and identified by comparing edited sequences with known sequences in GenBank using a BLASTN search to find a similarity organism sequences (<http://www.ncbi.nlm.nih.gov/blast/Blast.cgi>). A cut-off of 97% identity were considered for the same species.

Phylogenetic analysis of symbiotic bacteria

The nucleotide sequences from this study were aligned with known species and subspecies of *Xenorhabdus* and *Photorhabdus* using ClustalW in MEGA program Version 7.0 (Kumar et al., 2016). Maximum likelihood trees were constructed on IQ-Tree program v2.3.6 with 1,000 ultrafast bootstraps. The best-fit model selected by the IQ-Tree program for *Xenorhabdus* was TIM3e + I + G4 and TNe + I + R2 for *Photorhabdus* (Nguyen et al., 2014).

Bioassay of bacterial ethyl acetate extract against *Biomphalaria glabrata*

Rearing of *B. glabrata* (Say, 1818)

Biomphalaria glabrata snails were obtained from the Department of Helminthology, Faculty of Tropical Medicine, Mahidol university, Bangkok, Thailand.

The snails were maintained and bred in 120 L aquarium in laboratory at the department of Microbiology and Parasitology, faculty of Medical Science, Naresuan University. The aquarium filled with dechlorinated water, pH 7.0 and temperature 25 ± 3 °C under 12 h:12 h light: dark cycles. The snails were fed with fresh lettuce leaves (*Lactuca sativa* L.). Pigmented *B. glabrata* approximately 10-14 mm in size were used for experiment.

Preparation of symbiotic bacteria

Five selected *Xenorhabdus* and *Photorhabdus* isolates (Table 12) were subcultured on NBTA plate, incubated at room temperature (approximately 25-28 °C) in the dark for 4 days. A single colony of each selected isolate was cultured in a flask containing 500 ml of Tryptic Soy broth (TSB) at 28°C, 180 rpm for 72 h using a shaking incubator. To extract the crude compounds, 1000 mL of ethyl acetate was added to the 500 ml bacterial culture and mixed well. Subsequently, the mixture flask was placed at room temperature for at least 24 h. A rotary vacuum evaporator (Buchi, Flawil, Switzerland) was used to evaporated ethyl acetate from mixture to concentrate all bacterial extracts. This process was performed three times to maximize the crude ethyl acetate extract. The condensed bacterial extract of each isolate was weighted and dissolved in dimethyl sulfoxide (DMSO) to obtain different concentration: 20, 10, 5, 2.5, 1.25, 0.625 mg/mL. These stock solutions were stored in the freezer at -20°C. Before being used, each stock solution of bacterial extract was diluted in dechlorinated water to obtain final different concentrations (200, 100, 50, 25, 12.5 and 6.25 µg/mL).

Table 12 The selected isolates of *Xenorhabdus* and *Photorhabdus*

Isolate	Species
bAPY3.5_TH	<i>P. luminescens</i>
bACM14.2_TH	<i>P. akhurstii</i>
bALN19.5_TH	<i>P. laumondii</i> subsp. <i>laumondii</i>
bALN10.5_TH	<i>Xenorhabdus</i> sp.
bAST17.4_TH	<i>X. stockiae</i>

Evaluation of symbiotic bacteria against *B. glabrata*

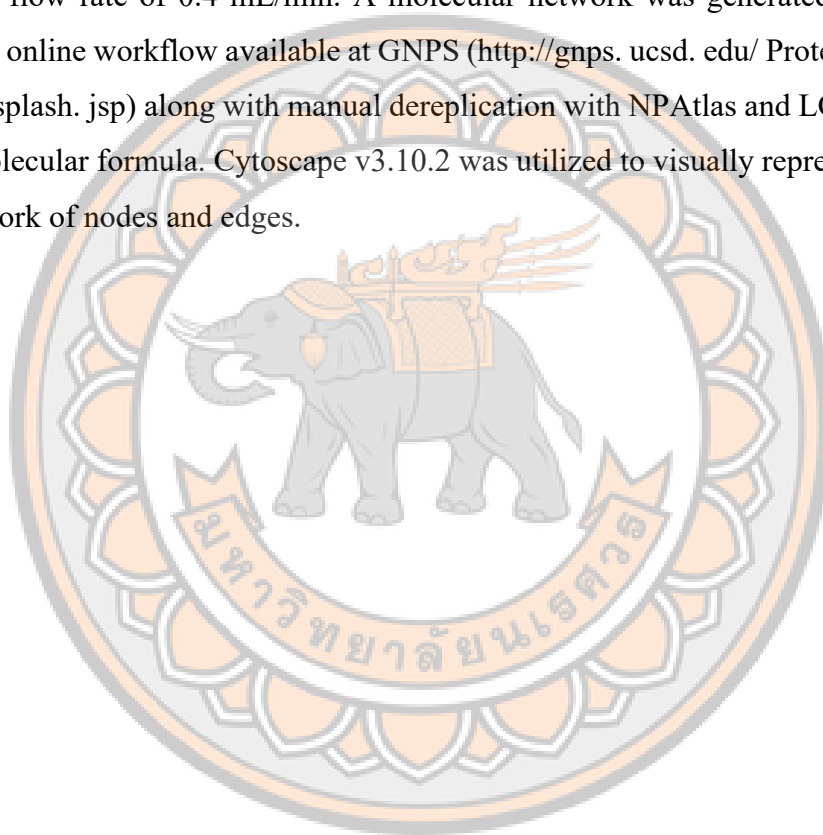
The bioassay of molluscicidal activity was determined according to the Guidelines for laboratory and field testing of molluscicides for control of schistosomiasis (World Health Organization, 2019). There were 6 final concentrations of each symbiotic bacteria extract (200, 100, 50, 25, 12.5 and 6.25 µg/mL), and 3 control groups were two negative controls (dechlorinated water and 1% DMSO) and a positive control (1.0 µg/mL niclosamide). Ten *B. glabrata* snails with shell diameters of 10 - 14 mm (Araújo et al., 2018) were set up for each concentration. The snails were exposed to 500 mL of dechlorinated water mixed with each bacterial extract in 600 mL beakers for 24 h. Subsequently, the snails were washed with dechlorinated water then placed in new beakers and fed with fresh lettuce. Monitoring the mortality of snails was performed at 24, 48, and 72 h. Snails that remain completely within their shells and show no movement were suspected to be dead. For confirmation of death, the soft tissue of snail was stimulated with a needle to determine if there was a contractile response and observed the heart movements under stereo microscope. The entire experiment was conducted in triplicate with different days. Statistical analysis of mortality rate was compared using ANOVA with Bonferroni's post hoc for multiple comparisons. The analyses were performed in GraphPad Prism10.3.1. The difference was considered statistically significant when the *P-value* was less than 0.05. The LC50 and LC90 with 95% confidence interval were calculated by regression analysis in Excel software.

Histochemical analysis

The snails were tested with LC₅₀ of all selected bacteria extract to investigate the histopathological change. After 3, 6, 12 and 24 h of exposure, snails were transferred to Davidson's Fixative for 72 h and shacked in 14% EDTA to decalcify the shell. Snail tissues were washed and dehydrated in a graded ethanol series. Dehydrated snails were cleared with xylene solution, then embedded each snail tissue in paraffin. Serial sections were cut into 5 µm thickness using rotary microtome (Leica RM2235, Wetzlar, Germany). The sections that mounted on glass slide were deparaffinized with xylene then stained with hematoxylin for 5 minutes, rinsed through tap water followed by eosin for 3 minutes. The slides with stained tissue of snail were mounted using Permount. The presence of histological alterations was observed under a light microscope (Olympus BX51, Tokyo, Japan). Images were captured by digital camera.

LC–MS/MS analysis of symbiosis bacteria extracts

The methanol crude extract of three symbiotic bacteria was prepared for analysis of the secondary metabolites. LC–MS/MS analysis was conducted using a Dionex Ultimate 3000 system coupled to an AmaZonX mass spectrometer (Bruker Daltonics). The data were obtained on a timsTOF fleX MALDI-2 with a column Acquity UPLC BEH C18 2.1 × 50 mm (solvent A = H₂O + 0.1% formic acid, solvent B = acetonitrile + 0.1% formic acid), gradient ranging from 5% to 95% over 16 min, with a flow rate of 0.4 mL/min. A molecular network was generated using the MS cluster online workflow available at GNPS (<http://gnps.ucsd.edu/Proteomics/Static/gnps-splash.jsp>) along with manual dereplication with NPAtlas and LOTUS based on the molecular formula. Cytoscape v3.10.2 was utilized to visually represent the data as a network of nodes and edges.



Chapter IV

Result

Isolation of entomopathogenic nematodes

A total of 1000 soil samples were collected from 200 sites from agriculture areas in northern Thailand. There were 565 soil samples from horticulture crops, 390 from field crops and 45 from forest. All of soils collected were loam 850 samples followed by 64, 47, 36 and 3 samples of clay, sandy loam, clay loam and sandy, respectively. The soil pH ranging between 3.8 – 7.0 and soil temperature range of 22 – 41 °C. To isolate the EPNs from soil samples, baiting technique and white-trap technique were performed. Sixty-nine soil samples showed positive for EPN with death waxworm that turned black, and red. When the death wax worms were transferred to the white trap, only fifty-one samples were positive (5.1% prevalence) for EPNs (Figure 16). Fourteen isolates were positive for the genus *Heterorhabditis* (1.4%), and 37 isolates were positive for the genus *Steinernema* (3.7%).

Most of the EPNs were isolated from loam (94.1%), followed by sandy loam (3.9%) and clay loam (2.0%). The soil parameters of the samples with EPNs were 23-39 °C (average = 30.03 °C) for temperature, 1-8 % RH (average = 4.14 % RH) for moisture, 46 - 490 meter (average = 230.25) for elevation and 4-7 (average = 6.13) for pH (Table 14).

The EPNs were mostly isolated from soil in horticulture crops (46/51), while few EPN isolates were found in soil from field crops (5/51). The horticulture crops with EPNs included vegetable gardens which most of EPNs were found. Following by dragon fruit gardens, papaya gardens, mango gardens, longan gardens, lemongrass gardens, banana plantations and butterfly pea gardens. The remaining EPNs were recovered from field crops, i.e., corn fields and Margaret flower fields. *Steinernema* were isolated from Corn fields, Vegetable garden, Dragon fruit garden, Papaya garden, Mango garden, Longan garden, Butterfly pea garden, and Lemongrass garden while *Heterorhabditis* were isolated from Corn fields, Margaret flower fields, Vegetable garden, Mango garden, Longan garden, Banana plantation, and Lemongrass garden.

Several EPN isolates were found in soil planted with longan, a tropical tree that produces edible fruit in Lamphun Province (Table 15).

Table 13 The association between soil texture and the presence of EPNs.

Soil texture	No. of soil positive for EPNs	No. of soil negative for EPNs	Total	% of EPNs positive
Loam	67	783	850	94.1
Clay	0	64	64	0
Sandy	0	3	3	0
Clay loam	1	35	36	2.0
Sandy loam	1	46	47	3.9
Total	69	931	1000	

Table 14 The association between soil parameters and the presence of EPNs.

Soil parameters	Soil samples positive for EPNs		Soil samples negative for EPNs	
	Min.–max.	Mean $\pm$ SD	Min.–max.	Mean $\pm$ SD
Temperature (°C)	23-39	30.03 $\pm$ 2.68	22-41	32.05 $\pm$ 2.68
pH	4-7	6.13 $\pm$ 0.74	3.8-7	5.32 $\pm$ 1.24
Moisture (1–8%)	1-8	4.14 $\pm$ 2.48	1-8	3.26 $\pm$ 2.64
Elevation (meter)	46 - 490	230.25 $\pm$ 135.99	45 - 490	275.23 $\pm$ 166.04

Table 15 Prevalence of entomopathogenic nematodes in agricultural areas in northern Thailand

Agricultural areas	Number of soil samples collected	Number of soil samples positive for EPNs		
		<i>Steinernema</i>	<i>Heterorhabditis</i>	EPNs prevalence (%)
Field crop				
Corn fields	105	2	1	2.8
Rice fields	90	0	0	0
Chilli fields	25	0	0	0
Pumpkin fields	10	0	0	0
Pineapple fields	25	0	0	0
Strawberry fields	5	0	0	0
Cassava plantations	45	0	0	0
Taro plantations	10	0	0	0
Sun hemp garden	10	0	0	0
Groundnut fields	20	0	0	0
Margaret flower fields	10	0	2	20
White cutter flower field	15	0	0	0
Sugarcane plantation	20	0	0	0
Horticulture crops				
Ivy gourd plantation	5	0	0	0
Vegetable garden	125	14	4	14.4
Mixed fruit garden	65	0	0	0
Marian Plum garden	10	0	0	0
Dragon fruit garden	10	1	0	10
Papaya garden	15	3	0	20
Mango garden	85	6	1	8.2
Longan garden	65	9	4	20
Jackfruit garden	10	0	0	0
Sapodilla garden	10	0	0	0
Cashew nut garden	5	0	0	0
Tamarind garden	10	0	0	0
Orange garden	5	0	0	0
Lime garden	25	0	0	0
Jujube garden	5	0	0	0
Turmeric garden	5	0	0	0

Table 15 (Cont.)

Agricultural areas	Number of soil samples collected	Number of soil samples positive for EPNs		
		<i>Steinernema</i>	<i>Heterorhabditis</i>	EPNs prevalence (%)
Banana plantation	55	0	1	1.8
Marigold garden	30	0	0	0
Butterfly pea garden	5	1	0	20
Lemongrass garden	20	1	1	10
Forest				
Teak plantation	25	0	0	0
Para rubber plantation	10	0	0	0
Betel palm plantation	10	0	0	0
Total	1000	3.7%	1.4%	5.1%

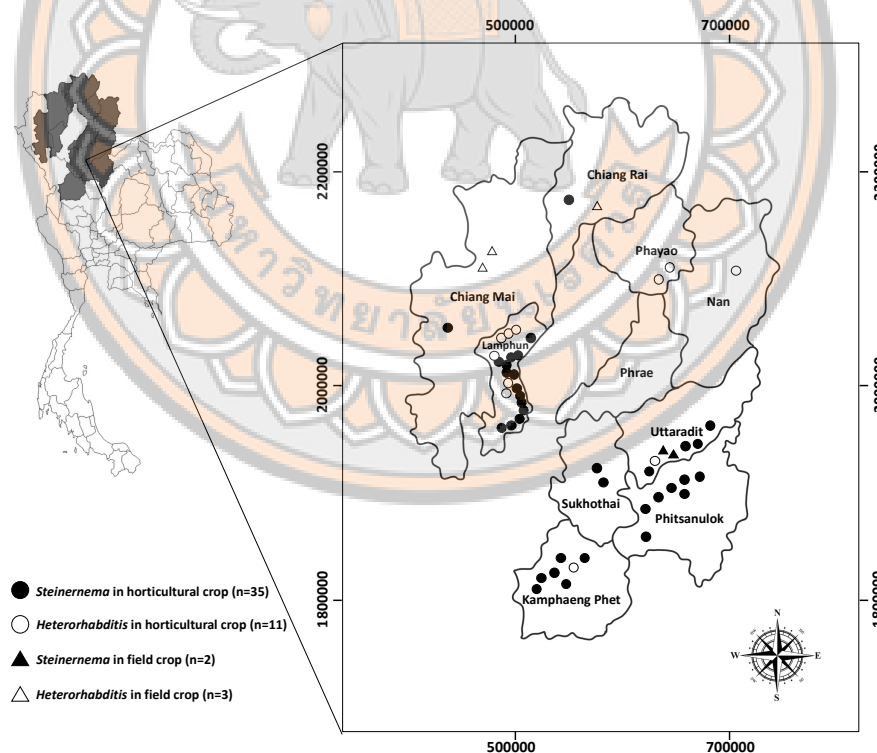


Figure 16 Map of soil collection sites in 10 province, northern Thailand. Total 1000 samples from 200 soil sites in agriculture areas were collected to search for EPNs.

Identification of entomopathogenic nematodes

Polymerase chain reaction (PCR) and DNA sequencing of a partial region of the 28S rDNA gene, D2/D3 region and an ITS were performed together with BLASTN search for identification (Table 16-19). PCR products of 28S rDNA gene, D2/D3 and ITS were approximately 870, 890 and 850 bp, respectively.

Among total 51 EPN isolates, nine EPN isolates were contaminated with fungi, and genomic DNA could not be obtained from them. Six isolates were sequenced but due to their short length and poor sequence data therefore, they were not included for analysis. Thus, 36 EPN isolates were molecularly identified with *Steinernema* (N = 27) and *Heterorhabditis* (N = 9). Based on the 28S rDNA nucleotide region, after a Basic Local Alignment Search Tool Nucleotide (BLASTN) search of 593 bp, seven *Steinernema* isolates were identified as *S. surkhetense* (accession nos. OP207742 - OP207748) with high similarity (98-100%) to *S. surkhetense* isolate CS44 (accession no. MH837096) (Table 16). Ten isolates of *Steinernema* (accession nos. OP207749 - OP207758) were analyzed based on 593 bp of the D2/D3 region. These 10 *Steinernema* isolates showed high similarities (97-99%) with 7 isolates of *S. lamjungense* (accession no. HM000102) and 3 isolates of *S. surkhetense* (accession no. MH837096) (Table 17). Moreover, ten isolates of *Steinernema* were identified as *S. surkhetense* 1 isolate (accession no. OP341541), *S. guangdongense* 3 isolates (accession nos. OP341537, OP341543 and OP341544) and *S. siamkayai* 6 isolates (accession nos. OP341535-OP341540 and OP341542) with similarity ranging from 96-100% (Table 18) after BLASTN search using 675 nucleotides of the internal transcribed spacer (ITS) region. For the genus *Heterorhabditis*, a partial region of the ITS sequences was determined for species identification. After a BLASTN search of 675 bp, 7 isolates of *Heterorhabditis* (accession nos. OP207759 - OP207764 and OP207767) and 2 isolates (accession nos. OP207765 and OP207766) showed sequence similarity to *H. indica* with 99-100% similarity (accession nos. MN922779, MW654494, KP970842, and KF247222) and *H. bacteriophora* with 99% similarity (accession no. MT135036), respectively (Table 19).

Table 16 BLASTN search of 593 bp in the partial region of 28S rDNA showed 7 isolates of *Steinernema* isolated from agricultural areas in Thailand

Code	Maximum identity to	BLASTN				
		Accession number	Total score	Query coverage	E value	Identity
eAKP16_2_TH	<i>Steinernema surkhetense</i> isolate CS44	MH837096	1020	100%	0	100.00%
eAKP19_5_TH	<i>Steinernema surkhetense</i> isolate CS44	MH837096	1013	100%	0	99.82%
eAKP20_2_TH	<i>Steinernema surkhetense</i> isolate CS44	MH837096	972	100%	0	98.22%
eAKP20_4_TH	<i>Steinernema surkhetense</i> isolate CS44	MH837096	1000	100%	0	99.46%
eAKP6_2_TH	<i>Steinernema surkhetense</i> isolate CS44	MH837096	979	100%	0	98.74%
eAST1_3_TH	<i>Steinernema surkhetense</i> isolate CS44	MH837096	1020	100%	0	100.00%
eAST17_4_TH	<i>Steinernema surkhetense</i> isolate CS44	MH837096	1020	100%	0	100.00%

Table 17 BLASTN search of 593 bp in the partial region of D2/D3 showed 10 isolates of *Steinernema* isolated from agricultural areas in Thailand

Code	Maximum identity to	BLASTN			
		Accession number	Total score	Query coverage	E value
eALN10_1_TH	<i>Steinernema lamjungense</i>	HM000102	944	100%	0
eALN10_4_TH	<i>Steinernema lamjungense</i>	HM000102	948	100%	0
eALN10_5_TH	<i>Steinernema lamjungense</i>	HM000102	948	100%	0
eALN11_5_TH	<i>Steinernema lamjungense</i>	HM000102	950	100%	0
eALN20_3_TH	<i>Steinernema surkhetense</i>	MH837096	1014	100%	0
eALN6_3_TH	<i>Steinernema surkhetense</i>	MH837096	1014	100%	0
eALN6_5_TH	<i>Steinernema surkhetense</i>	MH837096	1014	100%	0
eALN7_1_TH	<i>Steinernema lamjungense</i>	HM000102	948	100%	0
eALN7_4_TH	<i>Steinernema lamjungense</i>	HM000102	953	100%	0
eAUT20_5_TH	<i>Steinernema lamjungense</i>	HM000103	976	100%	0

Table 18 BLASTN search of 675 bp in the partial region of ITS showed 10 isolates of *Steinernema* isolated from agricultural areas in Thailand

Code	Maximum identity to	BLASTN			
		Accession number	Total score	Query coverage	E value Identity
eAPL10_3_TH	<i>Steinernema siamkayai</i>	AF331917	1064	100%	0 99.48%
eAPL10_5_TH	<i>Steinernema siamkayai</i>	AF331917	1064	100%	0 99.48%
eAPL11_5_TH	<i>Steinernema siamkayai</i>	AF331917	1053	99%	0 99.14%
eAPL12_2_TH	<i>Steinernema siamkayai</i>	AF331917	1064	100%	0 99.48%
eAPL7_2_TH	<i>Steinernema siamkayai</i>	AF331917	1064	100%	0 99.48%
eAPL7_4_TH	<i>Steinernema siamkayai</i>	AF331917	1064	100%	0 99.48%
eAUT20_2_TH	<i>Steinernema guangdongense</i> strain GDC339	AY170341	942	100%	0 96.21%
eAUT20_5_TH	<i>Steinernema guangdongense</i> strain GDC339	AY170341	937	100%	0 96.03%
eALN10_3_TH	<i>Steinernema guangdongense</i> strain GDC339	AY170341	944	100%	0 96.37%
eACM2_1_TH	<i>Steinernema surkhetense</i>	MF618312	1090	100%	0 99.83%

Table 19 BLASTN search of 637 bp in the partial region of ITS showed 9 isolates of *Heterorhabditis* isolated from agricultural areas in Thailand

Code	Maximum identity to	BLASTN			
		Accession number	Total score	Query coverage	E value Identity
eACM14_1_TH	<i>Heterorhabditis indica</i> isolate VS8	MN9222779	1027	100%	0 100.00%
eACM14_2_TH	<i>Heterorhabditis indica</i> isolate VS8	MN9222779	1027	100%	0 100.00%
eACR16_4_TH	<i>Heterorhabditis indica</i> isolate 58	MW654494	946	100%	0 99.61%
eALN18_2_TH	<i>Heterorhabditis bacteriophora</i>	MT135036	1081	100%	0 99.83%
eALN18_5_TH	<i>Heterorhabditis bacteriophora</i>	MT135036	1081	100%	0 99.83%
eANN2_5_TH	<i>Heterorhabditis indica</i> isolate EPN15	KP970842	1026	100%	0 100.00%
eAPY3_4_TH	<i>Heterorhabditis indica</i> isolate 58	MW654494	955	100%	0 99.81%
eAPY3_5_TH	<i>Heterorhabditis indica</i> isolate VS8	MN9222779	1022	100%	0 99.82%
eAUT13_2_TH	<i>Heterorhabditis indica</i> isolate C1CR-BBFN Warud	KF247222	1027	100%	0 100.00%

Phylogenetic analysis of entomopathogenic nematodes

A phylogenetic tree was created based on the maximum likelihood method using the Tamura-Nei parameter and 1000 bootstrap replicates. *Caenorhabditis elegans* (*C. elegans*) was used as the out-group. According to the phylogenetic tree created from sequences of 28S rDNA region, seven isolates in the present study and 16 sequences of *Steinernema* downloaded from the National Center for Biotechnology Information (NCBI) database, the phylogenetic tree showed that 7 isolates were grouped into *S. surkhetense* (accession no. MF621005) (Figure 17). The phylogenetic tree of the D2/D3 region divided *Steinernema* isolates in the present study into 2 groups. Group 1 contained 7 isolates of *Steinernema* in this study and *S. lamjungense* (accession no. MH000102), and Group 2 contained 3 *Steinernema* isolates in this study together with *S. surkhetense* (accession no. MF621005) (Figure 18). The maximum likelihood tree of the ITS region revealed 3 groups of *Steinernema* isolates in this study. Group 1 contained 6 isolates of *Steinernema* in this study and *S. siamkayai* (accession no. JN571085). Group 2 contained only 1 isolate of *Steinernema* in this study together with *S. surkhetense* (accession no. MF621005). Moreover, 3 *Steinernema* isolates (Group 3) were closely related to *S. guangdongense* (accession no. AY170341) (Figure 19). The phylogenetic tree of *Heterorhabditis* based on the ITS region was divided into 3 groups. Group 1 (5 isolates) was closely related to *H. indica* (accession no. KF247222). Group 2 contained 2 isolates that were grouped into *H. bacteriophora* (accession no. EU598237), and two isolates of Group 3 were closely related to *H. indica* (accession no. MW654494) (Figure 20).

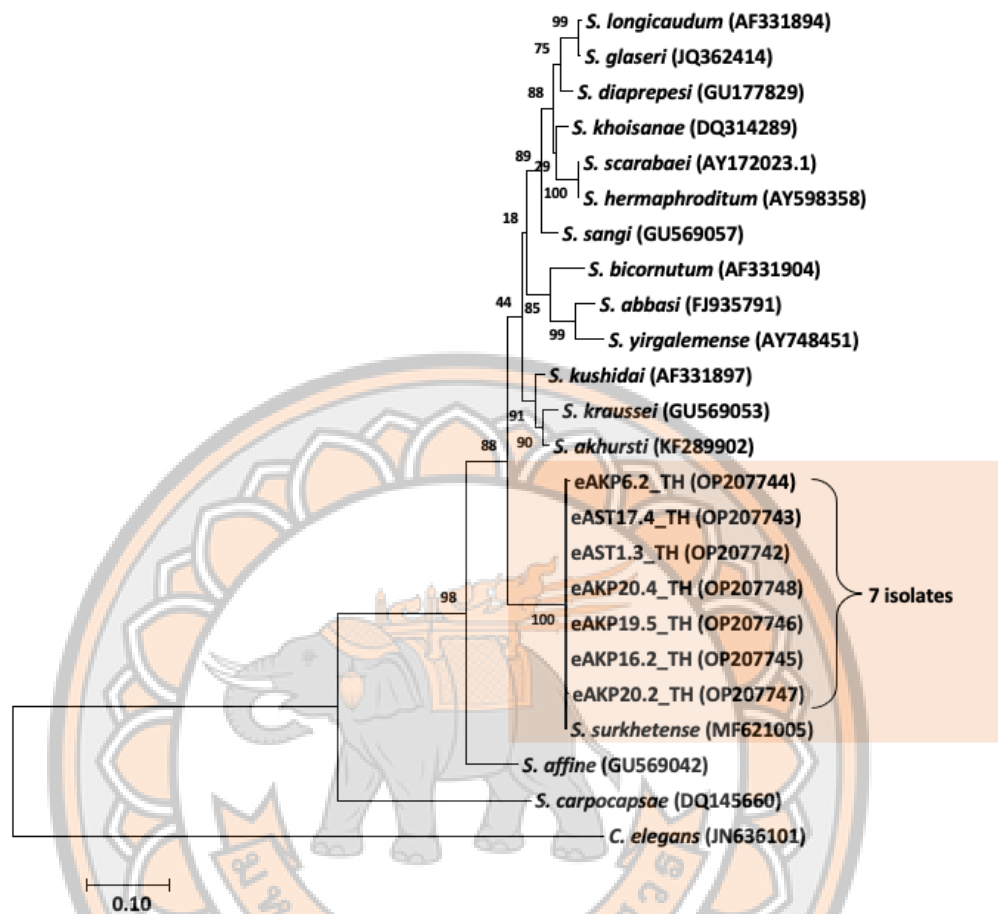


Figure 17 The phylogeny of *Steinernema* (maximum likelihood method, Tamura-Nei-parameter, 1000 Bootstrap replicates) based on 593 nucleotides of a partial region of 28S rDNA gene from 7 *Steinernema* isolates in the present study together with several *Steinernema* species retrieved from the NCBI database. *Caenorhabditis elegans* (*C. elegans*) was used as the out-group.

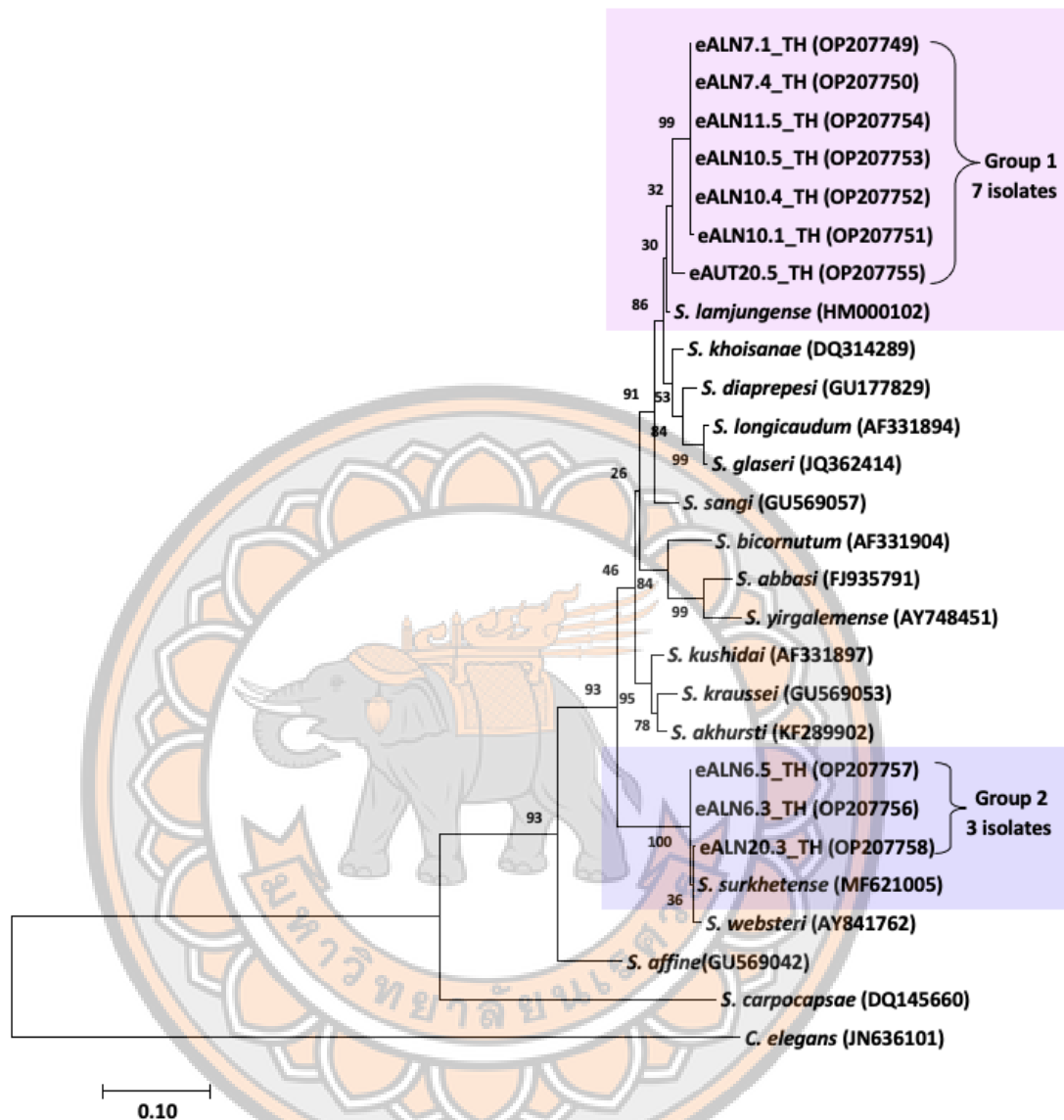


Figure 18 The phylogeny of *Steineria* (maximum likelihood method, Tamura-Nei-parameter, 1000 Bootstrap replicates) based on 593 nucleotides of a partial region of D2/D3 region from 10 *Steineria* isolates in the present study together with several *Steineria* species retrieved from the NCBI database. *Caenorhabditis elegans* (*C. elegans*) was used as the out-group.

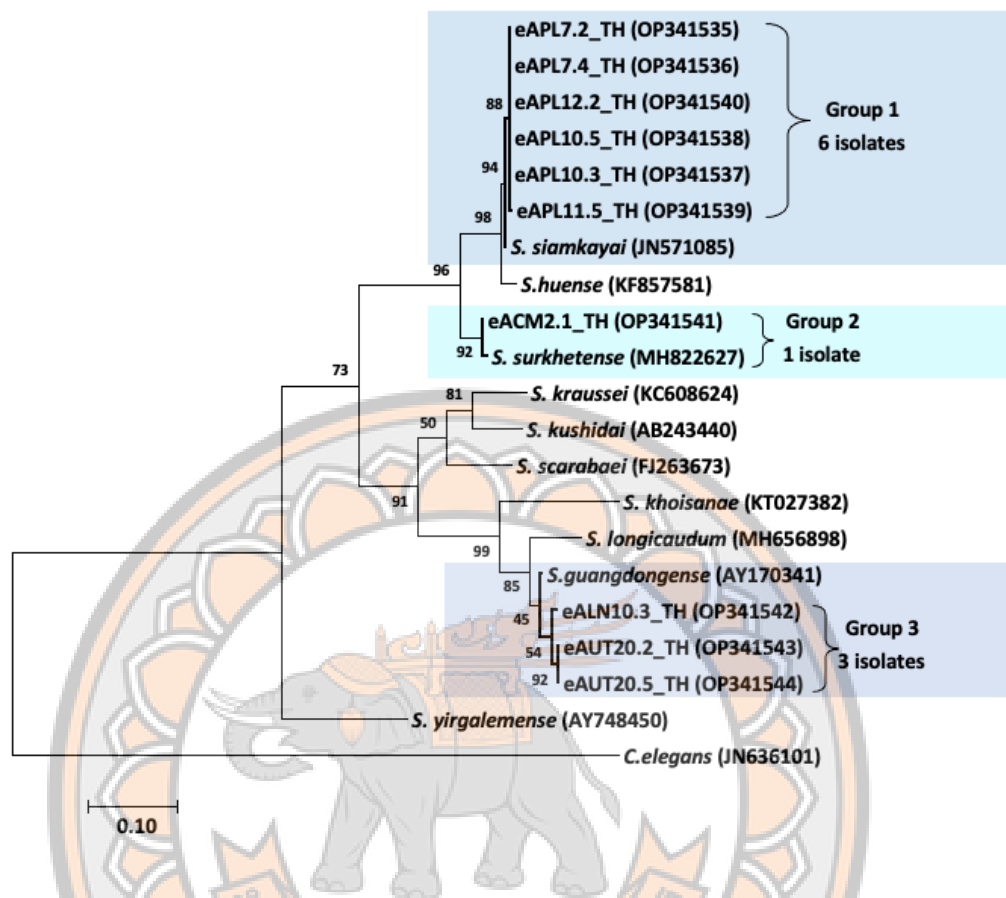


Figure 19 The phylogeny of *Steinernema* (maximum likelihood method, Tamura-Nei-parameter, 1000 Bootstrap replicates) based on 675 nucleotides of a partial region of ITS gene from 10 *Steinernema* isolates in the present study together with several *Steinernema* species retrieved from the NCBI database. *Caenorhabditis elegans* (*C. elegans*) was used as the out-group.

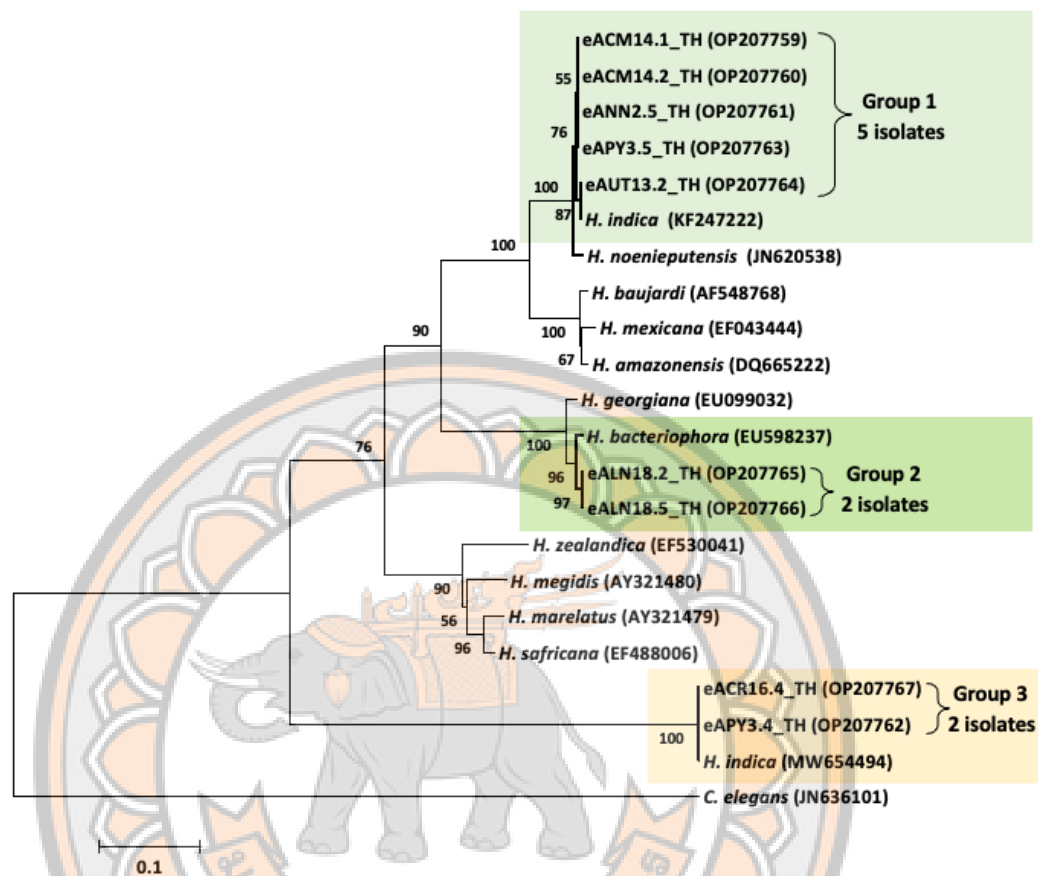


Figure 20 The phylogeny of *Heterorhabditis* (maximum likelihood method, Tamura-Nei-parameter, 1000 Bootstrap replicates) based on 637 nucleotides of a partial region of ITS gene from 9 *Heterorhabditis* isolates in the present study together with several *Heterorhabditis* species retrieved from the NCBI database. *Caenorhabditis elegans* (*C. elegans*) was used as the out-group.

Isolation of *Xenorhabdus* and *Photorhabdus*

Xenorhabdus and *Photorhabdus* bacteria were isolated from the hemolymph of the dead *G. mellonella* infected with EPNs. Hemolymph was streaked on NBTA agar to preliminary identify the symbiotic bacteria at the genus level. Based on colony morphology on NBTA agar, nineteen isolates of *Xenorhabdus* showed dark blue colonies on NBTA, whereas 12 isolates of *Photorhabdus* showed were light or dark green colonies on NBTA surface (Figure 21).

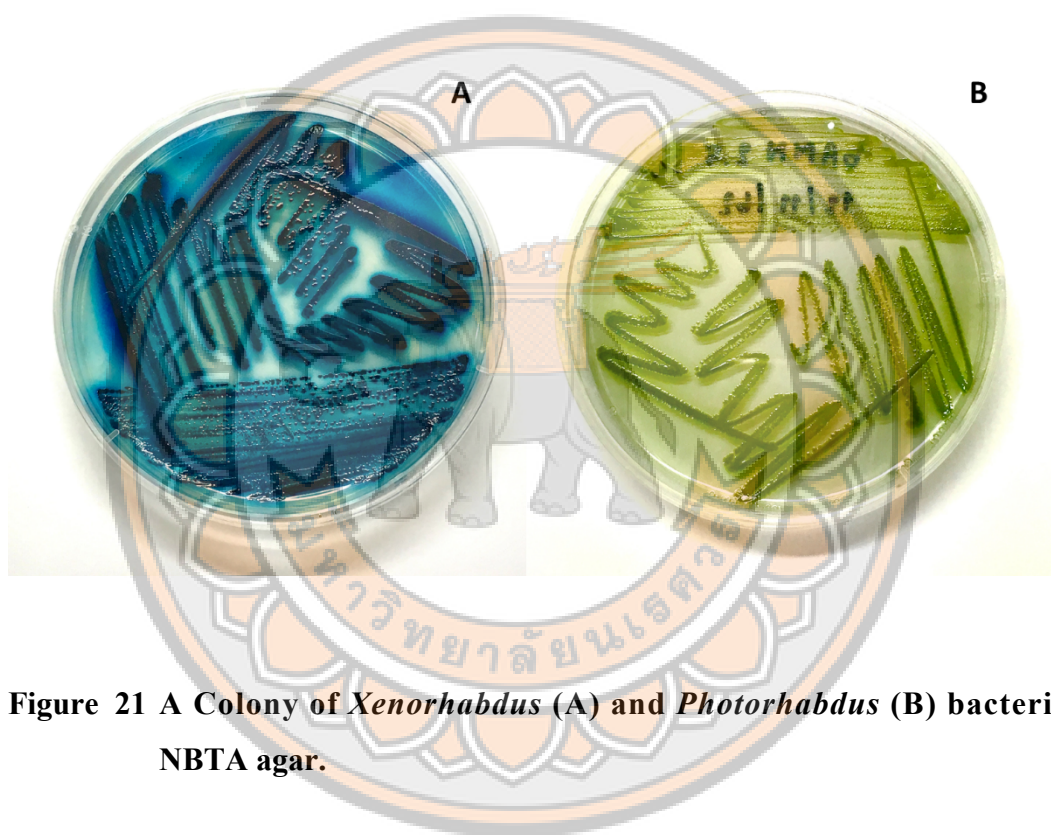


Figure 21 A Colony of *Xenorhabdus* (A) and *Photorhabdus* (B) bacteria on NBTA agar.

Identification of *Xenorhabdus* and *Photorhabdus*

A single colony of *Xenorhabdus* and *Photorhabdus* was performed for DNA extraction. Then PCR analysis of the *recA* gene sequence was amplified. PCR products had a range approximately 900 bp in size.

A total 31 isolates of symbiotic bacteria were identified as 19 and 12 isolates of *Xenorhabdus* (GenBank accession nos. OR506420 - OR506439) and *Photorhabdus* (GenBank accession nos. OR506440 - OR506451), respectively (Table 20-21). BLASTN search of *recA* sequences revealed that six isolates of *Xenorhabdus* showed sequence similarity to *X. stockiae* strain CS33 at above 99% identity. Three *Xenorhabdus* isolates were closely related to *X. stockiae* strain TH01, and another one isolate showed sequence similarity to *X. stockiae* strain 858516. The other 2 of *Xenorhabdus* isolates in this study showed sequence similarity to *X. griffinae* strain ID10 with 99.53 and 99.69 % identity. One isolate of *Xenorhabdus* was closely related to *X. indica* strain SabOM with 96.71% identity. The remaining 6 isolates of *Xenorhabdus* showed sequence similarity ranging from 95.77 - 96.08 % to *X. ehlersii* strain DSM16337 (Figure 22). Twelve isolates of *Photorhabdus* were identified as *P. luminescens* which included 4 isolates showed sequence similarity to *P. akhurstii*. Three *Photorhabdus* isolates were similar to *P. laumondii* subsp. *laumondii* and the other 4 isolates were closely related to *P. luminescens* strain C8404 (Figure 23). The sequences of *Photorhabdus* showed sequence similarity ranged from 98.50 – 100.00 %.

Table 20 BLASTN search of 654 bp in the partial region of *recA* showed 19 isolates of *Xenorhabdus* isolated from EPNs in agricultural areas in Thailand

Code	Maximum identity to	BLASTN			
		Accession number	Total score	Query coverage	E value Identity
bALN7_2_TH	<i>Xenorhabdus stockiae</i> strain CS33	MK401900	1157	100%	0 99.37%
bAPL10_3_TH	<i>Xenorhabdus stockiae</i> strain CS33	MK401900	1151	100%	0 99.22%
bAPL10_4_TH	<i>Xenorhabdus stockiae</i> strain CS33	MK401900	1162	100%	0 99.53%
bAST17_4_TH	<i>Xenorhabdus stockiae</i> strain CS33	MK401900	1151	100%	0 99.22%
bAUT12_4_TH	<i>Xenorhabdus stockiae</i> strain CS33	MK401900	1162	100%	0 99.53%
bAKP19_5_TH	<i>Xenorhabdus stockiae</i> strain CS33	MK401900	1157	100%	0 99.37%
bALN12_1_TH	<i>Xenorhabdus stockiae</i> strain TH01	FJ823425	1140	100%	0 98.90%
bALN20_3_TH	<i>Xenorhabdus stockiae</i> strain TH01	FJ823425	1151	100%	0 99.22%
bACM2_1_TH	<i>Xenorhabdus stockiae</i> strain TH01	FJ823425	1040	100%	0 96.08%
bACR12_1_TH	<i>Xenorhabdus stockiae</i> strain 858516	JX485977	1134	100%	0 98.75%
bAUT15_5_TH	<i>Xenorhabdus indica</i> strain SabOM	FJ536262	1062	100%	0 96.71%
bAKP19_4_TH	<i>Xenorhabdus griffinae</i> strain ID10	FJ823399	1168	50%	0 99.53%
bAUT20_2_TH	<i>Xenorhabdus griffinae</i> strain ID10	FJ823399	1168	100%	0 99.69%

Table 20 (Cont.)

Code	Maximum identity to	BLASTN				
		Accession number	Total score	Query coverage	E value	Identity
bALN1_1_TH	<i>Xenorhabdus ehlersii</i> strain DSM16337	FJ823398	1035	100%	0	95.92%
bALN10_3_TH	<i>Xenorhabdus ehlersii</i> strain DSM16337	FJ823398	1040	100%	0	96.08%
bALN10_5_TH	<i>Xenorhabdus ehlersii</i> strain DSM16337	FJ823398	1040	100%	0	96.08%
bALN11_5_TH	<i>Xenorhabdus ehlersii</i> strain DSM16337	FJ823398	1035	100%	0	95.92%
bALN6_3_TH	<i>Xenorhabdus ehlersii</i> strain DSM16337	FJ823398	1031	100%	0	95.77%
bALN7_1_TH	<i>Xenorhabdus ehlersii</i> strain DSM16337	FJ823398	1035	100%	0	95.92%

Table 21 BLASTN search of 601 bp in the partial region of *recA* showed 12 isolates of *Photorhabdus* isolated from EPNs in agricultural areas in Thailand.

Code	Maximum identity to	BLASTN			
		Accession number	Total score	Query coverage	E value Identity
bACM14_2_TH	<i>Photorhabdus akhurstii</i>	LN835348	1085	99%	0 99.33%
bAKP14_1_TH	<i>Photorhabdus akhurstii</i>	LN835348	1077	99%	0 99.17%
bALN9_3_TH	<i>Photorhabdus akhurstii</i>	LN835348	1079	99%	0 99.17%
bALN3_2_TH	<i>Photorhabdus akhurstii</i>	LN835348	1061	99%	0.0 99.32%
bAUT13_2_TH	<i>Photorhabdus akhurstii</i>	LN835348	1107	99%	0 100.00%
bALN14_4_TH	<i>Photorhabdus luminescens</i> strain C8404	FJ862004	1072	100%	0 98.84%
bALN9_1_TH	<i>Photorhabdus luminescens</i> strain C8404	FJ862004	1072	100%	0 98.84%
bANN2_5_TH	<i>Photorhabdus luminescens</i> strain C8404	FJ862004	1072	100%	0 98.84%
bAPY3_5_TH	<i>Photorhabdus luminescens</i> strain C8404	FJ862004	1072	100%	0 98.84%
bALN18_2_TH	<i>Photorhabdus laumondii</i> strain E21	FJ861999	1061	100%	0 98.50%
bALN18_5_TH	<i>Photorhabdus laumondii</i> strain E21	FJ861999	1061	100%	0 98.50%
bALN19_5_TH	<i>Photorhabdus laumondii</i> strain E21	FJ861999	1061	100%	0 98.50%

Phylogenic analysis of *Xenorhabdus* and *Photorhabdus*

The phylogenetic tree based on the maximum likelihood method exhibited 4 groups of *Xenorhabdus*. Group 1 contained ten isolates of *Xenorhabdus* in this study, which were clustered with *X. stockiae* strain TH01 (accession no. FJ823425). Group 2 contained only one isolate, which was grouped with *X. indica* strain SabOM (accession no. FJ536262). Group 3 contained two isolates of *Xenorhabdus* in this study together with *X. griffiniae* strain ID10 (accession no. FJ823399) received from the NCBI database. Group 4 contained six isolates of *Xenorhabdus* in this study, which were clustered with *X. ehlersii* strain DSM16337 (accession no. FJ823398) (Figure 22). Twelve isolates of *Photorhabdus* in this study were divided into three groups. Group 1 contained five isolates of *Photorhabdus* and the referenced *P. akhurstii* strain LB06 (accession no. LN835348). Group 2 contained four isolates of *Photorhabdus* in the present study and *P. luminescens* strain C8404 (accession no. FJ862004). Group 3 was composed of three isolates of *Photorhabdus* in this study, which were closely related to *P. laumondii* subsp. *laumondii* strain E21 (accession no. FJ861999) (Figure 23).

Tree scale: 0.1

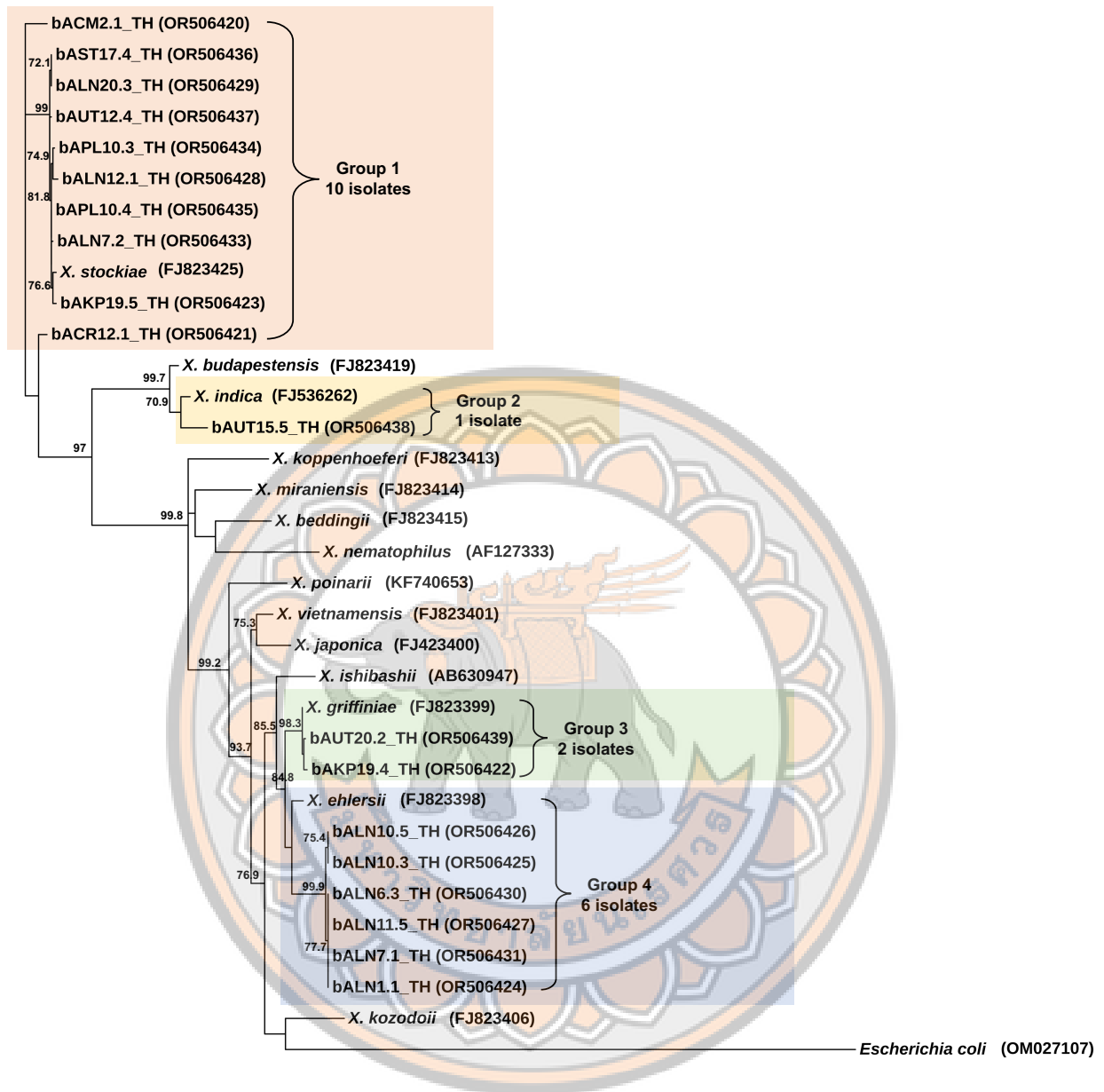


Figure 22 The phylogeny of *Xenorhabdus* (maximum likelihood method, 1000 Bootstrap replicates) based on 654 nucleotides of a partial region of *recA* gene from 19 *Xenorhabdus* isolates in the present study together with several *Xenorhabdus* species retrieved from the NCBI database. *Escherichia coli* (*E. coli*) was used as the out-group.



Figure 23 The phylogeny of *Photorhabdus* (maximum likelihood method, 1000 Bootstrap replicates) based on 601 nucleotides of a partial region of *recA* gene from 12 *Photorhabdus* isolates in the present study together with several *Photorhabdus* species retrieved from the NCBI database. *Escherichia coli* (*E. coli*) was used as the out-group.

Ultrastructural study of entomopathogenic nematodes

The morphology of the infective juvenile was observed using scanning electron microscopy (SEM) and the measurements of 20 IJs were made of the distance from the anterior end to the end of the body under light microscope (Table 22).

Heterorhabditis bacteriophora (eALN18.2_TH)

Body slender, exsheathed infective juveniles with body length 384.89 ± 26.71 μ m. The anterior end showing closed mouth. A head smooth, annulated along with tessellate pattern on cuticle. Lateral field was with 8 ridges at mid-body. The longitudinal ridges present in posterior part. The excretory pore lies in the final region. Tail is long and pointed (Figure 24).

Heterorhabditis indica (eACM14.2_TH)

Body thin, elongate and long $892.03 \pm 75.94 \mu\text{m}$. Anterior region rounded with four distinct of cephalic papillae and amphid prominent. Head section has a characteristic in the form of rings and continuous with body contour. Lateral field with 8 ridges. Tail long with pointed terminus (Figure 25).

Steinernema surkhetense (eALN6.3_TH)

The body size of IJs was $436.3 \pm 20.66 \mu\text{m}$. A head round and smooth with closed mouth. Two of four cephalic papillae prominent. Labial papillae were observed. Lateral fields with eight notorious ridges at midbody region. Tail was tapering to pointed tail end) (Figure 26).

Steinernema lamjungense (eALN11.5_TH)

The length of infective larvae is $946.57 \pm 57.97 \mu\text{m}$. Anterior end showing smooth head, labial and cephalic papillae were observed. The number of lateral ridges observed in IJs was eight. Tail elongate conoid with pointed terminus (Figure 27).



Figure 24 SEM pictures of infective juvenile *Heterorhabditis bacteriophora*. A) Anterior region showing smooth head along with tessellate pattern on cuticle; B)-C) tail with longitudinal ridges; D) Lateral field showing 8 ridges at mid-body; E-G) Entire body focusing on head and tail of IJs under LM.

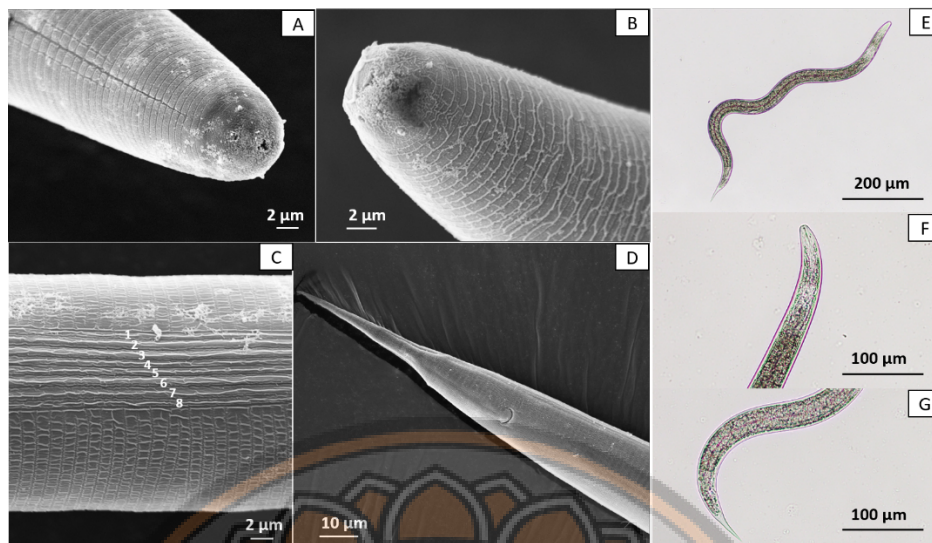


Figure 25 SEM pictures of infective juvenile *Heterorhabditis indica*. A-B) Anterior region showing cephalic papillae and amphid; C) Lateral field showing 8 ridges at mid-body; D) Tail long with pointed terminus; E-G) Entire body focusing on head and tail of IJs under LM.

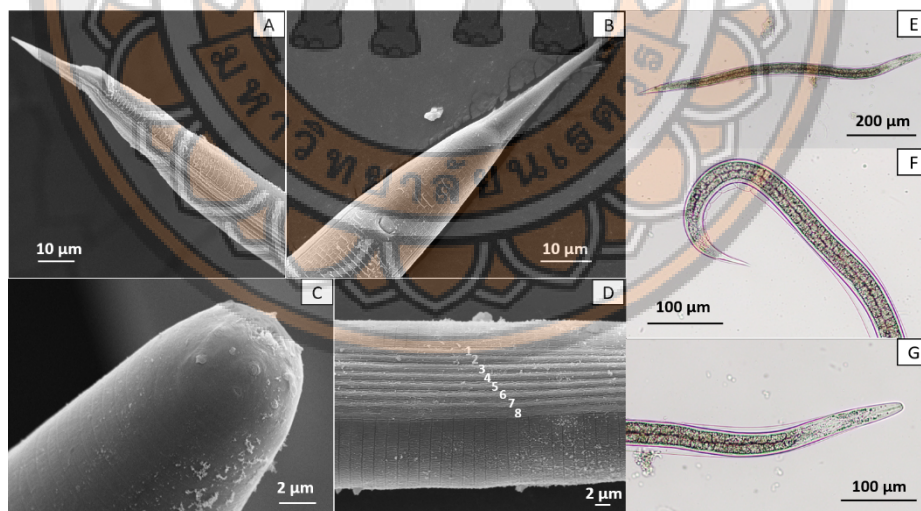


Figure 26 SEM pictures of infective juvenile *Steinernema surkhetense*. A-B) Tail was tapering to pointed tail end; C) Anterior region showing Labial papillae, cephalic papillae and amphid; D) Lateral fields with eight notorious ridges at midbody region; E-G) Entire body focusing on head and tail of IJs under LM.

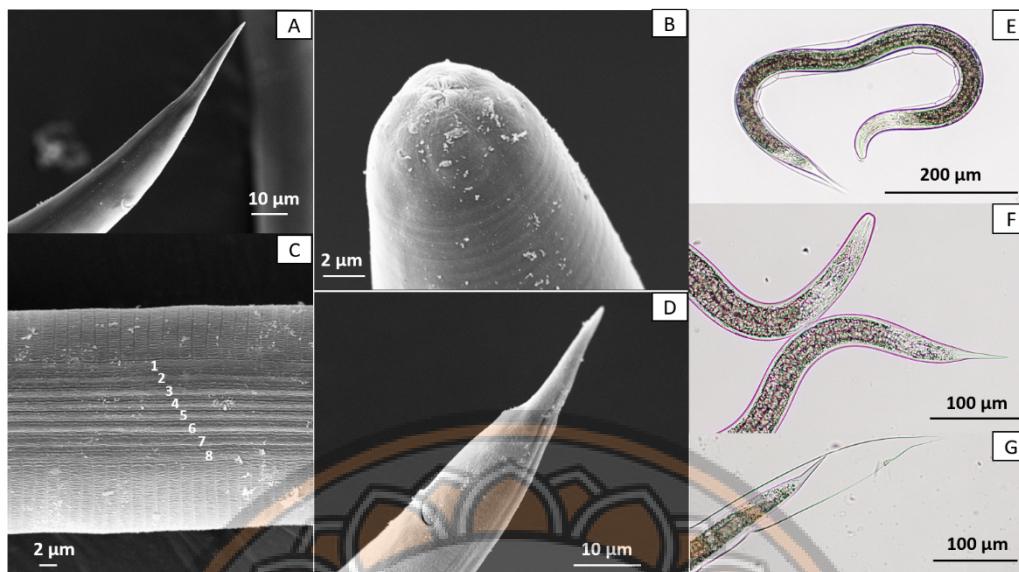


Figure 27 SEM pictures of infective juvenile *Steinernema lamjungense*. A, D) Tail was tapering to pointed tail end; B) Anterior region showing close mouth with labial papillae, cephalic papillae and amphid; C) Lateral fields with eight notorious ridges at midbody region; E-G) Entire body focusing on head and tail of IJs under LM.

Molluscicidal activity of *Photorhabdus* and *Xenorhabdus* against *B. glabrata*

Adult *B. glabrata* were exposed to crude extracts of 5 symbiotic bacteria: *X. stockiae* (bAST17.4_TH), *Xenorhabdus* sp. (bALN10.5_TH), *P. laumondii* subsp. *laumondii* (bALN19.5_TH), *P. akhurstii* (bACM14.2_TH) and *P. luminescens* (bAPY3.5_TH). The results were significant differences from the negative control for all extracts tested at concentrations 100 and 200 μg/mL (P -value < 0.001) (Table 23). All bacterial isolates showed good potential in controlling *B. glabrata* at concentrations of 200 μg/mL. Snail mortality reached 100% within 72 h. The positive control was exposed with 1% niclosamide causing 100% mortality at 24 h. Two from 300 snails of the negative controls, treated with 1% DMSO and DW, died. 100% mortality was also observed from two isolates of symbiotic bacteria (bAST17.4_TH and bALN19.5_TH) at a concentration of 100 μg/mL (Figure 28). The extract from *P. laumondii* subsp. *laumondii* (bALN19.5_TH) showed highest effectiveness for killing *B. glabrata* after 24 h exposure with LC values of 56.58 μg/mL and 92.24 μg/mL for LC₅₀ and LC₉₀,

respectively. At 72 h of exposure, the LC values were 54.52 (52.47 – 56.61) $\mu\text{g/mL}$ and 89.58 (87.35 – 91.84) $\mu\text{g/mL}$ for LC_{50} and LC_{90} , respectively. The extract from *X. stockiae* (bAST17.4) also showed high effectiveness with LC values at 72 h with 58.57 (57.18 – 60.05) $\mu\text{g/mL}$ and 94.61 (92.54 – 96.85) $\mu\text{g/mL}$ for LC_{50} and LC_{90} , respectively. The mortality rate of other isolates ranged between 73 – 86.7 % at 100 $\mu\text{g/mL}$ after 72 h. Testing 50 $\mu\text{g/mL}$ showed low activity against *B. glabrata* snail in all bacterial isolates.

Table 22 Morphometrics of four entomopathogenic nematodes infective juvenile stage. All measurements are in micrometers.

No.	EPNs species			
	eALN6.3_TH	eALN11.5_TH	eACM14.2_TH	eALN18.2_TH
	<i>S. surkhetense</i>	<i>S. lamgungense</i>	<i>H. indica</i>	<i>H. bacteriphora</i>
1	424.7	898.5	874.6	378.5
2	414	988.2	896.9	394.6
3	442.3	965.2	972.7	387.4
4	457.4	897.3	944.7	357.2
5	449.1	1023.7	937.5	408.1
6	423.9	887.2	986.9	368.7
7	438.5	975	946.1	342.3
8	412.1	964.6	894.8	386.9
9	410.3	856.5	831.3	421.9
10	456	889.5	956.3	425.1
11	483.4	997.8	810.3	360.9
12	402.9	1052.1	817.3	375.3
13	443	1003.5	1021	377
14	439.7	875.4	992.7	411.1
15	460.7	885.9	881.1	369.2
16	435	964.8	819.2	391.7
17	438	871	833	346

Table 22 (Cont.)

No.	EPNs species			
	eALN6.3_TH	eALN11.5_TH	eACM14.2_TH	eALN18.2_TH
	<i>S.</i>			
	<i>S. surkhetense</i>	<i>lamgungense</i>	<i>H. indica</i>	<i>H. bacteriphora</i>
18	445.9	978.8	741.9	360.7
19	406.2	988.9	805.8	441
20	442.9	967.5	876.5	394.3
Average	436.3	946.57	892.03	384.895
Min.	402.9	856.5	741.9	342.3
Max.	483.4	1052.1	1021	441
S.D.	20.66947915	57.97237182	75.94431547	26.71213946

Table 23 Lethal concentrations of symbiotic bacterial extract in *Biomphalaria glabrata* during 72 h.

Symbiotic bacterial	Lethal concentration and 95% CI* (µg/ml)	
	LC ₅₀	LC ₉₀
<i>P. luminescens</i> (bAPY3.5_TH)	96.40 (94.46 – 98.38)	163.56 (161.31 – 165.87)
<i>P. luminescens</i> subsp. <i>akhurstii</i> (bACM14.2_TH)	101.27 (98.83 – 103.48)	170.43 (167.63 – 172.90)
<i>P. luminescens</i> subsp. <i>laumondii</i> (bALN19.5_TH)	54.52 (52.47 – 56.61)	89.58 (87.35 – 91.84)
<i>Xenorhabdus</i> sp. (bALN10.5_TH)	101.27 (99.92 – 102.37)	170.43 (168.57 – 171.96)
<i>X. stockiae</i> (bAST17.4_TH)	58.57 (57.18 – 60.05)	94.61 (92.54 – 96.85)
* 95% confidence interval		

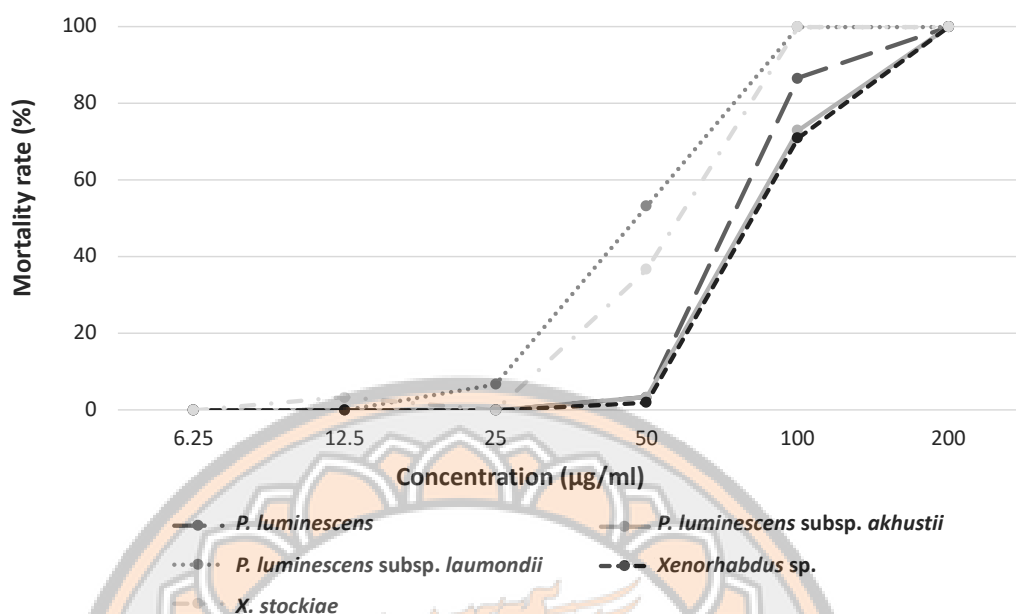


Figure 28 Mortality rates of *Biomphalaria glabrata* at different concentrations of symbiotic bacteria extract.

Histopathological change of *Biomphalaria glabrata*

Pathological changes were not observed in the head-foot region, digestive glands, and hermaphrodite glands of snails in the negative control group. The histopathological change of snail was observed after 3 h after exposure to niclosamide and *P. luminescens* subsp. *laumondii* (bALN19.5_TH). For another 4 species, histological alterations were observed after 12 h exposure.

Head-foot region

The foot of the snails was covered with columnar epithelial layer with the connective tissue layer containing mucous gland beneath. The foot was the first target of toxic activity. After exposure to bacteria extracts and niclosamide, the columnar epithelial of outer layer was ruptured, and the muscle fibers beneath epithelial layer were destructed and deformed. The empty spaces vacuoles were observed in the connective tissue. Moreover, there was the presence of dark brown pigment and the necrosis of the gland within connective tissue (Figure 29).

Digestive glands

The digestive gland of snail was lining with digestive simple columnar cells which was cylindrical in character and have basal nuclei. The tracts were covered with ciliated cells. There was no damage to cells or tissue, and no hemocytes were present. The presence of hemocytes were observed at the columnar epithelial layer in digestive gland after being treated with bacteria extracts and niclosamide. A decrease in ciliated cells was also observed. The morphology of cell and nucleus showed no differentiation after treated (Figure 30).

Hermaphrodite glands

The hermaphrodite gland of *B. glabrata* consisted of male (spermatocytes and sperms) and female (oocytes and mature ova) reproductive gametes in the vesicles known as acini. Each acinus was separated by thin vascular connective tissue. Snails treated with bacteria extracts and niclosamide caused the decreasing in number of spermatozoa and the degeneration of oocytes inside the acini when compared to control snail. The vascular connective tissue between acini was deformed. Sever necrotic changes and degeneration of acini were also observed (Figure 31).

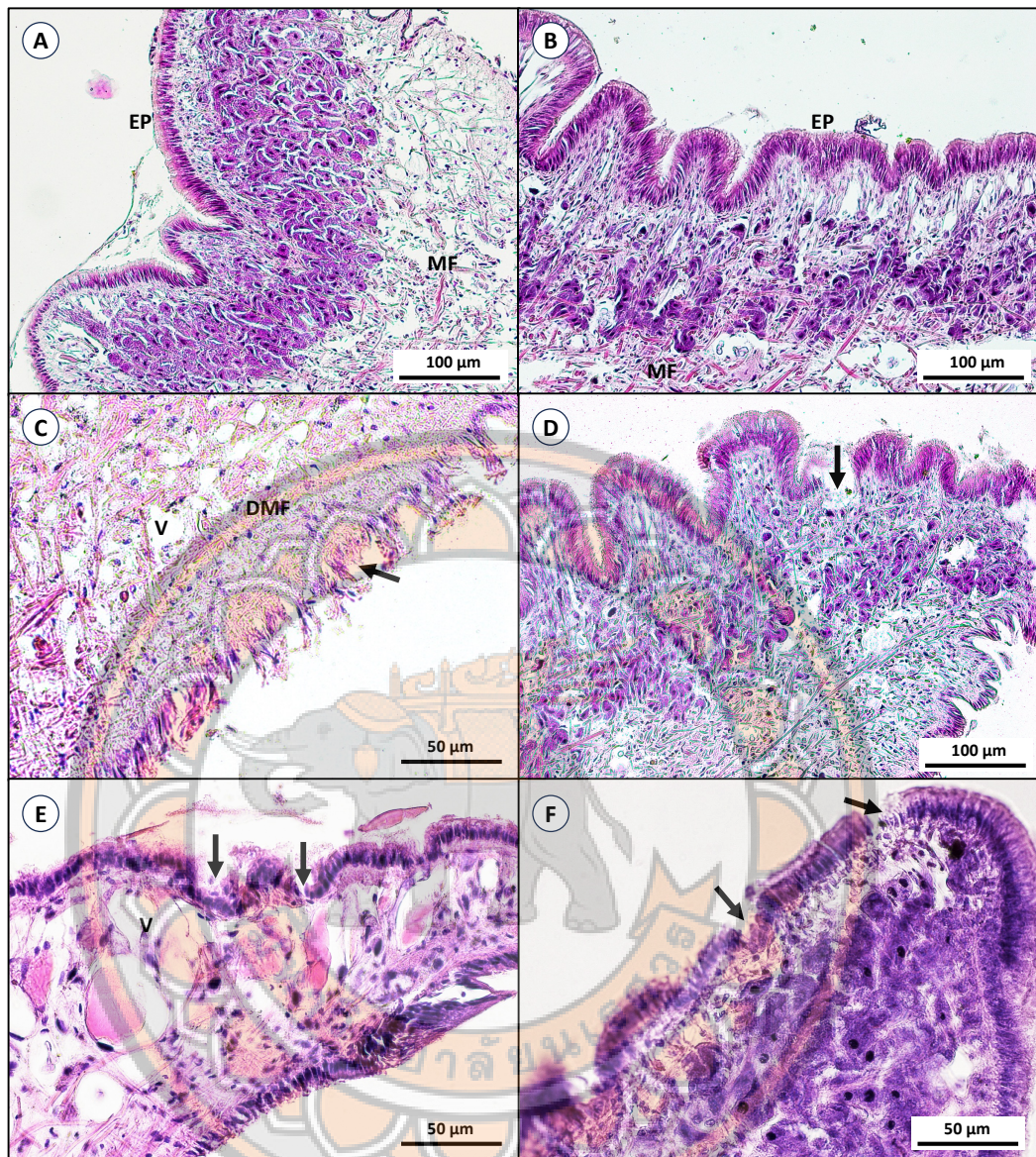


Figure 29 Histological section of *B. glabrata* head-foot region (Hematoxylin & Eosin stained); snails in the negative control exposed to dechlorinated water and DMSO showed sole of foot covered with regular columnar epithelium (EP) (A and B). Snails treated with niclosamide showed ruptured epithelial layer (arrow) and deformed muscle fiber (DMF) (C). Snail treated with LC₅₀ of bacteria extract with ruptured of epithelial layer (arrow) and empty spaces vacuoles (V) in connective tissue layer (D-F).

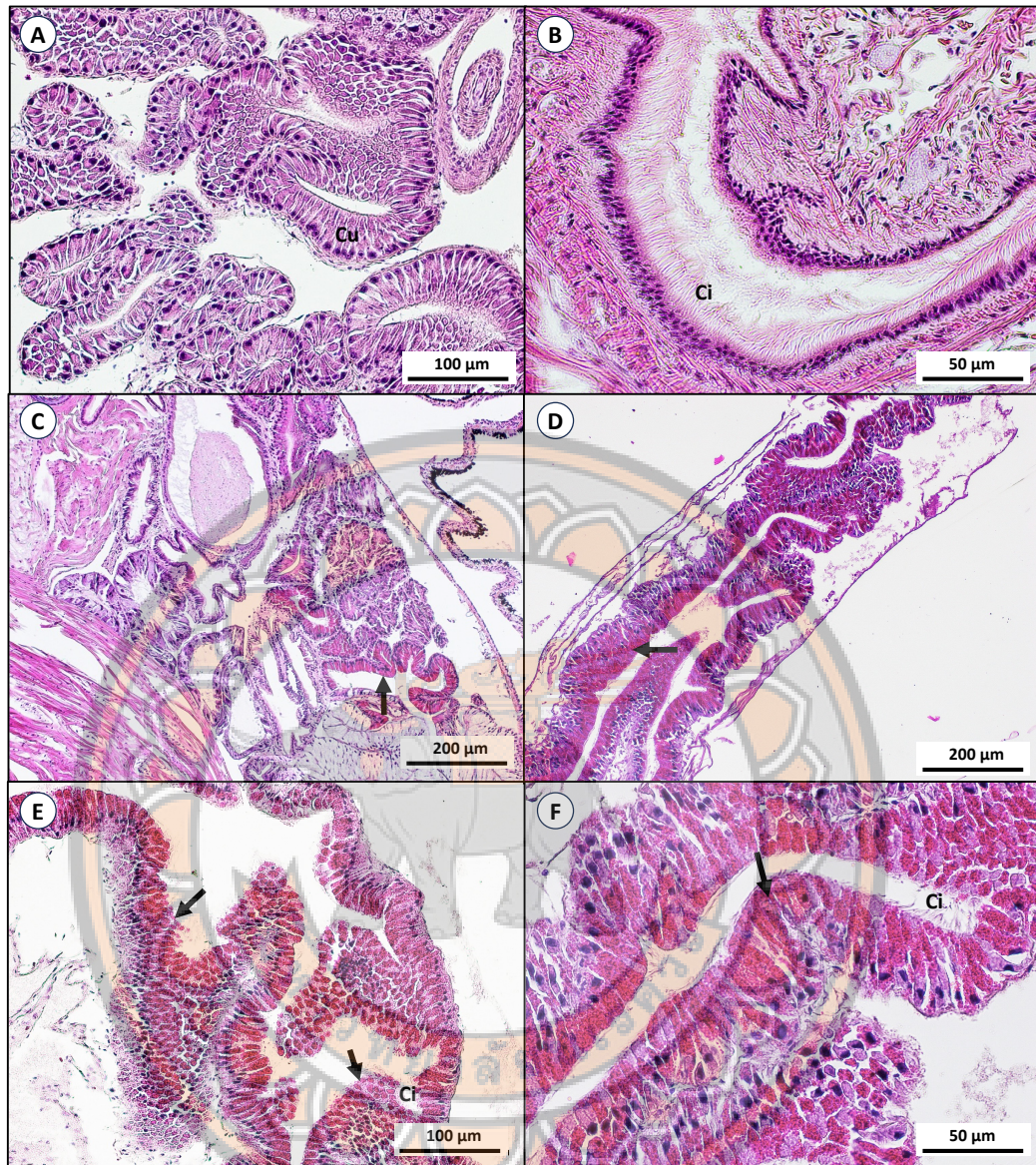


Figure 30 Histological section of *B. glabrata* digestive gland (Hematoxylin & Eosin stained); snails in the negative control with normal simple columnar cell (Cu) of digestive gland and cilia (Ci) covered in digestive tract (A, B). Snail treated with niclosamide and bacteria extract presented hemocyte (arrow) in tissue (C-F).

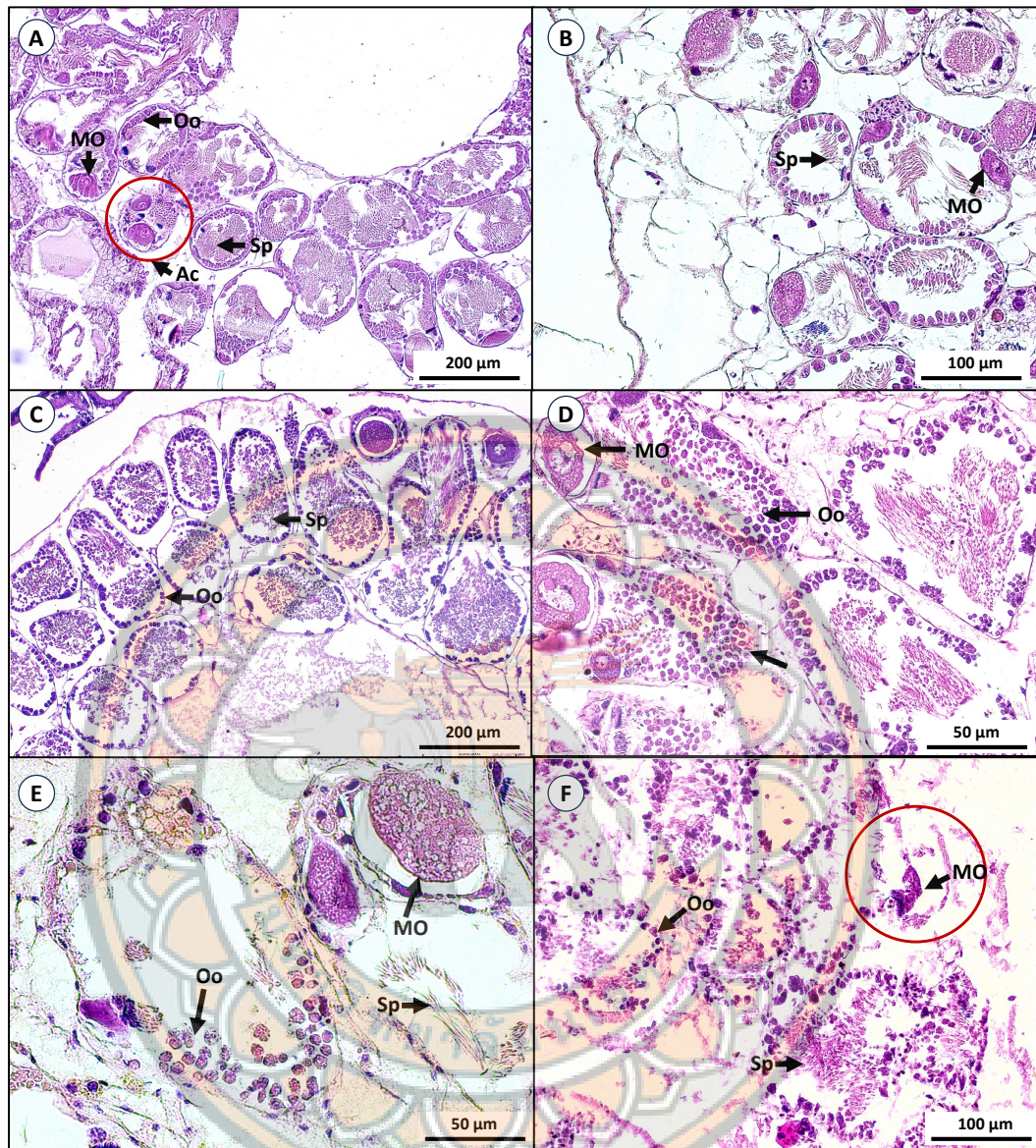


Figure 31 Histological section of *B. glabrata* hermaphrodite glands (Hematoxylin & Eosin stained); snails in the negative control with normal acini (Ac) containing plenty of spermatozoa (Sp), some oocytes (Oo) and mature ovum (MO) (A). Snail treated with niclosamide showed decreasing of spermatozoa and the degeneration of oocytes (B). Snail treated with bacteria extract showed mild (C), moderate (D) and severe affect (E, F), causing deformation and destruction in hermaphrodite gland.

Secondary metabolites in *Xenorhabdus* and *Photorhabdus* bacteria

Three bacterial isolates were submitted to liquid chromatography-tandem mass spectrometry (LC-MS/MS) to identify the secondary metabolites produced from each isolate (Table 24). *Xenorhabdus stockiae* (bAST17.4_TH) had a greater number of single nodes (355), compared to *P. luminescens* (bAPY3.5_TH) (244) and *P. laumondii* subsp. *laumondii* (bALN19.5_TH) (223). These metabolites were identified based on previous studies using GNPS, NPAtlas, and LOTUS databases. All isolates produced GameXPeptide. *Photorhabdus laumondii* subsp. *laumondii* (bALN19.5_TH) produced GameXPeptide A (Figure 32), while *P. luminescens* (bAPY3.5_TH) (Figure 34) and *X. stockiae* (bAST17.4_TH) (Figure 33) produced both GameXPeptide A and C. Prolylphenylalanine was also found in these three isolates. Rhabdopeptide and Xenofuranone were produced by *P. laumondii* subsp. *laumondii* (bALN19.5_TH) and *X. stockiae* (bAST17.4_TH). Furthermore, *P. laumondii* subsp. *laumondii* (bALN19.5_TH) was found to produce Versicoloritide A, Photopyrone D and Lumizone B (Figure 32). However, most of molecules produced by these bacteria are still unknown.

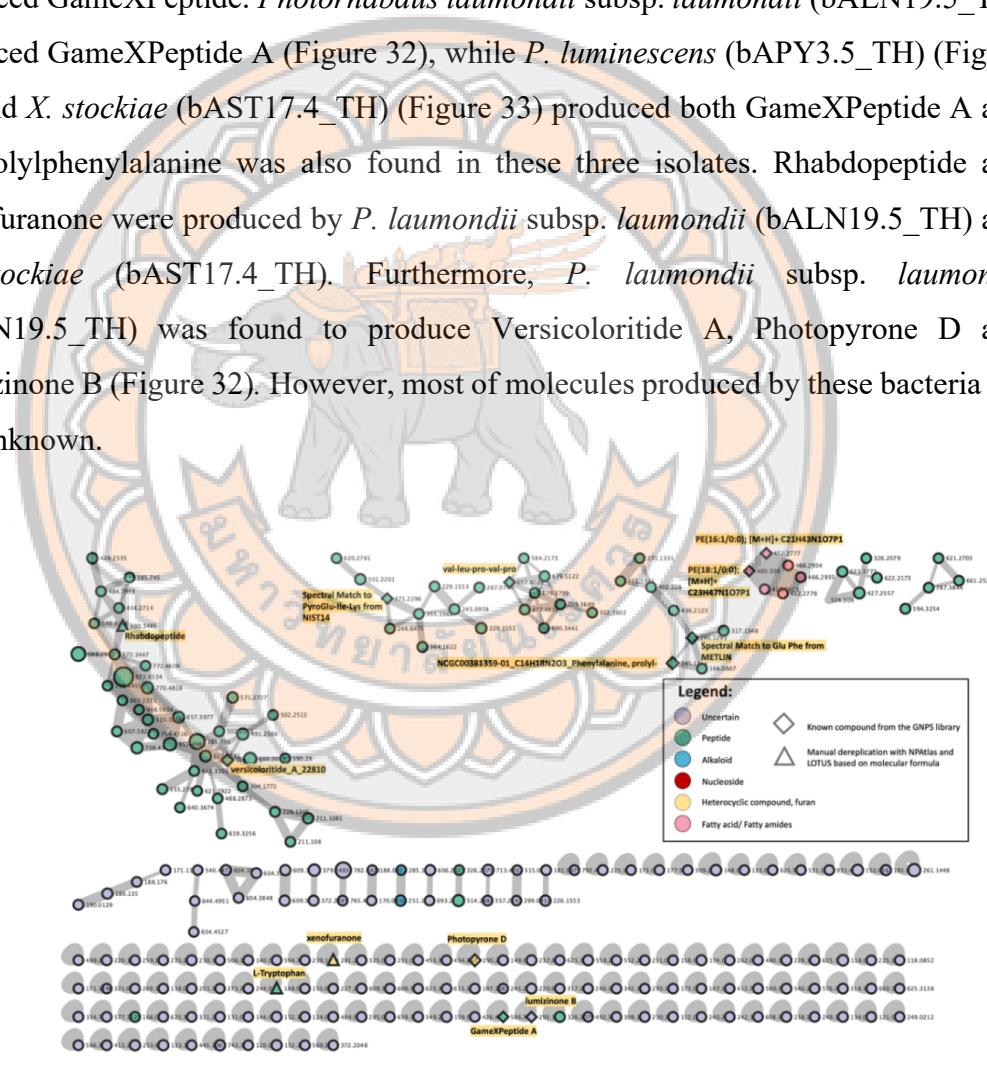


Figure 32 Network analysis of *Photorhabdus laumondii* subsp. *laumondii* (bALN19.5_TH) showing 223 nodes connected in networks with at least one neighbor and a single node. Thirteen known natural product compounds were highlighted in the network

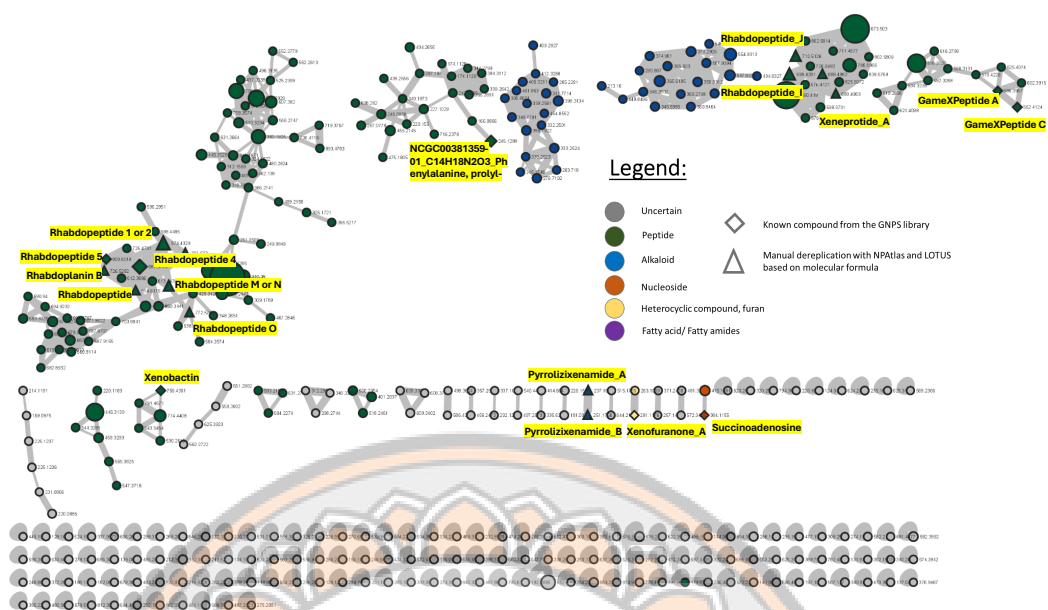


Figure 33 Network analysis of *Xenorhabdus stockiae* (bAST17.4_TH) showing 355 nodes connected in networks with at least one neighbor and a single node. Eighteen known natural product compounds were highlighted in the network

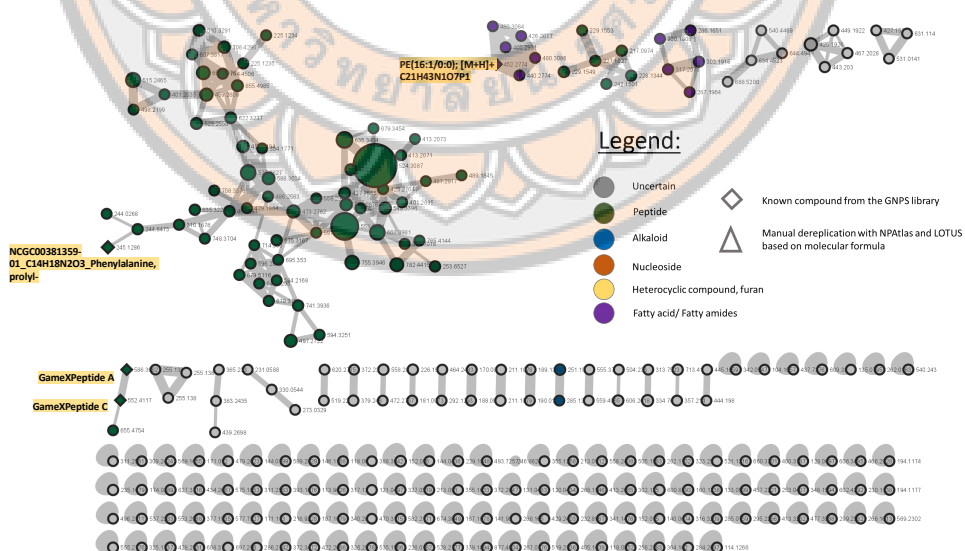


Figure 34 Network analysis of *P. luminescens* (bAPY3.5_TH) showing 244 nodes connected in networks with at least one neighbor and a single node. Four known natural product compounds were highlighted in the network

Table 24 The secondary metabolites derived from symbiotic bacteria *Xenorhabdus* and *Photorhabdus*. These metabolites were predicted from LC-MS/MS analysis of bacterial crude extracts.

No.	<i>P. luminescens</i> subsp. <i>laumondii</i> (bALN19.2)	<i>X. stockiae</i> (bAST17.4)	<i>P. luminescens</i> (bAPY3.5)
1.	Rhabdopeptide	Rhabdopeptide 1 or 2	PE(18:1/0:0); [M+H] ⁺ C23H47N1O7P1
2.	Versicoloritide A_22810	Rhabdopeptide 5	PE(16:1/0:0); [M+H] ⁺ C21H43N1O7P1
3.	Xenofuranone	Rhabdopeptide 4	prolylphenylalanine
4.	L-Tryptophan	Rhabdopeptide M or N	GameXPeptide C
5.	Photopyrone D	Rhabdopeptide	GameXPeptide A
6.	GameXPeptide A	Rhabdopeptide O	
7.	Lumizinone B	Rhabdoplanin B	
8.	Spectral Match to PyroGlu-Ile-Lys from NIST14	Xenobactin	
9.	val-leu-pro-val-pro	Rhabdopeptide_J	
10.	Spectral Match to Glu Phe from METLIN	Rhabdopeptide_I	
11.	prolylphenylalanine	Xeneprotide A	
12.	PE(18:1/0:0); [M+H] ⁺ C23H47N1O7P1	GameXPeptide A	
13.	PE(16:1/0:0); [M+H] ⁺ C21H43N1O7P1	GameXPeptide C	
14.		Pyrrolizixenamide A	
15.		Pyrrolizixenamide_B	
16.		Xenofuranone_A	
17.		Succinoadenosine	
18.		NCGC00381359- 01_C14H18N2O3_Phenylalanine, prolyl-	

Chapter V

Discussion and conclusion

Discussion

This study shows a 5.1% prevalence of EPNs recovered from agricultural areas in northern Thailand, which consisted of 14 isolates of *Heterorhabditis* (1.4% prevalence) and 37 isolates of *Steinernema* (3.7% prevalence). This finding showed low recovery of EPNs when compared to previous studies of EPNs in northern Thailand (Vitta et al., 2017; Ardpairin et al., 2020) whereas the recovery of EPNs was higher than a survey of EPNs from Nam Nao National Park of Thailand (Yooyangket et al., 2018). The EPNs were recovered mostly from horticultural crops (90.2%), and a few EPNs were recovered from field crop areas (9.8%). This result contrasted with a previous study of EPNs from agricultural areas (Ardpairin et al., 2020; de Brida et al., 2017b) which found EPNs mostly in field crops, followed by a few samples from horticultural crops. Half of the positive EPN samples were found in soil planted with corn (field areas) (Ardpairin et al., 2020), while the EPNs found in this study were mostly associated with soil planted with vegetables (35.3%) and longan gardens (25.5%). In this study, we collected EPNs from different types of agricultural areas. This may be the result of more positive samples in horticultural crops. We suggest that most farmers in horticultural crops may not use chemicals to eliminate insect pests and allow them to be hosts for EPNs. Alternatively, insecticides are usually applied in crop fields to eliminate all insects, which might be a reason for the lower prevalence of EPN in soil samples of field crops than in horticultural crops (Ardpairin et al., 2020).

In this study, most EPNs were found in loam soils (92.1%), which was similar to previous reports (Muangpat et al., 2017; Vitta et al., 2017; Ardpairin et al., 2020; Subkrasae et al., 2022; Yooyangket et al., 2018). Soil texture and soil parameters, including temperature, pH, and moisture, are important for EPN survival, movement, and infectivity. Even heterorhabditids are frequently found in sandy soils (Hominick, 2002). However, none EPN in this study were found in sandy or clay soil, consistent with the results of other reports showing that the texture of loam soil has more gaps and that the character of sand content is suitable for the movement and survival of EPNs

(Kary et al., 2009; Hazir et al., 2003). Soil parameters are important factor that supports the ability of EPNs to infect hosts and its survival in different environments. In this study, the temperature of the soil samples was 23-39 °C with pH 4-8 and moisture in the 1-8% RH ranges, indicating that EPNs in this study can live in a wide range of soil parameters which similar to previous study in Thailand (Thanwisai et al., 2012). In general, temperatures under 0 °C and above 40 °C would lead to EPN death (Rohde et al., 2010). However, we isolated EPN from soil temperature 39 °C which is nearly the temperature that led the nematode to death.

The isolated EPNs in this study were molecularly identified as *Steinernema* more than *Heterorhabditis* isolates, a result that was similar to previous studies (Vitta et al., 2017; Yooyangket et al., 2018; Thanwisai, 2012), possibly due to the ability of *Steinernema* to adapt to the environment (Kimenju et al., 2008). Steinernematid are almost generally isolated more often than heterorhabditids during randomly collected of soil sample because there are many more species of *Steinernema* than *Heterorhabditis*. One heterorhabditid is sufficient to multiply after invasion while at least two steinernematids including male and female, must invade before reproduction can occur. This means that *Steinernema* would be expected to have a higher characteristic abundance in the environment, therefore *Steinernema* were higher probability recovered (Hominick, 2002). Herein, we report that the most common species recovered from agricultural areas in northern Thailand was *S. surkhetense* (11 isolates). In 2020, *S. surkhetense* was also reported as a predominant EPN found in Phitsanulok Province, northern Thailand (Suwannaroj et al., 2020). This nematode was first identified in Nepal (Khatri-Chhetri et al., 2011c). In Thailand, *S. surkhetense* has been reported from Khon Kaen, Sakon Nakhon, Uttaradit, Kalasin, Lopburi, Phayao, Phetchaburi, Saraburi and Phitsanulok (Subkrasae et al., 2022; Suwannaroj et al., 2020). In our study, *S. surkhetense* was recovered from Lamphun, Chiang Mai, Sukhothai and Kamphaeng Phet, indicating that *S. surkhetense* is distributed in several soil environments throughout Thailand. In this study, six isolates of *S. siamkayai* were all found in the agricultural areas of Phitsanulok Province. Additionally, the first finding of this nematode in Thailand was isolated from soil samples in a sweet tamarind orchard in Lohmsak, Petchabun Province (Stock et al., 1998), which is close to Phitsanulok Province. Moreover, *S. siamkayai* has also been isolated from agricultural areas in

Nepal (Khatri-Chhetri et al., 2010). Recently, (Ardpairin et al., 2020) reported *S. siamkayai* isolated from agricultural areas in Phitsanulok Province, suggesting that *S. siamkayai* may frequently be found in agricultural areas. In this study, we reported the first finding of *S. lamjungense* isolated in Thailand, which was recovered from Lamphun Province. This nematode was first reported from Lamjung district, Nepal, in 2011 (Khatri-Chhetri et al., 2011b). However, one isolate of *S. lamjungense* (eALN11.5_TH) was then identified to be a new species *S. adamsi* n. sp, a member of the Longicaudatum clade. Which was confirmed by molecular analyses of the ITS rDNA and D2D3 of 28S rDNA sequences (Baniya et al., 2024). For *Heterorhabditis*, we found 7 isolates of *H. indica* and 2 isolates of *H. bacteriophora*. Both *Heterorhabditis* species have been previously reported in Thailand (Thanwisai, 2012). In addition, the most geographically widespread heterorhabditid nematodes was *H. bacteriophora*, which were mostly recovered from soil sampled at sea level (Hominick, 2002) but our soil sample were collected at above sea level. Our results are consistent with the results of a previous study in Thailand showing that the most common *Heterorhabditis* in northern Thailand was *H. indica*.

The morphology of the infective juvenile stage (IJs) was observed using SEM. *Heterorhabditis bacteriophora* isolate eALN 18.2_TH had a body length of $384.89 \pm 26.71 \mu\text{m}$, which was smaller than that of strain Azarbaijan (IRAZ8) at $530 \pm 10 \mu\text{m}$ (Nikdel et al., 2012), *H. bacteriophora* H39 at $562 \mu\text{m}$ (Sagun et al., 2015), and *H. bacteriophora* at $570 \mu\text{m}$ (Poinar, 1975). However, the longest individual in this study measured $441 \mu\text{m}$, which is close to the length of *H. bacteriophora* from Poland, with a mean length of $458.41 \mu\text{m}$ (range: $429.80\text{--}497.90 \mu\text{m}$) (Skrzypek et al., 2004). The tessellate pattern on cuticle with longitudinal ridge were presented like in those studies. *Heterorhabditis indica* showed an opposite trend, with a longer body length compared to other isolates ($892.03 \pm 75.94 \mu\text{m}$ vs. $528 \pm 26 \mu\text{m}$) (Houssou et al., 2014). The body length of *Steinernema lamjungense* (eALN11.5_TH) ranged from $856\text{--}1052 \mu\text{m}$, which is close to that of *S. lamjungense* n. sp. from Nepal ($690\text{--}950 \mu\text{m}$), and the lateral field with eight ridges was also observed (Hari Bahadur Khatri-Chhetri et al., 2011b). The IJs of *Steinernema surkhetense* (eALN6.3_TH) matched the description by Khatri-Chhetri et al., 2011b (436.3 ± 20.66 and $415 \pm 15 \mu\text{m}$). The lateral field in this study showed eight ridges, consistent with Khatri-Chhetri et al., 2011b. However, (Mráček et

al., 2019) suggested that the count of eight ridges may be incorrect, as SEM images showed only six ridges. The presence of six ridges in *S. surkhetense* was also described by (Bhat et al., 2017), which means our results do not agree with the previous study. Although *S. surkhetense* from Vietnam was described nine ridges at the mid body of IJs (Trinh et al., 2021).

The symbiotic bacteria, *Photorhabdus* and *Xenorhabdus* were isolated from EPNs isolated from soil sample in agricultural area in Thailand. Phylogenetic analysis of 31 isolates of *Photorhabdus* and *Xenorhabdus* showed the sequence closely related to *P. luminescens*, *P. luminescens* subsp. *akhurstii*, *P. luminescens* subsp. *laumondii*, *X. ehlersii*, *X. stockiae*, *X. indica*, and *X. griffinae*. The most abundant *Xenorhabdus* species found was *X. stockiae*, while *P. luminescens* and *P. luminescens* subsp. *akhurstii* were mostly found among the genus *Photorhabdus*. This result was consistent with the results of previous studies in Thailand, showing that *X. stockiae* and *P. luminescens* subsp. *akhurstii* were the most common species found in Thailand. In addition, all of bacteria found in this study were in line with the results of a previous study in Thailand (Fukruksa et al., 2017; Muangpat et al., 2017; Subkrasae et al., 2022; Suwannaroj et al., 2020; Thanwisai, 2012; Yimthin et al., 2021; Yooyangket et al., 2018). Most of *X. stockiae* in this study were isolated from *S. surkhetense* and one isolate was from *S. siamkayai*. *Xenorhabdus stockiae* was previously reported with *S. siamkayai* in Thailand since 2006 (Tailliez et al., 2006). Other species of EPNs host have been reported to be associated with *X. stockiae* were *S. surkhetense*, *S. huense* and *S. websteri*. (Fukruksa et al., 2017; Muangpat et al., 2017; T. Yooyangket et al., 2018; Suwannaroj et al., 2020; Thanwisai, 2012; Yimthin et al., 2021). Two isolates of *X. griffinae* were isolated from *S. surkhetense*. This species has been first isolated from *S. hermaphroditum* in Indonesia (Tailliez et al., 2006). And recently, Subkrasae et al. (2022) reported the association between *X. griffinae* and an unknown *Steinernema*. *Xenorhabdus indica* was only one isolate found with an unknown species of *Steinernema*. *Xenorhabdus ehlersii* was shown to be associated with *S. lamjungense* which is contrast to previous studies that reported *X. ehlersii* to be associated with *S. serratum* (Lengyel et al., 2005), *S. scarabaei* (Fukruksa et al., 2017) and *S. longicaudum* (Tailliez et al., 2006). Three species of *Photorhabdus* were isolated from *H. indica* and *H. bacteriophora*. *Heterorhabditis indica* is a host for *P. luminescens* and

P. luminescens subsp. *akhustii* in this study. The result is similar to previous reported in Thailand (Maneesakorn et al., 2011; Subkrasae et al., 2022; Thanwisai, 2012) and the reported in Australia, Cuba, the Dominican Republic, Israel, Jamaica, and Puerto Rico (Fischer-Le Saux et al., 1999; Abd-Elgawad, 2021). *Photorhabdus luminescens* subsp. *laumondii* was previously reported in association with *H. bacteriophora* (Fischer-Le Saux et al., 1999; Machado et al., 2018; Thanwisai, 2012). In the present study, all *P. luminescens* subsp. *laumondii* were also found to be associated with *H. bacteriophora*. The new associations between EPN and symbiotic bacteria which were observed in this study include *S. surkhetense* - *X. griffiniae* and *S. lamjungense* - *X. ehlersii*. In addition, a recent study identified some of the bacteria in this research as new species. *Xenorhabdus ehlersii* (bALN11.5_TH), which was isolated from *Steinernema adamsi* (eALN11.5_TH), was analyzed to determine its relationship to other *Xenorhabdus* species through phylogenomic analyses. The results confirmed that eALN11.5_TH represents a novel species, proposed as *Candidatus Xenorhabdus lamphunensis* (Heppert et al., 2024). Furthermore, *Xenorhabdus indica* (bAUT15.5_TH) was described as a new species, *Xenorhabdus siamensis* sp. nov., based on whole-genome phylogenomic reconstructions, core genome sequence identity values, and phenotypic characterization (Thanwisai et al., 2024).

The strategies that search for alternative molluscicide to decrease the snail and interrupt schistosome life cycle was provided by the WHO's Road map and planned for 2030 (Evan Secor, 2014). Herein, we reported the first evaluation of symbiotic bacteria, *Photorhabdus* and *Xenorhabdus* against *B. glabrata* snail. All crude extracts of symbiotic bacteria showed good activity against *B. glabrata*. Since there was no previous study of using these bacteria-derived compounds for control the snail. However, according to the WHO criterion, the crude extract obtained from plant is considered active in freshwater snails at concentration up to 100 µg/mL which causes 70–100 % mortality of snails at the 24 h exposure time (World Health Organization, 1983). Two species of bacteria tested (*P. luminescens* subsp. *laumondii* and *X. stockiae*) cause 100 % mortality at concentration 100 µg/ml. The most effective bacterium in this study is *P. luminescens* subsp. *laumondii* which showed the LC values of 56.58 µg/ml and 92.24 µg/ml for LC50 and LC90, respectively. In this study, *P. luminescens* subsp. *laumondii* was isolated from the nematode, *H. bacteriophora*. Recently, the

susceptibility of *B. glabrata* to infective juveniles of *H. bacteriophora* (isolate HP88) was reported under laboratory conditions. The result indicated that the infection induces breakdown of carbohydrate homeostasis in *B. glabrata* but did not cause lethality in the population of infected snails (Santos Amaral et al., 2022). This result also suggested that the nematode may not release the symbiotic bacteria when infected snails. Considering other substances have been evaluated to control *B. glabrata*, crude extract and ethyl acetate fraction of *Manilkara subsericea* leaves induced $80 \pm 4.13\%$ and $86.66 \pm 4.59\%$ mortality of adult snails at concentrations of 250 $\mu\text{g/ml}$ after 96h (Faria et al., 2018). Curcumin caused 53 % mortality in adult snail after exposed to 100 $\mu\text{g/ml}$ (Matos et al., 2020). The essential oil extracted from *Eryngium triquetrum* caused 100% in *B. glabrata* and the LC50 and LC90 values for the snails were 0.61 and 1.02 $\mu\text{g/ml}$ respectively for 24 h exposure (de Carvalho Augusto et al., 2020). The usnic acid (UA), isolated and purified from *Cladonia substellata* caused 87% and 100% mortality against *B. glabrata* at the concentrations of 3 $\mu\text{g/ml}$ and 4 $\mu\text{g/ml}$, respectively (Araújo et al., 2018). The possible mechanism of UA action diffuses through the cell membranes and affect to electron transport chain which can lead to biochemical and physiological changes to cell homeostasis and result in cell apoptosis (Joseph et al., 2009). Most of purified extract showed high efficacy against the snail at low concentration. However, the bacterial extract in this study showed molluscicidal activities at concentrations lower than the limits established by the WHO. Therefore, the extract from *Xenorhabdus* and *Photorhabdus* bacteria showed promising results as an alternative molluscicidal for control *B. glabrata*. The toxicity to other organism in environment was not evaluated in this study; however, *Xenorhabdus* and *Photorhabdus* are well known to pathogenic to the insect and not the infectious bacteria except for *Photorhabdus asymbiotica* that can also infect humans (Gerrard et al., 2004).

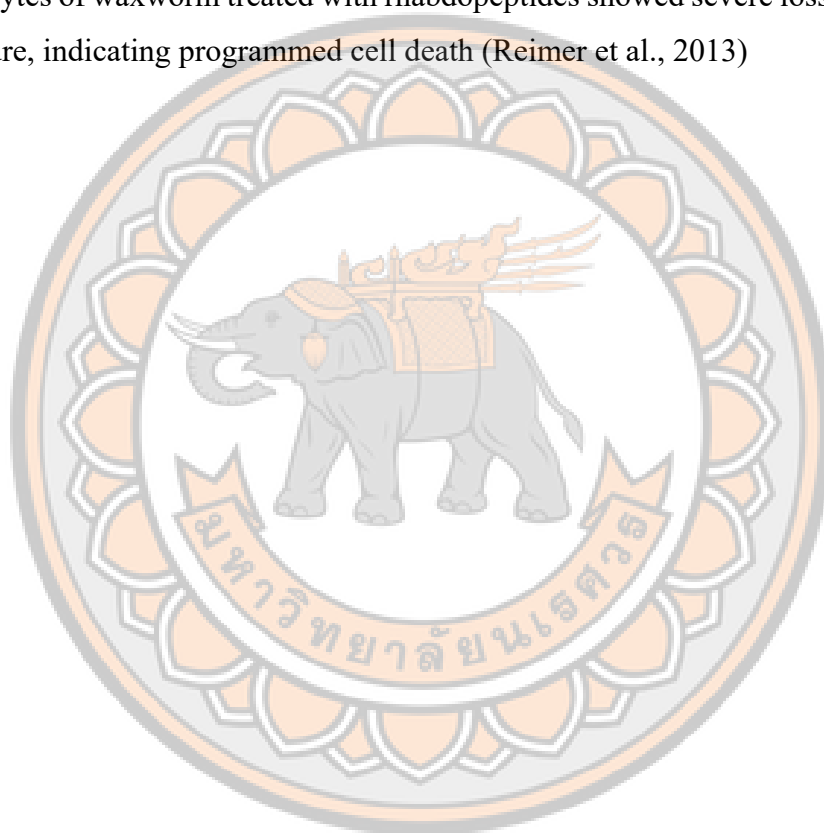
Ethyl acetate extract of *Xenorhabdus* and *Photorhabdus* affected cells in the snails. The first target of toxic activity was the head-foot region. After being treated with LC50 of bacteria extract, the foot of snails showed histological change with the destruction of epithelial covering layer and presenting of dark brown pigment. The results were similar to the previous report of (Abdel-Rahman, 2020). The land snail *Monacha* sp. was exposed to essential plant oils, the foot of snail showed slight shrinkage and rupture of muscular tissue, migration and precipitation of pigment cells

and slight distortion of epithelial covering. Same results were previously reported that the foot of *M. obstructa* snails treated with LC50 of *Jatropha* showed rupture of the epithelium covering the foot (Ibrahim et al., 2022). In addition, the foot of *B. siamensis goniomphalos* snails treated with camellia and mangosteen extracts showed the disrupting of columnar muscle fibers and creating gaps between epithelial cells and connective tissue. Moreover, protein and pigment cells were found to be concentrated (Aukkanimart et al., 2013).

The pathological change of digestive glands is used as a biomarker of induced toxicity in snails (Triebkorn & Köhler, 1996). In the present study, we found the hemocytes in the digestive cell. This result was also found in *B. glabrata* after exposed with *E. milii* latex and the hemocytes were presented in digestive gland, kidney, and mantle of the snail. The author suggested that this phytochemical stimulated the hemocytes proliferation (circulating and tissue-specific) in the tissue, which hyalinocytes were the most found, followed by granulocytes and blast cells. However, the hemocytes type does not influence the cellular immune response, but the number of hemocytes does (Friani et al., 2022).

The hermaphrodite glands in this study showed histopathological change included decreasing of spermatozoa and the degeneration of oocytes. In addition, severe necrotic changes and degeneration of acini were also observed. The same results were observed by (Abououf et al., 2018), who observed histopathological effects of plants extracts *A. arvensis* and *V. tinus* against *B. alexandrina*. The alterations in the hermaphrodite glands of the treated snails showed degeneration and necrotic changes in the acini. The present results also agreed with the studies on *B. glabrata* after exposure to *Eucalyptus camaldulensis* and niclosamide (Zhou et al., 1993). Also, when *B. alexandrina* and *B. truncates* were exposed to *P. canescens*, the hermaphrodite gland of these snails revealed that some ova organelles were deformed, and empty ova appeared. The sperms were totally distorted, and the reduction in the number of sperms was also observed. Moreover, acinar epithilum showed necrotic changes in form of partial destruction (Abdel-Hamid et al., 2014). LC-MS/MS analysis of 3 isolates which are most effective in controlling snails contained known compounds such as GameXPeptide, Rhabdopeptide, Xenofuranone and Photopyrone. All three isolates produced GameXPeptides which are a class of cyclic pentapeptides initially believed

to be widespread in *Photorhabdus* and *Xenorhabdus* and significant for these bacteria (Bode et al., 2012). Based on their structure, GameXPeptides were speculated to disrupt the insect immune response by interfering with the link between the innate and adaptive immune systems (Tobias et al., 2018). Rhabdopeptides, which were found in *X. stockiae* (bAST17.4) and *P. laumondii* subsp. *laumondii* (bALN19.5), have unknown activity, but it has structures similar to protease inhibitors. Therefore, this compound might degrade a range of proteins associated with immunity (Cai et al., 2017). In addition, the hemocytes of waxworm treated with rhabdopeptides showed severe loss of cytoskeletal structure, indicating programmed cell death (Reimer et al., 2013)



Conclusion

In conclusion, we isolated and identified EPNs from soil samples collected from agricultural areas of northern Thailand. The prevalence of EPNs was 5.1% which consisted of *Steinernema* 3.7% and *Heterorhabditis* 1.4%. The EPN species showed more variety with a newly recorded species (*S. lamjungense*) in Thailand. Moreover, one isolate from this study was then identified as *S. adamsi* n. sp. Most EPNs were isolated from loam in horticulture crops with a wide range of soil parameters.

The symbiotic bacteria show 3.1% prevalence including 1.9% and 1.2% of *Xenorhabdus* and *Photorhabdus*, respectively. Phylogenetic analysis of *Xenorhabdus* showed closely related to *X. stockiae*, *X. indica*, *X. griffithiae*, and *X. ehlersii*. *Photorhabdus* isolates showed sequence similarity to *P. luminescens* subsp. *akhurstii*, *P. luminescens*, and *P. luminescens* subsp. *laumondii*.

The morphology of the infective juvenile of EPNs 4 species were described with the body length and the characteristic using SEM. Most of them showed difference size from other studies however the morphological were similar to previous described.

Significantly, herein we reported the first investigation of bacterial extract to control *B. glabrata*, the intermediate host of *Schistosoma mansoni*. The result indicated that all crude extracts of 5 symbiotic bacteria showed good activity against *B. glabrata*, especially *P. luminescens* subsp. *laumondii* (bALN19.5_TH) and *X. stockiae* (bAST17.4_TH), which produced virulent secondary metabolites such as GameXPeptides and Rhabdopeptides. These extracts also caused the histopathological alterations in foot, digestive glands and hermaphrodite glands of the snails. Therefore, these extracts may be effective molluscides that could be used in the field to control the intermediate host snails.

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Appendix A Culture medium and chemicals preparation

Nutrient- bromothymol blue- triphenyl tetazolium chloride agar (NBTA)

Components for 1 L

Nutrient agar (Oxoid, Ltd, England)	28	g
Bromothymol blue	0.025	g
Filtered 0.004% tetrazolium chloride	500	μL (dissolved with 90%

alcohol, 0.22 μm filter)

Distilled water 1 L

Preparation

Dissolve all component with distilled water in flask, then sterilize by adding the flask into autoclave at 121 °C for 15 min. After that, let the medium in flask cool down until around 50°C, follow by adding tetrazolium chloride. Lastly, pouring the medium into sterilized petri dish. Let the medium cool down until become to an agar. The NBTA is ready to use or keep in 4°C fridge.

Luria-Bertani broth (LB)

Components for 1 L

Luria-Bertani broth (LB) powder (Caisson LABS, USA)	25	g
Distilled water	1	L

Preparation

Dissolve LB powder with distilled water in flask, then sterilize by adding the flask into autoclave at 121 °C for 15 min. After that, let the medium in flask cool down. The LB is ready to use or keep in 4°C fridge.

Tryptone soy agar (TSA)

Components for 1 L

Tryptone soya agar (TSA) (Oxoid, Ltd, England)	40	g
Distilled water	1	L

Preparation

Dissolve TSA with distilled water in flask, then sterilize by adding the flask into autoclave at 121 °C for 15 min. After that, let the medium in flask cool down. The LB is ready to use or keep in 4°C fridge.

10X TBE bufferComponents for 1 L

Tris base (Oxoid, Ltd, England)	108	g
Boric acid	55	g
Ethylenediaminetetraacetic acid (EDTA)	7.5	g
Distilled water	1	L

Preparation

Dissolve all component with 800 mL distilled water in flask, then adjust the volume until 1 L. The 10X TBE is ready to use or keep in RT.

1.2% agarose gelComponents for 100 ml

Agarose powder	1.2	g
1X TBE buffer	100	ml

Preparation

Dissolve agarose powder with 1X TBE buffer in flask, then heat by adding the flask in to microwave for 1 – 1.30 min. Let the dissolved gel cool down a little bit in RT and pour to gel tray.

0.8% agarose gelComponents for 100 ml

Agarose powder	0.8	g
1X TBE buffer	100	ml

Preparation

Dissolve agarose powder with 1X TBE buffer in flask, then heat by putting the flask into microwave for 1 – 1.30 min. Let the dissolved gel cool down a little bit in RT and pour to gel tray. Soil samples were collected from various ecologies roadsides, hot springs, and agricultural areas

Appendix B Soil sample collection in Thailand



Figure 35 Soil sample collection at Agricultural areas



Figure 36 Agricultural areas in Northern Thailand



Figure 37 Extrinsic factors and geographic coordinates measurement

Appendix C Soil sample information

Date	Time	Code	GPS	Temperature	Moisture	pH	Soil texture	Agricultural area
8/09/2018	08.00	AKP 1.1	N16 30 0.18	29	8	5.6	Clay	Rice field
		AKP 1.2	E100 00 52.7	29	4	6.2	Sandy	Rice field
		AKP 1.3		29	5	5.2	Loam	Rice field
		AKP 1.4	Elev = 61 m	29	6	5.6	Clay	Rice field
		AKP 1.5		28	1	5.6	Clay	Rice field
8/09/2018	08.10	AKP 2.1	N16 29 57.1	31	3.5	4.8	Loam	Lime garden
		AKP 2.2	E099 56 13.4	31	8	5.4	Loam	Lime garden
		AKP 2.3		31	8	4.5	Loam	Lime garden
		AKP 2.4	Elev = 51 m	31	8	4.2	Loam	Lime garden
		AKP 2.5		31	8	5	Loam	Lime garden

Appendix C Soil sample information (Cont.)

Date	Time	Code	GPS	Temperature	Moisture	pH	Soil texture	Agricultural area
8/09/2018	08.15	AKP 3.1	N16 29 55.3	31	7	5	Loam	Dragon fruit
		AKP 3.2	E099 56 14.0	31	7	4	Loam	Dragon fruit
		AKP 3.3		31	8	5	Loam	Dragon fruit
		AKP 3.4	Elev = 45 m	31	3	5	Loam	Dragon fruit
		AKP 3.5		31	6	5	Loam	Dragon fruit
8/09/2018	08.30	AKP 4.1	N16 29 04.4	28	25	6.5	Loam	-
		AKP 4.2	E100 56 50.9	28	24	4.5	Loam	-
		AKP 4.3		28	25	6.5	Loam	-
		AKP 4.4	Elev = 677 m	28	24	8.0	Loam	-
		AKP 4.5		28	25	5.0	Loam	-
8/09/2018	08.40	AKP 5.1	N16 25 33.7	29	7.5	4.2	Clay	Rice fields
		AKP 5.2	E099 50 24.3	28	1	6.6	Loam	Rice fields
		AKP 5.3		28	4	6.6	Clay	Rice fields
		AKP 5.4	Elev = 60 m	28	4	5.6	Clay	Rice fields
		AKP 5.5		29	3.5	6.6	Clay	Rice fields

Appendix C Soil sample information (Cont.)

Date	Time	Code	GPS	Temperature	Moisture	pH	Soil texture	Agricultural area
8/09/2018	08.50	AKP 6.1	N16 21 25.9	29	4	5.6	Loam	Vegetable garden
		AKP 6.2	E099 50 17.2	29	8	5.0	Loam	Vegetable garden
		AKP 6.3		29	8	5.0	Loam	Vegetable garden
		AKP 6.4	Elev = 52 m	30	5	6.0	Loam	Vegetable garden
		AKP 6.5		29	8	5.0	Loam	Vegetable garden
8/09/2018	09.00	AKP 7.1	N16 21 25.1	29	6.5	5.6	Loam	Corn fields
		AKP 7.2	E099 50 16.6	29	5.8	6.2	Loam	Corn fields
		AKP 7.3		29	5	6.4	Loam	Corn fields
		AKP 7.4	Elev = 55 m	29	6	6.5	Loam	Corn fields
		AKP 7.5		29	7	6.0	Loam	Corn fields
8/09/2018	09.10	AKP 8.1	N16 21 24.0	29	8	5.2	Loam	Vegetable garden
		AKP 8.2	E099 50 16.7	29	8	4.6	Loam	Vegetable garden
		AKP 8.3		29	8	5.2	Loam	Vegetable garden
		AKP 8.4	Elev = 61 m	29	8	5.2	Loam	Vegetable garden
		AKP 8.5		29	8	4.8	Loam	Vegetable garden

Appendix C Soil sample information (Cont.)

Date	Time	Code	GPS	Temperature	Moisture	pH	Soil texture	Agricultural area
8/09/2018	09.15	AKP 9.1	N16 21 22.2	30	4.5	5.0	Loam	Vegetable garden
		AKP 9.2	E099 50 15.4	31	6.5	5.2	Loam	Vegetable garden
		AKP 9.3		30	1.5	6.1	Loam	Vegetable garden
		AKP 9.4	Elev = 59 m	30	1	6.2	Loam	Vegetable garden
		AKP 9.5		30	5.5	6.4	Loam	Vegetable garden
8/09/2018	09.20	AKP 10.1	N16 21 21.7	30	4.5	6.2	Loam	Chilli fields
		AKP 10.2	E099 50 15.2	29	5	5.2	Loam	Chilli fields
		AKP 10.3		29	6.5	5.0	Loam	Chilli fields
		AKP 10.4	Elev = 57 m	29	3.5	6.2	Loam	Chilli fields
		AKP 10.5		29	5	6.0	Loam	Chilli fields

Appendix C Soil sample information (Cont.)

Date	Time	Code	GPS	Temperature	Moisture	pH	Soil texture	Agricultural area
8/09/2018	09.30	AKP 11.1	N16 21 22.6	29	8	6.2	Loam	Lime garden
		AKP 11.2	E099 50 18.7	29	4	6.4	Loam	Lime garden
		AKP 11.3		30	5	6.0	Loam	Lime garden
		AKP 11.4	Elev = 57 m	30	7.5	5.4	Loam	Lime garden
		AKP 11.5		30	6	6.2	Loam	Lime garden
8/09/2018	09.40	AKP 12.1	N16 21 19.9	29	1	6.6	Loam	Vegetable garden
		AKP 12.2	E099 50 25.9	29	6.5	6.2	Loam	Vegetable garden
		AKP 12.3		28	3.5	6.0	Loam	Vegetable garden
		AKP 12.4	Elev = 47 m	28	8	4.2	Loam	Vegetable garden
		AKP 12.5		28	5	6.4	Loam	Vegetable garden
8/09/2018	09.50	AKP 13.1	N16 21 20.7	28	3	6.4	Loam	Pumpkin fields
		AKP 13.2	E099 50 24.9	28	5	5.0	Loam	Pumpkin fields
		AKP 13.3		28	4.5	6.4	Loam	Pumpkin fields
		AKP 13.4	Elev = 50 m	28	4	6.2	Loam	Pumpkin fields
		AKP 13.5		28	2	6.4	Loam	Pumpkin fields

Appendix C Soil sample information (Cont.)

Date	Time	Code	GPS	Temperature	Moisture	pH	Soil texture	Agricultural area
8/09/2018	10.00	AKP 14.1	N16 20 56.9	30	3.5	5.3	Loam	Lemongrass
		AKP 14.2	E099 50 25.3	29	7	6.0	Loam	Lemongrass
		AKP 14.3		29	6	5.4	Loam	Lemongrass
		AKP 14.4	Elev = 54 m	29	5	6.2	Loam	Lemongrass
		AKP 14.5		28	7	6.4	Loam	Lemongrass
8/09/2018	10.10	AKP 15.1	N16 20 43.9	31	7	6.2	Loam	Lime garden
		AKP 15.2	E099 50 34.4	29	8	5.0	Loam	Lime garden
		AKP 15.3		30	8	5.0	Loam	Lime garden
		AKP 15.4	Elev = 57 m	29	7	5.2	Loam	Lime garden
		AKP 15.5		29	7.5	5.8	Loam	Lime garden

Appendix C Soil sample information (Cont.)

Date	Time	Code	GPS	Temperature	Moisture	pH	Soil texture	Agricultural area
8/09/2018	10.10	AKP 16.1	N16 20 42.5	28	8	4.0	Loam	Dragon fruit
		AKP 16.2	E099 50 35.1	28	7	5.4	Loam	Dragon fruit
		AKP 16.3		28	8	5.4	Loam	Dragon fruit
		AKP 16.4	Elev = 54 m	28	7	5.4	Loam	Dragon fruit
		AKP 16.5		28	8	4.8	Loam	Dragon fruit
8/09/2018	10.15	AKP 17.1	N16 20 00.0	30	7	5.2	Loam	Vegetable garden
		AKP 17.2	E099 52 01.0	30	6	4.4	Loam	Vegetable garden
		AKP 17.3		30	7	5.4	Loam	Vegetable garden
		AKP 17.4	Elev = 58 m	31	7	5.8	Loam	Vegetable garden
		AKP 17.5		30	8	5.2	Loam	Vegetable garden
8/09/2018	10.20	AKP 18.1	N16 19 58.7	32	6	5.4	Loam	Vegetable garden
		AKP 18.2	E099 52 01.8	30	8	6.0	Loam	Vegetable garden
		AKP 18.3		30	7	6.0	Loam	Vegetable garden
		AKP 18.4	Elev = 56 m	30	6	5.2	Loam	Vegetable garden
		AKP 18.5		30	5	6.4	Loam	Vegetable garden

Appendix C Soil sample information (Cont.)

Date	Time	Code	GPS	Temperature	Moisture	pH	Soil texture	Agricultural area
8/09/2018	12.20	AKP 19.1	N16 20 00.1	32	8	5.0	Loam	Vegetable garden
		AKP 19.2	E099 52 03.2	30	8	4.8	Loam	Vegetable garden
		AKP 19.3		30	7	4.8	Loam	Vegetable garden
		AKP 19.4	Elev = 55 m	29	8	4.6	Loam	Vegetable garden
		AKP 19.5		30	8	4.8	Loam	Vegetable garden
8/09/2018	12.25	AKP 20.1	N16 19 58.4	29	8	5.2	Loam	Papaya garden
		AKP 20.2	E099 52 03.8	29	8	5.6	Loam	Papaya garden
		AKP 20.3		30	7	5.0	Loam	Papaya garden
		AKP 20.4	Elev = 53 m	29	7	5.2	Loam	Papaya garden
		AKP 20.5		29	8	5.6	Loam	Papaya garden

Appendix C Soil sample information (Cont.)

Date	Time	Code	GPS	Temperature	Moisture	pH	Soil texture	Agricultural area
23/06/2019	07.14	APL 1.1	N16°57'36.4"	30	1.5	6.8	Clay loam	Rice fields
		APL 1.2	E100°07'47.8"	30	1.5	6.8	Clay loam	Rice fields
		APL 1.3		30	1.0	7.0	Clay loam	Rice fields
		APL 1.4	Elev = 41 m	30	1.0	7.0	Clay loam	Rice fields
		APL 1.5		30	1.0	7.0	Clay loam	Rice fields
23/06/2019	07.28	APL 2.1	N16°58'17.1"	30	1.0	7.0	Clay loam	Mixed fruit
		APL 2.2	E100°08'18.3"	30	1.0	7.0	Clay loam	Mixed fruit
		APL 2.3		30	1.5	6.8	Clay loam	Mixed fruit
		APL 2.4	Elev = 42 m	30	1.0	7.0	Clay loam	Mixed fruit
		APL 2.5		30	1.0	6.8	Clay loam	Mixed fruit
23/06/2019	07.42	APL 3.1	N17°00'18.5"	31	1.0	6.8	Loam	Mixed fruit
		APL 3.2	E100°10'33.7"	31	1.0	7.0	Loam	Mixed fruit
		APL 3.3		31	1.0	7.0	Loam	Mixed fruit
		APL 3.4	Elev = 48 m	31	1.0	6.8	Loam	Mixed fruit
		APL 3.5		31	1.0	6.8	Loam	Mixed fruit

Appendix C Soil sample information (Cont.)

Date	Time	Code	GPS	Temperature	Moisture	pH	Soil texture	Agricultural area
23/06/2019	07.52	APL 4.1	N17°01'04.8"	31	1.0	6.8	Loam	Mixed fruit
		APL 4.2	E100°10'23.0"	31	1.0	7.0	Loam	Mixed fruit
		APL 4.3		31	1.0	7.0	Loam	Mixed fruit
		APL 4.4	Elev = 47 m	31	1.0	6.8	Loam	Mixed fruit
		APL 4.5		31	1.0	6.8	Loam	Mixed fruit
23/06/2019	07.58	APL 5.1	N17°01'41.4"	32	1.0	6.8	Loam	Mixed fruit
		APL 5.2	E100°10'20.0"	31	1.0	6.8	Loam	Mixed fruit
		APL 5.3		32	1.0	6.8	Loam	Mixed fruit
		APL 5.4	Elev = 51 m	32	1.0	6.8	Loam	Mixed fruit
		APL 5.5		32	1.0	6.8	Loam	Mixed fruit

Appendix C Soil sample information (Cont.)

Date	Time	Code	GPS	Temperature	Moisture	pH	Soil texture	Agricultural area
23/06/2019	08.05	APL 6.1	N17°01'39.9"	32	1.0	7.0	Loam	Mixed fruit
		APL 6.2	E100°10'20.8"	32	1.0	7.0	Loam	Mixed fruit
		APL 6.3		32	1.0	7.0	Loam	Mixed fruit
		APL 6.4	Elev = 48 m	32	1.0	6.8	Loam	Mixed fruit
		APL 6.5		32	1.0	6.8	Loam	Mixed fruit
23/06/2019	08.12	APL 7.1	N17°02'02.6"	30	1.0	6.2	Loam	Vegetable garden
		APL 7.2	E100°10'30.8"	30	1.0	6.4	Loam	Vegetable garden
		APL 7.3		32	1.0	6.8	Loam	Vegetable garden
		APL 7.4	Elev = 54 m	31	1.0	6.4	Loam	Vegetable garden
		APL 7.5		32	1.0	6.8	Loam	Vegetable garden
23/06/2019	08.17	APL 8.1	N17°02'06.5"	32	1.0	6.8	Loam	Marigold garden
		APL 8.2	E100°10'34.1"	32	1.0	7.0	Loam	Marigold garden
		APL 8.3		32	1.0	7.0	Loam	Marigold garden
		APL 8.4	Elev = 66 m	32	1.0	6.8	Loam	Marigold garden
		APL 8.5		32	1.0	6.8	Loam	Marigold garden

Appendix C Soil sample information (Cont.)

Date	Time	Code	GPS	Temperature	Moisture	pH	Soil texture	Agricultural area
23/06/2019	08.22	APL 9.1	N16 21 22.2	31	1.0	6.8	Loam	Corn fields
		APL 9.2	E099 50 15.4	31	1.0	6.8	Loam	Corn fields
		APL 9.3		31	1.0	6.8	Loam	Corn fields
		APL 9.4	Elev = 45 m	31	1.0	6.8	Loam	Corn fields
		APL 9.5		32	1.0	6.8	Loam	Corn fields
23/06/2019	08.28	APL 10.1	N17°02'14.0"	32	1.0	7.0	Loam	Vegetable garden
		APL 10.2	E100°10'14.0"	32	2.0	6.8	Loam	Vegetable garden
		APL 10.3		32	1.0	6.8	Loam	Vegetable garden
		APL 10.4	Elev = 51 m	32	1.0	6.6	Loam	Vegetable garden
		APL 10.5		32	2.0	6.4	Loam	Vegetable garden

Appendix C Soil sample information (Cont.)

Date	Time	Code	GPS	Temperature	Moisture	pH	Soil texture	Agricultural area
23/06/2019	08.33	APL 11.1	N17°02'14.5"	32	1.0	6.8	Loam	Vegetable garden
		APL 11.2	E100°10'13.8"	31	1.0	6.8	Loam	Vegetable garden
		APL 11.3		31	1.0	6.8	Loam	Vegetable garden
		APL 11.4	Elev = 60 m	31	1.0	6.4	Loam	Vegetable garden
		APL 11.5		31	1.0	6.8	Loam	Vegetable garden
23/06/2019	08.42	APL 12.1	N17°02'12.8"	32	1.0	6.8	Loam	Vegetable garden
		APL 12.2	E100°10'01.0"	32	1.0	6.8	Loam	Vegetable garden
		APL 12.3		32	1.0	6.8	Loam	Vegetable garden
		APL 12.4	Elev = 48 m	32	1.0	6.8	Loam	Vegetable garden
		APL 12.5		31	1.0	6.8	Loam	Vegetable garden
23/06/2019	08.51	APL 13.1	N17°02'12.3"	31	1.0	6.8	Loam	Banana plantation
		APL 13.2	E100°10'01.4"	31	1.0	6.8	Loam	Banana plantation
		APL 13.3		31	1.0	6.8	Loam	Banana plantation
		APL 13.4	Elev = 52 m	30	1.0	6.8	Loam	Banana plantation
		APL 13.5		30	1.0	6.8	Loam	Banana plantation

Appendix C Soil sample information (Cont.)

Date	Time	Code	GPS	Temperature	Moisture	pH	Soil texture	Agricultural area
23/06/2019	09.01	APL 14.1	N17°02'34.6"	32	1.0	6.8	Loam	Mango garden
		APL 14.2	E100°09'28.4"	32	1.0	7.0	Loam	Mango garden
		APL 14.3		32	1.0	7.0	Loam	Mango garden
		APL 14.4	Elev = 53 m	32	1.0	7.0	Loam	Mango garden
		APL 14.5		32	1.0	6.6	Loam	Mango garden
23/06/2019	09.11	APL 15.1	N17°02'37.9"	32	1.0	6.8	Loam	Mango garden
		APL 15.2	E100°09'23.8"	31	1.0	7.0	Loam	Mango garden
		APL 15.3		31	1.0	7.0	Loam	Mango garden
		APL 15.4	Elev = 51 m	31	1.0	6.8	Loam	Mango garden
		APL 15.5		31	1.0	6.8	Loam	Mango garden

Appendix C Soil sample information (Cont.)

Date	Time	Code	GPS	Temperature	Moisture	pH	Soil texture	Agricultural area
23/06/2019	09.19	APL 16.1	N17°02'37.8"	30	1.0	6.8	Loam	Mango garden
		APL 16.2	E100°09'25.4"	30	1.0	6.8	Loam	Mango garden
		APL 16.3		30	1.0	6.8	Loam	Mango garden
		APL 16.4	Elev = 49 m	30	2.0	6.8	Loam	Mango garden
		APL 16.5		30	2.0	6.8	Loam	Mango garden
23/06/2019	09.28	APL 17.1	N17°03'19.2"	32	1.0	7.0	Loam	Corn fields
		APL 17.2	E100°10'27.8"	32	1.0	6.6	Loam	Corn fields
		APL 17.3		32	1.0	6.6	Loam	Corn fields
		APL 17.4	Elev = 44 m	32	1.0	6.8	Loam	Corn fields
		APL 17.5		31	1.0	7.0	Loam	Corn fields
23/06/2019	09.37	APL 18.1	N17°02'50.0"	31	1.0	6.8	Loam	Vegetable garden
		APL 18.2	E100°10'24.7"	31	1.0	6.8	Loam	Vegetable garden
		APL 18.3		32	1.0	6.6	Loam	Vegetable garden
		APL 18.4	Elev = 56 m	31	1.0	6.8	Loam	Vegetable garden
		APL 18.5		32	1.0	6.8	Loam	Vegetable garden

Appendix C Soil sample information (Cont.)

Date	Time	Code	GPS	Temperature	Moisture	pH	Soil texture	Agricultural area
23/06/2019	09.01	APL 19.1	N17°02'47.8"	31	1.0	6.8	Clay loam	Mango garden
		APL 19.2	E100°10'30.2"	30	1.0	7.0	Clay loam	Mango garden
		APL 19.3		30	1.0	7.0	Clay loam	Mango garden
		APL 19.4	Elev = 70 m	31	1.0	6.8	Clay loam	Mango garden
		APL 19.5		31	1.0	7.0	Clay loam	Mango garden
23/06/2019	09.53	APL 20.1	N17°02'48.4"	32	1.0	6.8	Loam	Turmeric garden
		APL 20.2	E100°10'48.6"	32	1.0	6.8	Loam	Turmeric garden
		APL 20.3		32	1.0	6.8	Loam	Turmeric garden
		APL 20.4	Elev = 52 m	31	1.0	6.8	Loam	Turmeric garden
		APL 20.5		31	1.0	6.8	Loam	Turmeric garden

Appendix C Soil sample information (Cont.)

Date	Time	Code	GPS	Temperature	Moisture	pH	Soil texture	Agricultural area
23/06/2019	09.19	AUT 1.1	N17°13'56.1"	32	1.0	6.6	Clay	Sugarcane
		AUT 1.2	E100°03'03.3"	32	1.0	6.4	Clay	Sugarcane
		AUT 1.3		32	1.0	6.8	Clay	Sugarcane
		AUT 1.4	Elev = 52 m	32	2.0	6.8	Clay	Sugarcane
		AUT 1.5		34	2.0	6.8	Clay	Sugarcane
23/06/2019	10.55	AUT 2.2	N17°14'59.6"	34	1.0	7.0	Loam	Mango garden
		AUT 2.3	E100°03'43.8"	33	1.0	6.8	Loam	Mango garden
		AUT 2.4		33	1.0	7.0	Loam	Mango garden
		AUT 2.5	Elev = 57 m	33	1.0	7.0	Loam	Mango garden
		AUT 2.6		33	1.0	7.0	Loam	Mango garden
23/06/2019	09.37	AUT 3.1	N17°14'59.4"	33	1.0	6.8	Loam	Banana plantation
		AUT 3.2	E100°03'42.9"	33	1.0	6.8	Loam	Banana plantation
		AUT 3.3		32	1.0	7.0	Loam	Banana plantation
		AUT 3.4	Elev = 54 m	32	1.0	6.8	Loam	Banana plantation
		AUT 3.5		32	1.0	6.8	Loam	Banana plantation

Appendix C Soil sample information (Cont.)

Date	Time	Code	GPS	Temperature	Moisture	pH	Soil texture	Agricultural area
23/06/2019	11.04	AUT 4.1	N17°14'58.1"	33	1.0	6.8	Loam	Marian Plum
		AUT 4.2	E100°03'46.0"	33	1.0	6.8	Loam	Marian Plum
		AUT 4.3		32	1.0	6.8	Loam	Marian Plum
		AUT 4.4	Elev = 58 m	32	3.0	6.8	Loam	Marian Plum
		AUT 4.5		32	1.0	6.8	Loam	Marian Plum
23/06/2019	09.53	AUT 5.1	N17°19'58.2"	33	1.0	6.4	Clay	Sugarcane
		AUT 5.2	E100°03'05.2"	33	1.0	6.6	Loam	Sugarcane
		AUT 5.3		33	1.0	6.8	Clay	Sugarcane
		AUT 5.4	Elev = 56 m	33	1.0	6.4	Clay	Sugarcane
		AUT 5.5		33	1.0	6.6	Clay	Sugarcane

Appendix C Soil sample information (Cont.)

Date	Time	Code	GPS	Temperature	Moisture	pH	Soil texture	Agricultural area
23/06/2019	11.30	AUT 6.1	N17°13'56.1"	32	1.0	6.6	Clay loam	Banana plantation
		AUT 6.2	E100°03'03.3"	32	1.0	6.4	Clay loam	Banana plantation
		AUT 6.3		32	1.0	6.8	Clay loam	Banana plantation
		AUT 6.4	Elev = 52 m	32	2.0	6.8	Clay loam	Banana plantation
		AUT 6.5		34	2.0	6.8	Clay loam	Banana plantation
23/06/2019	11.50	AUT 7.1	N17°24'40.0"	31	1.0	7.0	Loam	Mango garden
		AUT 7.2	E100°05'04.9"	32	1.0	6.8	Loam	Mango garden
		AUT 7.3		32	1.0	6.8	Loam	Mango garden
		AUT 7.4	Elev = 54 m	33	1.5	6.8	Loam	Mango garden
		AUT 7.5		34	1.0	6.6	Loam	Mango garden
23/06/2019	11.56	AUT 8.1	N17°24'58.4"	34	1.0	7.0	Loam	Corn fields
		AUT 8.2	E100°05'16.5"	34	1.0	6.6	Loam	Corn fields
		AUT 8.3		34	2.0	6.0	Loam	Corn fields
		AUT 8.4	Elev = 58 m	34	1.0	6.2	Loam	Corn fields
		AUT 8.5		34	1.0	6.6	Loam	Corn fields

Appendix C Soil sample information (Cont.)

Date	Time	Code	GPS	Temperature	Moisture	pH	Soil texture	Agricultural area
23/06/2019	12.01	AUT 9.1	N17°25'02.3"	35	1.0	6.6	Loam	Corn fields
		AUT 9.2	E100°05'14.3"	36	1.0	6.8	Loam	Corn fields
		AUT 9.3		35	1.0	6.8	Loam	Corn fields
		AUT 9.4	Elev = 60 m	36	3.0	6.8	Loam	Corn fields
		AUT 9.5		37	1.0	6.8	Loam	Corn fields
23/06/2019	12.08	AUT 10.1	N17°25'06.5"	33	1.0	6.8	Loam	Sugarcane
		AUT 10.2	E100°05'07.8"	34	1.0	6.6	Loam	Sugarcane
		AUT 10.3		34	1.0	7.0	Loam	Sugarcane
		AUT 10.4	Elev = 59 m	34	1.0	7.0	Loam	Sugarcane
		AUT 10.5		35	1.0	6.8	Loam	Sugarcane

Appendix C Soil sample information (Cont.)

Date	Time	Code	GPS	Temperature	Moisture	pH	Soil texture	Agricultural area
23/06/2019	12.17	AUT 11.1	N17°26'11.8"	35	2.0	6.4	Loam	Mango garden
		AUT 11.2	E100°05'38.5"	35	2.0	6.4	Loam	Mango garden
		AUT 11.3		36	2.0	6.4	Loam	Mango garden
		AUT 11.4	Elev = 61 m	35	2.0	6.4	Loam	Mango garden
		AUT 11.5		37	1.0	6.8	Loam	Mango garden
23/06/2019	12.22	AUT 12.1	N17°26'14.1"	34	1.0	7.0	Loam	Mango garden
		AUT 12.2	E100°05'39.4"	34	1.0	7.0	Loam	Mango garden
		AUT 12.3		33	1.0	7.0	Loam	Mango garden
		AUT 12.4	Elev = 56 m	33	1.0	6.8	Loam	Mango garden
		AUT 12.5		33	1.0	7.0	Loam	Mango garden
23/06/2019	12.29	AUT 13.1	N17°26'13.4"	37	1.0	7.0	Loam	Mango garden
		AUT 13.2	E100°05'40.4"	36	1.0	6.8	Loam	Mango garden
		AUT 13.3		35	2.0	7.0	Loam	Mango garden
		AUT 13.4	Elev = 57 m	35	1.0	6.8	Loam	Mango garden
		AUT 13.5		35	1.0	6.8	Loam	Mango garden

Appendix C Soil sample information (Cont.)

Date	Time	Code	GPS	Temperature	Moisture	pH	Soil texture	Agricultural area
23/06/2019	13.54	AUT 14.1	N17°33'28.5"	40	1.0	6.2	Loam	Corn fields
		AUT 14.2	E100°10'03.0"	39	1.0	6.8	Loam	Corn fields
		AUT 14.3		39	1.0	6.6	Loam	Corn fields
		AUT 14.4	Elev = 66 m	39	2.0	6.4	Loam	Corn fields
		AUT 14.5		38	1.0	6.6	Loam	Corn fields
23/06/2019	14.01	AUT 15.1	N17°33'27.6"	39	1.0	7.0	Loam	Corn fields
		AUT 15.2	E100°10'05.1"	39	1.0	7.0	Loam	Corn fields
		AUT 15.3		39	1.0	6.6	Loam	Corn fields
		AUT 15.4	Elev = 62 m	39	1.0	6.8	Loam	Corn fields
		AUT 15.5		39	1.0	7.0	Loam	Corn fields

Appendix C Soil sample information (Cont.)

Date	Time	Code	GPS	Temperature	Moisture	pH	Soil texture	Agricultural area
23/06/2019	14.06	AUT 16.1	N17°32'36.7"	37	1.0	6.6	Loam	Cassava
		AUT 16.2	E100°11'33.3"	37	1.0	7.0	Loam	Cassava
		AUT 16.3		37	1.0	7.0	Loam	Cassava
		AUT 16.4	Elev = 73 m	37	1.0	7.0	Loam	Cassava
		AUT 16.5		37	1.0	7.0	Loam	Cassava
23/06/2019	14.11	AUT 17.1	N17°32'31.1"	40	1.0	6.8	Loam	Groundnut fields
		AUT 17.2	E100°12'44.3"	40	1.0	7.0	Loam	Groundnut fields
		AUT 17.3		40	1.0	7.0	Loam	Groundnut fields
		AUT 17.4	Elev = 79 m	41	1.0	6.8	Loam	Groundnut fields
		AUT 17.5		42	1.0	7.0	Loam	Groundnut fields
23/06/2019	10.20	AUT 18.1	N17°32'08.1"	39	1.0	7.0	Loam	Cassava
		AUT 18.2	E100°12'22.0"	36	1.0	7.0	Loam	Cassava
		AUT 18.3		39	1.0	6.8	Loam	Cassava
		AUT 18.4	Elev = 95 m	37	1.0	7.0	Loam	Cassava
		AUT 18.5		40	1.0	6.8	Loam	Cassava

Appendix C Soil sample information (Cont.)

Date	Time	Code	GPS	Temperature	Moisture	pH	Soil texture	Agricultural area
23/06/2019	14.26	AUT 19.1	N17°30'42.3"	37	1.0	7.0	Loam	Teak plantation
		AUT 19.2	E100°13'27.9"	37	1.0	7.0	Loam	Teak plantation
		AUT 19.3		37	1.0	7.0	Loam	Teak plantation
		AUT 19.4	Elev = 84 m	28	1.0	7.0	Loam	Teak plantation
		AUT 19.5		40	1.0	7.0	Loam	Teak plantation
23/06/2019	14.38	AUT 20.1	N17°26'40.4"	35	1.0	7.0	Loam	Mango garden
		AUT 20.2	E100°14'01.8"	35	1.0	6.8	Loam	Mango garden
		AUT 20.3		36	1.0	7.0	Loam	Mango garden
		AUT 20.4	Elev = 84 m	34	1.0	7.0	Loam	Mango garden
		AUT 20.5		37	1.0	7.0	Loam	Mango garden

Appendix C Soil sample information (Cont.)

Date	Time	Code	GPS	Temperature	Moisture	pH	Soil texture	Agricultural area
09/07/2022	08.00	ALN 1.1	N18°28'13.3"	30	5.0	6.2	Loam	Mango garden
		ALN 1.2	E98°57'26.6"	30	6.0	4.2	Loam	Mango garden
		ALN 1.3		31	5.0	5.2	Loam	Mango garden
		ALN 1.4	Elev = 306 m	30	4.0	4.8	Loam	Mango garden
		ALN 1.5		30	2.5	6.0	Loam	Mango garden
09/07/2022	08.05	ALN 2.1	N17°32'12.9"	30	5.5	5.4	Loam	Longan garden
		ALN 2.2	E098°57'27.3"	30	3.0	6.0	Loam	Longan garden
		ALN 2.3		30	5.0	5.6	Loam	Longan garden
		ALN 2.4	Elev = 308 m	29	3.0	5.8	Loam	Longan garden
		ALN 2.5		29	3.0	6.4	Loam	Longan garden
09/07/2022	08.15	ALN 3.1	N18°28'13.1"	31	4.0	5.0	Loam	Mango garden
		ALN 3.2	E98°57'27.0"	31	7.0	5.0	Loam	Mango garden
		ALN 3.3		30	6.0	5.8	Loam	Mango garden
		ALN 3.4	Elev = 303 m	30	7.0	4.2	Loam	Mango garden
		ALN 3.5		29	7.0	4.4	Loam	Mango garden

Appendix C Soil sample information (Cont.)

Date	Time	Code	GPS	Temperature	Moisture	pH	Soil texture	Agricultural area
09/07/2022	08.26	ALN 4.1	N18°28'13.7"	30	1.0	6.6	Loam	Banana plantation
		ALN 4.2	E098°57'27.87"	30	1.0	6.4	Loam	Banana plantation
		ALN 4.3		30	1.5	6.0	Loam	Banana plantation
		ALN 4.4	Elev = 301 m	29	6.5	3.6	Loam	Banana plantation
		ALN 4.5		29	4.0	5.0	Loam	Banana plantation
09/07/2022	08.30	ALN 5.1	N18°28'13.5"	32	4.0	6.2	Loam	Longan garden
		ALN 5.2	E098°57'26.0"	32	2.5	5.6	Loam	Longan garden
		ALN 5.3		30	4.0	5.6	Loam	Longan garden
		ALN 5.4	Elev = 320 m	30	4.0	5.8	Loam	Longan garden
		ALN 5.5		30	5.0	5.0	Loam	Longan garden

Appendix C Soil sample information (Cont.)

Date	Time	Code	GPS	Temperature	Moisture	pH	Soil texture	Agricultural area
09/07/2022	08.40	ALN 6.1	N18°28'04.0"	29	3.0	6.4	Loam	Longan garden
		ALN 6.2	E98°58'16.4"	29	2.0	6.8	Loam	Longan garden
		ALN 6.3		29	2.5	6.8	Loam	Longan garden
		ALN 6.4	Elev = 327 m	29	3.5	6.8	Loam	Longan garden
		ALN 6.5		28	2.0	6.8	Loam	Longan garden
09/07/2022	08.45	ALN 7.1	N18°28'04.2"	30	2.5	6.4	Loam	Vegetable garden
		ALN 7.2	E098°58'17.2"	30	3.5	5.4	Loam	Vegetable garden
		ALN 7.3		30	5.0	5.8	Loam	Vegetable garden
		ALN 7.4	Elev = 324 m	30	3.0	6.4	Loam	Vegetable garden
		ALN 7.5		30	2.5	6.2	Loam	Vegetable garden
09/07/2022	08.55	ALN 8.1	N18°28'03.2"	29	5.2	6.4	Loam	Longan garden
		ALN 8.2	E098°58'17.3"	29	2.0	6.6	Loam	Longan garden
		ALN 8.3		29	3.0	5.8	Loam	Longan garden
		ALN 8.4	Elev = 332 m	29	2.0	6.6	Loam	Longan garden
		ALN 8.5		29	2.0	6.5	Loam	Longan garden

Appendix C Soil sample information (Cont.)

Date	Time	Code	GPS	Temperature	Moisture	pH	Soil texture	Agricultural area
09/07/2022	08.59	ALN 9.1	N18°28'04.2"	30	5.5	6.4	Loam	Vegetable garden
		ALN 9.2	E098°58'16.8"	30	5.5	6.4	Loam	Vegetable garden
		ALN 9.3		30	4.5	6.6	Loam	Vegetable garden
		ALN 9.4	Elev = 326 m	30	5.6	6.2	Loam	Vegetable garden
		ALN 9.5		30	5.8	6.2	Loam	Vegetable garden
09/07/2022	09.00	ALN 10.1	N18°28'03.3"	29	3.5	6.6	Loam	Longan garden
		ALN 10.2	E098°58'17.1"	29	4.5	6.2	Loam	Longan garden
		ALN 10.3		29	4.5	6.8	Loam	Longan garden
		ALN 10.4	Elev = 329 m	29	4.6	6.4	Loam	Longan garden
		ALN 10.5		29	4.0	6.8	Loam	Longan garden

Appendix C Soil sample information (Cont.)

Date	Time	Code	GPS	Temperature	Moisture	pH	Soil texture	Agricultural area
09/07/2022	09.00	ALN 11.1	N18°28'05.1"	29	4.0	6.8	Loam	Longan garden
		ALN 11.2	E098°58'17.7"	29	3.0	6.7	Loam	Longan garden
		ALN 11.3		30	4.5	6.6	Loam	Longan garden
		ALN 11.4	Elev = 318 m	29	3.0	6.8	Loam	Longan garden
		ALN 11.5		29	3.5	6.7	Loam	Longan garden
09/07/2022	09.10	ALN 12.1	N18°28'59.6"	30	8.0	6.2	Sandy loam	Lemongrass
		ALN 12.2	E098°58'30.3"	30	7.5	6.2	Sandy loam	Lemongrass
		ALN 12.3		30	8.0	4.2	Sandy loam	Lemongrass
		ALN 12.4	Elev = 311 m	30	8.0	6.0	Sandy loam	Lemongrass
		ALN 12.5		30	8.0	5.2	Sandy loam	Lemongrass
09/07/2022	09.15	ALN 13.1	N18°28'59.6"	29	6.0	5.6	Loam	Longan garden
		ALN 13.2	E098°58'30.0"	29	4.0	6.0	Loam	Longan garden
		ALN 13.3		29	5.0	6.0	Loam	Longan garden
		ALN 13.4	Elev = 311 m	30	5.0	5.6	Loam	Longan garden
		ALN 13.5		29	8.0	4.8	Loam	Longan garden

Appendix C Soil sample information (Cont.)

Date	Time	Code	GPS	Temperature	Moisture	pH	Soil texture	Agricultural area
09/07/2022	09.19	ALN 14.1	N18°29'00.4"	28	7.5	3.8	Loam	Longan garden
		ALN 14.2	E098°58'30.4"	29	6.0	5.2	Loam	Longan garden
		ALN 14.3		29	5.0	4.4	Loam	Longan garden
		ALN 14.4	Elev = 310 m	29	8.0	4.4	Loam	Longan garden
		ALN 14.5		29	8.0	5.0	Loam	Longan garden
09/07/2022	09.23	ALN 15.1	N18°29'00.0"	29	5.0	5.6	Sandy loam	Lime garden
		ALN 15.2	E098°58'30"	29	3.5	6.2	Sandy loam	Lime garden
		ALN 15.3		29	5.0	5.5	Sandy loam	Lime garden
		ALN 15.4	Elev = 308 m	28	4.0	6.2	Sandy loam	Lime garden
		ALN 15.5		28	4.5	6.2	Sandy loam	Lime garden

Appendix C Soil sample information (Cont.)

Date	Time	Code	GPS	Temperature	Moisture	pH	Soil texture	Agricultural area
09/07/2022	09.30	ALN 16.1	N18°28'59.8"	31	5.5	6.2	Loam	Pumpkin fields
		ALN 16.2	E098°58'30.5"	30	8.0	5.0	Loam	Pumpkin fields
		ALN 16.3		30	7.5	5.8	Loam	Pumpkin fields
		ALN 16.4	Elev = 312 m	30	8.0	5.2	Loam	Pumpkin fields
		ALN 16.5		29	8.0	5.4	Loam	Pumpkin fields
09/07/2022	09.34	ALN 17.1	N18°29'04.1"	30	5.0	6.2	Loam	Lime garden
		ALN 17.2	E098°58'28.2"	30	7.0	6.2	Loam	Lime garden
		ALN 17.3		30	6.5	6.0	Loam	Lime garden
		ALN 17.4	Elev = 308 m	30	6.0	6.2	Loam	Lime garden
		ALN 17.5		30	7.0	6.2	Loam	Lime garden
09/07/2022	09.40	ALN 18.1	N18°29'04.4"	29	8.0	5.8	Loam	Longan garden
		ALN 18.2	E098°58'28.9"	29	4.5	6.4	Loam	Longan garden
		ALN18.3		29	6.0	6.0	Loam	Longan garden
		ALN 18.4	Elev = 311 m	29	5.0	6.4	Loam	Longan garden
		ALN 18.5		29	7.0	6.2	Loam	Longan garden

Appendix C Soil sample information (Cont.)

Date	Time	Code	GPS	Temperature	Moisture	pH	Soil texture	Agricultural area
09/07/2022	09.46	ALN 19.1	N18°29'04.8"	28	6.5	6.8	Loam	Longan garden
		ALN 19.2	E098°58'29.6"	29	6.5	6.4	Loam	Longan garden
		ALN 19.3		29	5.0	6.6	Loam	Longan garden
		ALN 19.4	Elev = 307 m	29	8.0	4.2	Loam	Longan garden
		ALN 19.5		29	5.0	6.4	Loam	Longan garden
09/07/2022	09.55	ALN 20.1	N18°29'04.7"	30	6.0	6.6	Loam	Longan garden
		ALN 20.2	E098°58'28.1"	30	7.0	6.2	Loam	Longan garden
		ALN 20.3		29	5.0	6.6	Loam	Longan garden
		ALN 20.4	Elev = 311 m	29	4.5	6.6	Loam	Longan garden
		ALN 20.5		29	4.0	6.6	Loam	Longan garden

Appendix C Soil sample information (Cont.)

Date	Time	Code	GPS	Temperature	Moisture	pH	Soil texture	Agricultural area
08/07/2022	12.12	APE 1.1	N17°59'48.3"	35	1.0	6.2	Loam	Vegetable garden
		APE 1.2	E099°51'17.0"	33	7.0	5.0	Loam	Vegetable garden
		APE 1.3		33	1.5	6.0	Loam	Vegetable garden
		APE 1.4	Elev = 152 m	37	2.0	6.2	Loam	Vegetable garden
		APE 1.5		34	1.0	7.0	Loam	Vegetable garden
08/07/2022	12.20	APE 2.1	N17°59'48.0"	37	2.5	6.0	Loam	Rice fields
		APE 2.2	E099°51'16.9"	37	1.0	6.0	Loam	Rice fields
		APE 2.3		37	4.0	6.2	Loam	Rice fields
		APE 2.4	Elev = 152 m	35	5.0	6.6	Loam	Rice fields
		APE 2.5		35	2.0	6.0	Loam	Rice fields
08/07/2022	12.25	APE 3.1	N17°59'48.7"	34	4.0	6.6	Loam	Betel palm
		APE 3.2	E009°51'16.4"	30	2.0	6.6	Loam	Betel palm
		APE 3.3		32	2.0	7.0	Loam	Betel palm
		APE 3.4	Elev = 177 m	29	5.0	6.4	Loam	Betel palm
		APE 3.5		29	3.5	6.4	Loam	Betel palm

Appendix C Soil sample information (Cont.)

Date	Time	Code	GPS	Temperature	Moisture	pH	Soil texture	Agricultural area
08/07/2022	12.30	APE 4.1	N17°59'46.3"	30	3.0	6.2	Loam	Betel palm
		APE 4.2	E098°51'17.0"	29	3.0	4.8	Loam	Betel palm
		APE 4.3		29	3.0	5.8	Loam	Betel palm
		APE 4.4	Elev = 152 m	29	3.0	6.2	Loam	Betel palm
		APE 4.5		29	7.0	5.2	Loam	Betel palm
08/07/2022	12.35	APE 5.1	N17°59'48.0"	29	4.5	6.4	Loam	Banana plantation
		APE 5.2	E099°51'16.9"	29	7.0	5.4	Loam	Banana plantation
		APE 5.3		29	7.0	5.2	Loam	Banana plantation
		APE 5.4	Elev = 152 m	29	7.0	5.8	Loam	Banana plantation
		APE 5.5		29	7.0	5.8	Loam	Banana plantation

Appendix C Soil sample information (Cont.)

Date	Time	Code	GPS	Temperature	Moisture	pH	Soil texture	Agricultural area
08/07/2022	12.40	APE 6.1	N17°59'45.1"	32	1.0	6.6	Loam	Tamarind garden
		APE 6.2	E099°50'17.6"	33	1.0	6.4	Loam	Tamarind garden
		APE 6.3		33	1.0	6.6	Loam	Tamarind garden
		APE 6.4	Elev = 174 m	34	1.5	6.7	Loam	Tamarind garden
		APE 6.5		33	1.0	6.6	Loam	Tamarind garden
08/07/2022	12.45	APE 7.1	N17°59'44.9"	33	3.0	5.2	Loam	Corn fields
		APE 7.2	E099°50'12.6"	34	5.0	5.4	Loam	Corn fields
		APE 7.3		32	2.5	7.2	Loam	Corn fields
		APE 7.4	Elev = 172 m	34	5.0	5.5	Loam	Corn fields
		APE 7.5		33	5.0	5.2	Loam	Corn fields
08/07/2022	12.48	APE 8.1	N17°59'45.1"	33	3.0	6.0	Loam	Corn fields
		APE 8.2	E009°50'13.4"	34	2.0	6.0	Loam	Corn fields
		APE 8.3		34	2.0	5.2	Loam	Corn fields
		APE 8.4	Elev = 156 m	34	3.0	6.2	Loam	Corn fields
		APE 8.5		34	2.5	5.8	Loam	Corn fields

Appendix C Soil sample information (Cont.)

Date	Time	Code	GPS	Temperature	Moisture	pH	Soil texture	Agricultural area
08/07/2022	12.50	APE 9.1	N17°59'45.3"	33	1.5	6.5	Loam	Papaya garden
		APE 9.2	E099°50'13.1"	33	2.0	6.3	Loam	Papaya garden
		APE 9.3		33	1.0	6.4	Loam	Papaya garden
		APE 9.4	Elev = 158 m	33	7.0	5.9	Loam	Papaya garden
		APE 9.5		32	4.0	5.8	Loam	Papaya garden
08/07/2022	12.55	APE 10.1	N17°59'45.4"	30	1.0	6.0	Loam	Mixed fruit
		APE 10.2	E099°50'12.5"	30	3.0	6.4	Loam	Mixed fruit
		APE 10.3		30	1.0	6.6	Loam	Mixed fruit
		APE 10.4	Elev = 157 m	30	1.0	6.8	Loam	Mixed fruit
		APE 10.5		30	1.0	6.6	Loam	Mixed fruit

Appendix C Soil sample information (Cont.)

Date	Time	Code	GPS	Temperature	Moisture	pH	Soil texture	Agricultural area
08/07/2022	12.40	APE 6.1	N17°59'45.1"	32	1.0	6.6	Loam	Tamarind garden
		APE 6.2	E099°50'17.6"	33	1.0	6.4	Loam	Tamarind garden
		APE 6.3		33	1.0	6.6	Loam	Tamarind garden
		APE 6.4	Elev = 174 m	34	1.5	6.7	Loam	Tamarind garden
		APE 6.5		33	1.0	6.6	Loam	Tamarind garden
08/07/2022	12.45	APE 7.1	N17°59'44.9"	33	3.0	5.2	Loam	Corn fields
		APE 7.2	E099°50'12.6"	34	5.0	5.4	Loam	Corn fields
		APE 7.3		32	2.5	7.2	Loam	Corn fields
		APE 7.4	Elev = 172 m	34	5.0	5.5	Loam	Corn fields
		APE 7.5		33	5.0	5.2	Loam	Corn fields
08/07/2022	12.48	APE 8.1	N17°59'45.1"	33	3.0	6.0	Loam	Corn fields
		APE 8.2	E009°50'13.4"	34	2.0	6.0	Loam	Corn fields
		APE 8.3		34	2.0	5.2	Loam	Corn fields
		APE 8.4	Elev = 156 m	34	3.0	6.2	Loam	Corn fields
		APE 8.5		34	2.5	5.8	Loam	Corn fields

Appendix C Soil sample information (Cont.)

Date	Time	Code	GPS	Temperature	Moisture	pH	Soil texture	Agricultural area
08/07/2022	12.50	APE 9.1	N17°59'45.3"	33	1.5	6.5	Loam	Papaya garden
		APE 9.2	E099°50'13.1"	33	2.0	6.3	Loam	Papaya garden
		APE 9.3		33	1.0	6.4	Loam	Papaya garden
		APE 9.4	Elev = 158 m	33	7.0	5.9	Loam	Papaya garden
		APE 9.5		32	4.0	5.8	Loam	Papaya garden
08/07/2022	12.55	APE 10.1	N17°59'45.4"	30	1.0	6.0	Loam	Mixed fruit
		APE 10.2	E099°50'12.5"	30	3.0	6.4	Loam	Mixed fruit
		APE 10.3		30	1.0	6.6	Loam	Mixed fruit
		APE 10.4	Elev = 157 m	30	1.0	6.8	Loam	Mixed fruit
		APE 10.5		30	1.0	6.6	Loam	Mixed fruit

Appendix C Soil sample information (Cont.)

Date	Time	Code	GPS	Temperature	Moisture	pH	Soil texture	Agricultural area
08/07/2022	13.00	APE 11.1	N17°59'49.2"	32	1.0	7.0	Loam	Teak plantation
		APE 11.2	E099°47'45.8"	33	1.0	7.0	Loam	Teak plantation
		APE 11.3		33	1.0	6.9	Loam	Teak plantation
		APE 11.4	Elev = 164 m	34	1.0	6.9	Loam	Teak plantation
		APE 11.5		35	1.0	7.0	Loam	Teak plantation
08/07/2022	13.04	APE 12.1	N17°59'51.7"	39	1.5	6.8	Loam	Jujube garden
		APE 12.2	E099°47'44.2"	38	1.5	6.8	Loam	Jujube garden
		APE 12.3		38	1.5	6.8	Loam	Jujube garden
		APE 12.4	Elev = 174 m	38	1.3	6.8	Loam	Jujube garden
		APE 12.5		38	1.0	7.0	Loam	Jujube garden
08/07/2022	13.10	APE 13.1	N17°59'49.0"	35	1.5	6.8	Clay loam	Cassava
		APE 13.2	E009°47'45.9"	35	1.1	7.0	Clay loam	Cassava
		APE 13.3		35	1.1	6.9	Clay loam	Cassava
		APE 13.4	Elev = 164 m	35	1.1	6.8	Clay loam	Cassava
		APE 13.5		35	1.1	6.9	Clay loam	Cassava

Appendix C Soil sample information (Cont.)

Date	Time	Code	GPS	Temperature	Moisture	pH	Soil texture	Agricultural area
08/07/2022	13.16	APE 14.1	N17°59'49.2"	37	1.0	6.2	Loam	Cassava
		APE 14.2	E099°47'45.8"	38	1.5	6.7	Loam	Cassava
		APE 14.3		37	1.5	6.5	Loam	Cassava
		APE 14.4	Elev = 164 m	37	0.5	6.8	Loam	Cassava
		APE 14.5		37	1.0	6.8	Loam	Cassava
08/07/2022	13.19	APE 15.1	N17°59'51.6"	37	1.0	6.8	Loam	Rice fields
		APE 15.2	E099°47'43.9"	37	1.5	6.2	Loam	Rice fields
		APE 15.3		36	0.5	6.6	Loam	Rice fields
		APE 15.4	Elev = 176 m	36	1.0	6.6	Loam	Rice fields
		APE 15.5		37	1.0	6.6	Loam	Rice fields

Appendix C Soil sample information (Cont.)

Date	Time	Code	GPS	Temperature	Moisture	pH	Soil texture	Agricultural area
08/07/2022	13.25	APE 16.1	N17°59'51.7"	39	0	7.0	Sandy loam	Cassava
		APE 16.2	E099°47'44.2"	39	0.5	7.0	Sandy loam	Cassava
		APE 16.3		39	1.0	6.8	Sandy loam	Cassava
		APE 16.4	Elev = 174 m	38	0.5	6.8	Sandy loam	Cassava
		APE 16.5		38	0.5	6.8	Sandy loam	Cassava
08/07/2022	13.27	APE 17.1	N17°59'55.0"	38	0.5	6.9	Loam	Turmeric garden
		APE 17.2	E099°47'14.9"	39	0.5	6.8	Loam	Turmeric garden
		APE 17.3		39	0.5	6.8	Loam	Turmeric garden
		APE 17.4	Elev = 124 m	39	0.5	6.8	Loam	Turmeric garden
		APE 17.5		38	0.5	6.8	Loam	Turmeric garden
08/07/2022	13.30	APE 18.1	N17°59'55.8"	40	1.0	6.8	Loam	Cassava
		APE 18.2	E009°47'15.0"	41	1.0	6.8	Loam	Cassava
		APE 18.3		40	0	6.8	Loam	Cassava
		APE 18.4	Elev = 121 m	40	1.0	6.4	Loam	Cassava
		APE 18.5		39	0.5	6.8	Loam	Cassava

Appendix C Soil sample information (Cont.)

Date	Time	Code	GPS	Temperature	Moisture	pH	Soil texture	Agricultural area
08/07/2022	13.33	APE 19.1	N18°00'07.7"	37	0	6.6	Loam	Rice fields
		APE 19.2	E099°45'27.5"	38	0	6.3	Loam	Rice fields
		APE 19.3		37	1.0	6.4	Loam	Rice fields
		APE 19.4	Elev = 127 m	40	1.0	6.4	Loam	Rice fields
		APE 19.5		39	1.0	6.6	Loam	Rice fields
08/07/2022	13.35	APE 20.1	N18°00'07.1"	36	0	6.8	Loam	Orange garden
		APE 20.2	E099°45'28.9"	36	1.5	6.6	Loam	Orange garden
		APE 20.3		36	0.5	6.6	Loam	Orange garden
		APE 20.4	Elev = 131 m	36	0.5	6.8	Loam	Orange garden
		APE 20.5		36	0.5	6.8	Loam	Orange garden

Appendix C Soil sample information (Cont.)

Date	Time	Code	GPS	Temperature	Moisture	pH	Soil texture	Agricultural area
24/10/2019	10.40	ANN 1.1	N18°29'11.6"	30	1.0	7.0	Loam	Corn fields
		ANN 1.2	E100°30'29.2"	29	1.0	6.9	Loam	Corn fields
		ANN 1.3		29	1.0	7.0	Loam	Corn fields
		ANN 1.4	Elev = 404 m	29	1.0	7.0	Loam	Corn fields
		ANN 1.5		30	1.0	7.0	Loam	Corn fields
24/10/2019	10.50	ANN 2.1	N18°30'05.3"	28	2.0	6.0	Loam	Banana plantation
		ANN 2.2	E100°31'24.8"	28	1.0	6.8	Loam	Banana plantation
		ANN 2.3		28	2.0	6.8	Loam	Banana plantation
		ANN 2.4	Elev = 377 m	28	1.0	6.8	Loam	Banana plantation
		ANN 2.5		28	2.5	6.0	Loam	Banana plantation
24/10/2019	11.00	ANN 3.1	N18°31'24.1"	28	1.0	6.8	Loam	Teak plantation
		ANN 3.2	E100°34'32.2"	28	1.5	6.8	Loam	Teak plantation
		ANN 3.3		28	1.5	6.8	Loam	Teak plantation
		ANN 3.4	Elev = 292 m	30	1.0	7.0	Loam	Teak plantation
		ANN 3.5		29	1.0	7.0	Loam	Teak plantation

Appendix C Soil sample information (Cont.)

Date	Time	Code	GPS	Temperature	Moisture	pH	Soil texture	Agricultural area
24/10/2019	11.04	ANN 4.1	N18°32'13.5"	33	1.5	6.8	Loam	Corn fields
		ANN 4.2	E100°40'08.1"	33	1.5	6.8	Loam	Corn fields
		ANN 4.3		34	1.5	6.8	Loam	Corn fields
		ANN 4.4	Elev = 238 m	34	1.0	6.6	Loam	Corn fields
		ANN 4.5		34	1.5	6.8	Loam	Corn fields
24/10/2019	11.11	ANN 5.1	N18°32'13.5"	30	1.0	6.6	Clay	Rice fields
		ANN 5.2	E100°40'07.5"	30	2.0	6.6	Clay	Rice fields
		ANN 5.3		29	2.0	6.8	Clay	Rice fields
		ANN 5.4	Elev = 236 m	31	3.5	6.6	Clay	Rice fields
		ANN 5.5		28	4.0	5.8	Clay	Rice fields

Appendix C Soil sample information (Cont.)

Date	Time	Code	GPS	Temperature	Moisture	pH	Soil texture	Agricultural area
24/10/2019	11.16	ANN 6.1	N18°32'13.5"	30	1.0	6.6	Clay	Rice fields
		ANN 6.2	E100°40'07.5"	30	2.0	6.6	Clay	Rice fields
		ANN 6.3		29	2.0	6.8	Clay	Rice fields
		ANN 6.4	Elev = 236 m	31	3.5	6.6	Clay	Rice fields
		ANN 6.5		28	4.0	5.8	Clay	Rice fields
24/10/2019	11.20	ANN 7.1	N18°38'43.4"	32	1.0	7.0	Loam	Mixed fruit
		ANN 7.2	E100°44'26.2"	30	1.5	6.8	Loam	Mixed fruit
		ANN 7.3		30	3.5	6.4	Loam	Mixed fruit
		ANN 7.4	Elev = 197 m	32	7.0	6.8	Loam	Mixed fruit
		ANN 7.5		32	2.0	6.6	Loam	Mixed fruit
24/10/2019	11.25	ANN 8.1	N18°38'43.2"	33	2.5	6.8	Loam	Banana plantation
		ANN 8.2	E100°44'27.3"	33	4.5	6.2	Loam	Banana plantation
		ANN 8.3		32	4.5	6.2	Loam	Banana plantation
		ANN 8.4	Elev = 200 m	32	4.0	6.5	Loam	Banana plantation
		ANN 8.5		32	3.0	6.8	Loam	Banana plantation

Appendix C Soil sample information (Cont.)

Date	Time	Code	GPS	Temperature	Moisture	pH	Soil texture	Agricultural area
24/10/2019	11.30	ANN 9.1	N18°38'42.1"	33	1.0	6.8	Loam	Mixed fruit
		ANN 9.2	E100°44'24.5"	32	1.0	6.8	Loam	Mixed fruit
		ANN 9.3		32	1.0	6.8	Loam	Mixed fruit
		ANN 9.4	Elev = 190 m	31	2.0	6.4	Loam	Mixed fruit
		ANN 9.5		31	1.5	6.6	Loam	Mixed fruit
24/10/2019	11.36	ANN 10.1	N18°38'43.1"	32	1.5	6.8	Loam	Mixed fruit
		ANN 10.2	E100°24'23.8"	32	1.0	6.5	Loam	Mixed fruit
		ANN 10.3		32	2.0	6.8	Loam	Mixed fruit
		ANN 10.4	Elev = 190 m	32	2.0	6.3	Loam	Mixed fruit
		ANN 10.5		32	1.5	6.4	Loam	Mixed fruit

Appendix C Soil sample information (Cont.)

Date	Time	Code	GPS	Temperature	Moisture	pH	Soil texture	Agricultural area
24/10/2019	11.16	ANN 11.1	N18°39'03.7"	34	2.0	6.3	Loam	Mango garden
		ANN 11.2	E100°44'00.5"	34	3.5	6.6	Loam	Mango garden
		ANN 11.3		34	1.0	6.8	Loam	Mango garden
		ANN 11.4	Elev = 205 m	34	3.0	6.2	Loam	Mango garden
		ANN 11.5		34	3.0	6.6	Loam	Mango garden
24/10/2019	11.20	ANN 12.1	N18°39'04.4"	32	1.0	7.0	Loam	Mango garden
		ANN 12.2	E100°43'56.6"	32	1.0	7.0	Loam	Mango garden
		ANN 12.3		32	1.0	7.0	Loam	Mango garden
		ANN 12.4	Elev = 205 m	32	1.0	7.0	Loam	Mango garden
		ANN 12.5		32	1.0	7.0	Loam	Mango garden
24/10/2019	11.25	ANN 13.1	N18°39'05.6"	31	1.0	7.0	Loam	Longan garden
		ANN 13.2	E100°43'55.8"	30	1.0	7.0	Loam	Longan garden
		ANN 13.3		32	1.0	7.0	Loam	Longan garden
		ANN 13.4	Elev = 198 m	32	1.0	7.0	Loam	Longan garden
		ANN 13.5		32	1.0	7.0	Loam	Longan garden

Appendix C Soil sample information (Cont.)

Date	Time	Code	GPS	Temperature	Moisture	pH	Soil texture	Agricultural area
24/10/2019	11.30	ANN 14.1	N18°43'51.2"	29	1.0	7.0	Loam	Vegetable garden
		ANN 14.2	E100°45'03.4"	41	1.0	7.0	Loam	Vegetable garden
		ANN 14.3		29	1.0	7.0	Loam	Vegetable garden
		ANN 14.4	Elev = 193 m	35	1.0	7.0	Loam	Vegetable garden
		ANN 14.5		35	1.0	7.0	Loam	Vegetable garden
24/10/2019	11.36	ANN 15.1	N18°48'59.2"	37	1.5	6.2	Loam	Groundnut fields
		ANN 15.2	E100°44'45.2"	37	3.0	6.4	Loam	Groundnut fields
		ANN 15.3		37	2.0	6.8	Loam	Groundnut fields
		ANN 15.4	Elev = 197 m	37	2.0	6.8	Loam	Groundnut fields
		ANN 15.5		37	2.0	6.6	Loam	Groundnut fields

Appendix C Soil sample information (Cont.)

Date	Time	Code	GPS	Temperature	Moisture	pH	Soil texture	Agricultural area
24/10/2019	11.40	ANN 16.1	N18°43'59.2"	33	4.0	6.2	Loam	Corn fields
		ANN 16.2	E100°44'44.3"	33	3.0	6.4	Loam	Corn fields
		ANN 16.3		33	3.5	6.4	Loam	Corn fields
		ANN 16.4	Elev = 205 m	32	4.5	6.2	Loam	Corn fields
		ANN 16.5		32	3.0	6.4	Loam	Corn fields
24/10/2019	11.50	ANN 17.1	N18°43'43.7"	34	1.0	7.0	Loam	Jackfruit garden
		ANN 17.2	E100°43'11.0"	34	1.0	7.0	Loam	Jackfruit garden
		ANN 17.3		34	1.0	7.0	Loam	Jackfruit garden
		ANN 17.4	Elev = 225 m	34	1.0	7.0	Loam	Jackfruit garden
		ANN 17.5		34	1.0	7.0	Loam	Jackfruit garden
24/10/2019	11.55	ANN 18.1	N18°43'43.9"	34	1.0	7.0	Loam	Corn fields
		ANN 18.2	E100°43'12.8"	34	1.0	6.6	Loam	Corn fields
		ANN 18.3		34	1.0	7.0	Loam	Corn fields
		ANN 18.4	Elev = 229 m	34	1.0	7.0	Loam	Corn fields
		ANN 18.5		34	1.0	7.0	Loam	Corn fields

Appendix C Soil sample information (Cont.)

Date	Time	Code	GPS	Temperature	Moisture	pH	Soil texture	Agricultural area
24/10/2019	11.58	ANN 19.1	N18°43'37.9"	30	1.0	6.8	Sandy loam	Para rubber
		ANN 19.2	E100°42'54.8"	31	1.2	6.4	Sandy loam	Para rubber
		ANN 19.3		32	1.0	6.8	Sandy loam	Para rubber
		ANN 19.4	Elev = 213 m	32	1.0	6.6	Sandy loam	Para rubber
		ANN 19.5		32	1.0	6.8	Sandy loam	Para rubber
24/10/2019	12.00	ANN 20.1	N18°43'37.4"	36	1.0	6.8	Loam	Cashew nut
		ANN 20.2	E100°42'55.9"	36	1.0	7.0	Loam	Cashew nut
		ANN 20.3		36	1.0	7.0	Loam	Cashew nut
		ANN 20.4	Elev = 231 m	36	1.0	7.0	Loam	Cashew nut
		ANN 20.5		36	1.0	7.0	Loam	Cashew nut

Appendix C Soil sample information (Cont.)

Date	Time	Code	GPS	Temperature	Moisture	pH	Soil texture	Agricultural area
25/10/2019	09.00	APY 1.1	N19°03'07.7"	30	1.0	7.0	Sandy loam	Mixed fruit
		APY 1.2	E099°56'07.9"	30	2.0	7.0	Sandy loam	Mixed fruit
		APY 1.3		30	1.0	7.0	Sandy loam	Mixed fruit
		APY 1.4	Elev = 423 m	30	1.0	7.0	Sandy loam	Mixed fruit
		APY 1.5		30	1.0	6.8	Sandy loam	Mixed fruit
25/10/2019	09.05	APY 2.1	N19°00'51.7"	28	2.0	6.8	Loam	Mixed fruit
		APY 2.2	E099°54'36.7"	28	7.0	5.4	Loam	Mixed fruit
		APY 2.3		28	4.0	6.2	Loam	Mixed fruit
		APY 2.4	Elev = 484 m	28	3.0	6.6	Loam	Mixed fruit
		APY 2.5		28	7.0	5.2	Loam	Mixed fruit
25/10/2019	09.13	APY 3.1	N19°00'50.9"	30	3.0	6.4	Loam	Vegetable garden
		APY 3.2	E009°54'36.9"	29	1.5	6.6	Loam	Vegetable garden
		APY 3.3		29	5.0	6.2	Loam	Vegetable garden
		APY 3.4	Elev = 490 m	29	3.0	4.0	Loam	Vegetable garden
		APY 3.5		29	3.0	6.4	Loam	Vegetable garden

Appendix C Soil sample information (Cont.)

Date	Time	Code	GPS	Temperature	Moisture	pH	Soil texture	Agricultural area
25/10/2019	09.15	APY 4.1	N19°00'50.2"	29	2.0	6.8	Loam	Vegetable garden
		APY 4.2	E099°54'35.8"	29	2.0	6.8	Loam	Vegetable garden
		APY 4.3		29	1.0	7.0	Loam	Vegetable garden
		APY 4.4	Elev = 487 m	29	1.0	7.0	Loam	Vegetable garden
		APY 4.5		29	1.0	7.0	Loam	Vegetable garden
25/10/2019	09.20	APY 5.1	N19°00'49.6"	31	1.0	7.0	Loam	Corn fields
		APY 5.2	E099°54'36.2"	29	1.0	7.0	Loam	Corn fields
		APY 5.3		30	1.0	7.0	Loam	Corn fields
		APY 5.4	Elev = 482 m	30	1.0	7.0	Loam	Corn fields
		APY 5.5		31	1.0	7.0	Loam	Corn fields

Appendix C Soil sample information (Cont.)

Date	Time	Code	GPS	Temperature	Moisture	pH	Soil texture	Agricultural area
25/10/2019	09.25	APY 6.1	N19°00'20.2"	31	1.0	7.0	Loam	Banana plantation
		APY 6.2	E099°54'02.1"	31	1.0	7.0	Loam	Banana plantation
		APY 6.3		31	2.0	7.0	Loam	Banana plantation
		APY 6.4	Elev = 490 m	31	2.0	6.8	Loam	Banana plantation
		APY 6.5		31	3.0	6.8	Loam	Banana plantation
25/10/2019	09.29	APY 7.1	N19°00'19.7"	31	1.0	7.0	Loam	Corn fields
		APY 7.2	E099°54'00.7"	31	1.0	7.0	Loam	Corn fields
		APY 7.3		31	1.0	7.0	Loam	Corn fields
		APY 7.4	Elev = 491 m	31	1.0	7.0	Loam	Corn fields
		APY 7.5		31	1.0	7.0	Loam	Corn fields
25/10/2019	09.34	APY 8.1	N19°02'59.4"	31	2.0	6.8	Loam	Corn fields
		APY 8.2	E099°56'07.9"	31	1.5	6.8	Loam	Corn fields
		APY 8.3		32	2.0	7.0	Loam	Corn fields
		APY 8.4	Elev = 425 m	32	2.0	7.0	Loam	Corn fields
		APY 8.5		31	1.0	7.0	Loam	Corn fields

Appendix C Soil sample information (Cont.)

Date	Time	Code	GPS	Temperature	Moisture	pH	Soil texture	Agricultural area
25/10/2019	09.36	APY 9.1	N19°30'53.4"	29	1.0	6.8	Loam	Banana plantation
		APY 9.2	E099°55'43.4"	28	1.0	6.8	Loam	Banana plantation
		APY 9.3		28	1.0	7.0	Loam	Banana plantation
		APY 9.4	Elev = 420 m	28	1.5	7.0	Loam	Banana plantation
		APY 9.5		29	1.0	7.0	Loam	Banana plantation
25/10/2019	09.40	APY 10.1	N19°03'55.0"	29	2.0	7.0	Loam	Marigold garden
		APY 10.2	E099°55'42.6"	29	1.0	7.0	Loam	Marigold garden
		APY 10.3		29	1.0	7.0	Loam	Marigold garden
		APY 10.4	Elev = 421 m	29	2.0	7.0	Loam	Marigold garden
		APY 10.5		30	1.0	6.8	Loam	Marigold garden

Appendix C Soil sample information (Cont.)

Date	Time	Code	GPS	Temperature	Moisture	pH	Soil texture	Agricultural area
25/10/2019	09.55	APY 11.1	N19°09'20.1"	35	2.0	6.8	Loam	Rice fields
		APY 11.2	E099°56'45.9"	34	1.0	7.0	Loam	Rice fields
		APY 11.3		34	1.0	7.0	Loam	Rice fields
		APY 11.4	Elev = 392 m	34	1.0	7.0	Loam	Rice fields
		APY 11.5		34	8.0	5.0	Loam	Rice fields
25/10/2019	10.00	APY 12.1	N19°09'21.2"	32	8.0	5.2	Clay	Rice fields
		APY 12.2	E099°57'12.9"	32	8.0	5.4	Clay	Rice fields
		APY 12.3		32	8.0	5.2	Clay	Rice fields
		APY 12.4	Elev = 389 m	32	8.0	5.4	Clay	Rice fields
		APY 12.5		32	8.0	5.2	Clay	Rice fields
25/10/2019	10.05	APY 13.1	N19°09'50.8"	33	3.0	6.8	Sandy loam	Vegetable garden
		APY 13.2	E099°59'45.8"	33	6.0	6.2	Sandy loam	Vegetable garden
		APY 13.3		34	7.0	5.4	Sandy loam	Vegetable garden
		APY 13.4	Elev = 394m	34	8.0	6.4	Sandy loam	Vegetable garden
		APY 13.5		34	6.5	6.0	Sandy loam	Vegetable garden

Appendix C Soil sample information (Cont.)

Date	Time	Code	GPS	Temperature	Moisture	pH	Soil texture	Agricultural area
25/10/2019	10.07	APY 14.1	N19°11'14.4"	32	3.0	5.4	Clay	Rice fields
		APY 14.2	E100°01'08.5"	32	4.5	4.2	Clay	Rice fields
		APY 14.3		32	8.0	5.0	Clay	Rice fields
		APY 14.4	Elev = 386m	31	8.0	4.0	Clay	Rice fields
		APY 14.5		31	8.0	5.0	Clay	Rice fields
25/10/2019	10.11	APY 15.1	N19°11'13.1"	32	6.0	6.0	Clay	Rice fields
		APY 15.2	E100°01'08.7"	32	7.0	6.2	Clay	Rice fields
		APY 15.3		33	5.0	5.8	Clay	Rice fields
		APY 15.4	Elev = 386 m	33	8.0	4.0	Clay	Rice fields
		APY 15.5		33	6.0	6.0	Clay	Rice fields

Appendix C Soil sample information (Cont.)

Date	Time	Code	GPS	Temperature	Moisture	pH	Soil texture	Agricultural area
25/10/2019	10.15	APY 16.1	N19°12'49.4"	31	2.0	6.8	Sandy loam	Mixed fruit
		APY 16.2	E100°02'19.7"	31	2.5	6.6	Sandy loam	Mixed fruit
		APY 16.3		31	3.0	6.8	Sandy loam	Mixed fruit
		APY 16.4	Elev = 390 m	31	4.0	6.6	Sandy loam	Mixed fruit
		APY 16.5		31	2.5	6.8	Sandy loam	Mixed fruit
25/10/2019	10.20	APY 17.1	N19°12'48.3"	30	3.0	6.8	Sandy loam	Rice fields
		APY 17.2	E100°02'18.9"	30	3.5	6.6	Sandy loam	Rice fields
		APY 17.3		30	4.0	6.8	Sandy loam	Rice fields
		APY 17.4	Elev = 384 m	30	3.0	6.8	Sandy loam	Rice fields
		APY 17.5		30	3.0	6.8	Sandy loam	Rice fields
25/10/2019	10.25	APY 18.1	N19°12'57.4"	32	4.0	6.2	Clay loam	Teak plantation
		APY 18.2	E100°02'24.0"	31	4.0	6.0	Clay loam	Teak plantation
		APY 18.3		30	6.0	6.2	Clay loam	Teak plantation
		APY 18.4	Elev = 388m	30	5.0	6.6	Clay loam	Teak plantation
		APY 18.5		30	3.0	6.8	Clay loam	Teak plantation

Appendix C Soil sample information (Cont.)

Date	Time	Code	GPS	Temperature	Moisture	pH	Soil texture	Agricultural area
25/10/2019	10.30	APY 19.1	N19°12'57.0"	30	5.0	6.0	Clay	Rice fields
		APY 19.2	E100°02'24.8"	30	4.0	6.6	Clay	Rice fields
		APY 19.3		30	6.0	5.8	Clay	Rice fields
		APY 19.4	Elev = 309m	30	8.0	4.8	Clay	Rice fields
		APY 19.5		30	8.0	5.6	Clay	Rice fields
25/10/2019	10.34	APY 20.1	N19°03'06.9"	31	2.0	5.6	Clay	Rice fields
		APY 20.2	E099°56'08.0"	31	2.0	5.6	Clay	Rice fields
		APY 20.3		31	2.0	5.6	Clay	Rice fields
		APY 20.4	Elev = 435 m	31	3.0	6.4	Clay	Rice fields
		APY 20.5		30	1.5	6.8	Clay	Rice fields

Appendix C Soil sample information (Cont.)

Date	Time	Code	GPS	Temperature	Moisture	pH	Soil texture	Agricultural area
08/09/2019	07.58	AST 1.1	N16°51'12.0"	28	2.0	6.4	Loam	Butterfly pea
		AST 1.2	E99°57'62.3"	27	2.0	6.8	Loam	Butterfly pea
		AST 1.3		28	1.0	7.0	Loam	Butterfly pea
		AST 1.4	Elev = 45 m	27	2.0	6.6	Loam	Butterfly pea
		AST 1.5		28	2.0	6.6	Loam	Butterfly pea
08/09/2019	08.12	AST 2.1	N16°51'16.0"	28	1.0	6.6	Clay	Sugarcane
		AST 2.2	E099°56'30.8"	28	5.0	5.4	Clay	Sugarcane
		AST 2.3		28	2.0	5.2	Loam	Sugarcane
		AST 2.4	Elev = 48 m	28	1.0	5.2	Loam	Sugarcane
		AST 2.5		28	3.0	5.0	Clay	Sugarcane
08/09/2019	08.21	AST 3.1	N16°51'47.4"	29	1.0	6.8	Loam	Banana plantation
		AST 3.2	E099°56'20.3"	29	1.0	6.6	Loam	Banana plantation
		AST 3.3		29	1.0	6.8	Loam	Banana plantation
		AST 3.4	Elev = 43 m	28	1.0	6.4	Clay	Banana plantation
		AST 3.5		29	1.0	6.8	Loam	Banana plantation

Appendix C Soil sample information (Cont.)

Date	Time	Code	GPS	Temperature	Moisture	pH	Soil texture	Agricultural area
08/09/2019	08.30	AST 4.1	N16°52'48.8"	29	1.0	6.8	Clay	Marigold garden
		AST 4.2	E099°56'20.3"	29	2.0	6.2	Loam	Marigold garden
		AST 4.3		29	1.0	7.0	Sandy	Marigold garden
		AST 4.4	Elev = 43 m	29	1.0	6.8	Clay	Marigold garden
		AST 4.5		29	1.0	7.0	Clay	Marigold garden
08/09/2019	09.43	AST 5.1	N17°04'37.3"	30	1.0	6.8	Loam	Marigold garden
		AST 5.2	E099°49'51.7"	30	1.0	6.8	Loam	Marigold garden
		AST 5.3		31	1.0	6.8	Loam	Marigold garden
		AST 5.4	Elev = 54 m	31	1.0	6.8	Loam	Marigold garden
		AST 5.5		32	1.0	6.8	Loam	Marigold garden

Appendix C Soil sample information (Cont.)

Date	Time	Code	GPS	Temperature	Moisture	pH	Soil texture	Agricultural area
08/09/2019	09.54	AST 6.1	N17°04'57.9"	30	1.0	7.0	Loam	Banana plantation
		AST 6.2	E099°49'29.2"	30	1.0	6.8	Loam	Banana plantation
		AST 6.3		31	1.0	6.8	Loam	Banana plantation
		AST 6.4	Elev = 56 m	31	1.0	6.8	Loam	Banana plantation
		AST 6.5		32	1.0	7.0	Loam	Banana plantation
08/09/2019	10.03	AST 7.1	N17°05'07.6"	29	1.0	6.8	Loam	Mixed fruit
		AST 7.2	E099°49'10.7"	30	1.0	6.8	Loam	Mixed fruit
		AST 7.3		32	1.0	6.8	Loam	Mixed fruit
		AST 7.4	Elev = 40 m	30	1.0	6.8	Loam	Mixed fruit
		AST 7.5		31	1.0	6.6	Clay	Mixed fruit
08/09/2019	10.10	AST 8.1	N17°05'14.8"	29	1.0	6.2	Clay	Chilli fields
		AST 8.2	E099°49'01.4"	28	1.0	6.0	Loam	Chilli fields
		AST 8.3		29	1.0	6.0	Loam	Chilli fields
		AST 8.4	Elev = 97 m	30	1.0	5.8	Clay	Chilli fields
		AST 8.5		29	1.0	4.8	Clay	Chilli fields

Appendix C Soil sample information (Cont.)

Date	Time	Code	GPS	Temperature	Moisture	pH	Soil texture	Agricultural area
08/09/2019	10.20	AST 9.1	N17°05'14.5"	30	4.0	6.4	Loam	Groundnut fields
		AST 9.2	E099°49'02.6"	30	1.0	6.2	Loam	Groundnut fields
		AST 9.3		30	1.0	6.8	Loam	Groundnut fields
		AST 9.4	Elev = 52 m	30	1.0	6.8	Loam	Groundnut fields
		AST 9.5		30	1.0	6.8	Loam	Groundnut fields
08/09/2019	10.30	AST 10.1	N17°05'28.7"	30	1.0	6.0	Loam	Chilli fields
		AST 10.2	E099°48'51.1"	30	2.0	6.8	Clay	Chilli fields
		AST 10.3		30	1.0	5.8	Loam	Chilli fields
		AST 10.4	Elev = 53 m	30	1.0	6.4	Loam	Chilli fields
		AST 10.5		31	2.0	5.4	Clay	Chilli fields

Appendix C Soil sample information (Cont.)

Date	Time	Code	GPS	Temperature	Moisture	pH	Soil texture	Agricultural area
08/09/2019	10.42	AST 11.1	N17°04'57.9"	32	1.0	6.6	Clay	Vegetable garden
		AST 11.2	E099°49'29.2"	33	1.0	6.8	Loam	Vegetable garden
		AST 11.3	Elev = 56 m	35	2.0	6.8	Sandy loam	Vegetable garden
		AST 11.4		33	1.0	6.8	Clay loam	Vegetable garden
		AST 11.5		35	1.0	7.0	Clay loam	Vegetable garden
08/09/2019	10.50	AST 12.1	N17°06'27.9"	31	1.0	6.2	Loam	Chilli fields
		AST 12.2	E099°49'01.1"	30	1.0	5.4	Loam	Chilli fields
		AST 12.3	Elev = 50 m	31	1.0	6.6	Clay loam	Chilli fields
		AST 12.4		31	1.0	7.0	Loam	Chilli fields
		AST 12.5		31	1.0	6.2	Clay loam	Chilli fields
08/09/2019	10.59	AST 13.1	N17°06'48.3"	30	7.5	5.0	Loam	Corn fields
		AST 13.2	E099°49'05.0"	30	8.0	5.0	Clay	Corn fields
		AST 13.3	Elev = 53 m	30	8.0	5.0	Clay	Corn fields
		AST 13.4		31	7.5	5.8	Loam	Corn fields
		AST 13.5		31	6.5	5.7	Loam	Corn fields

Appendix C Soil sample information (Cont.)

Date	Time	Code	GPS	Temperature	Moisture	pH	Soil texture	Agricultural area
08/09/2019	11.13	AST 14.1	N17°07'07.5"	30	1.0	6.6	Loam	Marian Plum
		AST 14.2	E099°49'08.4"	30	1.0	6.8	Loam	Marian Plum
		AST 14.3		31	1.0	6.8	Loam	Marian Plum
		AST 14.4	Elev = 53 m	30	3.0	6.8	Clay loam	Marian Plum
		AST 14.5		30	1.0	6.8	Loam	Marian Plum
08/09/2019	11.23	AST 15.1	N17°07'41.7"	31	1.0	6.6	Loam	Corn fields
		AST 15.2	E099°48'18.9"	32	1.0	6.8	Loam	Corn fields
		AST 15.3		31	2.0	6.6	Loam	Corn fields
		AST 15.4	Elev = 51 m	30	1.0	6.6	Loam	Corn fields
		AST 15.5		32	1.0	6.8	Loam	Corn fields

Appendix C Soil sample information (Cont.)

Date	Time	Code	GPS	Temperature	Moisture	pH	Soil texture	Agricultural area
08/09/2019	11.30	AST 16.1	N17°07'41.1"	30	1.0	6.8	Loam	Groundnut fields
		AST 16.2	E099°49'20.1"	30	1.0	6.6	Loam	Groundnut fields
		AST 16.3		30	1.0	6.6	Loam	Groundnut fields
		AST 16.4	Elev = 53 m	30	2.0	6.4	Loam	Groundnut fields
		AST 16.5		30	1.0	6.8	Loam	Groundnut fields
08/09/2019	11.40	AST 17.1	N17°08'43.6"	30	1.0	6.6	Loam	Papaya garden
		AST 17.2	E099°49'26.3"	30	2.0	6.4	Loam	Papaya garden
		AST 17.3		30	1.0	7.0	Loam	Papaya garden
		AST 17.4	Elev = 46 m	30	3.0	6.2	Loam	Papaya garden
		AST 17.5		30	1.0	6.8	Loam	Papaya garden
08/09/2019	11.53	AST 18.1	N17°09'40.2"	30	2.0	6.0	Loam	Jackfruit garden
		AST 18.2	E099°49'45.0"	30	2.0	6.0	Loam	Jackfruit garden
		AST 18.3		30	2.0	6.4	Loam	Jackfruit garden
		AST 18.4	Elev = 51 m	31	8.0	5.0	Loam	Jackfruit garden
		AST 18.5		30	6.5	5.2	Loam	Jackfruit garden

Appendix C Soil sample information (Cont.)

Date	Time	Code	GPS	Temperature	Moisture	pH	Soil texture	Agricultural area
08/09/2019	12.05	AST 19.1	N17°11'02.0"	30	3.0	6.4	Sandy loam	Sapodilla garden
		AST 19.2	E099°51'09.3"	32	1.0	6.8	Sandy loam	Sapodilla garden
		AST 19.3		33	1.0	6.8	Loam	Sapodilla garden
		AST 19.4	Elev = 65 m	32	4.5	6.6	Sandy loam	Sapodilla garden
		AST 19.4		32	3.0	6.4	Loam	Sapodilla garden
08/09/2019	12.10	AST 20.1	N17°11'02.9"	30	1.0	6.6	Loam	Sapodilla garden
		AST 20.2	E099°51'13.5"	29	1.0	6.4	Loam	Sapodilla garden
		AST 20.3		32	3.0	6.6	Loam	Sapodilla garden
		AST 20.4	Elev = 58 m	31	3.0	6.4	Loam	Sapodilla garden
		AST 20.5		30	1.0	6.8	Loam	Sapodilla garden

Appendix C Soil sample information (Cont.)

Date	Time	Code	GPS	Temperature	Moisture	pH	Soil texture	Agricultural area
16/11/2020	08.20	ACM 1.1	N16°45'20.8"	26	1.0	7.0	Loam	Mango garden
		ACM 1.2	E100°11'28.8"	26	1.5	6.8	Loam	Mango garden
		ACM 1.3		26	2.0	6.7	Loam	Mango garden
		ACM 1.4	Elev = 47 m	27	1.0	7.0	Loam	Mango garden
		ACM 1.5		27	1.0	6.8	Loam	Mango garden
16/11/2020	08.23	ACM 2.1	N16°45'20.8"	26	2.0	6.8	Loam	Mango garden
		ACM 2.2	E100°11'28.8"	27	1.5	6.6	Loam	Mango garden
		ACM 2.3		26	1.0	6.8	Loam	Mango garden
		ACM 2.4	Elev = 47 m	27	2.0	6.6	Loam	Mango garden
		ACM 2.5		27	2.0	6.6	Loam	Mango garden
16/11/2020	08.28	ACM 3.1	N18°52'38.8"	32	2.0	6.8	Loam	Vegetable garden
		ACM 3.2	E98°58'47.1"	32	2.0	6.8	Loam	Vegetable garden
		ACM 3.3		32	1.5	6.8	Loam	Vegetable garden
		ACM 3.4	Elev = 317 m	32	1.5	7.0	Loam	Vegetable garden
		ACM 3.5		32	2.5	6.6	Loam	Vegetable garden

Appendix C Soil sample information (Cont.)

Date	Time	Code	GPS	Temperature	Moisture	pH	Soil texture	Agricultural area
16/11/2020	08.30	ACM 4.1	N18°52'39.7"	32	3.0	6.6	Loam	Marigold garden
		ACM 4.2	E98°58'45.4"	32	3.0	6.8	Loam	Marigold garden
		ACM 4.3		32	4.0	6.4	Loam	Marigold garden
		ACM 4.4	Elev = 314 m	32	2.0	6.8	Loam	Marigold garden
		ACM 4.5		32	2.0	6.6	Loam	Marigold garden
16/11/2020	08.35	ACM 5.1	N18°55'57.9"	29	4.5	5.8	Loam	Vegetable garden
		ACM 5.2	E09°58'39.3"	29	2.0	6.6	Loam	Vegetable garden
		ACM 5.3		29	3.0	6.6	Loam	Vegetable garden
		ACM 5.4	Elev = 318 m	29	3.0	5.8	Loam	Vegetable garden
		ACM 5.5		30	4.0	6.2	Loam	Vegetable garden

Appendix C Soil sample information (Cont.)

Date	Time	Code	GPS	Temperature	Moisture	pH	Soil texture	Agricultural area
16/11/2020	08.40	ACM 6.1	N18°53'57.9"	29	1.0	7.0	Loam	Vegetable garden
		ACM 6.2	E09°58'3.9"	29	1.0	7.0	Loam	Vegetable garden
		ACM 6.3		29	1.0	7.0	Loam	Vegetable garden
		ACM 6.4	Elev = 318 m	29	1.5	6.8	Loam	Vegetable garden
		ACM 6.5		29	2.0	6.6	Loam	Vegetable garden
16/11/2020	08.46	ACM 7.1	N18°55'57.9"	30	5.0	6.4	Loam	Corn fields
		ACM 7.2	E09°58'37.8"	30	2.5	6.6	Loam	Corn fields
		ACM 7.3		30	5.5	6.2	Loam	Corn fields
		ACM 7.4	Elev = 318 m	30	3.0	6.2	Loam	Corn fields
		ACM 7.5		30	2.5	6.6	Loam	Corn fields
16/11/2020	08.50	ACM 8.1	N18°55'57.5"	30	4.5	5.4	Loam	Vegetable garden
		ACM 8.2	E09°58'37.7"	30	5.0	5.2	Loam	Vegetable garden
		ACM 8.3		30	3.0	6.2	Loam	Vegetable garden
		ACM 8.4	Elev = 318 m	31	5.5	6.0	Loam	Vegetable garden
		ACM 8.5		30	7.5	5.2	Loam	Vegetable garden

Appendix C Soil sample information (Cont.)

Date	Time	Code	GPS	Temperature	Moisture	pH	Soil texture	Agricultural area
16/11/2020	09.03	ACM 9.1	N18°53'55.9"	30	1.5	6.8	Loam	Mango garden
		ACM 9.2	E98°59'04.6"	30	3.5	6.7	Loam	Mango garden
		ACM 9.3		30	2.0	6.6	Loam	Mango garden
		ACM 9.4	Elev = 320 m	30	2.0	6.8	Loam	Mango garden
		ACM 9.5		31	2.0	6.8	Loam	Mango garden
16/11/2020	09.12	ACM 10.1	N18°53'55.5"	29	1.5	6.8	Loam	Mango garden
		ACM 10.2	E098°59'04.2"	29	2.0	6.8	Loam	Mango garden
		ACM 10.3		29	2.0	6.6	Loam	Mango garden
		ACM 10.4	Elev = 320 m	29	1.5	6.8	Loam	Mango garden
		ACM 10.5		30	1.5	6.8	Loam	Mango garden

Appendix C Soil sample information (Cont.)

Date	Time	Code	GPS	Temperature	Moisture	pH	Soil texture	Agricultural area
16/11/2020	09.15	ACM 11.1	N18°53'49.5"	30	1.5	6.8	Loam	Chilli fields
		ACM 11.2	E098°59'23.4"	30	2.0	6.4	Loam	Chilli fields
		ACM 11.3		30	3.0	6.6	Loam	Chilli fields
		ACM 11.4	Elev = 314 m	30	5.0	6.5	Loam	Chilli fields
		ACM 11.5		30	4.0	6.4	Loam	Chilli fields
16/11/2020	09.20	ACM 12.1	N18°53'45.4"	30	9.0	4.5	Clay	Taro plantations
		ACM 12.2	E098°59'41.5"	30	9.0	5.2	Clay	Taro plantations
		ACM 12.3		30	9.0	5.0	Clay	Taro plantations
		ACM 12.4	Elev = 318 m	30	9.0	5.4	Clay	Taro plantations
		ACM 12.5		30	9.0	4.2	Clay	Taro plantations
16/11/2020	09.26	ACM 13.1	N18°53'07.5"	26	2.0	6.8	Sandy loam	Longan garden
		ACM 13.2	E098°59'35.6"	26	2.0	6.8	Sandy loam	Longan garden
		ACM 13.3		26	1.0	6.8	Sandy loam	Longan garden
		ACM 13.4	Elev = 315 m	26	2.0	6.7	Sandy loam	Longan garden
		ACM 13.5		26	1.5	6.8	Sandy loam	Longan garden

Appendix C Soil sample information (Cont.)

Date	Time	Code	GPS	Temperature	Moisture	pH	Soil texture	Agricultural area
16/11/2020	09.28	ACM 14.1	N18°53'06.5"	26	8.0	4.8	Sandy loam	Margaret flower
		ACM 14.2	E98°59'36.0"	27	6.5	5.0	Sandy loam	Margaret flower
		ACM 14.3		27	7.5	4.4	Sandy loam	Margaret flower
		ACM 14.4	Elev = 319 m	28	6.0	5.4	Sandy loam	Margaret flower
		ACM 14.5		28	9.0	4.7	Sandy loam	Margaret flower
16/11/2020	09.32	ACM 15.1	N18°53'18.2"	28	4.0	6.3	Sandy loam	Cutter flower
		ACM 15.2	E098°59'08.2"	28	4.5	6.6	Sandy loam	Cutter flower
		ACM 15.3		28	8.5	5.0	Sandy loam	Cutter flower
		ACM 15.4	Elev = 317 m	28	9.0	5.0	Sandy loam	Cutter flower
		ACM 15.5		28	4.0	6.2	Sandy loam	Cutter flower

Appendix C Soil sample information (Cont.)

Date	Time	Code	GPS	Temperature	Moisture	pH	Soil texture	Agricultural area
16/11/2020	09.40	ACM 16.1	N18°53'10.8"	30	4.0	5.6	Loam	Vegetable garden
		ACM 16.2	E098°59'21.2"	30	4.0	5.4	Loam	Vegetable garden
		ACM 16.3		30	2.5	6.5	Loam	Vegetable garden
		ACM 16.4	Elev = 313 m	30	4.5	6.2	Loam	Vegetable garden
		ACM 16.5		30	3.0	6.4	Loam	Vegetable garden
16/11/2020	09.45	ACM 17.1	N18°53'12.2"	29	6.0	5.4	Loam	Vegetable garden
		ACM 17.2	E098°59'22.9"	29	2.0	6.4	Loam	Vegetable garden
		ACM 17.3		29	5.0	6.4	Loam	Vegetable garden
		ACM 17.4	Elev = 313 m	29	9.0	5.2	Loam	Vegetable garden
		ACM 17.5		29	6.5	5.6	Loam	Vegetable garden
16/11/2020	09.49	ACM 18.1	N18°55'18.0"	32	2.0	6.6	Loam	Cutter flower
		ACM 18.2	E098°59'08.8"	32	2.0	7.0	Loam	Cutter flower
		ACM 18.3		32	2.0	6.8	Loam	Cutter flower
		ACM 18.4	Elev = 317 m	32	1.0	7.0	Loam	Cutter flower
		ACM 18.5		32	1.0	7.0	Loam	Cutter flower

Appendix C Soil sample information (Cont.)

Date	Time	Code	GPS	Temperature	Moisture	pH	Soil texture	Agricultural area
16/11/2020	09.55	ACM 19.1	N18°53'17.5"	29	6.0	5.4	Clay	Cutter flower
		ACM 19.2	E98°59'08.1"	29	6.0	5.6	Clay	Cutter flower
		ACM 19.3		29	9.0	4.8	Clay	Cutter flower
		ACM 19.4	Elev = 317 m	29	9.0	4.5	Clay	Cutter flower
		ACM 19.5		28	9.0	4.4	Clay	Cutter flower
16/11/2020	10.02	ACM 20.1	N18°53'17.1"	30	5.0	6.5	Loam	Margaret flower
		ACM 20.2	E098°59'08.2"	29	4.5	6.0	Loam	Margaret flower
		ACM 20.3		29	8.0	6.4	Clay	Margaret flower
		ACM 20.4	Elev = 317 m	29	7.0	5.4	Clay	Margaret flower
		ACM 20.5		29	5.0	5.2	Loam	Margaret flower

Appendix C Soil sample information (Cont.)

Date	Time	Code	GPS	Temperature	Moisture	pH	Soil texture	Agricultural area
17/11/2020	07.30	ACR 1.1	N19°57'15.6"	23	1.0	7.0	Sandy loam	Vegetable garden
		ACR 1.2	E099°50'17.1"	23	1.0	6.8	Sandy loam	Vegetable garden
		ACR 1.3		23	1.0	6.8	Sandy loam	Vegetable garden
		ACR 1.4	Elev = 408 m	23	1.0	6.8	Sandy loam	Vegetable garden
		ACR 1.5		23	1.0	7.0	Sandy loam	Vegetable garden
17/11/2020	07.33	ACR 2.1	N19°56'55.9"	22	1.5	6.8	Loam	Vegetable garden
		ACR 2.2	E099°49'37.9"	22	1.0	6.9	Loam	Vegetable garden
		ACR 2.3		22	1.8	6.9	Loam	Vegetable garden
		ACR 2.4	Elev = 403 m	22	1.0	7.0	Loam	Vegetable garden
		ACR 2.5		22	1.5	6.8	Loam	Vegetable garden
17/11/2020	07.36	ACR 3.1	N19°56'17.8"	24	1.5	6.8	Sandy loam	Pineapple fields
		ACR 3.2	E099°48'10.8"	23	1.0	7.0	Sandy loam	Pineapple fields
		ACR 3.3		24	1.0	6.9	Sandy loam	Pineapple fields
		ACR 3.4	Elev = 410 m	24	1.5	6.8	Sandy loam	Pineapple fields
		ACR 3.5		24	1.5	6.8	Sandy loam	Pineapple fields

Appendix C Soil sample information (Cont.)

Date	Time	Code	GPS	Temperature	Moisture	pH	Soil texture	Agricultural area
17/11/2020	07.40	ACR 4.1	N19°56'17.1"	25	1.0	7.0	Sandy loam	Pineapple fields
		ACR 4.2	E99°48'10.5"	25	1.0	7.0	Sandy loam	Pineapple fields
		ACR 4.3		23	1.0	7.0	Sandy loam	Pineapple fields
		ACR 4.4	Elev = 410 m	25	1.0	7.0	Sandy loam	Pineapple fields
		ACR 4.5		25	1.0	7.0	Sandy loam	Pineapple fields
17/11/2020	07.45	ACR 5.1	N19°55'00.6"	24	1.0	7.0	Sandy loam	Cassava
		ACR 5.2	E099°47'01.5"	24	1.0	7.0	Sandy loam	Cassava
		ACR 5.3		24	1.0	7.0	Sandy loam	Cassava
		ACR 5.4	Elev = 401 m	24	1.0	7.0	Sandy loam	Cassava
		ACR 5.5		24	1.0	7.0	Sandy loam	Cassava

Appendix C Soil sample information (Cont.)

Date	Time	Code	GPS	Temperature	Moisture	pH	Soil texture	Agricultural area
17/11/2020	07.50	ACR 6.1	N19°55'09.3"	23	1.5	6.8	Loam	Cassava
		ACR 6.2	E099°47'00.1"	23	1.5	6.8	Loam	Cassava
		ACR 6.3		23	1.0	6.9	Loam	Cassava
		ACR 6.4	Elev = 403 m	23	1.0	6.9	Loam	Cassava
		ACR 6.5		23	1.0	6.9	Loam	Cassava
17/11/2020	07.54	ACR 7.1	N19°54'52.0"	24	1.5	7.0	Loam	Cassava
		ACR 7.2	E099°46'55.4"	24	1.0	7.0	Loam	Cassava
		ACR 7.3		24	1.0	7.0	Loam	Cassava
		ACR 7.4	Elev = 398 m	23	1.0	7.0	Loam	Cassava
		ACR 7.5		23	1.0	7.0	Loam	Cassava
17/11/2020	08.02	ACR 8.1	N19°55'09.1"	23	3.0	6.6	Loam	Pineapple fields
		ACR 8.2	E099°47'00.1"	23	2.0	6.7	Loam	Pineapple fields
		ACR 8.3		23	1.2	6.8	Loam	Pineapple fields
		ACR 8.4	Elev = 403 m	23	3.0	6.6	Loam	Pineapple fields
		ACR 8.5		23	3.5	6.2	Loam	Pineapple fields

Appendix C Soil sample information (Cont.)

Date	Time	Code	GPS	Temperature	Moisture	pH	Soil texture	Agricultural area
17/11/2020	08.05	ACR 9.1	N19°55'09.6"	25	2.0	6.8	Loam	Corn fields
		ACR 9.2	E99°47'00.3"	25	1.0	7.0	Loam	Corn fields
		ACR 9.3		25	1.0	7.0	Loam	Corn fields
		ACR 9.4	Elev = 403 m	25	1.0	6.9	Loam	Corn fields
		ACR 9.5		24	1.0	6.9	Loam	Corn fields
17/11/2020	08.11	ACR 10.1	N19°51'43.1"	24	2.0	7.0	Loam	Pineapple fields
		ACR 10.2	E099°46'04.6"	24	1.5	6.9	Loam	Pineapple fields
		ACR 10.3		23	1.5	7.0	Loam	Pineapple fields
		ACR 10.4	Elev = 414 m	23	1.5	6.9	Loam	Pineapple fields
		ACR 10.5		23	1.5	6.9	Loam	Pineapple fields

Appendix C Soil sample information (Cont.)

Date	Time	Code	GPS	Temperature	Moisture	pH	Soil texture	Agricultural area
17/11/2020	08.15	ACR 11.1	N19°51'43.2"	24	1.0	7.0	Loam	Cassava
		ACR 11.2	E099°46'04.5"	24	1.0	7.0	Loam	Cassava
		ACR 11.3		24	2.0	7.0	Loam	Cassava
		ACR 11.4	Elev = 414 m	24	1.5	7.0	Loam	Cassava
		ACR 11.5		24	1.0	7.0	Loam	Cassava
17/11/2020	08.20	ACR 12.1	N19°50'24.8"	28	8.0	5.2	Loam	Vegetable garden
		ACR 12.2	E099°43'57.3"	28	5.0	5.6	Loam	Vegetable garden
		ACR 12.3		27	5.0	6.2	Loam	Vegetable garden
		ACR 12.4	Elev = 425 m	27	9.0	5.0	Loam	Vegetable garden
		ACR 12.5		27	9.0	5.0	Loam	Vegetable garden
17/11/2020	08.24	ACR 13.1	N19°50'03.7"	27	3.0	6.0	Loam	Pineapple fields
		ACR 13.2	E099°44'34.9"	26	2.0	6.8	Loam	Pineapple fields
		ACR 13.3		26	1.0	6.9	Loam	Pineapple fields
		ACR 13.4	Elev = 429 m	26	1.0	7.9	Loam	Pineapple fields
		ACR 13.5		28	2.0	7.8	Loam	Pineapple fields

Appendix C Soil sample information (Cont.)

Date	Time	Code	GPS	Temperature	Moisture	pH	Soil texture	Agricultural area
17/11/2020	08.26	ACR 14.1	N19°50'03.8"	26	2.0	6.8	Loam	Lemongrass
		ACR 14.2	E99°44'34.6"	25	1.5	6.8	Loam	Lemongrass
		ACR 14.3		25	2.0	6.8	Loam	Lemongrass
		ACR 14.4	Elev = 429 m	25	2.0	6.8	Loam	Lemongrass
		ACR 14.5		25	2.0	6.8	Loam	Lemongrass
17/11/2020	08.30	ACR 15.1	N19°47'35.7"	26	1.5	6.8	Loam	Corn fields
		ACR 15.2	E099°44'56.2"	25	1.5	6.8	Loam	Corn fields
		ACR 15.3		25	1.5	6.8	Loam	Corn fields
		ACR 15.4	Elev = 431 m	25	2.0	6.9	Loam	Corn fields
		ACR 15.5		25	1.5	6.9	Loam	Corn fields

Appendix C Soil sample information (Cont.)

Date	Time	Code	GPS	Temperature	Moisture	pH	Soil texture	Agricultural area
17/11/2020	08.35	ACR 16.1	N19°46'06.1"	23	1.0	7.0	Loam	Corn fields
		ACR 16.2	E099°44'12.9"	24	1.0	7.0	Loam	Corn fields
		ACR 16.3		23	1.0	7.0	Loam	Corn fields
		ACR 16.4	Elev = 405 m	24	1.0	7.0	Loam	Corn fields
		ACR 16.5		23	2.0	6.8	Loam	Corn fields
17/11/2020	08.37	ACR 17.1	N19°41'14.5"	25	2.0	6.4	Clay	Strawberry fields
		ACR 17.2	E099°43'29.6"	25	3.0	6.8	Clay	Strawberry fields
		ACR 17.3		25	2.0	6.6	Clay	Strawberry fields
		ACR 17.4	Elev = 447 m	25	2.5	6.8	Clay	Strawberry fields
		ACR 17.5		25	6.0	6.6	Clay	Strawberry fields
17/11/2020	08.39	ACR 18.1	N19°41'12.1"	23	1.0	6.8	Loam	Marigold garden
		ACR 18.2	E099°43'31.9"	23	2.0	6.6	Loam	Marigold garden
		ACR 18.3		23	5.0	6.1	Loam	Marigold garden
		ACR 18.4	Elev = 439 m	24	4.5	6.4	Loam	Marigold garden
		ACR 18.5		24	4.5	6.2	Loam	Marigold garden

Appendix C Soil sample information (Cont.)

Date	Time	Code	GPS	Temperature	Moisture	pH	Soil texture	Agricultural area
17/11/2020	08.41	ACR 19.1	N19°41'13.2"	24	1.0	7.0	Sandy loam	Para rubber
		ACR 19.2	E099°43'32.2"	24	1.0	7.0	Sandy loam	Para rubber
		ACR 19.3		24	1.0	7.0	Sandy loam	Para rubber
		ACR 19.4	Elev = 439 m	25	1.0	7.0	Sandy loam	Para rubber
		ACR 19.5		24	1.0	7.0	Sandy loam	Para rubber
17/11/2020	08.45	ACR 20.1	N19°40'28.4"	26	1.0	7.0	Loam	Sun hemp garden
		ACR 20.2	E099°43'48.8"	26	1.0	7.0	Loam	Sun hemp garden
		ACR 20.3		26	1.0	7.0	Loam	Sun hemp garden
		ACR 20.4	Elev = 426 m	27	1.0	7.0	Loam	Sun hemp garden
		ACR 20.5		27	1.0	7.0	Loam	Sun hemp garden