International Journal of Agricultural Technology 2025 Vol. 21(2):655-672
DOI: https://doi.org/10.63369/ijat.2025.21.2.655-672
Available online http://www.ijat-aatsea.com
ISSN 2630-0192 (Online)

Species of commercially-farmed crickets in Thailand

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Ruang-Rit, K., Poommarin, P. and Seritrakul, P. (2025). Species of commercially-farmed crickets in Thailand. International Journal of Agricultural Technology 21(2):655-672.

Abstract Result found that there are four species of commercially farmed crickets in Thailand with some farms raising two or more types of crickets. First, the "Thongdum Cricket" (Two-spotted cricket, *Gryllus bimaculatus*) is the most commonly raised in 92 farms. The "Thongdum Cricket" has two phenotypes as the red-coloured and black-coloured body. Genetic evidence from mitochondrial DNA sequences at the cytochrome oxidase I (COI) gene found that these two phenotypes are found in the same species. Second, the "House Cricket" (*Acheta domesticus*) commonly known in Thai as "Sa-ding" is raised in 65 farms. Third, the "Thongdaeng Cricket" (Ground Cricket*, Teleogryllus mitratus*) has yellow V-shape mark between the two eyes when viewed anteriorly and is raised in 33 farms. The name "Jing Krong" is often mistaken which instead of the correct one of "Thongdaeng Cricket". The genetic sequence information confirmed these species should be named "Thongdaeng Cricket". Last, "Thonglai Cricket" (Crazy red field cricket, *Gryllus locorojo*) has distinct M-shaped marking on the head, and the body is red-brown. This species is raised in 15 farms, and is often mistakenly called "Vietnam Cricket" because they were imported originally from Vietnam.

Keywords: Cricket, Cricket's species, Commercial cricket farming, Cricket farms in Thailand

Introduction

Cricket has been commercially farmed in Thailand for over 30 years. It was reported that there were approximately 20,000 cricket farms in Thailand for domestic consumption and export (Hanboonsong *et al*., 2013). A cricket is among the most protein-rich edible insects. Compared with the same 100g of other meat, the cricket has about 68g of protein, chicken has 21.3g of protein and beef has 30g of all (Singh, 2017).

The Bureau of Agricultural Commodity and Food Standards (ACFS), Ministry of Agriculture and Cooperatives has recently announced the guidance on the application of Thai agricultural standard TAS 8202 (G) – 2017 relating to "Good Agricultural Practices (GAP) for Cricket Farm", therefore it is now a good opportunity for Thai farmers to share knowledge and expertise in sustainable cricket cultivation. In 2015, the total annual output was about 7,500 tons, with a market value of about 1,000 million baht for crickets (Thansettakij, 2016).

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Crickets are increasingly being accepted by consumers around the world as an alternative protein source. The exports are both in the forms of dried crickets and cricket powder, exported to different markets such as the European Union, the United States, Japan, etc., and are likely to grow significantly. According to the forecast of Meticulous Research TM Analysis (2018), the demand for crickets from 2018 to 2023 in the USA has been increasing by about 32.5%, in the European Union such as Belgium, the Netherlands, Denmark, France and the UK by about 27.6-32.0%, including in Thailand itself by about 27.2%.

In Thailand, there are many types of commercial crickets. Although crickets have been domesticated for a long time, with the morphology of some crickets, farmers are still confused regarding the use of different names used for referring to the same species, or the name of the species to be called incorrectly. This causes miscommunication among academics, farmers, and the media who want to present information on cricket farming in Thailand. To realize the types of crickets that are commercially raised in Thailand and to identify species from the morphological and genetic characteristics of crickets, it is therefore necessary to study the types of crickets that are commercially farmed in Thailand so that they can be called correctly. The study aimed to investigate the distinct morphology of each cricket species to establish accurate classification and naming and ensure correct information for the public.

Materials and methods

Ethics statement

All animal procedure was done in accordance with the protocol reviewed and approved by Silpakorn University Institutional Animal Care and Use Committee (Project ID: 08/2564, approved on DATE 28 April, 2021).

Sample collection

Commercially-raised crickets were collected from farms in 46 provinces across the 6 geographical regions of Thailand from April 2021 to October 2022. For each farm, 30 samples were collected for both the nymph stage and adult stage for both male and female. The northern provinces are Chiangmai (CMI), Lamphun (LPN), Lampang (LPG), Nan (NAN), Phrae (PRE), Uttaradit (UTD). The central provinces are Sukhothai (STI), Phitsanulok (PLK), Pichit (PCT), Kamphaengphet (KPT), Suphanburi (SPB), Nakonpathom (NPT), Saraburi (SRI), Chainat (CNT), Lopburi (LRI), Phranakornsri Ayutthaya (AYA), Angthong (ATG), Singburi (SBR), and Samutprakarn (SPK). The northeastern provinces are Nakonratchasima (NMA), Chaiyaphum (CPM), Khonkaen (KKN), Udonthani (UDN), Sakonnakhon (SNK), Nakhonphanom (NPM), Kanlasin (KSN), Mahasarakham (MKM), Yasothorn (YST), Ubonratchatani (UBI), Surin (SRN), and Burirum (BRM). The eastern provinces are Rayong (RYG), Chonburi (CBI), Chachengsao (CCO), and Srakaew (SKW). The western provinces are Petchaburi (PBI), Prachuapkirikhan (PKN), Rachaburi (RBR), Kanchanaburi (KRI). The southern provinces are Chumphon (CPN), Patthalung (PLG), Nakhonsrithammarat (NRT), Songkhla (SKA), Ranong (RNG), Trang (TRG), and Suratthani (SRT). The collected samples were analyzed morphologically and then immediately stored in 70% ethanol for DNA extraction for genetic diversity analysis.

Sample classification by morphological evidence

Nymph and adult crickets for both male and female were photographed and examined for external morphological features such as the head, thorax, abdomen, body colour, body size, wing shape. These features were then compared to the reference database for each respective species.

Sample classification by genetic analysis

DNA extraction

For each farm, 3 alcohol-preserved crickets were randomly sampled per species and sex. One back leg was removed for DNA extraction per individual cricket, for a total of 3 legs per extraction. DNA was extracted using gSYNC^{TM} DNA Extraction Kit (Geneaid). Samples were homogenized using Disposable PELLET PESTLES® in solution from DNA Extraction Kit.

PCR and sequencing

The extracted genomic DNA was quantified using a spectrophotometer and then amplified by touchdown polymerase chain reaction (TDPCR) using primers specific for each of the mitochondrial gene, the COI gene region which is commonly used for insect identification. Materials and Touchdown PCR conditions are shown in Table 1 and 2.

PCR products were run on 1% agarose gel electrophoresis to verify correct amplicon sizes. Then the DNA samples were sent for sequencing (U2bio, Thailand) and the resulting nucleotide sequences were aligned with each other and analyzed using the basic local alignment search tool (BLAST) against the publicly-available database on GenBank, National Center for Biotechnology Information: NCBI. Then, DNA sequences were aligned and constructed the phylogenetic tree using Multiple Sequence Alignment by CLUSTALW developed by Kyoto University Bioinformatics Center.

Table 1. Primers used in this study

Target gene	Primer	Primer Sequence 5’ to 3’	Product size (bp.)	Reference	Organism
Cytochrome oxidase subunit 1	Degen_COI_F	YCGAACAGAAYTWGGACAACC	500	This study[1]	*G. bimaculatus, T. mitratus*
	Degen_COI_R	GCACCDGCTARKACTGGTAR			
	Ad_COI_F	TCGAACAGAACTTGGACAACC	500	This study[1]	*A. domesticus*
	Ad_COI_R	GCACCTGCTAGGACTGGTAA			
	Tm_COI_F	CGGACAGAACTAGGACAACCA	565	This study[1]	*T. mitratus*
	Tm_COI_R	CTCCCCCTGCAGGATCAAAG			
	GA_COI_F	AAGAAGCCTTTGGTACATTAGGAA	525	Weissman *et al.*, 2009	*G. locorojo*
	GA_COI_R	ATCGTCGTGGTATTCCTGCT			
Cytochrome b	Degen_CytB_F	YTTACCMACCCCATCWAATA	750	This study[1]	*G. bimaculatus*
	Degen_CytB_R	KGGTTGRATATGKAYTGGRG			
16S ribosomal RNA	Gryllus_16S_F	CGCCTGTTTATCAAAAACAT	537	Weissman *et al.*, 2012	*G. locorojo*
	Gryllus_16S_R	CCGGTTGAACTCAGATCA			

1/Primers were created in this research.

Table 2. Touchdown PCR conditions for each pair of primers

Primer		Denaturation	Annealing	Elongation	Number of cycles
Forward	**Reverse**				
Degen_COI_F	Degen_COI_R	30s at 94°C	30s at 65°C to 55°C	30s at 72°C	20
		30s at 95°C	30s at 55°C	30s at 72°C	10
Ad_COI_F	Ad_COI_R	30s at 95°C	30s at 60°C to 50°C	30s at 72°C	20
		30s at 95°C	30s at 50°C	30s at 72°C	15
Tm_COI_F	Tm_COI_R	30s at 95°C	30s at 62°C to 55°C	30s at 72°C	20
		30s at 95°C	30s at 55°C	30s at 72°C	15
GA_COI_F	GA_COI_R	30s at 94°C	30s at 60°C to 50°C	1 mins at 68°C	20
		30s at 95°C	30s at 50°C	1 mins at 68°C	10
Degen_CytB_F	Degen_CytB_R	30s at 94°C	30s at 65°C to 55°C	30s at 72°C	20
		30s at 95°C	30s at 55°C	30s at 72°C	10
Gryllus_16S_F	Gryllus_16S_R	30s at 94°C	30s at 60°C to 50°C	1 mins at 68°C	20
		30s at 95°C	30s at 50°C	1 mins at 68°C	10

Results

Cricket farms

The commercially-raised crickets were surveyed from 108 farms throughout 46 provinces across all regions of Thailand: 6 provinces in northern region, 13 provinces in central region, 12 provinces in northeastern region, 4 provinces in eastern region, 4 provinces in western region and 7 provinces in southern region (Figure 1). Results revealed that there are 4 types of crickets commercially raised among farms Thailand. The most abundant was Thongdum (92 farms), followed by Sa-ding (house cricket) (65 farms), Thongdaeng (33 farms) and Thongnlai (15 farms), with some farms raising two or more types of crickets (Figure 2).

Figure 1. Overall map of the surveyed area across Thailand from google map, Each pin indicates a farm

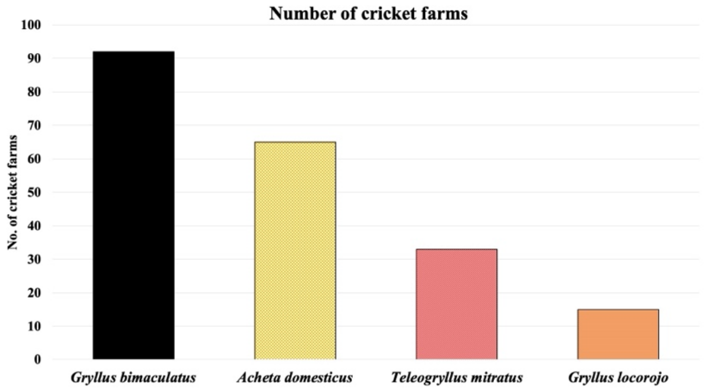

Figure 2. The number of farms rearing each cricket species

Characteristics and DNA analysis of commercially-farmed crickets in Thailand

Thongdum Cricket or Two-spotted cricket (*Gryllus bimaculatus*)

In Thailand, the two-spotted cricket (*G. bimaculatus*) is called Thongdum cricket. The head region, when viewed dorsally, is dark brown to black, without any marking. The pronotum is shiny black or dark brown. For both male and female, the wings vary in colour from black (Figure 3, Row A-B) to dark and lighter brown (Figure 3, Row C-D). The two spots are clearly visible at the base of the wings for the dark brown or black ones, but less obvious in the light brown ones.

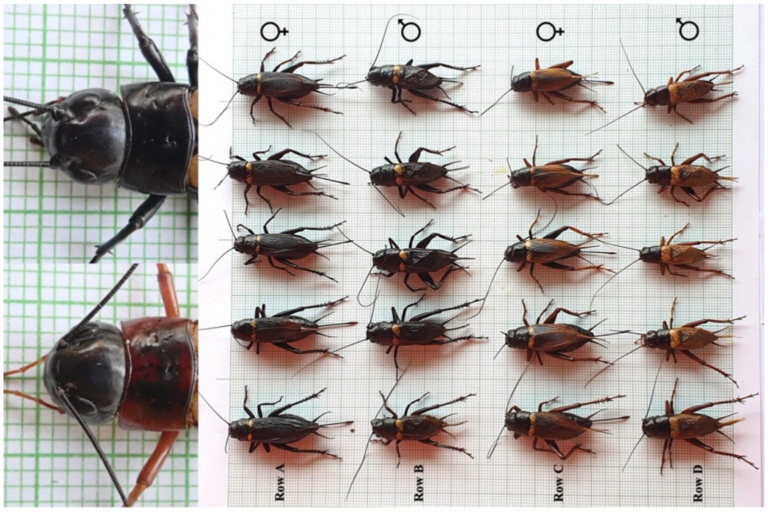

Figure 3. Colour characteristics of "Thongdum cricket" Head, Row A: black female, Row B: black male, Row C: brown female, Row D: brown male

When viewed from the ventral side, there are two yellow spots on the base of their wings (Figure 4, A). The mouth, thorax, and legs were dark brown to black, matching the colour of the wings (Figure 4, B-D). For those with black legs, the proximal side of the femur was lighter in colour, appearing red-yellowish (Figure 4, E). Nymphs have dark brown to black colour (Figure 4, F).

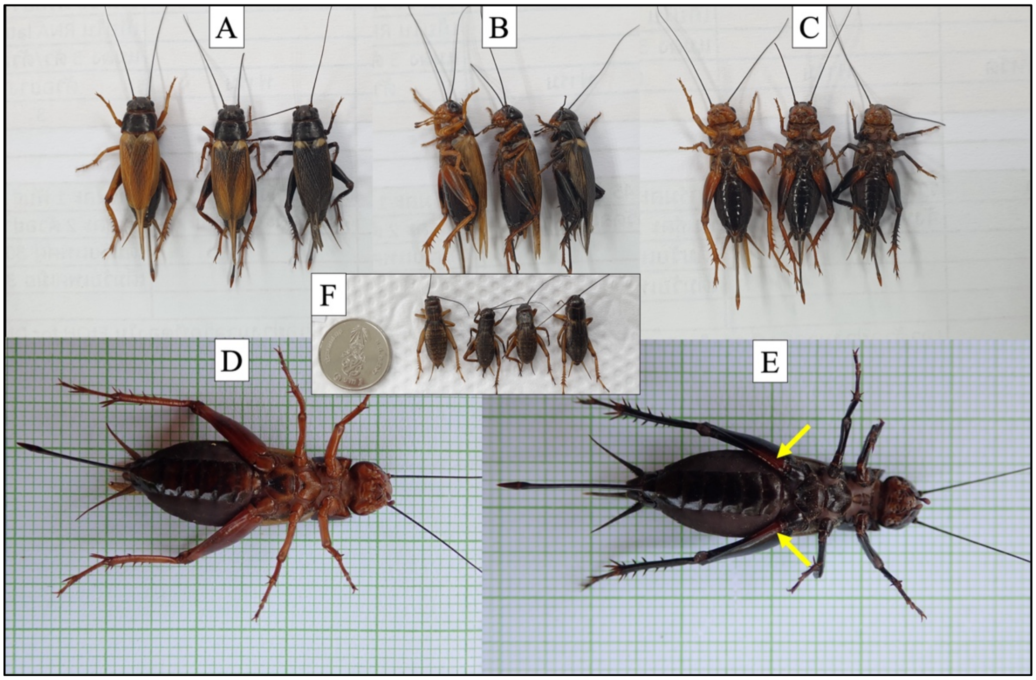

Figure 4. Wing colour characteristics of female *G. bimaculatus* when viewed dorsally (A), laterally (B), ventrally (C). Colour characteristics of legs and ventral side (D-E), and morphology of nymphs (F)

The DNA fragments covering the COI gene region were amplified, sequenced, and aligned to the reference sequence of *Gryllus bimaculatus* (Genbank accession number MW085273.1). The aligned DNA was nearly identical for both the black and brown Thongdum crickets, indicating that they were indeed *G. bimaculatus* (Supplementary figure 1).

The cytochrome oxidase I (COI) gene sequences from Thongdum crickets from the 26 provinces from all regions of Thailand were aligned and the phylogenetic tree was constructed from the aligned sequences (Figure 5). The phylogenetic tree topology revealed that there is no obvious pattern or clustering of the genotypes by provinces or regions. In other words, all sequences were distributed throughout all parts of Thailand.

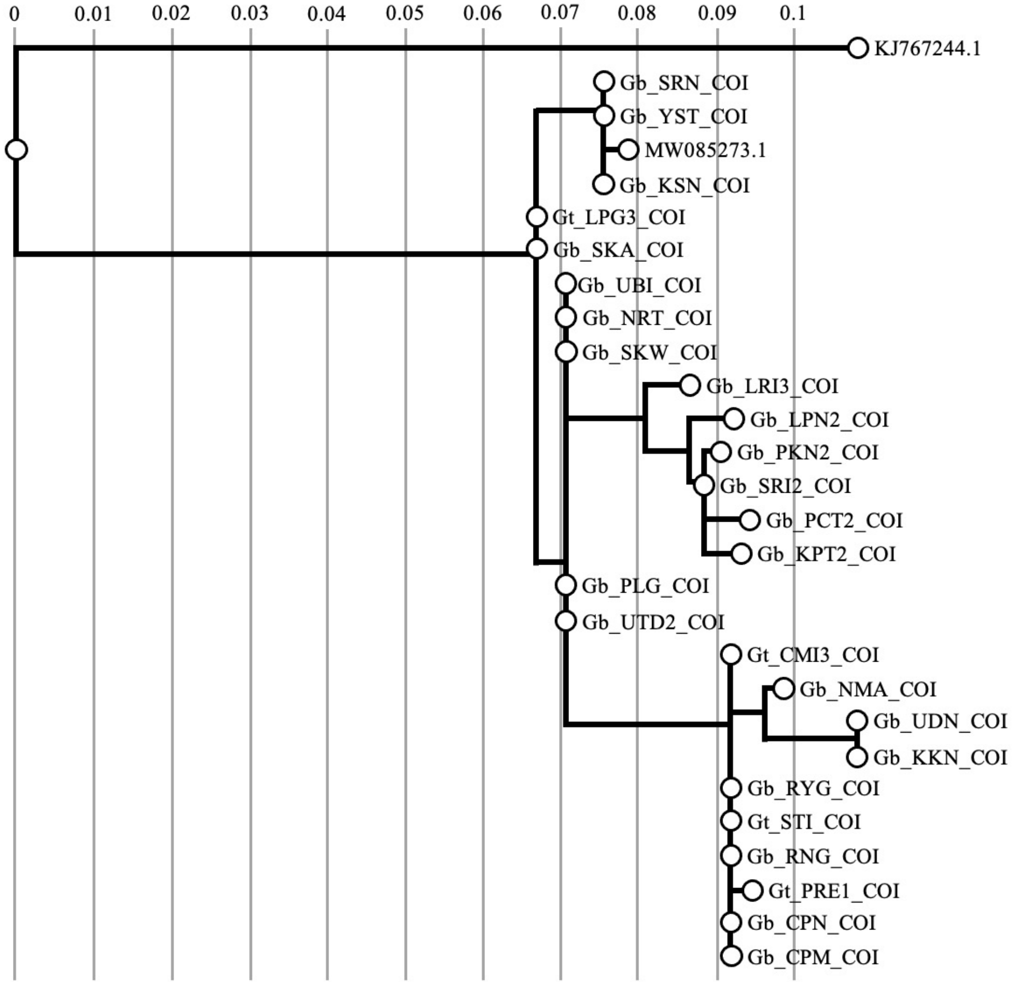

Figure 5. Phylogenetic tree constructed from the COI gene region of the black (Gb) and brown (Gt) Thongdum crickets, *G. bimaculatus* reference sequence (accession no. MW085273.1), and *D. melanogaster* as an outgroup (accession no. KJ76244.1)

Sa-ding or House Cricket (*Acheta domesticus*)

In Thailand, the house cricket is known by the farmers as Sa-ding which are relatively small, light yellow-brown in colour with light yellow legs appearing almost translucent. The fully-grown sizes of both the male and female are approximately 2 cm (Figure 6 A-D). The head region has a black stripe running across from left eye to right eye (Figure 6 E). When viewed dorsally, the first thoracic segment has a distinct marking composed of 5 separate spots, with the central one resembling the heart shape (Figure 6 F) in both adult and nymph stage. During the nymph stage, the abdomen has 7-8 black stripes on the dorsal side (Figure 6 G).

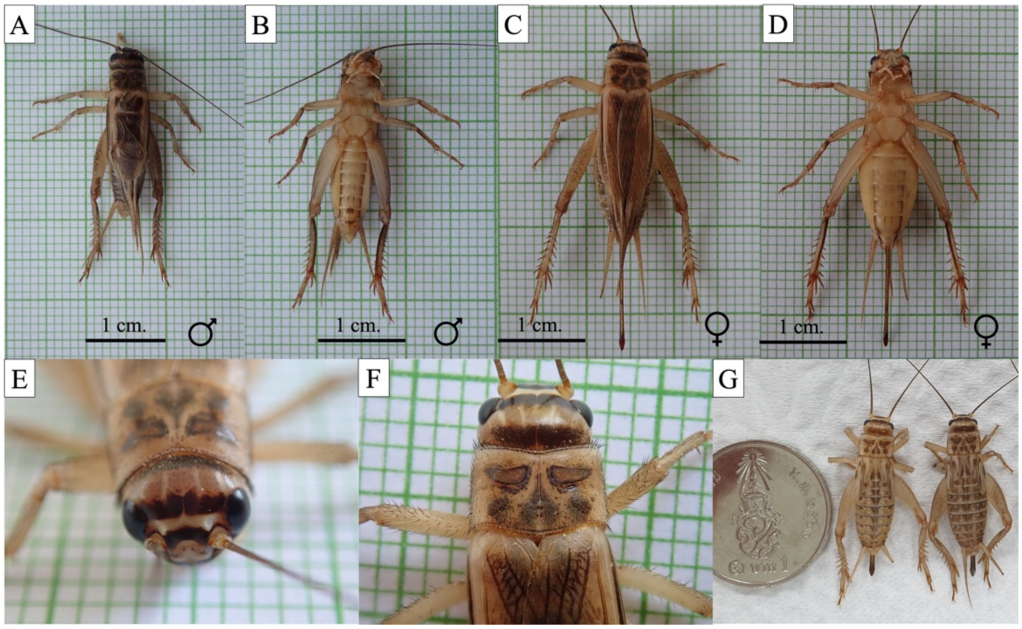

Figure 6. Morphology of the Sa-ding house cricket (*A. domesticus*) adult male (A-B) adult female (C-D) distinct marking on the head (E) and first segment of the thorax in adult (F) and nymph (G)

The DNA sequence of the Sa-ding COI gene region was analyzed by BLAST and found to be similar to the house cricket (accession number KR919594.1). Multiple sequence alignment of the COI gene from 18 provinces showed some genetic diversity among the Sa-ding house cricket, although there was no obvious clustering of the genotypes by province. The genotypes are randomly distributed among all the surveyed provinces (Figure 7).

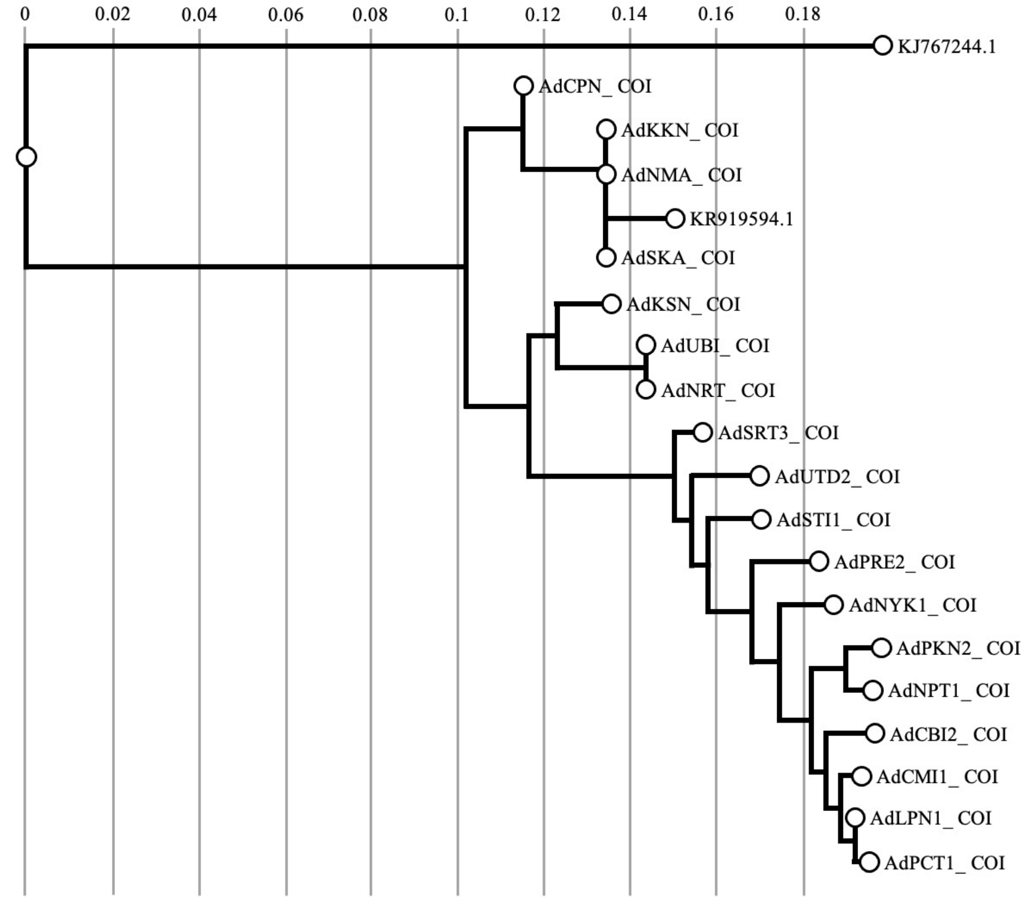

Figure 7. Phylogenetic tree constructed from multiple sequence alignment of the COI gene region of Sa-ding house crickets collected from 18 provinces in Thailand, reference *A. domesticus* sequence from database (accession no. KR919594.1) with the COI sequence of *D. melanogaster* (accession no. KJ76244.1) as an outgroup

Thongdaeng Cricket or Ground Cricket (*Teleogryllus mitratus*)

Thongdaeng (Thai translation: copper) is the formal name used by the Thai agricultural product standardization according to the Good Agricultural Practice (GAP) for cricket farm. This type of cricket is medium-sized but is the largest among the commercially-farmed crickets in Thailand, ranging from 2.8-3.5 cm in size (Figure 8 A-D). When viewed ventrally, the legs and abdomen are off-white (Figure 8 B, D). The nymph stage has a white stripe across the body (Figure 8 E), while the adult stage has dark brown wings. When viewed anteriorly, the boundary around the eyes is yellow or called eyebrow (Lu *et al*., 2018), with a pattern resembling the letter V and spots on the head at the base of the antennae (Figure 8 F-G). This eyebrow is also used as one of the characteristics to identify species of *Teleogryllus* (Lu *et al*., 2018).

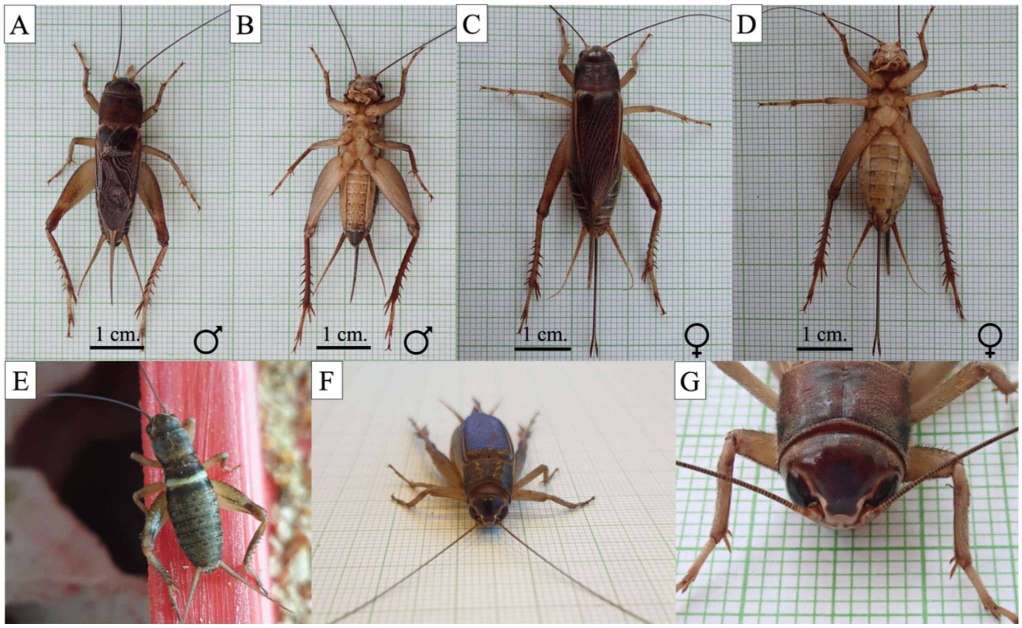

Figure 8. Morphology of the commercially raised “Thongdaeng cricket” *Teleogryllus mitratus* male (A-B), female (C-D), nymph (E), yellow V-shaped edges around the eyes or eyebrow (F-G)

Sequences of COI gene region from Thongdaeng crickets collected from 5 farms in Thailand were analyzed by BLAST. It was found that COI genes of the Thongdaeng crickets from KKN and TRG were entirely identical to *Telegryllus mitratus* from NCBI Genbank (accession number MF046156.1) while COI genes of the Thongdaeng crickets from SRT, NRT, and LPG was low level of genetic diversity. The genotypes appeared to be divided into two separate groups (Figure 9).

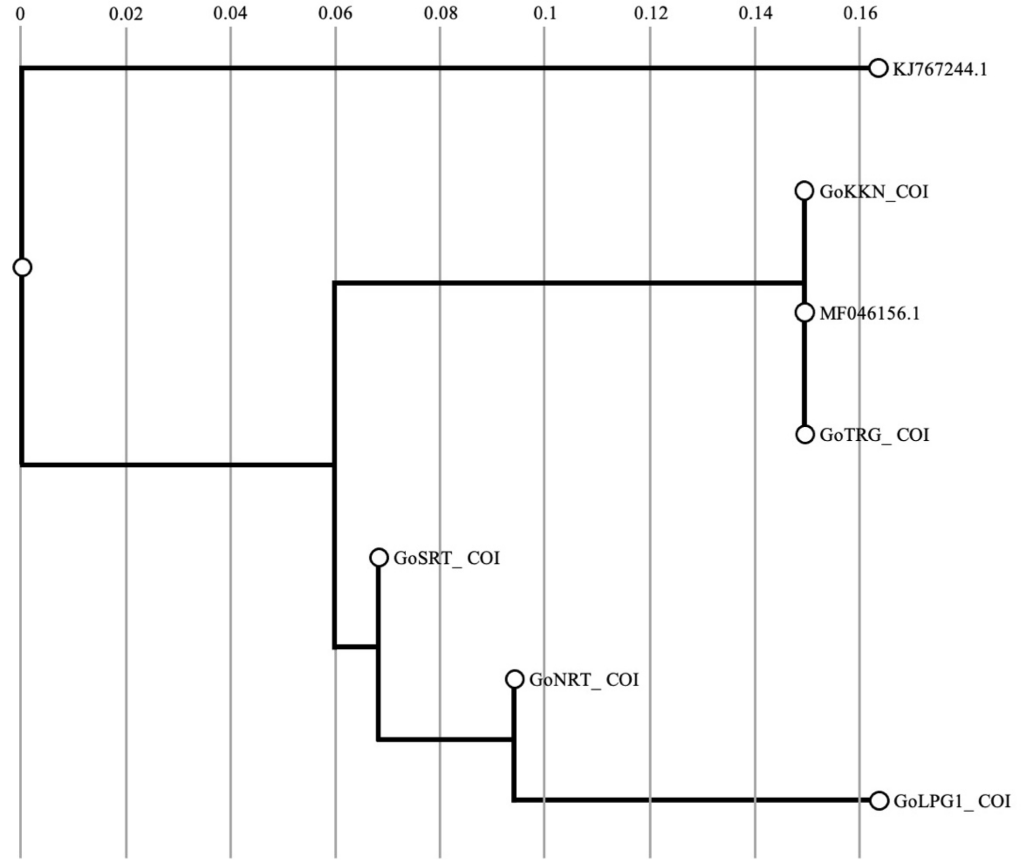

Figure 9. Phylogenetic tree constructed from multiple sequence alignment of the Thongdaeng COI gene region with the reference *Telegryllus mitratus* sequence from NCBI database (accession no. MF046156.1) and the COI sequence of *D. melanogaster* (accession no. KJ76244.1) as an outgroup

Thonglai Cricket or Crazy red field cricket (*Gryllus locorojo*)

During our sample collection, we collected the Thonglai cricket, which is morphologically similar to the Crazy red field cricket. This type of cricket is medium-sized at 2.5cm in length, similar to the Thongdum cricket. The wings, legs and thorax are all red-brow colour (Figure 10 A-D). When viewed ventrally, the central part of abdomen is almost black, while both edges are off-white (Figure 10 B-D). When viewed anteriorly, the pattern of the marking on the head resembles the letter “M” in both nymph and adult (Figure 10 E-F).

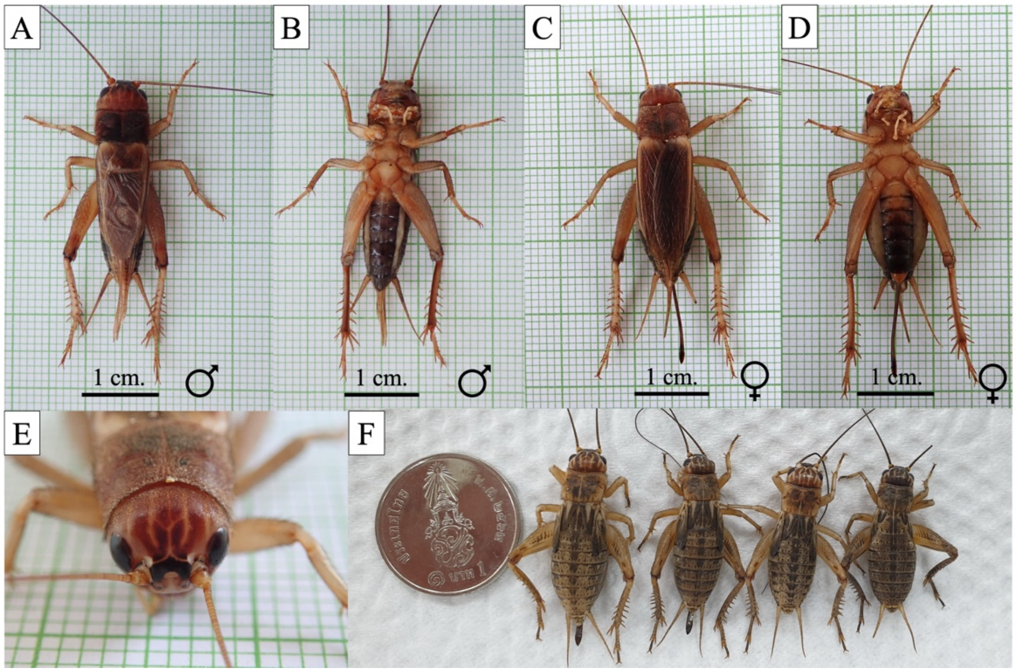

Figure 10. Thonglai cricket male (A-B) and female (C-D) the marking pattern on the anterior port of the head resembles the letter M (E) Thonglai cricket nymph (F)

To genetically identify the Thonglai cricket, we initially used the COI primers with degenerate bases to PCR amplify the sequence (accession number OQ346298.1, OQ346299.1, OQ346300.1, OQ346301.1, OQ346302.1, OQ346303.1, and OQ346304.1). However, the sequence of Thonnglai COI gene did not match a cricket species. Therefore, we utilized the 16S ribosomal RNA (16S rRNA) to identify the Thonglai cricket instead. The amplified 16S gene region (accession number OQ379919.1, OQ379918.1, OQ379917.1, OQ379916.1, OQ379915.1, OQ379914.1, and OQ379913.1) matched with the *Gryllus locorojo* in the NCBI Genbank database for 100% similarity (accession number JX269022). Subsequently, the 16S gene and COI gene sequences of Thonglai cricket from 7 provinces were analyzed by multiple sequence alignment and were found to be identical with no genetic diversity at this locus (Figure 11-12).

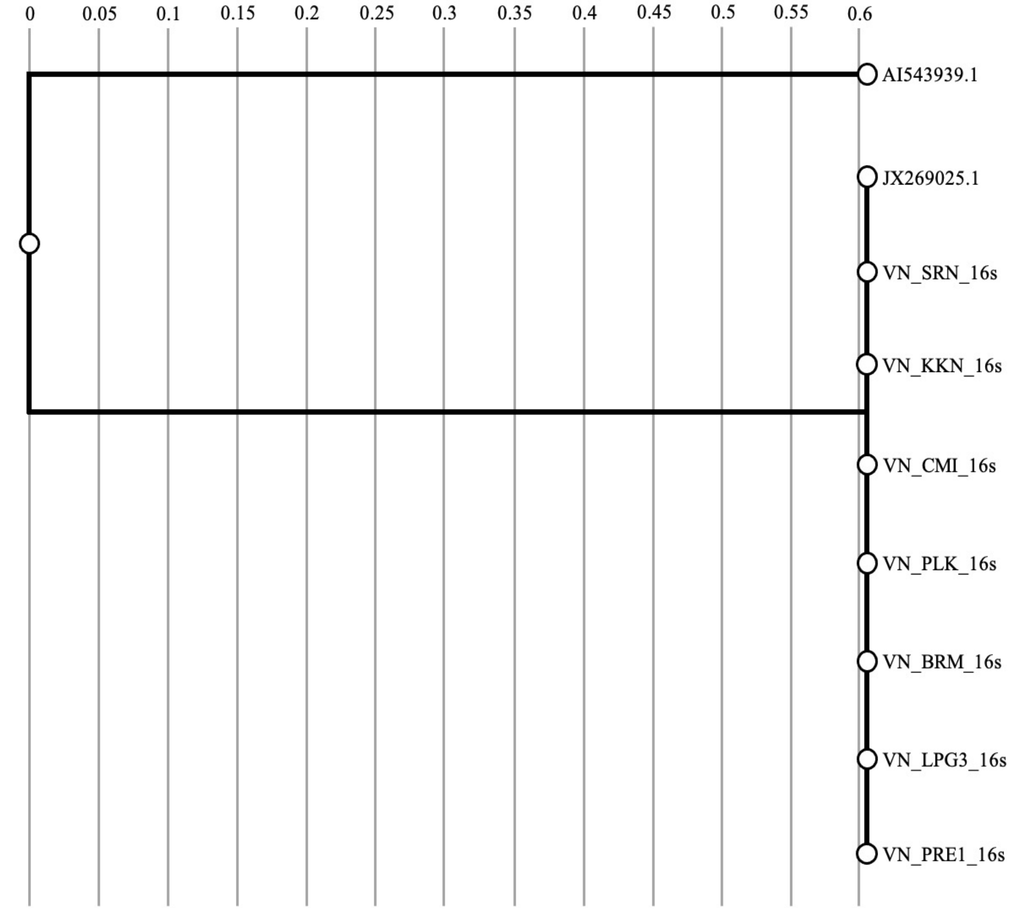

Figure 11. Phylogenetic tree constructed from multiple sequence alignment of the 16S ribosomal gene from the Thonglai cricket, compared to *G. locorojo* from database (accession no. JX269025) the fruit fly (*D. melanogaster*, accession no. AI543939.1) as an outgroup

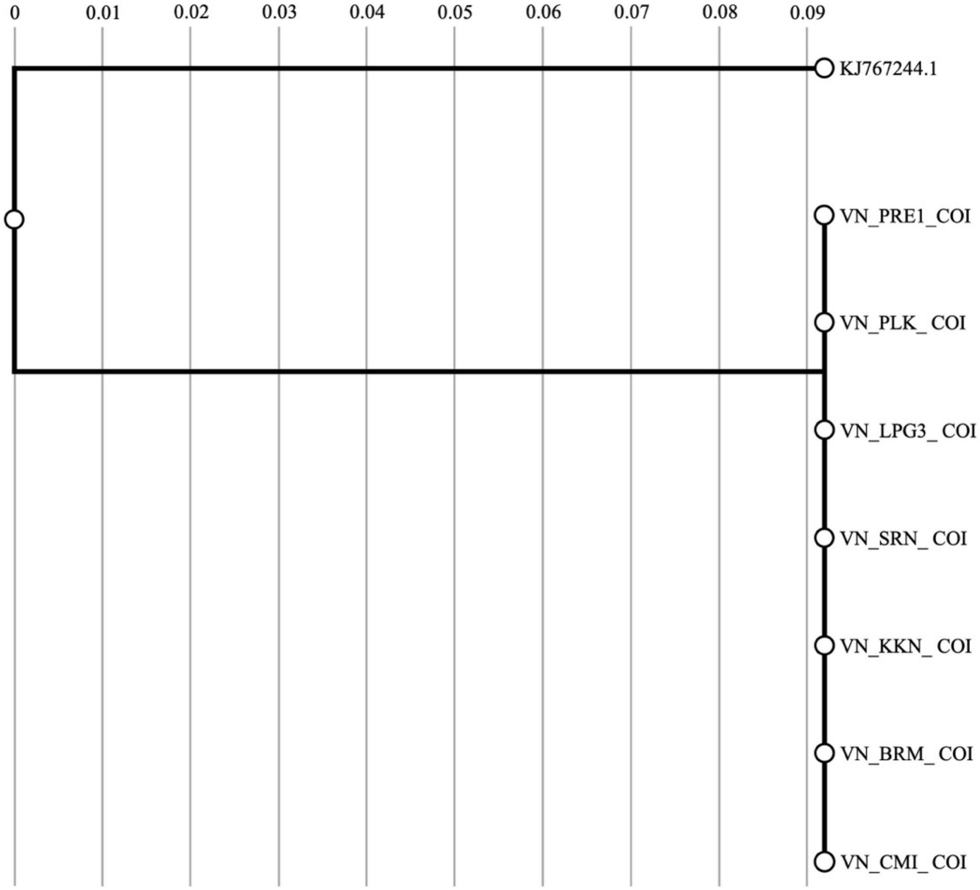

Figure 12. Phylogenetic tree constructed from multiple sequence alignment of the COI gene of Thonglai cricket from 7 locations in Thailand, compared to the fruit fly (*D. melanogaster*, accession no. KJ767244.1) as an outgroup

Discussion

The survey and field collection indicated that the Thongdum cricket (*G. bimaculatus*) is found to be the most abundant commercially-farmed cricket in Thailand. This is mainly because the Thongdum crickets have shortest life cycle of 35 days, thus allowing farmers to raise and harvest several rounds per year. Additionally, Thongdum was the first species of cricket that were farmed in Thailand in 1998, followed by the house cricket (*A. domesticus*) (Hanboonsong *et al*., 2013).

The house cricket is the second most abundant cricket farmed in Thailand and is gaining popularity among farmers because of its export potential as frozen or powdered form. However, farmers and relevant agencies in Thailand still call these cricket species by varying and incorrect common names, which are not always consistent with the scientific name, resulting in confusion. For example, the Thongdum cricket was frequently called both Thongdum and Thongdaeng cricket, depending on their black or copper-brown colour phenotypes, respectively (Hanboonsong *et al*., 2018; Otte and Cade, 1984; Weissman *et al*., 2012).

This leads to the misunderstanding and misidentification of Thongdaeng as *T. mitratus*. Moreover, the actual *T. mitratus*, which is in fact Thongdaeng, was mistakenly called Jing-Grong, which is the common name for another species (*Brachytrupes portentosus*). This is only one example of the mis-identification that leads to confusion among various media both online and off-line, as shown in this online magazine article: https://www.technologychaoban.com/bullet-news-today/article_101066 (Kusuma na Ayuthya, 2022). The only species that is identified most correctly and consistently is the house cricket, with the exception of the second common name Sa-ding, which is reserved for the house cricket (*A. domesticus*) only, and is never used with any other species of crickets.

Thonglai cricket is the most recently-imported cricket for commercial farming in Thailand. Some farmers indicated that the Thonglai cricket eggs were originally brought from Vietnam without any proper labeling or identification, so they were initially named Vietnam, or Thongdaeng Vietnam cricket because of their resemblance to the Thongdaeng cricket. Thus far, there was no previous report of genetic identification of this species of cricket. Therefore, many online sources and various exotic pet food shops still mistakenly called this species *G. assimilis*, due their similar appearance during nymphal stage. However, the calls of these two species are distinct, and *G. locorojo* has a much more pronounced red body and pattern at the head region. Therefore, this study is the first to identify the Thonglai cricket as *G. locorojo* both genetically and morphologically in Thailand and, to our knowledge, in Asia.

In addition to morphology, we also used the mitochondrial DNA sequence within the cytochrome oxidase I (COI) region to compare with the existing genetic database on Genbank (NCBI) using BLAST. Our COI results were able to unambiguously distinguish the Thongdum, Thongdaeng, and house cricket. However, we could not identify the Thonglai cricket using only the COI region due to the lack of this region on the database. Alternatively, we used the sequences at 16S ribosomal RNA (16S rRNA) for amplification and sequencing of the Thonglai cricket DNA. The BLAST result and nucleotide alignment showed that the 16S rRNA of Thonglai cricket matches with the reference number JX269022 which belongs to the *G. locorojo* (Weissman *et al*., 2012).

Using the sequences amplified from cricket samples collected from various provinces throughout Thailand, we were able to align the sequences and construct phylogenetic trees to determine whether the cricket genetic distribution match with their geographical origins. However, our phylogenetic trees revealed that there was no clear distribution pattern: all sequences were randomly distributed without any obvious geographical clusters. This is most likely due to the widespread trading and exchange of cricket eggs throughout the country over several decades. Additionally, we found that both the 16S rRNA sequences and COI sequences of the Thonglai cricket collected from 7 locations in Thailand possessed no genetic diversity (100% identical). This is in agreement with the report from Weissman *et al.* (2012) that showed identical sequence of *G. locorojo* collected from 8 sites across 3 continents. This shows that *G. locorojo* has high genetic homogeneity, due to low mutation rate, or they have not been farmed long enough for any mutation to accumulate.

Acknowledgements

This study was funded by National Research Council of Thailand (NRCT) grant number N24A640133. We would like to thank all 108 cricket farms in Thailand for collaboration, and our students for laboratory assistance. We also would like to thank the Faculty of Animal Science and Agricultural Technology, Silpakorn University, for providing research facility.

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(Received: 10 September 2024, Revised: 6 January 2025, Accepted: 10 January 2025)