Corrigendum: Detection of nosemosis in European honeybees (Apis mellifera) on honeybees farm at Kanchanaburi, Thailand (2019 IOP Conf. Ser.: Mater Sci Eng. 639 012048)

Samrit Maksong¹, Tanawat Yemor² and Surasuk Yanmanee³

¹Department of General Science, Faculty of Science and Technology
Kanchanaburi Rajabhat University, Thailand
²Department of Plant production technology, Faculty of agriculture and natural resources Rajamangala University of Technology Tawan-ok Chonburi, Thailand
³Department of Biology, Faculty of Science Naresuan University Phitsanulok, Thailand

Description of corrigendum e.g.,

Page 1:
In the Abstract section, the following text appears:

This study was aimed to the detection of Nosema in European honeybees at Kanchanaburi Province and identify species of Nosema by Polymerase Chain Reaction technique. The ventriculus of bees was individually checked to verify the presence of Nosema spores under light microscope. The number of spores per bee were quantified on a haemocytometer for infectivity. It was studied for three periods of the year. The first period was studied between October 2015 to January 2016, the second period from February to May 2016 and the third period from June to September 2016. The results showed that the highest infection rate in June-September 2019 was 100 and the lowest infection rate was 40 in October 2015 and January 2019. The average number of Nosema spores infection level in an individual was infectivity. The highest infectivity was 2.47x10⁶ spores/bee in February to May 2016 and the lowest infectivity was 0.04x10⁶ spores/bee in October 2015 to January 2016. Nosema ceranae was found in this study by Polymerase Chain Reaction technique.

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2.1 DNA extraction, amplification and sequencing
Genomic DNA was extracted from a whole Nosema spores from the A. mellifera colonies at apiaries of Wanna Bee Farm using DNA Extraction Kit (Qiagen) according to the manufacturer’s instructions. The 16S rRNA gene were amplified by polymerase chain reaction (PCR) using the following primers: Noce239L (5’–AGGGGCGAAA C TGACCTAT-3’) and Noce950R, (5’–GGGCATAACKG ACCTGT TTA-3’) [9]. PCR was carried out using 20 µl volumes containing 5 units of Taq DNA polymerase (biotchrabbit™), 2 µl of 10X PCR reaction buffer, 0.6 µl of 50 mM MgCl₂ , 0.4 µl of 10 mM of each dNTP, 30 pmol of each primer, and 2 µl of the extracted DNA. The amplification profile comprised initial denaturation at 94°C for 3 min, 35 cycles at 94°C for 50 s, 55°C for 30 s, and 72°C for 1 min, and a final extension at 72°C for 10 min. The amplicons were then run on a 1% agarose gel, stained with ethidium bromide (0.5 lg/ml) and visualized with a UV illuminator (AB1500 Printgraph and AE 6905H Image Saver HR, ATTO, Tokyo, Japan). PCR products of 16S rRNA gene were purified with the PCR Purification Extraction Kit (Qiagen) and sent for sequencing using Sanger method (Macrogen, South Korea). The revealed sequences were verified by Bioedit software (version 7.0.5; Ibis Therapeutics, Carlsbad, USA).

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Sequences of 16S rRNA gene were searched against the GenBank nucleotide database (National Center for Biotechnology Information, http://www.ncbi.nlm.nih.gov) for gene homology Nosema sequences and their highest homologies obtained from GenBank, including N. apis (accession number. FJ789796). The sequences were aligned using the CLUSTALX multiple alignment program [10]. Gap sites were excluded from the following analysis. Genetic distances were estimated with the Kimura two-parameter method [11]. Construction of neighbor-joining trees [12] and the bootstrap test with 1,000 replications were conducted with the MEGA version 7 programs [13].

2.3 Detection of Nosema infection
The presence of nosemosis in honeybee colonies was investigated in three different seasons in Thailand. The first period was done on winter season, during October 2015 - January 2016 February, second period on dry season (February - May 2016) and the third period on raining season (June - September 2016). The honey bees used in this study were collected from the Apis mellifera colonies maintained at apiaries of Wanna Bee Farm, located at Kaeng Sian Subdistrict, Mueang District, Kanchanaburi Province, Thailand. Honeybee was randomly collected from 20 colonies, 50 bees from each colony in different season to verify the presence of Nosema spp. spores and the number of spores per individual bee. For this, the abdomens were individually separated, placed into a 1.5 ml Eppendorf tube and then homogenized thoroughly in 200 ml of deionized H₂O. Ten microliters of the homogenate were loaded onto a hemocytometer and the number of spores counted.

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Results and discussion

3.1 Nosema species determination
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All 20 colonies of A. mellifera were monitor throughout the 3 different season period for Nosema infection level; there were significant differences in infection levels between periods. We observed the infections level were significantly lower in October 2015 - January 2016, a 40% of colonies was infection by Nosema. The infections level was increased in February-May 2016 with 80% of colonies were infected, while during June-September 2016 a 100% of colonies were infected with Nosema “Fig.3”. However, a total number of Nosema spores counted from individual honeybee were not corresponding with the infection level. The highest number spores counted per bee was found in bees sampled during the warmer months were collected in February-May 2016 with 2.47 x10^6 ± 4.27 spores per bee. The lower number spores counted per bee were showed in bees sampled in June-September 2016 with represent the average number of spores counted 0.27 x10^6 ± 0.43 spores per bee, whereas it was the lowest in bees collected in February - May 2016 with 0.04 x10^6 ± 0.08 spores per bee. There was a significant difference in number of spores counted in different periods sampled (Chi2 = 24.98, P<0.05), as expected “Fig.4”.

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on honeybees farm at Kanchanaburi, Thailand

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¹Department of General Science, Faculty of Science and Technology
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E-mail: Samrit@kru.ac.th

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technique. The ventriculus of bees was individually checked to verify the presence of Nosema
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1. Introduction
The European honeybees (*Apis mellifera*) are a highly valued resource worldwide and importance to
the ecosystem, not only as a honey or honeybees productivity but also as a pollinator of agricultural
and horticultural crops and wild flora. A peculiar decline in honeybee colonies has gained worldwide.
Much attention has been given to Colony Collapse Disorder (CCD). Several causes of these large-
scale losses have been reported, including honey bee parasites, pesticides, environment, antibiotics,
poor nutrition, and pathogens. Nosema are classified to class Microsporidia has a single cell spore-
forming microorganisms. They are intracellular obligate parasites of eukaryotes including fishes,
mammals, or especially in insects. The genus Nosema belongs to this group of fungi [1-4]. Nosema apis and Nosema ceranae are the two best known Nosema species because they were found in
honeybees. N. apis, the historical microsporidian parasite of European honeybees, whilst N. ceranae,
probably introduced into European honeybees from its Asian honeybees (*Apis cerana*) within the last
few decades, and they invade the ventriculus epithelial cells of adult honeybees, and the best known of
honey bee disease as nosemiasis or nosemosis. Honeybees become infected when they ingest Nosema
spores, the fecal-oral and oral-oral routes being the main modes of transmission between honeybees.
Once in the ventriculus, the parasites invade and develop within the cytoplasm of the mid gut
epithelium cells. This disease negatively affects productivity and survival of honeybee colonies, and that may lead to colony depopulation and collapse [2-6].

In this study, the detection of Nosema on honeybee colonies needs data concerning its prevalence. To the best of our knowledge, little is reported about the prevalence of Nosema in Thailand apiaries and no reported in Kanchanaburi province was west of Thailand [7, 8]. Therefore, in this research we evaluated the prevalence of Nosema infection in Kanchanaburi province was west of Thailand apiaries. First, identify species of Nosema by polymerase chain reaction (PCR) assay based on 16S rRNA-gene-targeted species-specific primers to differentiate N. ceranae from N. apis and the second were light microscopy was carried out to screen the presence of Nosemosis in adult worker honeybees.

2. Materials and Method

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2.4 *Nosema* spore morphological identification using light microscopy
The mid gut of 50 bees was macerate in 1.5 ml Eppendorf containing 1,000 µl deionized water, the suspension was centrifuged at 6000g for ten minutes and the homogenate examined under the light microscope using × 400 magnification and photographed. Measurement is present in micrometers and data are expressed as the mean followed by the range in parentheses.

3. Results and discussion

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3.3 Morphological identification of *Nosema* spp. spores

Spores of *Nosema* spp. were examined under the light microscope using 400x magnification. Light microscopy of the midgut content showed a large number of spores. *Nosema* spp. spores were oval to rod shaped with average length and width of 4-5.2 µm and 2-2.5 µm, N=50 “Fig.5” respectively. In the last few years, infection of honeybees (*A. mellifera*) by *N. ceranae* has been reported in a number of European countries, including Sweden, Germany, Denmark, France, Finland, Switzerland, Greece, Holland, United Kingdom, Hungary, Italy, Serbia, Slovenia, Poland and Spain, soon being confirmed in worldwide including Denmark, Finland, France, Germany, Greece, Italy, Serbia, Spain, Sweden, Switzerland, Republic of Croatia, Brazil, USA, Japan, Vietnam and Thailand [2, 7-8, 14].

Our result is the report on the *Nosema* disease in European honeybees at Kanchanaburi who west of Thailand and the result show that the highest infectivity was 2.47x10^6 spores/bee in February to May 2016 and the lowest infectivity was 0.04x10^6 spores/bee in October 2015 to January 2016 and
*N. ceranae* was found in this study by Polymerase Chain Reaction technique. Our findings, microscopy is still a valuable, relatively cheap and simple method to screen for the presence of *Nosema* infection in apiaries. Similar results were also reported by Reference [4, 15]

**Figure 1.** The amplified product of 16S rRNA of *N. ceranae* from *A. mellifera* runs on a 2% agarose gel. Lane 1 = Nosema 1, Lane 2 = Nosema 2 respectively; M =100 bp ladder

**Figure 2.** Phylogeny of *N. ceranae* based on 16s rRNA. The tree was generated by neighbor-joining analysis. Numbers on the nodes indicate probabilities based on 1,000 bootstrap replicates. A probability of more than 50% is shown. Branch lengths are proportional to genetic distance (scale bar)

**Figure 3.** The infection rate of European honeybees on honeybees farm at Kanchanaburi Province
Figure 4. The infectivity of European honeybees on honeybees farm at Kanchanaburi Province (Chi2 = 24.98, P<0.05)

Figure 5. Spores of *N. ceranae* under light microscope (A=400x), (B=1000x)
Figure 6. Comparisons of *Nosema* 16S rRNA sequences. The *Nosema* 16S rRNA sequences were aligned using ClustalX. Numbers on the top refer to nucleotide position. The nucleotide differences are denoted and similarities are shaded and (–) indicates gap. Abbreviations: *N. ceranae* Noce950R = *N. ceranae* isolated from *A. mellifera*; *N. ceranae* (DQ673615, *N. ceranae* France (DQ374655), *N. ceranae* Germany (DQ374656), *N. ceranae* (XR_002966746) = *N. ceranae* retrieved from GenBank accession number. DQ673615, DQ375655-6, and XR_002966746, respectively; *N. apis* (FJ789796) = *N. apis* retrieved from GenBank accession number. FJ789796
4. Conclusions
From our studies, light microscopy is a simple, cheap and valuable method to check the spread of Nosemosis in honeybee farm. However, morphological identification of *Nosema* spp. spores under light microscope is difficult to distinguish the species, because *N. ceranae* and *N. apis* are very similar. To identification to species level, molecular techniques such as PCR are needed to reliable. The average number of *Nosema* spores infection level in individual was infectivity. The highest infectivity in February to May 2016 and the lowest infectivity in October 2015 to January 2016.

5. References

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6. Acknowledgement
The authors would like to thank Mr. Manus Prachakal for his help. This work was supported by the Budget bureau of Thailand no. 227678 and special thanks also go to Faculty of Science and Technology, Kanchanaburi Rajabhat University.