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Haematophagous mites on poultry farms in the Republic of the Union of Myanmar

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Abstract

Haematophagous ectoparasites of poultry, such as *Ornithonyssus sylviarum,* northern fowl mites (NFMs), *Dermanyssus gallinae*, poultry red mites (PRMs), and *Ornithonyssus bursa*, tropical fowl mites (TFMs) are prevalent worldwide. Although poultry farming is a major industry in Southeast Asia, there are only a few reports concerning the prevalence of avian mites in this region. In this study, we sampled twenty farms in four major poultry farming areas in Myanmar. We detected the mites on six farms, and they showed morphological similarities to NFMs and TFMs. The nucleotide sequences of *cytochrome c oxidase subunit I* indicated that some mites were NFMs. This is the first report confirming the presence of NFMs and TFMs among the hematophagous mites infesting chickens on Myanmar poultry farms.

Keyword: Agriculture

1. Introduction

Infestation of haematophagous ectoparasites in the poultry industry is a global problem that leads to decreased productivity. *Ornithonyssus sylviarum*, northern fowl

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mites (NFMs) (Canestrini and Fanzago), *Dermanyssus gallinae*, poultry red mites (PRMs) (De Geer), and *Ornithonyssus bursa*, tropical fowl mites (TFMs) (Berlese) are harmful ectoparasites affecting poultry farming. Infestation by these mites induces stress in chickens, such as reduction in egg production and egg quality, anaemia, and diminished immunity; severe infestation often causes death by blood loss (Arce et al., 2018; Denmark and Cromroy, 2003; Lemke and Kissam, 1986; Sparagano et al., 2014). NFMs are the most common and most damaging ectoparasites of poultry in North America (Mullens et al., 2009) and TFMs are prevalent in tropical and temperate zones (Denmark and Cromroy, 2003). PRMs are endemic to European countries, Japan, and Brazil (Roy et al., 2010; Sparagano et al., 2014), and are prevalent in North America, particularly in the western region although they are not as important as NFMs. Although these avian mites are globally distributed, the predominant species varies per region.

The Republic of the Union of Myanmar (Myanmar) lies in the western region of mainland Southeast Asia. Poultry farms in Myanmar occur on the outskirts of urban areas, and the produce is mainly consumed in the cities. Although poultry farming is a major industry in Southeast Asia, including Myanmar, there are few reports concerning the distribution of avian mites. In this study, we captured mites from poultry farms at the outskirts of four large cities in Myanmar. We detected the hematophagous mites and classified the collected mite species, based on the morphological observations and nucleotide sequence analysis.

2. Materials and methods

2.1. Sample collection

We collected mites from twenty poultry farms around four large cities in Myanmar. iTraps-2 (Kondo Electronics Industry Co. Ltd., Osaka, Japan) were set up to capture chicken mites from the poultry houses. One to three days later, the iTraps-2 were collected and transferred to the laboratory at room temperature. The mites were captured from the poultry houses on twenty farms around the cities Mandalay (Ma 1−3), Pyin Oo Lwin (Py 1−5), Taunggyi (Ta 1−3), and Yangon (Ya 1−9).

2.2. Morphological observation

The mites were stored in 70% ethanol for a few days, followed by overnight incubation in 60% lactic acid at 45 °C. The mites were then gently washed with distilled water and transferred onto a small drop of Hoyer's medium on microscope slides. The prepared specimens were observed using a light microscope.

Upon morphological observation, we focused on differences in the anal plates to distinguish the PRMs from NFMs (Di Palma et al., 2012). The anal plate of NFMs

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is oval shaped, whereas PRMs have a triangle-shaped anal plate. In addition, NFMs have an open anus in front of the anal plate, whereas PRMs have the anus behind the anal plate. NFMs and TFMs were distinguished based on the shape of dorsal plates and the number of setae on the sternal plates (Denmark and Cromroy, 2003). The dorsal plate gets narrow toward the posterior end, and the shape of NFMs is indicated more sharply than the TFMs. The setae on the sternal plate of NFMs are present in two pairs, while those on TFMs are present in three pairs.

2.3. Nucleic acid extraction and cDNA synthesis

The mites were homogenized using a mortar and pestle in 200 μL of phosphate-buffered saline, and total cellular DNA was extracted using QIAamp DNA Mini Kit (QIAGEN, Hilden, Germany), according to the manufacturer's instructions. Samples were eluted in 200 μL of elution buffer and stored at -20 °C. For RNA extraction, FastGene RNA Premium Kit (NIPPON Genetics Co. Ltd., Tokyo, Japan) was used. After the chicken mites were homogenized in 350 μL of RL buffer, total cellular RNA was extracted according to the manufacturer's instructions. cDNA was synthesized using PrimeScript™ Reverse Transcriptase (Takara Bio Inc., Shiga, Japan), as directed by the manufacturer.

2.4. Polymerase chain reaction and sequence analysis

The DNA or cDNA samples were used as templates for polymerase chain reaction (PCR). A part of the mitochondrial gene *cytochrome c oxidase subunit I* (*COI*) was amplified by using the primers, RhipiCOIF (5′-CGA ATA AAT AAT ATA AGA TTT TGA-3′) and TyphloCOIR (5′-GCT AAT CAA GAA AAA ATT TTA AT-3′) (Roy et al., 2009). The PCR mixture contained 10 pmol of each primer, 1 U of Takara Ex Taq (Takara Bio Inc.) and, 200 μM of each deoxynucleotide. The PCR was performed with 1 cycle of 94 °C for 5 min for initial denaturation, followed by 40 cycles of 94 °C for 30 sec, 52 °C for 30 sec, and, 72 °C for 1 min each. The amplified fragments were separated on agarose gels (2.0%) and visualized under ultraviolet light after staining with ethidium bromide.

2.5. Sequence analysis

For sequencing analysis, the amplicons were purified using FastGene gel/PCR extraction kit (NIPPON Genetics Co. Ltd.), cloned into T-vector pMD20 (Takara Bio Inc.), and sequenced using GenomeLab™ GeXP Genetic Analysis System (Beckman Coulter, Fullerton, CA, USA). The resulting nucleotide sequence of the *COI* gene was aligned using MEGA software, version 7.0 (Kumar et al., 2016) and the phylogenetic tree was constructed with the same software, using the neighbour -joining method (Saitou and Nei, 1987).

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3. Results

3.1. Detection of chicken mites in Myanmar poultry houses

The mites were detected from the poultry houses on six farms around Taunggyi (Ta-3), Mandalay (Ma-1), and Pyin Oo Lwin (Py-1,2, 3,5) (Table 1). No mites were found in the poultry houses around Yangon (Table 1). In Py-1, village chickens were bred outside the poultry houses, and mites similar to those in the poultry houses of Py-1 were confirmed on the village chickens.

3.2. Classification of the mites by morphological observation

Only one specimen each was observed from Ma-1 and Py-3. These mites were lost during morphological analysis. Therefore, we analysed the morphology of chicken mites from four farms: Ta-3, Py-1,2,5. We prepared the mite specimens by sealing them in Hoyer's medium after treatment with 60% lactic acid for taxonomic classification. NFMs/TFMs and PRMs were differentiated based on anal plate shape and anus location (Di Palma et al., 2012). The anal plates of NFMs/TFMs are oval,

Table 1. Chicken mites detected in the poultry houses in Myanmar.

Area and year	Farm ID	Capture by traps	Number of mites captured	Mite species[1)]
Taunggyi (December 2017)	Ta-1	-	-	-
	Ta-2	-	-	-
	Ta-3	+	>30	TFM
Mandalay (February 2018)	Ma-1	+	1	N.D.[2)]
	Ma-2	-	-	-
	Ma-3	-	-	-
Pyin Oo Lwin (February 2018)	Py-1	+	>30	NFM
	Py-2	+	20–30	NFM
	Py-3	+	1	N.D.[2)]
	Py-4	-	-	-
	Py-5	+	1	NFM/TFM[3)]
Yangon (May 2018)	Ya-1	-	-	-
	Ya-2	-	-	-
	Ya-3	-	-	-
	Ya-4	-	-	-
	Ya-5	-	-	-
	Ya-6	-	-	-
	Ya-7	-	-	-
	Ya-8	-	-	-
	Ya-9	-	-	-

NFM: Northern fowl mite; TFM: Tropical fowl mite.

[1)] Mite species were determined based on morphological features and *cytochrome c oxidase subunit I* (*COI*) gene sequence.

[2)] N.D.: not determined. One mite was collected. However, the species was not determined because the mite was lost during analysis.

[3)] Mite morphology in Py-5 resembled NFMs and TFMs, based on the features of the anal plate and the anus. However, the *COI* gene sequence was not determined because there were too few mites for the analysis.

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whereas PRMs have a triangular anal plate. In addition, NFMs/TFMs have an open anus in front of the anal plate, whereas the PRM anus is behind the anal plate. The anal plates of the mites from Py-1,2,5 were oval, and their anuses opened in front of the anal plates, suggesting that the mites from these three farms were NFMs or TFMs (Fig. 1A).

3.3. Comparison of the COI gene

Next, we determined the genetic characteristics *COI*, because the partial sequences of the *COI* genes of NFM and PRM are available in GenBank. We amplified and sequenced the *COI* gene from the mites from Ta-3, Py-1,2, because we were able to obtain approximately 10−30 chicken mites from each farm for DNA extraction. The *COI* gene from Py-1,2 showed 100% and 99% similarities, respectively, to NFMs, whereas it showed a lower similarity to PRMs (Table 2). However, the similarities in the *COI* gene from Ta-3 and NFMs/PRMs were lower. The phylogenetic analysis revealed that the mites from Ta-3 were relatively closer to NFMs (Fig. 1B).

3.4. Sequencing and phylogenetic tree analysis

Since the mites in Ta-3 were not NFMs or PRMs, we asked if the mites in Ta-3 were TFMs. By closely examining their morphology, we reconfirmed that the features of the anal plate were similar to those of NFMs/TFMs (Fig. 1C, b). Although the morphological features of TFMs are similar to NFMs, they can be distinguished based on dorsal plate shape and the number of setae on the sternal plates (Denmark and Cromroy, 2003). The dorsal plate narrows posteriorly, and that of NFMs narrows more sharply. The setae on the sternal plate of NFMs are present in two pairs, while those on TFMs occur in three pairs. The dorsal plates of the Ta-3 mites narrowed posteriorly (Fig. 1C, arrow), and three pairs of setae were present on the sternal plates (Fig. 1C, a). Thus, the Ta-3 mites is TFMs.

4. Discussion

Hematophagous chicken mites are a major threat to the poultry industry. The prevalence of chicken mites varies from region to region. However, despite poultry farming being an important industry in Southeast Asia, the classification of mites affecting poultry in this region is lacking. Therefore, we sought to detect the mite species in Myanmar, which is in mainland Southeast Asia.

We found that NFMs were observed in the poultry houses in and around of Py-1,2, and TFMs were identified from Ta-3. PRMs, however, were not collected from any of the farms. Thus, NFMs and TFMs may be the predominant species in Myanmar. TFMs are prevalent in warm areas, such as tropical and temperate zones (Denmark and Cromroy, 2003), whereas NFMs are widely distributed in various areas (Murillo

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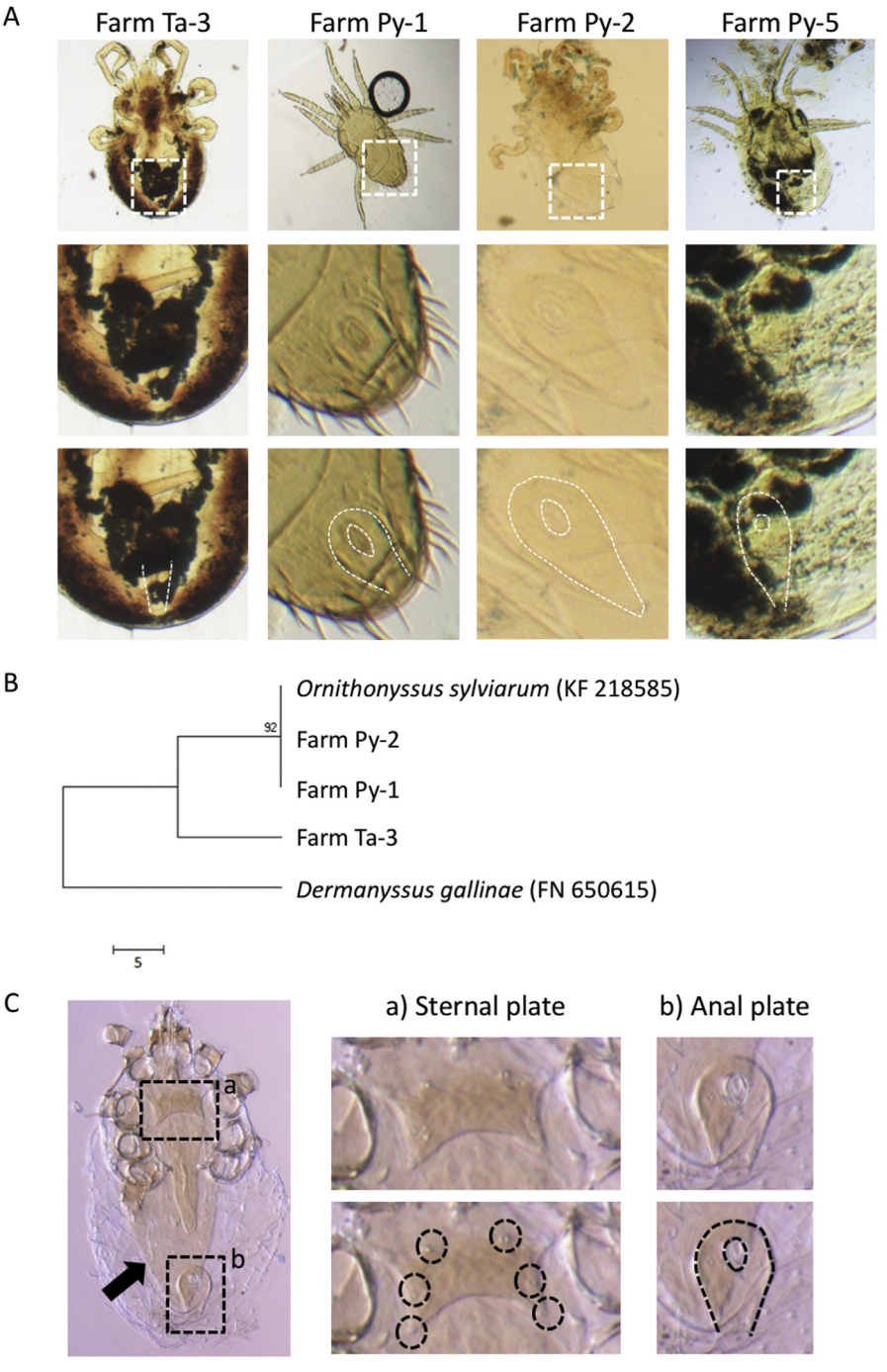

Fig. 1. A. Morphological observation of mites from four farms in Myanmar: Pyin Oo Lwin (Py-1, Py-2, and Py-5) and Taunggyi (Ta-3). The mites were sealed in Hoyer's medium after treatment with 60% lactic acid. The middle and lower panels represent the magnified images of the squares from the upper panel. The anal plate and the anus are indicated by the dotted lines on the lower panels. B. Phylogenetic analysis of the *COI* gene from the mite samples. The tree was constructed with MEGA software, version 7.0 (Kumar et al., 2016), using the neighbour-joining method (Saitou and Nei, 1987). The numbers next to the branches indicate the percentage of 1,000 bootstrap replicates. The scale bar indicates the number of nucleotide substitutions per site. C. Morphology of mites captured from Ta-3. The mites captured from

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Table 2. Homology between the *cytochrome c oxidase subunit I* (*COI*) gene among the mites from each farm with *Ornithonyssus sylviarum,* northern fowl mites (NFMs) and *Dermanyssus gallinae*, poultry red mites (PRMs).

Areas	Farm ID/species	Homology with *COI* (%)			
		Py-1	Py-2	NFM	PRM
Taunggyi	Ta-3	84.0	84.2	84.2	76.7
Pyin Oo Lwin	Py-1	-	99.8	100	78.0
Pyin Oo Lwin	Py-2	-	-	99.8	77.8
-	NFM	-	-	-	78.0

NFM and PRM nucleotide sequences obtained from GenBank (NFM: KF218580; PRM: FN 650615).

and Mullens, 2017). The climate in Pyin Oo Lwin is relatively cool throughout the year, which may affect the prevalence of chicken mites.

Myanmar farmers often raise village chickens outside the poultry houses. We found that the mites on cage-free village chickens in Py-1 were similar to those in the Py-1 poultry houses. Therefore, mites that usually infest village chickens may get transmitted from village chickens to farmed chickens via equipment or humans. In addition, the mites may enter the poultry houses via birds and rodents, since the poultry houses in Myanmar are usually open-sided houses and there are no bird nets on most of the farms. Therefore, to prevent mite infestations, biosecurity should be improved by enacting safety measures like excluding wildlife from poultry farms.

Declarations

Author contribution statement

Masaki Takehara: Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Shiro Murata: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Ken Katakura, Saw Bawm: Conceived and designed the experiments; Performed the experiments.

Myint Myint Hmoon, Shwe Yee Win, Lat Lat Htun, Ye Htut Aung, Mar Mar Win: Performed the experiments.

Ta-3 were sealed in Hoyer's medium after treatment with 60% lactic acid. The whole-body image of a mite captured from Ta-3. The dorsal plate gently narrowed posteriorly. The arrow indicates the dorsal plate. The centre and right panels represent the magnified images of the squares from the left panel. (a) The circles represent the pores of setae on the sternal plate. (b) The anal plate and anus are indicated by the dotted lines.

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Masayoshi Isezaki, Naoya Maekawa, Tomohiro Okagawa, Satoru Konnai, Sotaro Fujisawa: Analyzed and interpreted the data.

Kazuhiko Ohashi: Conceived and designed the experiments; Analyzed and interpreted the data.

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Competing interest statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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