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Mitochondrial DNA-Based Identification of Forensically Important Flesh Flies (Diptera: Sarcophagidae) in Thailand

Chutharat Samerjai [1,2,3], Kabkaew L. Sukontason [1], Narin Sontigun [1], Kom Sukontason [1], Tunwadee Klong-klaew [3], Theeraphap Chareonviriyaphap [4], Hiromu Kurahashi [5], Sven Klimpel [6], Judith Kochmann [6], Atiporn Saeung [1], Pradya Somboon [1] and Anchalee Wannasan [1,*]

1 Center in Insect Vector Study, Department of Parasitology, Faculty of Medicine, Chiang Mai University, Chiang Mai 50200, Thailand; chutharat.smj@gmail.com (C.S.); kabkaew.s@cmu.ac.th (K.L.S.); narinsontigun@gmail.com (N.S.); kom.s@cmu.ac.th (K.S.); atiporn.s@cmu.ac.th (A.S.); pradya.somboon@cmu.ac.th (P.S.)
2 Graduate School, Chiang Mai University, Chiang Mai 50200, Thailand
3 School of Allied Health Sciences, Walailak University, Nakhonsithammarat 80161, Thailand; somtunwa@gmail.com
4 Department of Entomology, Faculty of Agriculture, Kasetsart University, Bangkok 10900, Thailand; faasthc@ku.ac.th
5 Department of Medical Entomology, National Institute of Infectious Disease, Toyama 1-23-1, Shinjuku-ku, Tokyo 162-8640, Japan; MLB15110@nifty.com
6 Institute for Ecology, Evolution and Diversity, Faculty of Biological Sciences, Goethe University, Max-von-Laue-Str.13; Senckenberg Biodiversity and Climate Research Centre (SbiK-F), 60438 Frankfurt am Main, Germany; Klimpel@bio.uni-frankfurt.de (S.K.); judith.kochmann@senckenberg.de (J.K.)
* Correspondence: kdantra@gmail.com; Tel.: +66-8-943-46851

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Abstract: Flesh flies (Sarcophagidae) are necrophagous insects initially colonizing on a corpse. The species-specific developmental data of the flies collected from a death scene can be used to estimate the minimum postmortem interval (PMI_{min}). Thus, the first crucial step is to correctly identify the fly species. Because of the high similarity among species of flesh flies, DNA-based identification is considered more favorable than morphology-based identification. In this study, we demonstrated the effectiveness of combined sequences (2216 to 2218 bp) of cytochrome c oxidase subunit I and II genes (*COI* and *COII*) for identification of the following 14 forensically important flesh fly species in Thailand: *Boettcherisca nathani* Lopes, *Fengia ostindicae* (Senior-White), *Harpagophalla kempi* (Senior-White), *Liopygia ruficornis* (Fabricius), *Lioproctia pattoni* (Senior-White), *Lioproctia saprianovae* (Pape & Bänziger), *Parasarcophaga albiceps* (Meigen), *Parasarcophaga brevicornis* (Ho), *Parasarcophaga dux* (Thomson), *Parasarcophaga misera* (Walker), *Sarcorohdendorfia antilope* (Böttcher), *Sarcorohdendorfia inextricata* (Walker), *Sarcorohdendorfia seniorwhitei* (Ho) and *Seniorwhitea princeps* (Wiedemann). Nucleotide variations of Thai flesh flies were evenly distributed throughout the *COI-COII* genes. Mean intra- and interspecific variations ranged from 0.00 to 0.96% and 5.22% to 12.31%, respectively. Using Best Match (BM) and Best Close Match (BCM) criteria, identification success for the combined genes was 100%, while the All Species Barcodes (ASB) criterion showed 76.74% success. Maximum Likelihood (ML) and Bayesian Inference (BI) phylogenetic analyses yielded similar tree topologies of monophyletic clades between species with very strong support values. The achieved sequences covering 14 forensically important flesh fly species including newly submitted sequences for *B. nathani*, *F. ostindicae* and *S. seniorwhitei*, can serve as a reliable reference database for further forensic entomological research in Thailand and in other areas where those species occur.

Keywords: forensic entomology; molecular identification; *COI*; *COII*; flesh flies; Thailand

1. Introduction

Besides the Calliphoridae, Sarcophagidae (flesh flies) contain some of the most important carrion-breeding flies which colonize a human cadaver during the initial stages of decomposition [1]. In forensic investigations, the sarcophagids provide more precise PMI_{min} estimation than calliphorids because they are larviparous and deposit larvae directly on the cadaver and feed immediately [2]. Substantial entomological evidence has been presented for flesh flies, for example, *Bercaea africa* (Wiedemann) in Italy [3], *Liopygia ruficornis* (Fabricius) in Thailand [4] and Kuwait [5], *B. africa*, *Parasarcophaga dux* (Thomson), *Liopygia argyrostoma* (Robineau-Desvoidy), *Robineauella scoparia* (Pandelle), *Parasarcophaga similis* (Meade) in Switzerland [6], *Seniorwhitea princeps* (Wiedemann) in Malaysia [7], and *L. argyrostoma*, *B. africa*, *Heteronychia fertoni* (Villeneuve), *Boettcherisca peregrine* (Robineau-Desvoidy) in Iran [8,9].

Among 2510 known species in 173 genera of Sarcophagidae described worldwide [10], 86 species in 31 genera have been recorded in Thailand [11]. Most adults in the subfamily Sarcophaginae share some common morphological characteristics which include grey-black longitudinal stripes on the thorax, a checkerboard abdomen, and a strongly bristled body [12]. Morphological characteristics of immature and adult stages among flesh fly species are very similar, thus making identification difficult, particularly for non-expert taxonomists [13]. Since the developmental times of flesh flies are species specific, the correct identification at the species level is a primary step for estimating the PMI_{min} [14,15]. Therefore, a potential tool is needed which can discriminate the species regardless of life-history stage is needed [16].

Recently, DNA-based identification which requires only a small sample of any life stage, has been extensively used and has become a reliable routine tool in forensic entomology [17–19]. Among applied genetic markers, mitochondrial cytochrome c oxidase subunit I (*COI*) has been widely used as a species identifier because of its beneficial features for evolutionary genetics studies, such as a high copy number per cell, a high mutation rate, and haploid maternal inheritance [20,21]. Many studies have documented the robustness of *COI* as the DNA barcode for fly species discrimination [16,17,22]. Nervertheless, the usage of short fragments or even the entire sequence of *COI* is sometimes limited in resolving phylogenetic relationships and identifying cryptic species [16,23] or species complexes [24] of some flesh flies. Several investigations suggested that using *COI* alone, as a species identifier, should be done with care and to achieve a 100% identification success, multiple markers should be used in the analyses, especially for Sarcophagidae [24–26].

Sequences of forensically important flesh flies have been published from different regions of the world [16,22,27,28], but they are still insufficient in the Oriental regions [2,21]. To date, a reference DNA database of forensically important flesh flies in Thailand is missing and only two genetic studies involving the *COI* and nuclear *28S* rRNA genes for only five flesh fly species have been reported [29,30]. Therefore, this study aimed to evaluate the use of combining *COI* and *COII* genes to identify 14 forensically important Thai flesh fly species and to improve the regional databases as sequence data for some species have never been reported before.

2. Materials and Methods

2.1. Specimen Collection

From 2015 to 2016, flesh fly collections were carried out in 7 provinces of Thailand, including Chiang Mai, Lampang, Phitsanulok, Khon Kaen, Ubon Ratchathani, Songkla, and Satun (Figure 1). Collections were performed by sweeping method using 300 g of 1-day tainted beef offal as the attractive bait. After collections, specimens were frozen at −20 °C for 1 h and adult males were identified based