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Molecular Phylogeny of Indonesian Armyworm *Mythimna* Guenée (Lepidoptera: Noctuidae: Hadeninae) Based on CO I Gene Sequences

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Armyworm *Mythimna* Guenée is one of the most important pests on graminaceous crops and pastures in South East Asia (i.e. *M. separata* Walker is well known to cause serious damages on rice in Indonesia). Like of most other genera of moths, the systematic of this genus is still in dispute, especially on the taxonomy and classification within this genus due to their morphological characters that are very difficult to distinguish from one to others. Molecular approaches such as using CO I gene sequence to differentiate among species has been recommended since this gene has ability to reveal the character identity at the specific level. In order to populate the genetic characters of Indonesian *Mythimna*, to clarify the clasification within the genus *Mythimna* and to reveal the phylogenetic relationship among them, we analyzed 14 species of *Mythimna* and two species outgroups (*Spodoptera litura* dan *S. exigua*) based on nucleotide sequence variation across a 649 bp region in the CO I gene. Over entire 649 bp region 72% of the nucleotide positions were constant, 10.6% were uninformtive (i.e. any variants were found in a single sequence) and 16.9% were parsimony informative. The informative site constituted in the 3rd codon position was the highest, whereas in 2nd codon position was the lowest. The results also showed that the base composition of this region was low A + T biased. The results showed that the monophyly of *Mytimna* was supported by 95% bootstrap test at any tree building methods. The three subgenera based on morphology were recovered but *M.* (*Mythimna*) shown to be a paraphyletic group in term of *M.* (*Hyphilare*), and *M.* (*Pseudalteia*); *M.* (*Mythimna*) was branched off first then followed by *M.* (*Pseudaletia*) and *M.* (*Hyphilare*). However, all internal nodes were least support except for the monophyly of subgenus *M.* (*Hyphilare*). It indicates that the relationships among internal nodes proposed here were least valid due to the number of species included in the analysis which may not be enough to represent the real number of species in the nature. More investigation was needed by including more species and other genes.

Key words: *Mythimna*, phylogenetic relationships, CO I gene

INTRODUCTION

Armyworm is one of the most important pests on Graminaceous crops in Indonesia. There are two groups of armyworm, i.e. the genus *Spodoptera* and *Mythimna*. The last one comprises species involved in what are referred to as 'armyworm' outbreaks (Holloway *et al*. 1987; Chandler & Benson 1991). They have been given this common name due to their sporadic occurrence in large numbers in a manner similar to the African armyworm, *Spodoptera exempta* (Walker), which is a serious pest of many graminaceous crops (Brown 1972; Carnegie *et al.* 1974). Larvae of *Mythimna* spp. feed on pasture grasses, sugarcane, maize and rice but, unlike S. *exempta,* which is a true armyworm, they do not migrate from one feeding site to another in searching food. Both of them cause a serious damage on several crops. Compared with the genus *Spodoptera*, however, *Mythimna* larvae is more unique since they have specific-host plants, mostly on Gramineae.

The moths of *Mythimna* spp. are nocturnal and the larvae start feeding at the onset of darkness and are rarely seen during the day (Carnegie & Dick 1972). Several species have been reported as serious pests in Mauritiuss such as *Mythimna* (= *Leucania) loreyi* Duponchel, *M. tincta* Walker. *M. loreyi* has been reported to attack sugarcane and other graminaceous crops in Africa, Australia, Philippines, and Southern Europe (Calora 1966; Chandler & Benson 1991; Edwards 1992; Ganesha & Rajabale 1996). *M. curvula* Walker has been reported in Reunion on sugarcane and other grasses (Etienne 1976) and in Madagascar on sugarcane (Rungs 1955). Whereas *M. separata* Walker, *M. venalba* Moore, and *M. loreyi* has been reported as important pests of rice, maize, and sugarcane in Indonesia (Kalshoven 1981).

Mythimna is a large group and distributes widely in the world but the status of this genus is still in dispute, especially on their taxonomy and classification within this genus. There has been considerable taxonomic confusion regarding members of the genus *Mythimna*. Rungs (1955), Calora (1966), Edwards (1992), andYoshimatsu (1994) have studied the species from the Phillipines, Madagascar, Australia, Japan, and Taiwan, respectively. Detailed taxonomic studies have not been carried out in Indonesia except those that have been conducted by Holloway (1989) based on materials from Borneo.

Sugi (1982) divided this *Mythimna* complex based on Japanese moths into several genera, they are: *Mythimna*, *Aletia*, *Pseudaletia*, *Dysaletia*, *Analetia*, *Leucania*, *Acantholeucania*, and *Xypholeucania*. They share two distinctive features: in the male gentitalia the valve bases are united by a bubble-like structure (Calora 1966); in the female genitalia the ovipositor lobes are strongly sclerotised, with their distal margin distinctively oblique. This character is probably assosiated with oviposition within the leaf axils or between blades of the graminaceous host in neat row. The female bursa copulatrix lack of signa.

Holloway (1989) conducted his study based on Bornean materials and divided the genus *Mythimna* into several subgenera i.e. *Aletia*, *Pseudaletia*, *Acantholeucania*, and *Leucania*. On the other hand, Edwards (1996) treated *Leucania* as a dependent genus and *Acantholeucania* placed under sub genus *Leucania* and *Pseudoaletia* under subgenus *Mythimna*. They also treated several species such as *M. decisissima* and *M.consanguis* as unknown place.

The most intensive work on classification of this *Mythimna* group was conducted by Yoshimatsu (1994) by revising the classification of the genus based on Japan and Taiwan species. He divided the genus *Mythimna* into seven subgenera, they are: *Mythimna*, *Hephilare*, *Sablia*, *Pseudaletia*, *Acantholeucania*, *Anapoma,* and *Dysaletia*. He proposed these seven subgenera based on genitalia both of male and female characters. Each subgenus has been established based on uniquely derived characters as shown in Table 1.

He treated *Aletia* and *Leucnaia* under genus *Mythimna* based on these genitalia characters. Genus *Aletia* has been been recorded from Asia and America but European authors have used the genus name *Mythimna* in which they include species *Aletia*. In fact, the structure of the genitalia characters of these two type species, *M. turca* and *A. vitellina* are similar. Moreover, previously, *Leucania* has been established based on the absence of coronal spines on the cucullus in which these characters are variable and not realiable to be used as criterion of the genus (Yoshimatsu 1990). This treatment resulted in a tremendous change on the classification of Indonesian species (Holloway 1989) (Table 2).

There is no doubt that morphological character is very important to recognize the identity of species but it is not always easy when we deal with a complex species. A lot of internal characters are probably useful, especially the genital characters, not only to confirm the identity species but also to reconstruct the phylogenetic relationship among them. However, it is often difficult to score these characters due the complexity of their structures. The other problem is the objectivity of the observers, different observers will give different results when they work on the same sample specimens.

Molecular approach is one of the alternatives that can be applied to fill that gap. The huge numbers of characters resulted from a certain gene sequence are very powerful not only to differentiate among species within a species complex but also in resolving their phylogenetic relationships. Among them, CO I gene has been chosen as one of the candidate genes to be applied in DNA barcoding (a novel system designed to provide rapid, accurate, and automatable species identifications by using short, standardized gene regions as internal species tags) (Hebert &Gregory 2005; Herbert *et al*. 2010). Almost all requirements that are needed in DNA barcoding can be reached by this gene. This gene can be used to distinguish species in almost all animals. The length of this gene is relatively short about 650 bp. Compared with another mitochondrial gene, CO I gene is more conserved. Therefore, this gene is very suitable to identify a species since its sequence has a low variability (in general less than 1-2%), even for the closely related species its value is less than 1%. Another benefit of using this mitochondrial gene is that it is relatively easy to sequence than nuclear genes such as Wingless, EF-1α and ITS (Sutrisno 2003,

Table 1. Seven subgenera of *Mythimna* with the uniquely derived characters (Yoshimatsu 1994)

Subgenera	Uniquely derived characters
Mythimna	1. Valvula not strongly produced 2. Ampulla and harpe moderately large
Hyphilare	1. Tufts of black hairs on the mail basal abdominal segment
Sablia	1. Sacculus with a long process dorsoposteriorly 2. male abdominal sternite 1+2 with a media pouch
Pseudaletia	1. Valvula strongly produced 2. Ampulla and harpe very small
Acantholeucania	1. Valvulla with a long acute process postoventrally
Anapoma	1. Corona represented by two or three rows of spines on posterior portion of cucullus
Dysaletia	1. Corona represented by many sparse spines on cucullus

Table 2. Treatment of the member of the genus *Mythimna* by Holloway (1989) and Yoshimatsu (1994)

Species	References	
	Holloway (1989)	Yoshimatsu (1994)
albomarginata	*Mytimna* (*Aletia*) *albomarginata*	*Mythimna* (*Mythimna*) *albomarginata*
decississima	*Mythimna* (*Aletia*) *decississima*	Mythimna (*Hyphilare*) *decississima*
calorai	*Mythimna* (*Aletia*) *calorai*	*Mythimna*(*Hyphilare*)*epieixilus*
radiata	*Mythimna* (*Aletia*) *radiata*	*Mythimna* (*Mythimna*) *radiata*
yu	*Mythimna* (*Acantholeucania*) *yu*	*Mythimna* (*Mythimna*) *yu*
albicosta	*Mythimna* (*Pseudaletia*) *albicosta*	*Mythimna* (*Pseudaletia*) *pallidicosta*