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Phylogenetic Relationships Within *Arctornis* (Lepidoptera: Erebidae) Based on *COI* Gene Sequences

HARI SUTRISNO

Laboratory of Entomology, Division of Zoology, Research Center for Biology,
The Indonesian Institute of Sciences, Jalan Raya Bogor Km 46, Cibinong 16911, Indonesia

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Genus *Arctornis* is one of Tussock moths which are most diverse in tropics, particularly in Sundaland.Several species associate with cultivated plants and have potential to become pests. The systematic of this genus is still in dispute, especially on the monophyly and the relationship within this genus due to the fact that it is very large genus (137 described species). To clarify the monophyly of the genus *Arctornis*, and to reveal the phylogenetic relationship among the Indonesian species, we analyzed ten species of Indonesian *Arctonis* involving seven other species distributed around the world based on a 600 bp region in the *COI* gene. The results showed that the monophyly of *Arctornis* was supported by a high bayesian partition test at Maximum likelihood tree building method. The relationship among groups was supported by moderate to high bayesian partition values. Indeed, *COI* gene was very useful to characterize *Arctornis* species, especially to distinguish member of Indonesian species. Nevertheless, this should be taken with precaution because more species and more conserved genes should be involved in the future analysis to test the validity of the proposed phylogeny.

Key words: *Arctornis*, *COI* gene, phylogenetic relationship

INTRODUCTION

Genus *Arctornis* is one of Tussock moths which are the most diverse in tropics, particularly in Sundaland. At present, about 137 described species has been reported worldwide, and more than half (86 species) distributed in Sundaland and the others to Paleartic, New Guinea and Australia (Schintlmeister 1994; Holloway 1999). This genus includes as synonyms *Redoa*, following Heppner and Inoue (1992), Nielsen *et al*. (1996), and Holloway (1999). Some species has been reported as important pests. *Arctornis riguata* has been reported as one of the species that defoliate seriously the cultivated mango in the center of mango production (Probolinggo, East Java) during March-May 2011 (Sutrisno *et al*. 2013). In addition, *A. cyana* also has been reported to defoliate the durian leaf in Thailand (Lim 1997), while *A. submarginata* defoliatestea leaf in North East India (Sinu *et al*. 2013).

Like most of other genera of moths, the monophyly and the relationship within this genus are still in dispute due to the bulkness of this genus (137 described species) (Schintlmeister 1994; Holloway 1999). The monophyly of the genus and the relationship within this genus have never been tested; even the comprehensive studies on the systematic of this genus were very limited except the study on taxonomy that has been conducted by Schintlmeister (1994) and Holloway (1999) based on specimens from Sumatra and Borneo.

Indeed, morphological characters are very important to establish a phylogeny of taxa but it is not always easy when we deal with complex genus such as *Arctornis*. The complexity of the structure creates problems in scoring of the characters states it self. So, it is often that different researcher will get different results in observing the same character. Another problem is to find a male and female of the adults at the same time. The females of *Arctornis* are often difficult to be collected by using a light trap because they are not attracted to light sources (Sutrisno 2014). On the other hand, the huge number of characters resulted from a certain gene sequence is able to fill the gap of the disadvantages of morphological characters (Hebert *et al*. 2010). Molecular data is very powerful not only to differentiate among species within a large and varied genus but also to resolve the phylogenetic relationships among them, from lower to higher level. *COI* gene is one of the most useful genes not only for species identification but this gene or combined with other genes also often has been used in inferring the relationship among closely-related species in several groups of Lepidoptera (Sutrisno *et al*. 2006; Yamamoto & Sota 2007; Tsao & Yeh 2008; Kim *et*

*Corresponding author. Phone: +62-21-8765056,
Fax: +62-21-8765068, E-mail: sutrisnohari@yahoo.com

al. 2010). Therefore, this study was aimed to clarify the monophyly of the genus *Arctornis*, and to reveal the phylogenetic relationship among the Indonesian species, based on *COI* gene sequences.

MATERIALS AND METHODS

Moth Specimens. In order to clarify the monophyly of the genus and the relationship of Indonesian *Arctornis* within this genus, we used mitochondrial *COI* gene sequence to reconstruct the relationships among ten species of *Arctornis* which are distributed in different localities in Indonesia, and seven other species from the Genbank. *Lymantria beatrix* and *L. atemeles* were used as the outgroup in the phylogenetic analysis. All species used in this study were presented in Table 1. Adult moths were collected by using light traps.

Species Identification. Adult moths were identified based on external and internal characters. The genitalia slide was made by the custom method of boiling in 10% potassium hydroxide for about 10-11 min and then moths were observed under binocular stereoscopic microscope.

DNA Extraction and Sequencing of *COI* Gene. DNA extraction was conducted through a non-destructive method with modification from QIAGEN animal tissue protocol kit using spin column following Sutrisno (2012a). So far, this method is the best for museum specimen since the genitalia characters can be accessed without any damage after extraction process.

The complete sequence primers used were LepF1: 5' ATT CAA CCA ATC ATA AAG ATA TTG G 3', and LepR1: 5' TAA ACT TCT GGA TGT CCA AAA AATCA 3' (Hajibabaei *et al*. 2006). The amplification was conducted following the protocol used by previous authors (Hebert *et al*. 2010; Sutrisno 2008, 2011, 2012a,b). PCR products were sent to Macrogen for sequencing. The sequences were aligned by using BioEdit (Hall1999).

Base Composition Analysis. Base composition and the homogenity of the base frequency across taxa were calculated through the base frequency's option in PAUP* version 4.0b.10 for 32-bit Microsoft Windows (Swofford 2001). For the sequence divergence we selected K2P distance model.

Phylogeny Reconstruction. Maximum Likelihood (ML) tree building method was constructed by using MrBayes version 3.2 (Bayesian Analysis of Phylogeny) (Ronquist & Huelsenbeck 2003).The best model of nucleotide substitutions was calculated by using Kakusan version 4 (Tanabe 2007). The Bayesian partition test with 1,000,000 replications was used to test the statistical confidence of a particular clade/group in the tree building method.

RESULTS

Base Composition. Seventy sequences of *Arctornis* and two species out groups *Lymantria beatrix* and *L. atemeles* were aligned with no evidence of insertion and deletion. The conserved regions within *Arctornis* were found at position:23 TTAATTCGAGC 33, 134 ATTATAATTGG 144, 284 GGAACTGGATGAAC 297, and 302 TACCCCCCACT 312. Aligned sequences have been submitted to the Genbank with accession numbers presented in the Table 1.

Table 1. Species selected for molecular study

Species	No. Acc.Genbank	Voucher specimens/sources
A. phrika	AB930202	MZB: Lepi.131
A. perfecta	AB930203	MZB: Lepi.132
A. meridionalis	AB930204	MZB: Lepi.134
A. lumulosa	AB930206	MZB: Lepi.136
A. phasmatodes	AB930207	MZB: Lepi.137
Arctornis sp.A.	AB930208	MZB: Lepi.138
Arctornis sp.B.	AB930209	MZB: Lepi.139
A. secula	AB9302010	MZB: Lepi.140
A. calcariphallus	AB9302013	MZB: Lepi.161
A. riguata	AB9302015	MZB: Lepi.163
A. l-nigrum	JF415315.1; GI:326369612	ZSM Lep 21258
Arctornis sp. 1SEM 2008	FJ500003.1; GI:22240535	USNM:ENT:002692200
Arctornis sp. 2SEM 2008	FJ499963.1; GI: 222540455	USNM:00507173
Arctornis sp. 3SEM 2008	FJ500000.1; GI:222540529	USNM: 00267623
Arctornis sp. 4SEM 2008	HQ558298.1; GI:313178097	USNM:00704471
Arctornis RZ 2010	HQ006943.1; GI:323408660	RZ89
Arctornis sp. C.	JX970173	USNM ENTO 00733729
Lymantria beatrix	AB851471	MZB: Lepi.108
Lymantria atemeles	DQ116184.1	-

Table 2 shows the pattern of nucleotide substitution of *COI* gene. The highest rate of substitution was 35.37 (transitional substitution from C to T), while the lowest was 2.51 (transversional substitution from T to G or C to G). Moreover, the nucleotide frequencies were A+T rich (69%). The transition/ transversion rate ratios were k_1 = 1.602 (purines) and k_2 = 5.209 (pyrimidines). The overall transition/ transversion bias was R = 1.594.

There was almost no interspecific variation in the base composition in *COI* for the total nucleotides. The chi-square test indicated that there was no significant difference in the frequency of bases between taxa (X^2 = 20.548433; df = 39; P = 0.99339404).

Sequence Divergence. The mean of pairwise sequence divergences of *COI* gene based on K2P distance model within Group A, C, D, were 9, 12.8, and 9.9%,respectively, and the average distance among group was 14%. The closest relationship within group was a pairwise between species *Arctornis* sp. 1 SEM 2008 and *Arctornis* sp. 2 SEM 2008 (1.6%). The complete pairwise divergent of sequences within *Arctornis* is presented in Table 3.

Phylogeny. The Maximum Likelihood tree building method based on the best model of Gama-distributed rate (GTR_Gamma = 6.93126e+003) showed that Genus *Arctornis* fall into four groups (A, B, C, and D) but the relation among group A, B, and C was unclear showing paraphyly relationships. The monophyly of *Arctornis* was supported by a high bayesian partition value (97%), leaving the outgroup *Lymantria* species. The monophyly of each group was supported from moderate to high values (54-91%) (Figure1). The basal group was the position of group D.

DISCUSSION

The results showed that *COI* genes from 17 species of *Arctornis* was A+T rich which is consistent with mitochondrial genomes of other previously reported genera of Lepidoptera ranging from 62 up to74% (Kranthi *et al*. 2006; Sutrisno *et al*. 2006; Sutrisno 2011, 2012b). The average of A+T proportion in the

Table 2. Maximum composite likelihood estimate of the pattern of nucleotide substitution

	A	T	C	G
A	-	6.79	2.97	**4.01**
T	5.72	-	**15.47**	2.51
C	5.72	**35.37**	-	2.51
G	**9.17**	6.79	2.97	-

Rates of different transitional substitutions are shown in bold and those of transversionsal substitutions are shown in regular.

Table 3. Pairwise of sequence divergent within *Arctornis* based on Kimura two parameter model (K2P)

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
A. phrika	-																	
A. pasmatodes	0.095	-																
A. calcariphallus	0.102	0.101	-															
A. meridionalis	0.122	0.136	0.131	-														
Arctornis sp.C	0.117	0.132	0.144	0.148	-													
Arctornis inigrum	0.126	0.121	0.121	0.122	0.096	-												
Arctornis sp.A	0.120	0.147	0.121	0.121	0.122	0.110	-											
Arctornis sp.B	0.100	0.100	0.091	0.105	0.132	0.121	0.096	-										
A. perfecta	0.143	0.148	0.179	0.176	0.159	0.161	0.149	0.087	-									
A. secula	0.142	0.121	0.126	0.126	0.167	0.122	0.143	0.063	0.117	-								
A. nr. intacta 3 SEM	0.121	0.100	0.117	0.122	0.149	0.132	0.133	0.054	0.101	0.064	-							
A. submarginata	0.121	0.110	0.096	0.117	0.149	0.138	0.117	0.054	0.101	0.054	0.051	-						
A. nr. intacta 4 SEM	0.150	0.127	0.134	0.140	0.181	0.163	0.152	0.082	0.122	0.073	0.050	0.046	-					
A. nr. intacta 1 SEM	0.121	0.100	0.106	0.127	0.173	0.143	0.122	0.054	0.101	0.063	0.041	0.041	0.059	-				
A. nr. intacta 2 SEM	0.121	0.090	0.106	0.116	0.160	0.132	0.122	0.036	0.091	0.054	0.032	0.032	0.050	0.016	-			
Arctornis RZ 2010	0.179	0.132	0.139	0.121	0.167	0.127	0.163	0.107	0.156	0.139	0.146	0.146	0.153	0.151	0.133	-		
A. lumuna	0.131	0.115	0.116	0.142	0.142	0.115	0.112	0.067	0.111	0.105	0.096	0.091	0.112	0.096	0.081	0.087	-	
A. riguata	0.142	0.122	0.132	0.154	0.137	0.121	0.127	0.092	0.157	0.117	0.102	0.123	0.134	0.102	0.102	0.114	0.072	-

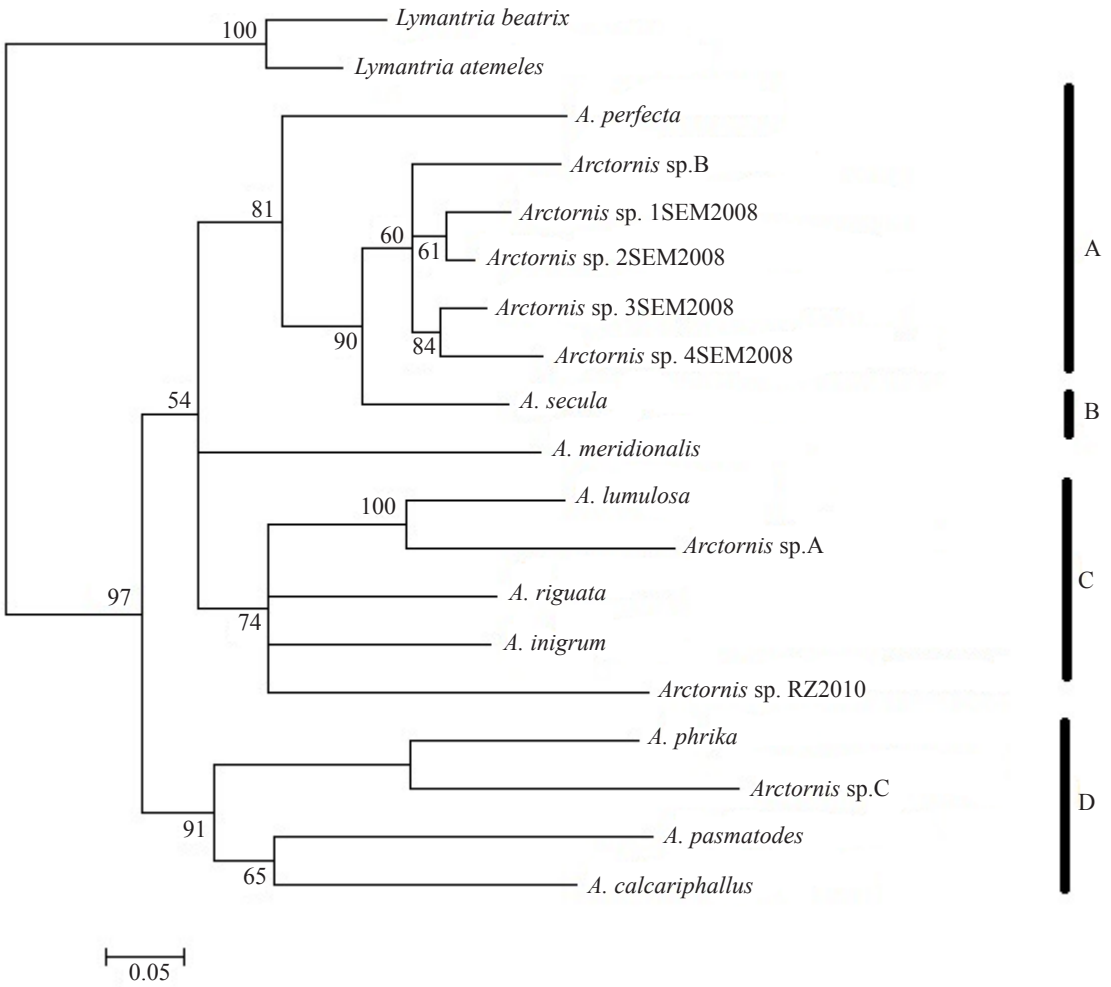

Figure 1. Maximum likelihood tree based on all substitution of *COI* gene (Bayesian partition supports are shown on each node).

present study (69.56%) was comparable with those found in other genera of Lepidoptera.

This study also showed that transition and transversion bias was moderate (R = 1.594). It indicates that transitions (Ts) occur more frequently than transversions (Tv), and Ts values are usually expected to exceed Tv values; however, it has been reported for some mitochondrial DNA that Tv values exceed Ts values (van Dorp 2004; Sutrisno *et al*. 2006; Roe & Sperling 2007).

The sequence divergence of *COI* gene within group was relatively high (9-12.8%), which indicates that each group within genus *Arctornis* is consisted of a large number of species and very diverse, especially within Group C. These values were higher than those found within group of *Glyphodes* (5.92-7.55%) and subgenera within *Mythimna* (5.32-8.82%) (Sutrisno *et al*. 2006; Sutrisno 2012b) but it was comparable to those of *Lymantria* (12.16%) (de Waard *et al*. 2010).

This study showed that *COI* gene alone was able to produce synapomorphies on each basal node when the best model of gama-distributed rate was used in the analysis. Previous study on very large genus *Lymantria* was failed to show that this genus is a good monophyletic group based on *COI* alone without using the best model of Gama-distributed rate (Sutrisno 2014). There is no doubt that combination of the *COI* and *EF-1α* will also increased resolution and supports most of the phylogenetic relationships as suggested by separate analysis of *Ectoedemia* s. str. (Lepidoptera: Nepticulidae) (van Nieukerken *et al*. 2012). However, amplification of the nuclear gene (*EF-1α*) from the museum specimens in this study was failed.

All findings in the present study suggest that monophyly of *Arctornis* was supported by high bayesian partition support (97%). This finding agrees with the previous hypothesis that this genus is a monophyletic group (Schintlmeister 1994; Holloway 1999). At least there are two aphomorphy characters to support the monophyly of this genus i.e. unusual articulated arm, or harpe, often very long, and slender arising from a pocket on the valve sacculus and the ornamentation of the valve margin. All members of this genus has very long and slender harpe except in *A. phrika* and *A. mallephrika*. The harpe in *A. phrika* as well as in *A. mallephrika* is not equally developed; it is possible that the harpe at the left side

is reduced (Figure 2) (Darmawan *et al*. 2013). This is very often occurs during evolutionary process even certain apomorphy character was lost in the member of the group for example in the genus *Hyalobathra*; a transparent window at the basal forewing (one of the apomorphies of this genus) was lost in one of the member of this genus (Sutrisno & Horak 2003). On the other hand, the ornamentation in the valva margin is presents in all members of this genus. The ornamentation is vary across the species within this genus and each species shows its specificity as is shown in the ornamentation of *A. perfecta* (Figure 3) (Darmawan *et al*. 2013).

The lack of species sampling in the analysis may resulted in a moderate bayesian partition value on a certain node in the ML tree building method. We believed that *Arctornis* included in this analysis is only a small part of the whole *Arctornis* in the world (< 10%). These problems can be resolved only by increasing the number of sample species in the analysis to reduce the distance sequences and also possibly by involving gene having slow evolutionary rate (Nei & Kumar 2000).

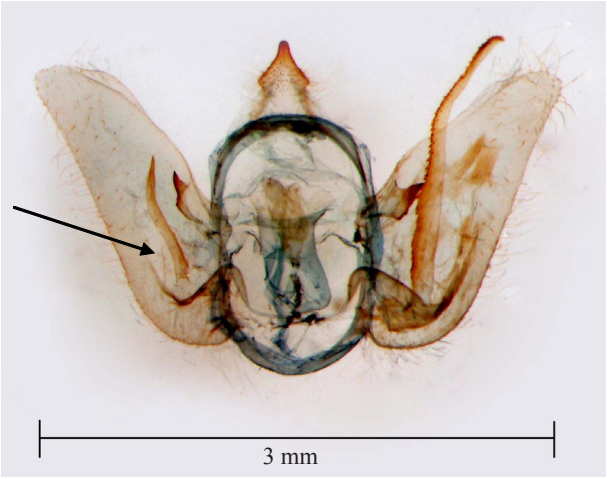

Figure 2. Male genitalia of *A. phrika*. Arrow shows a harpe reduced at left site (Darmawan *et al*. 2013).

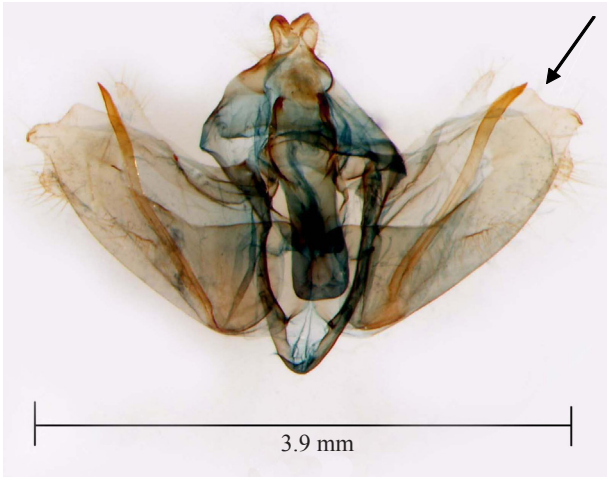

Figure 3. Male genitalia of *A. perfecta*. Arrow shows the ornamentation of margin valve (Darmawan *et al*. 2013).

Ten Indonesian species were evolved independently and distributed into four groups: A, B, C, and D. *A. lumulosa* was shown to be in a clade together with the potential mango pest, *A. riguata*. We should pay attention to those species regarding their potential to become pests in Indonesia in the future. All these data sequences are very useful as a reference information for molecular identification by non taxonomists (quarantine staffs and plant protectionists) in order to protect any potential species of this group that threaten our ecosystem.

Phylogenetic analysis of 17 species of *Arctornis* based on mitochondrial *COI* gene recovered four groups. A certain internal nodes gained only moderate supports. It indicates that the relationships among internal nodes proposed here were least valid due to the lack of sampling species. All evidences indicate that the relationship among these groups should be taken with caution. More species and more conserved genes are necessary to test the validity of the relationship proposed here.

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