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SHORT COMMUNICATION

PHYLOGENY OF *Holothuria leucospilota* (ECHINODERMATA: HOLOTHUROIDEA) AS INFERRED FROM CYTOCHROME C OXIDASE I mtDNA GENE SEQUENCES

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ABSTRACT

This study aimed to generate the genetic profile of *Holothuria (Mertensiothuria) leucospilota* (Brandt 1835) from Intan Besar Island, Langkawi, Kedah Darul Aman, Malaysia and then to determine the phylogenetic relationship between *H. leucospilota* and other members of genus *Holothuria* available in the GenBank using partial sequences of cytochrome c oxidase I (COI) mitochondrial DNA (mtDNA) gene. In this study, tree topologies of neighbour joining, maximum parsimony and maximum likelihood show that *H. leucospilota* has its own monophyletic clade clearly distinct from the other species and it is genetically closer to *Holothuria (Acanthotrapeza) coluber* Semper 1868.

Keywords: Molecular systematics; *Holothuria leucospilota*; partial sequences of cytochrome c oxidase I gene; phylogeny tree

Holothuria (Mertensiothuria) leucospilota (Brandt 1835) or locally known as *bat puntil* is currently considered as the most abundant sea cucumber species in Malaysia (Kamarudin *et al.* 2009). This coral reef-dwelling species is not in danger of extinction like the commercial *Stichopus* species such as *Stichopus horrens* (Selenka 1867) that are locally known as *gamat*. Previously, cytochrome c oxidase I (COI) mitochondrial DNA (mtDNA) gene sequences of *H. leucospilota* were unavailable online in the GenBank, National Center for Biotechnology Information (NCBI), U.S. National Library of Medicine until 19 September 2008; justifying the need for the present phylogenetic study. In addition, the genetic relationship between *H. leucospilota* and other members of genus *Holothuria* was also determined in this preliminary study.

Specimens of *H. leucospilota* were collected from Intan Besar Island, Langkawi, Kedah Darul Aman, Malaysia. Approximately 550 base pairs of COI mtDNA gene were amplified using universal primers (Palumbi *et al.* 1991) followed by DNA sequencing. Eight partial sequences of COI mtDNA gene of *H. leucospilota* from this study have been registered with the GenBank (accession no.: FJ223873 - FJ223880, submission date: 19 September 2008).

Three main methods namely neighbour joining, maximum parsimony and maximum likelihood were used for phylogenetic tree reconstruction. For the reconstruction of neighbour joining tree (Fig. 1A), pairwise genetic distances were calculated using Felsenstein (1984) model based on the unequal base frequencies and unequal ratio of transition to transversion. In general, current results suggest that monophyletic *H. leucospilota* is genetically closer to *Holothuria* (*Acanthotrapeza*) coluber Semper 1868 as compared to *Holothuria* (*Halodeima*) *atra* Jaeger 1833 (Figure 1). Besides, the taxonomic status of the unidentified species (GenBank accession no.: EU220818) is unresolved due to its inconsistent clustering position in all three methods and low bootstrap support (i.e. <55% bootstrap values).

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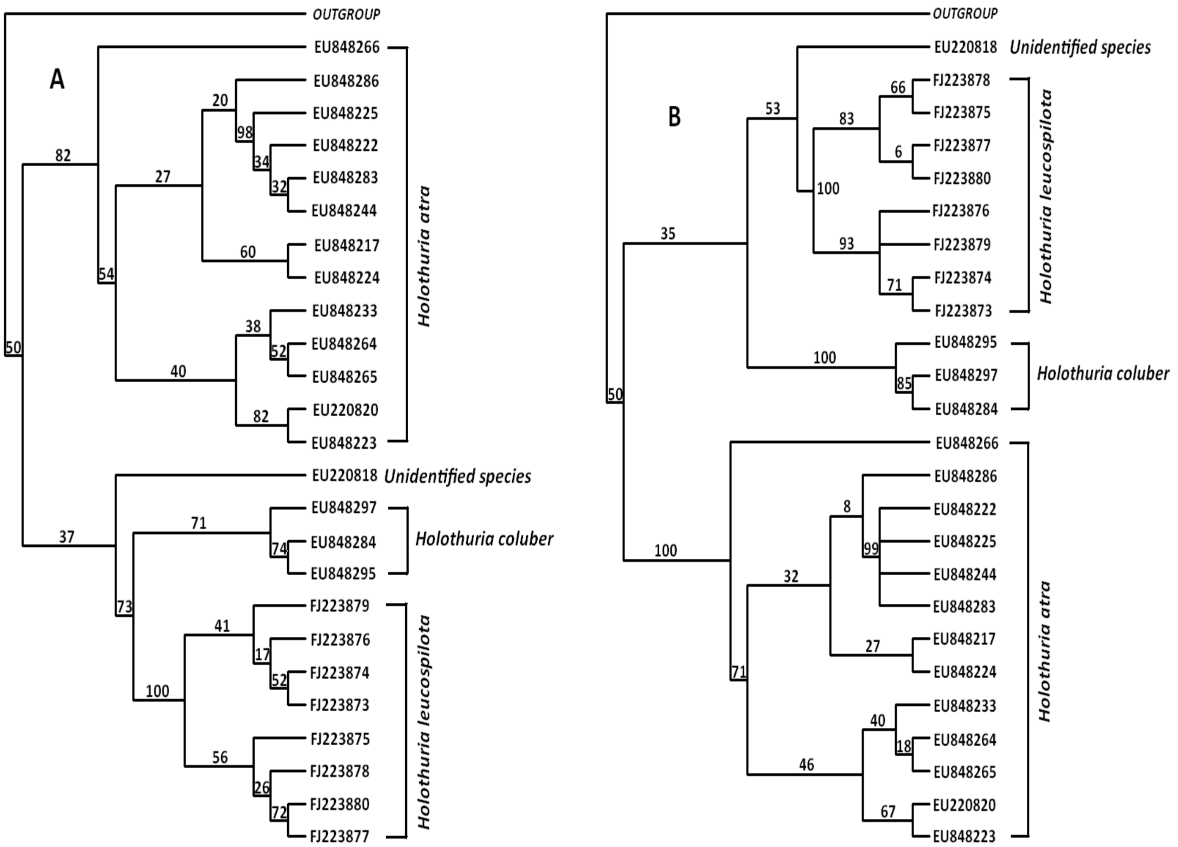

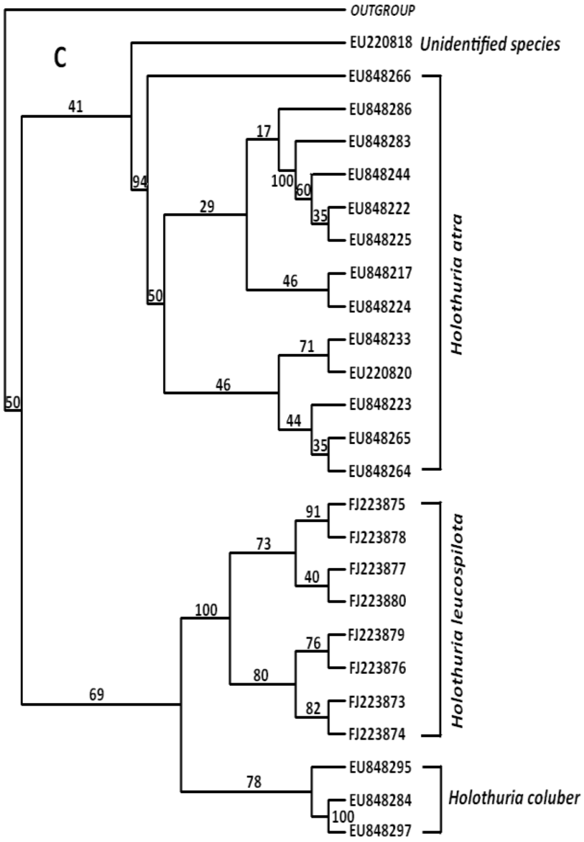

Figure 1. Topologies of neighbour joining tree (A), maximum parsimony tree (B) and maximum likelihood tree (C) of *Holothuria* species inferred from COI mtDNA gene using PHYLIP version 3.6b (Felsenstein 2004). Felsenstein (1984, F84) evolutionary model was incorporated for the neighbour joining tree, modified Templeton test using ordinary parsimony for the maximum parsimony tree, and Kishino-Hasegawa model for the maximum likelihood tree. Accession number of EU refers to sequences obtained from the GenBank. The majority rule consensus trees are each rooted with a sequence of *Cucumaria piperata*, a sea cucumber (GenBank accession number: U32211) with 1000 sequence replications and 100 data sets. Numbers.

Thus, inclusion of more specimens of the species is required to confirm its phylogenetic position within the genus *Holothuria*. Further studies encompassing broader geographical locations of *H. leucospilota* including the East Malaysian states of Sabah and Sarawak, as well as phylogenetic inferences from other *mt*DNA genes are currently being carried out in order to obtain a better view on the molecular phylogeny of *H. leucospilota*.

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