UNIVERSITI TEKNOLOGI MARA

# MOLECULAR SURVEY AND PHYLOGENETIC RELATIONSHIP OF ENDOSYMBIONTS IN *Diaphorina citri* FROM HLB-INFECTED *Citrus suhuiensis* AND HLB-FREE *Murraya paniculata*

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# ABSTRACT

*Diaphorina citri* is the psyllid vector of *Candidatus* Liberibacter asiaticus (*Ca.* Li. asiaticus), the causative agent of Huanglongbing (HLB), a debilitating citrus disease. Known to also feed on other host plants without causing any HLB symptoms, this psyllid also harbours other endosymbionts namely Syncytium, Mycetocyte, *Wolbachia sp.* and *Arsenophonus sp.* However, it is not certain whether *Candidatus* Liberibacter asiaticus and these other endosymbionts are obligate or transient endosymbionts or whether their presence is influenced by the disease status of the host plants or type of host plants the psyllid feeds on. Thus the aims of this study were to detect the presence of *Ca.* Li. asiaticus, Syncytium, Mycetocyte, *Wolbachia* sp. and, *Arsenophonus* sp. in *Diaphorina citri*, from HLB-infected *Citrus suhuiensis* and HLB-free *Murraya paniculata* and to determine the nucleotide variation and hence the phylogenetic relationship of each type of the endosymbionts of *Diaphorina citri* from HLB-infected and HLB-free plants. Detection of Mycetocyte, Syncytium, *Arsenophonus* sp. and *Ca.* Li. asiaticus endosymbionts was carried out by amplifying the partial 16S rDNA genes whereas detection of *Wolbachia* sp. was carried out by amplifying the *wsp* gene. Phylogenetic trees were constructed using Neighbour-Joining method. This study showed that Mycetocyte, Syncytium, *Wolbachia* sp., and *Arsenophonus* sp. are present in at least 66.7 % and 62.5% of the pysllid population feeding on *Citrus suhuiensis and Murraya paniculata* respectively. Thus they are transient rather than obligate endosymbionts. Their presence is also not influenced by the disease status and type of host plants the psyllids feed on. *Ca.* Li. asiaticus however, are only found in psyllids feeding on HLB-infected *Citrus suhuiensis.* Thus, their presence is influenced by the disease status of the host plants. Sequence analyses of the five endosymbionts from both HLB-infected and HLB-free plants revealed 14 haplotypes each with its own accession number. There is only one haplotype for *Ca.* Li. asiaticus and two haplotypes for Syncytium and *Wolbachia.* In *Mycetocyte* and *Arsenophonus* there are three and six haplotypes respectively. Phylogenetic analyses based on the partial 16S rDNA genes and *wsp* genes showed that there are no significant genetic variations or evolutionary changes between the endosymbionts of *Diaphorina citri* feeding on HLB-infected *Citrus suhuiensis* and *Murraya paniculata.* The data obtained from this study are the first on the prevalence of diversity and phylogenetic relationship endosymbionts in *Diaphorina citri* from HLB infected *Citrus suhuiensis* and HLB free *Murraya paniculata.*

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# CHAPTER ONE
# INTRODUCTION

## 1.1 Background of Study

Huanglongbing (HLB) or formerly known as citrus greening is one of the most devastating widespread citrus diseases and has become the major cause of economic loss to the citrus industry. The infected plants have a short life span, severe citrus yield and quality defect. This disease has destroyed an estimated of 60 million trees in Africa and Asia (Timmer *et al.*, 2003). The first record of HLB-like symptoms was first reported in India in the early 1900's which was referred as dieback and then as decline and death of citrus (Capoor, 1963; Husain & Nath, 1927). Then, in 1919, HLB was first discovered in Southern China (Lin, 1956). In the last decade, it has spread to 40 different countries in Asia, Africa, Oceania, South and North America (Halbert & Manjunath, 2004).

In Malaysia, *Diaphorina citri* was first recorded in Cameron Highland and Johor in 1978 (Yunus & Ho, 1980) although the symptoms of HLB were first noticed in Malaysia in the 1970s (Ko, 1988). The presence of HLB in Malaysia was confirmed in 1989 (Lim *et al.*, 1990). According to Lim *et al.*, (1990) in another report, HLB together with its vector were then rapidly distributed in the lowlands and has been recorded in many parts of the country. Between 1989 and 1992, HLB has spread rapidly and had destroyed citrus orchards in Peninsular and East Malaysia, including Sarawak (Teo *et al.*, 2000; Lim *et al.*, 1990). In the Samarahan Division of Southwest Sarawak in Malaysia, the citrus industry was completely destroyed by HLB in 1992 (Teo *et al.*, 2000).

In early 2000, the Department of Agriculture, Malaysia had initiated an intensive survey for HLB from the year 2001 to 2004. Zazali and Az1btain1at05) reported that major citrus growing areas in Peninsular Malaysia as well as Sabah and Sarawak were where most of the cultivated citrus varieties were infected with HLB. About 2,458 ha out of 3,526 ha of cultivation areas (69.7%) in Malaysia were found to be infected with the HLB disease. In Terengganu, HLB was distributed widely, increasing from 641 ha to 1262 ha from 2001 to 2004 (Zazali & Azizah, 2005). A study done by Sijam *et al.*