DNA BARCODING – DIFFERENTIATION OF VERBENACEAE *Lantana camara*, *Duranta erecta* AND *Stachytarpheta jamaicensis* USING *matK* and *trnH-psbA* AS MARKERS.

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ABSTRACT

DNA barcoding uses a short DNA sequence as a marker to identify which species an organism belongs to. In this experiment, identifying *Lantana camara, Duranta erecta* and *Stachytarpheta jamaicensis* belonging to the family Verbanaceae was attempted using *matK* gene and *trnH-psbA* loci. *matK* gene and *trnH-psbA* loci in DNA isolated from three plants of each species were amplified and sent for sequencing. Sequences of each gene were then analysed using bioinfomatic methods. Conflicting results were obtained i.e. the barcode gap and tree topology method failed but BLAST gave correct identification of the species. Since both tree topology and barcode gap are distance based methods in which sequence alignment is important, it may be that because of gaps or inversions of the alignment is incorrect. Thus it may be necessary to reanalyse the alignments or use alignment free analysis. Additionally further studies using other gene loci, and doing further replications could verify the results.

TABLE OF CONTENT

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

BLAST	Basic local Alignment search tool
bp	Base pair
DNA	Deoxyribonucleic acid
EDTA	Ethylene diamine tetraacetic acid
g	Gram
i.e.	'That is'
matK	Maturase K
MEGA 7	Molecular Evolutionary genetics analysis
min	Minute
MSA	Multiple sequence alignment
mL	Milliliter.
PCR	Polymerase chain reaction
s	Second
TAE	Tris-acetate-EDTA
TE	Tris/EDTA
TBE	Tris-Borate-EDTA
µL	Microlite

CHAPTER 1

INTRODUCTION

1.1 Background

Human exploitation of plants for production of food, paper, clothing, building materials and chemicals aimed at medication keep increasing each year. Humans consume around 20-25 percent of total plants on earth each year (The *National Aeronautics and Space Administration [NASA], 2010*). Because of the high consumption, problems have developed such as food shortage and loss of global biodiversity of plants (Convention on biological diversity, n.d.).

Society faces the challenge of providing safe food and medication as well as supporting biodiversity and functioning functioning. We must know what we eat, what we use and what we preserve. Species identification of plants can help prevent humans consuming plants which might be toxic or harmful, and helps to monitor and make decisions on species preservation (Taxonomy & Linneas, 2014). There are 390,900 species of plants known on earth (Morelle, 2016). Distinguishing between several species of plants can be difficult especially with the lack of expertise especially in tropical areas (Webb, Slik & Triono, 2010).

DNA barcoding is a way of species identification using DNA marker sequences (Valentini, Pompanon, & Taberlet, 2009). As only a short DNA fragment is needed, DNA barcoding can determine species composition in environmental samples which may be degraded, including fecal samples (Valentini et.al, 2009) and both processed food as well as raw food materials (Galimberti et al., 2013). However, DNA barcoding has its problems; in plants no single gene has been able to identify all plant species. The Consortium for the Barcode of Life (Li et al., 2011) proposed the use of *matk* and *rbcL*, but the non-coding *trnH-psbA* gene has a higher sequence divergence among species (Kress & Erickson, 2007), and the only way to determine if it is possible to use DNA barcodes on a particular taxonomic group is to test the

proposed loci on that particular taxonomic group. Adding to the need to test different plant taxons, may be that plant invaders can exhibit post-invasion genetic mutation (Jakobs, Weber, & Edwards, 2004) and also hybridization and/or introgression with closely related congeners present in the invasive range (Prentis et al., 2009; Meyerson & Cronin, 2013; Ndlovu, Richardson, Wilson, O'Leary, & Le Roux, 2013), which when combined with selection and genetic drift, may result in locally adapted genotypes (Pigliucci, Murren & Schlichting, 2006). Thus, species boundaries are often much harder to discern when individuals are sampled across geographical scales or through time (Baselga et al. 2013)

1.2 Aims

matk and *trnH-psbA* markers were tested for their ability to differentiate and identify plants in the Verbenaceae family, i.e., *Lantana camara*, *Stachitarpheta jamaicensis* and *Duranta erecta*.

CHAPTER 2

LITERATURE REVIEW

2.1 Species identification

Species identification is fundamental in all field of biological sciences. Development of sciences are highly related to the discovery of living organisms. Species identification is to assign an organism to a taxon. Among all types of living organisms, plant species identification is the most advanced field with most focus on evolutionary studies (Haider, 2011).

Why is it so important for us to identify the plants? It is because plant usage is widespread, including agriculture, food, and pharmaceutical uses. Species identification of plants help in the applications of agriculture seed production, processing of food, plant breeding for getting desirable traits, and other aspects (Haider, 2011).

In the past, plant species identification relied highly on morphological characters that is the physical and external structure of the plant (Wikipedia, 2016). Identification through traditional morphological methods cannot be neglected as it is the fundamental first steps in the identification process (Mehle & Trdan, 2012). However, it has it's own disadvantages. Two species can be morphologically similar, but can be genetically distinct (McGowan, n.d.). To solve this problem, scientist suggested using genes, which are more differentiated among species.

DNA barcoding is a method used for species identifications by using short sequence of gene as tags (Hebert & Gregory, 2005), and is a powerful system in the field of biodiversity and taxonomy research (Hajibabaei , Singer, Hebert & Hickey, 2007). With the development of DNA barcoding, species discovery has been accelerated rapidly (Hebert & Gregory, 2005). Genetic variation can be observed

through plants at all taxonomic levels of plants. The development of molecular techniques that generate gene markers and bioinfomatics tools to analyse these sequences has made it possible to identify plants accurately (Haider, 2011).

2.2 Verbenaceae

Verbenaceae, also known as verbena family, is a diverse group about 1200 species, mainly topical flowering plants, many of which are aromatic and have flowers often arranged in an elongate cluster (Wikipedia, 2016). This family contains trees, herbs, shrubs, lianas and some species are valuable the lumber production (NETindustries, n.d.).

2.2.1 *Lantana camara*

Lantana camara, (Figure 2.1) known as big-sage in Malaysia, is a species of flowering plant under the verbena family (Wikipedia, 2016). *L. camara* is a popular garden plant that has many uses in folk medicine. It produces a good yield of metabolites which can have useful biological activities. Traditional remedies use its leaves to relieve pain and swellings of the body by boiling and applying it onto the area. Its bark is used as an astringent lotion in leprous ulcers (National Tropical Botanical Garden, n.d.). In Brazil, plant extracts have been used to treat respiratory infections (Wikipedia, 2016).

Figure 2.1 Physical appearance of *Lantana camara*.

Flowers of *L. camara* can be in different colours, depending on location, maturity and age of the plants. The pre-existing colour of the flowers will attract the pollinators and once pollen grains present on the stigma, colour change occurs. This mechanism helps increase the pollination efficiency (Mohan & Mathur, 1984).

2.2.2 *Duranta erecta*

Duranta erecta, (Figure 2.2) also known as Golden dewdrop, is a species of flowering bush in Verbenaceae, which is native to Mexico. In tropical and warm subtropical gardens, it is often planted as an ornamental plant (Wikipedia, 2016).

Figure 2.2 Physical appearance of *Duranta erecta*

Duranta erecta grows into a bush or even a small tree. It can grow up to 6 m tall with equal width (Wikipedia, 2016). Its showy fruits and flowers make it desirable to be grown in a garden, but it's leaves and fruits are poisonous (Scanlan, Eagles, Vacher, Irvine, Ryan, & McKenzie, 2006). It can affect animals such as birds, cats, dogs and human especially children if the leaves and fruits are consumed. Symptoms of poisoning can be drowsiness, fever, nausea, vomitting, stomach ache and convulsions (Read, 2014).