

**PATTERNS OF BEE AND BUTTERFLY DIVERSITY IN
SOUTHEAST AND SOUTHERN EAST ASIAN
MEGACITIES**

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**FACULTY OF SCIENCE
UNIVERSITY OF MALAYA
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**PATTERNS OF BEE AND BUTTERFLY DIVERSITY
IN SOUTHEAST AND SOUTHERN EAST ASIAN
MEGACITIES**

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ABSTRACT

I investigated bee diversity and human perceptions of bees in four megacities – Greater Bangkok, Klang Valley, Pearl River Delta, and Singapore-Iskandar Malaysia. I sampled bees and conducted questionnaires at three different site types in each megacity: a botanical garden, central business district and peripheral suburban areas. Overall, the mean species richness and abundance of bees were significantly higher in peripheral suburban areas than central business districts ($p < 0.05$). Urban residents were unlikely to have seen bees but agreed that bees have a right to exist in their natural environment. Residents who did notice and interact with bees, were more likely to have positive opinions towards the presence of bees in cities. Additionally, I examined the species diversity of butterflies in urban parks in two cities – the Federal Territory of Kuala Lumpur, Malaysia and Shenzhen, South China. I investigated the relationships between butterfly species richness and three park variables: i) park age, ii) park size and iii) distance from the central business district. I conducted standardized butterfly sampling across different microhabitat types at each park: i) groves, ii) hedges, iii) flowerbeds and iv) unmanaged areas. I recorded 572 butterflies belonging to 60 species in Kuala Lumpur's urban parks. Although species richness was positively correlated with park age and size and negatively correlated with distance from the central business district; the correlations were not statistically significant. The highest species richness was recorded in the unmanaged microhabitat. In Shenzhen, I collected 1933 butterflies belonging to 74 species. Butterfly species richness showed weak negative correlations with park age and distance from the central business district but the positive correlation with park size was statistically significant ($p < 0.05$). Among microhabitat types, highest species richness was recorded in unmanaged areas.

ABSTRAK

Kepelbagaian lebah dan persepsi manusia terhadap lebah telah ditinjau di empat bandar mega — Greater Bangkok, Lembah Klang, Pearl River Delta, dan Singapura-Iskandar Malaysia. Kepelbagaian lebah dan soal selidik telah dijalankan di tiga jenis tapak yang berbeza dalam setiap bandar mega: taman botani, kawasan pusat perniagaan dan kawasan pinggir bandar. Secara keseluruhan, min kekayaan spesies dan kelimpahan lebah adalah lebih tinggi di kawasan pinggir bandar berbanding dengan kawasan pusat perniagaan ($p < 0.05$). Penduduk bandar ada kemungkinan tidak perasan kewujudan lebah di kawasan bandar tetapi bersetuju bahawa lebah mempunyai hak wujud dalam persekitaran semula jadinya. Penduduk-penduduk yang perasan akan kewujudan lebah dan pernah berinteraksi dengan lebah, lebih cenderung mempunyai pandangan yang positif terhadap kehadiran lebah di bandar. Selain itu, kepelbagaian spesies kupu-kupu di dalam taman-taman bandar di dua bandar — Wilayah Persekutuan Kuala Lumpur, Malaysia dan Shenzhen, Selatan China telah ditinjau. Hubungan antara kekayaan spesies kupu-kupu dan tiga pembolehubah taman: i) umur taman, ii) saiz taman dan iii) jarak dari kawasan pusat perniagaan telah disiasat. Persampelan kupu-kupu yang standard telah dijalankan merentasi pelbagai jenis mikrohabitat yang berbeza di setiap taman: i) kawasan teduh, ii) kawasan berpagar, iii) kawasan berbunga dan iv) kawasan tidak terurus. Sebanyak 572 kupu-kupu yang mewakili 60 spesies telah direkodkan di taman-taman bandar Kuala Lumpur. Walaupun korelasi kekayaan spesies dengan umur dan saiz taman adalah positif, dan korelasi dengan jarak dari kawasan pusat perniagaan adalah negatif; tetapi korelasi-korelasi tersebut adalah lemah dan tidak ketara secara statistik. Kekayaan spesies tertinggi dicatatkan di mikrohabitat yang tidak diurus. Sebanyak 1933 kupu-kupu yang mewakili 74 spesies telah dicatatkan di Shenzhen.

Korelasi antara kekayaan spesies kupu-kupu dengan umur taman dan jarak dari pusat perniagaan adalah negatif yang lemah, tetapi korelasi antara kekayaan spesies kupu-kupu dengan saiz taman ketara positif ($p < 0.05$). Di antara semua mikrohabitat, kekayaan spesies kupu-kupu yang tertinggi direkod di kawasan yang tidak terurus.

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LIST OF SYMBOLS AND ABBREVIATIONS

~	Approximate
X^2	Chi-square test
°C	Degree Celsius
%	Percentage
±	Plus-minus
AIC	Akaike Information Criterion
ANOVA	Analysis of variance
BIN	Barcode Index Number
BOLD	Barcode Of Life Datasystems
bp	Base pair
CCA	Canonical Correspondence Analysis
COI	Cytochrome c oxidase I
DBKL	Dewan Bandaraya Kuala Lumpur
<i>df</i>	Degrees of freedom
DNA	Deoxyribonucleic acid
DOI	Digital object identifier
ESA	East-Southeast Asia
e.g.	Latin phrase <i>exempli grātiā</i> (for example)
<i>et al.</i>	Latin phrase <i>et alia</i> (and other)
<i>F</i>	F-test
GPS	Global positioning system
<i>i.e.</i>	Latin phrase <i>id est</i> (that is)
km	kilometer
ln	Natural logarithm
m	Meter
mh ⁻¹	meter per hour
ml	Milliliter
mtDNA	mitochondrial DNA
numt	nuclear mitochondrial DNA
PCA	Principal component analysis
PCR	Polymerase chain reaction
<i>Q</i>	Tukey Q Test.
SEA	Southeast and East Asia
sp	Species
spp	Species pluralis
UK	United Kingdom
USA	United State of America

CHAPTER 1: GENERAL INTRODUCTION

The urban population in the East-Southeast Asia region (ESA) grew from 738 million to 969 million between the years 2000 and 2010 and it is estimated that cities in the region will be home to 1.8 billion people by 2050 (Schneider *et al.*, 2015). Additionally, in the last decade, the urban density of ESA cities reached a new milestone with a mean of 5850 person/km²; considered “high” by the World Bank (Schneider *et al.*, 2015).

ESA is one of the fastest urbanizing regions in the world during the last decade (2000-2010) with 34, 000 km² more urban land (Schneider *et al.*, 2015). As urban development replaces native or remnant habitat, and resources in surrounding areas are depleted to support urban economies, urbanization is regarded as a major threat to biodiversity (Czech *et al.*, 2000) and results in the biotic homogenization of a region (McKinney, 2002). However, there is still a lack of empirical studies regarding the impact of urbanization on biodiversity in ESA (Hernandez *et al.*, 2009).

Most flowering plants, including those utilised by humans for agriculture and beautification, require pollination, the transfer of pollen for reproduction. Plants have evolved a variety of methods for pollen transfer such as: utilising abiotic agents (wind and water), and animal vectors (Ollerton *et al.*, 2011). It is estimated that three-quarters of flowering plant species worldwide rely on animal pollinators, mostly insects, for pollination (National Research Council, 2007). These insect pollinators, and especially bees, are responsible for pollination of one-third of the crops that are consumed by humans (Klein *et al.*, 2007). Gallai *et al.* (2009) estimated that the annual economic value of insect pollination globally is €153 billion and €63.1 billion for ESA alone. Unfortunately, these important pollinators are declining globally (Winfree *et al.*, 2009)

which could have negative impacts on productivity of crops and sustainability of ecosystems (Potts *et al.*, 2010; Hooper *et al.*, 2012).

In urban environments bees and butterflies are important pollinating insects, offering important benefits to humans, especially pollination of plants, which contributes to improved mental wellbeing and productivity (Keniger *et al.*, 2013). There is recognition that lack of exposure to the natural environment can cause mental health problems (Miller, 2006; Brethour *et al.*, 2007). Generally, urbanization is an important factor driving the decline in pollinators (Brown & Paxton, 2009; Hernandez *et al.*, 2009). Based on a species-area model calibrated to biodiversity losses in highly urbanized Singapore, Sodhi *et al.* (2004) estimated that Southeast Asia will lose 20-40% of its butterfly species by 2100 due to land-use changes in the region. However, bees, one of the most important pollinator groups playing an important role in maintaining life on earth, have received little attention in ESA (Hernandez *et al.*, 2009). In view of the negative impact of urbanization on biodiversity, especially the extirpation/extinction of important native species such as bees and butterflies, conservation plans for the preservation of native species are urgently needed to provide a good quality of life for urban dwellers.

This thesis consists of three chapters with the overarching objective: To investigate patterns of bee and butterfly diversity in rapidly urbanizing areas in Southeast and Southern East Asia (also known as Tropical East Asia; Corlett 2014).

In Chapter 3, I addressed the following two questions: (a) How does bee diversity differ among urban sites in Southeast and East Asia megacities? (b) Do the human communities in Southeast and Southern East Asia megacities perceive and appreciate bees? This work is published in *Genome* as: Kong-Wah Sing, Wen-Zhi

Wang, Tao Wan, Ping-Shin Lee, Zong-Xu Li, Xing Chen, Yun-Yu Wang and John-James Wilson, 2016, "Diversity and human perceptions of bees (Hymenoptera: Apoidea) in Southeast Asian megacities". DOI: 10.1139/gen-2015-0159.

In Chapter 4, I examined the value of urban parks as refuges for tropical butterflies through investigating the relationships between butterfly species richness and the age, size and distance from the central business district of parks in Kuala Lumpur. This project has been published in *Urban Ecosystems* as: Kong-Wah Sing, Wan Faridah Ahmad Jusoh, Nor Rasidah Hashim and John-James Wilson, 2016, "Urban parks: refuges for tropical butterflies in Southeast Asia?", *Urban Ecosystems*, DOI: 10.1007/s11252-016-0542-4.

In Chapter 5, I investigated butterfly diversity in a young and rapidly growing megacity in Southern East Asia - Shenzhen asking: (a) Does butterfly species richness decrease with park age? (b) Does butterfly species richness increase with the park area? (c) Does butterfly species richness decrease along the rural-urban gradient? This work is published in *Genome* as: Kong-Wah Sing, Hui Dong, Wen-Zhi Wang, John-James Wilson, 2016, "Can butterflies cope with city life? Butterfly diversity in a young megacity in Southern China". DOI: 10.1139/gen-2015-0192.

CHAPTER 2: LITERATURE REVIEW

2.1 Urbanization in East-Southeast Asia

Today, 2.2 billion people live in the East-Southeast Asia (ESA) region, accounting for nearly one third of the world's 7 billion people (Schneider *et al.*, 2015). The global human population is projected to increase to over 9 billion in 2050, with much of this growth concentrated in developing countries, largely located in the tropics and sub-tropics (United Nations Population Division, 2011). The greatest growth is set to occur in urban areas, disproportionately impacting Asia where half of the population is expected to be living in urban areas by 2020 (United Nations Population Division, 2011). During the 18th and 19th centuries, ESA was one of the world's least urbanized regions (United Nations, 2002) with most of the population living in rural areas and undertaking agriculture (Huff & Angeles, 2011). In contrast, at the turn of 20th century, ESA experienced fast and intense urbanization. In the first decade of the 21st century, ESA was one of the fastest urbanizing regions in the world and the urban population grew from 738,415,036 to 968,624,426 (Schneider *et al.*, 2015).

Rapid growth and high densities of the human population are recognized as being among the key threats to biodiversity and ecosystem functioning (Kerr & Currie, 1995; Forester & Machlis, 1996; Kirkland & Ostfeld, 1999; Thompson & Jones, 1999; Cincotta, Wisnewski, & Engelman, 2000; Cincotta & Engelman, 2000; Abbitt *et al.*, 2000; McKinney, 2001; Harcourt, Parks and Woodroffe, 2001; Harcourt & Parks, 2003; Balmford *et al.*, 2001; Ceballos & Ehrlich, 2002; McKee *et al.*, 2003). On a global scale, Kerr and Currie (1995) found human population density was the anthropogenic factor most closely associated with the proportion of threatened bird species per nation. Using data of threatened bird and mammal species across 114 continental nations,

McKee *et al.* (2003) suggested the number of threatened species is positively correlated with human population growth.

While experiencing unprecedented urbanization in its history during the last two decades, ESA lost 32 million hectares of forest (Stibig *et al.*, 2014). The loss of tropical forests and land-use change are considered the major threat to Southeast Asia's biodiversity (Sodhi *et al.*, 2004; Sodhi *et al.*, 2010; Brickford *et al.*, 2012).

2.2. Pollinator declines and potential drivers

Birds, mammals and insects provide pollination services which maintain wild plant communities and commercial crops (Ashman *et al.*, 2004; Aguilar *et al.*, 2006; Klein *et al.*, 2007; Ricketts *et al.*, 2008). Many important crops (e.g. almond, apple, avocado, coffee, cucumber, melon, sunflower, water melon) rely on pollination by insects, and in particular bees (Dias *et al.*, 1999, Klein *et al.*, 2007). Pollinator declines began to receive widespread attention when it was reported that 25% (in central Europe) and 59% (across the USA) of managed honey bee colonies had disappeared mysteriously since the 1950s (Natural Research Council, 2007; vanEngelsdorp *et al.*, 2008; Potts *et al.*, 2010). While, Ghazoul (2005) questioned whether the loss of honey bee colonies in central Europe and the USA constitutes substantial evidence indicating a global pollination crisis, the author nevertheless suggested a pollination crisis is in progress.

Millions of dollars has been spent to investigate the potential drivers of the decline in honey bee numbers and to develop mitigation strategies in Europe and the USA (Pettis & Delaplane, 2010). Habitat loss and fragmentation, increasing pesticide application, decreased resource diversity, pathogens, and climate change have all been

proposed as drivers of the pollinator decline (Potts *et al.*, 2010; González-Varo *et al.*, 2013, Kerr *et al.*, 2015).

2.2.1 Land use change: habitat loss and fragmentation

Lands use change involving the conversion of natural land (e.g. forest) into human managed areas (e.g. agricultural fields, roads, buildings and impervious surfaces) is thought to be the most important factor driving pollinator declines (Brown & Paxton, 2009; Garibaldi *et al.*, 2011). Agricultural and urban expansion, which reduces floral resources and nesting opportunities negatively impacts on the populations of wild pollinators (Kleijn & Raemakers, 2008; Garibaldi *et al.*, 2011). In Europe, land use change, particularly agricultural intensification is thought to be responsible for the decline in rare and specialized bees and butterflies (Corbet, 2000; Saarinen *et al.*, 2003; Goulson & Darvill, 2004). Two independent quantitative review articles (Ricketts *et al.*, 2004; Winfree *et al.*, 2009) found a similar widespread pattern of losses of wild bees as a consequence of habitat loss and fragmentation. Other studies have found a negative correlation between natural habitat fragment size with diversity of bees (Steffan-Dewenter *et al.*, 2006) and butterflies (Soga & Kaike, 2013b).

2.2.2 Pesticides

Pesticides can cause mortality of pollinators by direct intoxication (Alston *et al.*, 2007; Gill *et al.*, 2012). Sublethal effect of pesticides on honey bees include impairment of physiology (Hatjina *et al.*, 2013), cognitive abilities (memory and learning; Ramirez-Romero *et al.*, 2005; Yang *et al.*, 2012), foraging (Romero *et al.*, 2005; Henry *et al.*, 2012; Schneider *et al.*, 2012), homing behaviour (Williamson & Wright, 2013; Fischer *et al.*, 2014) and reductions in queen fecundity (Dai *et al.*, 2010). Pollen and nectar of flowering crops contaminated with imidacloprid can cause impairment of natural

foraging behaviour and high worker losses in bumblebee (*Bombus terrestris*) colonies (Gill *et al.*, 2012). Sandrock *et al.* (2014a) revealed that honeybee colonies constantly exposed to thiamethoxam and clothianidin exhibited a decline in the numbers of adult bees and broods in hives, as well as a reduction in honey production and pollen collection. A 50% reduction in offspring production and a significantly male-biased offspring sex ratio in populations of Red Mason bees (*Osmia bicornis*) upon chronic neonicotinoid exposure, demonstrated that chronic, dietary neonicotinoid exposure also has severe detrimental effects on the reproductive output of solitary bees (Sandrock *et al.*, 2014b).

2.2.3 Pathogens

Studies have linked the declines in domesticated honey bees and wild pollinators with parasitic infections (Le Conte *et al.*, 2010; Cameron *et al.*, 2011; Evans & Schwarz, 2011). Le Conte and colleagues (2010) suggested *Varroa destructor* mites are the primary vector of many viruses (Picornavirales) responsible for losses of honey bee colonies. The parasitic mites live phoretically on adult bees (Oldroyd, 1999) and suppress host immunity through feeding on its hemolymph (Yang & Cox-Foster, 2005; Highfield *et al.*, 2009). The microsporidian, *Nosema* spp. (Paxton, 2010; Higes *et al.*, 2013), infects the gut epithelia of adult bees and was found to be significantly associated with declines of generalist bumblebee species (*Bombus occidentalis*) in North America (Cameron *et al.*, 2011). However, studies suggest that multiple co-infections of pathogens (bacteria, microsporidians, mites, viruses) is more likely to play a role in the decline of pollinators (Runckel *et al.*, 2011; Cornman *et al.*, 2012; Vanbergen *et al.*, 2013).

2.2.4 Climate change

Climate change affects the distribution of plants and pollinators causing pollinators with narrow climatic niches to become more susceptible to population declines and even extinction (Williams & Osborne, 2009; Forister *et al.*, 2010; Kerr *et al.*, 2015). Climate change can also result in asynchrony between plant flowering times and pollinator emergence (Memmott *et al.*, 2007; Burkle *et al.*, 2013). This particularly affects specialist pollinators because if they emerge before or after their host plant blooms, they will face starvation (Memmott *et al.*, 2007). Studies have shown that the fecundity and longevity of pollinators reduced when adults experienced food limitations with direct consequences for population densities and extinction risk (Memmott *et al.*, 2007).

2.2.5 Interactions between drivers

No single driver has emerged as the definitive cause of on-going honey bee colony losses and declines of wild pollinators, instead, interactive, and sometimes synergistic effects among proposed drivers most likely explain the phenomena (Potts *et al.*, 2010; Gill *et al.*, 2012; Goulson *et al.*, 2015). For example, honey bees reared in brood combs exposed to neonicotinoid insecticides have been shown to be more susceptible to infection by the parasitic microsporidan, *Nosema ceranae* (Wu *et al.*, 2012). Imidacloprid exposure increased the prevalence of *Nosema* infections in bee hives (Pettis *et al.*, 2012) and *Nosema*-induced mortality (Alaux *et al.*, 2010). Bees suffering immunosuppression by causes such as nutritional stress have reduced ability to cope with exposure to pesticides and pathogens (Oldyold 2007; Goulson *et al.*, 2015). However, studies examining the effects of multiple stressors on pollinator diversity are

scarce (González-Varo *et al.*, 2013; Goulson *et al.*, 2015), most likely due to the difficulties in conducting well-replicated experiments.

2.3 Urban green spaces and pollinators

Urban development is strongly associated with insect diversity declines and extirpations (McKinney, 2008; Jones & Leather, 2012; Bonebrake & Cooper, 2014), particularly through fragmentation and removal of foraging and nesting resources that are vital to pollinators (Hernandez *et al.*, 2009). For example, Fattorini (2011) recorded 32% of tenebrionid beetles, 45% of butterflies and 63% of Scarabaeidae have been extirpated from Rome as a result of habitat alteration due to urban development from 1885 to 1999. Although urbanization has negative impacts on insect fauna, urban habitats such as gardens and parks can attain remarkably high densities of wild bees (McFrederick & LeBuhn, 2006; Matteson *et al.*, 2008; Matteson & Langellotto, 2009) and otherwise declining species (Goddard *et al.*, 2009; Nielsen *et al.*, 2014), suggesting urban green spaces could provide important refuges for pollinators. Abundance and species richness of insect pollinators (e.g. bees and flies) were not significantly different amongst urban sites, farmland and nature reserves in the UK (Baldock *et al.*, 2015). In Singapore, Koh and Sodhi (2004) found urban parks adjoining forest had a higher number of butterfly species and abundance than forest remnants.

Bee species richness is dependent upon the diversity, quality, and quantity of foraging and nesting resources (Cane, 2005) and bee abundance was positively correlated with the “green” cover of urban golf courses and parks in northwestern Ohio, USA (Pardee & Philpott, 2014). However, other studies have reported that bee abundance decreased with an increase in green spaces in New York city and suggested that this is most likely due to differences in floral quality across different types of green

spaces (Matteson *et al.*, 2012), floral specialisation of certain bee genera (Cane *et al.*, 2006) and an increase in the area of impervious surfaces in the landscape surrounding the urban green spaces (Arhné, 2008).

Previous studies in urban environments have demonstrated that the features of habitat patches, such as their size (Mauro *et al.*, 2007) and degree of isolation (Koh & Sodhi 2004; Öckinger *et al.*, 2009; Lizée *et al.*, 2012) are significant predictors of butterfly species richness. In the city of Prague, Czech Republic, butterfly diversity was attributed to heterogeneity in the surrounding urban landscape (Kadlec *et al.*, 2008). Conditions surrounding patches, such as building density and the area of impervious surfaces were also thought to be influential factors accounting for butterfly species richness in urban areas (Jokimaki, 1999; Germaine & Wakeling, 2001; Matteson & Langelotto, 2009).

2.4 DNA barcoding: biodiversity inventory and conservation units

Our understanding of biodiversity patterns and attempts at wildlife conservation are hampered by lack of detailed species inventories i.e. fully knowing and appreciating what is there. A biodiversity inventory is simply a list of biological entities at a site (Stork & Davies, 1996), but is essential data for those tasked with understanding biodiversity patterns, managing and conserving biodiversity, e.g. providing justification for gazetted protected areas (Syaripuddin *et al.*, 2015). Biodiversity inventories take time and expertise. A taxonomically diverse inventory in an African rainforest required 10,120 scientists-hours to sample, sort and catalogue 2,000 species (Lawton *et al.*, 1998). Numerous “morphospecies” could not be assigned to described taxa making it difficult to know if these species were ever found before or again. Many small-bodied taxa with high richness were simply not inventoried because they are difficult to identify (Lawton *et al.*, 1998). This situation is frequently encountered during

biodiversity inventory in ESA due to lack of taxonomic expertise and the high proportion of undescribed taxa (May, 2010). Building on experience with Southeast Asian beetles, Balke *et al.* (2013) were optimistic for the implementation of DNA barcoding as a reliable, rapid tool for biodiversity inventory and proposed a framework through which to accelerate processes (Reidel *et al.*, 2013). This optimism is validated by a “guinea pig” from Costa Rica. Unparalleled biodiversity inventory of caterpillars in Área de Conservación Guanacaste included 2, 500 species after 25 years but grew rapidly after DNA barcoding was incorporated into the process in 2003 reaching 4, 500 species by 2009 (Janzen *et al.*, 2009). Informal names, coupled to genetic divergences, widely used for Southeast Asian bats further demonstrate the utility of DNA barcodes facilitating connections between inventoried taxa lacking formal description (Wilson *et al.*, 2014).

DNA barcoding has had a major influence on the “species problem” - how do we recognise the “units” worthy of inventory or monitoring in the first place (Adamowicz, 2015). While taxonomy is not conservation biology per se, nomenclature has widespread implications for the direction of conservation actions. Pertinent examples from ESA include the tiger, controversially reduced to two subspecies on the basis of cytochrome b sequences (and geography and morphology) (Wilting *et al.*, 2015), and the critically endangered *Batagur* terrapins, split into six species based upon cytochrome b sequences (Praschag *et al.*, 2007). DNA barcodes provide an equally valid source of data upon which to establish taxonomic hypotheses, and certainly provide superior levels of interoperability (Wilson *et al.*, 2014) than formal taxonomic names dubiously assigned on the basis of (incorrectly annotated; Goodwin *et al.*, 2015) museum specimens. It is worthwhile to remember that described taxa, whether

recognised on the basis of molecular or other characters, are “not facts, but testable hypotheses about the structure of biodiversity” (Pante *et al.*, 2015).

This thesis examined the effect of land-use on bee and butterfly diversity in rapidly urbanizing SEA through the use of DNA barcoding.

University of Malaya

CHAPTER 3: DIVERSITY AND HUMAN PERCEPTIONS OF BEES (HYMENOPTERA: APOIDEA) IN SOUTHEAST ASIAN MEGACITIES

Citation: Kong-Wah Sing, Wen-Zhi Wang, Tao Wan, Ping-Shin Lee, Zong-Xu Li, Xing Chen, Yun-Yu Wang, and John-James Wilson (2016) Diversity and human perceptions of bees (Hymenoptera: Apoidea) in Southeast Asian megacities, *Genome*, DOI: 10.1139/gen-2015-0159.

3.1 Introduction

The Southeast and East Asia (SEA) region is seeing the fastest rates of urbanization globally (Schneider *et al.*, 2015). During the last 20 years in countries such as China the proportion of the human population living in urban areas, has risen from 20% to more than one half (Schneider *et al.*, 2015). Considering that urbanization often requires the conversion of natural land cover to cover with human-constructed elements - buildings, roads, and impervious surfaces (McKinney, 2006), urbanization is considered one of the major threats to biodiversity globally (Cane *et al.*, 2006; Clergeau *et al.*, 2006; Williams & Kremen, 2007; McKinney, 2008). Southeast Asia has one of the highest concentrations of endemic species on Earth (Myers *et al.*, 2000; Sloan *et al.*, 2014), but has suffered the greatest losses in biodiversity of any tropical region while undergoing rapid economic development over the past 50 years (Sodhi *et al.*, 2004). Only 5% of the land cover of the island of Singapore, one of the region's economic powerhouses, is considered "natural" (Corlett 1992; Turner *et al.*, 1994; Yee *et al.*, 2011), and an estimated 75% of native species have been lost (Brook *et al.*, 2003).

Urban habitats, characterized by a high level of heterogeneity, are organized along an "urban gradient" extending from residential suburbs, bordering natural (e.g., forest) or agricultural land, to the central business districts (Young & Jarvis, 2001). Plant species richness is often higher in urban areas than in rural areas (Grimm *et al.*, 2008) because humans actively manage the plant communities present (Hope *et al.*,

2003; Grimm *et al.*, 2008). Conversely, animal species richness in urban areas is generally lower than in rural areas due to lack of suitable habitats, habitat fragmentation, and high levels of pesticides and pollutants (Grimm *et al.*, 2008). However, bird species richness is often highest at intermediate levels along the urban gradient (Blair, 1996; Marzluff, 2005) and there are mixed reports on the relative diversity of urban insects (Jones & Leather 2012). Abundance and species richness of carabid beetles in Pacé, France (Varet *et al.*, 2011), butterflies in Sheffield, UK (Dallimer *et al.*, 2012), ants in Silicon Valley, California (Vonshak & Gordon, 2015), and hoverflies in 12 large cities in the UK (Baldock *et al.*, 2015), showed no significant differences with comparable rural areas. Restrepo and Halffer (2013) recorded higher butterfly species richness in the Mexican cities of Xalapa and Coatepec than in nearby forest whereas Lee *et al.* (2015) found that the species richness of butterflies in four urban green spaces in Seoul, South Korea, were significantly lower than natural forest.

Urban wildlife can enhance human well-being (Keniger *et al.*, 2013) and is important from a social perspective, as personal exposure to “nature” in everyday life is a major determinant of sensitivity to environmental issues and views towards natural ecosystems (Miller, 2006). However, the presence of wildlife in urban areas can lead to human-wildlife conflicts (Hill *et al.*, 2007). While the human community can generally tolerate “nuisance” aspects of their co-existence with wildlife, aspects that result in economic loss (Hill *et al.*, 2007) or threats to safety, can negatively affect attitudes towards wildlife and may drive support of lethal control measures (Wittmann *et al.*, 1998; Hill *et al.*, 2007). Therefore, in urban areas, there is the opportunity and responsibility to facilitate positive interactions between humans and wildlife, particularly because these interactions determine how humans value non-human life (Savard *et al.*, 2000).

Bees represent a complex case for human-wildlife coexistence: the human benefits derived directly from bees, particularly luxury food and health products - honey, pollen, royal jelly and propolis, appear to be well-recognized (Schmidt 1997; Cortés *et al.*, 2011; Pimentel *et al.*, 2013). Wild bees retain important ecosystem services in urban areas - pollination of plants that can provide food for humans and other wildlife (Baldock *et al.*, 2015). Yet, at the same time, bees have consistently been misunderstood as aggressive insects under any circumstance (Vetter & Visscher, 1998; Greene & Breisch, 2005). Certainly, mass honey bee attacks can threaten human safety and can be fatal in extreme cases of anaphylactic shock (Franca *et al.*, 1994). However, bees are extremely unlikely to sting, and the sting is only used in defense (Vetter & Visscher, 1998). A questionnaire conducted in 92 veterinary clinics and hospitals in metropolitan Tucson, Arizona, revealed that honey bees were responsible for far fewer deaths (6) among companion (non-human) animals than domestic dogs (114 deaths) and snakes (36 deaths) (Johnston & Schmidt, 2001).

Bee species richness within cities has been found to be lower than in nearby rural areas (e.g., McIntyre & Hostetler, 2001; Eremeeva & Sushchev, 2005; Fetridge *et al.*, 2008 but see Baldock *et al.*, 2015). Nonetheless, urban green spaces such as parks and gardens can provide suitable habitat for many species of bees (Tommasi *et al.*, 2004; Frankie *et al.*, 2005; Cane *et al.*, 2006; McFrederick & LeBuhn, 2006; Matteson *et al.*, 2008; Matteson & Langellotto, 2009; Threlfall *et al.*, 2015). In New York City, Matteson *et al.* (2008) recorded 54 bee species in community gardens and Fetridge *et al.* (2008) collected 110 bee species from 21 residential gardens. Fifty-six bee species were recorded within urban Vancouver (Tommasi *et al.*, 2004) and 262 bee species have been collected within the city limits of Berlin (Saure, 1996). Several other studies of urban bee diversity have been conducted in temperate cities in Australia, Europe and North

America (e.g., San Francisco, McFrederick & LeBuhn, 2006; Ukiah, Frankie *et al.*, 2009a; Ukiah, Sacramento, Berkeley, Santa Cruz, San Luis Obispo, Santa Barbara, La Cañada Flintridge, Frankie *et al.*, 2009b; Grand Lyon, Fortel *et al.*, 2014; Melbourne, Threlfall *et al.*, 2015) but few studies exist for other regions (Hernandez *et al.*, 2009). In the urbanization hotspot of SEA, only two studies of urban bee diversity have been conducted, both in Singapore (Liow *et al.*, 2001; Soh & Ngiam, 2013).

Globally, bee populations are under threat and conservation is an important international priority (Kleijin *et al.*, 2015; Tang *et al.*, 2015). Conservation of bees in urban areas requires both scientific justification and public interest. Given the pressing issues of bee conservation and urbanization in SEA, coupled with the complex issues surrounding the coexistence of humans and bees, our objective was to address the following two questions: (a) How does bee diversity differ among urban sites in SEA megacities? Given the lack of taxonomic treatment for the bees of SEA we address this question through the use of DNA barcoding. (b) Do the human communities in SEA megacities perceive and appreciate bees?

3.2 Materials and methods

3.2.1 Locations and sampling site selection

No definitive definition exists, but generally, a megacity is a metropolitan area with a large and dense population. The term “mega-cities” has been used to describe metropolitan agglomerations of more than ten million inhabitants (City Population, 2015) and has been applied to both single metropolitan areas, and, two or more metropolitan areas that have converged, with the terms: conurbation, metropolis and metroplex, effectively synonyms for the latter usage. For the purpose of this study, we

use “megacity” as a general term for a metropolitan area, either one city or converging cities, with at least five million inhabitants.

This study was carried out at a botanical garden, a central business district and peripheral suburban areas (bordering natural or agricultural land) at each of four megacities in SEA: Greater Bangkok (Thailand), Klang Valley (Malaysia), Pearl River Delta (China), and Singapore-Iskandar Malaysia (Singapore/Malaysia) (Figure 3.1, Table 3.1). For the purpose of this study, in contrast with other treatments (e.g., City Population, 2015), we treat Hong Kong as part of Pearl River Delta and Singapore and Iskandar Malaysia as a single megacity. Despite the political borders between these metropolitan areas urban coverage is mostly contiguous. Permission for bee sampling was provided by the Agriculture, Fisheries and Conservation Department of Hong Kong Special Administrative Region, and by property owners, where applicable. No specific permits were required for other sampling localities.

3.2.2 Bee sampling

We sampled bees over continuous days (between 0800-1700) in each megacity (= 108 person-hours for each megacity), between June and November 2014, with our time in each megacity divided equally between each site type, i.e., three days (= 27 person-hours) each for the botanical garden, the central business districts and the peripheral suburban areas. A different transect (i.e. site) was sampled each day (see “virtual walks” below). Sampling was adjourned in the case of rain and continued the next day until the target person-hours for each site type were completed. The daily weather conditions throughout this study were similar (26-32°C). The tropical megacities (Greater Bangkok, Klang Valley, Singapore-Iskandar Malaysia) experience

high temperatures and humidity year-round while sampling was conducted during “summer” June-July in the subtropical Pearl River Delta.

Yellow bowl traps have been used previously for bee sampling in urban areas (Droege *et al.*, 2010; Banaszak-Cibicka & Zmihorski, 2012). Each sampling day, 15 yellow bowl traps (containing 300ml water and 4ml surfactant) were set, evenly spaced, along a 50m transect following protocols from The Bee Inventory Plot (see <http://online.sfsu.edu/beeplot/>). At the end of the sampling day any bees were removed from the bowls and stored in 99% ethanol until pinned for identification. Direct searching and hand-netting of bees (by KWS) along transects (approximately 600-1,000m) (Figure 3.1) was also conducted each day. We walked along the transect at a slow speed, pausing at potentially attractive resource patches (areas of vegetation, particularly blooming plants) and sampled any bees during an observational period of 10-15min. Once netted, bees were transferred to a jar containing ethyl acetate for a few minutes and then stored at 99% ethanol until pinned for identification. For a “virtual walk” along the transects see (1) Greater Bangkok: <https://www.google.com/maps/d/u/0/edit?mid=zCFbRfM-Xkys.kT8WL6vF5Bz0>, including Lumpini Park botanical garden (58ha); (2) Klang Valley: <https://www.google.com/maps/d/u/0/edit?mid=zCFbRfM-Xkys.kEIB2x7jFe2s>, including Lake Garden botanical garden (101ha); (3) Pearl River Delta: https://www.google.com/maps/d/u/0/edit?mid=zCFbRfM-Xkys.k5_p6eaDBT_I, including Fairy Lake botanical garden (590ha); (4) Singapore-Iskandar Malaysia: https://www.google.com/maps/d/u/0/edit?mid=zCFbRfM-Xkys.k7MZG_OYSzqQ, including Hutan Rimba botanical garden (32ha).

Table 3.1: Population (City Population, 2015) and area of the surveyed megacities.

Megacity	Population (million)	Area (km²)
Greater Bangkok	16.7	7,762
Klang Valley	7.0	2,805
Pearl River Delta	54.1	39,380
Singapore-Iskandar Malaysia	6.9	2,934

1. Greater Bangkok
2. Klang Valley
3. Singapore-Iskandar Malaysia
4. Pearl River Delta

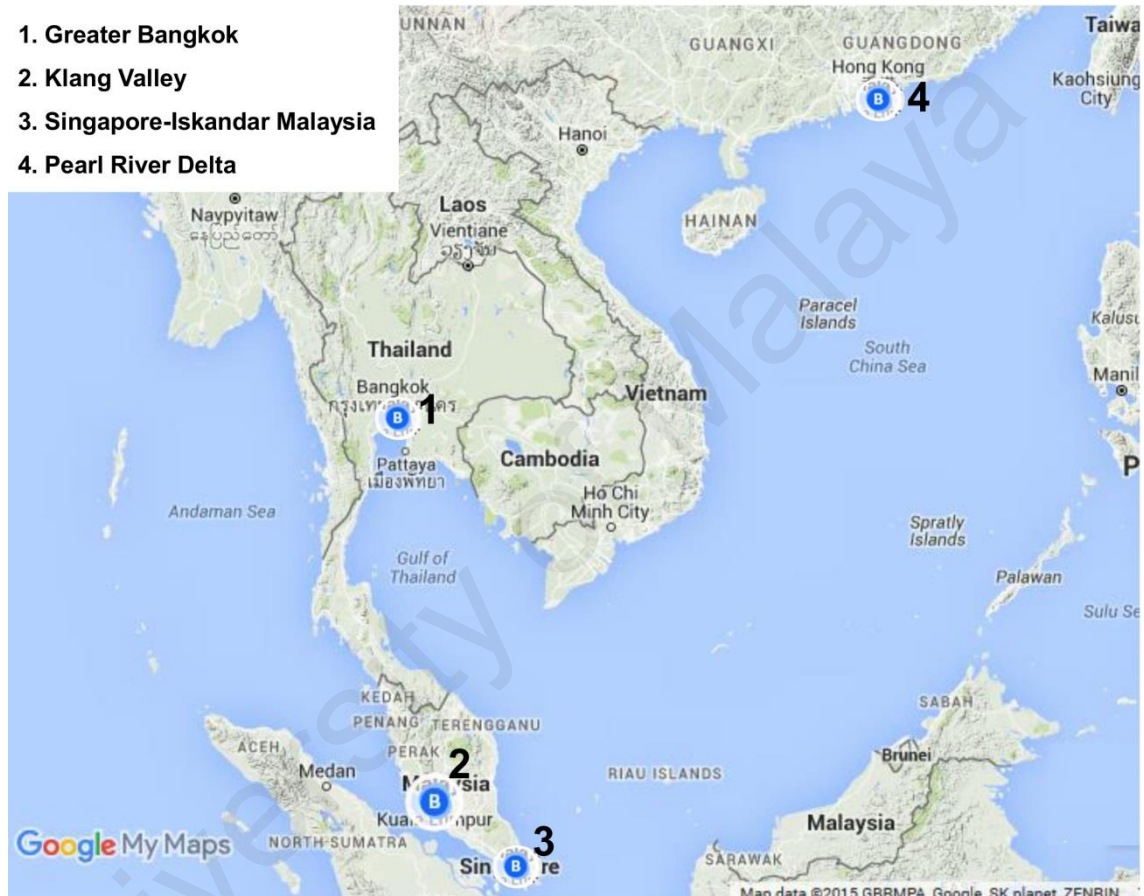


Figure 3.1: Megacities in Southeast and East Asia where bee sampling and human questionnaire surveys were conducted.

3.2.3 Bee diversity evaluation and analyses

Given the lack of formal taxonomic treatment for the bees of SEA (J. S. Ascher, N. Warrit and J. X. Q. Lee, personal communication, 2014), the collected bees were sorted into species on the basis of COI DNA barcodes (Floyd *et al.*, 2009) using the Barcode Index Number system (BINs; Ratnasingham & Hebert, 2013). BINs are Molecular Operational Taxonomic Units produced by Refined Single Linkage analysis of DNA barcodes across the Barcode Of Life Datasystems (BOLD) database (Ratnasingham & Hebert, 2007) and have been shown to correspond closely with traditional species limits characterized by morphology (Ratnasingham & Hebert, 2013; Hausmann *et al.*, 2013).

DNA was extracted from a single leg of each bee and the DNA barcode segment of COI mtDNA (~650bp), PCR-amplified and sequenced using standard protocols at the South China DNA Barcoding Center (following Wilson, 2012). During initial testing with one plate (95 DNA extracts) we found low PCR amplification success (~10%) with the standard insect DNA barcoding primers LCO1490 and HCO2198 (see Wilson, 2012). Consequently, we proceeded with primers BarbeeF and MtD09 (Francoso & Arias, 2013) for a first PCR pass and LCO1490 and HCO2198 (Folmer *et al.*, 1994) for a second pass. The DNA barcodes (and associated specimen data) were submitted to BOLD; (see BOLD project: Southeast Asia Megacities Bees, Project Code: SABEE) where they were automatically sorted into BINs. BINs are referred to as “species” below.

We assigned our new DNA barcodes to Linnaean species names when the BIN they belonged to contained DNA barcodes submitted by other BOLD users with Linnaean species names. Species which could not be assigned names using this method

(i.e., new BINs to BOLD, BINs with no formally named members, or BINS containing DNA barcodes with several different Linnaean names) we assigned genus or family names using a strict tree-based criterion (following Wilson *et al.*, 2011) based on the tree based identification (full database) option in BOLD. Species richnesses for each megacity and each site type within each megacity (botanical garden, central business district, suburban area) were determined. We performed one-way ANOVA to compare mean species richness and abundance between site types (4 megacities/replicates) and Tukey's range test to determine which site types were significantly different from the others.

3.2.4 Human perceptions questionnaire

We developed a questionnaire consisting of 25 questions covering respondent demographics, experience and interactions with bees, and attitudes towards bees. Pre-test surveys (30) were conducted to evaluate the comprehension of the target population and revealed that the respondents could understand all the questions. Consequently, the original pre-test questionnaire was retained for this study with minor modifications for clarity. The questionnaire was delivered face-to-face in situ during the 36 bee sampling days (see above) by an interviewer (KWS, PSL, or JJW, and with the help of a local volunteer in Greater Bangkok). Respondents were approached without any conscious bias during short breaks in bee sampling (e.g., while walking between potential resource patches).

The first part of the questionnaire contains demographic questions, including the respondents' sex, age, ethnicity, education level and place of origin. Respondents were also asked their history of staying in the current megacity (the location of the survey) if they answered they were not originally from that megacity. In the second part of the

questionnaire we asked about the frequency and locality of bee observations by the respondent and for the respondent to estimate, where possible, the number of bee types (species) to which their responses related. Respondents were also asked about their experience with bees and any financial loss due to bee stings. Eleven statements related to knowledge and opinions of bees in urban areas (“attitude statements”) were presented to the respondents who were asked to indicate whether they agreed, had no opinion, or disagreed with the statements. Our human perceptions questionnaire was approved by the University of Malaya Research Ethics Committee (Reference Number: UM.TNC2/RC/H&E/UMREC - 81).

To analyze the responses to the eleven attitude statements, we pooled the responses “Maybe” and “Don’t know”. We initially performed a Principle Components Analysis (PCA) with a Varimax rotation (following Hills *et al.*, 2007). However, due to low reliability values (Cronbach’s Alpha) regression analysis was not conducted. As an alternative, the responses to individual attitude statements were compared with respondent demographics and experiences with bees using Chi-square tests (following Cleargeau *et al.*, 2001). Comparisons which yielded expected counts of <5 were excluded as these can yield unreliable Chi-square test results.

3.3 Results

3.3.1 *Bee species composition*

We collected a total of 1698 individual bees – 574 from Klang Valley, 487 from Greater Bangkok, 368 from Pearl River Delta and 269 from Singapore-Iskandar Malaysia. Of these 1,698 individual bees only one was collected from the yellow bowl traps. A total of 1416 DNA barcodes were successfully generated from the 1698 individual bees (83%) and 1397 (82%) of these were of sufficient length and quality (<5

“N”s) to be assigned to BINs. Of these 128 BINs, 64 BINs (50%) were new to BOLD. The BINs could be assigned to four families: Apidae (76 BINs), Megachilidae (25 BINs), Halictidae (25 BINs) and Colletidae (2 BINs). Twenty-four BINs could be assigned to Linnaean species names, 117 BINs could be assigned to genus name and all BINs (128) could be assigned to family names. The most abundant species was *Apis* “ceranaAAA8457” (180 DNA barcodes) followed by *Apis florea* [BOLD:AAC3886] (153 DNA barcodes), *Apis* “ceranaAAM5455” (94 DNA barcodes), *Tetragonula* “ACV4063” (79 DNA barcodes) and *Ceratina smaragdula* [BOLD:AAF1368] (58 DNA barcodes). These five species accounted for 40% of the generated DNA barcodes. Thirty-three species comprised only a single DNA barcode.

3.3.2 Comparison of bee species richnesses and shared species between megacities

Klang Valley had the highest species richness (62 species), followed by Pearl River Delta (49 species), Greater Bangkok (40 species) and Singapore-Iskandar Malaysia (37 species) (Figure 3.2). *Ceratina smaragdula* [BOLD:AAF1368], *Megachile* “AAD3047” and *Xylocopa* “ACV4473”, were sampled in all four megacities. Thirty-five species were only found in Klang Valley, 30 species were only found in Pearl River Delta, 12 species were only found in Greater Bangkok and 9 were only found in Singapore-Iskandar Malaysia.

Twenty-one species were shared by Klang Valley and Singapore-Iskandar Malaysia, while nine species were shared by Pearl River Delta and Singapore-Iskandar Malaysia (Figure 3.2). The number of shared species between Greater Bangkok and the other three megacities was similar (13 species with Klang Valley, 16 species with Pearl River Delta and 15 species with Singapore-Iskandar Malaysia; Figure 3.2).

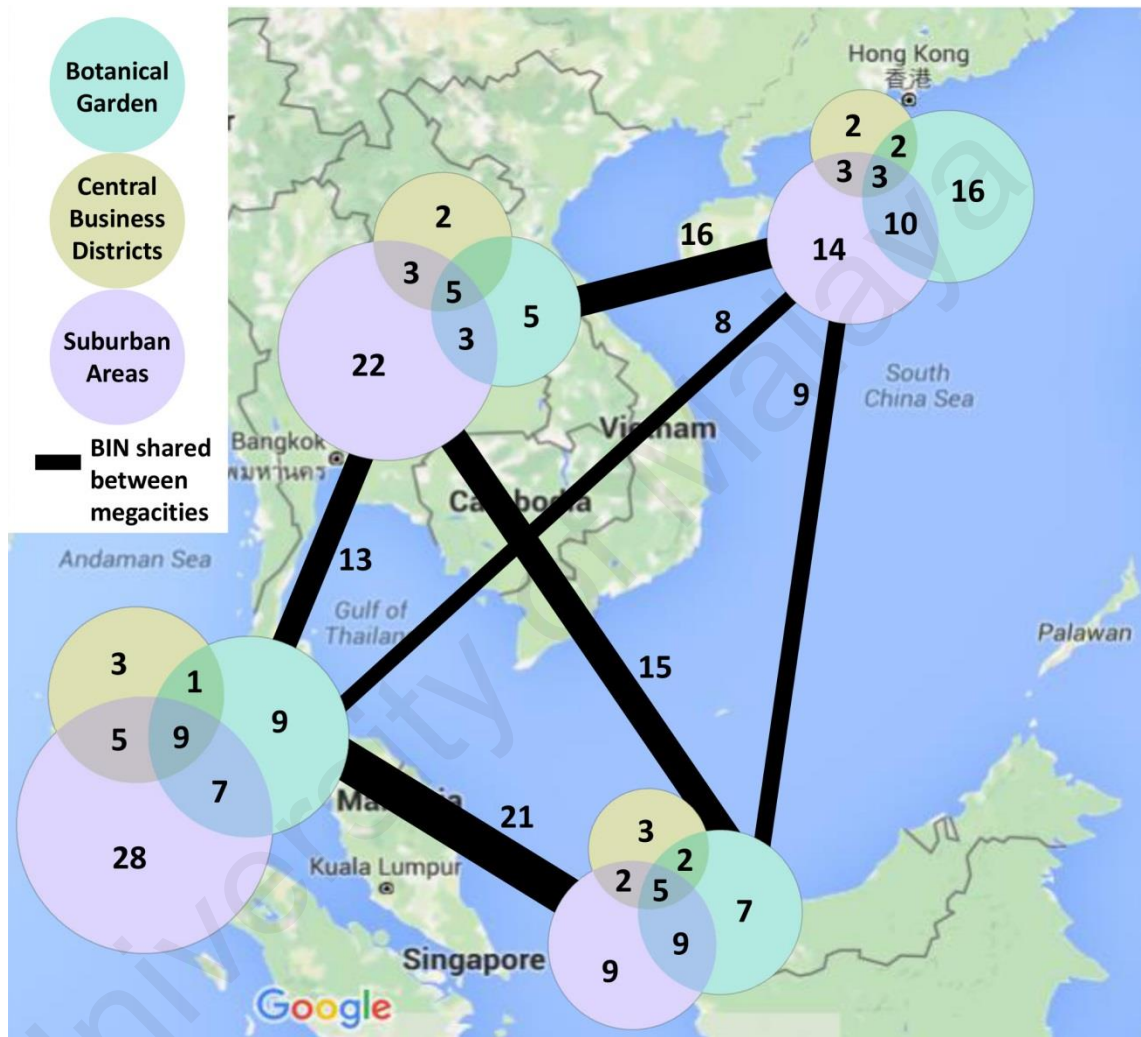


Figure 3.2: Number of species (BIN) collected from different site types and shared species (BIN) between megacities.

3.3.3 Comparison of bee abundances and species richnesses between central business districts, botanical gardens and suburban areas

Combined across all megacities, species richness in central business districts (50 species) was much lower than species richness in botanical gardens (92 species) and peripheral suburban areas (137 species). Bees (excluding the eusocial honey bees, *Apis* spp., and stingless bees, Meliponini) were more abundant in peripheral suburban areas (351 individuals from across the whole study) than botanical gardens (274 individuals) and central business districts (90 individuals). The mean species richness ($Q = 5.702$, $p = 0.0076$) and abundance ($Q = 4.541$, $p = 0.0262$) of bees in peripheral suburban areas were significantly higher than those in central business districts (Figure 3.3). There were no significant differences in the mean species richness ($Q = 2.753$, $p = 0.1815$) and abundance ($Q = 3.201$, $p = 0.1133$) between botanical gardens and central business districts or the mean species richness ($Q = 2.949$, $p = 0.148$) and abundance ($Q = 1.340$, $p = 0.626$) between botanical gardens and peripheral suburban areas (Figure 3.3).

3.3.4 Human perceptions

One hundred and eighty-five respondents completed our questionnaire: 55 from Klang Valley, 51 from Greater Bangkok, 46 from Pearl River Delta, and 33 from Singapore-Iskandar Malaysia. Eighty-eight female, 94 male and three respondents of unspecified gender completed the questionnaire. The respondents ranged in age from 13 to 79 years old; the mean age of the respondents was 35.4 and 57% were 20 to 39 years old. Chinese was the most common ethnic group among the respondents (n=70) followed by Malay (n=51), Thai (n=51), Indian (n=7) and others (n=6). Seventy percent of the respondents were born in cities. Eighty-four percent of respondents had received secondary education and 44% tertiary education. Two percent of respondents had not received formal education at any level.

Fifty-five percent of the respondents indicated they had seen bees at our sampling areas. Of 101 respondents who had seen bees, 84% had only seen one or two types of bees. Twenty-four percent (n=181) of respondents had seen bee nests in our sampling areas. Thirty percent of respondents had been stung by a bee and 8% had spent money to get treatment for bee stings. Fifty-one percent of the respondents indicated they knew friends or relatives who had been stung by a bee.

Ninety-six percent of respondents agreed with the statement “bees have a right to exist in their natural environment” (Table 3.2). Eighty-four percent disagreed that “bees are pests” and 69% that “bees cause damage to properties”. Seventy percent of the respondents agreed “bees are important for city plants”. Forty-one percent of respondents agreed “bees should be allowed to live in cities” while 52% agreed “bees in cities should be subject to greater control”. An equal number of respondents (40%) agreed and disagreed that they “like having bees around”.

Respondents who had seen bees were more likely to disagree that “bees are pests” ($X^2_{185, 2} = 6.1; p = 0.048$), and agree that “bees are important for city plants” ($X^2_{185, 2} = 6.2; p = 0.045$), than those who had not seen bees. When ages were categorized into three classes (<25, 25-44, ≥ 45 ; following Standardized Survey Classifications [see <http://www.pgagroup.com/standardized-survey-classifications.html>]), respondents aged 25-44 were more likely to disagree with the statement “I like having bees around” ($X^2_{185, 4} = 60.7; p = 0.000$), agree that “bees in cities should be subject to greater control” ($X^2_{185, 4} = 40.0; p = 0.000$) and “bees nest should be removed once they are found” ($X^2_{185, 4} = 37.0; p = 0.000$). Respondents aged ≥ 45 were more likely to agree that “people should be allowed to remove bees nests from their house” ($X^2_{185, 4} = 15.8; p = 0.0003$), than younger respondents. Respondents from Greater Bangkok and Pearl River Delta were more likely to agree “I like having bees

around” than those from Klang Valley and Singapore-Iskandar Malaysia ($X^2_{185,6} = 62.9$; $p = 0.000$). Respondents from Klang Valley, Singapore-Iskandar Malaysia and Greater Bangkok were more likely to agree that “bees in cities should be subject to greater control” than those from Pearl River Delta ($X^2_{185,6} = 39.6$; $p = 0.0000$). Respondents who had been stung by bees were less likely to agree “bees in cities should be subject to greater control” ($X^2_{185,2} = 6.1$; $p = 0.047$) (Table 3.3).

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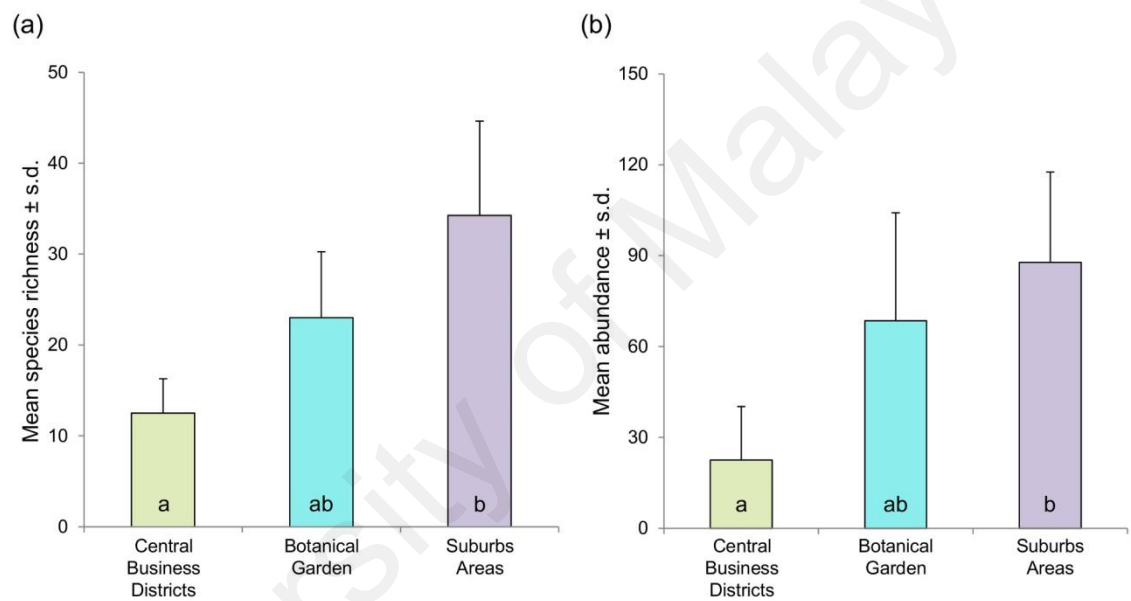


Figure 3.3: Mean \pm standard deviation of (a) species richness and (b) abundance of bees between sites in four megacities in Southeast and East Asia. Following Tukey's range test, means that did not differ significantly are shown with the same letter.

Table 3.2: Responses to eleven attitude statements about bees (n=185) during questionnaire survey conducted in four Southeast and East Asian megacities.

Attitude statements	Yes (%)	Don't know/Maybe (%)	No (%)
Bees have a right to exist in their natural environment	96.2	3.2	0.6
Bees should be allowed to live in cities	41.3	22.3	36.4
People should be allowed to remove bees nests from their house	62.5	22.3	15.2
Bees are important for city plants	70.7	19.5	8.8
I like having bees around	39.7	20.6	39.7
Bees cause damage to properties	7.0	24.5	68.5
Bees are pests	6.0	9.8	84.2
Bees in cities should be subject to greater controls	52.2	26.6	21.2
Keeping honey bees should be banned in cities	27.7	31.0	41.3
Bees are killed by insecticide use	52.7	25.0	22.3
Bees nests should be removed once they are found	29.9	29.9	40.2

Table 3.3: Distribution of responses to attitude statements regarding bees relative to the respondent demographics or experiences with bees.

Respondent knowledge and opinion of bees	Yes (%)	Don't know/ Maybe (%)	No (%)	X² test
People should be allowed to remove bees nests from their house				
<i>Age</i>				
<25	43	39	18	
25-44	67	15	18	X ² =15.8
≥45	75	17	8	p = 0.003
Bees are important for city plants				
<i>Have you ever seen bees here?</i>				
Yes	76	13	11	X ² =6.2
No	65	27	8	p = 0.045
I like having bees around				
<i>Age</i>				
<25	78	16	6	
25-44	16	20	64	X ² =60.7
≥45	40	27	3	p = 0.000
<i>Country</i>				
Greater Bangkok	78	16	6	
Klang Valley	20	22	58	
Pearl River Delta	41	26	33	X ² =62.9
Singapore/Iskandar Malaysia	9	18	73	p = 0.000
Bees are pests				
<i>Have you ever seen bees here?</i>				
Yes	6	5	89	X ² =6.1
No	7	15	78	p = 0.048

Table 3.3, continued

Respondent knowledge and opinion of bees	Yes (%)	Don't know/ Maybe (%)	No (%)	X² test
Bees in cities should be subject to greater controls				
<i>Age</i>				
<25	51	37	12	
25-44	64	27	9	X ² =40.0
≥45	33	15	52	p = 0.000
<i>Country</i>				
Greater Bangkok	51	37	12	
Klang Valley	67	22	11	
Pearl River Delta	33	15	52	X ² =39.6
Singapore/Iskandar Malaysia	58	33	9	p = 0.000
<i>Have you been stung by a bee?</i>				
Yes	39	34	27	X ² =6.1
No	59	22	19	p = 0.047
Bees nests should be removed once they are found				
<i>Age</i>				
<25	6	23	71	
25-44	44	35	21	X ² =37.0
≥45	29	29	42	p = 0.000

3.4 Discussion

A knowledge gap exists regarding the effect of land-use on bee diversity in rapidly urbanizing SEA (Brown & Paxton, 2009; Hernandez *et al.*, 2009). We attempted to start addressing this gap by conducting the first study looking at urban bee diversity across the SEA region. Effective biodiversity conservation in urban areas requires public interest, therefore, this study simultaneously examined human perceptions and attitudes towards bees.

During 36 days of sampling across four megacities, we sampled 1698 individual bees representing at least 128 species from four families, demonstrating urban areas in SEA can maintain diverse assemblages of bees. Although our sampling period was limited, the number of species collected in Singapore-Iskandar Malaysia (37) is similar to that reported in previous studies of the region – Liow *et al.* (2001) collected 45 morphospecies across eight lowland tropical forests with various degrees of anthropogenic disturbance, while Soh and Ngiam (2013) collected 40 morphospecies during an intensive study (February to June) across seven parks in Singapore. This suggests our bee sampling effort was sufficient to provide some broad insights into diversity patterns of bees in urban SEA. We employed two methods of bee sampling - yellow bowl traps and hand-netting. Yellow bowl traps are a low-cost, low labor-intensive, and easily standardized approach to bee sampling and have gained increased attention among melittologists following promising results in four North American ecoregions (Chihuahuan Desert, Coastal California, Columbia Plateau, and Mid-Atlantic; Droege *et al.*, 2010). Tang *et al.* (2015) have suggested bees collected with colored bowl traps can be made into “bee soup” for high-throughput monitoring of wild bee diversity and abundance via mitochondrial mitogenomics. Unfortunately, yellow bowl traps contributed just one (0.0006%) of the 1698 bees collected during our study.

Likewise, in Singapore, Soh (2015) recorded no bees after three sampling days with yellow bowl traps and Yee (2014) recorded only five bee species (*Amegilla* sp. n=3; *Apis andreniformis* n=1; *Ceratina* sp. n=23; *Hylaeus* sp. n=2; *Lasioglossum* sp. n=2) from yellow bowl traps after 90 sampling days in an urban botanical garden in Kuala Lumpur, Malaysia. The efficiency of bowl traps may be affected by their color (Campbell & Hanula, 2007; Wilson *et al.*, 2008; Gonçalves & Oliveira, 2013), spacing (Droege *et al.*, 2010), elevation (Campbell & Hanula, 2007; Tuell & Isaacs, 2011) and the degree of habitat heterogeneity (Droege *et al.*, 2010), but is unlikely to improve to the point of replacing the need for hand-netting, at least in the tropics (see Grundel *et al.*, 2011; for an alternate perspective from North America). In Brazil, Gonçalves *et al.* (2012) collected 57 bee species using malaise traps and yellow bowl traps with only two species contributed by the yellow bowl traps. Gonçalves *et al.* (2012) concluded that both trapping methods are inefficient compared to active capture, despite the efficiency of hand-netting being highly dependent on the motor skills and experience of the person wielding the net (Laroca & Orth, 2002).

DNA barcoding provides a means of analyzing diversity patterns of bees, and is particularly useful in the absence of a reliable, traditional, taxonomic framework. However, bee DNA barcoding has been plagued by reports of low PCR amplification success, particularly with the standard DNA barcoding primers (Yu *et al.*, 2012; Zhou *et al.*, 2013, Brandon-Mong *et al.*, 2015). This could be attributable to poor primer matching in certain groups of bees (Yu *et al.*, 2012; Zhou *et al.*, 2013; Schmidt *et al.*, 2015). Furthermore, production of clean and accurate DNA sequences is compromised by the presence of a poly-T region in the DNA barcode region in Hymenoptera (Zhou *et al.*, 2013). We experienced this challenge ourselves, obtaining a low PCR success rate with the Folmer *et al.* (1994) primers. However, a significant improvement in the PCR

success rate (84%) (and no *Wolbachia* or numt amplification) was achieved after using primer pair BarbeeF and MtD09 (Francoso & Arias, 2013). To date, 45,404 bee DNA barcodes have been deposited on BOLD. Based on the current composition of “named” bee DNA barcodes on BOLD, 19% of the species we sampled in SEA could be assigned to Linnaean species names. Ninety-one percent could be assigned to genus names. Half of the species we sampled were new to BOLD. Meshing traditional nomenclature with BINs will continue to remain a challenge, for bees as for other groups. The taxonomic muddle of the Asian honey bee *Apis cerana* is a particular case in point. Our DNA barcodes formed two BINs (BOLD:AAA8457 and BOLD:AAM5455) corresponding to two previously characterized (through morphology, biogeography and molecular data), *Apis cerana* “morphoclusters” - Indochinese (IV) *cerana* and Indomalayan (VI) *cerana* (Radloff *et al.*, 2010). Radloff *et al.* (2010) preferred to use these informal names rather than available Latin names as inconsistent and ambiguous previous usage of numerous *cerana* trinomials has rendered them useless for effective communication. Nevertheless, recording bee species richness and species distributions is crucial for effective conservation of bees in the rapid urbanizing SEA megacities. DNA barcodes are potentially much more useful at facilitating taxonomic connections between studies than morphospecies names such as “*Trigona* sp.1” (Soh & Ngiam, 2013), or even Latin names with a history of inconsistent and ambiguous usage. The BIN approach is further justified by other studies demonstrating BIN (Schmidt *et al.*, 2015) and DNA barcode divergences (Sheffield *et al.*, 2009; Carolan *et al.*, 2012; Magnacca and Brown 2012; Gibbs *et al.*, 2013) are highly congruent with traditional bee taxonomy and furthermore facilitate cryptic species recognition (Sheffield *et al.*, 2009; Williams *et al.*, 2012).

According to the Discover Life world checklist (Ascher & Pickering, 2015), 258 bee species have been recorded in Malaysia, 206 in Thailand and 92 in Singapore.

Using these figures, the species collected during our study are equivalent to 24% (in Klang Valley – Malaysia), 19% (Greater Bangkok – Thailand) and 14-40% (Singapore-Iskandar Malaysia – Singapore/Malaysia) of the species previously recorded for these regions. The only species found in all four megacities were the cosmopolitan *Ceratina* AAF1368 [BOLD:AAF1368; see the BIN page: http://boldsystems.org/index.php/Public_BarcodeCluster?clusteruri=BOLD:AAF1368], *Megachile* “AAD3047” and *Xylocopa* “ACV4473”. Ceratinini and *Xylocopa* bees are thought to be comparatively more adaptable to changing climates, and have flexible habitat preferences in comparison with other bee groups (Michener 1979; Rehan *et al.*, 2010). The similar number of species shared by Greater Bangkok with each of the other three megacities probably reflects the location of Thailand at the biogeographic transition zone between the Indo-Burmese (including Pearl River Delta) and Sundaland (including Klang Valley and Singapore-Iskandar Malaysia) faunal regions (see Hughes *et al.*, 2003; Woodruff & Turner, 2009). A common observation in our study and shared by Liow *et al.* (2001) and Soh and Ngiam (2013) in Singapore and Southern Peninsular Malaysia was the high abundance of honey bees (Apidae: Apini – *Apis cerana* and *Apis andreniformis*) and stingless bees (Apidae: Meliponini – *Geniotrigona thoracica* and *Tetragonula laeviceps*); it is common to find honey bees and stingless bees abundantly in tropical regions.

We found significant differences in bee species and abundance between the peripheral suburban areas and central business districts suggesting a negative correlation for bee diversity along gradients of urban intensity in SEA megacities. Although there have been no other similar studies from this region, our findings are consistent with those from other regions (North Asia – Eremeeva & Sushchev, 2005; North America – Petridge *et al.*, 2008; and Europe – Bates *et al.*, 2011; Banaszak-Cibicka & Żmihorski,

2012; Folter *et al.*, 2014) that reported bee species richness and abundance decreased with an increase in buildings and impervious surface and the loss of vegetation cover. Liow *et al.* (2001) suggested the distribution of bees in tropical forests was influenced by resource abundance, such as the density and flowering intensity of big trees. Similarly, most of the stingless bees, which rely on large trees for nesting (Inoue *et al.*, 1990), were collected in the peripheral suburban areas of Klang Valley, where large trees can still be found. In our study, the abundance and species richness of bees in urban botanical gardens did not differ significantly from the peripheral suburban areas. This finding is consistent with those from Australia, North America and Europe where researchers suggested green areas in cities, including botanical gardens (in Vancouver – Tommasi *et al.*, 2004; in Melbourne – Threlfall *et al.*, 2015) and residential gardens (California – Frankie *et al.*, 2005; UK – Gaston *et al.*, 2005; Melbourne – Threlfall *et al.*, 2015), can provide diverse food resources (native and exotic plants) and suitable nesting habitats for a diverse assemblage of bees. We recorded a relatively high species richness (30 species) at Fairy Lake Botanical Garden, Shenzhen in Pearl River Delta. Fairy Lake Botanical Garden is located in a peripheral area, but the other botanical gardens are located close to central business districts perhaps explaining the lack of significant differences between species richness and abundance at the botanical gardens and central business districts. We have not quantified the isolatedness of the sites in our study, and the effects of “corridors” certainly warrants further investigation in SEA megacities. Briffett *et al.* (2004) concluded that green corridors in Singapore provide functional habitats for some bird species, but their importance for bees needs to be assessed. Similarly, green roofs have been proposed as a potentially valuable site for bee conservation in North American cities with limited green space (Colla *et al.*, 2009;

MacIvor & Lundholm, 2011; Tonietto *et al.*, 2011), providing spatial and temporal contiguity of flowers (Tonietto *et al.*, 2011), but have yet to be investigated in SEA.

In addition to the provision of suitable habitats, a positive attitude towards wildlife amongst human society is essential for biodiversity conservation (e.g., Clucas *et al.*, 2008; Home *et al.*, 2009; Mulder *et al.*, 2009). Ninety-six percent of respondents to our questionnaire agreed that bees have the right to exist in their natural environment, suggesting the inhabitants of SEA megacities possess strong empathy for bees. This is despite almost half (45%) of the respondents having never seen bees. Of those respondents who had seen bees, the vast majority (84%) reported only having seen one or two types, in contrast to the ten species collected by us at our least species rich site, downtown Bangkok and Hong Kong (Pearl River Delta). Researchers in California, USA, also found the general public struggle to distinguish bee species due to the small size and diverse morphology of bees (Kremen *et al.*, 2011). Therefore, it is also likely that some responses to our questionnaire, including reports of bee stings, may relate to wasps, and this can negatively affect their perception of bees. Ironically, the respondents in Klang Valley, the megacity where we recorded the highest abundance and species richness of bees, were the least likely to report having seen bees (only 42%) whereas respondents in Singapore-Iskandar Malaysia, with the lowest species richness and abundance of bees amongst the megacities, were the most likely to report having seen bees (73%). This suggests that the degree of perception of bees is not related to abundance and species richness of bees in the megacity. Clergeau *et al.* (2001) conducted a study of human perceptions of birds in the city of Rennes, France, and likewise found 12% respondents (n=200) reported having never seeing birds even though they were abundant in the city.

Our analysis of the respondents' attitudes towards bees indicated that respondents aged 25 and above held more negative opinions of urban bees compared to younger respondents. Anecdotally, respondents in this group, who are likely to be parents or grandparents, commented that the presence of bees in urban areas increases the risk of children being stung by bees and they were more likely to agree that bees should be subject to greater control. Previous studies have suggested tolerance of nuisance aspects of wildlife coexistence can change to support of lethal control measures when there is a perceived threat to human safety (Wittmann *et al.*, 1998; Hill *et al.*, 2007). Interestingly, Langley (2005) calculated that of 533 human fatalities connected with Hymenoptera (excluding ants) in the United States, only 11 (2%) were persons aged 20 years and younger. Nevertheless bee attacks do occur and such incidents can receive high exposure in the media resulting in increased public fear (Johnston & Schmidt, 2001). Surprisingly, respondents who reported having seen bees and respondents who reported being stung by a bee generally demonstrated more positive opinions regarding the intrinsic value of bees and were less aggrieved by the negative aspects of coexistence with bees in urban areas. Respondents who had seen bees tended to agree bees are important for city plants and disagree that bees are pests compared to those who had never seen a bee. Also, respondents who had been stung by a bee were less likely to agree that bees should be subject to greater control.

CHAPTER 4: URBAN PARKS: REFUGES FOR TROPICAL BUTTERFLIES IN SOUTHEAST ASIA?

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4.1 Introduction

Of the 7 billion humans alive today, 3.9 billion are living in urban areas (United Nations Population Division, 2011). The majority of future human population growth will take place in cities (United Nations Population Division, 2011). Expansion and development of urban areas is not uniform and does not proceed in the same way in all regions but always requires conversion of natural habitats to impervious surfaces and buildings, whether houses or high-rise apartment blocks, roads or other transport systems (McKinney, 2008). Consequently, urbanisation is considered one of the major threats to global biodiversity (Czech *et al.*, 2000; Cane *et al.*, 2006; Clergeau *et al.*, 2006; Williams & Kremen, 2007; McKinney 2008). Southeast Asia has one of the highest concentrations of endemic species globally (Myers *et al.*, 2000) yet while undergoing rapid economic development has suffered the greatest losses in biodiversity of any tropical region over the past 50 years (Sodhi *et al.*, 2004). Only 5% of the land area of the island of Singapore, the region's economic powerhouse, is considered as covered by natural vegetation (Corlett, 1992; Turner *et al.*, 1994; Yee *et al.*, 2011) and an estimated 34–87% of all native species have been lost (Brook *et al.*, 2003).

Urban habitats are organised along gradients, extending from the boundaries with rural areas (e.g. forests or agriculture), through the suburbs, to the central business districts (Young & Jarvis, 2001). The high level of spatial heterogeneity, characteristic of urban areas, can have opposing impacts on different components of biodiversity (McKinney, 2008). Whereas urbanisation is always associated with a loss of total

species richness (Goddard *et al.*, 2010), the highest species richnesses of wild bees in Grand Lyon, France (Fortel *et al.*, 2014), and birds and butterflies in Santa Clara County, California (Blair, 1996; Blair & Launer, 1997), were recorded at intermediate levels of urbanisation, a pattern that is typically observed for plants (McKinney, 2008).

Urban planning, when feasible, will often incorporate green spaces (e.g. public parks) as these can provide improved air quality and opportunities for human recreation and well-being associated with being in or near green spaces (White *et al.*, 2013). Some urban habitats, such as road verges, brownfield sites or recreational parks, may function as surrogates for habitats already absent from intensively managed lands (Valtonen *et al.*, 2007; Lundholm & Richardson, 2010). These habitat types may in fact represent a “last stand” for some range-restricted species, particularly in the tropics and subtropics, trapped within expanding urban areas (Mattoni *et al.*, 2001).

A general finding of previous research is that internal habitat qualities of urban parks, such as the diversity and heterogeneity of microhabitats (e.g. tree species diversity), can have a stronger influence on the urban species richness of birds and invertebrates than either site area (i.e. size) or isolation (Nielsen *et al.*, 2014). The positive relationship generally observed between increased size of urban parks and increased species richness (Nielsen *et al.*, 2014) could be attributable to the fact larger parks tend to encompass greater habitat diversity and microhabitat heterogeneity than smaller ones (e.g. Fernandez-Juricic & Jokimäki, 2001; Cornelis & Hermy, 2004; Smith 2007; Khera *et al.*, 2009). The negative influence on urban bird diversity of isolation of parks within the “urban matrix” is overridden in explaining bird species richness, by the effects of park size and habitat heterogeneity (Nielsen *et al.*, 2014).

A linked factor that remains particularly contentious regarding its relationship with species richness is the urban park's age (McIntyre, 2000). One hypothesis suggests that older urban parks contain higher species richness; diversity should increase with the age of an urbanised area due to increased opportunity for colonisation (Fernandez-Juricic, 2000). Alternatively, in relatively young urban areas (15 years and younger), diversity may decline from the youngest sites to the oldest, due to the presence of “early successional” taxa, including Lepidoptera, in recently cleared sites (McIntyre, 2000). However, the local extinction of species following habitat loss or degradation can occur with a substantial delay in young urban areas, an effect known as “extinction debt” (Kuussaari *et al.*, 2009; Soga & Koike, 2013a), masking potential signals.

Butterflies, day-flying Lepidoptera, have frequently been the focus of studies of urban biodiversity (e.g. Blair & Laune, 1997; Clark *et al.*, 2007; Di Mauro *et al.*, 2007; Öckinger *et al.*, 2009; Bergerot *et al.*, 2011; Soga & Koike, 2012; Bonebrake & Cooper, 2014; Lee *et al.*, 2015; Tam & Bonebrake, 2015) including in Southeast Asia (Koh & Sodhi, 2004). Butterflies are thought to react rapidly to environmental changes due to their high mobility and short generation time (McIntyre, 2000), and patterns of butterfly diversity are reflected in other distantly related taxonomic groups (e.g. bats; Syaripuddin *et al.*, 2015). Furthermore, standardised sampling protocols for butterflies have been established and butterflies are particularly valuable “ambassadors” of biodiversity conservation for public outreach (Wilson *et al.*, 2015). However, data concerning urban butterfly diversity is valuable in itself, as populations of butterflies are dwindling globally (New, 1997). Tropical butterflies, such as those in Southeast Asia, are disappearing at the fastest rates due to loss of suitable habitat (Brook *et al.*, 2003; Koh, 2007).

Kuala Lumpur is the relatively young capital city and urban centre of Malaysia (Hashim & Yaacob, 2011). Unlike Singapore, an island where land available for development is limited, Kuala Lumpur is experiencing rapid urban sprawl across the Klang Valley conurbation (Cox, 2013; Figure 4.1). However, unlike many urban cores, the city of Kuala Lumpur (the Federal Territory of Kuala Lumpur) also continues to experience strong population growth; between 1980 (the first census since its designation as Federal Territory) and 2010, the city experienced a population increase of 77% (Cox, 2013). Given the location of Kuala Lumpur and the Klang Valley in a highly threatened biodiversity hotspot (Sodhi *et al.*, 2004), understanding the biodiversity carrying potential of urban habitats and the associated influencing variables is critical, but so far has received little attention (e.g. Karuppanan *et al.*, 2013; Baharuddin *et al.*, 2014; Syaripuddin *et al.*, 2015).

In this study we examined the species diversity of butterflies in Kuala Lumpur city parks. In particular we asked: (1) Does butterfly species richness increase with the park size, and how is this influenced by the presence of different microhabitat types? (2) Does butterfly species richness decrease with proximity to the central business district? (3) Does butterfly species richness decrease with park age?

4.2 Materials and methods

4.2.1 Study sites

Ten urban parks (of 12) managed by the Kuala Lumpur City Hall (known locally as DBKL) and open to the public were selected for this study (Table 4.1). Within each park, we categorised areas as one of four microhabitats: a) groves; b) hedges; c) flowerbeds and d) unmanaged areas. During the sampling period we also recorded the absence or presence of blooming plants in the parks. We obtained details of each park -

total park size, age of park and distance from the central business district (i.e. distance to the Petronas Twin Towers) through interviews with park managers, unpublished reports from DBKL and maps (Table 4.1).

4.2.2 Butterfly sampling

Butterfly sampling was conducted in the months of October and November 2014, with three sampling days at each park (30 sampling days in total). Kuala Lumpur experiences little annual fluctuation in temperature ($26 \pm 2^\circ\text{C}$) and humidity (79-90%) and mild seasonality, with a “dry” season from May to August and a “rainy” season from November to February, although the “seasons” are increasingly unpredictable (Akhiri & Yong, 2011; Tangang *et al.*, 2012). Our sampling was carried out during the inter-monsoon period with diurnal-type weather conditions characterised by late afternoon and evening showers with light, variable winds (Malaysian Meteorological Department, 2015). Measurements of relative humidity, temperature and average wind speed were taken using a weather meter (Kestrel 3000) each day, before and after sampling, at the central point of each park. We chose an active and centred search method (also known as “timed-surveys”) instead of standard Pollard walk methods to allow a full search of different microhabitat areas, and avoid biases due to differences between parks (e.g. size, shape) (see Dallimer *et al.*, 2012; Kadlec *et al.*, 2012). Our search for butterflies centred on the greenest areas (most vegetated area) in the parks for 180-minute periods. We rotated the sequence of microhabitat sampling daily to avoid bias. Sampling times were standardised as calm weather days (mean temperature 31°C ; relative humidity: 68%; and wind speed $< 0.7 \text{ m h}^{-1}$) between 09:30 and 15:00 to correspond with the peak flight activity period of butterflies (e.g. Pollard, 1977; Pollard & Yates, 1993; Koh & Sodhi, 2004).



Figure 4.1: The Federal Territory of Kuala Lumpur and its location within the Klang Valley conurbation and peninsular Malaysia.

Table 4.1: Ten parks in the Federal Territory of Kuala Lumpur where butterfly sampling was conducted.

Site	GPS coordinates	Age of park (year)	Area (ha)	Distance to central business district (km)	Micro-habitats present	Presence of blooming plants
Taman Botani Perdana (TBP)	N3.1446, E101.6838	126	101.1	3.7	G/H/F/U	Yes
Taman Metropolitan Batu (TMB)	N3.2140, E101.6779	13	24.0	7.3	G/H/F/U	Yes
Taman Rekreasi Alam Damai (RAD)	N3.0671, E101.7397	6	10.0	10.6	G/H/U	Yes
Taman Rekreasi Bukit Jalil (RBJ)	N3.0504, E101.6792	16	20.2	12.5	G/H/F/U	Yes
Taman Rekreasi Pudu Ulu (RPU)	N3.1228, E101.7320	6	25.9	4.4	G/H/F/U	Yes
Taman Rimba Kiara (TRK)	N3.1392, E101.6324	19	15.7	9.0	G/H/F/U	Yes
Taman Tasik Ampang Hilir (TAH)	N3.1525, E101.7435	6	16.0	3.5	G/H	No
Taman Tasik Manjalara (TTM)	N3.1931, E101.6277	10	10.6	10.1	G/H/F/U	Yes
Taman Tasik Permaisuri (TTP)	N3.0972, E101.7194	25	49.4	6.7	G/H/F/U	Yes
Taman Tasik Titiwangsa (TTT)	N3.1798, E101.7074	34	46.1	2.2	G/H/F	Yes

(G) Grove; (H) Hedge; (F) Flowerbed and (U) Unmanaged

4.2.3 Butterfly identification

Butterflies were caught using a sweep net and one leg (left hind leg) of each butterfly was gently removed to provide a tissue sample for DNA extraction. This non-lethal tissue sampling method has no effect on butterfly survival and reproduction (see Koscinski *et al.*, 2011; Crawford *et al.*, 2013; Marschalek *et al.*, 2013). DNA was extracted from each sampled butterfly leg, using a modified alkaline-lysis protocol (Ivanova *et al.*, 2009) and the DNA barcode fragment of COI mtDNA was amplified using LCO1490/HCO2198 primers as first pass, MLepF/LepR primers as second pass and mlCOLintF/HCO2198 (Leray *et al.*, 2013) as the final pass following standard protocols (Wilson, 2012). The PCR products were sequenced using the reverse primer by a local company (MYTACG Bioscience) and the DNA barcodes compared against the Barcode of Life Datasystems (BOLD; Ratnasingham & Hebert, 2007) to obtain species assignments on basis of > 98% sequence similarity. This is possible due to the existing DNA barcode reference library for the common butterfly species of peninsular Malaysia (Wilson *et al.*, 2013). A few DNA barcodes did not share > 98% similarity with any BOLD records and were assigned to genera (3 DNA barcodes) or family (2 DNA barcodes) based on the strict tree-based criterion of Wilson *et al.* (2011). Information on specimens and DNA barcodes are available on BOLD in the public dataset: CBPMY.

We obtained information about each species' caterpillar host-plants from Robinson *et al.* (2015)'s database of lepidopteran host-plants. We classified each species as either: a) "host-plant specialist" when the host-plants recorded in the database belonged to only a single family; b) "host-plant generalist" when host plants recorded in the database included more than one family or c) "unclassified" for species not present in the database.

4.2.4 Data analysis

The species richness of butterflies in Kuala Lumpur urban parks was assessed across the study by constructing the species accumulation curves (individual-based rarefaction) using PAleontological STatistics software (PAST; Hammer *et al.*, 2001). The predicted species richness (using individual-based rarefaction and Chao 2) was calculated for each park using EstimateS (Colwell *et al.*, 2004). Chao 2 is appropriate for determining species richness of mobile organisms such as insects (Hellman & Fowler, 1999; Brose & Martinez, 2004). Correlations between species richness (all recorded species and host-plant specialists separately) and park age, size and distance from the central business district were performed using Pearson's correlation coefficients using SPSS Version 21 (IBM Corp, 2012) and scatterplots were plotted using R version 3.1.2 (R Core Team, 2014). The Kruskal-Wallis test was used to compare species richness between different microhabitat types. Canonical Correspondence Analysis (CCA) was performed with PAST to determine the similarity of the butterfly assemblages observed in each park and the relative influence of the age, area and distance from the central business district on the park's butterfly assemblage and on the distribution of individual species.

4.3 Results

4.3.1 Species richness across Kuala Lumpur parks

In total we sampled 572 butterflies belonging to 60 species from five butterfly families (Figure 4.2). When species were ranked in the order of abundance, *Zizina otis* was the most abundant species with 135 individuals (23.6% of all individuals sampled). *Ypthima huebneri* (17.3%), *Eurema hecabe* (6.3%), *Ypthima baldus* (5.9%) and *Appias olferna* (4.9%) were also abundant with more than 27 individuals sampled of each

species. Of the 60 collected species, 35 (58.3%) were sampled fewer than three times. In total, 58 (96.7%) of the species sampled were considered common species in peninsular Malaysia (Corbet and Pendlebury 1992). The two rare species belonged to the genus *Taractrocera* (Hesperiidae) (Corbet and Pendlebury 1992); 4 species from *Taractrocera* are known from peninsular Malaysia and all are rare. *Appias olferna* and *Zizina otis* were the only species sampled in all ten parks, and nearly half of the sampled species (48.3%) were only sampled in a single park. The highest butterfly species richness was observed in Taman Tasik Permaisuri (TTP) (Figure 4.3) but Taman Rekrasi Alam Damai (RAD) had the highest predicted species richness (42, based on the Chao 2 estimator) (Figure 4.3). Taman Tasik Ampang Hilir (TAH), the park closest to central business district, had the lowest species richness, with only nine species sampled (Figure 4.3).

4.3.2 Correlations between species richness and park variables

The correlations between species richness (all recorded species) and size ($F = 2.776$, $p = 0.134$, $df = 8$; Figure 4.4), distance from the central business district ($F = 0.065$, $p = 0.806$, $df = 8$; Figure 4.4), and park age ($F = 1.466$, $p = 0.261$, $df = 8$; Figure 4.4) were not statistically significant (at $p < 0.05$). The correlations between the species richness of host-plant specialist species (17 species total) and area ($F = 8.855$, $p = 0.018$, $df = 8$; Figure 4.4), and park age ($F = 8.199$, $p = 0.021$, $df = 8$; Figure 4.4) were statistically significant (at $p < 0.05$). The correlation between the species richness of host-plant specialist species and distance of the park from the central business district ($F = 1.121$, $p = 0.321$, $df = 8$; Figure 4.4) was not statistically significant (at $p > 0.05$). The CCA did not detect any significant relationships between the distribution of individual species and the park size, distance from the central business district and/or age. In the CCA biplot (Figure 4.5) the first two ordination axes explain 40.0% and 23.2% of the

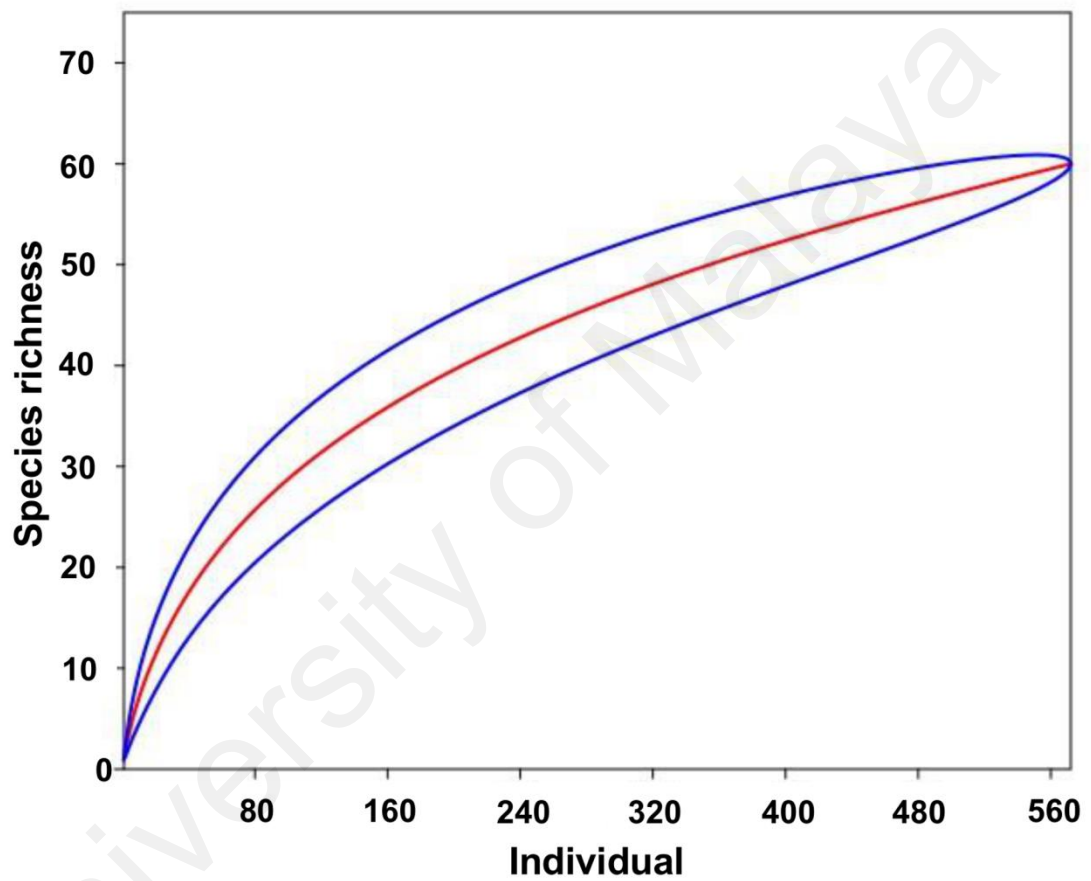


Figure 4.2: Rarefaction curve of species richness of butterflies in Kuala Lumpur urban parks. Blues lines represent the 95% confidence interval of the subsampled iteration.

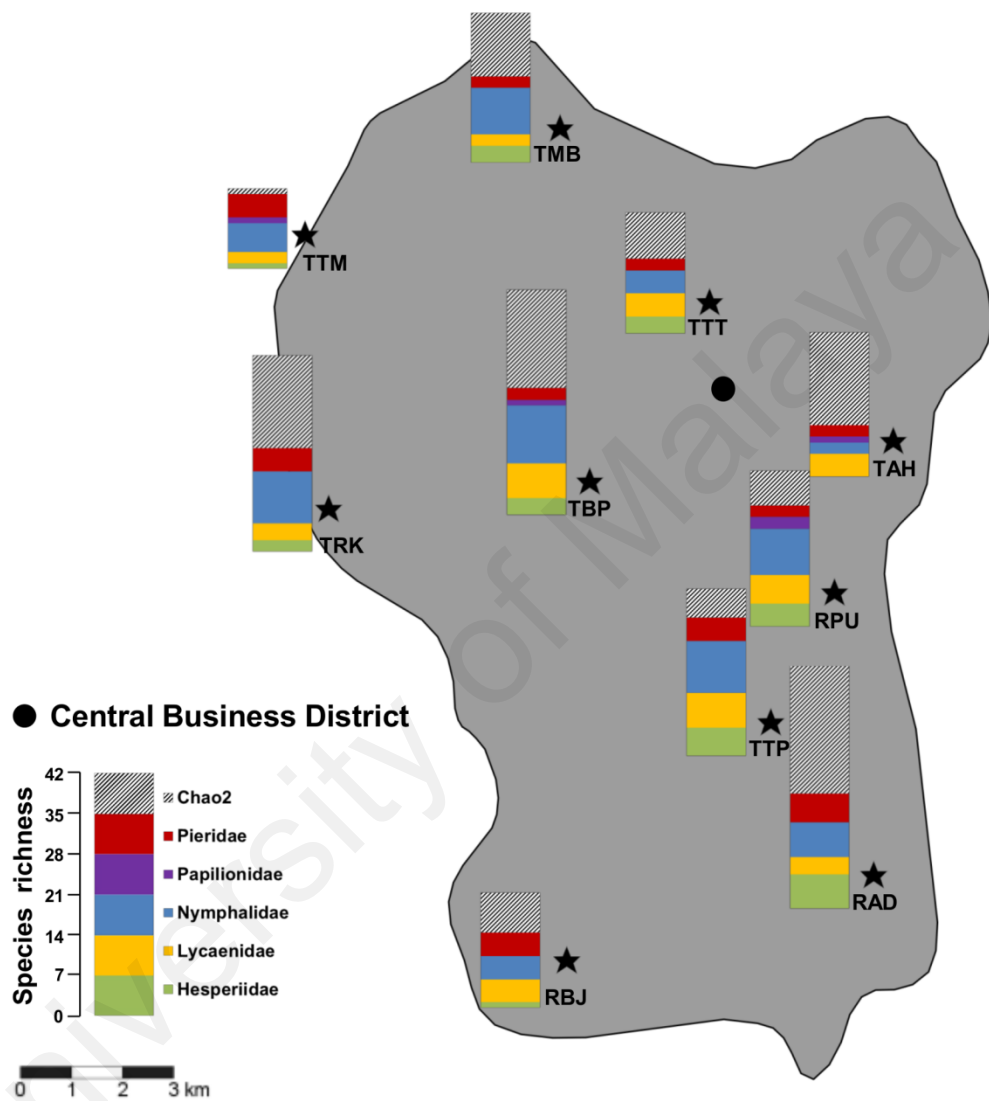


Figure 4.3: Butterfly species richness observed in ten Kuala Lumpur city parks (codes follow Table 4.1). Predicted species richness (in addition to the species richness observed) was calculated using Chao 2.

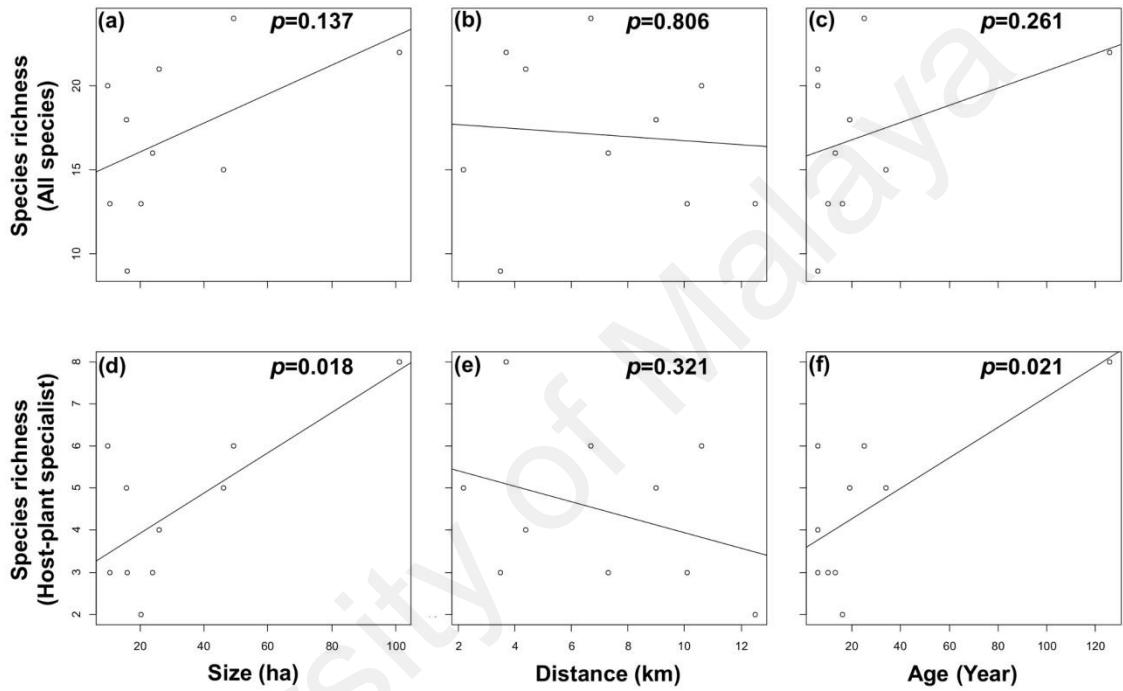


Figure 4.4: Scatterplots of butterfly species richness (all recorded species) and (a) park age, (b) park size and (c) distance from the central business district; species richness (host-plant specialist species) and (d) park age, (e) park size, (f) distance from the central business district.

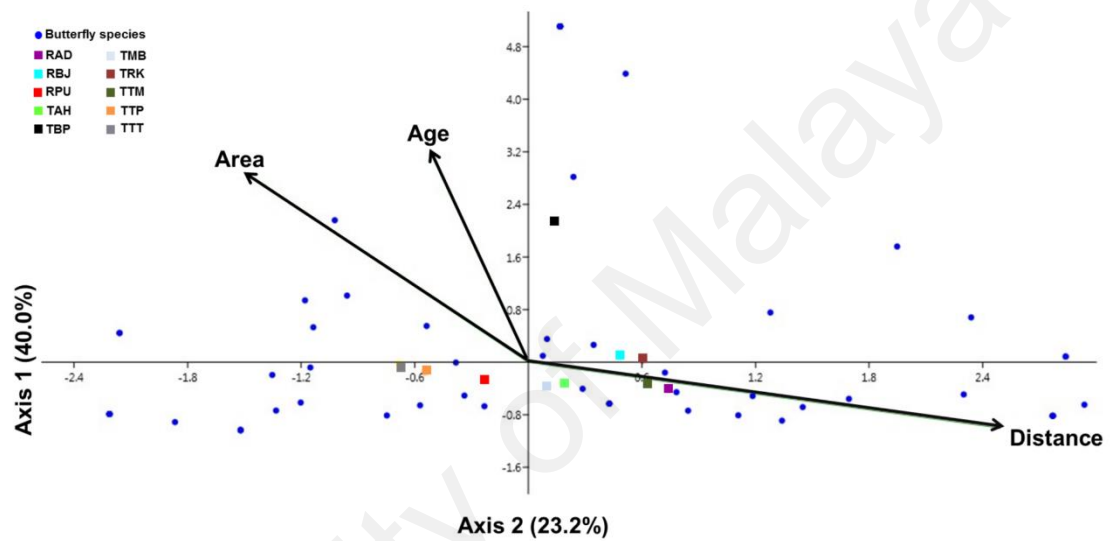


Figure 4.5: Canonical correspondence analysis biplot: species and park variables. The arrows are oriented towards the direction of steepest increase of the park variable. The length of an arrow indicates the importance of the park variable in the model, the direction of an arrow indicates how well the park variable is correlated with the axes, the angle between the arrows indicates the correlation between variables (smaller angle indicated higher correlation), and the location position of a park (following the codes in Table 4.1) relative to arrows indicates the variables of the park.

variance, respectively. Following 999 permutations the overall CCA ($p = 0.63$) and the first two axes ($p = 0.08$) were not significant.

4.3.3 Species richness across park microhabitats

Eleven species were sampled in all four microhabitats. Thirty-nine species (65% of the 60 species recorded across the entire study) were sampled in the unmanaged microhabitat (Figure 4.6). Groves had the second highest species richness with 36 species (60%) followed by flowerbeds with 27 species (45%) and hedges, with 26 species (43%) (Figure 4.6). The two rare species (as judged by Corbett and Pendlebury 1992) from the genus *Taractrocera* were only sampled in the unmanaged and flowerbed microhabitats. The difference in the species richnesses between microhabitats (Figure 4.7) was not statistically different ($p > 0.05$).

4.4 Discussion

Sixty butterfly species were sampled in ten parks in the Federal Territory of Kuala Lumpur, representing approximately 5% of the known butterfly fauna of peninsular Malaysia (Wilson *et al.*, 2013). Almost all sampled butterflies (97%) were from widely distributed, “common” species based on information in Corbet and Pendlebury’s (1992) checklist of the region’s butterflies. This suggests species with a wide geographic distribution are more likely to persist in urban parks, because these species are able to exploit a broader range of ecological niches (Jones *et al.*, 2001, Harcourt *et al.*, 2002). This pattern is exemplified by recently arrived species to peninsular Malaysia, such as *Appias olferna* (see Corbet & Pendlebury, 1992) and *Acraea terpsicore* (see Braby *et al.*, 2013), which were found in high abundance in most of the sampled parks. This further suggests that increased urbanisation during the past few decades in Southeast Asia has provided favourable conditions for colonisation by

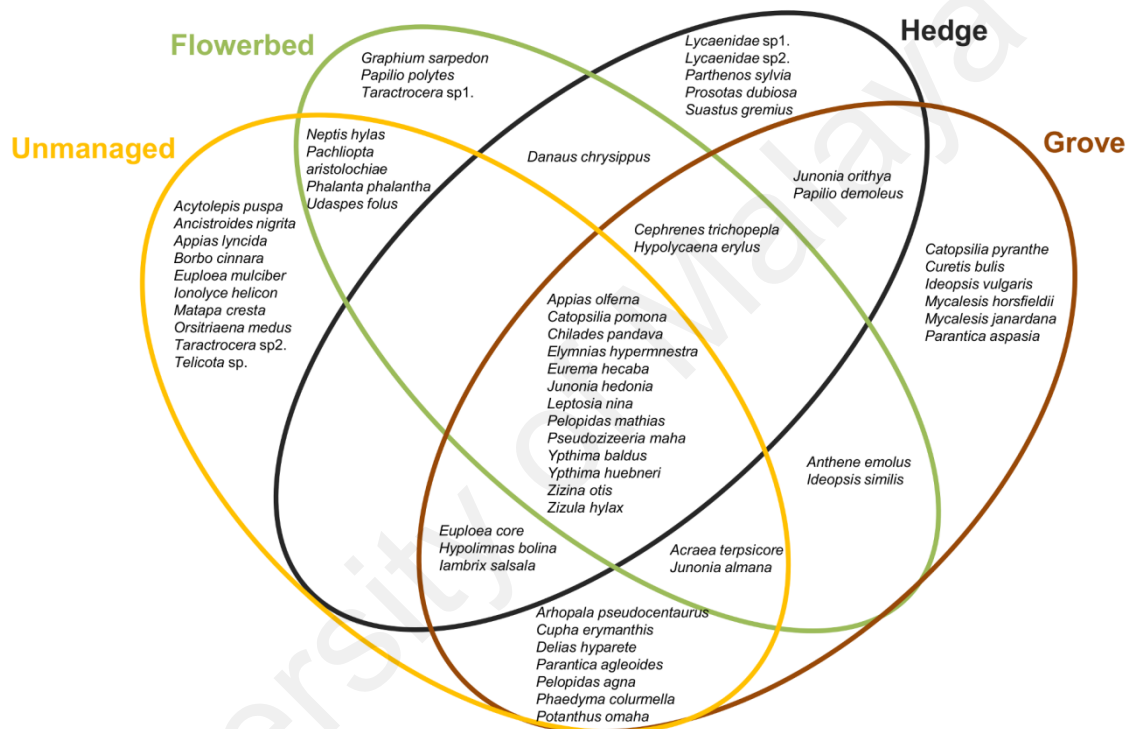


Figure 4.6: Butterfly species observed at four microhabitats across ten Kuala Lumpur city parks.

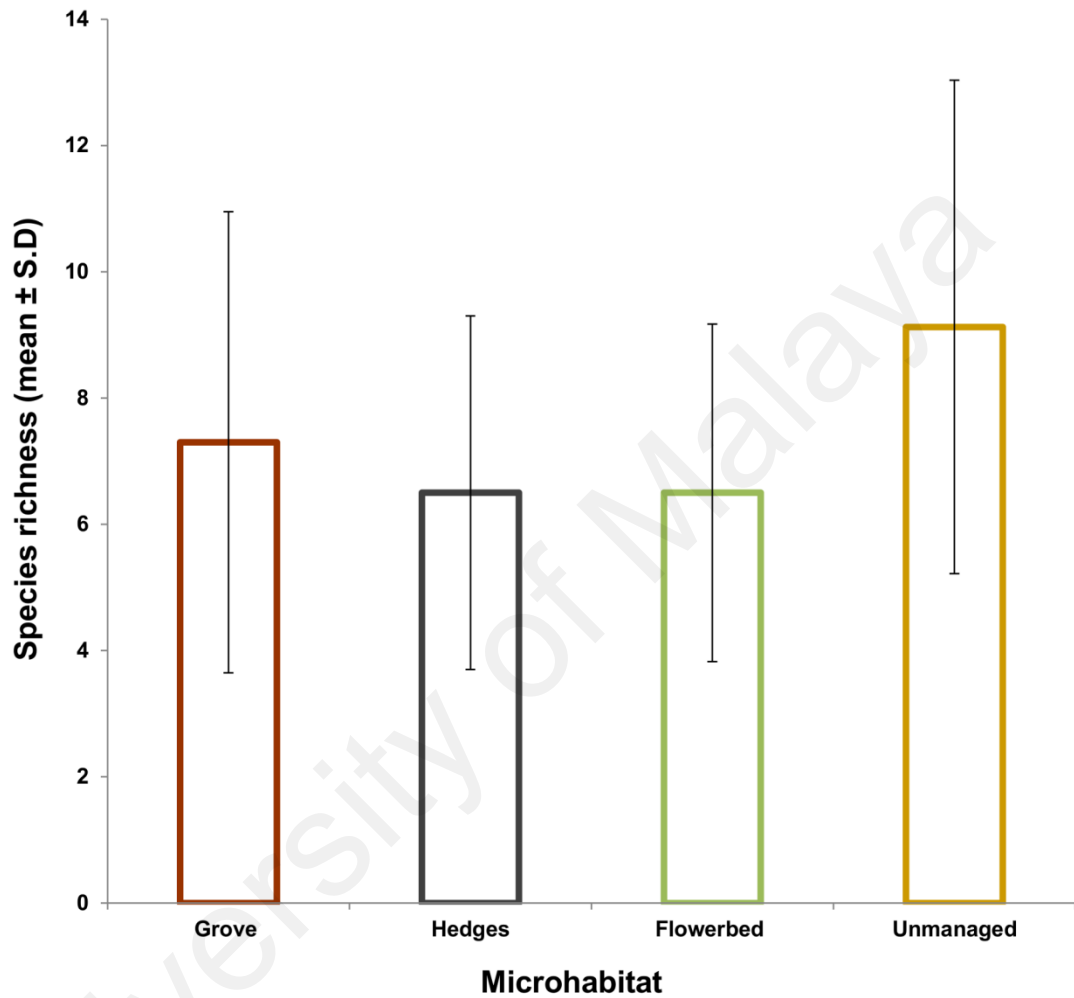


Figure 4.7: Mean butterfly species richness observed at four microhabitats across the ten Kuala Lumpur city parks. There was no statistically significant difference between microhabitats ($p > 0.05$).

these “invasive”, widely distributed species (Braby *et al.*, 2013). Furthermore, of the 56 butterfly species observed by Koh and Sodhi (2004) in forest reserves, forest fragments, isolated urban parks and urban parks adjoining forest in Singapore, 21 were sampled by us in Kuala Lumpur. All of the 21 species shared between these studies have been classified as “urban adapters” based on habitat specialisation of the adult butterfly and host plant specificity of the larvae (Koh & Sodhi, 2004). “Urban adapters” are considered generalist species whereas “urban avoiders” mostly are specialists found in narrow ecological niches (McKinney, 1997, Purvis *et al.*, 2000).

In our study, an average of 17 butterfly species were sampled from each city park, roughly equivalent to the species richnesses observed in Singapore parks after excluding HesperIIDae and Lycaenidae (Koh & Sodhi, 2004), and Hong Kong parks (Tam & Bonebrake, 2015). Surprisingly, 31 butterfly species were observed in four urban parks in the subtropical megacity of Seoul, South Korea (Lee *et al.*, 2015). However, in Seoul, one park was located nearby to natural forest, one park had significant natural forest remnants and the study was conducted over 120 days (Lee *et al.*, 2015) compared to 30 days in Kuala Lumpur. Most studies have reported lower butterfly species richnesses in urban parks compared with equivalent forest (e.g. Blair & Laune, 1997; Koh & Sodhi, 2004; Lee *et al.*, 2015), ruderal sites (e.g. Öckinger *et al.*, 2009) and even residential areas (e.g. Blair & Laune, 1997). At Ulu Gombak Forest Reserve, a reserve secondary forest 15 km from the Kuala Lumpur central business district, a comparable butterfly survey to that we conducted in each Kuala Lumpur park (i.e. 3 days) recorded 48 butterfly species (Syaripuddin *et al.*, 2015). Forty percent of the sampled species at Ulu Gombak Forest Reserve were rare or forest specialists (based on Corbett & Pendlebury, 1992). Similarly, the lack of rare species across Kuala Lumpur, Singapore (Koh & Sodhi, 2004) and Hong Kong (Tam & Bonebrake, 2015)

urban parks suggests tropical urban parks are poor substitutes to forest, even in comparison to (suburban) secondary forest reserves, for maintaining populations of rare butterflies.

Di Mauro *et al.* (2007) found that garden size was significantly correlated with the diversity of butterflies in the Washington, D.C., metropolitan area and suggested this was because more blooming plants are found in larger gardens. The butterfly species richnesses in Kuala Lumpur parks showed a positive relationship with park size but the weak correlation was not significant. We observed the highest species richnesses in the two largest parks (TBP and TTP) but lower species richness was observed from the third largest park (TAH) where we recorded no blooming plants and noticed a low diversity of plant species. Furthermore, the species richnesses of host-plant specialist species did show a significant positive correlation with park size. Koh and Sodhi (2004) reported the lack of a significant correlation between park size and butterfly species richness in Singapore, and suggested that the low plant diversity or generally small areas of the parks probably contributed to the low butterfly species richnesses observed. Positive correlations between urban park size and species richness of amphibians, birds, butterflies, carabid beetles, reptiles, plants, and snails are well-documented (Nielsen *et al.*, 2014). But studies encompassing countries across several continents have consistently identified a threshold size of 10 ha above which size is a less important determinant of species richness (reviewed by Nielsen *et al.*, 2014). In our study, the parks surveyed were all equal to or greater than (10 to 101.1 ha) this threshold size limiting our investigation of this variable.

It is likely that both the effect of the park planting scheme and the presence of early successional plants in unmanaged microhabitats contribute to the strongest pattern (although not statistically significant) that we observed which was highest butterfly

species richness in parks containing all the four microhabitat types. Similarly, Chong *et al.* (2014) reported higher species richness of butterflies in habitat with greater natural vegetation in Singapore. Parks that lacked areas of unmanaged microhabitat had the lowest butterfly species richnesses (although not statistically significant) suggesting that this microhabitat type is crucial for promoting butterfly diversity in urban parks. Unmanaged areas, often at an early-successional stage with a high diversity and quality of plants, provide suitable foraging habitat for butterflies (Swanson *et al.*, 2011; Chong *et al.*, 2014). For example, *Acrea terpsicore*, a species recently reported in Australia (Braby *et al.*, 2013) and sampled in five Kuala Lumpur parks, is a pioneer species favouring early successional plants such as *Hybanthus enneaspermus* (Violaceae) and *Passiflora foetida* (Passifloraceae) (Braby *et al.*, 2014). Alternatively, frequently disturbed sites, such as those intensely mowed or managed, have been found to sustain less diverse populations and abundance of butterflies due to destruction of host plants and potential foraging patches (Stork *et al.*, 2003; Tam & Bonebrake, 2015). Therefore, in addition to a beneficial (to butterflies) planting strategy park managers may consider setting aside an area of park as “unmanaged” or infrequently disturbed (i.e. semi-natural) if they wish to promote butterfly diversity in their parks. Our findings are in agreement with Nielsen and colleagues (2014) conclusion that internal habitat quality, diversity of habitats and microhabitat heterogeneity, is a more decisive driver for species richness generally, than either park size or park isolation.

Snep *et al.* (2006) have suggested that the butterflies present in urban areas are mostly immigrants from the surrounding landscapes. Thus, butterfly communities in urban parks are thought to be strongly influenced by park isolation (Lizée *et al.*, 2012). Other studies have found that park isolation overrides park size as a predictor of butterfly species richness (Koh & Sodhi, 2004; Öckinger *et al.*, 2009; Lizée *et al.*,

2012), with a pattern of decreasing species richness in parks along a rural-urban gradient explained by the composition of the surrounding urban matrix acting as an environmental filter excluding butterfly species, particularly those with specialised habitat requirements (Öckinger *et al.*, 2009). To investigate this pattern in Kuala Lumpur, we used proximity to the central business district as a rough proxy for park isolation. No clear pattern linking butterfly species richness with the distance of the park from the central business district was discovered. However, all the sampled parks in Kuala Lumpur, could be considered to be at the intense end of a long, sprawling, urbanisation gradient, with comparable levels of isolation. Further surveys in parks in the outlying suburbs of the Klang Valley conurbation may be a better approach to reveal any correlation between butterfly species richness and distance of parks from the central business district and park isolation effects. For example, a strong negative relationship was observed between the species richness of butterflies (categorized as feeding specialists, seasonal specialists and urban avoiders) and isolation of forest fragments in the urban matrix of Tokyo, Japan (Soga & Koike, 2013b).

Although overall butterfly species richness showed a weak, and non statistically significant, positive relationship with park age, the correlation of species richness of host-plant specialist species with park age was strong and statistically significant. Kuala Lumpur urban parks have a wide and uneven range of ages: the oldest, Taman Botani Perdana, was established 126 years ago, but half of the parks surveyed were established less than 20 years ago. In a study of urban gardens in New York City, Matteson and Langellotto (2010) found a negative correlation between butterfly species richness and garden age, a pattern which may be explained by the presence of new food sources and young leaves for butterflies during the early succession process in recently disturbed land. However, private gardens are generally much smaller than public parks, and

public parks likely encompass areas under different management regimes, effectively creating areas of different “ages” (i.e. different times since the most recent disturbance). Likewise, Nielsen *et al.* (2014) surmised that changing design fashions and management levels result in no consistent connection between park age and plant species richness. This suggests park managers may be able to influence butterfly diversity and combat outstanding extinction debts (Soga & Koike, 2013a), even in small parks, by careful attention to their planting and management schemes (Josephitis, 2014).

In addition to the variables discussed above, other park elements may influence butterfly species richness but were not investigated in our study. In particular, butterfly species richness has been shown to exhibit a negative relationship with the number of people using urban parks and the amount of park roads (Clark *et al.*, 2007). This suggests human disturbance variables (e.g. noise and traffic of vehicles) have negative effects on butterfly communities (Clark *et al.*, 2007; Chong *et al.*, 2014). The availability of sunlight had a significant influence on butterfly species richness in gardens in New York city (Matterson & Langellotto, 2010), and in particular, the number and design of buildings in and around urban parks, may cause shading that not only severely limits plant growth but also passive basking by butterflies (Matterson & Langellotto, 2010); an essential behaviour to maintain body temperature and adult activity levels (Turner *et al.*, 1987).

Similarly to other studies, our findings suggest that the diversity of habitats and microhabitat heterogeneity contained in urban parks is the most decisive factor driving overall species richness (Nielsen *et al.*, 2014). Management schemes and techniques for conserving butterflies in urban parks are well-established in temperate countries (Shwartz *et al.*, 2013; Smith & Cherry, 2014) but are currently lacking for tropical countries. Our study indicated that large, unmanaged areas should be incorporated into

park management schemes to maximise butterfly species richness. However, unmanaged areas, although beneficial to butterfly diversity, may cause social conflict. Such areas in tropical parks could be perceived as a breeding ground and resting area for mosquitoes (see Mangudo *et al.*, 2015) initiating insecticide application in the parks (Tzoulas *et al.*, 2007). Anecdotally, we did experience more intense attacks from mosquitoes while sampling butterflies in the unmanaged microhabitats. Insecticide usage will directly increase the cost of park management and may result in negative effects for non-target taxa (Boyce *et al.*, 2007). After pyrethrin insecticide application in Davis City, California, Boyce *et al.* (2007) recorded 15% mortality for alfalfa butterflies (*Colias eurytheme*), indicating the sensitivity of butterflies to insecticides commonly used to control mosquito populations. However, other pest control options exist, including *Bacillus thuringiensis israelensis*, an environmentally safe, Diptera-specific insecticide for control of mosquito larvae (Roh *et al.*, 2007). Further studies are required to understand how to effectively incorporate unmanaged areas into urban parks to promote biodiversity conservation while also considering disease vector-control measures.

As biodiversity conservation becomes more of a public concern in rapidly developing Southeast Asia (Wilson *et al.*, 2015), public investment in improving the butterfly “friendliness” of urban parks may be forthcoming. However, it remains to be seen if these practices can be effective in improving the ability of parks to sustain populations of rare butterflies in the face of other urban landscape and urbanisation variables.

CHAPTER 5: CAN BUTTERFLIES COPE WITH CITY LIFE? BUTTERFLY DIVERSITY IN A YOUNG MEGACITY IN SOUTHERN CHINA

Citation: Kong-Wah Sing, Hui Dong, Wen-Zhi Wang, John-James Wilson (2016) Can butterflies cope with city life? Butterfly diversity in a young megacity in Southern China, *Genome*, DOI: 10.1139/gen-2015-0192.

5.1 Introduction

China is currently one of the world's fastest urbanizing countries (Schneider *et al.*, 2015). A prime example of China's rapid urbanization is Shenzhen, one of the component cities of the Pearl River Delta megacity in subtropical Southern China. The location of Shenzhen has been a site of human habitation for a few centuries but designation as a Special Economic Zone in 1979 started a phase of unprecedented urban development. In 34 years, the human population of Shenzhen grew from 300,000 to 10.6 million (UN DESA, 2012) and the built-up area increased from 64, 625 ha in 1996 to 84, 115 ha in 2004 (Li *et al.*, 2010). Today, Shenzhen is categorized as a developed, level-one city, with the same status as three other Chinese cities – Beijing, Guangzhou and Shanghai (Ye *et al.*, 2012). However, in contrast to other cities in China, famous for their pollution, Shenzhen is an “ecological garden city”, with half of its total area under a form of environmental protection that prohibits construction (Jim, 2009). Shenzhen has been awarded the titles “China's Best 10 Cities for Greening”, “National Garden City”, “Nations in Bloom”, “National Greening Pioneer” and was shortlisted in the United Nations Environment Program's Global 500 Laureate Roll of Honor (Shenzhen Municipal E-government Resources Center, 2015).

Shenzhen has 218 parks and 5,000 ha of scenic forests (van Dijk, 2009). In contrast to the declines in biodiversity generally observed along rural-urban gradients, plant species richness is often higher in urban areas than in rural areas because humans actively manage the plant communities present (Hope *et al.*, 2003; Grimm *et al.*, 2008).

While the number of native plant species in Shanghai fell by 43-53% (Xu *et al.*, 1999; Yang *et al.*, 2002;) during a period of urban development (1980-2000), in Shenzhen, during a similar period (1985-2001) the number of plant species increased 406% (from 58 to 294) with an increase in both native and non-native species (Ye *et al.*, 2012).

The survival and diversity of butterflies are strongly associated with plant diversity, being affected by the availability of larval host plants, nectar as an energy source for adult butterflies, and diverse vegetation structures (Thomas *et al.*, 2001; Koh & Sodhi, 2004; Pywell *et al.*, 2004; Pöyry *et al.*, 2005; Öckinger *et al.*, 2006; Chong *et al.*, 2014). However, butterflies are sensitive to urbanization and, in contrast to plant diversity, butterfly diversity generally declines along rural-urban gradients (Blair, 1999; Öckinger *et al.*, 2009). Rome experienced the highest rates of extirpation of butterflies, over the city's long history, during a period of urbanization between 1871 and 1930 (Fattorini, 2011). In the San Francisco Bay Area, the extinction of iconic species such as the Xerces blue (*Glaucopsyche xerces*) by the early 1940s has been attributed to urban development (Connor *et al.*, 2002). *Hesperilla flavescens flavia* and *Jalmenus lithochroa* were extirpated from the city of Adelaide during urbanization in the late twentieth century (New & Sands, 2002).

Considering the unprecedented speed of urban development in Shenzhen, the large number of parks, and the close association between butterfly and plant diversity, we investigated butterfly diversity in Shenzhen city parks. In particular we asked: (1) Does butterfly species richness decrease with park age? (2) Does butterfly species richness increase with the park area? (3) Does butterfly species richness decrease along the rural-urban gradient?

5.2 Materials and methods

5.2.1 Study sites

Ten urban parks of various sizes, roughly evenly spread throughout Shenzhen city, managed by the Shenzhen government authorities and open to the public were selected for butterfly sampling (Figure 5.1; Table 5.1). We categorized areas in each park into four microhabitats plots: a) groves; b) hedges; c) flowerbeds; and d) unmanaged areas (Figure 5.2). Based on literature (Chen *et al.*, 2013), interviews with park managers and Google maps, we recorded the following variables for each park: park age (since year of establishment), total park area, and distance to the central business district (i.e., Shenzhen City Hall and Civic Center).

5.2.2 Butterfly sampling

Butterfly sampling was conducted between June and July 2015, with three sampling days at each park comprising of 180 minutes of sampling per day. Butterfly sampling, using sweep nets by two experienced butterfly collectors, was conducted during calm weather days between 09:00 and 15:00 to correspond with the peak flight activity period of butterflies (Koh & Sodhi, 2004). We followed an active and centered search method (also known as “timed-surveys”) to allow a thorough search of different microhabitat plots, and avoid biases due to differences in size and shape between parks (following Dallimer *et al.*, 2012; Kadlec *et al.*, 2012). During each sampling day, butterflies were sampled in the four microhabitat plots with our time equally divided between microhabitat types present (i.e., 45 minutes for each microhabitat type per sampling day). To avoid sampling bias, we rotated the sequence of microhabitat sampling each day (Sing *et al.*, 2016). The exception was Tanglangshan Suburb Park

Table 5.1: Information of ten parks in the Shenzhen city where butterfly sampling was conducted.

Park	GPS coordinates	Age of park (year)	Area (ha)	Distance to central business district (km)	Micro-habitats present
Donghu Park (DHP)	N22.558, E114.147	49	55.1	9.5	G/H/F/U
Honghu Park (HHP)	N22.569, E114.12	28	57.5	6.7	G/H/F/U
Huanggang Shuangyong Park (HSP)	N22.552, E114.059	18	15.0	4.0	G/H/F/U
Liahuashan Park (LHP)	N22.557, E114.058	18	180.6	0.9	G/H/F/U
Litchi Park (LCP)	N22.546, E114.102	33	27.7	4.7	G/H/F/U
Meilin Park (MLP)	N22.573, E114.036	13	620.8	2.8	G/H/F/U
Shenzhen Bay Leisure Greenway (SBL)	N22.522, E114.021	4	21.3	12.3	G/H/F/U
Shenzhen Central Park (SCP)	N22.551, E114.074	16	100.0	2.6	G/H/F/U
Shenzhen University Park (SUP)	N22.537, E113.931	32	282.0	13	G/H/F/U
Tanglangshan Suburb Park (TLS)	N22.574, E114.01	12	991.1	8.1	U

(G) Grove; (H) Hedge; (F) Flowerbed and (U) Unmanaged



Figure 5.1: The locations of ten urban parks in Shenzhen where butterfly sampling was conducted and the location of Shenzhen with the Pearl River Delta (inset). Park codes refer to Table 5.1.

which consists solely of unmanaged area, therefore, the 180 minutes of sampling per day were spent along a transect spanning the park.

5.2.3 Butterfly identification

All sampled butterflies were brought back to the laboratory and identified based on wing morphology using butterfly guide books (Li & Zhu, 1992; Chao, 2000) and DNA barcoding (Wilson, 2012). DNA was extracted from a single leg of each sampled butterfly, and the DNA barcode fragment of COI mtDNA amplified and sequenced using the primers LCO1490 and HCO2198 (Folmer *et al.*, 1994) at the Southern China DNA Barcoding Center. The DNA barcodes (and associated specimen data) were submitted to Barcode of Life Datasystems (BOLD; Ratnasingham & Hebert, 2007) where they were automatically sorted into Barcode Index Numbers (BINs; Ratnasingham & Hebert, 2013). All the submitted data can be obtained from BOLD under the Shenzhen City Butterflies Project (Project Code: SCBP; http://www.boldsystems.org/index.php/MAS_Management_OpenProject?code=SCBP).

The generated DNA barcodes were assigned to Linnaean species names when their BIN included DNA barcodes submitted by other BOLD users with Linnaean species names. In the case of conflicts, i.e., DNA barcodes with different Linnaean species names were found in the same BIN, we used a consensus approach and additionally cross-checked the validity of the names against usage in recent literature. We assigned DNA barcodes belonging to BINs that were new to BOLD (or had no formally named members) genus names (12 DNA barcodes) or family names (6 DNA barcodes) using the BOLD identification engine “Tree Based Identification” option and a strict tree-based criterion (following Wilson *et al.*, 2011). Ninety butterflies that failed

to generate DNA barcodes were assigned to Linnaean species or genus names based on their wing morphology.

We obtained information about species rarity from Chan *et al.* (2011)'s checklist for the butterflies of Hong Kong using a modified classification pooling "Very rare", "Rare" and "Uncommon" under "Rare"; and "Common" and "Very common" under "Common".

5.2.4 Data analysis

The predicted species richness (using individual-based rarefaction and Chao 1) was calculated for each park separately using EstimateS (Colwell *et al.*, 2004). A Canonical Correspondence Analysis (CCA) was performed with PAleontological STatistics software (PAST; Hammer *et al.*, 2001) to determine the similarity of the butterfly assemblages observed in each park and the relative influence of the park age, park area and distance from the central business district on butterfly diversity and on the distribution of individual species. A natural logarithm (ln) transformation was performed to normalize data prior to further analyses. We calculated Pearson correlation coefficients using R 2.6.1 (R Core Team, 2004) to identify significant correlations between species richness and park age, park area and distance from the central business district. One-way ANOVA was used to compare mean species richness between different microhabitat types. We examined the interaction effect of park size and microhabitat type on butterfly species richness using generalized linear models (Poisson distribution, log link function). Models were simplified by forward selection based on AIC (Akaike Information Criterion) values. The model with the lowest AIC value was selected as the most informative model (Fortel *et al.*, 2014).

5.3 Results

5.3.1 Species richness across Shenzhen urban parks

In total, we sampled 1,933 individual butterflies from ten urban parks in Shenzhen. 1,843 DNA barcodes (95%) were successfully generated and assigned to 72 BINs. Of these 72 BINs, 9 BINs (13%) were new to BOLD. Two additional species (*Faunis eumeus* and *Limenitis* sp.) were recognized on the basis of wing morphology from the 90 individual butterflies that failed to generate DNA barcodes. Consequently, the total butterfly species recorded was 74 species with 63 species (85%) assigned to Linnaean species names. Twenty-nine belonged to the family Nymphalidae, thirteen to Papilionidae, ten to Hesperidae, ten to Lycaenidae, ten to Pieridae and two to Riodinidae. The most abundant species were *Pseudozizeeria maha* (810 individuals), *Luthrodes pandava* (293 individuals), *Catopsilia pomona* (121 individuals) and *Pieris canidia* (111 individuals). These four species accounted for 69% of the total individuals sampled. Fifty-two species (70%) were represented by fewer than 10 sampled individuals and for nineteen species (26%) we sampled only a single individual. *Catopsilia pomona*, *Elymnias hypermnestra*, *Luthrodes pandava* and *Pseudozizeeria maha* were the only species sampled in all ten parks. Twenty-nine species (39%) were only sampled in a single park. Fifty-seven of the butterfly species sampled in this study and assigned to Linnaean species names have been recorded in Hong Kong (Chan *et al.*, 2011). Of these 57 species, 42 are Common and 15 are Rare (including *Lethe chandica* only recently known from Hong Kong; Chan *et al.*, 2011).

The highest butterfly species richness was observed in Tanglangshan Suburb Park which also had the highest predicted species richness (69, based on the Chao 1 estimator; Table 5.2). Huanggaong Shuangyong Park, the smallest park, had the lowest

species richness with only ten species sampled (Table 5.2). The eigenvalues for the first two axes of the CCA ordinations were 0.316 and 0.189 (Figure 5.3), respectively. The butterfly community in the two largest parks was positively associated with park area (Figure 5.3), whereas, the butterfly community in the youngest park was negatively associated with park age but positively associated with distance to central business district (Figure 5.3). The correlations between species richness and park age ($p = 0.859$) and distance from the central business district ($p = 0.951$) were not statistically significant (at $p < 0.05$; Figure 5.4). The correlation between species richness and park size was statistically significant ($p = 0.001$; Figure 5.4).

5.3.2 Species richness across park microhabitats

Sixteen species were sampled in all four microhabitats (Figure 5.5). Sixty-two species (84% of the 74 species sampled across the entire study) were sampled in the unmanaged microhabitat (Figure 5.5). Hedges had the second highest species richness with 37 species (50%) followed by groves with 32 species (43%) and flowerbeds with 25 species (34%) (Figure 5.5). Twenty-six species (35% of the 74 total recorded species) were only sampled in the unmanaged microhabitat (Figure 5.5). The difference in the species richness between microhabitats (Figure 5.6) was not statistically different ($p = 0.285$). However, for butterfly species richness the most informative model (General Linear Model) included both park size with microhabitat type (AIC = 201.35; $p = 0.000$).



Figure 5.2: The four microhabitats plots in Shenzhen urban parks: a) groves; b) hedges; c) flowerbeds; and d) unmanaged areas.

Table 5.2: The total observed and Chao 1 estimated species richness (95% confidence interval) in ten Shenzhen urban parks.

Park	Total observed	Chao 1(95% confidence interval)
Donghu Park (DHP)	25	42 (27-108)
Honghu Park (HHP)	18	16 (13-38)
Huanggang Shuangyong Park (HSP)	10	16 (11-48)
Liahuashan Park (LHP)	25	30 (24-64)
Litchi Park (LCP)	19	39 (18-115)
Meilin Park (MLP)	36	39 (35-57)
Shenzhen Bay Leisure Greenway (SBL)	15	13 (12-20)
Shenzhen Central Park (SCP)	15	15 (13-32)
Shenzhen University Park (SUP)	22	27 (17-82)
Tanglangshan Suburb Park (TLS)	41	69 (47-143)

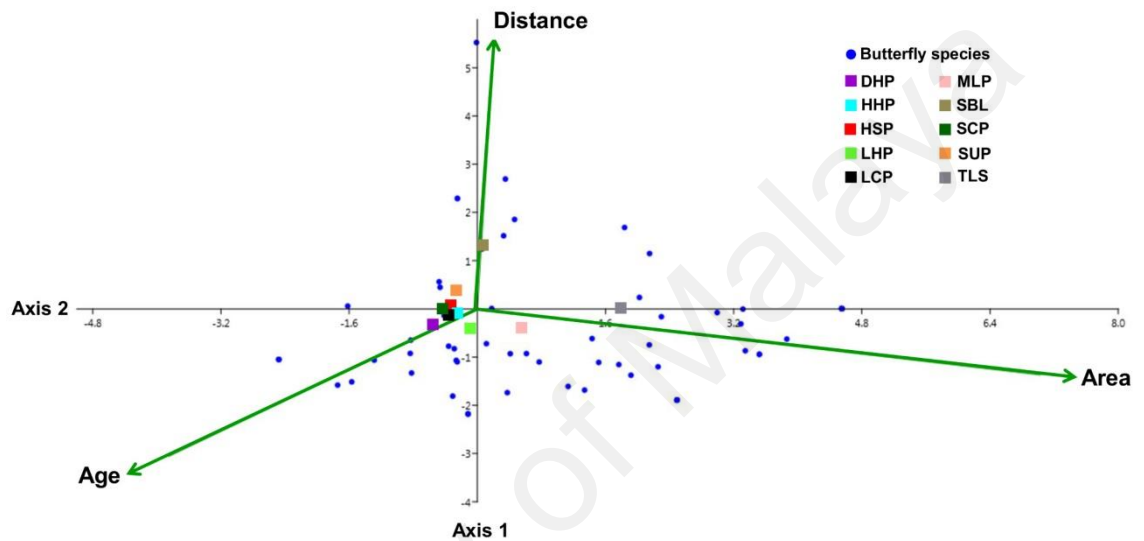


Figure 5.3: Canonical correspondence analysis (CCA) ordination diagram showing the distribution of butterfly species sampled in parks and park variables (arrows). The arrows are oriented towards the direction of steepest increase of the park variable. The length of an arrow indicates the importance of the park variable in the model, the direction of an arrow indicates how well the park variable is correlated with the axes, the angle between the arrows indicates the correlation between variables (smaller angle indicated higher correlation), and the position of a park (following code from Table 5.1) relative to arrows indicates the variables of the park. Park codes refer to Table 5.1.

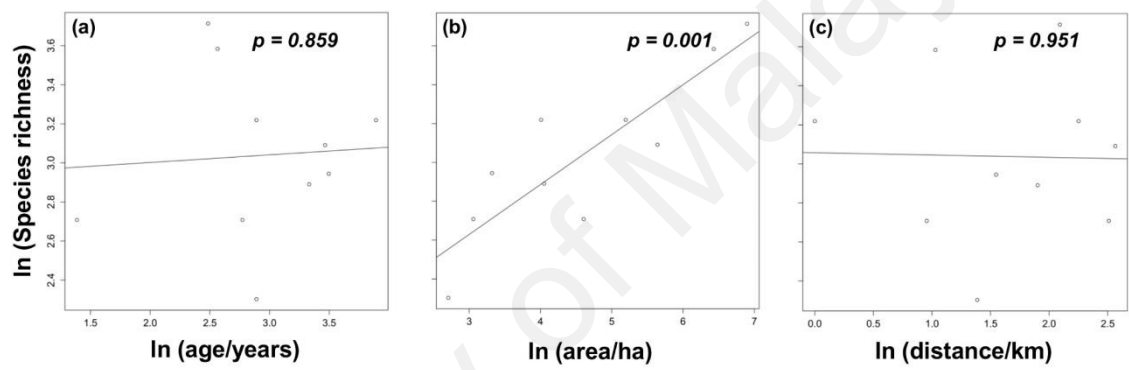


Figure 5.4: Scatterplots of observed butterfly species richness and (a) park age, (b) park area and (c) distance from the central business district.

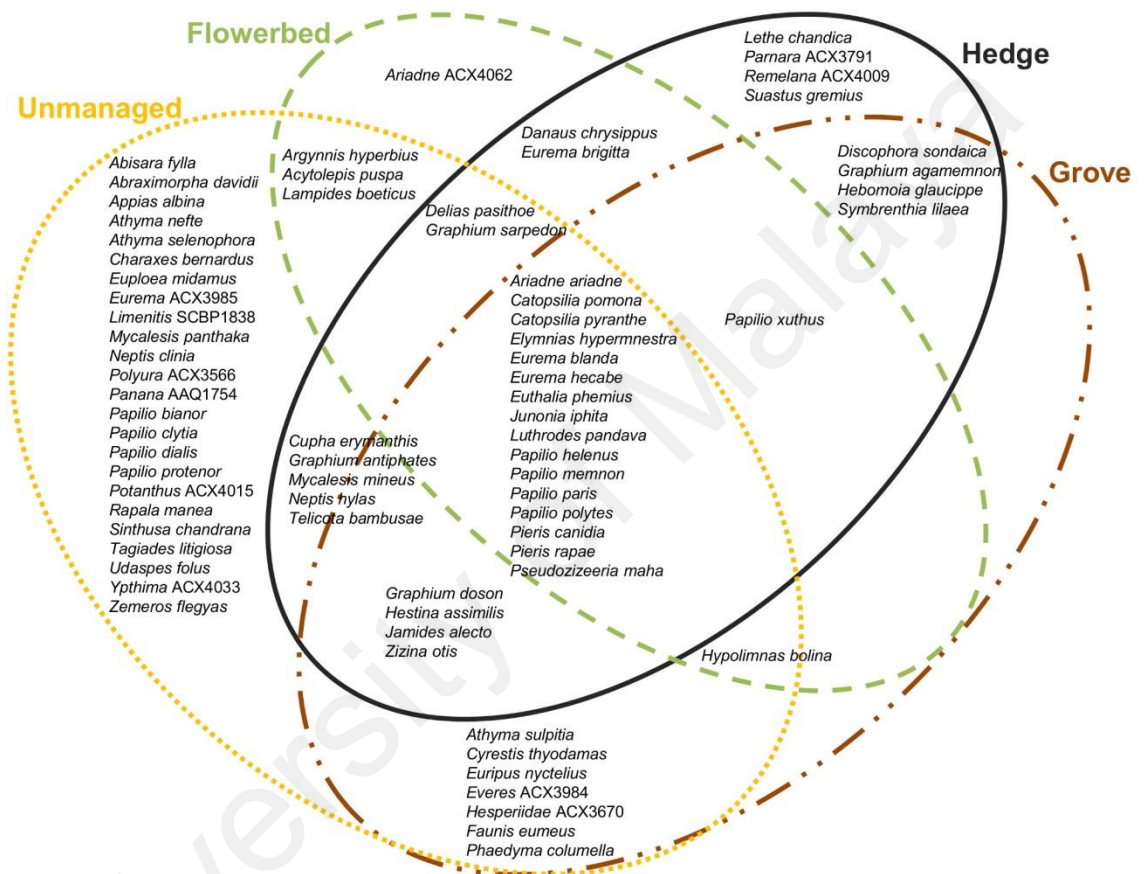


Figure 5.5: Seventy-four butterfly species recorded at four microhabitats across ten urban parks in Shenzhen.

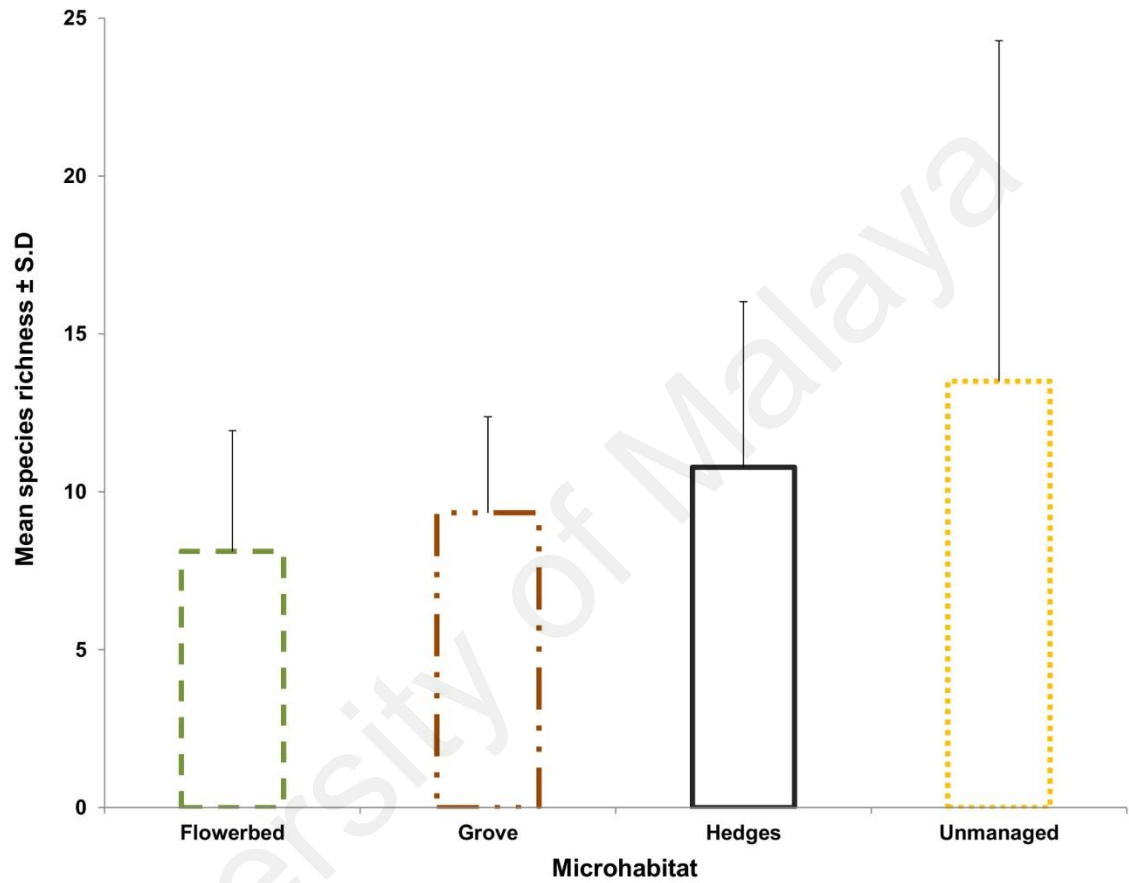


Figure 5.6: Mean butterfly species richness observed at four microhabitats across the ten Shenzhen urban parks (no statistically significant difference between microhabitats at $p = 0.285$).

5.4 Discussion

Of the 74 species sampled in Shenzhen parks, 84% were assigned to Linnaean species names based on the current composition of the BOLD reference library. This included species from the families HesperIIDae and Lycaenidae that are difficult to identify using wing morphology (Koh and Sohdi 2004). Although the number of butterfly species in China (1,223; Chao 2000) is similar that found in Peninsular Malaysia (1,100; Wilson *et al.*, 2013) the number of available DNA barcodes for butterflies from China in BOLD (331) is three times lower than from Peninsular Malaysia (1,247). Consequently, most of the DNA barcodes generated for this study were identified based on matches to DNA barcodes from Peninsular Malaysia for which a DNA barcode reference library is available (Wilson *et al.*, 2013). This study increased the number of DNA barcodes available in BOLD for butterflies from China five-fold.

Butterflies are among the most intensively studied insects, and certainly amongst the most DNA barcoded, with 120,388 records in BOLD. For the vast majority of cases, *a priori* defined butterfly species can also be delimited unambiguously based on DNA barcodes (Dincă *et al.*, 2011; Wilson *et al.*, 2013; Dincă *et al.*, 2015). Nevertheless, taxonomic uncertainties during the assembly of reference DNA barcode libraries, challenges the use of DNA barcoding for routine species identification (i.e., the assignment of unknown specimens to Linnaean species names) (Collins & Cruickshank, 2012). In our study, one quarter of the total BINs sampled (18 of 72) were BINs which included DNA barcodes submitted by other BOLD users under multiple Linnaean species names. For example, there were 284 DNA barcodes in BOLD from the BIN, BOLD:AAA2224; 283 (99.6%) were named *Pieris rapae* and one *Pieris extensa*. The single specimen identified as *P. extensa* (an unpublished GenBank record from Yunnan) in the BIN, BOLD:AAA2224, could be either a misidentification or contamination as *P.*

rapae and *P. extensa* are morphologically distinguishable “good” species. In these situations, we assigned our DNA barcode to the Linnaean species name used for the majority of records, which for this example and most cases (18 in total for our dataset), also corresponded to the name we had assigned our specimens based on wing morphology. We feel the vast majority of such cases are the result of different researchers working on the same taxa, but relying on different literature for morphological identifications (Becker *et al.*, 2011), rather than cases of “DNA barcode sharing” (Hausmann *et al.*, 2013). BINs that consist of more than one Linnaean species name can have various causes, from misidentifications or nomenclatural issues, to complex cases (e.g. oversplitting or incomplete lineage sorting) requiring additional studies in order to resolve the status of certain taxa. In a few cases, species pairs sharing DNA barcodes are either very closely related or known to hybridize regularly, consequently, it is not possible to identify them exclusively through DNA barcoding (Dincă *et al.*, 2011). However, cases of introgressive hybridization have seldom been reported for butterflies (Wilson *et al.*, 2013). Furthermore, Smith *et al.* (2012) reported no obvious association between DNA barcode sharing and *Wolbachia* infection after screening 539, 174 DNA barcodes from Lepidoptera (a finding consistent with Linares *et al.*, 2009).

Elias and colleagues (2007) suggested the inclusion of closely related (congeneric) species or geographical populations of the same species, in DNA barcoding analyses can compromise identification accuracy. More recently, Ashfaq *et al.* (2013) reported that the addition of conspecific DNA barcodes from other regions (countries) increases intraspecific distances, but the relationship between geographical distance and the level of intraspecific divergence was not strong which was consistent with the findings of Lukhtanov *et al.* (2009), Bergsten *et al.* (2012) and Gaikwad *et al.*

(2012). A notable example from Shenzhen were 6 DNA barcodes belonging to *Danaus chrysippus* [BOLD:ABX5122], a BIN with representatives from Spain (11), Kenya (8), India (9), Madagascar (6), Pakistan (6), Tanzania (6), South Africa (5), Malaysia (4), Algeria (3), Italy (3), Tunisia (3), Democratic Republic of the Congo (2), Egypt (2), Israel (2), Morocco (2), Philippines (2), Cameroon (1), Japan (1), Malawi (1), and Taiwan (1), yet with a maximum intraspecific distance of 1.49%. It is possible that DNA barcodes generated in this study will eventually be transferred to different Linnaean species names, which by their nature as scientific hypothesis, are transitory. The data generated for this project (e.g. DNA sequences, images, collection locality) are readily available in raw format for re-analysis, incorporation into a larger dataset, comparisons, and other forms of meta-analysis. This is a major advantage of DNA barcoding approach used, in contrast to typical studies in this field that rely on morphological identification of butterflies “on the wing”, with limited metadata provided.

During 30 days of sampling across ten urban parks in Shenzhen, we sampled 1,933 butterflies representing 74 species from six families, demonstrating a young, subtropical, megacity landscape such as Shenzhen can provide suitable habitat for many butterfly species. Although our sampling period was limited, the number of butterfly species collected in our study approached an asymptote and the observed species richness in seven (70%) of the surveyed parks was similar (different by two to six species) to the predicted species richness (Chao 1) suggesting our sampling effort was sufficient to provide some broad insights into diversity patterns across the parks. Furthermore, the total species count is similar to that reported in studies from other cities in the Pearl River Delta. Li and colleagues (2009) sampled 73 species during an intensive study (May 2005-December 2006) across four different sites with various

degrees of human disturbance in Guangzhou (approximately 100km from Shenzhen) but only 43 species were collected in the urban center. Tam and Bonebrake (2015) reported 58 species (June-November 2013) across 13 urban parks in Hong Kong (approximately 27 km from Shenzhen).

Fifty-seven butterfly species that we sampled in Shenzhen parks have also been reported from Hong Kong (Chan *et al.*, 2011) and represent approximately one quarter (24%) of the known butterfly species of Hong Kong (Chan *et al.*, 2011). Three quarter of these species (74%) were classified as Common. This is similar to the findings from Guangzhou where 70% of the species sampled in urban green spaces were Common (Li *et al.*, 2009), and Hong Kong where 79% of the species recorded in urban parks were Common (Tam & Bonebrake, 2015). In contrast, in Kuala Lumpur, Malaysia, 97% of the butterfly species sampled in urban parks were considered common species with good dispersal abilities (Sing *et al.*, 2016).

The butterfly species richness in Shenzhen parks showed a positive relationship with park size and the correlation was statistically significant ($p = 0.001$). Similarly, Giuliano (2004) reported park size was positively associated with the species richness of butterflies and moths in New York City parks. Di Mauro *et al.* (2007) found that garden size was significantly correlated with the species diversity of generalist butterflies in the Washington, D.C. metropolitan area and suggested this was because larger gardens probably contain more resources such as nectar and host plants for butterflies. This is consistent with our observation of the highest butterfly species richness in the two largest parks (Tanglangshan Suburb Park and Meilin Park) and similar species richness in two parks (Litchi Park and Honghu Park) where the number of plant species has been reported to be similar (120 species; Ye *et al.*, 2012).

The butterfly species richness in Shenzhen parks showed a negative relationship with park age and distance to the central business district but the correlations were weak and not statistically significant. Shenzhen urban parks have a narrow range of ages: the oldest, Donghu Park was established 49 years ago, but half of the parks surveyed were established less than 20 years ago. Matteson and Langellotto (2010) found a negative correlation between butterfly species richness and the age of gardens in New York City, a pattern which may be explained by the presence of new food sources and young leaves for butterflies during the early succession process in recently disturbed land (McIntyre, 2000). However, the species richness of fruit-feeding nymphalids has been reported to increase with age of secondary forest fragments on Sulawesi, Indonesia, as the temperature and humidity are regulated by the increased canopy density (Veddeler *et al.*, 2005). Although several studies have suggested the pattern of species distribution along rural-urban gradients are affected by the surrounding landscape matrix (Öckinger *et al.*, 2009; Lizée *et al.*, 2012; Syaripuddin *et al.*, 2015), we found no clear association between the park species richness and the distance of the park from the urban core (the central business district) similar to findings in Guangzhou (Li *et al.*, 2009) and Kuala Lumpur (Sing *et al.*, 2016).

Within the studied urban parks, it is likely that both park size and the presence of early successional plants in unmanaged microhabitats contribute to the strongest pattern that we observed, and this interaction was the most informative model. This was supported by the high observed butterfly species richness (41) in Tanglangshan Suburb Park – the largest park and the only park that was comprised solely of the unmanaged microhabitat type. Unmanaged areas, often with a high diversity and quality of (often native) early-successional plants, provide suitable foraging habitat for butterflies (Swanson *et al.*, 2011; Chong *et al.*, 2014). Alternatively, intensive managed sites, such

as those frequently mowed, are reported to sustain low populations and abundance of butterflies due to destruction of potential host plants and foraging patches (Stock *et al.*, 2003; Tam & Bonebrake, 2015). Our study is consistent with others in suggesting that in order to promote urban butterfly diversity it is necessary to make urban parks as large as possible and to set aside area of parks as “unmanaged” or with limited human management (Giuliano, 2004). In those areas where management is necessary, planting native butterfly host and nectar plants is the optimal management strategy (Tam & Bonebrake, 2015).

Without historical records of butterfly diversity from Shenzhen, we are unable to make a comparison between the current butterfly assemblages and those existing before urbanization. However, when compared to other Asian cities (Kuala Lumpur – 60, Sing *et al.*, 2016; Seoul – 31, Lee *et al.*, 2015, Singapore – 56, Koh & Sodhi, 2004; and neighboring Guangzhou – 43, Li *et al.*, 2009; and Hong Kong – 58, Tam & Bonebrake, 2015) the total butterfly species richness (74) recorded in Shenzhen parks does suggest the “ecological garden city” outlook may have been successful in maintaining butterfly diversity. In particular, the number of rare species was higher in Shenzhen urban parks (14) compared to Hong Kong parks (6; Tam & Bonebrake, 2015) suggesting urban parks in Shenzhen may, at least presently, have conservation value for rare butterfly species.

CHAPTER 6: CONCLUSION

This is the first study examining patterns of bee and butterfly diversity in megacities in the Southeast and Southern East Asia region. Results from this study suggest that urbanization has negative impacts on bee and butterfly diversity. Bee species richness and abundance diversity declined along the urban gradient and a lack of rare butterflies were reported in urban parks. These findings are similar with previous studies where the urban matrix acts as an environmental filter excluding species that are intolerant to human disturbance (particularly those with specialized feeding and/or habitat requirements), while generalist species may prosper.

The continued expansion of urban areas in ESA is unavoidable due to the rapid growth of the human population. This study revealed that bee species richness showed a negative trend along the urban gradient in tropical ESA megacities. Therefore, highlighting and promoting techniques in urban garden design and plant management that can improve bee restoration and conservation are urgently needed. Presently, urban residents do have empathy for bees but are unlikely to notice them. Those who do notice and interact with bees, even though being stung, are likely to have more positive opinions towards the presence of bees in cities. Therefore, raising awareness about the presence of bees in cities and providing the general public with correct information about bees (see Kasina *et al.*, 2009) could be the key to minimizing human-bees conflict and promoting coexistence of bees and humans in megacities.

Butterfly species richness in urban parks showed a strong positive correlation with park size. Among microhabitat types, highest butterfly species richness was recorded in unmanaged areas. These findings were consistent across two different cities with different urban development histories suggesting that to promote urban butterfly diversity it is necessary to make parks as large as possible and to set aside areas for

limited management. The measure of park isolation (distance from the center business district) in our studies were rather simplistic and we suggest other metrics such as degree of impervious surface and green spaces (see Matterson & Langellotto, 2010) should include in future research.

Understanding the causes and consequences of biodiversity declines in urban areas is a priority in urban ecological research. Consequently, collecting accurate information on pollinator populations (e.g. bees and butterflies) in data deficient areas such as the rapidly urbanizing ESA region will allow researchers to identify vulnerable populations and species and so better target conservation measures. However, tropical ESA is a megadiverse region with an acute taxonomic impediment. Urban biodiversity conservation and restoration is hampered by lack of detailed species inventories i.e. fully knowing and appreciating what is there. This study demonstrated that DNA barcodes can be used for taxonomic assessments and offer potential to mitigate the challenges of biodiversity inventory and species assessments in areas where they are most needed, such as those with unprecedented changes in land-use.

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LIST OF PUBLICATIONS AND PAPERS PRESENTED

1. Sing KW, Wang WZ, Wan T, Lee PS, Li ZX, Chen X, Wang YY, Wilson JJ. (2016) Diversity and human perceptions of bees (Hymenoptera: Apoidea) in Southeast Asian megacities. *Genome*, doi: 10.1139/gen-2015-0159.
2. Sing KW, Jusoh WFA, Hashim NR, Wilson JJ. (2016) Urban parks: refuges for tropical butterflies in Southeast Asia? *Urban Ecosystem*, doi: 10.1007/s11252-016-0542-4.
3. Sing KW, Dong H, Wang WZ, Wilson JJ. (2016) Can butterflies cope with city life? Butterfly diversity in a young megacity in Southern China. *Genome*, DOI: 10.1139/gen-2015-0192.
4. Sing KW, Jusoh WFA, Hashim NR, Wilson JJ. (2015). Urban parks: refuges for tropical butterflies? *Genome* 58: 281. Paper presented at the 6th International Barcode of Life Conference, Canada. (Awarded Best Oral Presentation)
5. Sing KW, Wang WZ, Wan T, Lee PS, Li ZX, Chen X, Wang YY, Wilson JJ. (2016) Diversity and human perceptions of bees (Hymenoptera: Apoidea) in Southeast Asian megacities. Paper presented at the 20th Biological Sciences Graduate Congress, Thailand. (Awarded Best Poster Presentation)