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Diversity and human perceptions of bees (Hymenoptera: Apoidea) in Southeast Asian megacities

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Abstract:

Urbanization requires the conversion of natural land cover to cover with human-constructed elements and is considered a major threat to biodiversity. Bee populations, globally, are under threat; however, the effect of rapid urban expansion in Southeast Asia on bee diversity has not been investigated. Given the pressing issues of bee conservation and urbanization in Southeast Asia, coupled with complex factors surrounding human-bee coexistence, we investigated bee diversity and human perceptions of bees in four megacities. We 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; however, there were no significant differences in the mean species richness and abundance between botanical gardens and peripheral suburban areas or botanical gardens and central business districts. 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, even though being stung, were more likely to have positive opinions towards the presence of bees in cities.

Keywords: Bees; DNA barcoding; Ecosystem services; Human perceptions; Pearl River Delta; Pollination

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 and 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/industrial suburbs, bordering natural (e.g., forest) or agricultural land, to the central business districts (Young and 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 higher 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 and 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 and 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 Halffter (2013) recorded higher butterfly species richness in the Mexican cities of

Xalapa and Coatepec than in nearby forest, whereas the species richness of butterflies in urban green spaces in Seoul, South Korea, was significantly lower than natural forest (Lee et al. 2015).

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). 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 and Visscher 1998; Greene and 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 and 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 and Schmidt 2001).

Bee species richness within cities has been found to be lower than in nearby rural areas (e.g., McIntyre and Hostetler 2001; Eremeeva and 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 and LeBuhn 2006; Matteson et al. 2008; Matteson and Langellotto 2009; Threlfall et al. 2015). In New York City, Matteson et al. (2008) recorded 54 bee species in community gardens and Fetrige 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 and 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 and 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 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?

Materials and methods

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) (Fig. 1, Table 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.

Bee diversity

(i) Sampling

We sampled bees over continuous days (between 0800-1700) in each megacity for a total of 81 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 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 and 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 600-1000m) (Fig. 1) was also conducted each day. We walked along transects 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 10-15 min. 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.kElB2x7jFe2s>, 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).

(ii) 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 and 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 and Hebert 2007) and have been shown to correspond closely with traditional species limits characterized by morphology (Ratnasingham and 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 of 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 and 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 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) were 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 of 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.

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 contained 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 (i.e. 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 Cleavage et al. 2001). Comparisons which yielded expected counts of <5 were excluded as these can yield unreliable Chi-square test results.

Results

Bee diversity

(i) 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 1698 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), Halictidae (25 BINs), Megachilidae (25 BINs), and Colletidae (2 BINs). Twenty-four BINs could be assigned Linnaean species names, 117 BINs could be assigned genus name and all BINs (128) could be assigned 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* “AAF1368” (58 DNA barcodes). These five species accounted for 40% of the generated DNA barcodes. Thirty-two species comprised only a single DNA barcode.

(ii) Comparison of 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) (Fig. 2). *Ceratina* “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 (Fig. 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; Fig. 2).

(iii) Comparison of 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 (93 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 ($Q= 5.702$, $p= 0.008$) and abundance ($Q= 4.541$, $p= 0.026$) of bees in peripheral suburban areas were significantly higher than those in central business districts (Fig. 3). There were no significant differences in the mean species richness ($Q= 2.753$, $p= 0.182$) and abundance ($Q= 3.201$, $p= 0.113$) 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 (Fig. 3).

Human perceptions

(i) Respondent demographics

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.

(ii) Knowledge and interactions

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.

(iii) Respondent attitudes

Ninety-six percent of respondents agreed with the statement “bees have a right to exist in their natural environment” (Table 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), 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” ($X^2_{185,4} = 15.8$; $p = 0.003$), 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 ($X^2_{185,6} = 39.6$; $p = 0.000$). 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).

Discussion

A knowledge gap exists regarding the effect of land-use on bee diversity in rapidly urbanizing SEA (Brown and 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 and Hanula 2007; Wilson et al. 2008; Gonçalves and Oliveira 2013), spacing (Droege et al. 2010), elevation (Campbell and Hanula 2007; Tuell and 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 and 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 and Arias 2013). To date, 45404 bee DNA barcodes have been deposited on BOLD. Based on the current composition of “named” bee DNA barcodes on BOLD and our assignment criteria, 19% of the species we sampled in SEA could be assigned Linnaean species names. Ninety-one percent could be assigned 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 rapidly 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 and 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 and 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”, *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 and 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 (*Apis* spp.) and stingless bees (Meliponini); it is common to find honey bees and stingless bees abundantly in tropical regions.

We found significant differences in bee species richness 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 and Sushchev 2005; Europe – Bates et al. 2011, Banaszak-Cibicka and Żmihorski 2012, Folter et al. 2014; and North America – Fretwell et al. 2008) 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, in our study 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 diverse assemblages of bees. We recorded 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 transects 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 rooves have been proposed as a potentially valuable site for bee conservation in North American cities with limited green space (Colla et al. 2009; MacIvor and Lundholm 2010; 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 sites, downtown Bangkok and Hong Kong (Pearl River Delta). Researchers in California, USA, 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 affect perceptions 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 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 and were more likely to agree that bees should be subject to greater control. 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. 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 and 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. Respondents who had been stung by a bee were less likely to agree that bees should be subject to greater control.

The continued expansion of urban areas in SEA is unavoidable due to the rapid growth of the human population. Our findings revealed that bee species richness showed a negative trend along the urban gradient in SEA megacities. Therefore, highlighting and promoting techniques in urban garden design and plant management that can improve bee restoration and conservation is 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.

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Draft

Tables

Table 1. Population (City Population, 2015) and area of the surveyed megacities.

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

Draft

Table 2. Responses to eleven attitude statements about bees (n=185) during questionnaire survey conducted in four Southeast and East Asian megacities.

Attitude statementsj	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. 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 (%)	X ² 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 Valleyjn	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

Have you ever seen bees here?

Yes	6	5	89	$X^2=6.1$
No	7	15	78	$p=0.048$

Bees in cities should be subject to greater controls

Age

<25	51	37	12	
25-44	64	27	9	$X^2=40.0$
≥ 45	33	15	52	$p=0.000$

Country

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	$p=0.000$

Have you been stung by a bee?

Yes	39	34	27	$X^2=6.1$
No	59	22	19	$p=0.047$

Bees nests should be removed once they are found

Age

<25	6	23	71	
25-44	44	35	21	$X^2=37.0$
≥ 45	29	29	42	$p=0.000$

Figure captions

Fig. 1 Megacities in Southeast and East Asia where bee sampling and human questionnaire surveys were conducted.

Fig. 2 Number of species (BIN) collected from different site types and shared species (BIN) between megacities.

Fig. 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.

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Fig. 1 Megacities in Southeast and East Asia where bee sampling and human questionnaire surveys were conducted.
232x184mm (300 x 300 DPI)

Fig. 2 Number of species (BIN) collected from different site types and shared species (BIN) between megacities.
172x157mm (300 x 300 DPI)

Fig. 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.
172x92mm (300 x 300 DPI)

Taxa	Taxonomic assignment method	Bangkok		
		CBD	Botanical	Suburb
Apidae ACV4001	Not applicable			
Apidae ACV3728	Not applicable			
Tetragonula ACV4636	tree-based	1		
Tetragonula ACV3527	tree-based	1		
Tetragonula ACV4063	tree-based	38	32	8
Tetragonula ACV4048	tree-based			
Tetragonula ACV4832	tree-based			
Tetragonula ACO7213	tree-based			
Tetragonula ACV4793	tree-based			
Tetragonula ACV4543	tree-based			
Tetragonula ACK8366	no conflict			
Apidae ACV4749	Not applicable			
Apidae ACV4526	Not applicable			
Apidae ACV3453	Not applicable			
Tetragonula ACV3528	tree-based			
Tetragonula ACV3462	tree-based			
Tetragonula ACV6019	tree-based			
Tetragonula ACR1716	tree-based			
Lepidotrigona ACR1544	tree-based			
Apidae ACV4228	Not applicable			
Apidae ACV4226	Not applicable			
Apidae ACV4392	Not applicable			
Apidae ACV4227	Not applicable			
Apis ceranaAAA8457	no conflict			
Apis ceranaAAM5455	no conflict	6	20	15
Apis dorsata	no conflict	1	7	5
Apis ACP2245	no conflict			
Apis mellifera	no conflict		3	
Apis florea	no conflict	38	25	90
Apis AAC5729	conflict, Genus tree-based			
Heriades AAI1851	no conflict		3	8
Euaspid ACV4603	tree-based			
Coelioxys ACV4189	tree-based			
Coelioxys apicata	no conflict			1
Coelioxys AAS0200	no conflict			
Coelioxys ACV4584	tree-based			
Megachile spissula	no conflict			
Megachile AAK6923	no conflict			1
Megachile ACV4377	tree-based			
Megachile ACV4656	tree-based			
Megachile AAJ2853	conflict, Genus tree-based			2
Megachile ABW1749	no conflict			3
Megachile AAJ3003	conflict, Genus tree-based		12	2
Megachile AAX3240	no conflict			1
Megachile AAJ3088	conflict, Genus tree-based			12

Megachile ACV3939	tree-based		1
Megachile ACV4362	tree-based		
Megachile AAK6946	conflict, Genus tree-based		3
Megachile laticeps	no conflict	1	2
Megachile rufovittata	no conflict		
Megachile kohtaoensis	no conflict		1
Megachile AAD3047	conflict, Genus tree-based	1	
Megachile AAN4635	no conflict		4
Megachile ACV4003	tree-based		
Megachile ACV4107	tree-based		
Braunsapis ACV4999	tree-based		
Braunsapis ACV4344	tree-based		
Braunsapis ACV3999	tree-based		
Braunsapis puangensis	no conflict		
Braunsapis AAM5520	no conflict	1	
Braunsapis AAW7140	no conflict		
Braunsapis ACV3464	tree-based		
Braunsapis mixta	no conflict	1	
Ceratina ACV3793	tree-based		2
Ceratina ACV3929	tree-based		
Ceratina dentipes	no conflict		1
Ceratina ACV4779	tree-based		
Ceratina unimaculata	no conflict		
Ceratina AAF1368	conflict, Genus tree-based		34
Ceratina ACV5698	tree-based		
Ceratina ACV4725	tree-based		
Ceratina ACV3648	tree-based		
Ceratina ACV4021	tree-based		
Ceratina bowringi	no conflict	1	5
Ceratina ACV4239	tree-based		2
Ceratina cognata	no conflict		1
Ceratina ACV3956	tree-based		
Ceratina ACV4515	tree-based		
Ceratina coptica	no conflict		
Ceratina ACV3751	tree-based		
Thyreus ACV3778	tree-based		
Thyreus ACV4821	tree-based		2
Thyreus ACV4361	tree-based		
Hymenoptera ACV4586	Not applicable		
Hylaeus ACV3695	tree-based		
Lasioglossum ACV3656	tree-based		
Lasioglossum ACV4415	tree-based		
Lasioglossum AAJ3142	no conflict		
Lasioglossum ACV4592	tree-based		
Lasioglossum scitulum	no conflict		
Lasioglossum ACV4772	tree-based	1	
Lasioglossum ACV3602	tree-based		

Sphecodes ABX2299	no conflict			
Hymenoptera ACV3647	Not applicable			
Lasioglossum AAJ3182	no conflict			
Lasioglossum AAW9931	no conflict			
Lasioglossum kumejimense	no conflict			
Lasioglossum AAX0164	no conflict	1	2	11
Lasioglossum AAF3895	no conflict			
Austronomia AAF2432	consensus			1
Nomia pavonura	no conflict			
Curvinomia strigata	no conflict			
Curvinomia iridescens	no conflict			3
Pseudapis siamensis	no conflict			
Gnathonomia ACV5985	tree-based			
Lipotriches ACS0821	tree-based			
Apidae ACV3744	Not applicable			
Apidae ACV4384	Not applicable			
Hoplonomia ACV4299	tree-based			
Hoplonomia incerta	no conflict			
Amegilla AAJ4645	no conflict			
Amegilla AAJ2255	no conflict			
Amegilla ACV4813	tree-based			
Amegilla ACV4570	tree-based			
Amegilla ACV4640	tree-based			
Amegilla ACV4492	tree-based			4
Amegilla AAE5642	no conflict			
Amegilla ACV3771	tree-based			
Xylocopa ACV3716	tree-based			
Xylocopa ACV4695	tree-based			
Xylocopa AAJ9217	no conflict			1
Xylocopa ACV4334	tree-based			
Xylocopa ACV4335	tree-based			1
Xylocopa ACV4573	tree-based			
Xylocopa ACV4668	tree-based			1
Xylocopa AAH9472	conflict, Genus tree-based	2		7
Xylocopa ACV4473	tree-based	1		7
Xylocopa AAJ9218	no conflict			

Hong Kong			Kuala Lumpur			Singapore																																															
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