Can butterflies cope with city life? Butterfly diversity in a young megacity in Southern China

Journal:	<i>Genome</i>
Manuscript ID	gen-2015-0192.R2
Manuscript Type:	Article
Date Submitted by the Author:	06-Mar-2016
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Keyword:	Butterflies, DNA barcoding, Shenzhen, urban parks, urbanization

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Can butterflies cope with city life? Butterfly diversity in a young megacity in Southern China

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Abstract:

During thirty years of unprecedented urbanization, plant diversity in Shenzhen, a young megacity in Southern China, increased dramatically. Although strongly associated with plant diversity, butterfly diversity generally declines with urbanization but has not been investigated in Shenzhen. Considering the speed of urbanization in Shenzhen and the large number of city parks, we investigated butterfly diversity in Shenzhen parks. We measured butterfly species richness in four microhabitats (groves, hedges, flowerbeds and unmanaged areas) across ten parks and examined the relationship with three park variables: i) park age; ii) park size and iii) distance from the central business district. Butterflies were identified based on wing morphology and DNA barcoding. We collected 1933 butterflies belonging to 74 species from six families; 20% of the species were considered rare. 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 ($p = 0.001$). Among microhabitat types, highest species richness was recorded in unmanaged areas. Our findings are consistent with others in 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. In comparison to neighbouring cities, Shenzhen parks have high butterfly diversity.

Keywords: Butterflies; DNA barcoding; Shenzhen; urban parks, urbanization

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 and 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 and 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?

Materials and methods

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 (Fig. 1; Table 1). We categorized areas in each park into four microhabitats plots: a) groves; b) hedges; c) flowerbeds; and d) unmanaged areas (Fig. 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).

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

Butterfly identification

All sampled butterflies were brought back to the laboratory and identified based on wing morphology using butterfly guide books (Li and Zhu 1992; Chou 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 and Hebert 2007) where they were automatically sorted into Barcode Index Numbers (BINs; Ratnasingham and 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 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 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".

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).

Results

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 among the 90 individual butterflies that failed to generate DNA barcodes. Consequently, the total butterfly species recorded was 74 species with 63 species (85%) assigned 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 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 2).

Huanggaong Shuangyong Park, the smallest park, had the lowest species richness with only ten species sampled (Table 2). The eigenvalues for the first two axes of the CCA ordinations were 0.316 and 0.189 (Fig. 3), respectively. The butterfly community in the two largest parks was positively associated with park area (Fig. 3), whereas, the butterfly community in the youngest park was negatively associated with park age but positively associated with distance to central business district (Fig. 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 $p < 0.05$; Fig. 4). The correlation between species richness and park size was statistically significant ($p = 0.001$; Fig. 4).

Sixteen species were sampled in all four microhabitats (Fig. 5). Sixty-two species (84% of the 74 species sampled across the entire study) were sampled in the unmanaged microhabitat (Fig. 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%) (Fig. 5). Twenty-six species (35% of the 74 total recorded species) were only sampled in the unmanaged microhabitat (Fig. 5). The difference in the species richness between microhabitats (Fig. 6) was not statistically different ($p = 0.285$). However, for butterfly species richness the most informative model (GLM) included both park size with microhabitat type (AIC = 201.35; $p = 0.000$).

Discussion

Of the 74 species sampled in Shenzhen parks, 84% were assigned Linnaean species names based on the current composition of the BOLD reference library. This included species from the families Hesperiiidae 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 to 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 Linnaean species names to unknown specimens) (Collins and 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 the 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 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%. Although 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) is 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 100 km 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 and 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 and 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 and 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 and Sodhi 2004; and neighboring Guangzhou – 43, Li et al. 2009; and Hong Kong – 58, Tam and 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 and Bonebrake 2015).

suggesting urban parks in Shenzhen may, at least presently, have conservation value for rare butterfly species.

Acknowledgements

This study was funded by Shenzhen Science and Technology Innovation Council (Grant No.: KQC201105310009A), University of Malaya (Grant No.: RP003D-13SUS) and South China DNA Barcoding Center. We would like to thank Prof. Liu Zhi Gang from University of Shenzhen and Liang Zhi Yu from Shenzhen Urban Management Bureau for their generous help in many ways. We also thank Cheah Men How, Kuang Jun Hao, Lin Hai Hang, Lin Xi Hong, Luo Jie, Wang Yun Yu, and Zeng Fan Shen for their assistance with field sampling, DNA sequencing and data analysis.

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Table

Table 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)	Microhabitats 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

Table 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)

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 1.
172x73mm (300 x 300 DPI)

Draft

The four micro-habitats plots in Shenzhen urban parks: a) groves; b) hedges; c) flowerbeds; and d) unmanaged areas.

172x116mm (300 x 300 DPI)

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 1) relative to arrows indicates the variables of the park. Park codes refer to Table 1.

172x83mm (300 x 300 DPI)

Scatterplots of observed butterfly species richness and (a) park age, (b) park area and (c) distance from the central business district.
172x55mm (300 x 300 DPI)

Draft

Seventy-four butterfly species recorded at four microhabitats across ten urban parks in Shenzhen.
172x131mm (300 x 300 DPI)

Mean butterfly species richness observed at four microhabitats across the ten Shenzhen urban parks (no statistically significant difference between microhabitats at $p = 0.285$).
172x135mm (300 x 300 DPI)