

DNA barcoding reveals insect diversity in the mangrove ecosystems of Hainan Island, China

Lu Liu, Zixiao Guo, Cairong Zhong, and Suhua Shi

Abstract: Insect diversity is an indicator of environmental conditions. Frequent outbreaks of mangrove pests have threatened the fragile mangrove ecosystem in China and the sustainable utilization of mangrove resources. The understanding of mangrove pests, as well as a fundamental knowledge of insect diversity, in mangrove forests in China has been hindered by the difficulty of morphological species delimitation because captured insect specimens are either larvae or incompletely preserved adults. DNA barcoding technology uses only a small amount of DNA to conduct species identification. Taking advantage of this, we investigated the entomofauna of mangrove forests on Hainan Island by using a barcode combining cytochrome *c* oxidase subunit I (COI) and cytochrome-b (Cytb). We collected 627 specimens at six localities around the island, which were identified as 219 insect species belonging to 11 orders and 72 families. Lepidoptera, Coleoptera, and Hymenoptera are the most species-rich and abundant taxa. We also identified 13 mangrove pests, 5 parasitoids, and 12 species of predators.

Key words: DNA barcoding, Hainan Island, mangrove, insect, pest.

Résumé : La diversité des insectes est un indicateur des conditions environnementales. De fréquentes éclosions de ravageurs de la mangrove ont menacé cet écosystème fragile en Chine de même que l'utilisation durable des ressources de la mangrove. Les difficultés à délimiter les espèces sur une base morphologique, soit parce que les spécimens capturés sont des larves ou des adultes incomplètement préservés, ont limité la compréhension des ravageurs et des connaissances fondamentales sur la diversité des insectes dans les forêts de mangrove en Chine. La technologie de codage à barres de l'ADN utilise une faible quantité d'ADN pour identifier les espèces. Tirant profit de cela, les auteurs ont étudié l'entomofaune des forêts de mangrove de l'île de Hainan au moyen d'un code à barre combinant la sous-unité I de la cytochrome *c* oxydase (COI) et le cytochrome b (Cytb). Les auteurs ont prélevé 627 spécimens dans six sites sur l'île. Ces échantillons ont été identifiés comme étant membres de 219 espèces d'insectes appartenant à 11 ordres et 72 familles. Les lépidoptères, les coléoptères et les hyménoptères étaient les taxons les plus riches en espèces et les plus abondants. Les auteurs ont également identifié 13 ravageurs de la mangrove, 5 parasitoïdes et 12 espèces de prédateurs. [Traduit par la Rédaction]

Mots-clés : codage à barres de l'ADN, île de Hainan, mangrove, insecte, ravageur.

Introduction

The mangrove ecosystem, found in intertidal zones of tropical and sub-tropical coasts (Thomas et al. 2017), plays a vital role in providing a variety of plant and animal products, as well as protection from floods, sediment trapping, and nutrient uptake and transformation (Polidoro et al. 2010; Atkinson et al. 2016; Ellison 2008). It is supported by woody plants and harbors a rich fauna. However, mangrove ecosystems worldwide are threatened by human activities such as logging, aquaculture, and coastal development (Alongi 2002; Friess and Webb 2014), as well as global climate change (Guo et al. 2018).

Approximately 20%–35% of mangrove forests have been cleared since 1980 (Richards and Friess 2016; Mayaux et al. 2005; Duke et al. 2007). Urgent investigation and management are needed to protect this valuable resource.

Insects play an important role in the mangrove ecosystem biodiversity. Mangrove plants and insects have long coexisted, with insects feeding on pollen, nectar, and other exudates while pollinating flowers (Jones and Jones 2001; Liu et al. 2017). Insects are also a major food source for birds and other animals in mangrove forests and thus are important for energy flow, matter conver-

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Table 1. Sample collection times and sites.

Sampling site	Sampling time	No. of orders	No. of families	No. of species
Dongzhai Harbor	April, July, and November 2017	11	59	160
Bamen Bay, Wenchang	July 2017	8	25	40
Yalong Bay, Sanya	July 2017	4	8	11
Yulin Harbor, Sanya	July 2017	2	3	5
Bailu Park, Sanya	July 2017	6	6	7
Danzhou	November 2017	3	7	16

sion, and the natural balance (Z.G. Li et al. 2014). Insect species representation is an important indicator of overall ecosystem health (Li et al. 2017) and can decrease sharply when the surrounding environment deteriorates. Furthermore, insect pest eruptions can cause direct harm to mangroves and thus have devastating secondary effects.

The past few years have seen continuous outbreaks of mangrove pests that have affected the mangrove ecosystem function (Li et al. 2012). One of these insects, the leaf roller, was first recorded on leaves of *Kandelia candel* and *Aegiceras corniculatum* in Fujian province, affecting 30%–40% of the trees (Lin and Wei 1981). Jia et al. (2001) found seven pests that harmed or potentially harmed mangrove forests in the Futian Mangrove Reserve of Shenzhen. Mangrove forests in India, Japan, and Thailand have been reported to suffer from insect infestation (Offenberg et al. 2004; Van et al. 2005). Fan and Qiu (2004) discovered that *Oligochroa cantonella*, *Pseudocatharylla duplicella*, and *Euricnia* sp. are the main pests of *Avicennia marina* in Beibu Gulf, Guangxi, China. Investigation into insect diversity is essential for mangrove conservation.

However, surveys are hindered by the difficulty in morphological identification of species because many captured specimens are at immature stages (such as eggs, larvae, or pupae) or incomplete adult carcasses (Chang et al. 2017). Needing only a small bit of DNA extracted from a tiny fragment of tissue (Iftikhar et al. 2016), DNA barcoding has advantages to morphology-based delimitation in dealing with such situations. DNA barcoding uses short DNA sequences, typically from one or more standard markers, to facilitate the identification of unknown specimens and guide species discovery (Birch et al. 2017). DNA barcoding is useful in species delimitation, especially in distinguishing cryptic species (Iftikhar et al. 2016; Zhao et al. 2016; Ball and Armstrong 2006) and discovering unrecorded species (Ball and Armstrong 2006). Since its emergence, DNA barcoding has acquired increasing global popularity as a tool for rapid identification of biological species (Miller et al. 2016).

In this study, we employed DNA barcoding technology to survey the entomofauna and identify pests of the man-

grove ecosystem on Hainan Island, China. The DNA barcode data collected will provide a resource for the understanding and management of the Hainan Island mangrove ecosystem. This information can be used to develop strategies to avoid perennial outbreaks of dominant species that harm mangrove plants.

Materials and methods

Sample collection and morphological delimitation

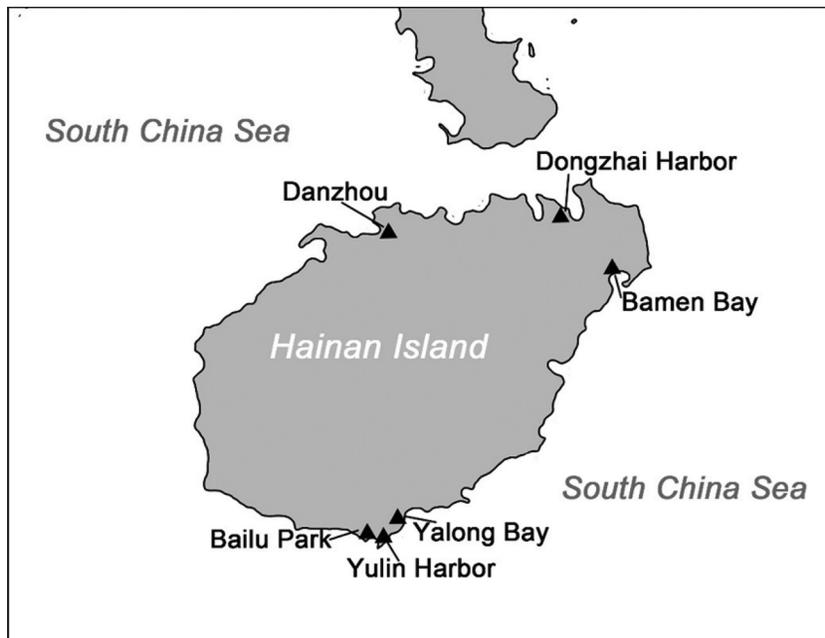
We collected insects at six localities on Hainan Island between latitudes 3.30°N and 20.07°N and longitudes 108.15°E to 120.05°E (detailed information in Table 1 and Fig. 1). We used the five-point sampling method during the day. We swept 50 times at each of five points (east, west, south, north, and central) at each location. The diameter of the net was 50 cm, depth was 70 cm, and the net was made of white nylon yarn. We also scanned the foliage, flowers, fruits, branches, and aerial roots of mangrove plants for insects and used two trap lamps to collect insects at night. Harvested insects were preserved in 100% ethanol or dried overnight in an oven. The morpho-species identity of the samples was determined using morphological characters with the help of two experts in insect classification from the Institute of Zoology, Chinese Academy of Sciences: Dayong Xue and Hongxiang Han.

DNA extraction, amplification, and sequencing

Total DNA was extracted from the muscle of one hind leg of each specimen using the StarSpin Animal DNA Kit in accordance with the manufacturer's instructions. Primers used to amplify 18S rDNA, cytochrome *c* oxidase subunit I (COI), and cytochrome-b (Cytb) genes are shown in Table S2¹. The 30 µL polymerase chain reaction (PCR) mixture contained 3 µL of template DNA, 15 µL 2× EasyTaq PCR Super-Mix, 1.5 µL of each primer (10 µM each), and 9 µL of ddH₂O. The thermal profile was as follows: 94 °C for 3 min; 35 cycles at 94 °C for 30 s, 55 °C for 30 s, and 72 °C for 90 s; and final extension at 72 °C for 8 min. The quality of PCR products was determined using 1% agarose gel electrophoresis. PCR products were sequenced by Applied Biosystems 3730xl DNA Analyzer in TsingKe (Guangzhou, China).

¹Supplementary data are available with the article through the journal Web site at <http://nrcresearchpress.com/doi/suppl/10.1139/gen-2018-0062>.

Fig. 1. Map of the study area showing collection localities.



Bidirectional consensus sequences were assembled using DNAMAN (J.M. Li et al. 2014) and aligned using MEGA 6.0 (Zhang et al. 2018). Parameters including the sequence length, base content, polymorphic sites, and parsimony-informative sites were calculated using MEGA 6.0 (Xing et al. 2018). The aligned COI sequences were 658 bp long, and those of Cytb were 455 bp long. The sequences of 18S rDNA from different taxa have variable lengths, with a highly variable region in the middle, flanked by two conservative regions. We aligned the sequences based on their secondary structures, and then removed unalignable regions before calculating genetics distances and barcode gaps.

Barcode evaluation

A similarity-based method, a genetic distance-based method, and a tree-based method were used to evaluate seven barcodes including the three single loci and four combinations (18S rDNA + COI, 18S rDNA + Cytb, COI + Cytb, and 18S rDNA + COI + Cytb). For the similarity-based method, best match (BM) and best close match (BCM) functions in program TaxonDNA-1.0 were used to assess the accuracy of the seven barcodes, following the criteria described in Xu et al. (2018). For the genetic distance method, we first computed intra- and interspecific divergences under the Kimura-two-parameter (K2P) model (Kimura 1980) using MEGA 6.0 (pairwise deletion), and then found the maximum intraspecific distance and minimum interspecific distance for each species using an in-house R script (<https://github.com/Mackyll/Rscript-used-to-find-genetic-distance/blob/master/Script.R>). Histograms were used to present the distribution of genetic distances by TaxonDNA. We also used scatter plots to capture barcoding gaps, in which the maximum intras-

pecific distance and minimum interspecific distance of a species is plotted as dots. Dots above the 1:1 slope were categorized as showing barcoding gaps (Xu et al. 2018). The number of species that have barcoding gaps were counted for each barcode. For the tree-based method, we constructed Neighbour-joining (NJ) trees using MEGA 6.0 with parameters set as pairwise deletion, K2P model, and 1000 bootstrap replicates. We identify a species if all conspecific individuals are clustered in a monophyletic clade with bootstrap value $\geq 50\%$.

Species delimitation using DNA barcodes

COI sequences were blasted against the National Center for Biotechnology Information (NCBI) database using BLAST for species identification. If the highest identity score was $\geq 95\%$, the BLAST match with the highest identity score and lowest e-value was assigned to the query. If the highest identity score was between 90% and 95%, Cytb and 18S rDNA sequences were further used in BLAST queries. Information from sequence similarity and morphological identification were combined to determine species identity. If the highest percent identity score was $< 90\%$, the sample was only confidently identified to the class, family, or genus level, to avoid any incorrect taxonomic identification.

Results

Sequence variation and barcode evaluation

We collected 627 specimens and obtained a total of 451 COI sequences, 214 Cytb, and 371 18S rDNA. The COI gene multiple sequence alignment was gap-free and trimmed to 658 bp, with 478 variable sites identified, of which 441 were parsimony informative. The Cytb alignment was trimmed to 455 bp, yielding 362 variable sites (331 pari-

Table 2. Summary of K2P genetic distances (%) calculated across taxonomic levels using three single markers and four combinations.

	K2P genetic distance (%)						
	18S rDNA	COI	Cytb	18S rDNA+COI	COI+Cytb	18S rDNA+Cytb	18S rDNA+COI+Cytb
Within species	0.08±0.42	1.36±2.98	0.66±0.81	0.65±1.43	1.39±3.03	0.61±1.35	0.52±0.79
Between species	5.90±6.10	22.31±7.85	23.80±7.20	12.41±5.52	21.73±6.80	11.50±5.60	14.40±5.70
Within family	0.31±0.68	9.89±7.19	10.84±7.49	4.63±2.90	9.29±6.80	3.42±2.74	5.16±3.77
Between family	7.58±5.73	26.15±7.06	26.32±6.43	14.85±4.56	24.40±6.30	13.3±5.3	17.00±5.20
Within order	1.26±1.77	16.35±8.94	17.56±8.50	7.48±4.15	16.61±8.24	6.55±3.36	9.49±4.70
Between order	9.10±4.80	29.96±4.17	30.30±5.10	17.35±2.47	29.6±4.40	15.7±2.90	20.10±2.10

mony informative). Both genes tended to have high AT content (69.3% for COI and 73.4% for Cytb; Table S3¹). The high AT content in insect mitochondrial genes has been well documented (Fрати et al. 1997). The 18S rDNA sequence alignment is rich in insertion-deletion variants among orders. Thus, we justified the start and end points of the alignment rather than trimming it to the same length.

High genetic distances, averaging 22% and 24%, were estimated between species for COI and Cytb (Table 2). The 18S rDNA was much more conservative, as indicated by low genetic distances (average 6%) estimated between species. For the four combinations, COI + Cytb have the highest genetic distances between species (average 22%), followed by 18S rDNA + COI + Cytb (average 14%), 18S rDNA + COI (average 12%), and 18S rDNA + Cytb (average 12%). Distribution histograms of the genetic distances within and between species were plotted for each barcode, but no barcoding gap was detected (Fig. S1¹). However, scatter plots of Cytb, COI + Cytb, and 18S rDNA + COI + Cytb showed barcoding gaps in all species (Fig. 2). In other barcodes, some of the sampled species showed barcoding gaps, namely, 99% in 18S rDNA + COI, 97% in COI, 93% in 18S rDNA + Cytb, and only 26% in 18S rDNA.

Based on the BM model, Cytb and the four combinations described above identified almost all species successfully, followed by COI (99%) and 18S rDNA (39%) (Table 3). Based on the BCM model, the best-performing barcodes were 18S rDNA + COI + Cytb (100%), followed by COI + Cytb (99%) and 18S rDNA + Cytb (99%), 18S rDNA + COI (98%), COI (97%), Cytb (95%), and 18S rDNA (38%). The tree-based method indicated that all species were successfully identified using barcodes using Cytb, COI + Cytb, 18S rDNA + Cytb, and 18S rDNA + COI + Cytb. Moreover, 97%, 95%, and 23% of the species could be successfully identified using 18S rDNA + COI, COI, and 18S rDNA, respectively. Summarizing the three methods, we suggest that both COI and Cytb have the power to barcode our sampled insects and thus recommend the combination of COI + Cytb for routine use.

Insect diversity in Hainan Island mangroves

The insect community we sampled from the Hainan Island mangrove forests consists of 11 orders, 72 families, with 162 samples identified to generic and species levels

and 57 to the family level (Table 4; Table S1¹). NJ trees of the samples using different barcodes are displayed in Fig. 3 and Fig. S2¹. Lepidoptera is the most diverse order, with 25 families (35% of all) and 134 species (61%). The second most diverse order is Coleoptera, with 11 families (15%) and 17 species (8%). Each of the following four orders: Hymenoptera, Diptera, Orthoptera, and Hemiptera, contributed more than two species. These top six orders account for 92% of families and 96% of species. The remaining orders (Mantodea, Odonata, Blattodea, Psocodea, and Trichoptera) are represented by no more than two species each.

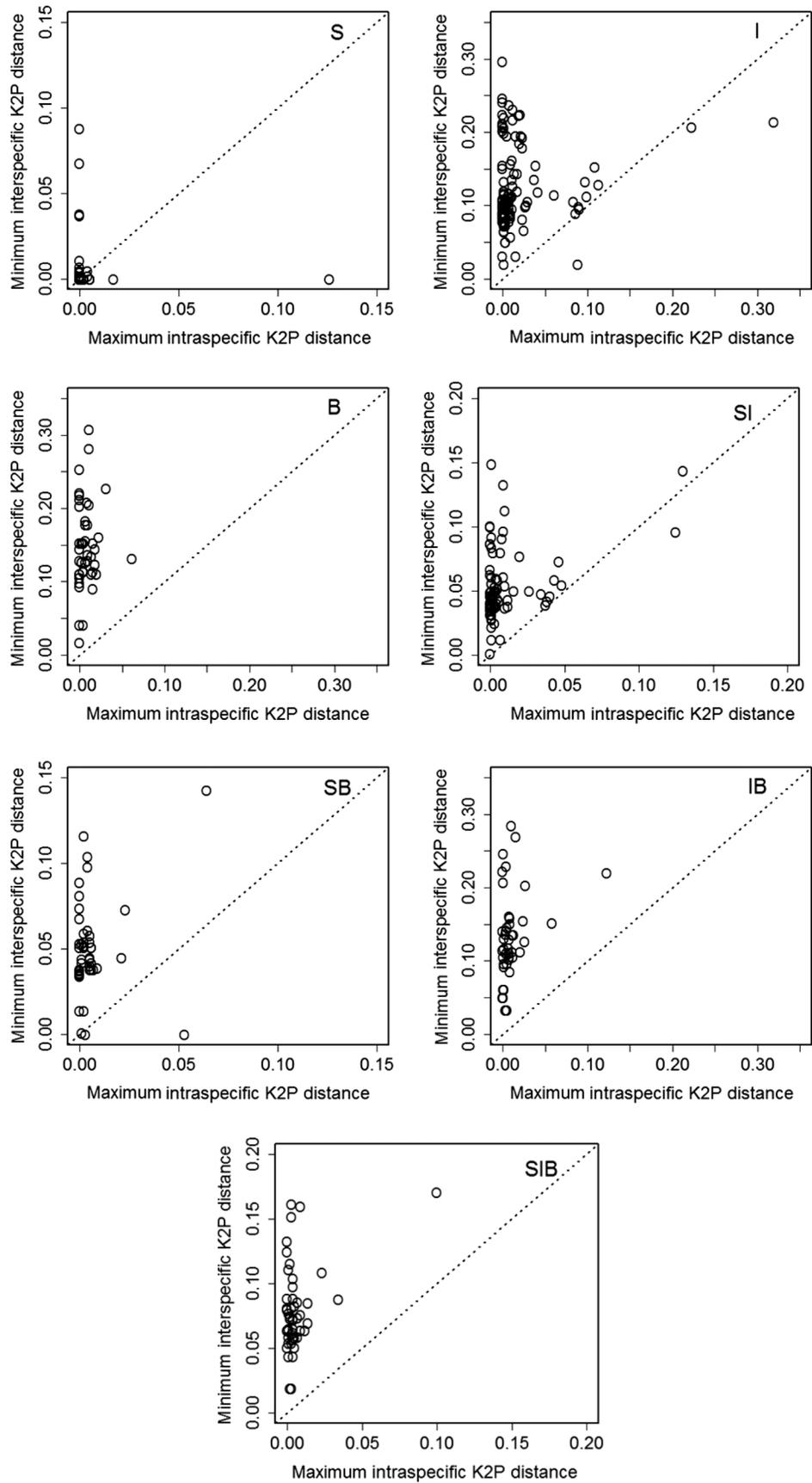
Insect community composition among sampling locations

We compared insect diversity and composition among the six surveyed locations and found notable among-site variation (Fig. 4). Dongzhai Harbor is the richest in diversity at all levels with Bamen Bay the runner-up. The three locations in Sanya (Yalong Bay, Yulin Harbor, and Bailu Park), as well as Danzhou, are much lower in diversity (Table 1). Among the orders, Lepidoptera, Hymenoptera, and Diptera are the most abundant at all sampling locations that they are found at (Fig. S3¹). The identity of the most abundant family depends on the location. Noctuidae and Pyralidae are the dominant families in Dongzhai Harbor, while Pyralidae and Leptoceridae are dominant in Bamen Bay. In the less diverse sites, Noctuidae and Crambidae are common in Yalong Bay, Formicidae and Noctuidae in Bailu Park, and Formicidae and Crambidae in Danzhou.

Seasonal dynamics in insect communities

Our collection of samples during three seasons from Dongzhai Harbor (April, July, and November; Table 5) allows us to assess seasonal effects on community diversity and composition. There is significant seasonal temperature variation on Hainan Island. The temperatures in April and July are similar (24–32 °C and 25–34 °C, respectively) but are much lower in November (13–19 °C). Eighty nine species belonging to nine orders and 43 families were collected in April. Lepidoptera and Coleoptera were the most abundant orders, while Noctuidae and Papilionidae were the dominant families. In July, we identified 48 species belonging to eight orders and

Fig. 2. Scatter plots of the maximum intraspecific K2P distance versus minimum interspecific K2P distance for three single markers and four combinations (S, 18S rDNA; I, COI; B, Cytb).



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Genome 2018.61:797-806.

Table 3. Identification success rates based on “best match” and “best close match” models in the TaxonDNA program.

DNA region	Best match (%)			Best close match (%)		
	Correct	Ambiguous	Incorrect	Correct	Ambiguous	Incorrect
18S rDNA	38.98	58.12	2.88	38.26	58.12	2.88
COI	99.41	0	0.58	97.38	0	0.28
Cytb	100	0	0	94.57	0	0
18S rDNA+COI	99.59	0	0.40	98.38	0	0
COI+Cytb	100	0	0	99.11	0	0
18S rDNA+Cytb	100	0	0	99.09	0	0
18S rDNA+COI+Cytb	100	0	0	100	0	0

Table 4. Species composition of insects from the Hainan mangrove forest.

Order	Family	Species
Lepidoptera	25 34.72%	134 61.19%
Coleoptera	11 15.28%	17 7.76%
Hymenoptera	8 11.11%	20 9.13%
Orthoptera	8 11.11%	13 5.94%
Diptera	7 9.72%	20 9.13%
Hemiptera	7 9.72%	7 3.20%
Blattodea	2 2.78%	2 0.91%
Mantodea	1 1.39%	2 0.91%
Odonata	1 1.39%	2 0.91%
Psocodea	1 1.39%	1 0.46%
Trichoptera	1 1.39%	1 0.46%

32 families. Lepidoptera, Hymenoptera, and Diptera were the most abundant orders, while Limacodidae and Pyralidae were the dominant families. Finally, 52 species belonging to five orders and 27 families were found in November. Lepidoptera was the most abundant order, while Pyralidae, Geometridae, and Amatidae were the dominant families.

Pest identification

Of the 13 mangrove pests collected on Hainan Island (Table 6), 10 were previously found to attack mangroves. The remaining three have not been reported in the literature to our knowledge. Two of the three were only identified as belonging to the families Notodontidae and Geometridae, because we were only able to obtain larval samples. The larvae of Notodontidae (unidentified) and Geometridae (unidentified) were found feeding on the leaves of *Lumnitzera littorea*. We managed to capture adults of the third pest and identified it to belong to the genus *Lobesia*. The larvae of *Lobesia* sp. were found in the *Sonneratia caseolaris* fruit. Although these pests could cause direct harm to mangrove plants, the damage seemed not serious. We also recorded 17 insects that are natural enemies of pest insects (Table S4¹), five species were parasitoids and 12 were predators. Some predators and mangrove plants are symbiotic. For example, as we observed in Bamen Bay, *Sonneratia alba* provides habitat for *Polyrhachis dives* to build nests which provides protection from pests. The second similar example is *Oecophylla*

smaragdina and *Bruguiera gymnorrhiza* we observed in Yalong Bay.

Discussion

DNA barcoding can be used for bio-assessment, also called biomonitoring, which measures species richness and taxonomic composition to evaluate the health of ecological systems (White et al. 2014). DNA barcodes usually need to exhibit high interspecific and low intraspecific divergence, showing distinct DNA barcoding gaps (Kress et al. 2005). In this study, 18S rDNA show no barcoding gaps in most species (59%), because it is too conservative to identify insect species. The two mitochondrial genes COI and Cytb are far more powerful in distinguishing insect species. COI is the core barcode for animals, while Cytb has the potential to become an important barcode. In animal barcoding, conservative nuclear markers such as the 18S rDNA or the 28S rDNA are used to assess specimens to higher taxa (e.g., orders; Stein et al. 2013). Mitochondrial DNA evolves faster, thus mitochondrial markers are typically used to define within-order relationships.

In our survey, 219 insect species belonging to 11 orders and 72 families were collected in the Hainan mangrove forest. A previous study by He et al. (2007) reported 434 insect species in mangrove forests of China, whose composition is not much different from those in coastal shrubs or croplands. In a single location, most previous surveys reported 100–300 species (Jiang and Hong 1993; Jiang 1997; Veenakumari et al. 1997; Jiang et al. 2000; Veenakumari and Prashanth 2009; Bao et al. 2013; Li et al. 2017). Comparing with terrestrial forests, the insect diversity is quite low in mangrove forests (Yi et al. 2014). Following the low mangrove species diversity (Duke 2017), the insect representation may be constrained by extreme conditions including high salinity, strong UV light, and inundation fluctuations (Jiang 1997). The low plant species diversity itself could be a cause of the low insect variety due to the close relationships between hosts and pests. Notably, many mangrove plants are rich in tannins, making them less palatable to insects (Li et al. 2012). The decrease and destruction of woodland, grass, and other vegetation around the mangrove forests may

Fig. 3. COI tree constructed from genetic distance using Neighbor-joining method. Insects of different orders are colour coded.

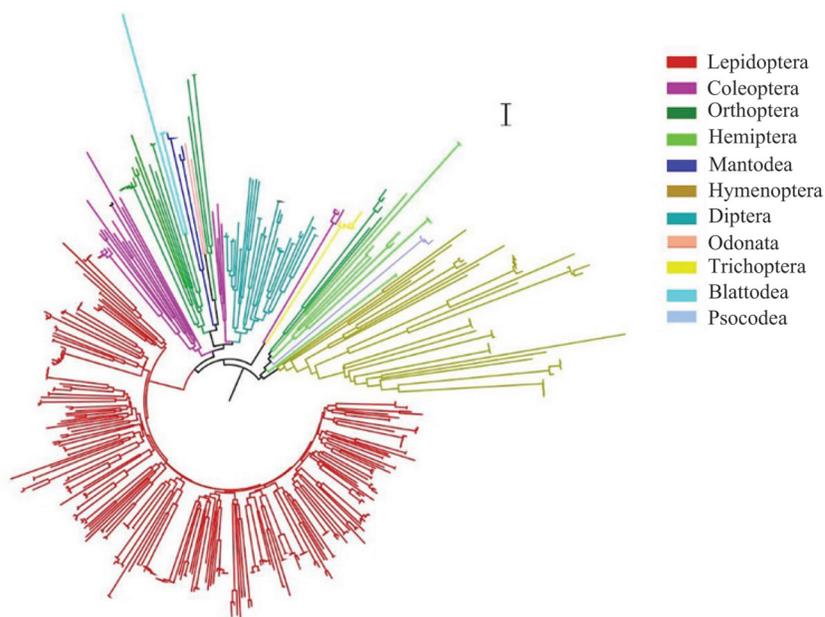
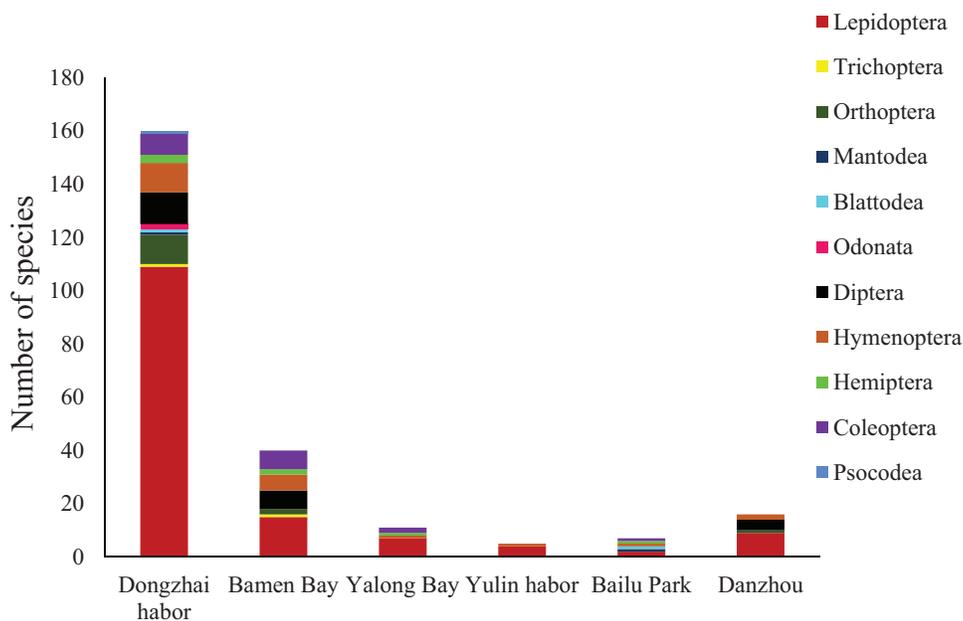


Fig. 4. Relative abundance of insect communities across sample sites.



also interrupt insects' life cycle and contribute to limits on species proliferation (Li et al. 2012).

The composition of insect communities in ecosystems is closely related to the host plant and habitat types (Z.G. Li et al. 2014). The composition of insect community in the mangrove forests on Hainan Island is similar to other places in China, e.g., Qi'ao Island (Z.G. Li et al. 2014), Futian National Nature Reserve (Li et al. 2016), and the Zhangjiang Estuary (Ding 2009), and that in Andaman Island and Nicobar Island, India (Veenakumari and Prashanth 2009; Veenakumari et al. 1997). Lepidoptera, Coleoptera, Hymenoptera, Diptera, Orthoptera, and

Hemiptera are the dominant taxa in both the species diversity and number of individuals. The species composition varies among locations and seasons, but the dominant species come from one or more of these six orders. As climate conditions, particularly temperature, change over seasons the insect community composition also changes slightly as we observed in Dongzhai Harbor.

Particular attention has been paid to pest insects for the management and conservation of mangroves. The low insect diversity of the mangrove community actually enlarges the risk of pest outbreaks when environmental conditions are suitable for specific pest species. Taking

Table 5. Insect community composition across seasons in Dongzhai Harbour.

Order	April		July		November	
Lepidoptera	61	68.54%	29	60.42%	41	78.85%
Coleoptera	7	7.87%	2	4.17%	0	0.00%
Hymenoptera	6	6.74%	3	6.25%	3	5.77%
Orthoptera	3	3.37%	3	6.25%	5	9.62%
Diptera	6	6.74%	6	12.50%	2	3.85%
Hemiptera	2	2.25%	3	6.25%	0	0.00%
Blattodea	1	1.12%	0	0.00%	0	0.00%
Mantodea	0	0.00%	1	2.08%	0	0.00%
Odonata	2	2.25%	0	0.00%	0	0.00%
Psocodea	1	1.12%	0	0.00%	0	0.00%
Trichoptera	0	0.00%	1	2.08%	1	1.92%

Table 6. Phytophagous insects associated with Hainan mangroves.

Species	Host plant	Locality	Month
<i>Bocula sejuncta</i>	<i>Pongamia</i>	Dongzhai Harbor	April
<i>Chasmina candida</i>	<i>Hibiscus tiliaceus</i> and <i>Thespectia populnea</i>	Dongzhai Harbor	April
<i>Orgyia australis</i>	<i>Aegicerus corniculatum</i>	Dongzhai Harbor	April
<i>Delias pasithoe</i>	<i>Sonneratia caseolaris</i>	Dongzhai Harbor	April
<i>Dysphania militaris</i>	<i>Carallia diplopetala</i> and <i>Bruguiera gymnorrhiza</i>	Dongzhai Harbor	April and July
<i>Hymenoptychis sordida</i>	<i>Avicennia marina</i> and <i>Avicennia</i> sp.	Dongzhai Harbor	July
<i>Procalyptis parooptera</i>	<i>Ceriops</i>	Dongzhai Harbor	April and July
Notodontidae	<i>Lumnitzera littorea</i>	Dongzhai Harbor	July
Geometridae	<i>Lumnitzera littorea</i>	Dongzhai Harbor	July
<i>Ricania</i> sp.	<i>Avicennia marina</i> , <i>Rhizophora stylosa</i> , and <i>Sonneratia apetala</i>	Bamen Bay	July
<i>Dysdercus decussatus</i> Boisduval	<i>Hibiscus tiliaceus</i>	Bamen Bay	July
<i>Lobesia</i> sp.	<i>Sonneratia caseolaris</i>	Bamen Bay	July
<i>Platypleura hilpa</i>	<i>Kandelia candel</i> and <i>Avicennia marina</i>	Yalong Bay	July

advantage of DNA barcoding for identification of pest young instars, as well as of fragmentary cuticular body parts, we found several pests in the Hainan mangrove forests. This information will inform future biocontrol and management.

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