

## Preliminary survey and molecular identification of *Geocoris ochropterus* (Fieber) (Hemiptera: Geocoridae)

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### ABSTRACT

Big-eyed bugs *Geocoris* spp. are a natural enemy species presented in many parts of the world that have been extensively researched because of the benefits that they bring to plants. In Vietnam, big-eyed bugs are found in many places, especially in farming complexes or large farms. In Ho Chi Minh City, *Geocoris* sp. was found in Pham Van Coi Commune, Cu Chi District, Ho Chi Minh City, with bug density fluctuations across seasons at an average of  $5.33 \pm 2.67$  individuals per point. The density of the big-eyed bug in Cu Chi District was 03 times higher than the density recorded in Ham Tan District, Binh Thuan Province. Morphological and molecular-based *COI* genes identified the specimens from Cu Chi, Ho Chi Minh City, and Ham Tan, Binh Thuan are *Geocoris ochropterus*. From the research results, these two sampling sites will be selected to further evaluate the rate of natural increase of the big-eyed bug *Geocoris ochropterus*.

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### 1. Introduction

Big-eyed bugs *Geocoris* spp. belong to the family Geocoridae, order Hemiptera are small insects found in many parts of the world such as the United States (Crocker & Whitcomb, 1980; Kóbor, 2018), Africa (Hesse, 1947; Tamaki & Weeks, 1972), and Asia (Cao & Dang, 2011; Kapadia & Puri, 1991). The big-eyed bug *Geocoris ochropterus* was discovered and named in 1844 by Fieber (Kóbor, 2018; Le & Nguyen, 2023). In 1992, Sannigrahi and Mukhopadhyay (1992) comprehensively studied the feeding ability, hunting behavior, molting time through stages, and survival rate of *Geocoris ochropterus*. The feeding behaviors of *Geocoris ochropterus* are varied, the bug sucks fluids from inside its prey, leaving the exoskeleton behind. By lifting and holding the prey for a short time, the bug secretes digestive enzymes through its salivary glands that digest the sucked substances from the prey. Ramirez (2011) found that adults and larvae of big-eyed bugs fed on a variety of small-sized prey including aphids, spiders, insect eggs, small pupa, and young caterpillars.

Big-eyed bugs *Geocoris* spp. have the potential for effective biological control (Hagler & Cohen, 1991). In Arkansas, *Geocoris punctipes* and *Geocoris uliginosus* are among the most important and effective predators of cotton pests from June to September, feeding on aphids and larvae (Bell & Whitcomb, 1964). Lingren, Ridgway, and Jones (1968) recorded that big-eyed bug larvae ate an average of 47 spiders, while adult bugs ate about 83 red spiders per day. Research results indicated that the larval stage consumed 1,600 spiders before developing into adults.

Around 25 big-eyed bug *Geocoris* spp. are found in the United States and Canada (Ramirez, 2011), while Hagler and Sanchez (2020) reported about 19 species present in North America. *Geocoris punctipes* occurs throughout Florida and many other places such as Western New Jersey, Southern Indiana, Southern Colorado, Southwestern Texas, Arizona, California and Mexico. *Geocoris punctipes* are the most common species on cotton plants and is also found in ornamental gardens, vegetable gardens, and in strawberry greenhouse systems (Mead, 2001). *Geocoris bullatus* is widely distributed in the United States and Canada and abundant in Southern Florida from the northern border to Key West, while *Geocoris uliginosus* is widely distributed throughout the United States and Southern Canada, usually living in grasslands (Mead, 2001). When the bugs lack food, they feed on other carnivorous insects or plant tissues to survive but the potential for damage to plants is negligible. These bugs provide great benefits to gardens, crops, and plants.

In Vietnam, the big-eyed bug *Geocoris* sp. was first recorded in Ha Hoa District, Phu Tho Province on tea trees (Vu, Truong, & Nguyen, 2015). Pham (2013) also identified the big-eyed bug *Geocoris* sp. in tea-growing areas of Thai Nguyen, Phu Tho, Yen Bai, Tuyen Quang, Ha Giang, Hoa Binh, Nghe An, and Lam Dong Provinces between 2006 and 2011. In 2018, Nguyen, Mai, and Tran (2018) studied on the morphological and growth characteristics of the big-eyed bug *Geocoris* sp. on cotton plants in Ninh Thuan Province. The report on suitable food sources for breeding the big-eyed bug *Geocoris ochropterus* by Nguyen, Nguyen, Le, Nguyen, and Nguyen (2021) recorded the species in Cu Chi District, Ho Chi Minh City. The big-eyed bug *Geocoris* sp. is distributed everywhere in Vietnam and we focused on investigating the density and identification of big-eyed bugs in two places: Tan Ha Commune, Ham Tan District, Binh Thuan Province, and Pham Van Coi Commune, Cu Chi District, Ho Chi Minh City.

## **2. Method**

### **2.1. Sampling locations**

#### *2.1.1. Cu Chi District, Ho Chi Minh City*

Cu Chi is a suburban district located northwest of Ho Chi Minh City. The sub-equatorial tropical monsoon climate is divided into two distinct seasons; the rainy season from May to November and the dry season from December to April. The average annual temperature is 26.6°C, with average humidity high at 79.5% (People's Committee of Cu Chi District). The big-eyed bugs specimens in Cu Chi District were collected and evaluated at the Aquaculture and Plant Breeding Center-Sagri Seed Farm, Pham Van Coi Commune, Cu Chi District, Ho Chi Minh City, with location coordinates shown in supplement data **Table S1**.

#### *2.1.2. Ham Tan District, Binh Thuan Province*

Ham Tan is a coastal district located in the south of Binh Thuan, Vietnam. The sub-equatorial monsoon tropical climate is sunny and windy, with no winter as the driest area in the country. Big-eyed bugs were collected around Tan Loc Phat Seed Co., Ltd. Binh Thuan branch in Dong Hoa village, Tan Ha Commune, Ham Tan District, Binh Thuan Province, with location coordinates shown in supplement data **Table S1**.

### **2.2. Sampling method**

Big-eyed bugs were collected in insecticide-free vegetable farms and weed-growing areas in Cu Chi and Ham Tan Districts. Methods of investigation, collection, and evaluation of the quantitative of *Geocoris* sp. were carried out according to the experimental method in Plant Protection

& Investigation following Vietnamese standards QCVN01-38: 2010/BNNPTNT for classification of morphological identification and molecular biology (Ministry of Agriculture and Rural Development, 2010). Sweep nets were used to collect big-eyed bugs at the survey sites as insecticide-free vegetable growing areas and weed bushes every 30 days. The number of survey points is 10; The number of survey samples of a site is 10 trees/point. The density of big-eyed bugs *Geocoris* sp. was monitored at each site, and the data were processed using Excel and SPSS 20 software.

### **2.3. Morphological identification**

Adult big-eyed bugs were brought to the laboratory and five mature individuals from each sampling site were randomly selected to analyze the morphological characteristics and compare image differences between adult males and females with Kóbor (2018). The big-eyed bugs were examined under a stereo microscope Olympus SZ51 (Japan), Labomed Luxeo 2S (USA) with a magnification of 35X using an optical microscope Olympus CX23 (Japan).

### **2.4. Molecular biology method using COI gene**

Big-eyed bugs were identified by the molecular method of the *COI* fragment sequence. Samples were sent for sequencing and results were compared with sequences in the GenBank database (NCBI, USA) to identify of big-eyed bug. Big-eyed bug specimens from Cu Chi and one from Ham Tan were randomly selected for morphological analysis. The DNA extraction process was performed as follows. First, a part of the big-eyed bug's leg was ground in 200 $\mu$ L of 50mM NaOH and incubated at 95°C for 15 minutes before adding 20 $\mu$ L Tris-HCl 200mM. The DNA was then diluted and the quality was assessed by measuring the Optical Density (OD). Standard DNA quality requires an OD ratio of  $A_{260}/A_{280}$  between 1.8 and 2.0 and is free of impurities. The *COI* gene sequences were amplified using the forward and reverse primer pairs LCO1490 (5' – GGTCAACAAATCATAAAGATATTGG – 3') and HCO2198 (5' – TAAACTTCAGGGTGACCAAAAATCA – 3') (Folmer, Black, Hoeh, Lutz, & Vrijenhoek, 1994), size of the amplified *COI* gene fragment about 658bp (Hebert, Cywinska, Ball, & deWaard, 2003).

PCR amplification of the *COI* gene region was carried out with the following thermal cycle: denaturation at 95°C for 05 minutes, followed by 35 cycles of 95°C for 30 seconds, 52°C for 30 seconds, 72°C for 02 minutes, and the stretching phase at 72°C for 10 minutes. The PCR product was then mixed with GelRed and electrophoresed on 1.5% agarose gel at 100V for 30 minutes. The electrophoresis results were scanned and photographed under UV light. PCR results should show a clear band with no breaks or sub-bands at the correct size. All samples were sent for sequencing at 1<sup>st</sup> Base (Malaysia). Product sequences were compared with GenBank data (NCBI, USA) to identify the species. A genetic clustering tree was built from the identified sequences by the Maximum Likelihood method with the General Time Reversible model based on 1,000 bootstrap samples using MEGA-X software.

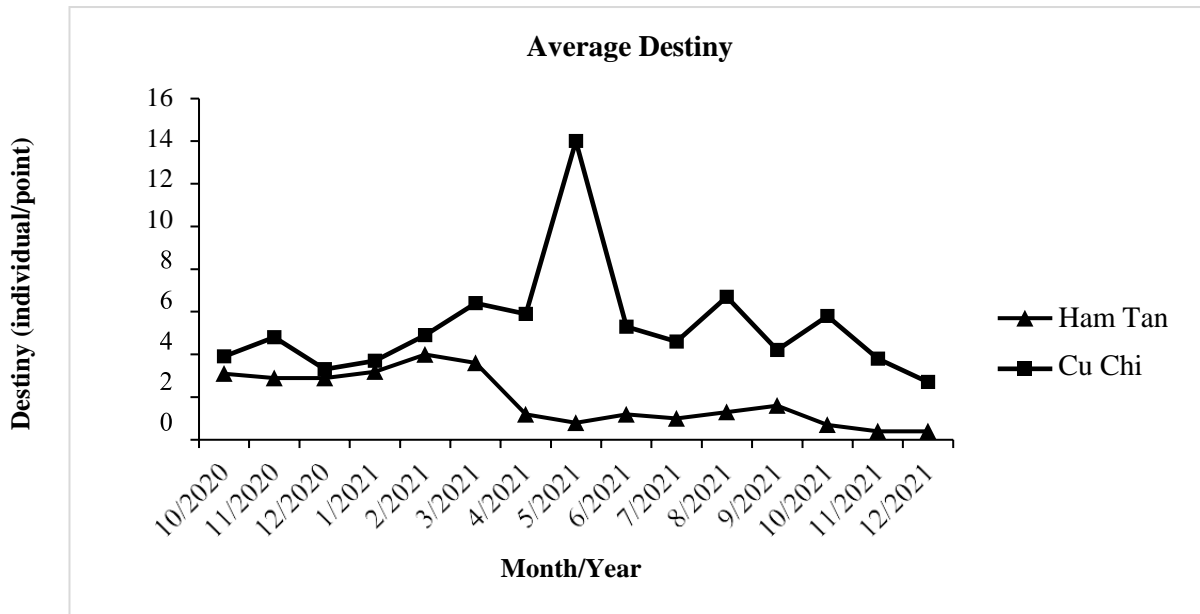
### **Statistical analysis**

The density of big-eyed bugs using an ANOVA model implemented in SPSS 22.0, followed by the Chi-square test.

### 3. Results and discussion

#### 3.1. Density of big-eyed bugs over the months of investigation

The density of the big-eyed bug *Geocoris* sp. at two locations, Cu Chi and Ham Tan Districts, showed significant differences and monthly fluctuations during the survey period from October 2020 to December 2021, as shown in **Figure 1**.



**Figure 1.** Average monthly density of big-eyed bugs *Geocoris ochropterus* in Ham Tan and Cu Chi Districts from October 2020 to December 2021

Density of big-eyed bugs in Cu Chi District fluctuated over the survey months and was higher than in Ham Tan District. In May 2021, the density of big-eyed bugs increased due to entering the breeding season of bugs and encountering suitable weather conditions to grow and develop., while in Ham Tan District, density of big-eyed bugs tended to decrease throughout the survey months.

In October and November 2020, density of big-eyed bugs in the two areas differed by about 1 individual/point, while during December and January, the density was similar in both areas. In March and April, the number of big-eyed bugs in Cu Chi (6.4 and 5.9 individuals/point) was almost twice the number recorded in Ham Tan (3.6 and 2.2 individuals/point).

In the May 2021 survey, the number of big-eyed bugs in Cu Chi (14.2 individuals/point) was 10 times higher than the density in Binh Thuan (1.2 individuals/point), while in June and July 2021, survey density in Cu Chi District (5.3 and 4.6 individuals/point) was 04 times higher than in Ham Tan District (1 and 1.3 individuals/point). In August, September, October, November, and December 2021, the densities of big-eyed bugs in Cu Chi District were 5.2, 2.6, 8.3, 9.5, and 6.8 times higher than that in Ham Tan District, respectively.

The T-test was used to compare the average density of big-eyed bugs in Cu Chi and Ham Tan Districts over the survey period, with results shown in **Table 1**.

**Table 1**

The density of big-eyed bugs in the two sampling locations by T-test

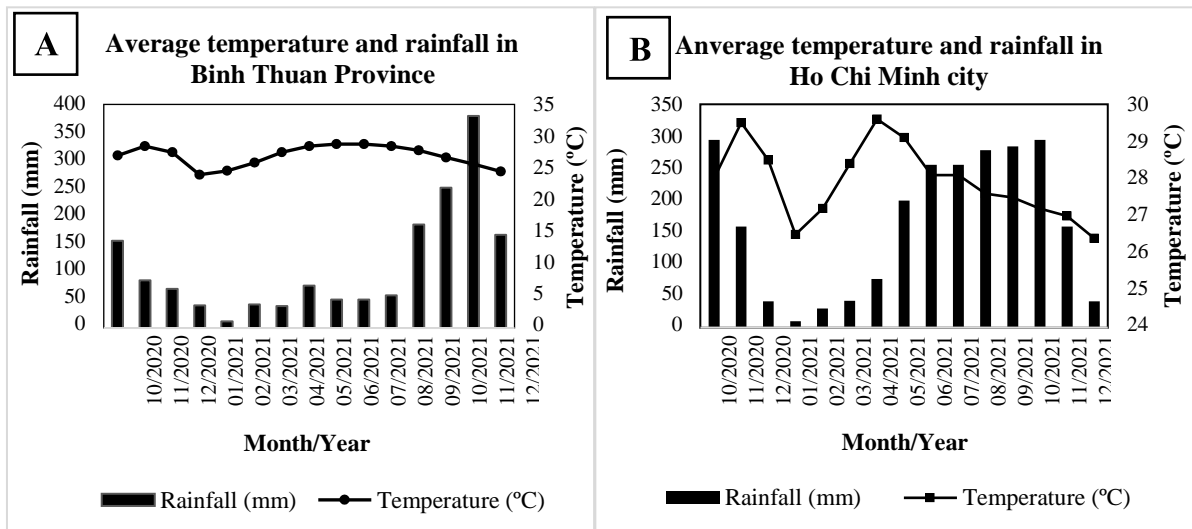
No.	Sampling location	Average density of big-eyed bug (individuals/point)
1	Cu Chi District	5.33*
2	Ham Tan District	1.89

\*: statistically significant difference

There was a significant difference in the density of the two survey sites, with  $T_{\text{start}} = 4.54 < T_{\text{Critical two-tail}} (0.05, df = 28) = 2.05$ . Average density of big-eyed bugs in Cu Chi District was higher than in Ham Tan District.

Big-eyed bugs were obtained in Cu Chi District on weed bushes, *Turnera* sp. flowers and *Bidens pilosa* flowers in the morning, while during the heat of the day, they mainly gathered in *Bidens pilosa* flowers because *Turnera* sp. flowers only bloom in the morning.

Regarding the influence of weather on the density of big-eyed bugs in the two places, we have two mixed charts shown in Figure 2. The data in the two charts are referenced at the official website of the National Centre for Hydrometeorological Forecasting - NCHMF.



**Figure 2.** Average temperature and rainfall in Ham Tan District, Binh Thuan Province (A) and Cu Chi District, Ho Chi Minh City (B)

The average rainfall in Ham Tan District is lower than in Cu Chi because the Tan Ha commune area is more than 20km from the coast, with mountains and forests. The hot and sunny climate, less affected by the Northeast monsoon with little rain and wind, contributes to lower humidity than Pham Van Coi Commune, which affects the reproduction and development of the big-eyed bug population. At the beginning of 2021, the climate was cool, with little rain as the best time for big-eyed bugs to breed. According to Boyd and Boethel (1998), pesticide residues on the leaves of some pesticides are lethal to bugs. In Ham Tan, Binh Thuan, the weather is mainly hot and sunny, with little rain. Cultivation of fruits and vegetables uses pesticides for plants, so the big-eyed bug population here is underdeveloped, difficult to find, and very low density.

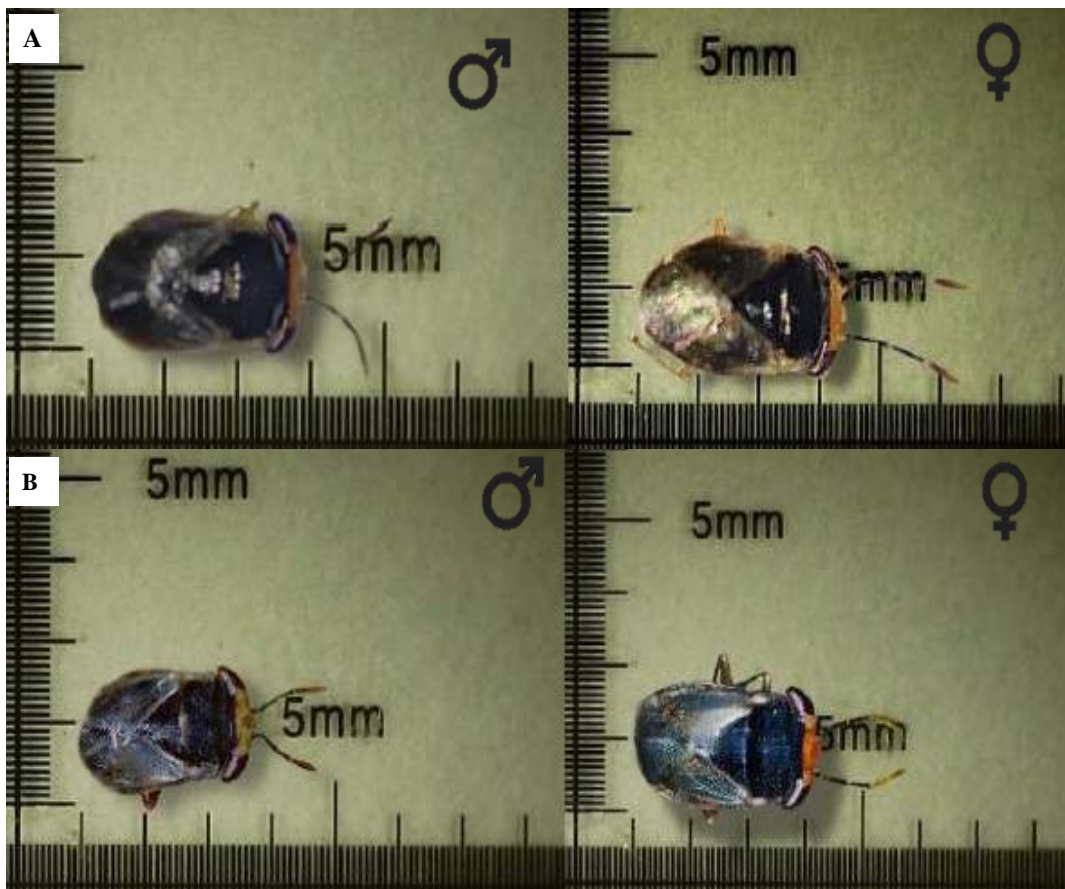
**Table 2**

The average size of adult big-eyed bugs in Cu Chi and Ham Tan Districts

Sampling location	Size (mm)			
	Average length		Average width	
	Male (n = 20)	Female (n = 20)	Male (n = 20)	Female (n = 20)
Cu Chi District, Ho Chi Minh City	3.75 ± 0.15	4.46 ± 0.20**	1.95 ± 0.09**	2.15 ± 0.13
Ham Tan District, Binh Thuan Province	3.67 ± 0.23 <sup>ns</sup>	4.11 ± 0.19	1.79 ± 0.13	2.03 ± 0.26 <sup>ns</sup>

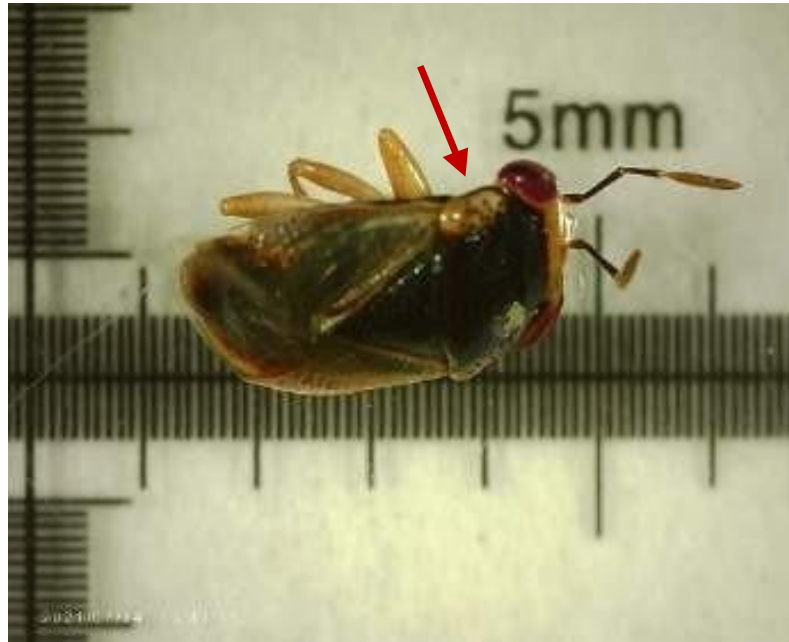
Values represent mean ± SD. Asterisks indicate significant differences against the control using Dunnett's two- sided t-test \*\* P < 0.01

### 3.2. Morphological identification

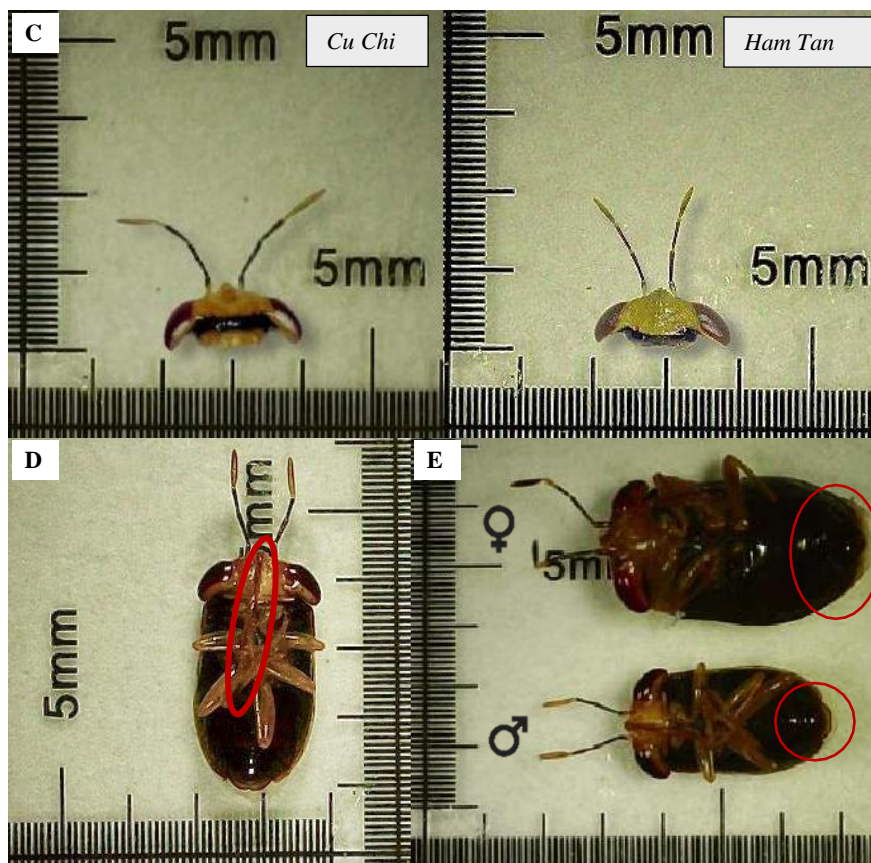


**Figure 3.** Big-eyed bug *Geocoris* sp. obtained in Cu Chi (A) and Ham Tan (B)

Big-eyed bugs were collected at Cu Chi and Ham Tan. The male was yellow between the eyes and the female varied from dark yellow to orange. The scutellum was black, with the dorsal plate of the anterior thoracic (pronotum) segment predominantly black (**Figure 3A and 3B**), with the sides and bottom of the dorsal segment ocher (Kóbor, 2018) (**Figure 4**).

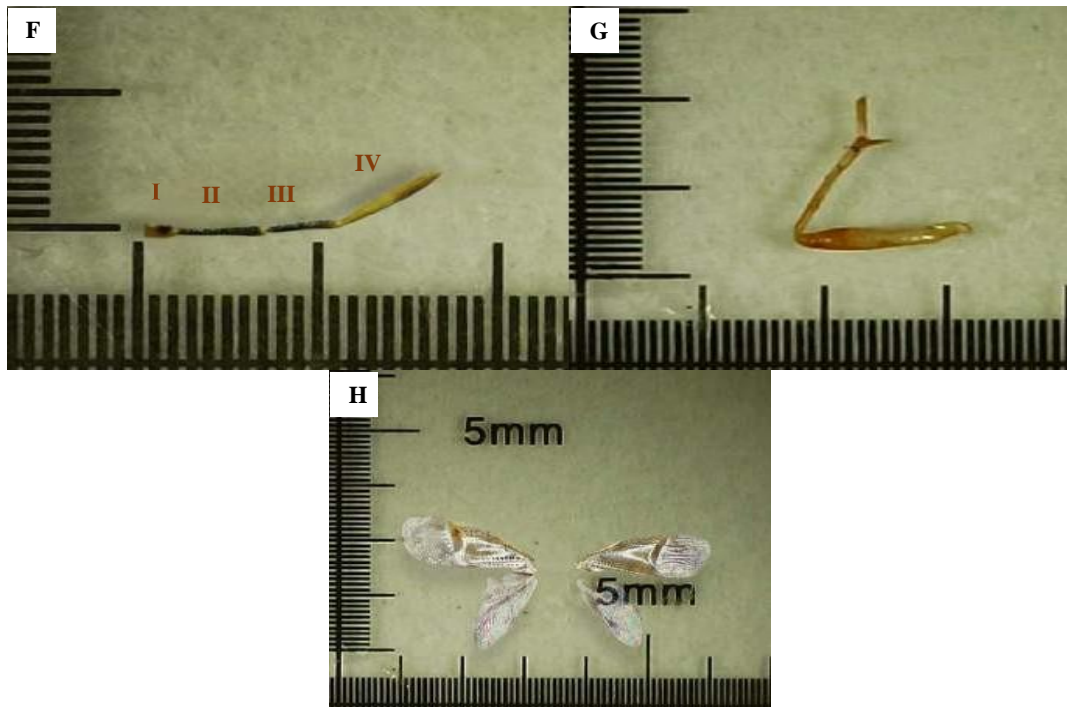


**Figure 4.** Side view of the big-eyed bug *Geocoris* sp.



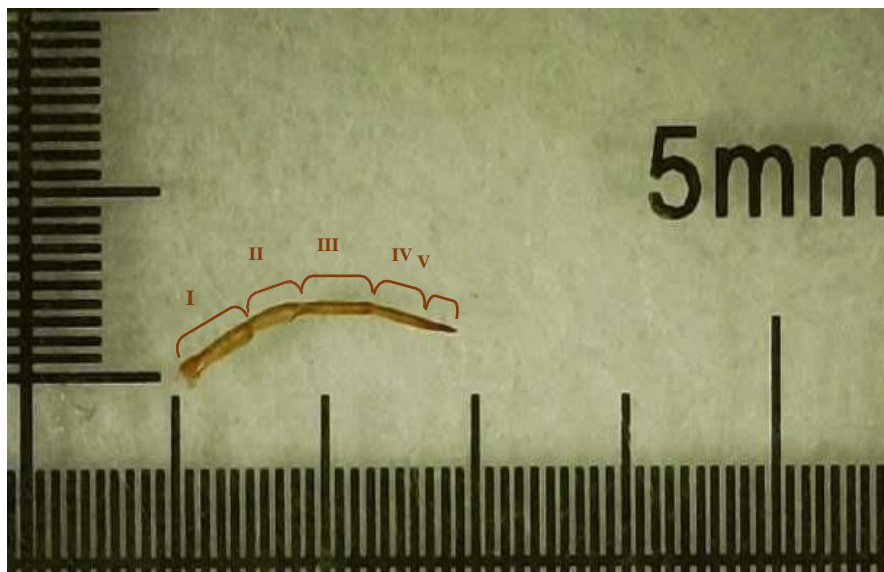
**Figure 5.** The head (C) and underside of big-eyed bug *Geocoris* sp. adults (D and E)

The big-eyed bug is characterized by large, curved compound eyes (**Figure 5C**). Between the eyes are the antennae, with the stinger folded below the abdomen (**Figure 5D**). The abdomen of the pregnant female is more rounded than the male (**Figure 5E**).



**Figure 6.** The antennae (F), back leg (G) and wings (H) of the big-eyed bug *Geocoris* sp.

The antenna of the big-eyed bug has IV segments, I and IV are ocher in color, and II and III are darker (Kóbor, 2018) (**Figure 6F**). The fourth segment is the largest, longest and has many small hairy branches. The back leg has three main segments that are yellow in color (**Figure 6G**) and the wings are double-layered, with the lower layer transparent and the upper layer dark yellow (**Figure 6H**).



**Figure 7.** Stinger of big-eyed bug *Geocoris* sp.

The stinger of the big-eyed bug is used to suck fluid from food and prey and consists of five earthy-yellow parts gradually smaller in size from the tail to the head. The 5th part is the shortest and smallest and is used to pierce and suck juice from the prey (**Figure 7**).

The adult female is larger than the male. The average sizes of the adult male and female at the two locations are shown in Table 3.

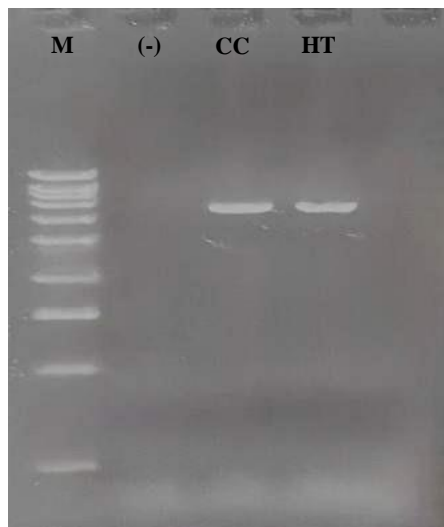
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Ham Tan District, Binh Thuan Province	3.67 ± 0.23 <sup>ns</sup>	4.11 ± 0.19	1.79 ± 0.13	2.03 ± 0.26 <sup>ns</sup>

Values represent mean ± SD. Asterisks indicate significant differences against the control using Dunnett's two-sided t-test \*\* P < 0.01

### 3.3. Identification by the molecular biology method

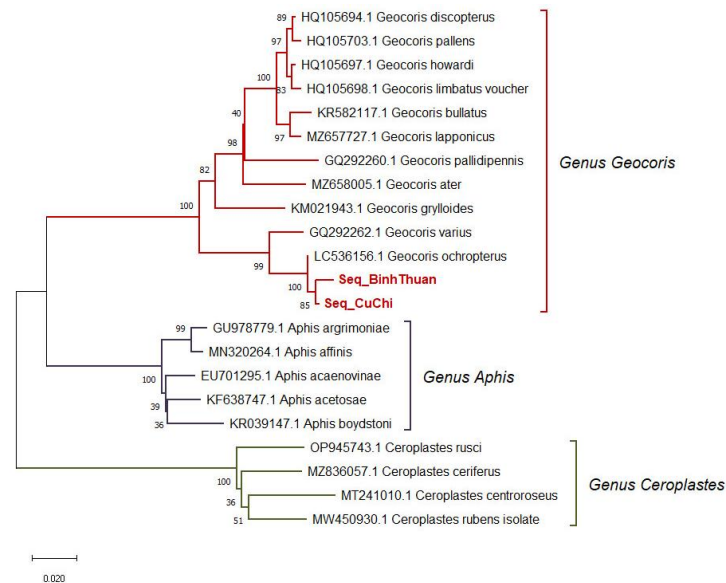


**Figure 8.** Results of PCR product electrophoresis of the *COI* segment of two samples of *Geocoris* sp.

Note: Marker 100bp (M), negative (-), big-eyed bug in Cu Chi (CC), big-eyed bug in Ham Tan (HT)

After DNA extraction, the *COI* gene fragment was amplified by PCR and checked by electrophoresis on 1.5% agarose gel for 35 minutes with a potential difference of 100V. Results showed a good amplification response; the products were single, clear bands with size of around 658bp (**Figure 8**).

The PCR products were then sent for two-way sequencing at 1<sup>st</sup> Base Company, with the sequencing results analyzed using SnapGene and BioEdit software. When compared with the sequence in the GenBank database using the BLAST tool, the big-eyed bug obtained from Cu Chi had 99.84% similarity, while the big-eyed bug obtained from Ham Tan had 94% similarity compared with *Geocoris ochropterus* (LC536156.1). Results of *genetic tree analysis*, with out-groups used for classification as the genera *Aphis* and *Bacillus* are shown in supplement data **Table S2**. Both genera belong to the order Hemiptera, the same order as the big-eyed bug. Both big-eyed bug samples collected in Cu Chi and Ham Tan Districts belonged to the genus *Geocoris* and species *Geocoris ochropterus* (**Figure 9**).



**Figure 9.** Genetic clustering tree built by Neighbor - Joining Tree method with Maximum Composite Likelihood model. Numbers shown in the tree are bootstrap values of 1,000 repetitions

#### 4. Conclusions

The density of big-eyed bugs in Cu Chi District, Ho Chi Minh City was higher than that in Ham Tan District, Binh Thuan Province. Topography, weather, and habitat affect the population of the big-eyed bug *Geocoris ochropterus*.

Big-eyed bugs collected from Cu Chi District, Ho Chi Minh City and Ham Tan District, Binh Thuan Province were evaluated for morphological characteristics and supported by molecular biology identification. Big-eyed bugs collected at both sites belonged to the species *Geocoris ochropterus*. This result showed that the *COI* gene fragment was an effective marker in assisting insect identification.

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#### Competing interests

The authors state that there are no conflicts of interest related to this publication.

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