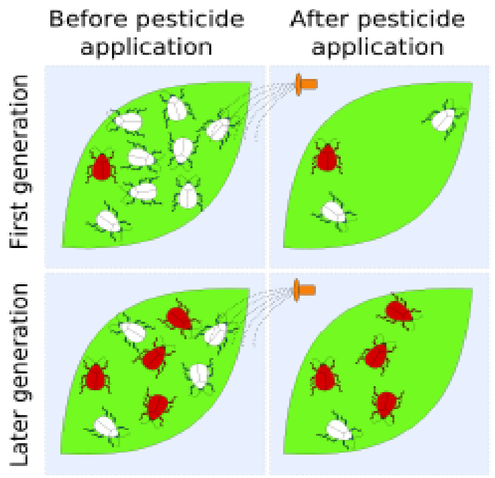
Rencontres du Vietnam
VIETNAM SCHOOL OF BIOLOGY (VSOB-4)
Metagenome Analysis in One Health Practice – From 16S to Shotgun
September 3 - 7, 2025 at ICISE, Quy Nhon, Binh Dinh, Vietnam

Identification of diet resources of big-eyed bug *Geocoris ochropterus* (Fieber) (Hemiptera: Geocoridae) by multiplex PCR and shotgun metagenomic approaches

Assoc.Prof.Dr. Nguyen Bao Quoc

Faculty of Biological Sciences, Nong Lam University

Current Situation of Chemical Pesticide Use



Resistance	Environmental Polluton	Human health

SOLUTION

- Integrated Pest Management Measures
- Using Biologically Derived Products

INTRODUCTION TO THE BIG-EYED BUG *Geocoris*

- Family: Geocoridae
- Order: Hemiptera
- Genus: *Geocoris*

Adult of the big eyed bug

☞ A beneficial species because it preys on numerous harmful insects

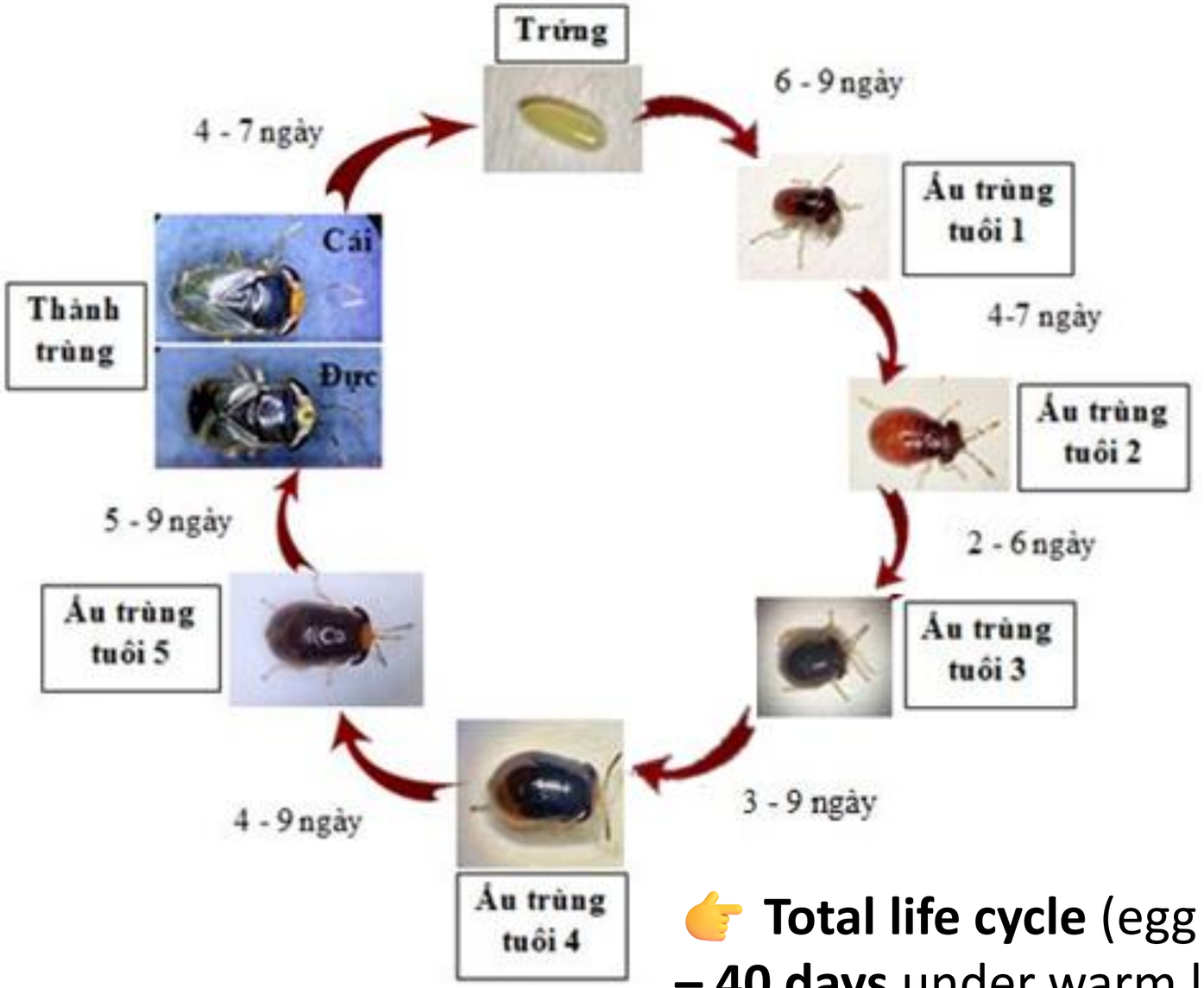
grasshopper nymph

Aphid

Diamondback moth

Thrips

LIFE CIRCLE OF THE BIG-EYED BUG *Geocoris ochropterus*



👉 **Total life cycle** (egg → adult) usually takes about **25 – 40 days** under warm laboratory or field conditions.

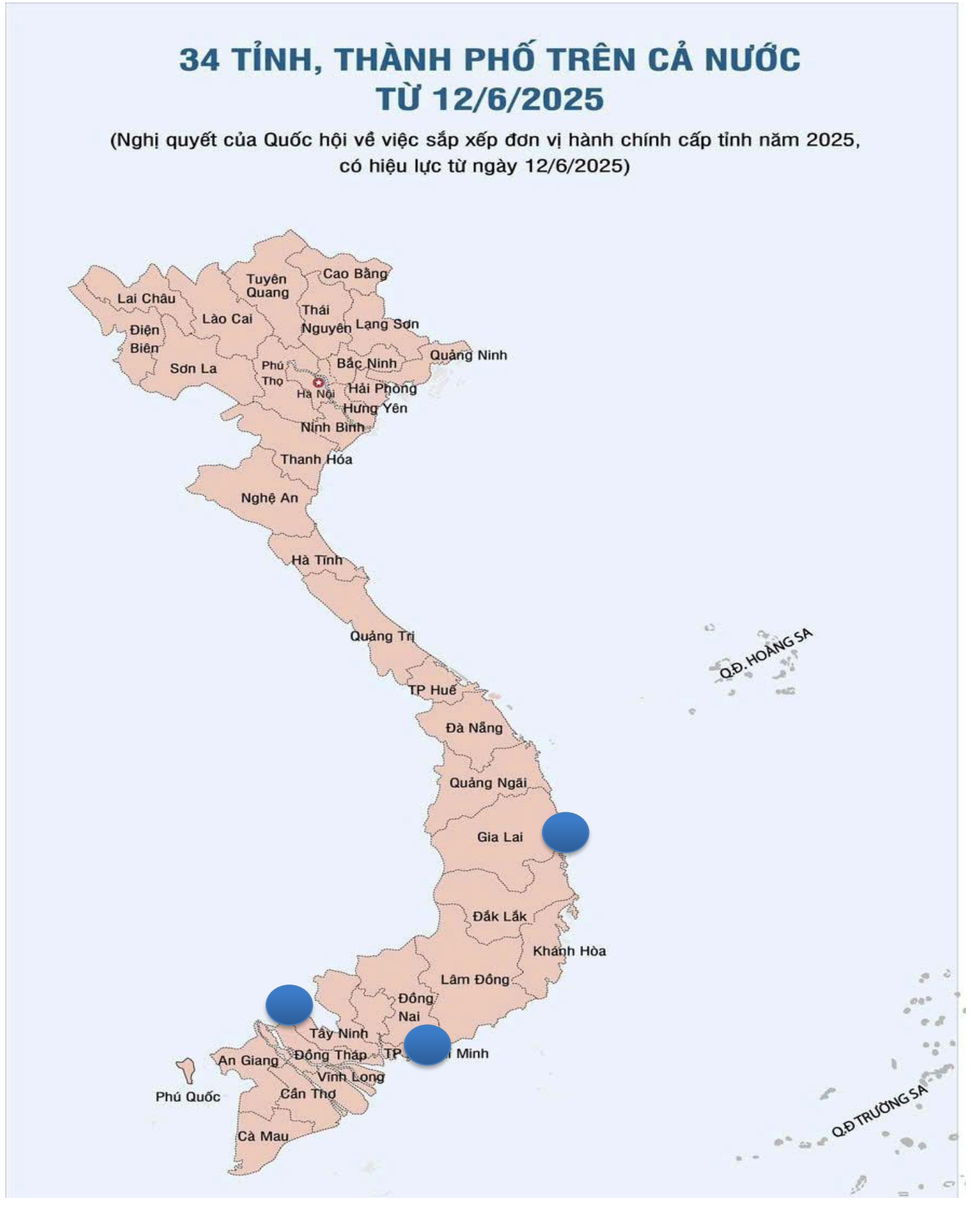
INTRODUCTION TO THE BIG-EYED BUG *Geocoris*

- There are about 147 species belonging to the genus *Geocoris* (Hemiptera: Geocoridae)

- Important and common species such as : *G. bullatus*, *G. ochropterus*, *G. pallens*, *G. proteus*, *G. punctipes*, *G. uliginosus*, *G. varius.*

Peter Asiimwe, Lydia Brown, Tim Vandervoet, Peter Ellsworth (University of Arizona) & Steven Naranjo (USDA-ARS, ALARC)

INTRODUCTION TO THE BIG-EYED BUG *Geocoris*



Geocoris ochropterus (Hemiptera, Lygaeidae) (Chau et al., 2021)

- *Geocoris ochropterus* was found in Gia Lai and reported in 2011 (Cao and Dang, 2011).
- *Geocoris* sp. was observed on cotton plants in Ninh Thuan Province (Nguyen Van Chinh, 2012, unpublished data)
- *Geocoris* sp. was found in fields and grasslands in several provinces of the Mekong Delta, and *Geocoris ochropterus* was recorded in Cu Chi on chili, okra fields, grasslands, etc

Geocoris: A NATURAL ENEMY OF MANY INSECT PESTS

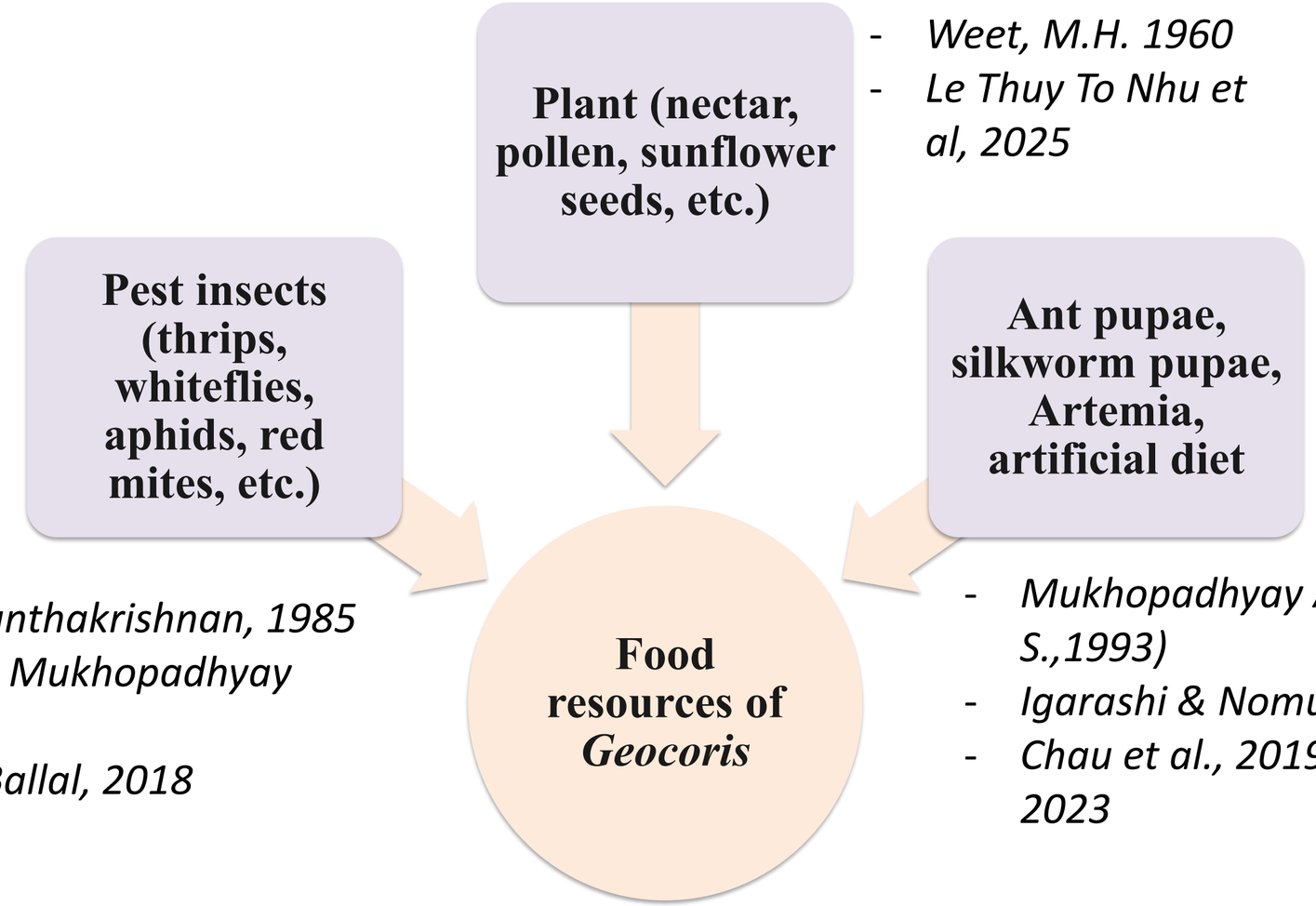
Thrips

whitefly

Lepidopteran eggs

Mites

Geocoris: CONSUMES A WIDE VARIETY OF FOOD SOURCES

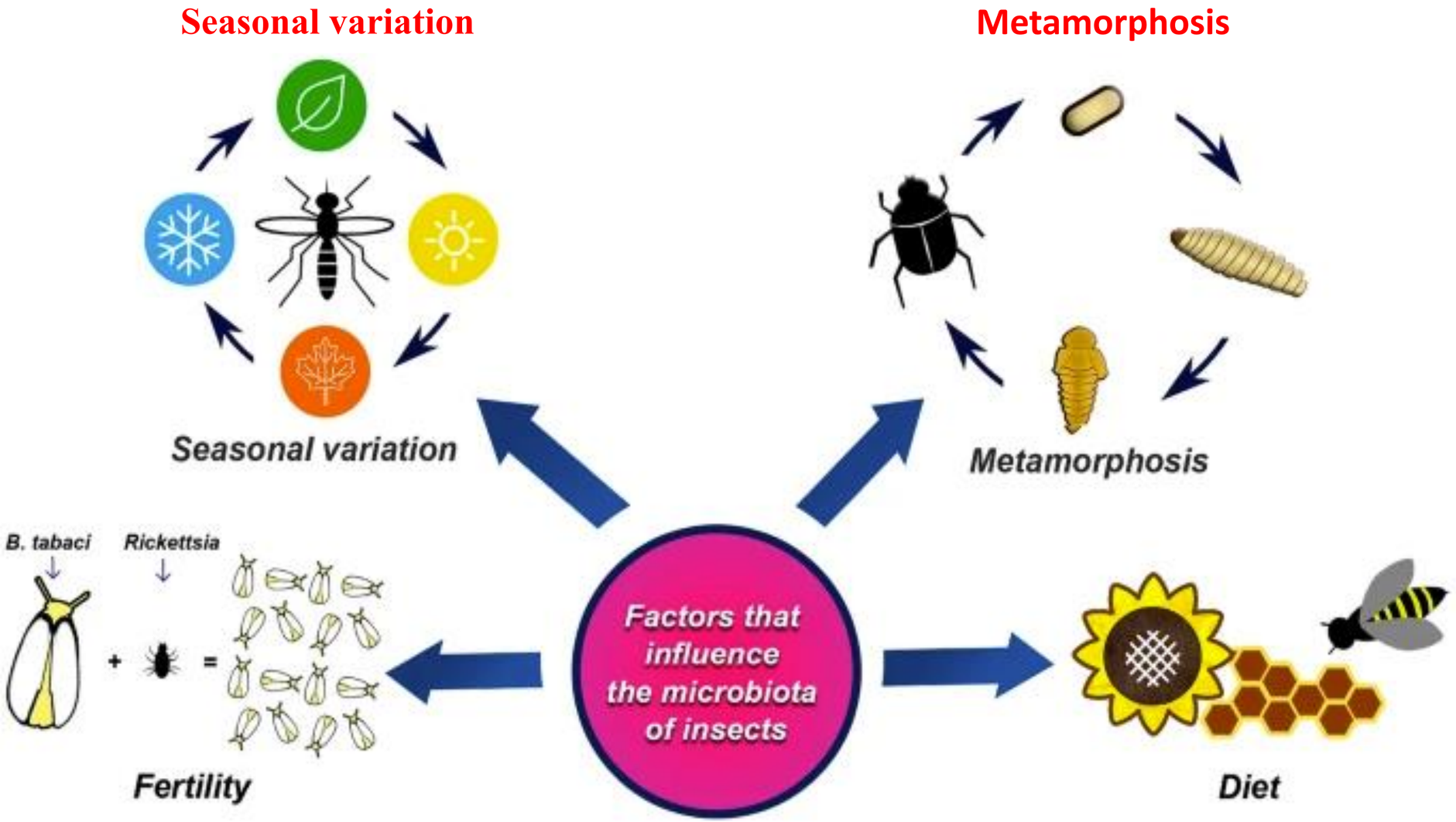


- *Weet, M.H. 1960*
- *Le Thuy To Nhu et al, 2025*

- *Kumar & Ananthakrishnan, 1985*
- *Sannigrahi S. Mukhopadhyay A.,1992)*
- *Varshney & Ballal, 2018*

- *Mukhopadhyay A. & Sannigrahi S.,1993)*
- *Igarashi & Nomura (2013)*
- *Chau et al., 2019, 2021, 2022, 2023*

UNDERSTANDING INSECT MICROBIOTA BY METAGENOMICS



Antonio Huerta-García & Jorge Álvarez-Cervantes, 2024

AIMS OF THE STUDY

- To evaluate the effects of temperature and different types of food on the growth and development of the big-eyed bug *Geocoris ochropterus* under laboratory conditions.

- To assess the prey spectrum from plants and other insects of the big-eyed bug using the Shotgun Metagenomics method

EFFECTS OF TEMPERATURE AND DIET ON *Geocoris ochropterus*

Chỉ tiêu		Nguồn thức ăn	Nhiệt độ (°C)			Trung bình hàng
			10	20	30	
Thời gian phát triển (ngày)	Lần lột xác thứ 4	*Zinnia elegans*	-	10.42 ± 0.55^{b}	9.50 ± 0.31^{b}	9.96 ± 0.64^{B}
		Mật ong 10%	-	9.00 ± 0.35^{a}	8.33 ± 0.47^{a}	8.70 ± 0.52^{A}
		Nhộng kiến và *Zinnia elegans*	-	9.09 ± 0.19^{a}	8.28 ± 0.30^{a}	8.69 ± 0.49^{A}
		Rệp sáp và *Zinnia elegans*	-	8.50 ± 0.52^{a}	8.03 ± 0.84^{a}	8.27 ± 0.70^{A}
		Trung bình cột	-	9.25 ± 0.83^{B}	8.55 ± 0.77^{A}	-
				F= 2.517; P= 0.036		
	Lần lột xác thứ 5	*Zinnia elegans*	-	-	-	-
		Mật ong 10%	-	10.42 ± 0.65^{b}	9.67 ± 0.47^{b}	10.04 ± 0.67^{B}
		Nhộng kiến và *Zinnia elegans*	-	9.40 ± 0.12^{a}	8.67 ± 0.30^{a}	9.04 ± 0.44^{A}
		Rệp sáp và *Zinnia elegans*	-	9.37 ± 0.13^{a}	8.67 ± 0.47^{a}	9.02 ± 0.49^{A}
		Trung bình cột	-	9.73 ± 0.62^{B}	9.00 ± 0.62^{A}	-
				F= 4.690; P= 0.004		
Tổng thời gian sống sót (ngày)		*Zinnia elegans*	6.00 ± 0.76^{b}	48.08 ± 3.99^{b}	43.60 ± 4.64^{b}	32.56 ± 19.81^{C}
		Mật ong 10%	10.32 ± 1.63^{b}	25.33 ± 3.83^{c}	25.20 ± 1.24^{c}	20.28 ± 7.65^{D}
		Nhộng kiến và *Zinnia elegans*	17.83 ± 3.77^{a}	81.25 ± 2.55^{a}	66.76 ± 5.58^{a}	55.28 ± 28.35^{A}
		Rệp sáp và *Zinnia elegans*	15.07 ± 1.71^{b}	54.59 ± 3.62^{b}	48.67 ± 3.45^{b}	39.44 ± 18.23^{B}
		Trung bình cột	12.31 ± 5.08^{C}	52.31 ± 20.70^{A}	46.06 ± 15.64^{B}	-
				F= 3.752; P= 0.001		

Development time through molts and total survival duration

- The development time of the nymphal stage of *G. ochropterus* is influenced by different diet combinations.
- When the temperature decreases, each molting stage of the big-eyed bug *G. ochropterus* may take longer
- When the temperature decreases, each molting stage of the big-eyed bug *G. ochropterus* may take longer
- Ant pupae and *Zinnia elegans* (C) had the longest survival days

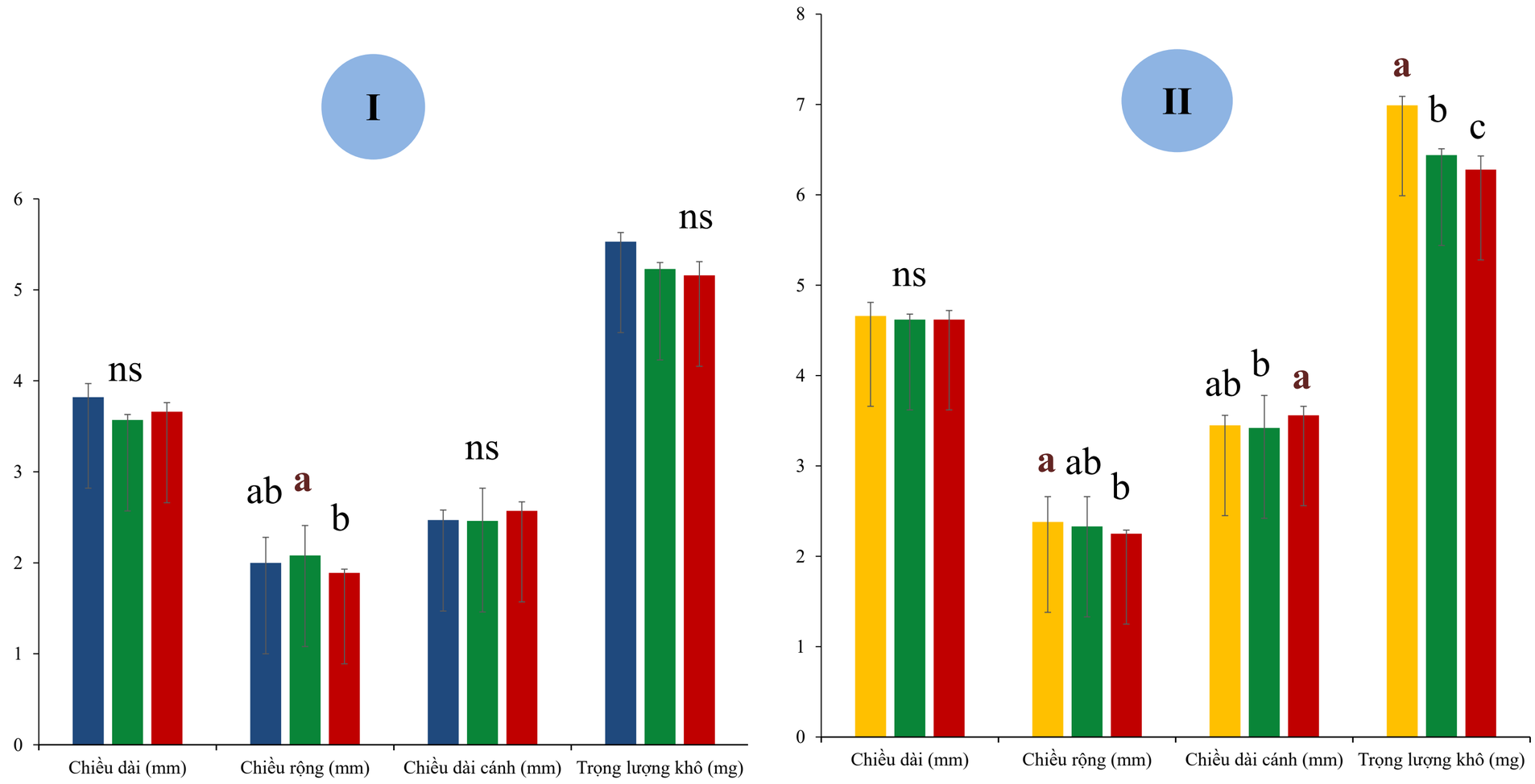
SURVIVAL RATE ACROSS MOLTING STAGES

Chỉ tiêu		Nguồn thức ăn	Nhiệt độ (°C)			Trung bình hàng
			10	20	30	
Tỷ lệ sống sót (%)	Lần lột xác thứ 3	*Zinnia elegans*	-	54.32 ± 10.08^{b}	50.99 ± 8.56^{b}	35.53 ± 26.08^{B}
		Mật ong 10%	6.34 ± 11.31^{ns}	65.96 ± 13.86^{b}	58.37 ± 6.93^{b}	43.55 ± 29.28^{B}
		Nhộng kiến và *Zinnia elegans*	18.98 ± 16.97	88.72 ± 0.00^{a}	88.72 ± 0.00^{a}	65.47 ± 35.22^{A}
		Rệp sáp và *Zinnia elegans*	11.39 ± 13.85	60.90 ± 5.66^{b}	58.37 ± 6.93^{b}	43.55 ± 25.16^{B}
		Trung bình cột	9.50 ± 13.15^{B}	67.47 ± 15.65^{A}	64.11 ± 16.05^{A}	
		F= 4.727; P= 0.000				
	Lần lột xác thứ 4	*Zinnia elegans*	-	37.06 ± 6.82^{c}	46.15 ± 6.32^{c}	28.16 ± 20.66^{C}
		Mật ong 10%	-	58.37 ± 6.93^{b}	58.37 ± 6.93^{b}	39.34 ± 28.34^{B}
		Nhộng kiến và *Zinnia elegans*	-	88.72 ± 0.00^{a}	88.72 ± 0.00^{a}	59.57 ± 42.67^{A}
		Rệp sáp và *Zinnia elegans*	-	58.37 ± 6.93^{b}	55.83 ± 6.93^{bc}	38.49 ± 27.76^{B}
		Trung bình cột	-	60.63 ± 19.66^{A}	62.27 ± 17.21^{A}	
		F= 21.737; P= 0.000				
	Lần lột xác thứ 5	*Zinnia elegans*	-	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}	0.00 ± 0.00^{C}
		Mật ong 10%	-	50.77 ± 0.00^{b}	48.46 ± 5.16^{b}	33.50 ± 23.77^{B}
		Nhộng kiến và *Zinnia elegans*	-	83.66 ± 11.31^{a}	73.55 ± 13.85^{a}	52.83 ± 39.16^{A}
		Rệp sáp và *Zinnia elegans*	-	51.22 ± 12.10^{b}	50.77 ± 0.00^{b}	34.42 ± 25.11^{B}
		Trung bình cột	-	46.73 ± 31.14^{A}	43.51 ± 27.80^{A}	
		F= 13.704; P= 0.000				

- 100% of *G. ochropterus* nymphs died at the 4th instar at 10°C.
- At 20°C and 30°C, the survival rates across molts were similar and higher than at 10°C.
- At 20°C, the highest survival at the final molt was observed with ant pupae and *Zinnia elegans* diets

Survival rates showed significant differences among diets and temperatures when nymphs reached the 1st, 2nd, 3rd, 4th, and 5th instars at 10°C, 20°C, and 30°C.

BODY SIZE AND WEIGHT OF ADULTS



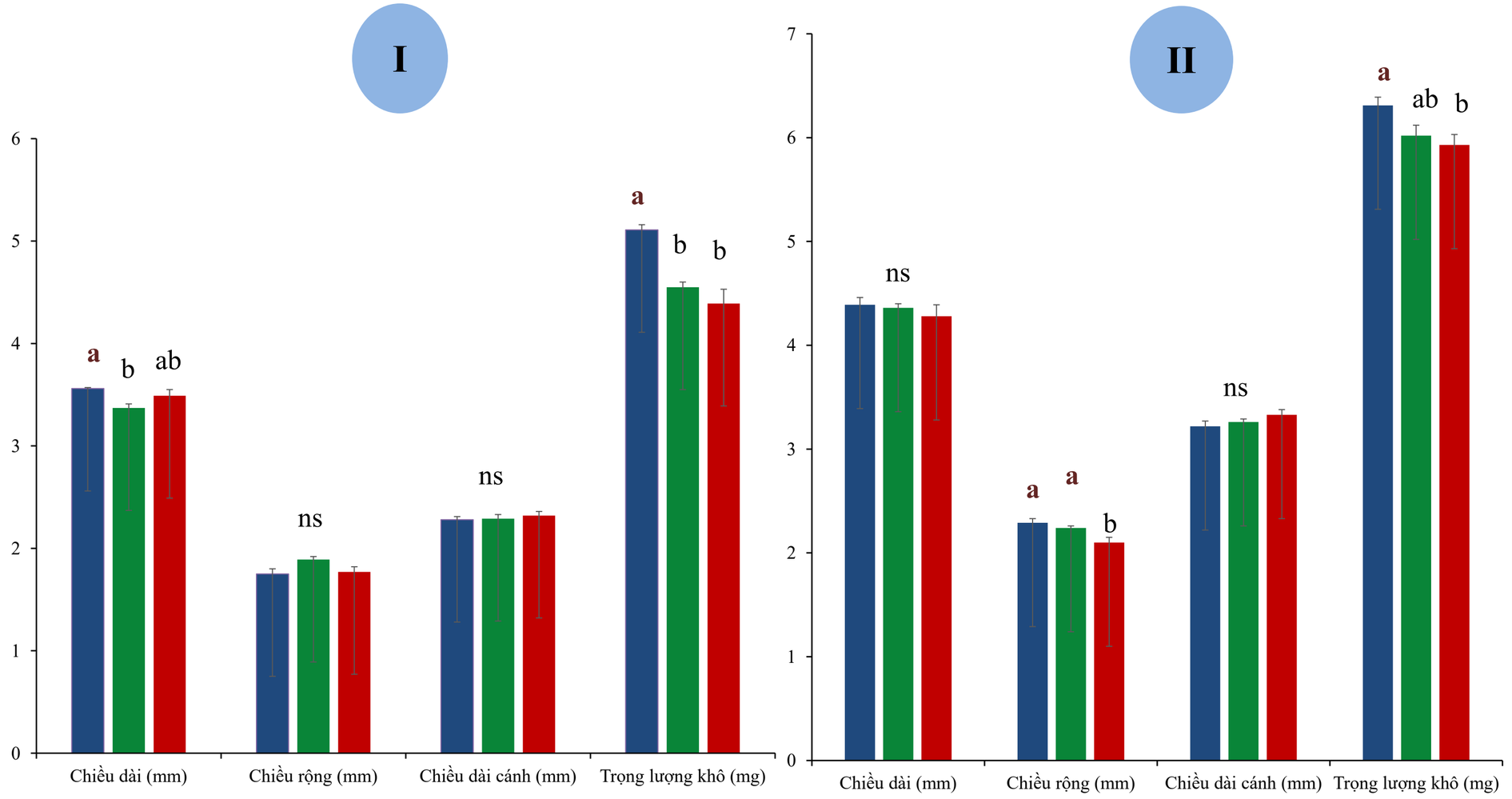
Body size of male (I) and female (II) big-eyed bugs under different food sources at 20ºC.

Yellow column: Ant pupae + *Zinnia elegans*;

Green column: Mealybugs + *Zinnia elegans*;

Red column: 10% honey solution.

BODY SIZE AND WEIGHT OF ADULTS



Body size of male (I) and female (II) big-eyed bugs under different food sources at 30ºC.

Yellow column: Ant pupae + *Zinnia elegans*;

Green column: Mealybugs + *Zinnia elegans*;

Red column: 10% honey solution

IMPORTANT MESSAGES

1. The importance of supplementary nutritional sources (such as flowers):

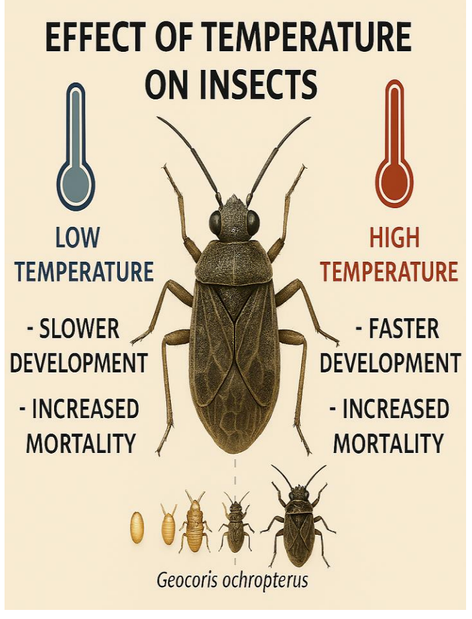
- Flowers provide nutrients that support the development of natural enemies.
- Enhance the effectiveness of biological control by sustaining the life cycle of natural enemies.

2. **Significance of studying the combination of flowers and food sources:**

- Understanding the effects of flower + food combinations on the development of the big-eyed bug (*G. ochropterus*) under different temperatures.
- Contributing to the optimization of biological control strategies

3. **Effects of temperature on insects:**

- Temperature strongly influences metabolism, growth, reproduction, and survival.
- For *G. ochropterus*, temperature regulates the entire life cycle from egg to adult.
- Optimal temperature: rapid development and high survival.
- Extreme temperature: slow development and increased mortality.

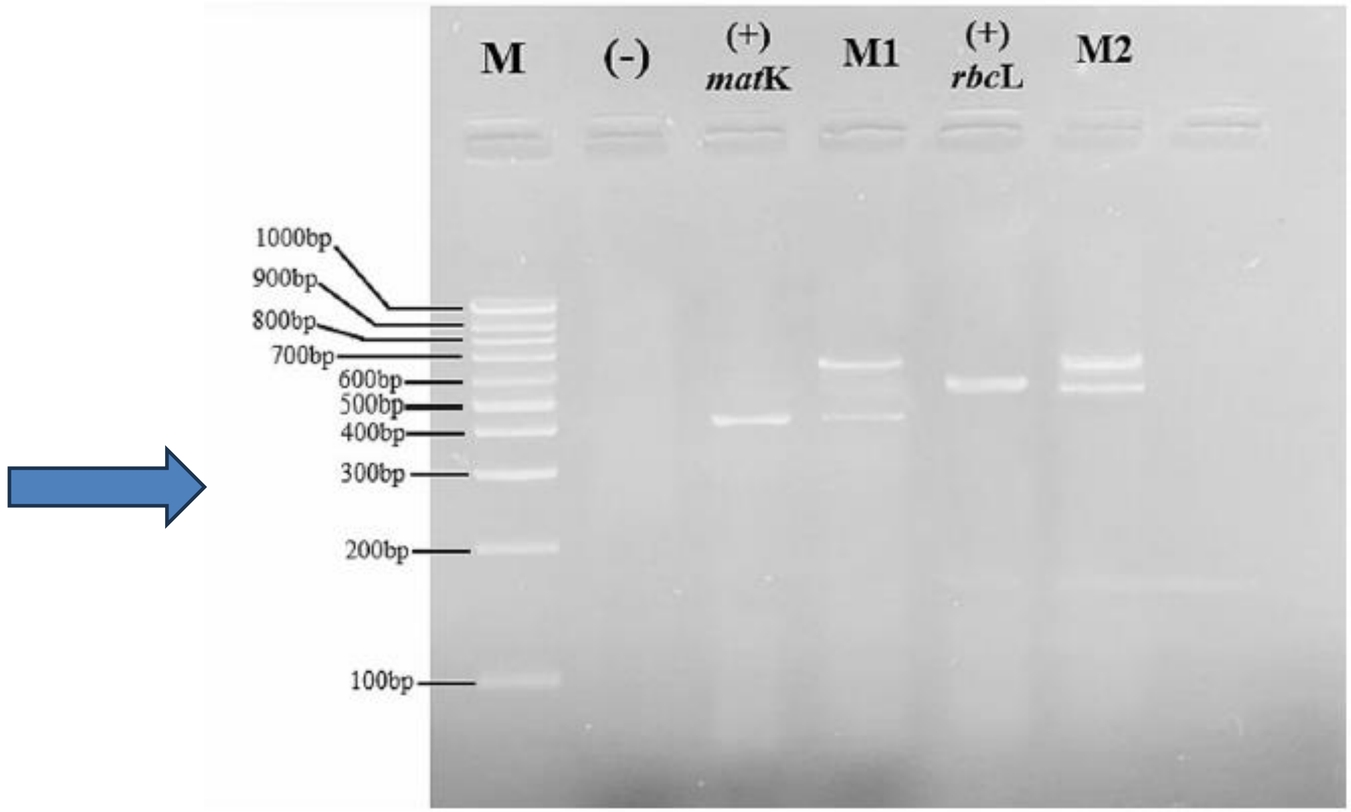


4. **Advantages of ant pupae and rough marigold flowers**:

- Ant pupae contain 31 trace elements, various vitamins, a high protein content (42–67%), and essential amino acids.
- Rough marigold flowers provide a rich source of nectar and serve as a suitable shelter for big-eyed bugs.
- Combining these two sources is an optimal solution for mass rearing big-eyed bugs to enhance biological control effectiveness

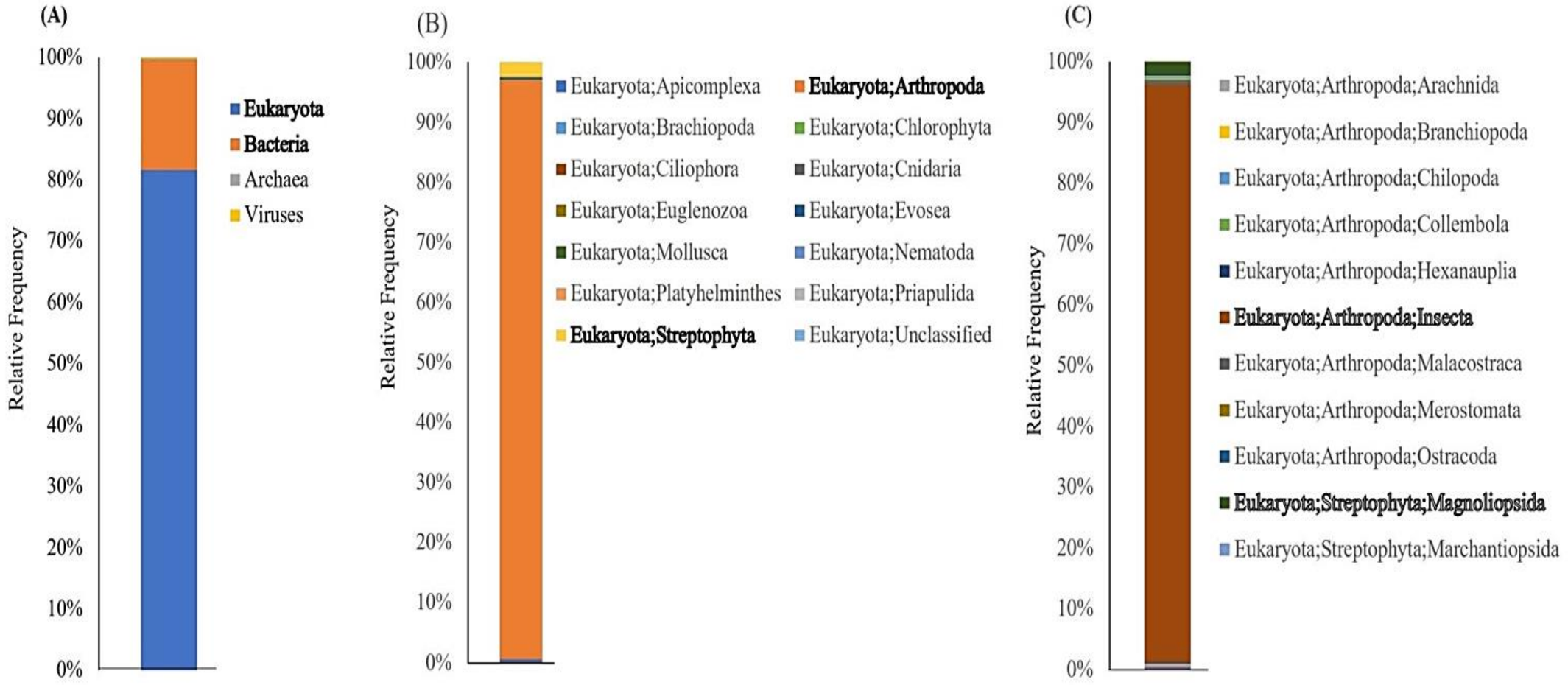
Assessment of the dietary spectrum of the big-eyed bug *Geocoris ochropterus* in nature using Multiplex PCR reaction

Intestine of the big-eyed bug



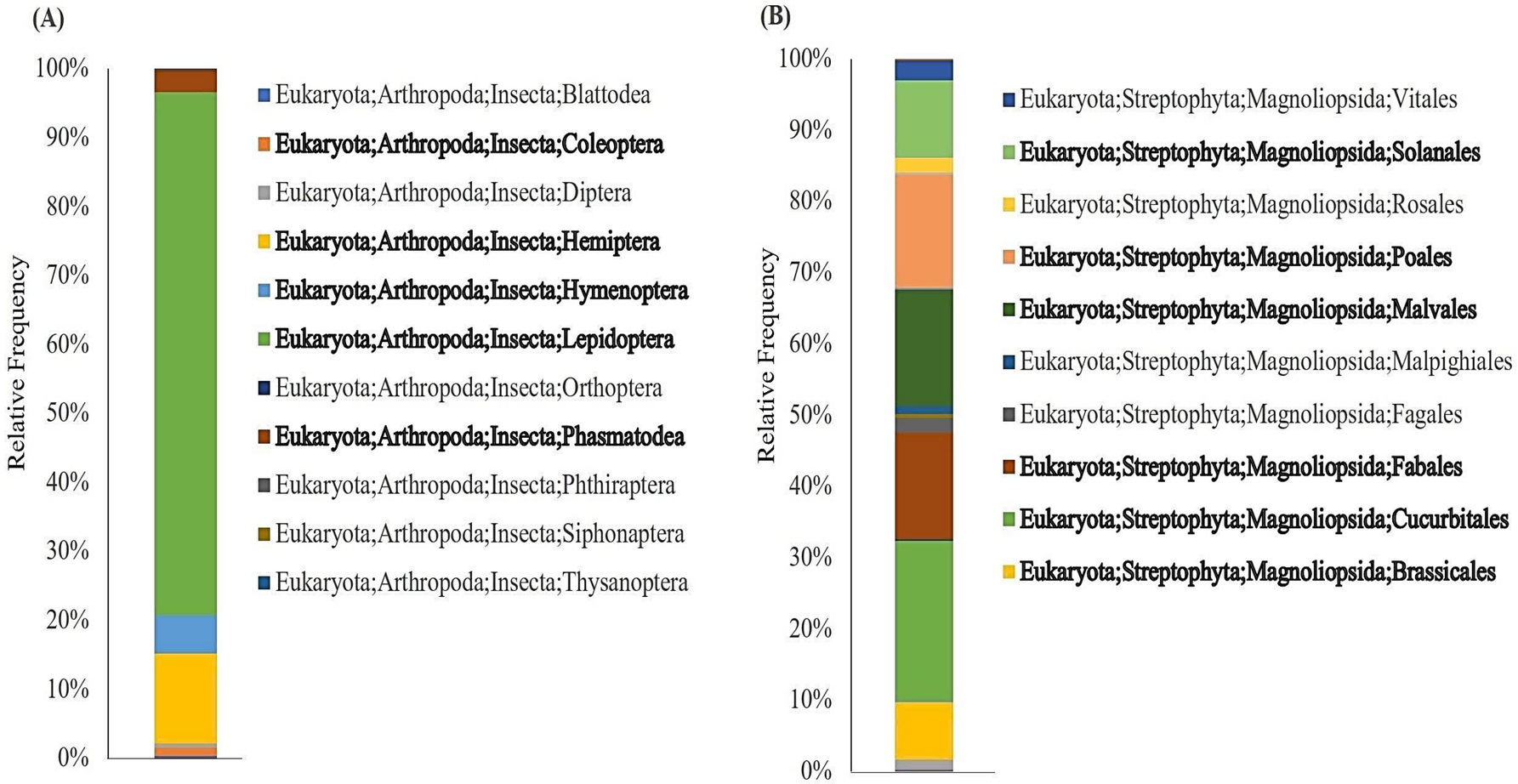
Multiplex PCR results indicate the presence of dietary compo nents in the gut of *G. ochropterus*. Lane (1) 100 bp DNA ladder; Lane (2) negative control; Lane (3) positive control with DNA of *Zinnia elegan* and matK primers; Lane (4) PCR products of M1 sample were amplified with LCO1490F/HCO2189R and matK-816 F/1254R prim ers; Lane (5) positive control with DNA of *Zinnia elegan* and rbcL primers; Lane (6) PCR products of M2 sample were amplified with LCO1490F/HCO2189R and rbcLaF/R primers

Assessment of the dietary spectrum of the big-eyed bug *Geocoris ochropterus* in nature using Shotgun Metagenomics



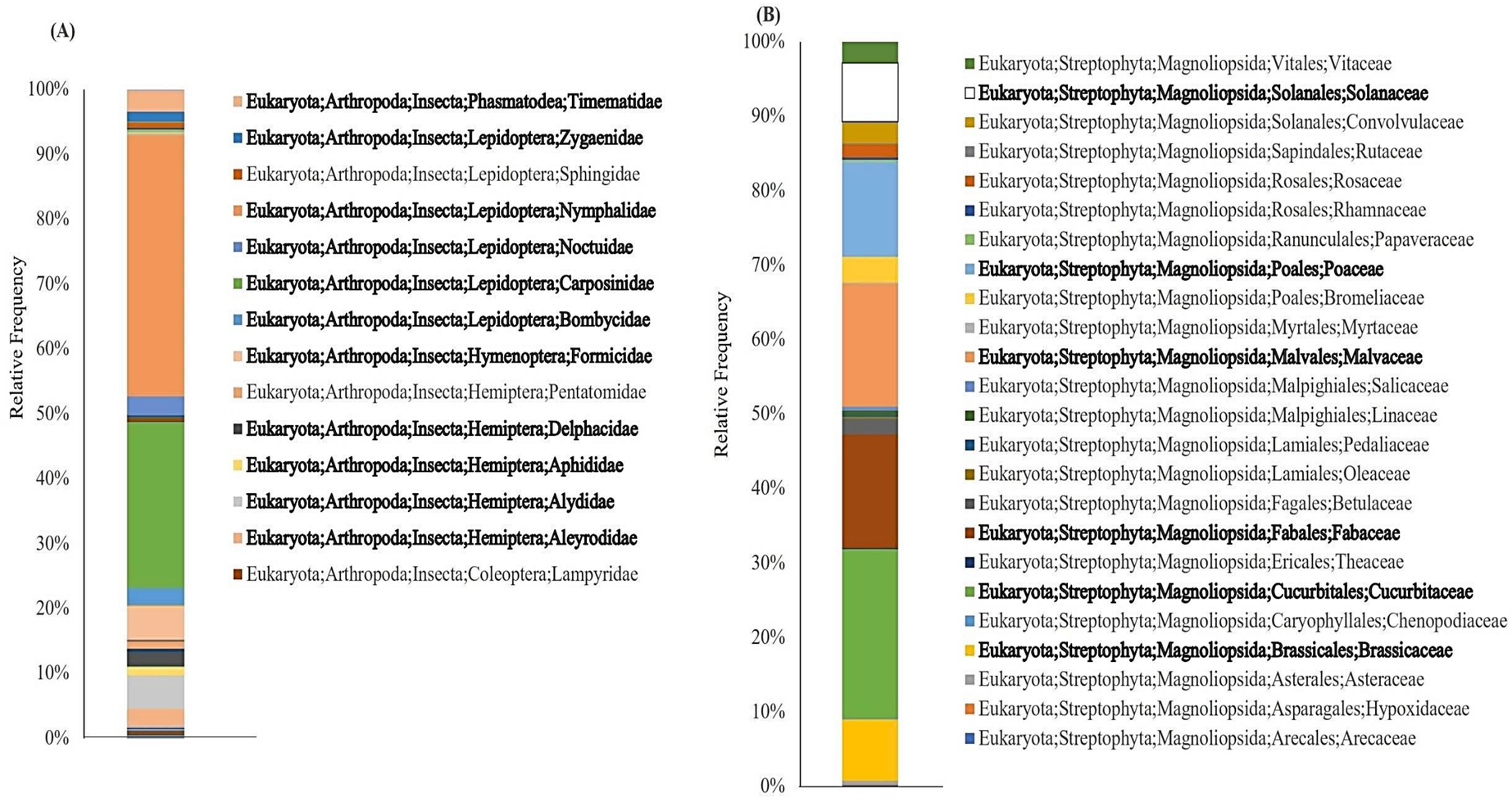
The microbial abundance is represented at the taxonomic levels of domain, phylum, and class. Bold texts indicate dominant groups in the taxonomic system, including domains, phyla, and classes

Assessment of the dietary spectrum of the big-eyed bug *Geocoris ochropterus* in nature using Shotgun Metagenomics



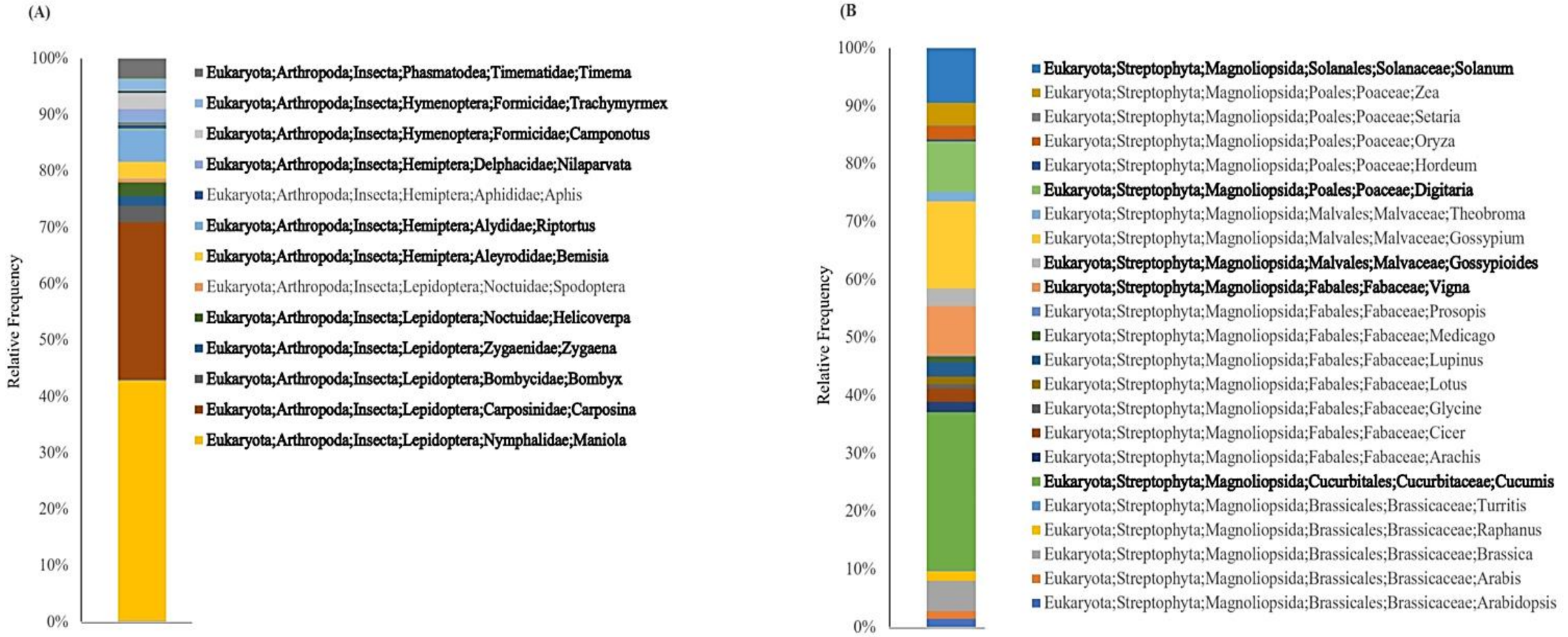
Microbial abundance is represented at the taxonomic level of order. Bold text indicates dominant groups at the order level, with read counts exceeding 1,000 for the class Insecta and 100 for the class Magnoliopsida

Assessment of the dietary spectrum of the big-eyed bug *Geocoris ochropterus* in nature using Shotgun Metagenomics



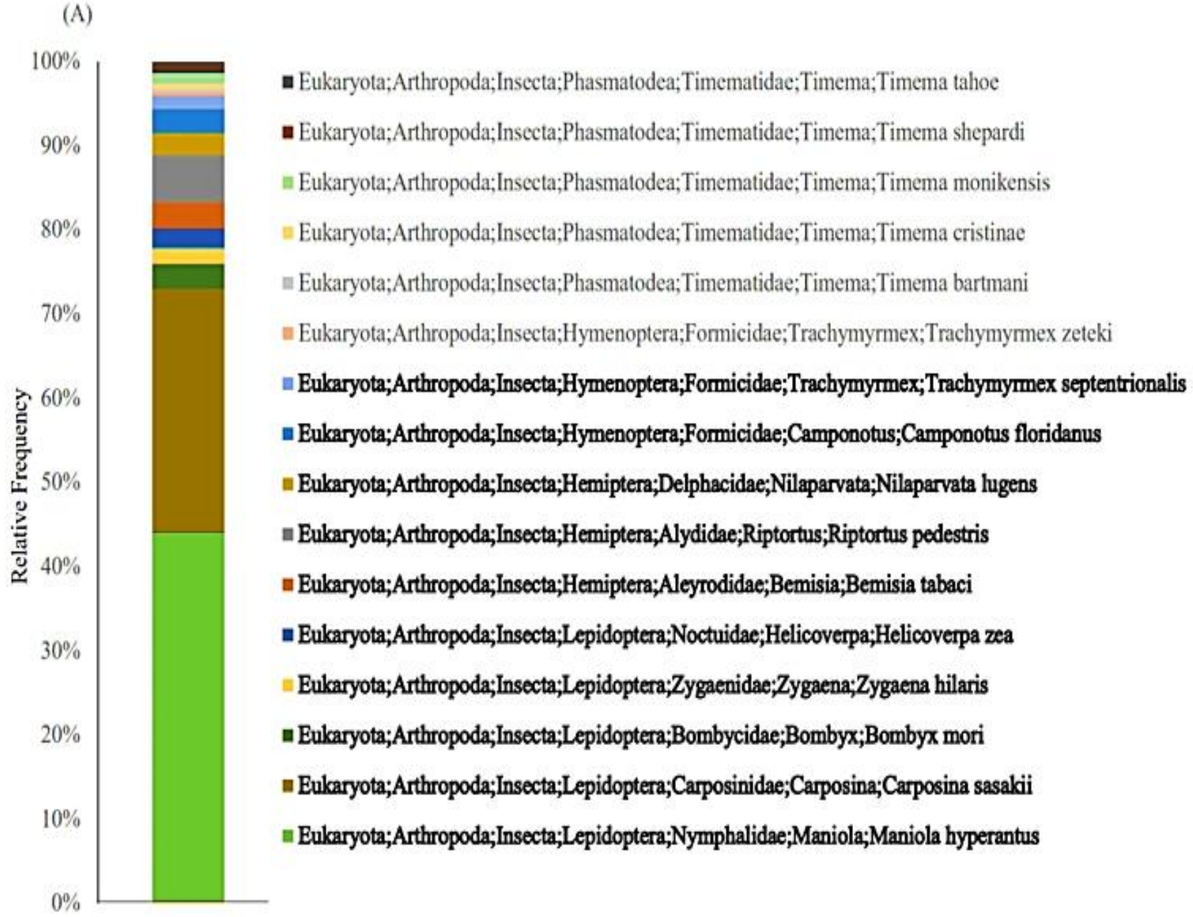
Microbial abundance is represented at the taxonomic level of family. Bold text indicates dominant families, with read counts exceeding 1,000 for the dominant insect orders and 100 for the dominant plant orders

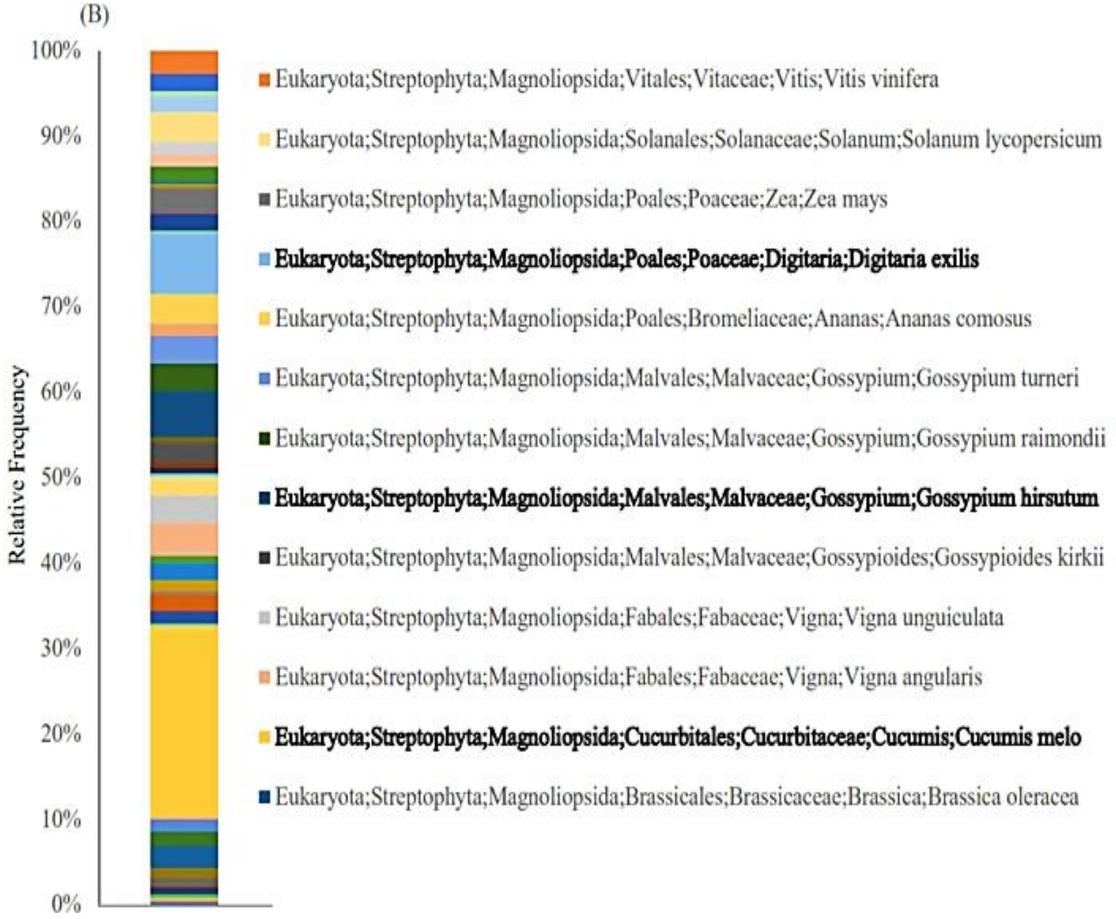
Assessment of the dietary spectrum of the big-eyed bug *Geocoris ochropterus* in nature using Shotgun Metagenomics



Microbial abundance is represented at the taxonomic level of genus. Bold text indicates dominant genera, with read counts exceeding 1,000 for the dominant insect families and 100 for the dominant plant families

Assessment of the dietary spectrum of the big-eyed bug *Geocoris ochropterus* in nature using Shotgun Metagenomics





Microbial abundance is represented at the species level. Bold text indicates dominant species, with read counts exceeding 1,000 for the dominant insect genera and 100 for the dominant plant genera

CONCLUSIONS

1. Optimal rearing temperature and conditions:

- A temperature range of 20ºC to 30ºC is suitable for rearing big-eyed bugs.
- Combining rough marigold flowers with ant pupae provides effective and convenient nourishment.
- Rough marigold flowers with mealybugs are also a good food combination for rearing.

2. Impact of floral & food sources on development:

- The combination of floral (plant) and animal food sources influences the growth and development rate of *G. ochropterus*.
- Simultaneous access to both sources ensures nutritional balance, supporting optimal development

3. The diverse predatory nature of the big-eyed bug

- *G. ochropterus* is capable of consuming both animal- and plant-based food sources.
- Flexible adaptability under different environmental conditions, which is particularly beneficial in diverse farming systems.
- Identifying insect and plant food sources in the gut of *G. ochropterus* provides insights into IPM strategies.
- The detection of pest species such as *Bemisia tabaci* and *Helicoverpa zea* in the gut further confirms the biological control potential of the big-eyed bug

PUBLICATION

Journal of Applied Entomology

WILEY

JOURNAL OF APPLIED ENTOMOLOGY

ORIGINAL ARTICLE

Effect of the Combination Floral and Diet Resources on Development of Big-Eyed Bug *Geocoris ochropterus* (Fieber) (Hemiptera: Geocoridae) at Different Temperature

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Keywords: big-eyed bugs | diets | *Geocoris ochropterus* | natural enemy | temperature | *Zinnia elegans*

ABSTRACT

The big-eyed bug, *Geocoris ochropterus* Fieber, is a polyphagous predator. Although there have been many publications on the rearing of big-eyed bugs by using other insects, their ability to use flowers as a source of nutrition or habitat has not been previously reported. The diets included *Zinnia elegans* (A), 10% honey solution (B) ant pupae and *Zinnia elegans* (C), mealybug and *Zinnia elegans* (D) treatments at 10°C, 20°C, and 30°C. Significant differences in survival rates were observed among the diets when the nymphs reached the first and second instars at 10°C. Significant differences in growth and development indicators of big-eyed bugs were found among the treatments during the second, third, fourth and fifth moults at 30°C. Additionally, body size and dry weight of adult *G. ochropterus* grown on different diets were measured at 20°C and 30°C. At 20°C, male body size parameters did not significantly differ among the diets, except for head width. At 30°C, body length and dry weight of males showed significant differences among the diets, while head width and dry weight of females also varied significantly among diets. The study indicates that the combination of floral resources and diet can affect the development of *G. ochropterus* at different temperatures.

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ORIGINAL ARTICLE

Identification of diet resources of big-eyed bug *Geocoris ochropterus* (Fieber) (Hemiptera: Geocoridae) by multiplex PCR and shotgun metagenomic approaches

Nguyen Bao Quoc[1] · Le Thuy To Nhu[2] · Nguyen Ngoc Bao Chau[2]

Received: 8 March 2025 / Accepted: 27 May 2025

Abstract

Background Big-eyed bugs (*Geocoris* spp.) are important generalist predators in agricultural ecosystems, playing a crucial role in natural pest control.

Methods To better understand their dietary sources, we assessed the plant and animal food sources in the gut of *Geocoris ochropterus* using multiplex PCR and shotgun metagenomic analysis. The PCR assays targeted genetic markers from both animal (COI) and plant (matK and rbcL) DNA.

Results Results revealed the presence of both animal and plant-derived DNA in the gut samples, indicating that *Geocoris ochropterus* feeds on a mixed diet. Additionally, the results of shotgun metagenomic sequencing of the gut microbiota showed a predominance of Eukaryota, with over 80% of sequences belonging to this domain, while a diverse range of taxonomic groups were identified, including arthropods, plants, bacteria, and fungi. Arthropods particularly insects from the orders Lepidoptera, Hemiptera, Hymenoptera, Coleoptera, Phasmatodea and plants belonging to the orders Brassicales, Cucurbitales, and Poales constituted the most abundant dietary components. At the genus level, notable taxa included *Maniola* (family Nymphalidae), *Carposina* (Carposinidae), *Helicoverpa* (Noctuidae), and *Solanum* (Solanaceae). Species-level analysis confirmed the dominance of several insect species, including *Maniola hyperanthus*, *Carposina sasakii*, and *Bombyx mori*, alongside plant species such as *Cucumis melo*, *Gossypium hirsutum*, and *Digitaria exilis*.

Conclusions These findings provide a comprehensive characterization of the diet of *Geocoris ochropterus*, highlighting its role as a generalist predator with a diverse diet consisting of both insect and plant food sources. This study contributes to the understanding of *Geocoris ochropterus* as a potential biocontrol agent in agricultural systems.

Keywords Big-eyed bugs · *Geocoris* sp. · *Geocoris ochropterus* · Natural enemy · Density of the big-eyed bug

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Bangkok, Thailand

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THANK YOU FOR YOUR ATTENTION!

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