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Genetic assessment of *Amblyseius eharai* and *Typhlodromus* sp. on Citrus in Vietnam using COI mtDNA barcoding

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Abstract

The study documents recorded presence of *Amblyseius eharai* (Amitai and Swirskii) and *Typhlodromus* sp. on citrus plants in Vietnam, expanding the known distribution of these Phytoseiidae mites. Genetic analysis using the COI gene showed that *A. eharai* from Vietnam is closely related to populations from Asia and Georgia, suggesting a shared lineage with adaptations due to geographic separation. *Typhlodromus* sp. from Vietnam formed its own clade within the genus, significantly differentiated from *Typhlodromus pyri* (Scheuten) and *Typhlodromus recki* (Wainstein), implying a potentially unique or undescribed species adapted to Vietnam's environment. These findings highlight the importance of molecular markers in Phytoseiidae taxonomy and recommend further studies with additional genetic markers and morphological comparisons to clarify these species' identities and evolutionary relationships.

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Introduction

Phytoseiid mites are a small group within the Mesostigmata, measuring approximately 0.4 mm (Tixier *et al.*, 2012; Seeman and Nahrung, 2018). They represent the most diverse group within this order, encompassing over 90 genera and more than 2,700 recognized species (Demite *et al.*, 2018; Chant and McMurtry, 2007; Demite *et al.*, 2023). Most species within this family are natural predators of harmful mites and small pest insects, although pollen feeding is also common, and some species may consume nematodes, fungal spores, and plant tissues. When associated with economically significant plants, many phytoseiid species serve as valuable biological control agents against pests such as mites, eriophyid mites, whiteflies, thrips, and mealy bugs (Tixier *et al.*, 2006a; b; 2007; 2008; 2010a; b; 2012a; 2017; Pekas *et al.*, 2017; Zemek and Prenerova, 1997; Huffaker *et al.*, 1970; McMurtry *et al.*, 1970; 2013; 2015). Due to their extensive species diversity, the taxonomy of phytoseiids has received considerable attention (Chant and McMurtry, 2007; Demite *et al.*, 2023). Taxonomic assessments not only aid in accurate species identification but also enhance our understanding of their diversity, ecological roles, evolutionary relationships, and geographical distribution.

DNA barcoding, a technique that utilizes a conserved DNA segment, allows for the quick and accurate identification of species. Researchers often employ this method to elucidate phylogenetic relationships between species or to resolve taxonomic ambiguities (Salomone and Bernini, 2002; Navajas and Fenton, 2000). The cytochrome oxidase I (COI) gene, located in the mitochondrial genome, has proven effective as a DNA barcode across most animal phyla, except Cnidaria, enabling the distinction of closely related species (Hebert *et al.*, 2003). Identifying pest and beneficial species at the species level is essential for crop protection. Historically, Tetranychidae and Phytoseiidae taxonomy relied primarily on morphological characteristics (Lindquist *et al.*, 2009), but their small size and limited morphological structures complicate accurate identification. Consequently, mite researchers have adopted DNA markers over recent decades, and this approach, combined with sequence similarity ratios and phylogenetic analysis, has facilitated precise species identification (Navajas and Fenton, 2000; Hurtado *et al.*,2008; Pérez-Sayas *et al.*, 2015; Gómez-Martínez *et al.*, 2020).

The COI gene has been applied to various taxonomic groups, such as ants associated with aphids (Siddiqui *et al.*, 2019) and fish species (Ward *et al.*, 2005). It also serves as a reliable marker for constructing barcode datasets for Acari identification (Pérez-Sayas *et al.*, 2022), including predatory mites like phytoseiids (Li *et al.*, 2012), eriophyoid mites (Guo *et al.*, 2015), and water mites (Klimov *et al.*, 2022).

Despite the availability of complete genomes for model species and the reduced costs of next-generation sequencing (NGS), single genes like the mitochondrial Cytochrome C Oxidase I (COI) gene remain preferred for phylogenetic and taxonomic analyses. COI is widely used across various taxa, even contributing to DNA barcode databases for species identification (Hebert *et al.*, 2003). However, despite the abundance of genetic data and genomic resources, gene fragments remain only partially available for some mite species, particularly primary biological control agents like the predatory phytoseiid mites, which appear to be somewhat overlooked (though recent studies have emerged). For instance, by September 2021, the public sequence database (GenBank) contained over one million Acari sequences, but less than 10% represented agriculturally significant orders, and fewer than 1% were phytoseiid mites. Of these, only 49 complete mitochondrial genomes were available—24 from the superorder Acariformes and 25 from Parasitiformes, with just two belonging to the Phytoseiidae family (Pérez-Sayas *et al.*, 2022).

Materials and methods

Predator mite collection

Predatory mites were collected from Binh Duong and Tien Giang Provinces during field surveys conducted

in June 2024. Leaves and buds were carefully examined with a handheld magnifying glass, and when predatory mites were detected, the plant parts were collected in paper or plastic bags and transported to the laboratory for further examination. Mites were directly collected from leaves under a stereomicroscope and transferred into vials containing either (i) 70% alcohol for morphological studies or (ii) 100% alcohol for genetic analysis. Morphological classification followed the method of Chant and McMurtry (2007).

DNA extraction from predatory mites

DNA was extracted from predatory mites collected from citrus trees using the DNeasy Tissue Kit (69504, Qiagen), following the manufacturer's instructions. Samples were stored at -20°C.

Viral DNA amplification by PCR

The partial COI mtDNA gene was amplified using degenerate primers (Tixier *et al.*, 2012b) (Table 1). The PCR reaction was conducted in a 25 µl solution containing 12.5 µl of Ampliqon® 2x master mix, 1 µl of each primer (10 pm), 2 µl of template DNA, and 9.5 µl of sterilized distilled water. The samples were denatured at 94°C for 4 minutes, followed by 40 cycles of denaturation at 94°C for 30 seconds, primer annealing at 60°C for 30 seconds, and extension at 72°C for 1 minute, with a final extension at 72°C for 10 minutes. A single band was visualized after electrophoresis on a 1% agarose gel.

Table 1. Primer pairs used for amplification

Primers	Sequence	Reference
COI mtDNA-F	TTTCAACWAATCATAAAGATATTGG	Tixier *et al.* (2012b)
COI mtDNA-R	TAAACTTCWGGRTGWCCAAARAATCA	

Table 2. Collection data of phytoseiid sp.

Phytoseiidae species	Genbank accession numbers	Locality
Amblyseius eharai	MW346235.1	Georgia
Amblyseius eharai	MW346236.1	Georgia
Amblyseius eharai	MW346298.1	Georgia
Amblyseius eharai	JX080345.1	China
Amblyseius eharai	JX080344.1	China
Amblyseius eharai	JX080343.1	China
Amblyseius eharai	JX080342.1	China
Amblyseius eharai	JX080331.1	China
Amblyseius eharai	-	Viet Nam
Neoseiulus longispinosus	MK577645.1	Viet Nam
Amblyseius herbicolus	JX080330.1	China
Amblyseius herbicolus	JX080326.1	China
Amblyseius largoensis	JX080349.1	China
Amblyseius largoensis	JX080346.1	China
Amblyseius largoensis	MK577645.1	Viet Nam
Typhlodromus sp.	KM831280.1	Canada
Typhlodromus sp.	KM824591.1	Canada
Typhlodromus sp.	-	Viet Nam
Typhlodromus pyri	MG414506.1	Canada
Typhlodromus pyri	MG410411.1	Canada
Typhlodromus pyri	JF279181.1	Poland
Typhlodromus pyri	JF279180.1	Italy
Typhlodromus pyri	JF279179.1	Italy
Typhlodromus pyri	JF279178.1	Hungary
Typhlodromus pyri	JF279175.1	Hungary
Typhlodromus pyri	JF279174.1	Austria
Typhlodromus pyri	JF279173.1	Austria
Typhlodromus pyri	JF279171.1	France
Typhlodromus pyri	JF279168.1	USA
Typhlodromus pyri	JF279167.1	USA
Typhlodromus pyri	JF279164.1	France
Typhlodromus pyri	JF279161.1	France
Typhlodromus pyri	EF372611.1	USA
Typhlodromus pyri	FM210180.1	-
Typhlodromus phialatus	KU342791.1	Lleida
Typhlodromus phialatus	JF279183.1	France
Typhlodromus phialatus	KP642062.1	Lleida
Typhlodromus phialatus	KP642061.1	Girona
Typhlodromus recki	MW074348.1	-
Typhlodromus recki	MT828363.1	Italy

Phylogenetic analyses

PCR products were purified using the ExoSAP-IT PCR Clean-up kit and then used as sequencing templates. Nucleotide sequences were determined using the 3730XL DNA Analyzer. COI mtDNA sequences were compared between two Vietnamese predatory mite isolates and other isolates from the NCBI GenBank database (Table 2), with alignment performed using CLUSTAL W (Tamura *et al.*, 2011). The Tamura & Nei model was used as the genetic distance model, and the neighbor-joining method was applied for phylogenetic tree construction (Saitou and Nei, 1987). Bootstrap analyses with 1,000 replications assessed confidence in branching order.

Results and discussion

Amblyseius eharai (Amitai & Swirski)

Specimens were collected from the districts of Cho Gao, Chau Thanh, My Tho, Cai Be, and Cai Lay in Tien Giang Province, with 30 females and 10 males found on *Citrus grandis* L., and 4 females on *Citrus reticulata* Blanco.

Distribution (previous records)

This species has previously been recorded in China (in the provinces of Jiangsu, Zhejiang, Jiangxi, Hubei, Hunan, Fujian, Guangdong, Guangxi, Hainan, and Hong Kong), Taiwan, the Matsu Islands, South Korea, and Japan (across numerous prefectures such as Akita, Miyagi, Yamagata, Fukushima, Saitama, Ibaraki, Chiba, Tokyo, Kanagawa, Niigata, Toyama, Ishikawa, Fukui, Nagano, Shizuoka, Gifu, Shiga, Kyoto, Mie, Nara, Wakayama, Hyogo, Tottori, Shimane, Okayama, Hiroshima, Yamaguchi, Tokushima, Ehime, Kochi, Fukuoka, Oita, Nagasaki, Kumamoto, Miyazaki, Kagoshima, and Okinawa), as well as Malaysia (Ho *et al.*, 2003). However, this study represents the first record of *A. eharai* in Vietnam on any host plants.

Description

The dorsal shield of *A. eharai* is smooth and sleek, with 17 pairs of setae: 6D, 2M, and 9L. The L9 setae are very long, L4 and M2 are long, D1 and L1 are moderately long, while the remaining setae are short and fine.

Measurements (in µm)

The dorsal shield measures 338–340 in length and 180–197 in width. Specific setae lengths are as follows: j1 = 38–39, j3 = 48–50, j4 = 8–11, j5 = 6–8, j6 = 8–12, J2 = 10–12, J5 = 9–11, z2 = 12–16, z4 = 10–14, z5 = 6–9, Z1 = 10–12, Z4 = 106–115, Z5 = 300–305, s4 = 107–110, S2 = 13–16, S4 = 10–16, S5 = 14, r3 = 11–12, and R1 = 11–15. The distances between St1–St3, St2–St3, and St5–St3 are 65, 67–69, and 68–79, respectively.

The ventral shield measures 113–116 in length and 58–65 in width, with an anal width of 68–74. The spermatheca measures 15–18 in length, and other appendages measure as follows: Sge I = 46–47, Sge II = 36–38, Sge III = 50–52, Sti III = 43–46, St III = 33–35, Sge IV = 152–160, Sti IV = 113–121, and St IV = 66–70.

Remarks

Amblyseius eharai closely resembles *Amblyseius herbicolus* (Chant), with the primary distinguishing features being the shape of the posterior margin of the sternal shield and the length and shape of the spermatheca's cervix. In *A. herbicolus*, the posterior sternal shield border is straight, whereas in *A. eharai*, it has a truncated median projection. The cervix of the spermatheca is longer in *A. herbicolus* (23–29 µm), with the distal two-thirds gradually expanding to 2–2.5 times the basal diameter. In *A. eharai*, it is shorter (18–24 µm) and flares distally to 2–3 times the narrowest diameter (McMurtry and Moraes, 1984). Due to these minor morphological differences and the fact that *A. eharai* had not been reported previously in Vietnam, molecular markers were employed to verify the identity of the Vietnamese specimens. Additionally, genetic evaluation was performed for *Amblyseius largoensis* and *Neoseiulus longispinosus* (using sequences from GenBank), both of which have been recorded on citrus trees in Vietnam.

To assess genetic distance, the *A. eharai* specimens from Vietnam were grouped with *A. eharai* samples from China (JX080344.1, JX080331.1, JX080342.1, JX080345.1, JX080344.1), referred to as the *Amblyseius eharai* Asia group, and compared with the *A. eharai* group from Georgia and other species groups.

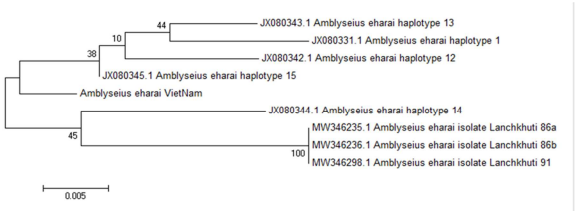

Fig. 1. A phylogenetic tree was constructed from partial *COI* sequences of *Amblyseius eharai* using the neighbor-joining analysis method

In the phylogenetic tree (Fig. 1), *Amblyseius eharai* from Vietnam forms an independent clade with a high bootstrap value (99), indicating significant genetic differentiation. The genetic distance between

A. eharai from Asia (including the Vietnamese sample and Chinese haplotypes) and *A. eharai* from Georgia is 0.050 ± 0.013 (Table 3), a small distance suggesting a close genetic relationship. This finding implies that despite minor differences, *A. eharai* populations from Vietnam, China, and Georgia share the same evolutionary lineage, with possible geographic adaptation contributing to genetic variation.

While *Amblyseius herbicolus* is described as morphologically similar to *A. eharai*, the genetic analysis results indicate a genetic distance of 0.663 (Table 3) between *A. eharai* Asia and *A. herbicolus*, highlighting a significant genetic distinction. This suggests that *A. herbicolus*, despite its morphological resemblance to *A. eharai*, is not closely related evolutionarily and does not belong to the same lineage. The observed morphological similarity may be due to convergent evolution, where similar traits develop in unrelated species as adaptations to similar ecological conditions.

In the phylogenetic tree, *Amblyseius largoensis* and *Neoseiulus longispinosus* occupy completely separate branches from the *A. eharai* clades, with high bootstrap values (97-100), indicating substantial genetic differences. The genetic distance between *A. eharai* Asia and *A. largoensis* is 0.679, a significant distance that underscores the lack of a close evolutionary relationship between these species. Although they may share the same habitat and ecological roles, *A. largoensis* and *A. eharai* belong to distinct evolutionary lineages within the Phytoseiidae family, consistent with findings from Tixier *et al.*, 2021.

Table 3. Matrix of Tamura & Nei genetic distance among *Amblyseius eharai* and phytoseiid species

	A. eharai Asia	*A. eharai* Geogria	*N. longispinosus*	*A. herbicolus*	*A. largoensis*
A. eharai Asia		0.013	0.226	0.201	0.201
A. eharai Geogria	0.050		0.234	0.200	0.196
N. longispinosus	0.778	0.805		0.308	0.364
A. herbicolus	0.663	0.650	1.012		0.083
A. largoensis	0.679	0.662	1.217	0.318	

Lower triangular matrix values were mean genetic distances, upper triangular matrix values were standard errors.

Neoseiulus longispinosus, a common biological control agent on citrus in Vietnam, also does not show a close genetic relationship with either *A. eharai* Asia or *A. eharai* Georgia (with genetic distances of 0.778 and 0.805, respectively). Although these species coexist in tropical environments and fulfill similar ecological roles, *N. longispinosus* and *A. eharai* represent different evolutionary lineages within the phylogenetic tree. This suggests that while these species may be geographically co-located and play important roles in biological control, they do not share a close genetic relationship.

Typhlodromus sp.

Sample information

Collected in Binh Duong Province, with 30 females and 10 males on *Citrus reticulata* Blanco.

Description

This species is distinguished by the presence of setae pairs S4, JV3, and JV4, with dorsal setae pairs of approximately equal lengths, except for Z4/Z5. The r3 and R1 pairs on the dorsal surface in the zZ and sS series are shorter than the distances between them (El-Banhawy *et al.*, 2009).

Dorsal shield measurements (in µm)

Length: 351

Width: 236

Setae lengths: j1 = 15, j3 = 13 (10–15), j4 = 9 (8–10), j5 = 10, j6 = 11 (10–13), J2 = 12 (10–12), J5 = 10, r3 = 15, R1 = 15, s4 = 14 (13–15), s6 = 15, S2 = 15, S4 = 17 (15–18), S5 = 14 (13–15), z2 = 13, z3 = 13, z4 = 13, z5 = 10, Z4 = 14 (13–15), Z5 = 31 (30–33)

Distance between setae (in µm): st1–st1 = 41 (40–43), st2–st2 = 49 (48–50), st3–st3 = 55, st4–st4 = 56 (55–58), st5–st5 = 58

Leg setae (in µm): Sge IV = 13 (12–13), Sti IV = 12 (12–13), St IV = 23 (23–24), Stt IV = 21 (20–23)

Ventral shield: Length 113 (110–115), width 96 (90–100)

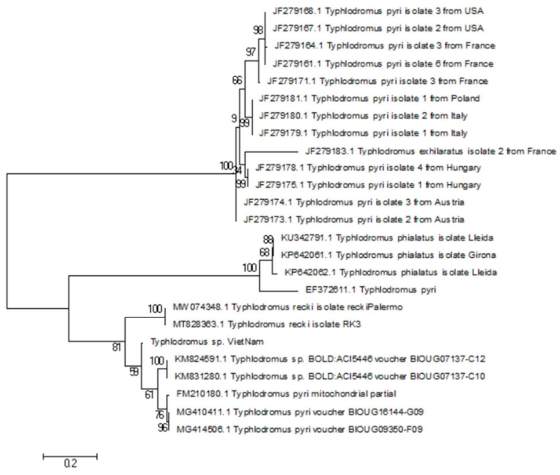

Fig. 2. A phylogenetic tree was constructed from partial COI sequences of *Typhodromus* sp. using the neighbor-joining analysis method

Remarks

This study marks the first time *Typhlodromus* sp. has been recorded on citrus plants in Vietnam. Phylogenetic analysis (Fig. 2) reveals that *Typhlodromus* sp. from Vietnam forms a distinct clade within the genus *Typhlodromus*, with a high bootstrap value (100), indicating significant divergence from other recorded species worldwide (data obtained from GenBank). The Vietnamese clade of *Typhlodromus* sp. is closely related to samples of *Typhlodromus pyri* (MG410411.1, MG414506.1), *Typhlodromus recki* (MW074348.1, MT828363.1), and samples from the *Typhlodromus* sp. BOLD: ACI5446 clade (KM824591.1, KM831280.1). However, *Typhlodromus* sp. Vietnam displays considerable differentiation from these groups, with bootstrap values ranging from 81 to 100, indicating stable genetic differences.

The genetic distance (Table 4) provides further insight into the evolutionary relationship between *Typhlodromus sp*. Vietnam and other species within the genus. The genetic distance between *Typhlodromus* sp. Vietnam and closely related samples in the phylogenetic tree, such as *Typhlodromus recki* (MW074348.1), is 0.675, and with *Typhlodromus pyri* (MG410411.1), it is 0.906. Other *Typhlodromus pyri* samples in the same group (KM824591.1, KM831280.1) show genetic distances ranging from 0.287 to 0.879, confirming the genetic differentiation between *Typhlodromus* sp. Vietnam and other *Typhlodromus pyri* samples. This suggests that *Typhlodromus* sp. Vietnam may not be *Typhlodromus pyri*, with the observed differentiation possibly arising from population isolation or adaptation to specific climatic conditions (Tixier *et al*., 2020b; Queiroz *et al*., 2021).

Table 4. Matrix of Tamura & Nei genetic distance among *Typhlodromus* family

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
1		0.000	0.066	0.019	0.019	0.022	0.831	0.831	0.831	0.842	0.842	0.842	0.842	0.903	0.903	0.871	0.902	0.903	0.698	0.006	0.066	0.609	0.605	0.609	1.179
2	0.000		0.066	0.019	0.019	0.022	0.831	0.831	0.831	0.842	0.842	0.842	0.842	0.903	0.903	0.871	0.902	0.903	0.698	0.006	0.066	0.609	0.605	0.609	1.179
3	0.276	0.276		0.071	0.071	0.050	1.022	1.022	1.022	1.079	1.079	1.042	1.042	1.103	1.103	1.073	1.096	1.103	0.824	0.067	0.000	0.713	0.707	0.713	1.360
4	0.077	0.077	0.287		0.000	0.023	0.851	0.851	0.851	0.866	0.866	0.867	0.867	0.923	0.923	0.891	0.918	0.923	0.733	0.018	0.071	0.642	0.641	0.642	1.219
5	0.077	0.077	0.287	0.000		0.023	0.851	0.851	0.851	0.866	0.866	0.867	0.867	0.923	0.923	0.891	0.918	0.923	0.733	0.018	0.071	0.642	0.641	0.642	1.219
6	0.095	0.095	0.214	0.096	0.096		0.879	0.879	0.879	0.897	0.897	0.901	0.901	0.951	0.951	0.918	0.945	0.951	0.675	0.024	0.050	0.579	0.574	0.579	1.331
7	1.244	1.244	1.428	1.296	1.296	1.340		0.000	0.000	0.020	0.020	0.016	0.016	0.025	0.025	0.022	0.025	0.025	1.301	0.831	1.022	1.198	1.202	1.198	0.059
8	1.244	1.244	1.428	1.296	1.296	1.340	0.000		0.000	0.020	0.020	0.016	0.016	0.025	0.025	0.022	0.025	0.025	1.301	0.831	1.022	1.198	1.202	1.198	0.059
9	1.244	1.244	1.428	1.296	1.296	1.340	0.000	0.000		0.020	0.020	0.016	0.016	0.025	0.025	0.022	0.025	0.025	1.301	0.831	1.022	1.198	1.202	1.198	0.059
10	1.297	1.297	1.554	1.317	1.317	1.350	0.074	0.074	0.074		0.000	0.014	0.014	0.028	0.028	0.024	0.028	0.028	1.304	0.841	1.079	1.159	1.157	1.159	0.051
11	1.297	1.297	1.554	1.317	1.317	1.350	0.074	0.074	0.074	0.000		0.014	0.014	0.028	0.028	0.024	0.028	0.028	1.304	0.841	1.079	1.159	1.157	1.159	0.051
12	1.329	1.329	1.466	1.339	1.339	1.351	0.057	0.057	0.057	0.044	0.044		0.000	0.027	0.027	0.022	0.027	0.027	1.266	0.841	1.042	1.203	1.207	1.203	0.051
13	1.329	1.329	1.466	1.339	1.339	1.351	0.057	0.057	0.057	0.044	0.044	0.000		0.027	0.027	0.022	0.027	0.027	1.266	0.841	1.042	1.203	1.207	1.203	0.051
14	1.261	1.261	1.496	1.338	1.338	1.397	0.098	0.098	0.098	0.112	0.112	0.112	0.112		0.000	0.011	0.004	0.000	1.288	0.902	1.103	1.281	1.282	1.281	0.066
15	1.261	1.261	1.496	1.338	1.338	1.397	0.098	0.098	0.098	0.112	0.112	0.112	0.112	0.000		0.011	0.004	0.000	1.288	0.902	1.103	1.281	1.282	1.281	0.066
16	1.253	1.253	1.431	1.328	1.328	1.374	0.084	0.084	0.084	0.094	0.094	0.091	0.091	0.031	0.031		0.012	0.011	1.251	0.871	1.073	1.244	1.246	1.244	0.064
17	1.261	1.261	1.522	1.361	1.361	1.421	0.098	0.098	0.098	0.112	0.112	0.112	0.112	0.006	0.006	0.038		0.004	1.287	0.902	1.096	1.281	1.282	1.281	0.066
18	1.261	1.261	1.496	1.338	1.338	1.397	0.098	0.098	0.098	0.112	0.112	0.112	0.112	0.000	0.000	0.031	0.006		1.288	0.902	1.103	1.281	1.282	1.281	0.066
19	1.095	1.095	1.186	1.217	1.217	1.078	1.845	1.845	1.845	2.017	2.017	1.864	1.864	1.925	1.925	1.799	1.925	1.925		0.716	0.824	0.044	0.045	0.044	1.610
20	0.012	0.012	0.282	0.071	0.071	0.103	1.223	1.223	1.223	1.275	1.275	1.308	1.308	1.261	1.261	1.253	1.261	1.261	1.128		0.067	0.610	0.606	0.610	1.179
21	0.276	0.276	0.000	0.287	0.287	0.214	1.428	1.428	1.428	1.554	1.554	1.466	1.466	1.496	1.496	1.431	1.522	1.496	1.186	0.282		0.713	0.707	0.713	1.360
22	0.989	0.989	1.088	1.072	1.072	1.003	1.734	1.734	1.734	1.813	1.813	1.833	1.833	1.922	1.922	1.798	1.922	1.922	0.193	0.971	1.088		0.009	0.000	1.407
23	1.008	1.008	1.119	1.091	1.091	1.031	1.707	1.707	1.707	1.814	1.814	1.807	1.807	1.909	1.909	1.785	1.909	1.909	0.200	0.990	1.119	0.022		0.009	1.408
24	0.989	0.989	1.088	1.072	1.072	1.003	1.734	1.734	1.734	1.813	1.813	1.833	1.833	1.922	1.922	1.798	1.922	1.922	0.193	0.971	1.088	0.000	0.022		1.407
25	1.719	1.719	1.798	1.698	1.698	1.809	0.235	0.235	0.235	0.209	0.209	0.210	0.210	0.249	0.249	0.248	0.253	0.249	2.361	1.692	1.798	2.081	2.068	2.081	

Lower triangular matrix values were mean genetic distances, upper triangular matrix values were standard errors.

1. MG410411.1 (*Typhlodromus pyri* voucher BIOUG16144-G09), 2. MG414506.1 (*Typhlodromus pyri* voucher BIOUG09350-F09), 3. MW074348.1 (*Typhlodromus recki* isolate reckiPalermo), 4. KM824591.1 (*Typhlodromus* sp. BOLD voucher BIOUG07137-C12), 5. KM831280.1 (*Typhlodromus* sp. BOLDvoucher BIOUG07137-C10), 6. *Typhlodromus* sp. from Vietnam, 7. JF279181.1 (*Typhlodromus pyri* isolate 1 from Poland), 8. JF279180.1 (*Typhlodromus pyri* isolate 2 from Italy), 9. JF279179.1 (*Typhlodromus pyri* isolate 1 from Italy), 10. JF279178.1 (*Typhlodromus pyri* isolate 4 from Hungary), 11. JF279175.1 (*Typhlodromus pyri* isolate 1 from Hungary), 12. JF279174.1 (*Typhlodromus pyri* isolate 3 from Austria), 13. JF279173.1 (*Typhlodromus pyri* isolate 2 from Austria), 14. JF279168.1 (*Typhlodromus pyri* isolate 3 from the USA), 15. JF279167.1 (*Typhlodromus pyri* isolate 2 from the USA), 16. JF279171.1 (*Typhlodromus pyri* isolate 3 from France), 17. JF279164.1 (*Typhlodromus pyri* isolate 3 from France), 18. JF279161.1 (*Typhlodromus pyri* isolate 6 from France), 19. EF372611.1 (*Typhlodromus pyri*), 20. FM210180.1 (*Typhlodromus pyri* mitochondrial partial), 21. MT828363.1 (*Typhlodromus recki* isolate RK3), 22. KU342791.1 (*Typhlodromus phialatus* isolate Lleida), 23. KP642062.1 (*Typhlodromus phialatus* isolate Lleida), 24. KP642061.1 (*Typhlodromus phialatus* isolate Girona), 25. JF279183.1 (*Typhlodromus exhilaratus* isolate 2 from France).

Conclusion

This study provides new insights into the distribution and genetic relationships of *Amblyseius eharai* and *Typhlodromus* sp. in Vietnam, particularly on citrus plants. This marks the first recorded presence of *A. eharai* and *Typhlodromus sp.* on citrus in this region, expanding our understanding of Phytoseiidae species distribution.

Phylogenetic analysis reveals that *Amblyseius eharai* from Vietnam is closely related to *A. eharai* populations from Asia and Georgia, although genetic differences may reflect geographic isolation and adaptation. For *Typhlodromus* sp. Vietnam, genetic analysis shows this species occupies a distinct clade within the genus *Typhlodromus*, with high bootstrap values indicating stable genetic differentiation from other *Typhlodromus* samples worldwide. The substantial genetic distance between *Typhlodromus* sp. Vietnam and samples like *Typhlodromus pyri* and *Typhlodromus recki* suggests that *Typhlodromus* sp. Vietnam may represent an undescribed species or subspecies with unique adaptations to Vietnam's climatic and ecological conditions.

These findings underscore the importance of molecular markers in Phytoseiidae taxonomy and genetic diversity assessment, particularly for species with similar morphological traits. Future studies should consider analyzing additional genetic markers and conducting comprehensive morphological comparisons to further clarify the identities and evolutionary relationships of *Amblyseius eharai* Vietnam and *Typhlodromus sp.* Vietnam within the Phytoseiidae family.

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