The Phylogenetic Relationships among Some Common Species of Amblyseiinae (Acari: Phytoseiidae) in China Orchard Based on the Mitochondrial *CO1* Gene

Zhiwen Zou, Jianfei Xi, Fen Chen, Rui Xu, Tianrong Xin and Bin Xia*

College of Life Science, Nanchang University, Nanchang 330031, China

ABSTRACT

Amblyseiinae is the largest subfamily in Phytoseiidae, with more than 1,500 nominal species. However, the relationships of this subfamily was confused. In order to improve the classification system of Amblyseiinae, the mitochondrial CO1 gene sequence of seven Amblyseiinae species, collected from four provinces of China, were sequenced, and another seven Amblyseiinae species mt CO1 gene sequenced were download from GenBank. And the phylogenetic relationship between the Amblyseiinae genus and the subspecies was studied. The results showed that four species of *Kampimodromus* and two species of *Euseius* clustered into two different branches, but the branch of 8 species of *Amblyseius* and *Neoseiulus* was chaotic. It indicated that the species of *Neoseiulus* and *Amblyseius* should be assigned carefully. The results could not only reveal its phylogenetic relationship and provide molecular evidence for the evolution, but also provide a better way to identify phytoseiid species in citrus.

INTRODUCTION

China is an important source area of citrus, with many cultivars, such as *Citrus unshiu*, *C. kinokuni*, *C. junos*, *C. ichangensis*, *C. sinensis*, *etc*., being native to Southern China. Citrus are widely cultivated along the Jiangxi province, making it the largest producer of citrus in China (Ammerman *et al*., 1965).

Due to the rapid propagation and strong adaptability, agricultural pest mites have been a serious threat to citrus fruit, decreasing the quality and yield of crops (Xin, 1988). However, the widespread use of synthetic chemicals to control these arthropod pests indirectly results in death for a large number of their natural enemies. This diminishes the success of natural enemies as a natural means of population suppression for harmful mite pests.

Amblyseiinae (Acari: Phytoseiidae) (Krantz, 1978) often lives with predatory mites and phytophagous spider mites, eriophyoid, tarsonemid, aphids, coccid, and so on. It is one of the common natural enemies of harmful mites, and widely used as biological control agents (Wu *et al*., 2009; Xu *et al*., 2010). The artificial breeding and industrial production of some phytoseiid mites (such as *Phytoseiulus persimilis* Athias-Henriot, *Neoseiulus fallacis* Garman, *Neoseiulus cucumeris* Oudemans, *Neoseiulus barkeri*

Hughes, *Amblyseius eharai* Amitai et Swirski, *etc*.) has been achieved (Mailloux *et al*., 2010). However, the phylogenetic system of phytoseiid has not been perfected, and the classification system of phytoseiini mites, especially the subfamily Amblyseiinae, is still unclear.

Amblyseiinae is the largest subfamily in Phytoseiidae, with more than 1,500 nominal species. The first described species of Phytoseiidae was *Zercon obtusus* Koch (Koch, 1839), which was named with *Amblyseius obtusus*. *Amblyseius* was first established as a genus in 1914, but was not initially accepted by many scholars (Berlese, 1914). Oudemans (1936) placed those species originally described by Berlese (1914) into *Tyohlodromus*. Vitzthum (1941) built the classification system of phytoseiini mites. Since that, Phytoseiini taxonomists confirmed *Amblyseius* and placed the species have setae Z2, Z4, Z1, S2, S4 and S5 short/minute, approximately subequal into this genus. Later, the subfamily Amblyseiinae was first built in 1961 and included 19 genera (Muma, 1961). This divided Phytoseiidae into 3 subfamilies: Phytoseiinae, Typhlodrominae and Amblyseiinae (Chant and McMurtry, 1994). A series of detailed reviews about Amblyseiinae (Chant and McMurtry, 2003a, b, 2004a, b, 2005a, b, c, 2006a, b) emerged from 2003 to 2006, which cataloged and/or described 1,499 species in 61 genera and 9 tribes.

Sequences of multiple genes or genomes have been used in phylogenetic studies in recent years and provided insights into the higher-level relationships in insects (Misof *et al*., 2014). Mitochondrial genomes, usually 16

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* Corresponding author: xiabin9@163.com

kb in size with 37 genes for animals (Boore, 1999; Guo *et al.*, 2017), have been shown to be a useful marker for inferring higher-level phylogeny (Bourguignon *et al.*, 2015; Li *et al.*, 2015).

In the past decade, the phylogeny of various mite groups has been studied using molecular markers such as mitochondrial (mt) gene (Dabert, *et al.*, 2010), mt genome (Chen *et al.*, 2014; Gu *et al.*, 2014) and nuclear gene sequences (Kreipe *et al.*, 2015; Pepato and Klimov, 2015). In this study, in order to improve the classification system of Amblyseiinae, the mitochondrial CO1 gene sequence of seven Amblyseiinae species, collected from four provinces of China, were sequenced. In addition, the phylogenetic relationship between the Amblyseiinae genus and the subspecies was studied. The results could not only reveal its phylogenetic relationship and provide molecular evidence for the evolution, but also provide a better way to identify phytoseiid species in citrus.

MATERIALS AND METHODS

Sample collection and identification

Seventeen geographic populations in Amblyseiinae were collected from Jiangxi, Guangdong, Hunan province, and Shanghai city, respectively (Table I; Fig. 1). Samples of each site were mounted as vouchers, using modified Berlese medium (Amrine and Manson, 1996) for morphological checking with a Zeiss A2 (microphoto camera AxioCam MRc) microscope. All of the specimens and vouchers were deposited in Department of Ecology, Nanchang University, China. Other samples were preserved in 70% ethanol at -20°C until DNA extraction.
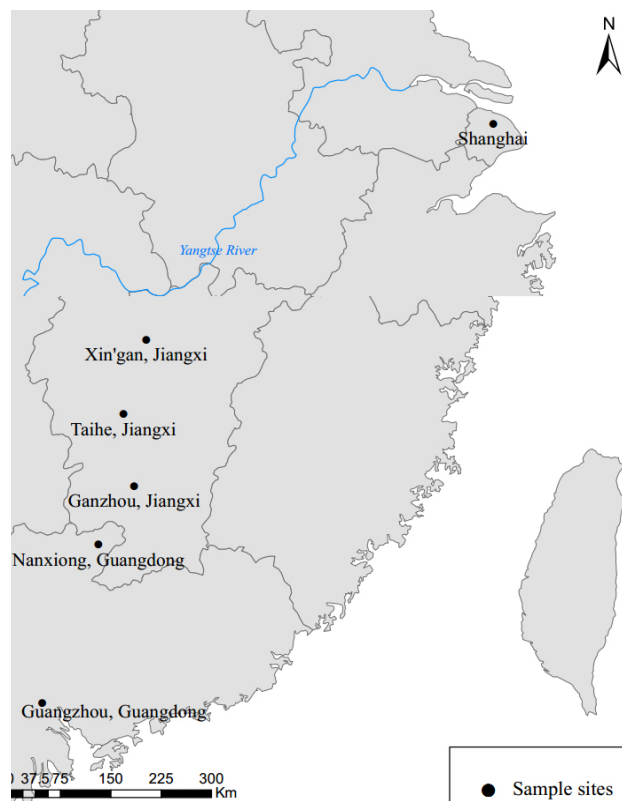

Fig. 1. Sampling sites of Amblyseiinae

Table I.- Sampled taxa for sequenced mt CO1 gene.

Families	Genus	Species	Location	Host	Code
Amblyseiinae	*Amblyseius*	*A. eharai*	Nanchang, Jiangxi	Citrus	AE1
			Xingan, Jiangxi	Tangerine	AE2
			Taihe, Jiangxi	Kumquat	AE3
			Yongxiu, Jiangxi	Citrus	AE4
			Guangzhou, Guangdong	Citrus	AE5
			Shanghai	Citrus	AE6
			Changsha, Hunan	Citrus	AE7
			Sihui, Guangzhou	Citrus	AE8
	Euseius	*E. nicholsi*	Nanchang, Jiangxi	Citrus	EN1
			Changsha, Hunan	Citrus	EN2
			Guangzhou, Guangdong	Citrus	EN3
		A. orientalis	Nanchang, Jiangxi	Citrus	AO1
			Xingan, Jiangxi	Chinese honey orange	AO2
		N. cucumeris	Guangzhou, Guangdong	Citrus	NC
	Scapulaseius	*S. asiaticus*	Nanxiong, Guangdong	Osmanthus fragrans	SA
		A. tsugawai	Jing 'an, Jiangxi	Citrus	AT
	Neoseiulus	*N. barkeri*	Ganzhou, Jiangxi	Navel orange	NB

Data collection

The mt genome sequences of seven species of Amblyseiinae and one outgroup species were retrieved from GenBank (Table II). The dataset includes four *Kampimodromus*, three *Neoseiulus*, and one *Euseius* mite species. The *Metaseiulus occidentalis* was used as outgroup.

Table II.- Sequences data for mt CO1 gene of some Amblyseiinae from GenBank.

Families / Genus	Species	Accession	Code
Amblyseiinae			
Kampimodromus	*K. corylosus*	EF372610	KC
	K. aberrans	EF372606	KA
	K. langei	EF372609	KL
	K. ericinus	EF372607	KE
Neoseiulus	*N. womersleyi*	AB500133	NW
	N. californicus	AB500131	NCA
Euseius	*E. finlandicus*	FJ404592	EF
Phytoseiinae			
Metaseiulus	*M. occidentalis*	EF221760	MO

DNA extraction, gene amplification and sequencing

The total DNA was extracted from a single adult mite using the improved Chelex-100 (Walsh *et al.*, 1991). A 453-bp fragment of COI was initially amplified by PCR with the primer pair (Navajas *et al.*, 1996), 5'- TGATTTTTTGGTCACCCAGAAG -3' and 5'-TACAGCTCCTATAGATAAAAC -3', then sequenced on an Illumina Hiseq 2000 platform at the Beijing Genomics Institute, Beijing.

Short PCRs were performed in 25-µL reactions containing: 2.5 µL of 10 × Buffer (Mg $^{2+}$ Free), 2.5 µL of MgCl$_2$ (25mmol/L), 1.0 µL of dNTPs Mixture (10mmol/L), 1.0 µL of each primer(10mmol/L), 0.4 µL of Taq DNA polymerase (5U/µL), 5.0 µL of template DNA, and 11.6 µL water. PCR cycling conditions were: 5 min denaturation at 95 °C; 30 cycles of 1 min denaturation at 95 °C, 45 s annealing at 45 °C and 1 min extension at 72 °C; 5 min final extension at 72 °C; and then held at 4 °C. After amplification, 5 µL of the PCR reaction was analyzed by electrophoresis on a 1% agarose gel and visualized by GelRed staining. The PCR product was purified, then the strand of the amplified fragments CO1 were sequenced by Beijing Tiangen, Beijing.

Sequence and phylogenetic analyses

The DNAStar (Lasergene, v.7.1.0) was used for editing and assembling the raw data into sequence contigs. The fragment gene of mt CO1, was identified by BLASTn searches of NCBI based on highly conserved sequence

motifs. Sequences obtained and cited from others published data were aligned using CLUSTAL W and numbers or parsimony-informative sites were calculated using MEGA5 software (Thompson *et al.*, 1994). The base composition, nucleotide differences, transformation and transversion, genetic distance, etc. of the sequence were analyzed. All of the above analyses of the CO1 sequences were conducted using MEGA version 5 software (Tamura *et al.*, 2011).

Phylogenetic analysis using the CO1 DNA fragment data was conducted using the Bayesian tree estimate methods and the Maximum likelihood (ML) method with 1,000 bootstrap replicates. Sequences for *Metaseiulus occidentalis* was used as a single outgroup. Maximum likelihood (ML) trees of the mt DNA genes was constructed with Paup* (v.4. ob.10; Swofford, 2002). The default parameters were used and the confidence values of the ML tree were evaluated via a bootstrap test with 1000 iterations (Felsenstein, 1985). Nodes supported by bootstrap values (BSP) ≥ 70% were considered strongly supported (Hillis and Bull, 1993). The Bayesian inference (BI) analysis using MrBayes version 3.12 (Ronquist and Huelsenbeck, 2003) was conducted using phylogeny. The dataset was run for 3 million generations, with tree sampled every 100 generations. After 2.5 million generations, the average standard deviation was below 0.01 in most Bayesian trees. The consensus tree was edited with FigTree1.4.0. Nodes supported by posterior probabilities (BPP) P 95% were considered strongly supported.

RESULTS

mt CO1 gene sequence

The CO1 sequence of seven species in Amblyseiinae were sequenced in this study. All sequences were 453 bp long. The base compositions are shown in Tables III and IV. In the 17 geographic populations, average content of T (42.4%) is the most abundant. C content is the lowest (13.4.0%), and T content of SA (45.9%) is the highest while that of NB is the lowest (37.1%). C content ranges from 18.3% (NB) to 18.7% (NC). A+T content is higher than 70% within 8 AE geographic populations (AE1~AE8), and is less than 70% in the others, which shows a difference in different geographic populations.

Sequence variable sites

The 242 conservative sites, 211 variable sites and 173 parsimony informative sites were detected in the CO1 gene fragment of 14 species in Amblyseiinae, while 262 conservative sites, 191 variable sites and 150 parsimony informative sites were detected in 7 species of this study.

There were 411 conservative sites, 42 variable sites and 9 parsimony informative sites, among 8 geographic

populations of AE (AE1 ~ AE8); 427 conservative sites, 26 variable sites and no parsimony informative site for the three geographic populations of EN (EN1~EN3); 443

conservative sites, 10 variable sites and no parsimony informative site detected for 2 geographic populations of AO.

Table III.- The content of COI gene of seven species in Amblyseiinae.

Species*	COI					
	T/%	C/%	A/%	G/%	A+T/%	Total
AE1	41.3	15.0	29.8	13.9	71.1	453.0
AE2	41.7	14.6	30.2	13.5	71.9	453.0
AE3	41.3	15.0	30.0	13.7	71.3	453.0
AE4	41.9	14.3	30.0	13.7	71.9	453.0
AE5	41.5	13.9	30.7	13.9	72.2	453.0
AE6	41.9	14.3	30.2	13.5	72.1	453.0
AE7	41.9	14.3	29.6	14.1	71.5	453.0
AE8	41.5	13.9	29.8	14.8	71.6	453.0
EN1	43.3	11.9	26.0	18.8	69.3	453.0
EN2	44.2	11.5	25.4	19.0	69.6	453.0
EN3	42.8	12.4	26.0	18.8	68.8	453.0
AO1	43.0	11.5	26.0	19.4	69.0	453.0
AO2	42.8	11.7	26.0	19.4	68.8	453.0
NC	44.2	11.3	25.4	19.2	69.6	453.0
SA	45.9	11.7	23.6	18.8	69.5	453.0
AT	43.3	13.2	26.3	17.2	69.6	453.0
NB	37.1	18.3	29.8	14.8	66.9	453.0
Avg.	42.4	13.4	27.8	16.4	70.2	453.0

*For full names of species, see Table I and II.

Table IV.- Nucleotides composition of COI gene partial sequences of the 17 populations in Amblyseiinae.

Species	First base frequency (%)					Second base frequency (%)					Third base frequency (%)				
	T-1	C-1	A-1	G-1	Pos#1	T-2	C-2	A-2	G-2	Pos#2	T-3	C-3	A-3	G-3	Pos#3
AE1	37	10.5	32.9	19.7	152.0	44	20.7	16.0	19.3	150.0	43	13.9	40.4	2.6	151.0
AE2	37	10.5	32.9	19.7	152.0	44	20.7	16.7	18.7	150.0	44	12.6	41.1	2.0	151.0
AE3	37	10.5	32.9	19.7	152.0	44	20.7	16.0	19.3	150.0	43	13.9	41.1	2.0	151.0
AE4	38	9.9	32.9	19.7	152.0	44	20.7	16.0	19.3	150.0	44	12.6	41.1	2.0	151.0
AE5	39	8.6	32.2	20.4	152.0	44	20.7	16.0	19.3	150.0	42	12.6	43.7	2.0	151.0
AE6	38	9.9	32.9	19.7	152.0	44	20.7	16.0	19.3	150.0	44	12.6	41.7	1.3	151.0
AE7	38	9.2	32.2	20.4	152.0	44	20.7	16.0	19.3	150.0	44	13.2	40.4	2.6	151.0
AE8	39	8.6	32.2	20.4	152.0	44	20.7	16.0	19.3	150.0	42	12.6	43.7	2.0	151.0
EN1	33	9.9	31.6	25.7	152.0	43	21.3	15.3	20.0	150.0	54	4.6	31.1	10.6	151.0
EN2	35	7.9	31.6	25.7	152.0	43	21.3	15.3	20.0	150.0	54	5.3	29.1	11.3	151.0
EN3	34	8.6	31.6	25.7	152.0	43	21.3	15.3	20.0	150.0	51	7.3	31.1	10.6	151.0
AO1	34	8.6	32.2	25.0	152.0	44	20.7	14.7	20.7	150.0	51	5.3	31.1	12.6	151.0
AO2	34	8.6	32.2	25.0	152.0	43	21.3	14.7	20.7	150.0	51	5.3	31.1	12.6	151.0
NC	36	7.2	28.9	28.3	152.0	44	20.7	15.3	20.0	150.0	53	6.0	31.8	9.3	151.0
SA	35	10.5	28.9	25.7	152.0	45	20.0	15.3	20.0	150.0	58	4.6	26.5	10.6	151.0
AT	36	7.9	31.6	24.3	152.0	44	21.3	16.0	18.7	150.0	50	10.6	31.1	8.6	151.0
NB	27	15.1	36.2	21.7	152.0	43	21.3	16.7	18.7	150.0	41	18.5	36.4	4.0	151.0
Avg.	35	9.6	32.1	22.9	152.0	44	20.9	15.7	19.6	150.0	48	9.9	35.6	6.5	151.0

*For full names of species, see Table I and II.

Genetic distance analysis

Genetic distance is shown in Table V. The results indicated that the intraspecific genetic distance ranged from 0.003 to 0.116 in 8 geographical populations of AE, of which had the smallest genetic distance was between AE2 and AE3, and AE2 and AE4, whereas the largest was between AE7 and AE8.

The intraspecific genetic distance ranged from 0.016 to 0.062 in 3 geographical populations of EN, of which the smallest genetic distance was between EN1 and EN3 and the largest one between EN2 and EN3. The intraspecific genetic distance between 2 geographical populations of AO was 0.015. The interspecific genetic distance between

KC and KE was the smallest (0.119), whereas the largest was 0.429 between NCA and NW.

Phylogenetic tree

Figures 2 and 3 shoe that the topological structure of phylogenetic trees constructed based on the two different phylogenetic analyses are the same. Samples of *Neoseiulus*, *Amblyseius*, *Scapulaseius*, *Kampimodromus* and *Euseius* are isolated from the outgroup *Metaseiulus occidentalis* (statistical value is 0.98). Likewise, *N. barkeri* and *N. womersleyi* were recovered separate from the other taxa (statistical value is 0.59).
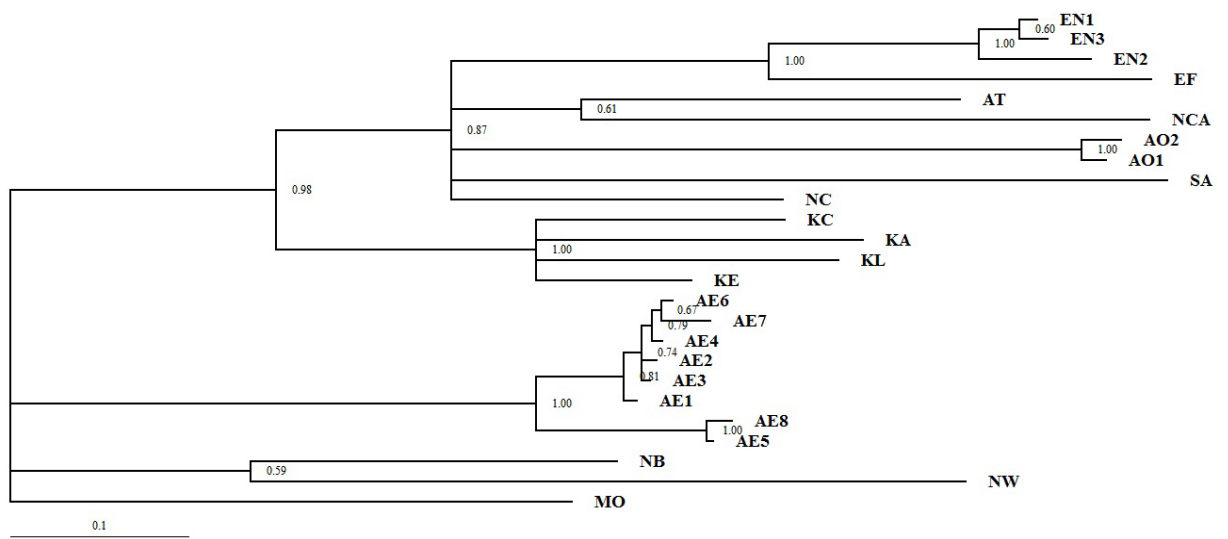

Fig. 2. BI trees based on CO1 gene of Amblyseiinae.

Fig. 3. ML trees based on CO1 gene of Amblyseiinae.

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Table V.- Pairwise distances and transition/transversion (above the diagonal) based on COI gene.

	AE1	AE2	AE3	AE4	AE5	AE6	AE7	AE8	AT1	AO1	AO2	NB1	NC1	SA1	EN1	EN2	EN3	KC	KA	KL	KE	NW	NCA
AE1																							
AE2	0.009																						
AE3	0.012	0.003																					
AE4	0.012	0.003	0.006																				
AE5	0.092	0.096	0.096	0.092																			
AE6	0.016	0.006	0.009	0.009	0.088																		
AE7	0.032	0.022	0.025	0.025	0.104	0.022																	
AE8	0.104	0.108	0.108	0.104	0.009	0.100	0.116																
AT1	0.353	0.353	0.353	0.347	0.365	0.353	0.353	0.384															
AO1	0.344	0.338	0.338	0.332	0.327	0.338	0.350	0.344	0.325														
AO2	0.355	0.349	0.349	0.343	0.337	0.349	0.361	0.355	0.299	0.015													
NB1	0.333	0.345	0.345	0.351	0.317	0.351	0.357	0.335	0.377	0.353	0.353												
NC1	0.310	0.321	0.327	0.316	0.309	0.316	0.321	0.326	0.300	0.268	0.258	0.362											
SA1	0.369	0.369	0.376	0.363	0.380	0.363	0.357	0.400	0.254	0.337	0.326	0.423	0.260										
EN1	0.316	0.316	0.322	0.311	0.300	0.311	0.322	0.317	0.274	0.267	0.262	0.305	0.227	0.264									
EN2	0.317	0.317	0.323	0.311	0.306	0.311	0.329	0.323	0.285	0.257	0.248	0.295	0.222	0.270	0.052								
EN3	0.311	0.322	0.328	0.316	0.300	0.316	0.340	0.317	0.274	0.283	0.272	0.300	0.232	0.280	0.016	0.062							
KC	0.294	0.294	0.294	0.289	0.284	0.289	0.289	0.300	0.294	0.336	0.330	0.333	0.224	0.288	0.207	0.221	0.217						
KA	0.315	0.315	0.315	0.309	0.332	0.326	0.320	0.349	0.253	0.268	0.272	0.351	0.239	0.248	0.264	0.289	0.274	0.168					
KL	0.310	0.304	0.304	0.299	0.299	0.304	0.315	0.316	0.277	0.248	0.253	0.373	0.254	0.288	0.220	0.253	0.234	0.169	0.190				
KE	0.278	0.278	0.278	0.273	0.249	0.273	0.300	0.264	0.288	0.298	0.303	0.334	0.220	0.293	0.255	0.259	0.265	0.119	0.160	0.147			
NW	0.288	0.288	0.288	0.283	0.288	0.283	0.315	0.304	0.372	0.379	0.391	0.323	0.371	0.414	0.311	0.323	0.323	0.277	0.347	0.328	0.290		
NCA	0.346	0.352	0.352	0.346	0.340	0.346	0.364	0.359	0.243	0.330	0.314	0.417	0.257	0.293	0.314	0.320	0.320	0.253	0.248	0.258	0.258	0.429	
EF	0.352	0.364	0.358	0.358	0.381	0.358	0.371	0.402	0.317	0.344	0.338	0.346	0.263	0.354	0.198	0.210	0.207	0.315	0.308	0.291	0.296	0.367	0.385

Four species of *Kampimodromus* and two species of *Euseius* are clustered into two different branches. KC and KE, KL and KA had close genetic relationship in *Kampimodromus*, whereas SA had close genetic relationship with *Neoseiulus* and *Amblyseius*.

The branch of 8 species of *Amblyseius* and *Neoseiulus* was chaotic, in which NB clustered into a branch with NW and isolated with others. AT and NCA clustered into a branch firstly and then gathered together with AO. NC had close genetic relationship with SA. It was the same for the branch conditions of the phylogenetic trees constructed by ML analysis and BI analysis, the trend was generally consistent.

It could be known from the branch of 5 genera of ingroup that the geographic populations of AE (AE1~AE8), EN (EN1~EN3) and AO (AO1 and AO2) clustered into a clade, respectively. As the phylogenetic tree constructed based on 2 different methods revealed that within EN, EN1 had closer phylogenetic relationships with EN3 compared with the EN2. The phylogenetic relationship within 8 geographical population of AE, AE2, AE3 and AE4 are close in Jiangxi province. They are also close between AE5 and AE8 in Guangdong province.

DISCUSSION AND CONCLUSION

The different geographical populations of *A. eharai*, *E. nicholsi* and *A. orientalis* were showed a certain degree of genetic differentiation. Based on the genetic distances and phylogenetic trees, the phylogenetic relationships between the populations agreed with their geographic distribution. Many studies have shown that geologic diversity will result in significant genetic variation among different populations, especially in populations of Amblyseiinae, that lack strong migratory ability (Ma *et al*., 2010; Meng, 2008).

The precise identification in Phytoseiidae is difficult due to little effective morphological features required to distinguish the closely related species. Therefore, there is not a perfect classification system to date for the family, subfamily, or genus in Phytoseiidae which is recognized by most taxonomists (Athias-Henriot, 1977, 1978; Tsolakis *et al*., 2012).

Four species of *Kampimodromus* in this study have been clustered into one branch, as well as two species of *Euseius*, which fit their morphological system. The adscription of the classification of *Amblyseius* and *Neoseiulus* has, however, been confusing for a long time. The genera were erected in 1914 and 1948, respectively, but the researchers took a long time to accept these within the taxonomic community. Moreover, there were some taxonomists that had different opinions on the classification. *Neoseiulus* was topically merged into *Amblyseius* or another genus (Muma, 1961; Wainsstein, 1962; Schicha, 1987), but after the 1990s, *Amblyseius* and *Neoseiulus* were treated as two independent genera and eventually (Moraes *et al*., 2004) divided into different subtribes, Neoseiulini (Chant and McMurtry, 2003a) and Amblyseiini (Chant and McMurtry, 2004). Wu *et al*. (2010) did not adopt this system and instead divided *Amblyseius* into two subgenera, *Amblyseius* and *Neoseiulus*, respectively. For instance, *N. barkeri* was merged into *Amblyseius* (Wu, 1980a), then recombined with *Neoseiulus* (Wu, 1986). However, it was regarded as a member of two genera by different scholars at the same time (Chant and McMurtry, 2003; Wu *et al*., 2009). *N. cucumeris* was also rearranged several times, which was firstly placed into *Amblyseius* (Chant, 1959), then was divided into *Typhlodromopsis* (Muma, 1961), but later was replaced in *Neoseiulus* in 1986 (Moraes *et al*., 1986). *A. tsugawai* and *A. orientalis* followed the same process, from *Amblyseius* (Amblyseiinae) to *Typhlodromus* (Typhlodrominae) (Hirschmann, 1962), and then shifted back to *Amblyseius* again (Chant and McMurtry, 1994; Moraes *et al*., 1986). *S. asiaticus* was grouped with *Typhlodromus* (Evans, 1953), then to *Amblyseius* and *Neoseiulus*, and ultimately to *Scapulaseius* (Wu, 1980b; Wu *et al*., 2010). Similar to their complex morphological taxonomic relationship, the molecular evolutionary relationship was also unclear in the present paper. They did not cluster into single group. *A. tsugawai*, *A. orientalis*, *S. asiaticus*, *N. cucumeris* and *N. californicus* were clustered with three *Euseius* species, while *N. womersleyi*, *N. barkeri* and *A. eharai* were far away with other phytoseiid mites. Judging from our results, the species of *Neoseiulus* and *Amblyseius* should be assigned carefully. In fact, the two genera were differentiated only based on whether the length of Z5 seta was longer than the half width of dorsal plate or not (Wu *et al*., 2009). More molecular data would be helpful to distinguish the two genera.

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REFERENCES

Ammerman, C.B., Hendrickson, R., Hall, G.M., Easley, J.F. and Loggins, P.E., 1965. The nutritive value of various fractions of citrus pulp and the effect of drying temperature on the nutritive value of citrus pulp. *Proc. Fla. State Hort. Soc.*, **78**: 307.

Amrine, J. and Manson, D., 1996. Preparation, mounting and descriptive study of eriophyoid mites. *World Crop Pests*, **6**: 383-396. https://doi.org/10.1016/S1572-4379(96)80023-6

Athias-Henriot, C., 1977. Nouvelles notes sur les Amblyseiini III. Sur le genre Cydnodromus: Redéfinition, composition [Parasitiformes, Phytoseiidae]. *BioControl*, **22**: 61-73.

Athias-Henriot, C., 1978. Définition deDictyonotus nov. gen., avec description de deux espèces nouvelles de France méridionale [Gamasides, Phytoseiidae]. *Entomophaga*, **23**: 189-194. https://doi.org/10.1007/BF02371727

Boore, J.L., 1999. Animal mitochondrial genomes. *Nucl. Acids Res.*, **27**: 1767-1780. https://doi.org/10.1093/nar/27.8.1767

Bourguignon, T., Lo, N., Cameron, S.L., Šobotník, J., Hayashi, Y., Shigenobu, S., Watanabe, D., Roisin, Y., Miura, T. and Evans, T.A., 2015. The evolutionary history of termites as inferred from 66 mitochondrial genomes. *Mol. Biol. Evolut.*, **32**: 406-421. https://doi.org/10.1093/molbev/msu308

Chant, D.A., 1959. Phytoseiid mites (Acarina: Phytoseiidae). *Mem. entomol. Soc. Canada*, **91(S12)**: 5-166. https://doi.org/10.4039/entm9112fv

Chant, D.A. and McMurtry, J.A., 1994. A review of the subfamilies Phytoseiinae and Typhlodrominae (Acari: Phytoseiidae). *Int. J. Acarol.*, **20**: 223-310. https://doi.org/10.1080/01647959408684022

Chant, D.A. and Mcmurtry, J.A., 2003a. A review of the subfamily Amblyseiinae Muma (Acari: Phytoseiidae): Part I. Neoseiulini new tribe. *Int. J. Acarol.*, **29**: 3-46. https://doi.org/10.1080/01647950308684319

Chant, D.A. and Mcmurtry, J.A., 2003b. A review of the subfamily Amblyseiinae Muma (Acari: Phytoseiidae): Part II. The tribe Kampimodromini Kolodochka. *Int. J. Acarol.*, **29**: 179-224. https://doi.org/10.1080/01647950308684331

Chant, D.A. and Mcmurtry, J.A., 2004a. A review of the subfamily Amblyseiinae Muma (Acari: Phytoseiidae): Part III. the tribe Amblyseiini Wainstein, subtribe Amblyseiina n. subtribe. *Int. J. Acarol.*, **30**: 171-228. https://doi.org/10.1080/01647950408684399

Chant, D.A. and Mcmurtry, J.A., 2004b. A review of the subfamily Amblyseiinae Muma (Acari: Phytoseiidae): part IV. Tribe Amblyseiini Wainstein, subtribe Arrenoseiina Chant and McMurtry. *Int. J. Acarol.*, **30**: 291-312. https://doi.org/10.1080/01647950408684388

Chant, D.A. and Mcmurtry, J.A., 2005a. A review of the subfamily Amblyseiinae Muma (Acari: Phytoseiidae): Part V. The tribe Amblyseiini Wainstein: Subtribe Proprioseiopsina Chant and McMurtry. *Int. J. Acarol.*, **31**: 3-22. https://doi.org/10.1080/01647950508684424

Chant, D.A. and Mcmurtry, J.A., 2005b. A review of the subfamily Amblyseiinae Muma (Acari: Phytoseiidae): Part VI. The tribe Euseiini n. tribe, subtribes Typhlodromalina n. subtribe, Euseiina n. subtribe, and Ricoseiina n. subtribe. *Int. J. Acarol.*, **31**: 187-224. https://doi.org/10.1080/01647950508683673

Chant, D.A. and Mcmurtry, J.A., 2005c. A review of the subfamily Amblyseiinae Muma (Acari: Phytoseiidae): Part VII. Typhlodromipsini n. tribe. *Int. J. Acarol.*, **31**: 315-340. https://doi.org/10.1080/01647950508684412

Chant, D.A. and Mcmurtry, J.A., 2006a. A review of the subfamily Amblyseiinae Muma (Acari: Phytoseiidae): Part VIII. The tribes Macroseiini Chant, Denmark and Baker, Phytoseiulini, n. tribe, Afroseiulini, n. tribe, and Indoseiulini Ehara and Amano. *Int. J. Acarol.*, **32**: 13-25. https://doi.org/10.1080/01647950608684439

Chant, D.A. and Mcmurtry, J.A., 2006b. A review of the subfamily Amblyseiinae Muma (Acari: Phytoseiidae): Part IX. An overview. *Int. J. Acarol.*, **32**: 125-152. https://doi.org/10.1080/01647950608684453

Chen, D.S., Jin, P.Y., Zhang, K.J., Ding, X.L., Yang, S.X., Ju, J.F., Zhao, J.Y. and Hong, X.Y., 2014. The complete mitochondrial genomes of six species of *Tetranychus* provide insights into the phylogeny and evolution of spider mites. *PLoS One*, **9**: e110625. https://doi.org/10.1371/journal.pone.0110625

Dabert, M., Witalinski, W., Kazmierski, A., Olszanowski, Z. and Dabert, J., 2010. Molecular phylogeny of acariform mites (Acari, Arachnida): Strong conflict between phylogenetic signal and long-branch attraction artifacts. *Mol. Phylogen. Evolut.*, **56**: 222-241. https://doi.org/10.1016/j.

ympev.2009.12.020

De Moraes, G.J., McMurtry, J.A., Denmark, H.A. and Campos, C.B., 2004. A revised catalog of the mite family Phytoseiidae. *Zootaxa,* **434**: 1-494. https://doi.org/10.11646/zootaxa.434.1.1

Ehara, S., 1958. Three predatory mites of the genus *Typhlodromus* from Japan (Phytoseiidae). *Annot. Zool. Japan*, **31**: 53-57.

Evans, G.O., 1953. On some mites of the genus Typhlodromus Scheuten, 1857, from S.E. Asia. *Annls. Mag. Nat. Hist.*, **6**: 449-467. https://doi.org/10.1080/00222935308654444

Felsenstein, J., 1985. Confidence limits on phylogenies: An approach using the bootstrap. *Evolution*, **39**: 783-791. https://doi.org/10.2307/2408678

Gu, X.B., Liu, G.H., Song, H.Q., Liu, T.Y., Yang G.Y. and Zhu, X.Q., 2014. The complete mitochondrial genome of the scab mite *Psoroptes cuniculi* (Arthropoda: Arachnida) provides insights into Acari phylogeny. *Parasites Vectors*, **7**: 340. https://doi.org/10.1186/1756-3305-7-340

Guo, B.Y., Yu, C., Zhang, C., Lv, Z.M., Xu, K.D., Ping, H.L. and Shi, H.L., 2017. Characterization of complete mitochondrial genome and phylogeny of *Sepia lycidas* (Sepioidea, Sepiidae). *Pakistan J. Zool.,* **50**: 1497-1508. http://dx.doi.org/10.17582/journal.pjz/2018.50.4.1497.1508

Hillis, D.M. and Bull, J.J., 1993. An empirical test of bootstrapping as a method for assessing confidence in phylogenetic analysis. *System. Biol.*, **42**: 182-192. https://doi.org/10.1093/sysbio/42.2.182

Hirschmann, W., 1962. *Gangystematik der parasitiformes. Teil 1. Rumpfberhaarung und Ruckenflachen. Acarologie Schritenreihe fur Vergeichende Milbenkunde*. Hirschmann, Furth/Bay, pp. 1-20.

Klompen, H., Lekveishvili, M. and Black, W.C., 2007. Phylogeny of parasitiform mites (Acari) based on rRNA. *Mol. Phylogen. Evolut*., **43**: 936-951. https://doi.org/10.1016/j.ympev.2006.10.024

Krantz, G.W. and Walter, D.E. (eds.), 2009. *A manual of acarology*, 3rd edition. Texas Tech University Press.

Kreipe, V., Corral-Hernández, E., Scheu, S., Schaefer, I. and Maraun, M., 2015. Phylogeny and species delineation in European species of the genus *Steganacarus* (Acari, Oribatida) using mitochondrial and nuclear markers. *Exp. appl. Acarol.*, **66**: 173-186. https://doi.org/10.1007/s10493-015-9905-4

Li, H., Shao, R., Song, N., Song, F., Jiang, P., Li, Z. and Cai, W., 2015. Higher-level phylogeny of paraneopteran insects inferred from mitochondrial genome sequences. *Scient. Rep*., **5**: 8527. https://doi.org/10.1038/srep08527

Ma, J., Li, T., Long, W.M., An, W.W., Guo, Y.P. and Ma, E.B., 2010. Genetic diversity of different geographical populations of oxya chinensis based on aflp analysis. *Yi Chuan*, **32**: 163-169. https://doi.org/10.3724/SP.J.1005.2010.00163

Mailloux, J., Le Bellec, F., Kreiter, S., Tixier, M.S. and Dubois, P., 2010. Influence of ground cover management on diversity and density of phytoseiid mites (Acari: Phytoseiidae) in Guadeloupean citrus orchards. *Exp. appl. Acarol.*, **52**: 275-290. https://doi.org/10.1007/s10493-010-9367-7

Meng, X., 2008. *Population genetic structure of* Chilo suppresalisa *(Walker) (Lepidoptera: Crambidae) in China inferred from microsatellile markers and mt DNA gene sequence*. Zhejiang Univerist, Zhejiang, Hangzhou.

Misof, B., Liu, S., Meusemann, K., Peters, R.S., Donath, A., Mayer, C., Frandsen, P. B., Ware, J., Flouri, T. and Beutel, R.G., 2014. Phylogenomics resolves the timing and pattern of insect evolution. *Science,* **346**: 763-767. https://doi.org/10.1126/science.1257570

Moraes, G.J.D., Mcmurtry J.A. and Denmark, H.A., 1986. *A catalog of the mite family Phytoseiidae. References to taxonomy, synonymy, distribution and habitat.* Empresa Brasileira de Pesquisa Agropecuaria, Bras'ilia.

Muma, M.H., 1961. Subfamilies, genera, and species of Phytoseiidae (Acarina: Mesostigmata). *Bull. Fla. State Mus. Biol. Sci.*, **5**: 267-302.

Murrell, A., Dobson, S.J., Walter, D.E., Campbell, N.J., Shao, R. and Barker, S.C., 2005. Relationships among the three major lineages of the Acari (Arthropoda: Arachnida) inferred from small subunit rRNA: Paraphyly of the Parasitiformes with respect to the Opilioacariformes and relative rates of nucleotide substitution. *Inverteb. System.*, **19**: 383-389. https://doi.org/10.1071/IS05027

Navajas, M., Gutierrez, J., Lagnel, J. and Boursot, P., 1996. Mitochondrial cytochrome oxidase I in tetranychid mites: a comparison between molecular phylogeny and changes of morphological and life history traits. *Bull. entomol. Res*., **86**: 407-417. https://doi.org/10.1017/S0007485300034994

Oudemans, A., 1936. Neues über Anystidae (Acari). *Arch. Naturgesch*., **5**: 364-446.

Pepato, A. and Klimov, P., 2015. Origin and higher-level diversification of acariform mites-evidence from nuclear ribosomal genes, extensive taxon sampling, and secondary structure alignment. *BMC*

Evolut. Biol., **15**: 178. https://doi.org/10.1186/s12862-015-0458-2

Ronquist, F. and Huelsenbeck, J.P., 2003 MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics*, **19**: 1572-1574. https://doi.org/10.1093/bioinformatics/btg180

Schicha, E., 1987. *Phytoseiidae of Australia and neighboring areas.* Indira Publishing House, Bhopal, India.

Song, H., Sheffield, N.C., Cameron, S.L., Miller, K.B. and Whiting, M.F., 2010. When phylogenetic assumptions are violated: base compositional heterogeneity and among-site rate variation in beetle mitochondrial phylogenomics. *System. Ent*., **35**: 429-448. https://doi.org/10.1111/j.1365-3113.2009.00517.x

Swofford, D.L., 2002. *Paup: Phylogenetic analysis using parsimony* (and other methods) 4.0. B5.

Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M. and Kumar, S., 2011. MEGA5: Molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Mol. Biol. Evolut*., **28**: 2731-2739. https://doi.org/10.1093/molbev/msr121

Thompson, J.D., Higgins, D.G. and Gibson, T.J., 1994. CLUSTAL W: Improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucl. Acids Res*., **22**: 4673-4680. https://doi.org/10.1093/nar/22.22.4673

Tsolakis, H., Tixier, M.S., Kreiter, S. and Ragusa, S., 2012. The concept of genus within the family Phytoseiidae (Acari: Parasitiformes): Historical review and phylogenetic analyses of the genus Neoseiulus Hughes. *Zool. J. Linn. Soc*., **165**: 253-273. https://doi.org/10.1111/j.1096-3642.2011.00809.x

Vitzthum, H.G., 1941. *Acarina in Bronn's Klassen und Ordnungen des Tierreichs 5*. Akad. Verlag, M.B.H., Leipzing, pp. 764-768.

Wainstein, B.A., 1962. Revision du genre Typhlodromus Sceuten, 1857 et systematique de la familie des Phytoseiidae (Berlese, 1916) (Acarina: Parasitiformes). *Acarologia*, **4**: 5-30.

Walsh, P.S., Metzger, D.A. and Higuchi, R., 1991. Chelex 100 as a medium for simple extraction of DNA for PCR-based typing from forensic material. *Biotechniques*, **10**: 506-513.

Wei, S.J., Li, Q., Achterberg, K. and Chen, X.X., 2014. Two mitochondrial genomes from the families Bethylidae and Mutillidae: Independent rearrangement of protein-coding genes and higher-level phylogeny of the Hymenoptera. *Mol. phylogen. Evolut*., **77**: 1-10. https://doi.org/10.1016/j.ympev.2014.03.023

Wu, W.N., 1980a. A new species of the genus *Phytoseius* (Acarina: Phytoseiidae). *Zool. Res*., **1**: 243-246.

Wu, W.N., 1980b. Notes on the genus *Amblyseius* Berlese from China (Acarina: Phytoseiidae). *Nat. Enem. Insects*, **2**: 39-50.

Wu, W.N., 1986. A new species and a new record of *Amblyseius* from Fujian (Acarina: Phytoseiidae). *Wuyi Sci. J*., **6**: 121-124.

Wu, W.N., Liang, L.R., Fang, X.D. and Ou, J.F., 2010. Phytoseiidae (Acari: Mesostigmata) of China: A review of progress, with a checklist. *Progr. Chinese Acarol. Zoosymp*., **4**: 288-315.

Wu, W.N. and Ou, J.F., 2001. The obtusus species group of the genus *Amblyseius* (Acari: Phytoseiidae), with descriptions of two new species in China. *System. appl. Acarol*., **6**: 101-108. https://doi.org/10.11158/saa.6.1.13

Wu, W.N., Ou, J.F. and Huang, J.L., 2009. *Fauna sinica, invertebrate*, Vol. 47, *Arachnida, Acari, Phytoseiidae*. China Press, Kuala Lumpur, Malaysia.

Xin, J.L., 1988. *Agricultural acarology*. Agriculture Press, Beijing, pp. 465.

Xu, X.N., Jiang, X.H. and Wang, E.D., 2010. Application of phytoseiid mites in China and an analysis of its problems. *Zoosymposia*, **4**: 316-328.