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RESEARCH ARTICLE

MOLECULAR IDENTIFICATION AND PHYLOGEOGRAPHIC RELATIONSHIPS OF *PORCELLIO LAEVIS* AND *PYCNOSCELUS SURINAMENSIS* BY USING MITOCHONDRIAL *CO1* SEQUENCE IN QENA GOVERNORATE, EGYPT

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ABSTRACT

Arthropods are the most diverse animal phylum and prevalent in nearly all the environments on the Earth. Soil macroarthropods play key roles in several supporting and regulating ecosystem services. Two widely-distributed species of terrestrial arthropods (*Porcellio laevis* and *Pycnoscelus surinamensis*) were collected from four areas in Qena governorate (Egypt), identified, and their phylogeographic relationships were carried out using mitochondrial cytochrome C oxidase subunit 1 (*CO1*) sequence. The *CO1* sequence of *P. laevis* had a nucleotide length between 641 base pair (bp) and 664 bp; while in *P. surinamensis* it varied from 648 bp to 654 bp. The average A+T content in *P. laevis* and *P. surinamensis* was 62.37% and 62.55%, respectively. The pairwise genetic distances among *P. laevis* specimens varied from 0.00 to 0.0235, with the most closely related locations being Naga Hammadi and El-Taramsa. The genetic distance of *P. surinamensis* ranged from 0.0000 to 0.0045, with the most related sites being South Valley University farm and Qus city.

INTRODUCTION

Arthropods are arguably the best-known organisms on Earth and have garnered the attention in several manners. Their phylogenetic relationships have been examined for decades, and the evolutionary implications of arthropod phylogeny predate the emergence of transcriptomic and genomic data that enhanced our understanding of the relationships among chelicerates, myriapods, crustaceans, and insects[1].

Within arthropods, soil isopods are regarded as potential bioindicators of soil quality in agroecosystems[2]. They are widespread and, in the majority of instances, the dominant component of detritivores in temperate areas[3-5]. Terrestrial isopods are anticipated to exhibit restricted gene flow within natural populations due to their low dispersal rates, brood their young in a pouch, and dependence on moist microhabitats[6,7]. The consequence of diminished gene flow is that subpopulations may exhibit genetic divergence. The cosmopolitan terrestrial isopod *Porcellio laevis*, part of family Porcellionidae, is widely dispersed over the

globe including regions such as some parts of America, Western Asia, Australia, Japan, and some Pacific islands leading to a complex synonymy[8].

The insect fauna constitutes the largest proportion of both arthropod biodiversity and the total animal populations on Earth. Cockroaches (Blattodea) are an insect order that includes numerous highly adaptable species, several of which are peridomestic pests introduced in various countries throughout the world[9]. The species *Pycnoscelus surinamensis* is a member of family Blaberidae. It is an invasive species in India, recognized for its medical and economic significance and has recently invaded large areas of the tropical and sub-tropical regions due to human activities[10-12]. The Surinam cockroach is recognized as a plant pest, a biological vector, and for its biological associations[13].

Species identification is an essential aspect of understanding and portraying biodiversity[14]. DNA barcoding is highly effective at discriminating a constrained set of species including those found in restricted habitats agriculture species and invasive species[15,16]. The evaluation of the genetic divergences of a species is a crucial metric for its conservation, sustenance, and further genetic enhancement[17].

Phylogeography was created as a hybrid discipline linking historical demographic events to external or environmental factors that have impacted the spatial distribution of genetic diversity within and among populations over extensive temporal ranges[18]. The mutation rate of mito-chondrial DNA in animals surpasses that most nuclear genes utilized in phylogenetic and phylogeographic research. Thus, it enables the identification of divergences within species with high resolution for the past few million years. The mitochondrial cytochrome c oxidase subunit 1 (*CO1*) gene facilitates inferring genetic changes among populations and recently elected as the standardized instrument for molecular taxonomy and identification across all animal phyla[19]. Thus, the current study aimed to molecularly identify the widely-distributed species of terrestrial arthropods (*P. laevis* and *P. surinamensis*) collected from four locations in Qena governorate and achieve their phylogeographic relationships using mitochondrial cytochrome C oxidase subunit 1 (*CO1*) sequence.

MATERIAL AND METHODS

Sampling

Two widely-distributed species of terrestrial macroarthropods were collected from Egypt; three samples of *P. laevis* and four samples of *P. surinamensis*. The two species were identified morphologically as *P. laevis*[20] and *P. surinamensis*[21]. *P. laevis* specimens were collected from three locations: Naga Hammadi city (26º05' N and 32º23' E), South Valley University farm in Qena city (26º19' N and 32º73' E), and El-Taramsa Village (26º14' N and 32º70' E). Specimens of *P. surinamensis* were obtained from four sites: Naga Hammadi city, South Valley University farm, El Taramsa Village, and Qus city (25º95' N and 32º78' E). Hand sorting method was carried out to catch the two species from the collected soil samples using a metal cube measuring 20×20×20 cm. The collected specimens were kept at –20°C until next DNA extraction. All animal experiments were carried out according to the Institutional Animals Ethics Committee (Faculty of Science, South Valley University, Qena, Egypt; Ethical reference number: 005/07/24).

DNA extraction and polymerase chain reaction (PCR) conditions

The preserved leg tissues of the specimens were utilized to extract the genomic DNA using the Qiagen DNA Mini kit (Hilden, Germany) following the manufacturer's instructions. The primers LCO1490 and HCO2198 were utilized to amplify the mitochondrial *CO1* gene[22]. The PCR reactions contained 1.0 µL of each forward and reverse primers, 20 µL of PCR master mix and 1.0 µL of genomic DNA in a total reaction volume of 40 µL. The PCR procedure included an initial denaturation

phase lasting 3 minutes at 94°C. Thirty-five cycles of denaturation for 1.0 minute at 94°C, annealing for 1.0 minute at 50°C, and extension for 1.0 minute at 72°C were conducted. A concluding extension at 72ºC for 7 minutes was performed. Agarose gel (1.5%) containing ethidium bromide was utilized to segregate the amplified products, with a 100 base pair (bp) DNA ladder serving as a DNA marker.

PCR sequencing

Macrogen Inc. (Seoul, Korea) performed all DNA sequencing using the same primer used for amplification. To gain accession numbers, the sequences were submitted to the National Center for Biotechnology Information (GenBank/NCBI). Muscles were used to align the sequences[23] with the default settings. The nucleotide frequencies, AT and CG contents, and phylogenetic tree analyses were carried out with MEGA version 7.0[24], employing the neighbor joining (NJ) and UPGMA tree construction methods, as well as 1000 bootstrap iterations[25], to enhance the depiction of phylogenetic relationships. Kimura two parameter distances were used to calculate sequence divergences[26].

RESULTS

CO1* sequencing, genetic distances, and phylogenetic tree of *P. laevis

The *CO1* sequencing of *P. laevis* obtained from Naga Hammadi city, South Valley University farm and El-Taramsa village, yielded nucleotide length varied from 641 bp to 664 bp. Naga Hammadi site possesses the shortest sequences (641 bp), whereas the longest sequence was recorded at El-Taramsa village. An accession numbers from OR223804 to OR223806 of the *CO1* partial nucleotide sequences were obtained as they submitted in the GenBank. Adenine (A), cytosine (C), guanine (G), and thymine (T) bases gave nucleotide frequencies averages of 25.36%, 15.95%, 21.68%, and 37.01%, respectively. The mean content of A+T was 62.37%, surpassing that of C+G. The pyrimidines (C+T) bases showed an average content of 52.96%, exceeding that of purines. Additional information regarding nucleotide frequencies, A+T content, pyrimidine content, and their averages is provided in Table (1). The final alignments comprised 667 bp, of which 562 were conserved sites and 89 were varied sites (Figure 1).

As the *CO1* sequences of *P. laevis* from the three locations were presented to BLAST/N at (NCBI), we noticed 11 related species: two species (*P. laevis* and *P. imbutus*) from family Porcellionidae, one species (*Hemilepistus elongatus*) from family Agnaridae, one species (*Amphisopus lintoni*) from family Amphisopidae, one species (*Halophiloscia couchii*) from family Halophilosciidae, three species (*Idotea urotoma*, *Idotea resecata*, and *Synisoma capito*) from family Idoteidae, three species (*Mongoloniscus sinensis*, *Trachelipus kytherensis*, and *Trachelipus rathkii*) from family Trachelipodidae; and three out-group species (*Niphargus croaticus, Niphargus fontanus*, and *Niphargus zagrebensis*) from family Niphargidae (Table 2).

The current specimens of *P. laevis* showed pairwise genetic distances varied

Table 1: Accession number, nucleotide frequencies, and their averages of mitochondrial *CO1* gene in three sites of *P. laevis*; bp: base pair.

Sites	Accession Number	Length (bp)	Nucleotide (%)				A+T (%)	C+T (%)
			A	T	C	G		
Naga Hammadi City	OR223804.1	641	25.74	36.82	16.22	21.22	62.56	37.44
South Valley University	OR223805.1	651	24.73	37.02	15.51	22.74	61.75	38.25
El-Taramsa Village	OR223806.1	664	25.60	37.20	16.12	21.08	62.80	37.20
Average (%)		652	25.36	37.01	15.95	21.68	62.37	37.63

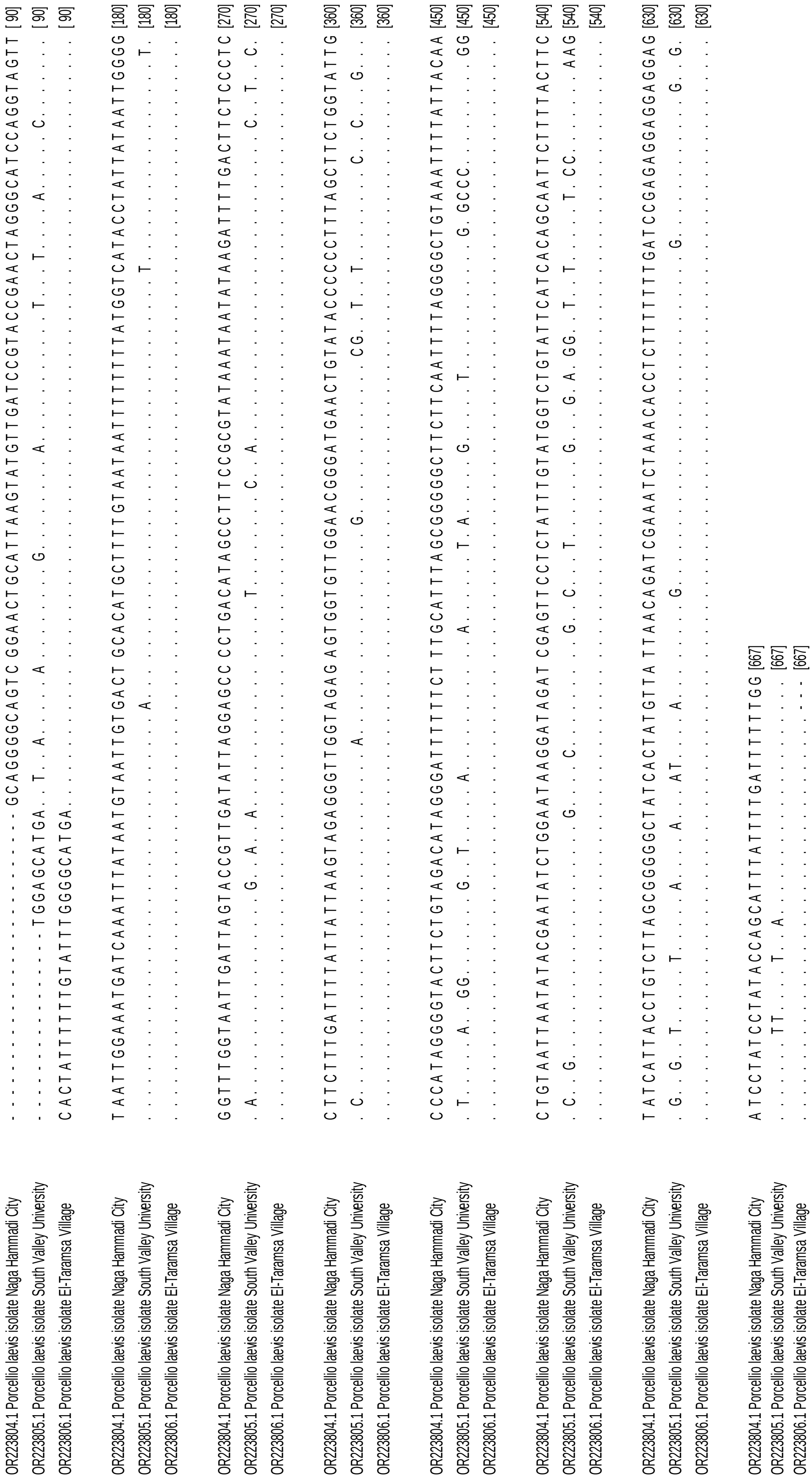

Figure 1: Alignment of partial sequences of mitochondrial *CO1* gene in *P. laevis*. Dots indicate the identical nucleotides.

Table 2: The understudied *P. laevis* and *P. surinamensis* with their related species, in addition to the out-group species from the GenBank/NCBI based on mitochondrial *CO1* genes sequences.

Species	Accession Number	Species	Accession Number
P. laevis isolate Naga Hammadi City	OR223804.1	*P. surinamensis* isolate Naga Hammadi City	OR223807.1
P. laevis isolate South Valley University	OR223805.1	*P. surinamensis* isolate South Valley University	OR223808.1
P. laevis isolate El-Taramsa Village	OR223806.1	*P. surinamensis* isolate El-Taramsa Village	OR223809.1
P. imbutus	FN824125.1	*P. surinamensis* isolate Qus City	OR223810.1
Hemilepistus elongates	ON212528.1	*P. surinamensis*	MW535114.1
Amphisopus lintoni	JX519295.1	*Blattella germanica*	HM996892.1
Halophiloscia couchii	KJ668172.1	*P. indicus*	MF149711.1
Idotea urotoma	KU530527.1	*Blaberus discoidalis*	KF372514.1
Mongoloniscus sinensis	KT424028.1	*Cosmozosteria trifasciata*	OM109219.1
Idotea resecata	JX545468.1	*Pseudophoraspis recurvata*	MH755954.1
P. laevis	MN689283.1	*Eublaberus posticus*	MF136387.1
Synisoma capito	FJ905097.1	*Phortioeca nimbata*	KF372533.1
Trachelipus kytherensis	EF027430.1	*Hemiblabera pabulator*	MK936717.1
Trachelipus rathkii	MK852336.1	*Appias albino*	KJ422876.1
Niphargus croaticus	KT007325.1	*Appias paulina*	KF226289.1
Niphargus fontanus	KC315631.1	*Appias indra*	KJ422880.1
Niphargus zagrebensis	OK156940.1		

from 0.0000 to 0.0235. The most associated sites were Naga Hammadi city and El-Taramsa village, with a genetic distance of 0.00. On the other hand, the least related locations were both "Naga Hammadi and El-Taramsa village" and South Valley University farm, where its genetic distance was 0.0235. The current *P. laevis* pairwise distances and the other related species of isopods varied from 0.00 to 0.0354, and the mean distance value was 0.26%. Among the 11 related isopod species, *P. laevis* (MN689283.1) exhibited the closest genetic relationship to the understudied species, then *Mongoloniscus sinensis* (KT424028.1), whilst *Amphisopus lintoni* (JX519295.1) was the most distantly associated species (Table 3).

For the phylogenetic tree analysis utilizing *CO1* sequencing, *P. laevis* specimens from the three locations were analyzed alongside sequences from the 11 related species and the out-group species sourced from GenBank/NCBI (as presented in Table 2). To enhance the depiction of phylogenetic relationships, we employed multiple phylogenetic methods (NJ and UPGMA) utilizing the *CO1* gene. The techniques exhibited virtually identical relationships, with modest variations in support values, and identified four primary features: the out-group species constituted a distinct cluster, the present *P. laevis* of El-Taramsa village constituted a sister clade to *P. laevis* of Naga Hammadi city, *P. laevis* (MN689283.1) and *Mongoloniscus sinensis* (KT424028.1) were near to *P. laevis* of El-Taramsa village and Naga Hammadi city, and *P. laevis* of South Valley University farm constituted a distinct clade, different from the remainder of *P. laevis* (Figures 2 and 3).

Table 3: Pairwise distances based on mitochondrial *CO1* gene among three *P. laevis* sites and the related species, in addition to the out-group.

		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
1	OR223804.1_Porcellio_laevis_isolate_Naga_Hammadi_City		0.0235	0.0000	0.0354	0.0312	0.0277	0.0266	0.0185	0.0307	0.0278	0.0000	0.0321	0.0258	0.0271	0.0343	0.0346	0.0332
2	OR223805.1_Porcellio_laevis_isolate_South_Valley_University	0.1757		0.0235	0.0371	0.0349	0.0283	0.0285	0.0215	0.0346	0.0287	0.0235	0.0336	0.0323	0.0327	0.0402	0.0414	0.0367
3	OR223806.1_Porcellio_laevis_isolate_El-Taramsa_Village	0.0000	0.1757		0.0354	0.0312	0.0277	0.0266	0.0185	0.0307	0.0278	0.0000	0.0321	0.0258	0.0271	0.0343	0.0346	0.0332
4	JX519295.1_Amphisopus_lirtoni	0.2995	0.3220	0.2995		0.0298	0.0290	0.0287	0.0335	0.0348	0.0375	0.0354	0.0345	0.0308	0.0333	0.0343	0.0345	0.0349
5	KJ668172.1_Halophiloscia_couchii	0.2616	0.2987	0.2616	0.2675		0.0273	0.0281	0.0293	0.0302	0.0358	0.0312	0.0341	0.0290	0.0326	0.0319	0.0384	0.0400
6	ON212528.1_Hemilepistus_elongatus	0.2259	0.2458	0.2259	0.2565	0.2333		0.0239	0.0257	0.0288	0.0267	0.0277	0.0293	0.0247	0.0289	0.0351	0.0369	0.0344
7	KU530527.1_Idotea_urotoma	0.2292	0.2425	0.2292	0.2562	0.2263	0.1976		0.0260	0.0197	0.0320	0.0266	0.0216	0.0271	0.0292	0.0361	0.0351	0.0344
8	KT424028.1_Mongoloniscus_sinensis	0.1198	0.1595	0.1198	0.2848	0.2469	0.2132	0.2220		0.0336	0.0318	0.0185	0.0331	0.0277	0.0302	0.0367	0.0356	0.0322
9	JX545468.1_Idotea_resecata	0.2490	0.2981	0.2490	0.3110	0.2460	0.2443	0.1446	0.2754		0.0344	0.0307	0.0248	0.0304	0.0302	0.0370	0.0402	0.0388
10	FN824125.1_Porcellio_imbutus	0.2262	0.2405	0.2262	0.3329	0.2984	0.2182	0.2631	0.2528	0.2939		0.0278	0.0311	0.0309	0.0338	0.0433	0.0450	0.0424
11	MN689283.1_Porcellio_laevis	0.0000	0.1757	0.0000	0.2995	0.2616	0.2259	0.2292	0.1198	0.2490	0.2262		0.0321	0.0258	0.0271	0.0343	0.0346	0.0332
12	FJ905097.1_Synisoma_capito	0.2662	0.2864	0.2662	0.3038	0.2846	0.2516	0.1630	0.2688	0.1950	0.2632	0.2662		0.0279	0.0304	0.0392	0.0430	0.0372
13	EF027430.1_Trachelipus_kytherensis	0.2126	0.2812	0.2126	0.2688	0.2329	0.2011	0.2256	0.2220	0.2552	0.2736	0.2126	0.2363		0.0236	0.0345	0.0406	0.0347
14	MK852336.1_Trachelipus_rathkii	0.2182	0.2768	0.2182	0.2892	0.2626	0.2376	0.2537	0.2500	0.2511	0.2851	0.2182	0.2607	0.1874		0.0332	0.0349	0.0338
15	KT007325.1_Niphargus_croaticus	0.3017	0.3613	0.3017	0.3174	0.2768	0.3112	0.3278	0.3358	0.3277	0.3921	0.3017	0.3401	0.2976	0.2913		0.0197	0.0194
16	KC315631.1_Niphargus_fontanus	0.3072	0.3743	0.3072	0.3132	0.3368	0.3329	0.3247	0.3217	0.3703	0.4127	0.3072	0.3862	0.3515	0.3115	0.1437		0.0215
17	OK156940.1_Niphargus_zagrebensis	0.2960	0.3315	0.2960	0.3205	0.3469	0.3007	0.3095	0.2897	0.3428	0.3749	0.2960	0.3281	0.3070	0.3068	0.1431	0.1645	

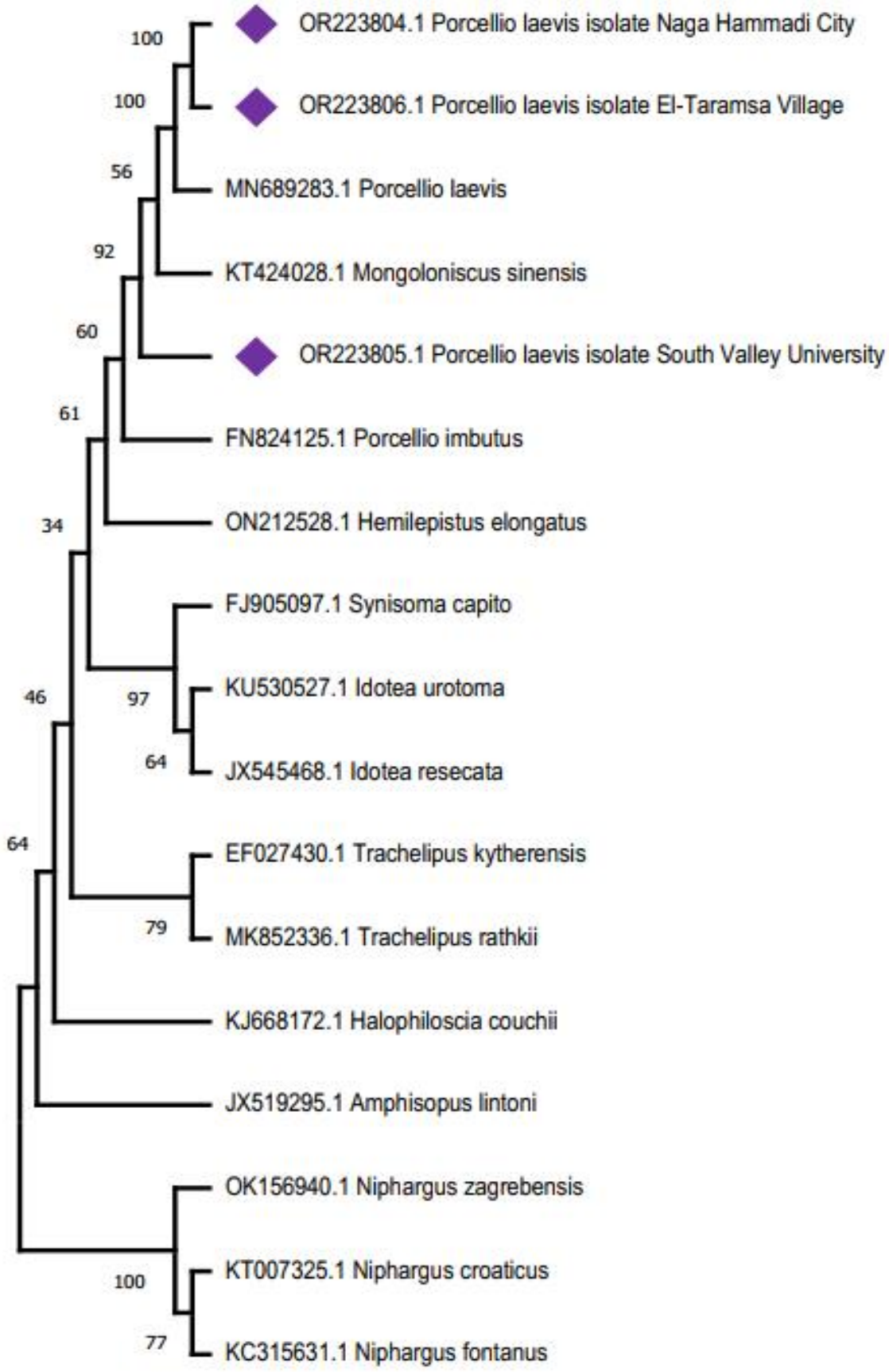

Figure 2: Phylogenetic tree using the neighbor joining method among *P. laevis* specimens from three sites based on mitochondrial *CO1* gene.

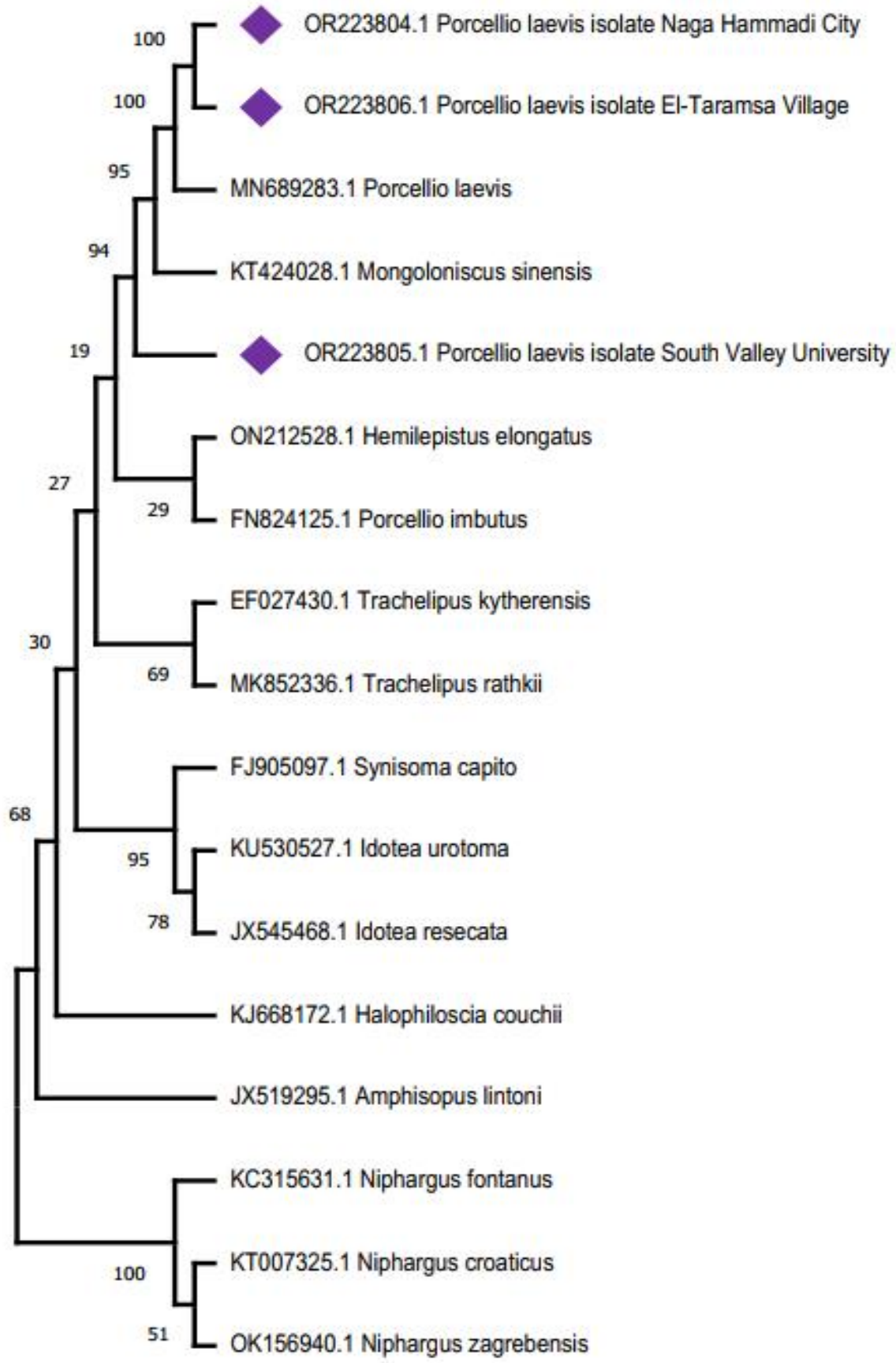

Figure 3: Phylogenetic tree using UPGMA method among *P. laevis* specimens from three sites based on mitochondrial *CO1* gene.

CO1* sequencing, genetic distances, and phylogenetic tree of *P. surinamensis

The sequencing of the *CO1* gene in *P. surinamensis*, collected from sites Naga Hammadi, South Valley University farm, El-Taramsa village, and Qus city showed nucleotide lengths varied from 648 bp to 654 bp. The findings revealed that South Valley University farm and Qus possess the shortest nucleotide sequences (648 bp). As the *CO1* partial nucleotide sequences were submitted to the GenBank, the accession numbers from OR223807 to OR2238010 were stated. The A, C, G, and T bases gave 27.55%, 19.75%, 17.71%, and 35.00% mean nucleotide frequencies, respectively. The average content of A+T was 62.55%, exceeding that of C+G content. The mean content of pyrimidines bases (C+T) was 54.75%, which surpassing that of purines, and more details were provided in Table (4). The resulting alignments consisted of 656 bp; 641 bp of them were conserved locations and the other 13 were variable sites (Figure 4).

The *CO1* sequences of *P. surinamensis* were analyzed using BLAST/N at NCBI identifying nine related species within order Blattodea comprising seven species from family Blaberidae (*Eublaberus posticus*, *P. surinamensis*, *P. indicus*, *Pseudophoraspis recurvata*, *Blaberus discoidalis*, *Phortioeca nimbata*, and *Hemiblabera pabulator*), one species (*Blattella germanica*) from family Ectobiidae, and one species (*Cosmozosteria trifasciata*) belonging to family Blattidae; in addition to the out-group species (*Appias albina*, *Appias paulina*, and *Appias indra*) from the family Pieridae (Table 2).

Pairwise genetic distances among *P. surinamensis* from the four sites varied from 0.0000 to 0.0045. The locations with the closest genetic relationship were South Valley University farm and Qus, with a genetic distance of 0.0000, whilst the sites with the greatest genetic divergence were El-Taramsa village and Naga Hammadi city, as the genetic distance was 0.0045. Among the present species *P. surinamensis* and its allied Blattodea species, the pairwise distances varied from 0.0060 to 0.0281, and the average distance value was 0.21%. From the nine associated species, the genetically closest one to the current species was *P. surinamensis* (MW535114.1), whereas *Phortioeca nimbata* (KF372533.1) was the most distantly regarded species (Table 5).

The phylogenetic tree analysis utilizing *CO1* sequencing involved the four *P. surinamensis* species, together with the sequences from the nine related species and the out-group ones received from GenBank/NCBI (as mentioned in Table 5). To elucidate phylogenetic relationships, we employed multiple phylogenetic methods (NJ, and UPGMA) based on *CO1* gene. The techniques demonstrated virtually identical relationships, with modest variations in favor values, and identified three basic features: the out-group species constituted a distinct cluster, *P. surinamensis* from South Valley University farm, Qus city and Naga Hammadi site constituted a related group, and *P. surinamensis* from El-Taramsa village form a distinct clade, apart from other *P. surinamensis* (Figures 5 and 6).

Table 4: Accession number and nucleotide frequencies and their averages of mitochondrial *CO1* gene in four sites of *P. surinamensis*; bp: base pair.

Sites	Accession Number	Length (bp)	Nucleotide (%)				A+T (%)	C+T (%)
			A	T	C	G		
Naga Hammadi City	OR223807.1	653	27.11	34.92	19.91	18.07	62.03	37.97
South Valley University	OR223808.1	648	27.62	35.19	19.75	17.44	62.81	37.19
El-Taramsa Village	OR223809.1	654	27.98	35.02	19.72	17.28	63.00	37.00
Qus City	OR2238010.1	648	27.47	34.88	19.60	18.06	62.35	37.65
Average (%)		650.75	27.55	35.00	19.75	17.71	62.55	37.45

Figure 4: Alignment of partial sequences of mitochondrial *CO1* gene in *P. surinamensis*. Dots indicate the identical nucleotides.

Table 5: Pairwise distances based on mitochondrial *CO1* gene among four *P. surinamensis* sites and the related species, in addition to the out-group.

		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1	OR223807.1_Pycnoscelus_surinamensis_isolate_Naga_Hammadi_City		0.0023	0.0023	0.0045	0.0078	0.0092	0.0130	0.0248	0.0270	0.0253	0.0259	0.0281	0.0276	0.0352	0.0368	0.0372
2	OR223810.1_Pycnoscelus_surinamensis_isolate_Qus_City	0.0033		0.0000	0.0037	0.0073	0.0088	0.0127	0.0243	0.0264	0.0247	0.0253	0.0276	0.0271	0.0348	0.0363	0.0369
3	OR223808.1_Pycnoscelus_surinamensis_isolate_South_Valley_University	0.0033	0.0000		0.0037	0.0073	0.0088	0.0127	0.0243	0.0264	0.0247	0.0253	0.0276	0.0271	0.0348	0.0363	0.0369
4	OR223809.1_Pycnoscelus_surinamensis_isolate_El-Taramsa_Village	0.0117	0.0083	0.0083		0.0060	0.0076	0.0113	0.0238	0.0255	0.0239	0.0250	0.0268	0.0262	0.0334	0.0345	0.0352
5	MW535114.1_Pycnoscelus_surinamensis	0.0330	0.0295	0.0295	0.0204		0.0057	0.0110	0.0223	0.0249	0.0242	0.0236	0.0267	0.0256	0.0318	0.0335	0.0331
6	HM996892.1_Blattella_germanica	0.0462	0.0426	0.0426	0.0332	0.0187		0.0119	0.0230	0.0240	0.0235	0.0225	0.0267	0.0254	0.0321	0.0321	0.0327
7	MF149711.1_Pycnoscelus_indicus	0.0742	0.0704	0.0704	0.0600	0.0561	0.0664		0.0219	0.0281	0.0265	0.0239	0.0288	0.0253	0.0344	0.0332	0.0324
8	KF372514.1_Blaberus_discoidalis	0.2231	0.2178	0.2178	0.2092	0.1927	0.1982	0.1825		0.0299	0.0225	0.0211	0.0253	0.0230	0.0298	0.0307	0.0333
9	OM1092191_Cosmozosteria_trifasciata	0.2359	0.2304	0.2304	0.2189	0.2132	0.2023	0.2389	0.2709		0.0248	0.0301	0.0261	0.0296	0.0301	0.0327	0.0355
10	MH755954.1_Pseudophoraspis_recurvata	0.2281	0.2226	0.2226	0.2112	0.2085	0.1948	0.2345	0.1878	0.2089		0.0256	0.0268	0.0268	0.0326	0.0312	0.0321
11	MF136387.1_Eublaberus_posticus	0.2276	0.2223	0.2223	0.2163	0.2022	0.1910	0.2053	0.1663	0.2751	0.2195		0.0247	0.0239	0.0319	0.0314	0.0348
12	KF372533.1_Phortioeca_nimbata	0.2475	0.2419	0.2419	0.2330	0.2328	0.2299	0.2475	0.2246	0.2351	0.2325	0.2193		0.0265	0.0294	0.0335	0.0353
13	MK936717.1_Hemiblabera_pabulator	0.2318	0.2264	0.2264	0.2175	0.2173	0.2144	0.2118	0.1874	0.2575	0.2294	0.2045	0.2321		0.0373	0.0385	0.0373
14	KJ422876.1_Appias_albina	0.3191	0.3128	0.3128	0.2986	0.2781	0.2814	0.3007	0.2719	0.2828	0.2901	0.2880	0.2594	0.3346		0.0143	0.0163
15	KF226289.1_Appias_paulina	0.3531	0.3463	0.3463	0.3276	0.3102	0.2965	0.3064	0.2774	0.3016	0.2859	0.2880	0.3062	0.3575	0.0927		0.0156
16	KJ422880.1_Appias_indra	0.3531	0.3463	0.3463	0.3313	0.3067	0.3033	0.2996	0.3030	0.3180	0.2967	0.3143	0.3226	0.3459	0.1094	0.1008	

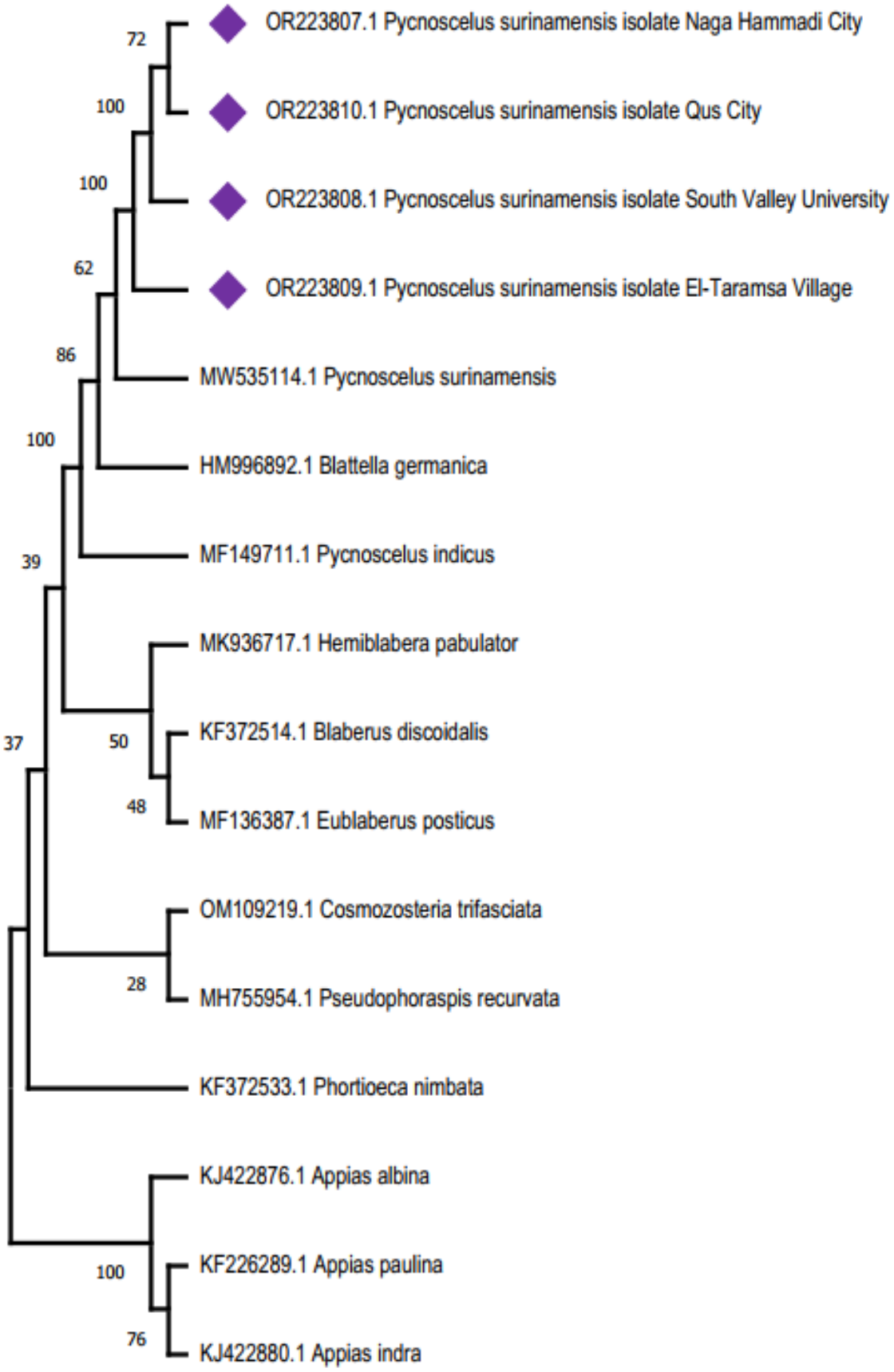

Figure 5: Phylogenetic tree using the neighbor joining method among *P. surinamensis* specimens from four sites based on mitochondrial *CO1* gene.

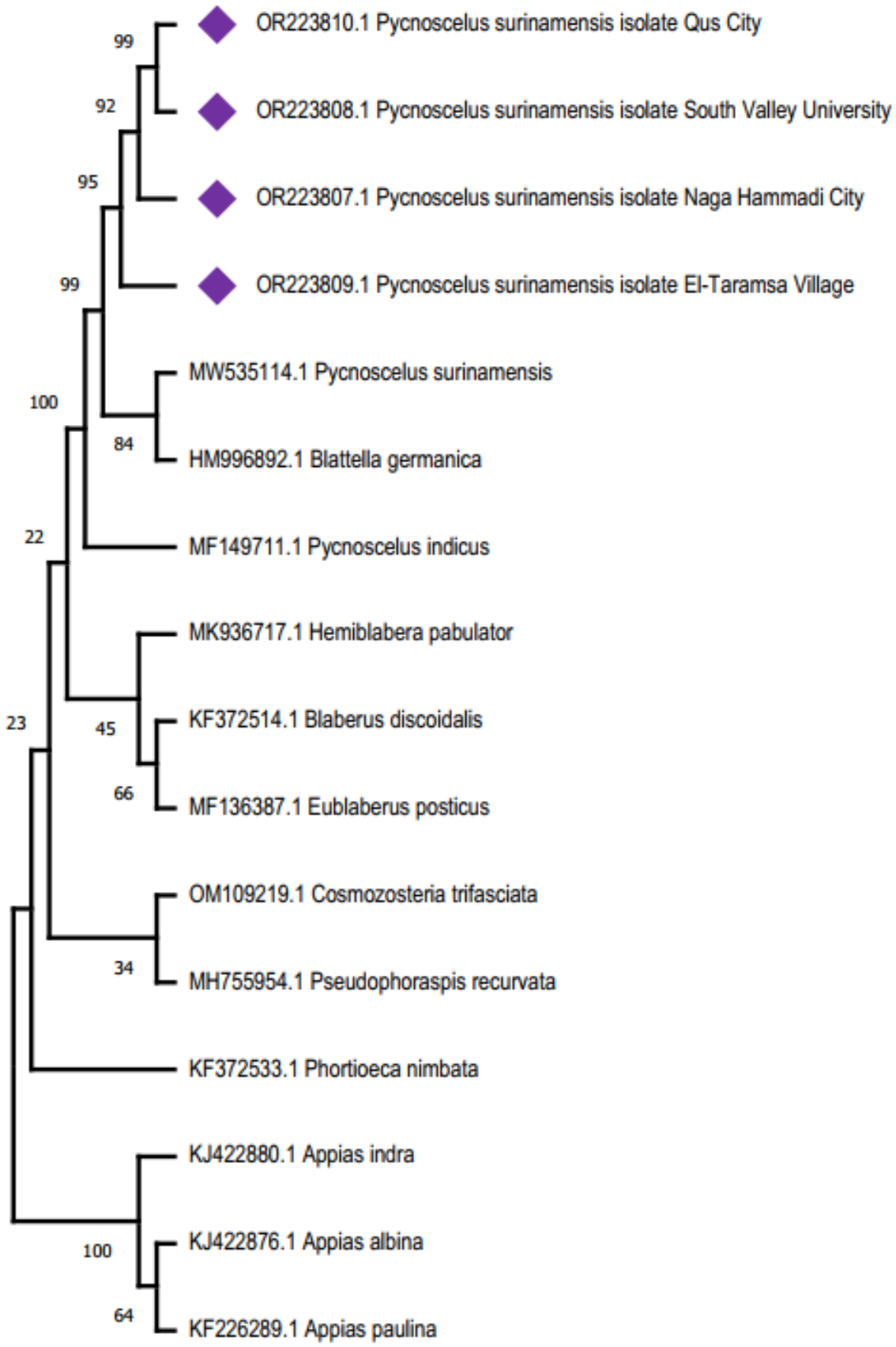

Figure 6: Phylogenetic tree using UPGMA method among *P. surinamensis*specimens from four sites based on mitochondrial *CO1* gene.

DISCUSSION

The current study used the sequences of the *CO1* gene to describe and identify the prevalent of species "*P. laevis* and *P. surinamensis*" at sites Naga Hammadi city, South Valley University farm in Qena city, El-Taramsa Village, and Qus city. Our finding based on *CO1* are correct given that they aligned with multiple GenBank sequences deposited by a lot of authors through different studies and were designated as *P. laevis* and *P. surinamensis*. Through a combined morphological identification and molecular methods, we sought to achieve the understudied species that were identified as *P. laevis* and *P. surinamensis*.

Among arthropods, terrestrial isopods are considered as beneficial organisms in most soil ecosystems[27]. The systematic of terrestrial isopods is complex and ambiguous[28]. Within some terrestrial isopods, great genetic diversity has been observed with an absence of obvious morphological variations, raising the possibility that they represent species complexes[29]. *CO1* is the principal gene employed in DNA barcoding investigations, essential for diagnosing existing species and discovering new ones[30].

The current investigation of *P. laevis* demonstrated that the mean A+T content was 62.37%, surpassing the C+G level. This finding agrees with Allam *et al.*[31] who stated that the mean content of A+T bases in *P. laevis* populations was 63.3%. Raupach *et al.*[32] recorded a high A+T content with an average sequence composition; A = 24.6%, C = 18.1%, G = 21.5%, and T = 35.8% within European woodlice species (Oniscidea). Zhang *et al*.[33] examined the phylogeny of a terrestrial isopods and concluded that the average A+T of *Mongoloniscus sinensis* is around 75.32% with a typical range of 54.4% to 71.2%.

The present *P. laevis* collected from the three locations showed pairwise genetic distances ranged from 0.000 to 0.0235. Allam *et al.*[31] stated that the pairwise distances in their understudied *P. laevis* collected from Qeft, Deshna, and Hurghada cities varied from 0.017 to 0.033. From our study, the most related species were found in Naga Hammadi and El-Taramsa village, while the least related species were located in the same sites in comparison to South Valley University farm. Despite El-Taramsa village and South Valley University farm being situated in the same city, the genetic distance was 0.0235. While Naga Hammadi city and El-Taramsa village are located in different cities, the genetic distance was 0.00. These findings were in agreement with those presented in the study of Rigaud *et al*.[34] on *Armadillidium vulgare* in Western France. They declared that some locations were being more than 300 km apart, however no genetic variations was recorded. While Zhang and Kang[35] reported one of the vital factors related with population differentiation is the geographical isolation; as the geographical distance between groups increases, the likelihood of gene flow diminishes resulting in increased difference. Porres *et al*.[2] concluded that the population genetic structure may be affected by more recent events including environment stressors such as land-use change or persistent soil contamination.

The current eventual alignments of *P. laevis* comprised of 667 bp; of them 562 conserved sites and 89 variable sites. Allam *et al.*[31] revealed that the sequencing of *CO1* gene in the three *P. laevis* populations contained 660 bp; 497 for the conserved locations, 152 for the variable sites, and 1.0 for thrift informative ones. Dangerfield and Telford[36] reported that the genetic structure of a species depends on its evolutionary and population histories and the level of environmental variations.

Insects have been a major focus of DNA barcoding due to their remarkable diversity and their economic, agricultural, and epidemiological importance[14]. In the present *P. surinamensis* collected from the four sites, the mean content of A+T was 62.55%, which was exceeding that of C+G. This result aligns with Ma *et al.*[37] who determined that the nucleotide composition of the *Periplaneta australasiae and Neostylopyga rhombifolia* (family:

Blatidae) exhibited a high A+T content of 74.9%. Li *et al.*[38] studied the wood-feeding cockroach C*ryptocercus meridianus* and concluded that the base composition of the whole genome was 45.20%, 9.74%, 16.06%, and 29.00% for A, G, C, and T, respectively; it showed a high A+T content (74.2%). Ma *et al.*[39] examined *Periplaneta americana* and reported that A+T (65.1%) was significantly more prevalent than G+C (34.8%), which is typical for insects[40].

The four current species *P. surinamensis* showed pairwise genetic distances varied from 0.0000 to 0.0045, and the genetic distances with the regarded species of order Blattodea ranged from 0.0060 to 0.0281. Zangl *et al*.[9] indicated that the pairwise distances within *P. surinamensis* populations from Central Europe varied from 0% to 3.9%, while ranged from 0% to 11.9% among the *P.* species included their study.

The sequencing of *P. surinamensis* across the four sites revealed that the most related species was *P. indicus*. This agrees with Bourguignon *et al*.[41] and Roth[42] who reported that *P. surinamensis* is the thelytokous descendant of its bisexually reproducing progenitor *P. indicus*. *P. surinamensis* is known to possess numerous parthenogenetic clonal forms from various global places, and the evolution of thelytokous parthenogenesis remains inadequately researched, rendering the *Pycnoscelus* taxon complex to comprehend. Consequently, the contribution of molecular data in this taxon is invaluable for delineating the species complex and their evolutionary history[43].

In conclusion, the current study is a contribution to elucidate the molecular data of two common species "*P. Laevis* and *P. Surinamensis*" of terrestrial arthropods in Qena governorate (Egypt) and their phylogeography, which has a value for determining the relationships between species through the *CO1* gene.

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CONFLICT OF INTEREST
There are no conflicts of interest

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التعريف الجزيئي وعلاقات جغرافية الأعراق لكل من "*Porcellio laevis*" و "*Pycnoscelus surinamensis*" باستخدام تسلسل *CO1* الميتوكوندري في محافظة قنا، مصر

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تعتبر مفصليات الأرجل من أكثر الشعب الحيوانية تنوعًا وانتشارًا في جميع البيئات تقريبًا علي كوكب الأرض. وتلعب مفصليات الأرجل كبيرة الحجم في التربة دورًا رئيسيًا في العديد من المهام الداعمة والتنظيمية للنظام البيئي. تم جمع نوعين موزعين على نطاق واسع من المفصليات الأرضية (*Porcellio laevis* و *Pycnoscelus surinamensis*) من أربع مناطق في محافظة قنا (مصر)، وتم التعرف عليهما وإجراء علاقات جغرافية الأعراق لهما باستخدام تسلسل الوحدة الفرعية 1 للسيتوكروم سي أوكسيديز الميتوكوندري (*CO1*). وكان طول النيوكليوتيدات في تسلسل *CO1* لمفصليات الأرجل "*Porcellio laevis*" يتراوح بين 641 و 664 زوجا من القواعد، بينما تراوح ما بين 648 و 654 زوجا قاعديا في تسلسل *CO1* لمفصليات الأرجل "*Pycnoscelus surinamensis*". وبلغ متوسط محتوي قواعد الأدينين والثايمين في "*Porcellio laevis*" و "*Pycnoscelus surinamensis*" 62.37% و 62.55%، علي التوالي. وتراوحت المسافات الجينية الزوجية في عينات "*Porcellio laevis*" من 0.00 إلي 0.0235، وكانت المواقع الأكثر ارتباطًا هي نجع حمادي والترامسة، بينما تراوحت المسافات الجينية في "*Pycnoscelus surinamensis*" من 0.0026 إلي 0.0045، وكانت المواقع الأكثر ارتباطًا هي مزرعة جامعة جنوب الوادي ومدينة قوص.