

RESEARCH ARTICLE

**PHYLOGEOGRAPHIC AND GENETIC DIVERSITY OF *PORCELLIONIDES PRUINOSUS* AND *PORCELLIO LAEVIS* BY USING THE MITOCHONDRIAL CYTOCHROME C OXIDASE SUBUNIT 1 SEQUENCE**

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**ABSTRACT**

Among Oniscidea suborder, Porcellionidae is the richest and widely-distributed Family. However, the phylogeny investigations of its taxa were not completely covered. Therefore, a phylogeographic population and genetic diversity study was carried out here on two cosmopolitan terrestrial isopod species (*Porcellionides pruinosus* and *Porcellio laevis*) by utilizing mitochondrial cytochrome c oxidase subunit 1 (*COI*) gene. The sequencing of *COI* among the understudied populations showed nucleotide length ranged from 646 base pairs (bp) to 660 bp. The average contents of nucleotide bases A+T in *Porcellionides pruinosus* and *Porcellio laevis* were 63.8% and 63.3%, respectively. Also, the average content of pyrimidines (C+T) bases in *Porcellionides pruinosus* and *Porcellio laevis* was higher than the purines content. Nucleotide bases A+T contents were higher than nucleotide bases C+G contents in all populations. The pairwise genetic distances among the understudied *Porcellionides pruinosus* populations ranged from 0.000 to 0.002. The most related populations were in Qeft and Dshna, where the genetic distance was 0.000. The pairwise genetic distances among the understudied populations of *Porcellio laevis* ranged from 0.017 to 0.033. The most related populations were in Qeft and Hurghada, where the genetic distance was 0.017. Furthermore, *Porcellio laevis* populations need more investigations, as it seems to be polyphyletic comparing to *Porcellionides pruinosus* populations.

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**INTRODUCTION**

Terrestrial isopods are essential representatives of soil fauna. They have a significant role in mineralizing organic matter, as well as in decomposing leaf litter<sup>[1-3]</sup>. Generally, terrestrial isopod systematics is complicated and controversial<sup>[4]</sup>. The Oniscidea is greatly

distributed, with the exception of Antarctica, it is found on every continent. Oniscidea is a varied suborder of the terrestrial isopods included more than 4000 species<sup>[5]</sup>. However, due to they have low spread rates, need moist microhabitats, and brood their young in a pouch, they are expected to have

limited gene flow within and among populations<sup>[6,7]</sup>. The spread of different isopod species was related to available habitats and habitat features at an accurate spatial scale, although some species may display different responses to environmental heterogeneity, which reflects a big inter-specific diversity within the group<sup>[8]</sup>. The family “Porcellionidae” is one of the richest family in suborder Oniscidea, it has about 19 genera included 333 species<sup>[9]</sup>. Porcellionidae were highly acclimated to terrestrial environments and can be occurred in a wide scale of habitats, from tropical rainforests to deserts. They were originally reported from the circum-Mediterranean region, Atlantic islands, Arabian Peninsula, and East Africa. For the time being, they found over the world<sup>[10,11]</sup>.

According to Schmalzfuss<sup>[5]</sup>, both *Porcellionides pruinosus* (Brandt, 1833) and *Porcellio laevis* (Latreille, 1804) are belong to Porcellionidae family. *Porcellionides pruinosus* is an extremely spread species of terrestrial isopods, and it is a cosmopolitan woodlouse<sup>[12-13]</sup>. The systematic status of *P. pruinosus* is still confused and remains inconspicuous<sup>[14]</sup>. *Porcellio laevis* is a large and distinctive woodlouse. It is a cosmopolitan species, dispersal greatly over the world, including Western Asia, Japan, North and South America, Australia, and some Pacific islands, resulting in a complex synonymy<sup>[5]</sup>.

Lee *et al.*<sup>[15]</sup> reported that great genetic variation has been found with an absence of apparent morphological divergence in at least some of the terrestrial isopods that have been studied<sup>[13,16-18]</sup>. This increases the potential that they represent complex species. The assessment of the genetic variation of a species is an important gauge for its preservation, maintenance, and further genetic improvement<sup>[19]</sup>. The population genetic structure is due to many factors like ecological and geographical, which may lead to divergence and division of the populations. Moreover, gene flow, genetic drift, and the equilibrium between them

can also lead to population genetic structure<sup>[20,21]</sup>.

The mitochondrial genome has been used in wide scale in evolutionary and population genetics studies, by virtue of the high number of copies inside the cell, the ease of isolating it from the nuclear genome, and the small size and rapid accumulation of mutations<sup>[22-24]</sup>. Mitochondrial DNA has several features as introns lack, restricted recombination, has uniparental inheritance (mainly in animal phyla), and rise evolutionary rate<sup>[25,26]</sup>. The mitochondrial cytochrome c oxidase subunit 1 (*COI*) protein-coding gene has been used in a wide scale as a practical and standardized species-level barcode for the majority of the animal kingdom<sup>[27]</sup>. The evolution rate of mitochondrial DNA and its amino acid sequence is very conserved to *COI* gene across the phyla and becomes very popular in various molecular tools in many genetic investigations<sup>[28]</sup>.

Although the Porcellionidae family is considered among the richest families of Oniscidea, the phylogeny investigations of its taxa were not completely examined<sup>[29]</sup>. Thus, the objective of this study was to estimate the phylogeographic population and genetic diversity of two widely distributed species of family Porcellionidae (*Porcellionides pruinosus* and *Porcellio laevis*) using the *COI* gene.

## MATERIAL AND METHODS

### Sampling

Specimens were collected between October 2019 and March 2020 from four localities from Egypt (Qeft, Qena, Dshna, and Hurghada), belonging to Qena and The Red Sea Governorates (Table 1). The two isopod species were identified as described previously<sup>[30]</sup>. The specimens were stored at  $-20^{\circ}\text{C}$  until used for genomic DNA extraction.

### DNA extraction

The genomic DNA was extracted from the preserved leg tissues using the DNA

extraction method of QIAamp DNA Mini kit (Qiagen, Hidden, Germany) by following the manufacturer's guidelines.

### Polymerase chain reaction (PCR) conditions

Mitochondrial *COI* gene was amplified using the primers LCO1490 and HCO2198<sup>[31]</sup>. The PCR reactions comprised of 10 pmol of each forward and reverse primers, 20 µL PCR master mix (OnePCR™ ready-to-use, Catalogue Number: MB203-0100, GeneDireX, Miaoli County, Taiwan), and 50-90 ng of genomic DNA in a final reaction volume of 40 µL. The PCR program was performed with (a) an initial denaturation at 94°C for 180 seconds; (b) 35 cycles including denaturation, annealing, and an extension for 60 seconds at 94°C, 60 seconds at 50°C, and 60 seconds at 72°C, respectively; (c) a final extension at 72°C for 7 minutes<sup>[31]</sup>. Agarose gel (1.5%) contained ethidium bromide was used to separate the amplified products; 100bp DNA Ladder ready-to-use (Catalogue Number:

DM001-R500, GeneDireX) was used as a DNA marker.

### The Sequencing of PCR

All DNA sequencing was achieved by Macrogen (Seoul, South Korea) using the same primer used for amplification. The sequences were submitted to the National Center for Biotechnology Information (GenBank/NCBI) for obtaining accession numbers. The generated sequences were compared to NCBI *COI* sequences of *Porcellionides pruinosus* and *Porcellio laevis* and revealed 99-100% identity. Sequence alignment was performed using MUSCLE<sup>[32]</sup> with default settings. The nucleotide frequencies, AT and CG contents, as well as the phylogenetic trees analyses were performed with MEGA version 7.0 18<sup>[33]</sup>, using maximum likelihood (ML), neighbor joining (NJ), and minimum evolution (ME) methods of trees construction, as well as using 1000 bootstrap iterations<sup>[34]</sup>. Calculation of sequence divergences was occurred by utilize Kimura two-parameter distances<sup>[35]</sup>.

**Table 1:** Localities and number of individuals of *Porcellionides pruinosus* and *Porcellio laevis*.

Governorate	City	Number of Individuals		Geographic Locations	
		<i>Porcellionides pruinosus</i>	<i>Porcellio laevis</i>	Latitudes	Longitudes
Qena	Qena	20	-	26°09'15.48"N	32°42'38.25"E
Qena	Qeft	30	50	25°59'52.50"N	32°48'59.89"E
Qena	Deshna	20	30	26°07'17.03"N	32°28'06.85"E
The Red Sea	Hurghada	-	20	27°15'28.83"N	32°48'41.90"E

## RESULTS

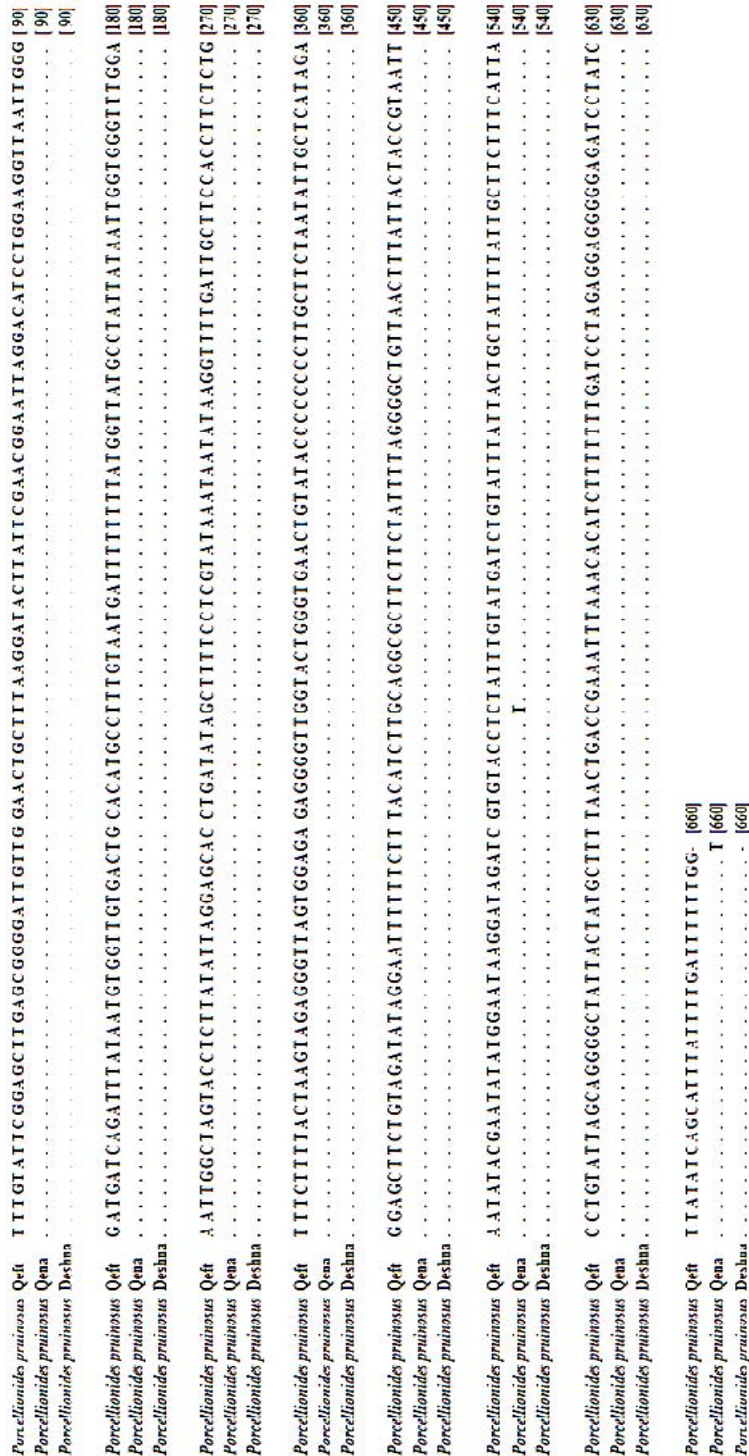
### *Porcellionides pruinosus* populations

The sequencing of mitochondrial *COI* gene in the three populations of *Porcellionides pruinosus* produced nucleotide length ranged from 659 base pairs (bp) to 660 bp. The partial nucleotide sequences of *COI* were deposited in the GenBank under accession numbers (MW449532-MW449534). The average nucleotide frequencies of adenine (A), cytosine (C), guanine (G), and thymine (T) were 24.3, 14.8, 21.4, and 39.5%,

respectively. The average A+T content was 63.8%, which was higher than the C+G content. Also, the average content of pyrimidines (C+T) bases was 54.3, which was higher than the purines content. More details about nucleotide frequencies, A+T contents, pyrimidines contents, and their averages were given in (Table 2). The final alignments consisted of 660 bp; out of them 658, 1, and 1 were conserved sites, variable sites, and parsimony informative sites, respectively (Figure 1).

**Table 2:** Accession number and nucleotide frequencies and their averages of mitochondrial cytochrome c oxidase subunit 1 (*COI*) gene in three populations of *Porcellionides pruinosus*.

Population	Accession Number	Base Pair Length	Nucleotide (%)				A+T Content (%)	Pyrimidines (C+T) Content (%)
			A	T	C	G		
Qeft	MW449532.1	659	24.3	39.5	14.8	21.4	63.8	54.3
Qena	MW449533.1	660	24.2	39.7	14.7	21.4	63.9	54.4
Deshna	MW449534.1	659	24.3	39.5	14.8	21.4	63.8	54.3
Average (%)	-		24.3	39.5	14.8	21.4	63.8	54.3



**Figure 1:** Alignment of partial sequences of mitochondrial cytochrome c oxidase subunit 1 (*COI*) gene in *Porcellionides pruinosus* populations. Dots indicate the identical nucleotides, while A, T, C, and G indicate different nucleotides.

The sequences of *COI* in the three populations of *Porcellionides pruinosus* were subjected to BLAST/N at (NCBI) and revealed 12 related species from order Isopoda; representing six species (*Porcellionides pruinosus*, *Porcellionides myrmecophilus*, *Porcellionidae* sp., *Porcellio scaber*, *Porcellio spinicornis*, and *Porcellio* sp.) of family Porcellionidae, three species (*Haloniscus longiantennatus*, *Haloniscus Searle*, and *Haloniscus* sp.) of family Scyphacidae and one species (*Armadillidium nasatum*, *Ligia dilatata*, and *Thysanoessa raschii*) from each of the

families Armadillidiidae, Ligia, and Euphausiidae, respectively; in addition to the out-group species (*Orchomenella cavimanus*, *Orchomenella obtusa*, and *Orchomenella rotundifrons*) of family Lysianassidae, order Amphipoda (Table 3). Among all the 12 related isopod species, the close genetic species to the understudied populations was *Porcellionides pruinosus* (KR424606.1), then *Porcellionidae* sp. (HM385141.1), while *Haloniscus searlei* (EU364621.1) was the most distantly species.

**Table 3:** The understudied *Porcellionides pruinosus* and *Porcellio laevis* populations with their related Oniscidea species, in addition to the out-group species from the GenBank/NCBI based on mitochondrial cytochrome c oxidase subunit 1 (*COI*) genes sequences.

Species	Accession Number	Species	Accession Number
<i>Porcellionides pruinosus</i> , Qeft	MW449532.1	<i>Porcellio laevis</i> , Deshna	MW449535.1
<i>Porcellionides pruinosus</i> , Qena	MW449533.1	<i>Porcellio laevis</i> , Qeft	MW449536.1
<i>Porcellionides pruinosus</i> , Deshna	MW449534.1	<i>Porcellio laevis</i> , Hurghada	MW449537.1
<i>Porcellionides pruinosus</i>	KR424606.1	<i>Porcellio laevis</i>	HM385125.1
<i>Porcellionidae</i> sp.	HM385141.1	<i>Porcellio imbutus</i>	FN824125.1
<i>Haloniscus longiantennatus</i>	EU364578.1	<i>Porcellio</i> sp.	MH279740.1
<i>Haloniscus</i> sp.	KT236011.1	<i>Porcellionidae</i> sp.	HM385141.1
<i>Porcellionides myrmecophilus</i>	FN824129.1	<i>Idotea resecata</i>	JX545468.1
<i>Thysanoessa raschii</i>	KP241406.1	<i>Idotea urotoma</i>	KU530527.1
<i>Porcellio spinicornis</i>	MG316309.1	<i>Idoteidae</i> sp.	MH242805.1
<i>Porcellio</i> sp.	MH279740.1	<i>Synisoma capito</i>	FJ905097.1
<i>Armadillidium nasatum</i>	FN824097.1	<i>Mongoloniscus sinensis</i>	KT424028.1
<i>Ligia dilatata</i>	MH173101.1	<i>Mongoloniscus koreanus</i>	LC017825.1
<i>Porcellio scaber</i>	MF750666.1	<i>Trachelipus rathkii</i>	MK852336.1
<i>Haloniscus searlei</i>	EU364621.1	<i>Trachelipus kytherensis</i>	EF027399.1
Out-group		<i>Halophiloscia couchii</i>	KJ668172.1
<i>Orchomenella franklini</i>	HM054022.1	<i>Halophiloscia hirsuta</i>	KJ668163.1
<i>Orchomenella obtusa</i>	MG935152.1	<i>Haloniscus longiantennatus</i>	EU364584.1
<i>Orchomenella minuta</i>	MG319744.1	<i>Haloniscus</i> sp.	EU364586.1
		<i>Amphisopus lintoni</i>	JX519295.1
		<i>Ligia perkinsi</i>	AY051336.1
		<i>Sphaeromatidae</i> sp.	KY263277.1
		<i>Tylos</i> sp.	KF007669.1
		Out-group	
		<i>Orchomenella obtusa</i>	MG935152.1
		<i>Orchomenella cavimanus</i>	GU109257.1
		<i>Orchomenella rotundifrons</i>	MF124141.1

Among the understudied *Porcellionides pruinosus* populations pairwise genetic

distances ranged from 0.000 to 0.002. The most related populations were in Qeft and

Deshna, where the genetic distance was 0.000. Pairwise genetic distances among the understudied populations and other related

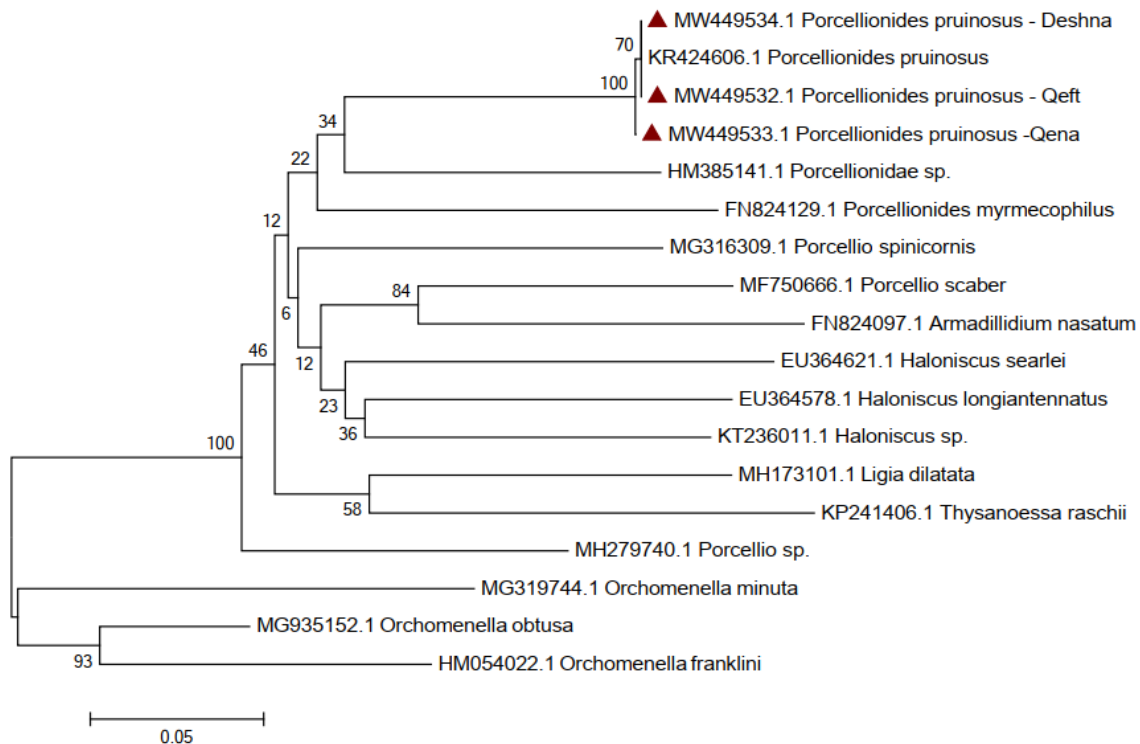
species of order Isopoda ranged from 0.000 to 0.041. Overall, the mean distance value was 0.248% (Table 4).

**Table 4:** Pairwise distances based on mitochondrial cytochrome c oxidase subunit 1 (*COI*) gene among three *Porcellionides pruinosus* populations and related isopod species, in addition to the out-group.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
1 MW449532.1, <i>Porcellionides pruinosus</i> , Qeft	0.002	0.000	0.000	0.000	0.026	0.021	0.024	0.025	0.026	0.025	0.029	0.024	0.028	0.027	0.025	0.027	0.033	0.033
2 MW449533.1, <i>Porcellionides pruinosus</i> , Qena	0.002	0.002	0.002	0.002	0.026	0.021	0.024	0.025	0.027	0.025	0.028	0.024	0.028	0.027	0.025	0.026	0.033	0.033
3 MW449534.1, <i>Porcellionides pruinosus</i> , Deshma	0.000	0.002	0.000	0.000	0.026	0.021	0.024	0.025	0.026	0.025	0.029	0.024	0.028	0.027	0.025	0.027	0.033	0.033
4 KR424606.1, <i>Porcellionides pruinosus</i>	0.000	0.002	0.000	0.000	0.026	0.021	0.024	0.025	0.026	0.025	0.029	0.024	0.028	0.027	0.025	0.027	0.033	0.033
5 FN824129.1, <i>Porcellionides mymecophilus</i>	0.217	0.214	0.217	0.217	0.207	0.204	0.205	0.206	0.208	0.203	0.203	0.207	0.203	0.209	0.204	0.202	0.204	0.206
6 HM385141.1, <i>Porcellionidae</i> sp.	0.177	0.174	0.177	0.177	0.207	0.206	0.206	0.205	0.206	0.203	0.209	0.205	0.208	0.207	0.203	0.207	0.203	0.204
7 MF750666.1, <i>Porcellio scaber</i>	0.233	0.230	0.233	0.233	0.232	0.249	0.249	0.249	0.249	0.249	0.249	0.249	0.249	0.249	0.249	0.249	0.249	0.249
8 MG316309.1, <i>Porcellio spinicornis</i>	0.223	0.220	0.223	0.223	0.226	0.212	0.229	0.229	0.229	0.229	0.229	0.229	0.229	0.229	0.229	0.229	0.229	0.229
9 MH279740.1, <i>Porcellio</i> sp.	0.238	0.241	0.238	0.238	0.233	0.213	0.211	0.199	0.203	0.203	0.209	0.205	0.203	0.208	0.203	0.207	0.203	0.203
10 EU364578.1, <i>Haloniciscus longiantennatus</i>	0.209	0.212	0.209	0.209	0.253	0.251	0.266	0.217	0.260	0.260	0.260	0.260	0.260	0.260	0.260	0.260	0.260	0.260
11 EU364621.1, <i>Haloniciscus searlei</i>	0.258	0.254	0.258	0.258	0.280	0.260	0.251	0.224	0.252	0.244	0.244	0.244	0.244	0.244	0.244	0.244	0.244	0.244
12 KT236011.1, <i>Haloniciscus</i> sp.	0.211	0.208	0.211	0.211	0.243	0.224	0.246	0.246	0.227	0.205	0.220	0.220	0.220	0.220	0.220	0.220	0.220	0.220
13 FN824097.1, <i>Armadillidium nasatum</i>	0.246	0.249	0.246	0.246	0.274	0.242	0.202	0.266	0.255	0.247	0.250	0.254	0.254	0.254	0.254	0.254	0.254	0.254
14 MH173101.1, <i>Ligia dilatata</i>	0.246	0.243	0.246	0.246	0.253	0.229	0.275	0.232	0.260	0.250	0.268	0.230	0.282	0.282	0.282	0.282	0.282	0.282
15 KP241406.1, <i>Thysanoessa raschii</i>	0.218	0.215	0.218	0.218	0.314	0.277	0.266	0.286	0.249	0.257	0.292	0.274	0.340	0.233	0.233	0.233	0.233	0.233
16 MG935152.1, <i>Orchomenella obtusa</i>	0.248	0.245	0.248	0.248	0.288	0.241	0.263	0.259	0.239	0.294	0.290	0.321	0.276	0.271	0.284	0.284	0.284	0.284
17 HM054022.1, <i>Orchomenella franklini</i>	0.314	0.310	0.314	0.314	0.304	0.295	0.309	0.342	0.305	0.353	0.351	0.324	0.343	0.293	0.329	0.329	0.329	0.329
18 MG319744.1, <i>Orchomenella minuta</i>	0.307	0.303	0.307	0.307	0.318	0.301	0.348	0.329	0.277	0.359	0.374	0.368	0.373	0.355	0.361	0.361	0.361	0.361

To carry out the phylogenetic tree analysis using *COI* sequencing, the three *Porcellionides pruinosus* populations were submitted to the analysis together with the 12 related isopod species sequences and the out-group species from GenBank/NCBI (previously mentioned in Table 4). For more illustrative phylogenetic relations, we used more than one phylogenetic method (ML, NJ, and ME) based on *COI* gene. The methods showed nearly the same relations with some differences in support values and

revealed 4 main features: (1) Species of out-group formed a separate cluster. (2) The understudied *Porcellionides pruinosus* populations with *Porcellionides pruinosus* (KR424606.1) formed one main clade. (3) Both *Porcellionides pruinosus* populations of Deshna and Qeft formed a sister clade with *Porcellionides pruinosus* (KR424606.1). (4) *Porcellionides pruinosus* population of Qena formed a basal clade with the rest of *Porcellionides pruinosus* populations (Figures 2-4).

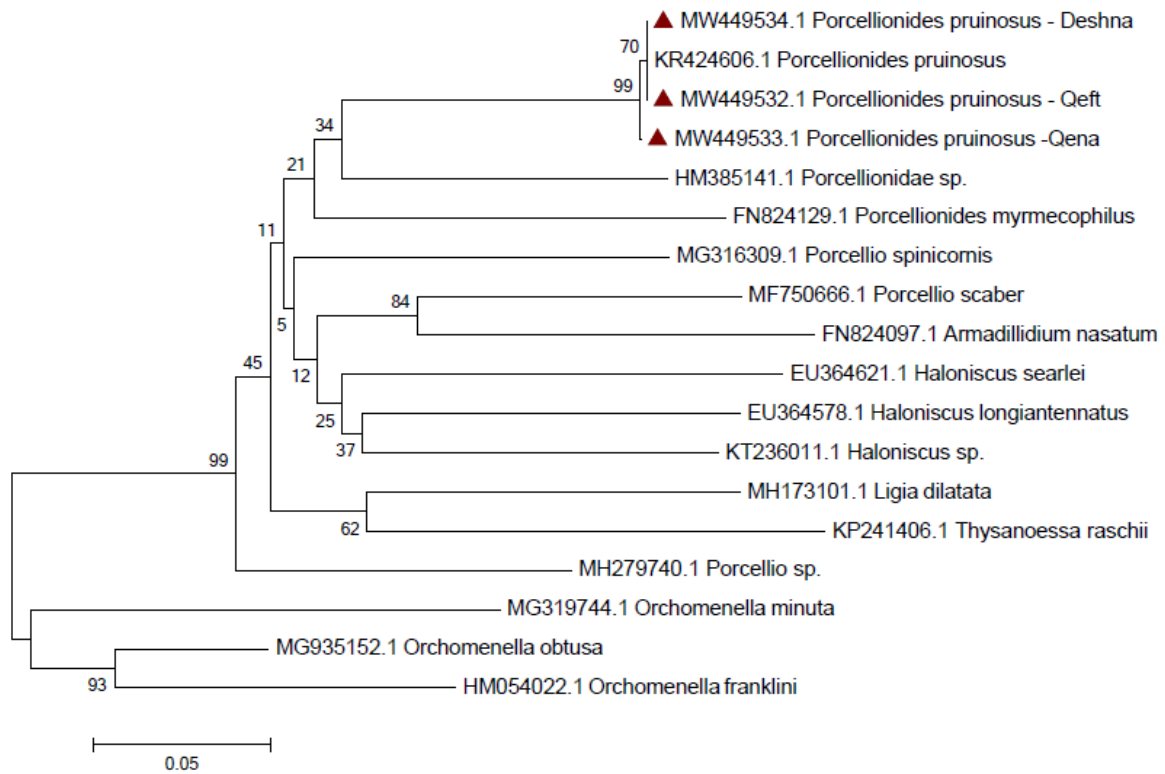


**Figure 2:** Phylogenetic tree using the maximum likelihood method among three *Porcellionides pruinosus* populations based on mitochondrial cytochrome oxidase subunit 1 (*COI*) gene.

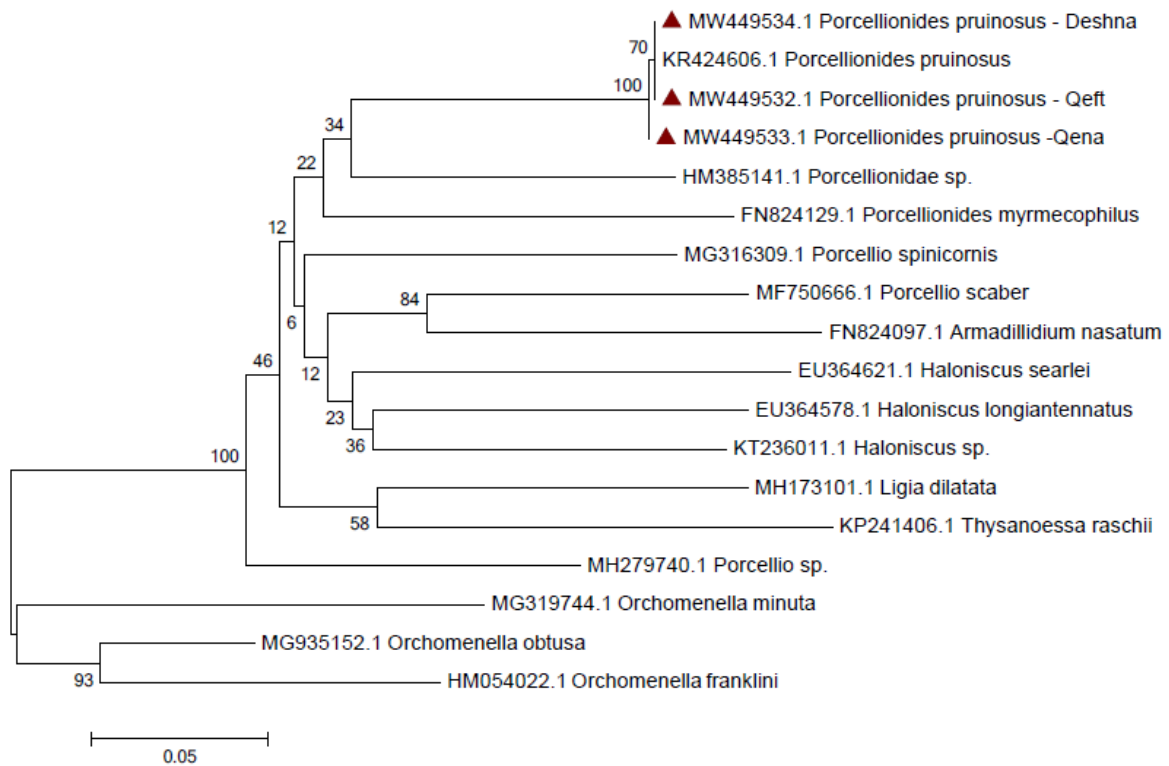
### *Porcellio laevis* populations

The sequencing of *COI* gene in the three *Porcellio laevis* populations produced nucleotide length ranged from 646 bp to 660 bp. The partial nucleotide sequences of *COI* were submitted in the GenBank with the accession numbers (MW449535-MW449537). The results indicated that Deshna population has the shortest nucleotide sequences (646 bp). The average nucleotide frequencies of A, C, G, and T were 25.7, 15.8, 20.9, and 37.6%,

respectively. The average A+T content was 63.3%, which was higher than the C+G content. Also, the average content of pyrimidines (C+T) bases was 53.4, which was higher than the purines content. More details about nucleotide frequencies, A+T contents, pyrimidines contents, and their averages were given in (Table 5). The final alignments consisted of 660 bp; out of them 497, 152, and 1 were conserved sites, variable sites, and Parsimony informative sites, respectively (Figure 5).



**Figure 3:** Phylogenetic tree using the neighbor joining method among three *Porcellionides pruinosus* populations based on mitochondrial cytochrome oxidase subunit 1 (*COI*) gene.

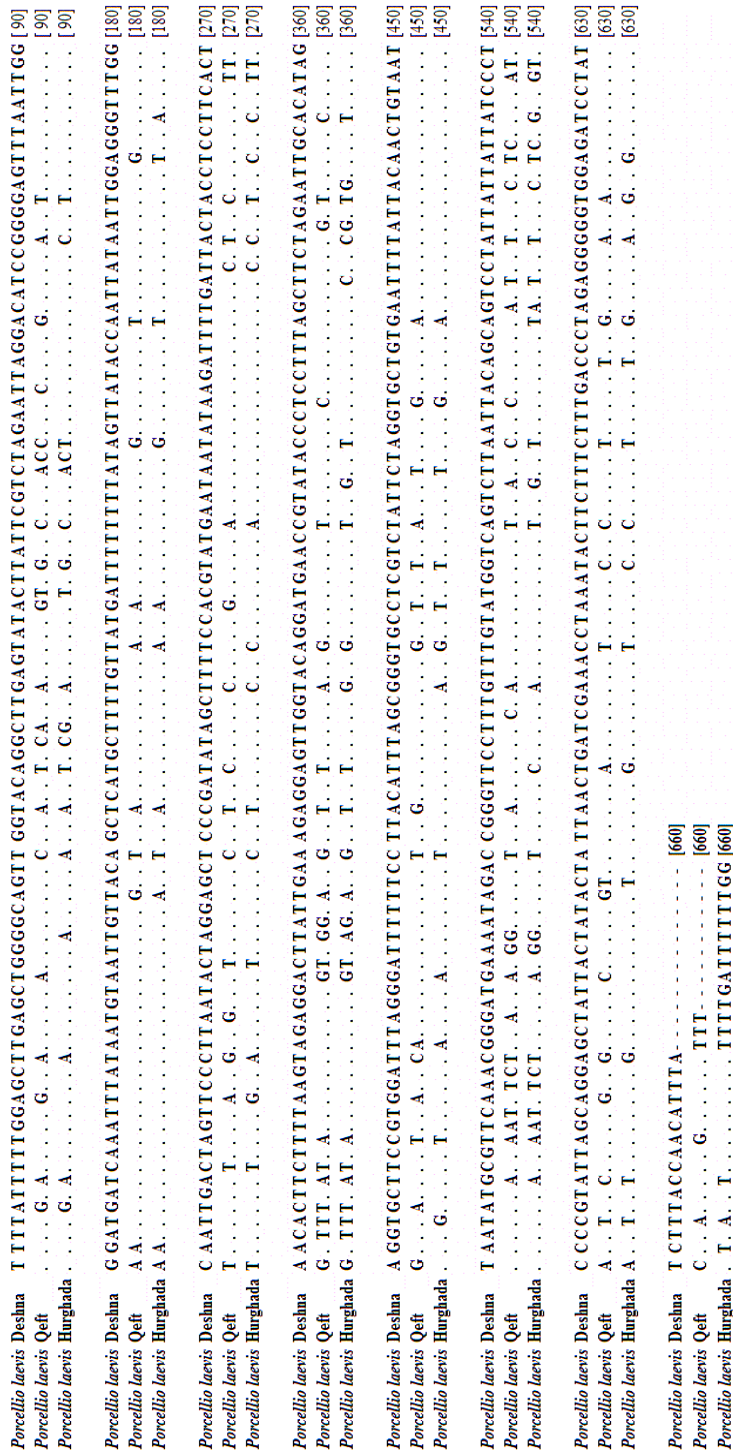


**Figure 4:** Phylogenetic tree using the minimum evolution method among three *Porcellionides pruinosus* populations based on mitochondrial cytochrome oxidase subunit 1 (*COI*) gene.



**Table 5:** Accession numbers, nucleotide frequencies, and their averages mitochondrial cytochrome oxidase subunit 1 (*COI*) gene in three *Porcellio laevis* populations.

Population	Accession Number	Base Pair Length	Nucleotide (%)				A+T Content (%)	Pyrimidines (C+T) Content (%)
			A	T	C	G		
Deshna	MW449535.1	646.0	26.2	36.8	17.0	20.0	63	53.8
Qeft	MW449536.1	649.0	25.7	36.9	16.0	21.4	62.6	52.9
Hurghada	MW449537.1	660.0	25.3	39.1	14.4	21.2	64.4	53.5
Average %	-		25.7	37.6	15.8	20.9	63.3	53.4



**Figure 5:** Alignment of partial sequences of mitochondrial cytochrome oxidase subunit 1 (*COI*) gene in *Porcellio laevis* populations. Dots indicate the identical nucleotides, while A, T, C, and G indicate different nucleotides.

The sequences of *COI* in the three *Porcellio laevis* populations were subjected to BLAST/N at (NCBI) and revealed 20 related species, from order Isopoda; representing four species (*Porcellio laevis*, *Porcellio imbutus*, *Porcellio* sp., and *Porcellionidae* sp.) of family Porcellionidae, four species (*Idotea resecata*, *Idotea urotoma*, *Idoteidae* sp., and *Synisoma capito*) of family Idoteidae, four species (*Mongoloniscus sinensis*, *Mongoloniscus koreanus*, *Trachelipus rathkii*, and *Trachelipus kytherensis*) of family Trachelipodidae, two species (*Halophiloscia couchii* and *Halophiloscia hirsuta*) of family Halophilosciidae, two species (*Haloniscus longiantennatus* and *Haloniscus* sp.) of family Scyphacidae, and one species (*Amphisopus lintoni*, *Ligia perkinsi*, *Sphaeromatidae* sp., and *Tylos* sp.) from each of the families Amphisopidae, Ligiidae, Sphaeromatidae, and Tylidae, respectively; in addition to the out-group species (*Orchomenella obtusa*, *Orchomenella cavimanus*, and *Orchomenella rotundifrons*) of family Lysianassidae, order Amphipoda (Table 3).

Pairwise genetic distances among the understudied populations of *Porcellio laevis* ranged from 0.017 to 0.033. The most related populations were in Qeft and Hurghada, where the genetic distance was 0.017. The Pairwise genetic distances among the understudied *Porcellio laevis* populations and the related species of order Isopoda ranged from 0.002 to 0.037. Overall, the mean distance value was 0.251%. Among all 20 related isopod species, the closely genetic species to the understudied populations was *Porcellio laevis* (HM385125.1), while *Amphisopus lintoni* (JX519295.1) was the distantly species (Table 6).

To carry out the phylogenetic tree analysis using *COI* sequencing, the three *Porcellio laevis* populations were submitted to the analysis together with the 20 related isopod species sequences and the out-group species

from GenBank/NCBI (previously mentioned in Table 3). For more illustrative phylogenetic relations, we used more than one phylogenetic method (ML, NJ, and ME) based on *COI* gene. The methods showed nearly the same relations with some differences in support values and revealed 4 main features: (1) Species of the out-group formed a separate cluster. (2) The understudied *Porcellio laevis* of Qeft population formed a sister clade with *Porcellio laevis* (HM385125.1). (3) Both *Porcellio laevis* populations of Deshna and Hurghada formed a separate clade. (4) *Porcellio laevis* populations of Deshna formed a separate clade far from the rest of *Porcellio laevis* populations (Figures 6-8).

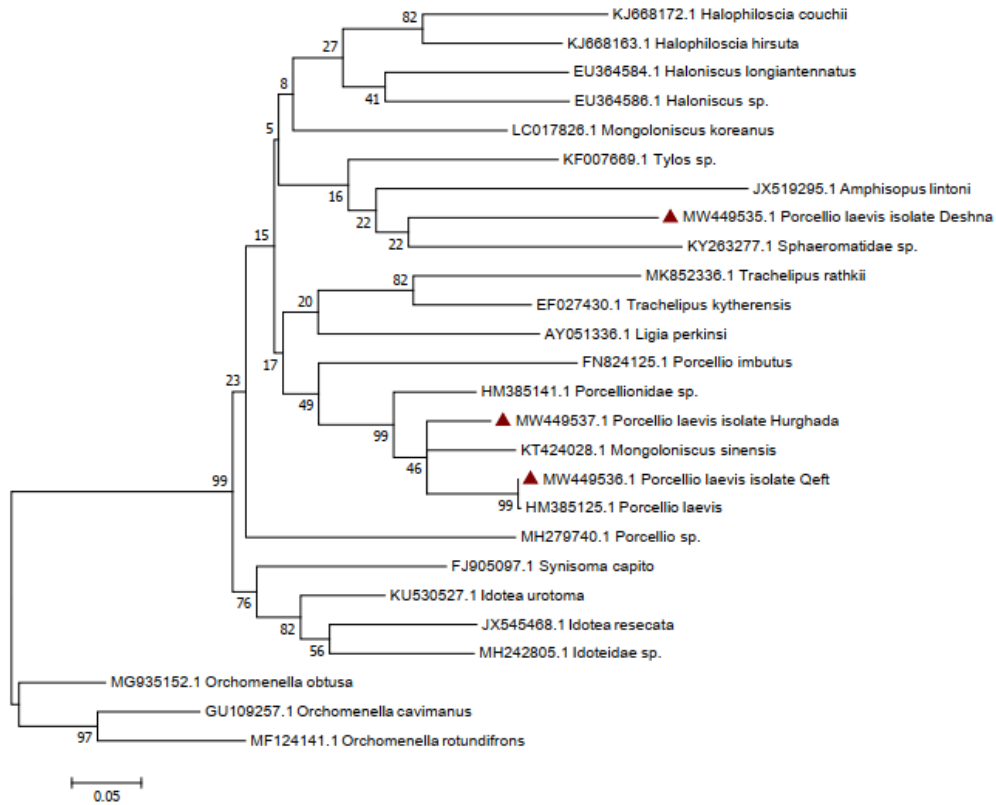
## DISCUSSION

*COI* is the core gene utilized by DNA barcoding studies and plays an important role to facilitate both the diagnosing of known species, as well as the discovery of new species<sup>[27]</sup>. Several studies illustrated the effects of DNA barcodes in identification of species<sup>[36-39]</sup>. The present study revealed that the partial sequencing of *COI* among populations of the two isopod species "*Porcellionides pruinosus* and *Porcellio laevis*" ranged from 646 bp to 660 bp. This concurs with many studies that used *COI* in invertebrates<sup>[29,41-43]</sup>. Wetzer<sup>[40]</sup> reported that isopod mitochondrial DNA shows a high ratio of A+T in two ribosomal genes (12S rRNA and 16S rRNA). In addition, the third codon position of mitochondrial *COI* exhibits a high proportion of A+T content. The average content of A+T in *Porcellionides pruinosus* and *Porcellio laevis* in the present investigation was 63.8% and 63.3%, respectively. The contents of A+T were higher than C+G contents in all populations; these agree with Chen *et al.*<sup>[42]</sup> who found that the average composition of A+T was 63.5% in 9 genera of Scelimeninae using *COI* gene.

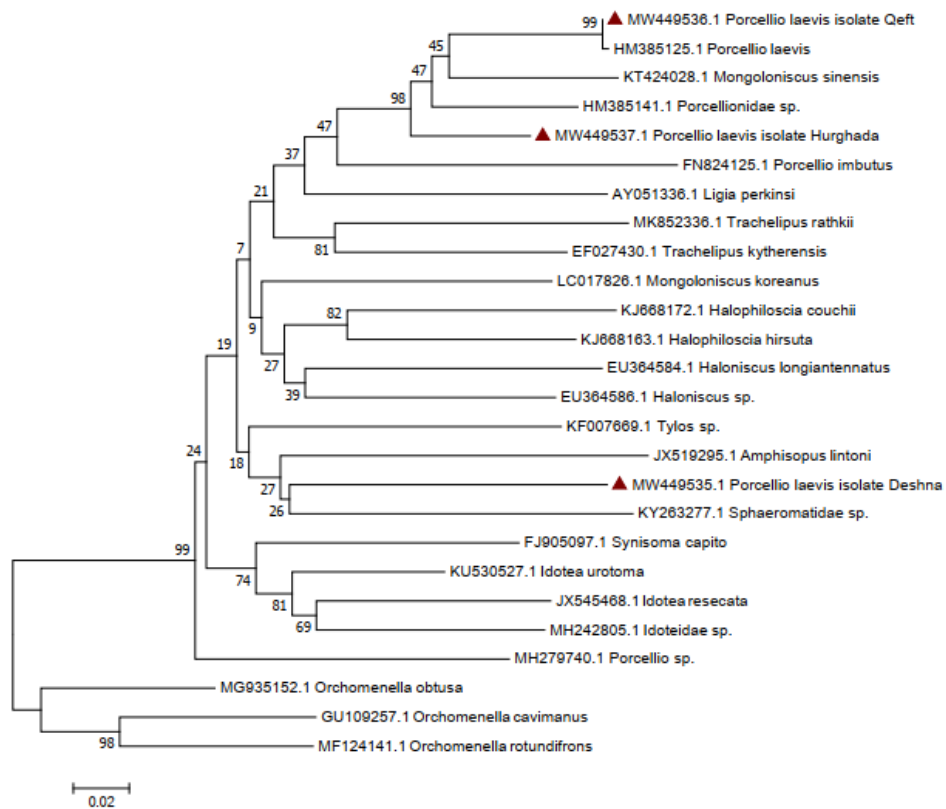
The global species *Porcellionides pruinosus* (Brandt, 1833) display geographical diversity of its morphological characteristics and its reproduction pattern.

**Table 6:** Pairwise distances based on mitochondrial cytochrome oxidase subunit 1 (COI) gene among three *Porcellio laevis* populations and the related isopod species, in addition to the out-group.

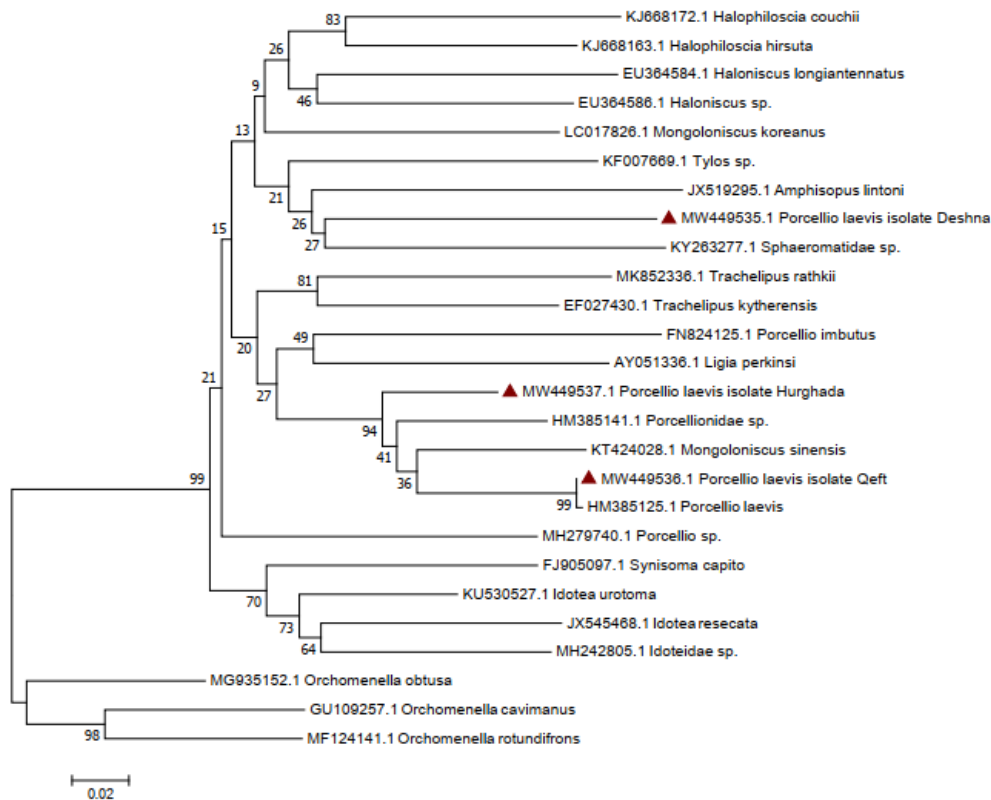
1	MW449535.1, <i>Porcellio laevis</i> , Doshma	0.033	0.032	0.034	0.033	0.030	0.031	0.033	0.025	0.032	0.031	0.037	0.026	0.030	0.029	0.028	0.030	0.031	0.028	0.033	0.028	0.027	0.029	0.037	0.037	
2	MW449536.1, <i>Porcellio laevis</i> , Qeef	0.280	0.017	0.002	0.026	0.026	0.017	0.027	0.026	0.030	0.030	0.018	0.027	0.025	0.030	0.028	0.031	0.027	0.034	0.028	0.031	0.031	0.031	0.031	0.032	0.033
3	MW449537.1, <i>Porcellio laevis</i> , Hurgada	0.266	0.108	0.017	0.023	0.025	0.017	0.027	0.023	0.028	0.025	0.016	0.025	0.025	0.024	0.026	0.025	0.030	0.026	0.030	0.023	0.027	0.027	0.026	0.027	0.029
4	HM385125.1, <i>Porcellio laevis</i>	0.283	0.002	0.111	0.026	0.026	0.017	0.027	0.026	0.030	0.030	0.018	0.027	0.025	0.025	0.031	0.028	0.031	0.027	0.034	0.028	0.031	0.031	0.031	0.032	0.033
5	FN824125.1, <i>Porcellio imburus</i>	0.295	0.224	0.181	0.224	0.029	0.026	0.032	0.030	0.033	0.030	0.031	0.029	0.031	0.030	0.034	0.032	0.032	0.027	0.035	0.026	0.033	0.030	0.033	0.034	0.034
6	MH279740.1, <i>Porcellio</i> sp.	0.256	0.218	0.196	0.221	0.261	0.026	0.028	0.026	0.025	0.027	0.028	0.028	0.028	0.028	0.028	0.029	0.028	0.024	0.031	0.029	0.031	0.028	0.027	0.033	0.033
7	HM385141.1, <i>Porcellionidae</i> sp.	0.266	0.119	0.114	0.122	0.209	0.213	0.028	0.026	0.027	0.027	0.017	0.026	0.028	0.024	0.029	0.029	0.031	0.029	0.030	0.027	0.029	0.028	0.026	0.030	0.030
8	JX545468.1, <i>Idotea resicata</i>	0.289	0.244	0.228	0.247	0.295	0.237	0.244	0.018	0.020	0.023	0.032	0.030	0.030	0.029	0.028	0.028	0.031	0.029	0.033	0.028	0.029	0.026	0.031	0.033	0.033
9	KU530527.1, <i>Idotea uretoma</i>	0.220	0.227	0.182	0.227	0.264	0.216	0.214	0.145	0.019	0.020	0.027	0.025	0.029	0.026	0.027	0.023	0.030	0.025	0.028	0.027	0.024	0.023	0.024	0.030	0.031
10	MH242805.1, <i>Idoteidae</i> sp.	0.276	0.259	0.236	0.259	0.305	0.214	0.225	0.166	0.147	0.024	0.028	0.027	0.028	0.030	0.031	0.030	0.033	0.029	0.031	0.030	0.029	0.030	0.027	0.031	0.033
11	FJ905097.1, <i>Synisoma capito</i>	0.269	0.261	0.218	0.264	0.264	0.235	0.237	0.196	0.164	0.198	0.030	0.027	0.029	0.027	0.032	0.029	0.026	0.025	0.033	0.030	0.025	0.025	0.028	0.032	0.032
12	K1424028.1, <i>Mongoloniscus sinensis</i>	0.300	0.115	0.097	0.118	0.251	0.236	0.113	0.274	0.220	0.245	0.267	0.026	0.030	0.026	0.028	0.029	0.031	0.030	0.033	0.029	0.034	0.030	0.028	0.031	0.031
13	LC017826.1, <i>Mongoloniscus koreanus</i>	0.223	0.226	0.216	0.226	0.252	0.256	0.222	0.269	0.208	0.232	0.229	0.218	0.028	0.025	0.025	0.024	0.027	0.025	0.029	0.029	0.031	0.026	0.028	0.037	0.036
14	MK852336.1, <i>Trachelipus rathkii</i>	0.266	0.213	0.209	0.216	0.283	0.262	0.251	0.249	0.252	0.251	0.259	0.251	0.250	0.023	0.030	0.027	0.027	0.030	0.032	0.026	0.032	0.031	0.031	0.033	0.035
15	EF027430.1, <i>Trachelipus bytherensis</i>	0.264	0.207	0.210	0.210	0.272	0.245	0.202	0.253	0.224	0.255	0.234	0.223	0.200	0.188	0.029	0.026	0.028	0.027	0.030	0.025	0.029	0.026	0.030	0.035	0.032
16	KJ668172.1, <i>Halophiloscia couchii</i>	0.254	0.259	0.223	0.263	0.297	0.247	0.255	0.244	0.224	0.272	0.283	0.245	0.222	0.260	0.231	0.023	0.026	0.026	0.032	0.032	0.031	0.028	0.031	0.037	0.036
17	KJ668163.1, <i>Halophiloscia lirsata</i>	0.258	0.240	0.214	0.240	0.285	0.264	0.253	0.251	0.185	0.262	0.256	0.252	0.207	0.244	0.223	0.178	0.024	0.025	0.027	0.027	0.028	0.031	0.026	0.031	0.034
18	EU364584.1, <i>Haloniscus longiantennatus</i>	0.263	0.247	0.250	0.251	0.283	0.254	0.253	0.263	0.262	0.276	0.229	0.259	0.238	0.253	0.235	0.231	0.208	0.023	0.031	0.027	0.032	0.028	0.032	0.036	0.037
19	EU364586.1, <i>Haloniscus</i> sp.	0.266	0.234	0.217	0.234	0.224	0.207	0.242	0.253	0.204	0.249	0.237	0.250	0.214	0.278	0.228	0.220	0.210	0.196	0.029	0.028	0.030	0.024	0.033	0.035	0.038
20	JX519295.1, <i>Amphisopus litonoi</i>	0.245	0.297	0.265	0.301	0.331	0.283	0.259	0.309	0.254	0.288	0.302	0.283	0.256	0.287	0.267	0.269	0.237	0.284	0.241	0.030	0.029	0.029	0.029	0.036	0.035
21	AY051336.1, <i>Ligia perkinsi</i>	0.298	0.241	0.194	0.245	0.226	0.251	0.234	0.260	0.228	0.268	0.279	0.246	0.256	0.229	0.227	0.276	0.244	0.235	0.242	0.266	0.029	0.025	0.030	0.034	0.034
22	KY263277.1, <i>Sphaeromatidae</i> sp.	0.237	0.288	0.252	0.288	0.323	0.268	0.261	0.270	0.209	0.259	0.223	0.314	0.270	0.296	0.261	0.294	0.257	0.284	0.274	0.261	0.279	0.026	0.031	0.036	0.036
23	KF007669.1, <i>Tylos</i> sp.	0.224	0.270	0.235	0.274	0.282	0.248	0.251	0.232	0.195	0.255	0.232	0.260	0.223	0.277	0.239	0.257	0.284	0.239	0.208	0.272	0.225	0.230	0.028	0.032	0.034
24	MG935152.1, <i>Orchomenella obtusa</i>	0.273	0.278	0.236	0.282	0.326	0.239	0.236	0.285	0.221	0.244	0.245	0.254	0.253	0.283	0.276	0.288	0.249	0.303	0.299	0.277	0.287	0.290	0.268	0.020	0.021
25	GU109257.1, <i>Orchomenella cavimanus</i>	0.357	0.288	0.240	0.292	0.334	0.295	0.266	0.305	0.268	0.280	0.292	0.266	0.339	0.308	0.324	0.328	0.281	0.319	0.317	0.339	0.316	0.339	0.312	0.156	0.019
26	MF124141.1, <i>Orchomenella rotundifrons</i>	0.343	0.290	0.249	0.290	0.303	0.298	0.272	0.288	0.270	0.286	0.283	0.276	0.322	0.325	0.296	0.341	0.307	0.338	0.336	0.322	0.303	0.315	0.318	0.164	0.140



**Figure 6:** Phylogenetic tree using the maximum likelihood method among three *Porcellio laevis* populations based on mitochondrial cytochrome oxidase subunit 1 (*COI*) gene.



**Figure 7:** Phylogenetic tree using the neighbor joining method among three *Porcellio laevis* populations based on mitochondrial cytochrome oxidase subunit 1 (*COI*) gene.



**Figure 8:** Phylogenetic tree using the minimum evolution method among three *Porcellio laevis* populations based on mitochondrial cytochrome oxidase subunit 1 (*COI*) gene.

In addition, it has a dialectical taxonomic history and wide ecological plasticity<sup>[14]</sup>. Pairwise genetic distances among *Porcellionides pruinosus* populations, in our present study, ranged from 0.000 to 0.002. This closely genetic relation was consistent with the findings of Delhoumi *et al.*<sup>[14]</sup>, who reported that the lowest genetic distances among populations of *Porcellionides pruinosus* in Tunisia, was found among Sbiba, Elfeija, Monastir, Chebba, and Sned.

The study of Rigaud *et al.*<sup>[44]</sup> on *Armadillidium vulgare* populations in western France reported that although some sites being more than 300 km apart, but there was not any genetic isolation by distance. Also, Lee *et al.*<sup>[15]</sup> found no *COI* genetic diversity among *Spherillo grossus* populations at Batemans Bay, Burleigh Heads, Woy Woy, Wollongong, and Urunga regions in Australia, although more than one

specimen being collected and sequenced at each site. Likewise, Chen *et al.*<sup>[42]</sup> showed a close genetic distance (0.004) of the two geographical populations of *Paragavialidium sichuanensis* in Zhougongshan and Bifengxia. A phylogeographic study on *Porcellio scaber* (Latreille, 1804) in Europe was found that human passive dispersal has been a significant factor in shaping genetic variation across the continent<sup>[45]</sup>.

In the present study, pairwise genetic distances among *Porcellio laevis* populations based on mitochondrial *COI* ranged from 0.017 to 0.033, and the highest level of the genetic variation was in accordance with several studies on terrestrial isopods. Cooper *et al.*<sup>[46]</sup> using *COI* sequences in genus *Haloniscus* (Chilton, 1919), cave-dwelling oniscid found in Western Australia, revealed that p-distances of up to 10.7% were found between sites. Hurtado *et al.*<sup>[17]</sup> during their

study on the rocky-shore oniscid species “*Ligia occidentalis* (Dana, 1853)” found that *COI* p-distances were up to 26% between populations in the Gulf of California. In the same species<sup>[18]</sup>, a pairwise distance was of up to 26.7% between population, and of up to 25% between populations was found<sup>[47]</sup>. In all these cases, the high ratios of genetic variation were considered as an indicator of species-level divergences between lineages<sup>[15]</sup>.

The present findings showed that, the final alignments in *Porcellio laevis* consisted of 660 bp; out of them 497, 152, and 1 were conserved sites, variable sites, and parsimony informative sites, respectively. Dimitriou *et al.*<sup>[29]</sup> used *COI* in five Porcellionidae genera and reported that the alignment length, conserved sites, variable sites, and Parsimony informative sites were 655, 214, 434, and 303 bp, respectively. The present phylogenetic tree analysis based on mitochondrial *COI* in *Porcellio laevis* populations revealed that Dëshna population formed a separate clade far from the Qeft and Hurghada populations. Thus, the *Porcellio laevis* populations need more investigations, as it seems to be polyphyletic comparing with *Porcellionides pruinosus* populations. Brix *et al.*<sup>[48]</sup> declared, despite the uniform morphology, genetic divergence refers to the presence of overlooked or cryptic species in isopods. In addition to that, Dimitriou *et al.*<sup>[29]</sup> proclaim the extremely high genetic distances that reached up to 50.3 in mitochondrial DNA and 44.2 in nucleus DNA, which supported the vast divergence among taxa within Porcellionidae. Also, the genus *Porcellio* needs to be revised, as it seems to be polyphyletic, including at least two separate groups.

In conclusion, the present study investigated the phylogeographic population and genetic diversity of two terrestrial isopods *Porcellionides pruinosus* and *Porcellio laevis* based on *COI* sequence. The results of *COI* sequences illustrated closely genetic relationship between Qeft

and Dëshna *Porcellionides pruinosus* populations, while the most related *Porcellio laevis* populations were Qeft and Hurghada. The *COI* Sequence seems to be useful in exposing differences in *Porcellionides pruinosus* and *Porcellio laevis* specimens from different localities in Egypt, it is clear that there is an environment effect on the genetic structure

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#### CONFLICT OF INTEREST

The authors have no potential financial conflict of interest.

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العلاقات الجغرافية والتنوع الوراثي لكل من "*Porcellionides pruinosus*"  
و "*Porcellio laevis*" باستخدام تسلسل الوحدة الفرعية-1  
للسيتوكروم سي أوكسيديز الميتوكوندري

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تُعتبر الفصيلة "*Porcellionidae*" الأكثر ثراءً بالأنواع والموزعة على نطاق واسع من بين فصائل تحت رتبة "*Oniscidea*". ومع ذلك فإن دراسة تطور نسالة السلالات بينها لم يتم تغطيته بالكامل. لذلك، تم هنا دراسة التعداد الجغرافي والتنوع الجيني على نوعين من متماثلات الأرجل الأرضية المنتشرة عالمياً (*Porcellionides pruinosus* و *Porcellio laevis*)، من خلال استخدام جين الوحدة الفرعية-1 للسيتوكروم سي أوكسيديز الميتوكوندري (*COI*). وأظهر تسلسل جين "*COI*" للعشائر التي خضعت للدراسة أن طول النيوكليوتيدات يتراوح بين 646 و 660 زوجاً من القواعد. وبلغ متوسط محتوى قواعد النيوكليوتيدات من الأدينين + الثايمين في "*Porcellionides pruinosus*" و "*Porcellio laevis*" 63.8% و 63.3%، علي التوالي. أيضاً، كان متوسط محتوى قواعد البيريميدين (C+T) في "*Porcellionides pruinosus*" و "*Porcellio laevis*" أعلى من محتوى قواعد البيرين. وفي كل العشائر كان محتوى الأدينين + الثايمين أعلى من الجوانين + السيتوسين. وترواحت المسافات الوراثية بين أفراد عشائر "*Porcellionides pruinosus*" التي تم دراستها بين 0.000 و 0.002، وكانت العشائر الأكثر قرابة هي في منطقتي قفط ودشنا، حيث كانت المسافة الوراثية 0.000. وترواحت المسافات الوراثية بين أفراد عشائر "*Porcellio laevis*" التي تم دراستها بين 0.017 و 0.033، وكانت العشائر الأكثر قرابة هي في منطقتي قفط والغردقة، حيث كانت المسافة الوراثية 0.017. علاوة على ذلك، تحتاج عشائر "*Porcellio laevis*" إلى مزيد من الدراسة، حيث إنها تبدو متعدد الأنماط مقارنة بعشائر "*Porcellionides pruinosus*".