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The Mitochondrial Genome of the Freshwater Crab *Potamon fluviatile*, the First Sequenced Representative of the Subfamily Potaminae and Its Phylogenetic Position within Potamidae

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Abstract

Here we report the mitogenome of *Potamon fluviatile* collected from the Maltese islands, representing the first such study on species from the subfamily Potaminae. The mitogenome was analysed through next-generation sequencing and annotated. The genome was found to contain 37 genes that include 13 protein coding genes, 22 tRNA genes, two rRNA genes and a non-coding region. The gene order was compared to that of other Potamidae species, while protein coding and rRNA genes were used to evaluate the phylogenetic position of *Potamon* with other species of freshwater crabs. The phylogenetic analysis shows that the subfamily Potaminae, here represented by *P. fluviatile*, forms a distinct clade from its sister subfamily Potamiscinae, with the two sharing common ancestry within the family Potamidae. This study contributes to the genetic resources available for the genus *Potamon*.

Introduction

Potamon fluviatile (Herbst, 1785), is a primary freshwater crab that is found to occur in the southern European continent, in a number of hydrogeographically isolated freshwater bodies in Italy; the Balkan Peninsula; the Turkish Thrace region; and some neighbouring islands, including the Maltese Islands (Cumberlidge, 2008; Ng et al., 2008; Jesse et al., 2009; Harlıoğlu, Farhadi & Harlıoğlu, 2018). Consequently, this species even though widespread, its distribution is highly fragmented even on small-scale given that it is restricted to fresh watercourses (Vella & Vella, 2020). Additionally, it is highly threatened by anthropogenic activities mainly because of the pressures imposed on freshwater bodies including pollution, pesticides, alien species and water usage (Barbaresi, Cannicci, Vannini & Fratini, 2007; Cumberlidge et al., 2009; Freyhof & Brooks, 2011; Vella, Vella & Mifsud, 2017, Gozlan, Karimov, Zadereev, Kuznetsova & Brucet, 2019; Grzybowski & Glińska-Lewczuk, 2019). These threats have led IUCN to enlist this species as Nearly Threatened on a global scale with populations showing negative trends (Cumberlidge, 2008, Cumberlidge et al., 2009). In the Maltese archipelago, this is the only species of freshwater crabs and is locally protected, while it is considered as a flagship species for freshwater habitats and also for invertebrates as *P. fluvitale* is considered as the national invertebrate (Laws of Malta, 2021).

Potamon fluviatile is a member of the family Potamidae Ortmann, 1896. The latter is a large family of primary freshwater crabs containing two subfamilies, Potaminae Ortmann, 1896 and Potamiscinae Bott, 1970 (Ng et al., 2008). In the past few years, attention has been given to the taxonomy and systematics of this

family due to its conservation value and high species diversity, with works leading to the discovery of new species (Daniels et al., 2006; Ng et al., 2008; Yeo et al., 2008; Shih, Yeo & Ng, 2009; Leelawathanagoon, Lheknim & Ng, 2010). Huang, Shih & Mao, 2016; Naruse, Chia & Zhou, 2018; Zou, Bai & Zhou, 2018; Gao et al., 2019; Wang, Zhou & Zou, 2019; Wang, Zhou & Zou, 2020; Tan, Zhou & Zou, 2021). In 2008, a checklist of the subfamily Potaminae enlisted 34 species (Ng et al., 2008), with the genus *Potamon* being its largest genus with 17 species, and since then a new species has been described by Jesse et al., (2010). While some of these species, including *P. fluviatile*, have been DNA barcoded for systematic studies or included in population studies (Jesse et al., 2009; Jesse et al., 2010; Jesse et al., 2011; Vella and Vella, 2020), yet data on Potamidae mitochondrial genomes is limited to the subfamily Potamiscinae, while none from the subfamily Potaminae have ever been studied for the complete set of mitochondrial genes and gene order.

In Potamiscinae the mitogenomes range from 15,318 bp in *Nanhaipotamon hongkongense* (Wang et al., 2020), to at least 20,227 bp in the incomplete mitogenome of *Parapotamon spinescens* (Zhang et al., 2020) (Table 1). Some of these Potamiscinae mitogenomes exhibit different gene arrangements, including tandem duplication or random loss events in non-coding sequences, single gene duplication, tRNA remolding, transposition and inversion transposition of genes, leading to at least nine distinct mitogenomic gene order patterns, with eight of them deviating from the typical brancyuran mitogenome ground pattern (Zhang et al. 2020). These interesting variations in the mitogenomes of Potamiscinae species have led to better understand the evolutionary history of different freshwater crabs at subfamily level (Zhang et al. 2020), yet the absence of studies on Potaminae limits the understanding of the phylogenetic relationships within the family Potamidae.

The current study uses the mitochondrial genome sequence to elucidate its organization, gene order and codon usage in *P. fluviatile* and at the same time compare it with that of other freshwater crabs. This phylogenetic contribution is essential as it uses a large set of genes to investigate the systematic position and the taxonomic relationship between the two subfamilies within Potamidae, that is Potaminae and Potamiscinae, adding knowledge on the systematics of primary freshwater crabs.

Table 1. The mitogenome composition of different Potamidae species.

Species	GenBank	Mitogenome length bp (GC%)	Protein-coding genes length bp (GC%)	Ribosomal RNA genes length bp (GC%)	Reference
Potaminae					
Potamon fluviatile	OL944387	16,037 (26.6)*	11,174 (28.6)	2,120 (22.2)	current work
Potamiscinae					
Aparapotamon similium	MK950854	19,236 (27.2)	11,145 (30.2)	2,146 (25.3)	Lie et al., 2019
Apotamonautes hainanensis	MN737137	17,011 (26.6)	11,158 (28.0)	2,236 (22.8)	Zhang et al., 2020
Bottapotamon lingchuanense	MN117717	17,612 (27.7)	11,186 (30.4)	2,146 (25.4)	Wang et al., 2021
Candidiopotamon okinawense	MN737145	17,211 (27.7)	11,155 (29.9)	2,133 (23.5)	Zhang et al., 2020
Chinapotamon maolanense	MT134100	17,130 (26.6)	11,122 (29.6)	2,143 (23.0)	Cui et al., 2020
Geothelphusa dehaani	AB187570	18,197 (25.1)*	11,137 (28.5)	2,136 (23.2)	Segawa et al., 2005
Huananpotamon lichuanense	KX639824	15,380 (26.8)	11,127 (28.5)	2,144 (22.3)	Bai et al., 2018
Indochinamon bhumibol	MT872370	16,351 (29.7)	11,142 (32.0)	1,893 (26.5)	Naktang et al., 2021
Longpotamon depressum	MW182411	16,537 (26.7)	11,143 (28.8)	2,095 (22.9)	Wang et al., 2021
Longpotamon exiguum	MW182410	17,324 (26.2)*	11,149 (29.1)	2,134 (23.2)	Wang et al., 2021
Longpotamon kenliense	MK584299	18,499 (25.5)	11,170 (29.1)	2,121 (23.3)	Wang et al., 2020
Longpotamon parvum	MN737134	19,637 (26.0)	11,161 (30.5)	2,139 (24.6)	Zhang et al., 2020
Longpotamon xiushuiense	KU042041	18,460 (25.5)	11,172 (29.0)	2,138 (23.0)	unpublished
Longpotamon yangtsekiense	KY785879	17,885 (25.0)	11,155 (28.2)	2,120 (22.7)	Yuhui et al., 2017
Lophopotamon yenyuanense	MN737139	18,869 (27.1)	11,154 (30.3)	2,123 (25.0)	Zhang et al., 2020
Nanhaipotamon hongkongense	MW125541	15,318 (27.3)	11,136 (29.0)	2,122 (23.7)	Wang et al., 2021
Neilupotamon sinense	MN737143	18,894 (32.6)	11,169 (36.0)	2,159 (29.3)	Zhang et al., 2020
Neilupotamon xinganense	MN117718	16,965 (32.9)	11,150 (35.6)	2,156 (28.4)	Tan et al., 2020
Parapotamon spinescens	MN737144	20,227 (22.8)*	11,143 (26.8)	2,144 (22.3)	Zhang et al., 2020
Potamiscus montosus	MN737133	16,299 (27.3)	11,148 (29.3)	2,156 (24.1)	Zhang et al., 2020
Potamiscus motuoensis	MN737138	18,257 (28.2)	11,152 (31.3)	2,126 (25.7)	Zhang et al., 2020
Potamiscus yiwuensis	MN737136	16,307 (27.4)	11,148 (29.4)	2,157 (24.2)	Zhang et al., 2020
Potamiscus yongshengensis	MN737142	17,821 (29.4)	11,148 (32.0)	2,130 (25.7)	Zhang et al., 2020
Sinolapotamon patellifer	MK883709	16,547 (23.6)	11,142 (25.6)	2,196 (21.3)	Ji et al., 2019
Sinopotamon yaanense	KY785880	17,126 (26.6)	11,151 (29.3)	2,120 (23.8)	Yuhui et al., 2017
Tenuilapotamon latilum	MN737132	19,582 (26.6)*	11,158 (30.3)	2,140 (24.5)	Zhang et al., 2020
Tenuipotamon yuxiense	MN737140	18,404 (28.9)	11,145 (31.7)	2,125 (26.0)	Zhang et al., 2020
Terrapotamon thungwa	MW697087	16,156 (26.8)	11,136 (28.6)	2,132 (23.5)	unpublished
mean values		17,561±1299 (27.1±2.1)	11,151±14 (30.1±2.2)	2,132±53 (24.2±1.8)	

* mitogenome contains a gap or was not circularized due to a gap in the sequence.

Materials and Methods

Sample Collection and DNA Extraction

A specimen of *P. fluviatile* collected from the freshwater stream at Baħrija valley (35°53'40.40"N, 14°20'20.11"E) was tissue sampled for this study. This live specimen was sampled through the collection of one walking leg as indicated in Permit NP 0176/18/33A and has already contributed towards a population study on this species in the Maltese archipelago (Vella and Vella, 2020). Upon collection the tissue sample was stored in 100% ethanol and soon after the total genomic DNA was extracted using GF-1 Tissue DNA Extraction Kit (Vivantis Technologies, Malaysia) following the manufacturer's manual. The purified DNA was stored at −20°C.

Library Construction, Mitogenome Assembly and Annotation

A DNA library of the whole genome was constructed and sequenced using 2 × 150 bp paired-end sequencing through Illumina HiSeq 2500 (Illumina, USA). DNA sequences were paired, trimmed at ≥ Q40, and reads shorter than 100 nucleotides were discarded. The final data set was *de novo* assembled using Geneious R10 (Kearse et al., 2012). Once the mitogenome was obtained, the PCGs were identified through homology with other Potamidae species obtained from GenBank (refer to Table 1). These genes were then translated using the invertebrate mitochondrial genetic code and checked for the presence of the predicted start codons, for the positions of the stop codons, and for possible insertions and deletions that would have caused a frameshift using Geneious R10 (Kearse et al., 2012). The software tRNAscan-SE 2.0 (Chan & Lowe, 2019) and ARWEN (Laslett & Canback, 2008) were used to determine the genomic position and the secondary structure for each tRNA, that were identified through their cloverleaf secondary structure and anticodon sequence. The annotated mitogenome was then validated against published mitogenomes of other Potamidae species (refer to Table 1). Nucleotide composition statistics, relative synonymous codon usage, and analyses of AT-skew and GC-skew were also carried out.

Mitogenome Phylogenetic Analysis

The sequence obtained in this study was used to investigate the phylogenetic relationships between *P. fluviatile*, other Potamidae species and other freshwater crabs from the family Gecarcinucidae, using the concatenated data of PCGs and rRNA genes data. Non-coding sequences and tRNAs were not included during this analysis due to their variability arising from tandem duplication and random loss events, gene duplication, tRNA remolding, transpositions and inversion transpositions even between closely related taxa (Zhang et al., 2020). Sequences were aligned using ClustalW (Thompson et al., 1994), while MEGA v10 (Kumar et al., 2018) was used to construct a maximum-likelihood phylogenetic tree (Figure 1) using 1,000 bootstrap and GTR+G+I as it was identified as the best substitution model through the same software.

Results

Mitogenome Organization

The whole genome sequencing for *P. fluviatile* produced 7.9 × 10^7 pair-end reads. After *de novo* assembly, the sequence of the mitochondrial genome was achieved with an average coverage of 2,796 (SD ±276). This mitochondrial genome (GenBank accession no. OL944387) was 16,037 bp, and was partially incomplete due to the presence of a gap arising from tandem repeats in the non-coding region meaning that it could not be circularized unambiguously between 12S rRNA gene and the tRNA-Ile. This work represents the first mitochondrial genome for the subfamily Potaminae. The overall base composition is 35.8% A, 16.2% C, 10.3% G and 37.6% T, with a GC content of 26.6%. As expected the mitogenome contains 37 coding genes that is, 2 ribosomal RNA (12S and 16S) genes, 22 tRNA genes and 13 PCGs. Most of these genes (23) are encoded on the H-strand, while the ND5, ND4, ND4L, ND1, 8 tRNA genes [Gln, Cys, Tyr, His, Phe, Pro, Leu^1, Val] and the two rRNA genes are encoded on the L-strand (Table 2).

The AT and GC skews were calculated using (A−T)/(A+T) and (G−C)/(G+C) respectively. The overall AT skew for the genes was -0.12, indicating a higher T content than A content, while the GC skew was 0.06, indicating that the G content is only slightly higher than C content. The AT and the GC skews of the genes on the H-strand was -0.14 for both skews, while for the genes on the L-strand the values were -0.10 and 0.31 respectively. The latter indicates that the genes encoded on the L-strand have a high proportion of G content when compared to the C content, in fact this GC skew is also reflected in the rRNA genes given that both occur on the L-strand (Table 2).

Protein-coding Genes

The mitogenome contains 11,174 bp that code for PCGs, which add up to a total of 3,724 amino acids. The gene length varies between 1,729 bp for ND5 to 159 bp for ATP8. Most of the PCGs begin with ATG except for ND5 and ND6 that begin with ATT, and ATP6 and ND3 that use ATA as the start codon. For these PCGs the most common stop codon is TAA, although some use TAG (CO3, ND3, ND4) and the incomplete stop codon T— (ND5, CYTB). Overlapping nucleotides between PCGs are present between: ATP6 and ATP8 genes; between ND4L and ND4 genes; and the stop codon of ND6 with CYTB. While the longest non-coding intergenic region is 31 bp

and occurs between the ND5 and ND4 genes. The most commonly used amino acids are Leu (15.5%), Ser (9.8%) and Ile (9.3%) (Table 3). The relative synonymous codon usage for the PCGs is summarized in Table 3. All PCGs have a negative AT skew, except ND1, ND4, ND4L and ND5, which are encoded on the L-strand. All genes have a negative GC skew, except CO1 for which the GC skew value is zero.

Transfer RNAs and Ribosomal RNAs

The tRNAs in *P. fluviatile* vary in size from 62 bp in tRNA-Ala, tRNA-Asn, tRNA-Arg and tRNA-Gly, to 72 bp in tRNA-Val (Table 2), with an overall 25.3% GC content, 0.04 AT-skew and 0.13 GC-skew. The two rRNA genes are separated by tRNA-Val gene, and vary in length from 817 bp for the 12S rRNA to 1,303 bp for the 16S rRNA gene. Their overall GC content is 22.2%, with a 0.02 and 0.30 AT-skew and GC-skew respectively (Table 2).

Phylogenetic Analysis

This analysis shows that Potaminae, here represented by the currently sequenced *P. fluviatile,* is clustered within the family Potamidae, and is a sister to the subfamily Potamiscinae, a relationship that was confirmed with high statistical support.

Table 2. Characteristics of the *Potamon fluviatile* mitochondrial genome coding genes.

Gene	Position (from – to)	Length (bp)	Start / Stop codon	Amino acids	Anticodon	GC%	AT skew[a]	GC skew[b]	IGN	Strand
tRNA-Ile	956 – 1,025	70			GAT	30.0	0.04	0.05		H
tRNA-Gln	1,025 – 1,093	69			TTG	24.6	-0.04	0.65	-1	L
tRNA-Met	1,108 – 1,177	70			CAT	30.0	0.02	-0.14	+14	H
ND2	1,178 – 2,188	1,011	ATG / TAA	336		25.4	-0.19	-0.35	0	H
tRNA-Trp	2,186 – 2,251	66			TCA	15.2	0.18	-0.20	+3	H
tRNA-Cys	2,250 – 2,312	63			GCA	27.0	0.04	0.18	-2	L
tRNA-Tyr	2,313 – 2,376	64			GTA	28.1	-0.09	0.33	0	L
CO1	2,377 – 3,915	1,539	ATG / TAA	512		33.1	-0.16	0.00	0	H
tRNA-Leu[2]	3,911 – 3,976	66			TAA	30.3	0.17	0.20	-5	H
CO2	3,996 – 4,682	687	ATG / TAA	228		29.5	-0.09	-0.12	+19	H
tRNA-Lys	4,685 – 4,749	65			TTT	40.0	-0.03	0.00	+2	H
tRNA-Asp	4,751 – 4,816	66			GTC	21.2	-0.15	0.00	+1	H
ATP8	4,817 – 4,975	159	ATG / TAA	52		21.4	-0.23	-0.53	0	H
ATP6	4,972 – 5,643	672	ATA / TAA	223		30.1	-0.16	-0.21	-4	H
CO3	5,643 – 6,434	792	ATG / TAG	263		33.1	-0.18	-0.10	-1	H
tRNA-Gly	6,434 – 6,495	62			TCC	25.8	0.09	0.00	-1	H
ND3	6,493 – 6,849	357	ATA / TAG	118		26.6	-0.15	-0.14	-3	H
tRNA-Ala	6,849 – 6,910	62			TGC	24.2	0.02	0.20	-1	H
tRNA-Arg	6,913 – 6,974	62			TCG	29.0	0.00	-0.11	+2	H
tRNA-Asn	6,945 – 7,036	62			GTT	30.6	0.02	0.16	0	H
tRNA-Ser[1]	7,035 – 7,103	69			TCT	24.6	0.08	0.06	-2	H
tRNA-Glu	7,126 – 7,192	67			TTC	17.6	0.00	0.00	+22	H
tRNA-His	7,208 – 7,272	65			GTG	24.6	0.06	0.25	+15	L
tRNA-Phe	7,272 – 7,337	66			GAA	22.7	0.10	0.47	-1	L
ND5	7,337 – 9,065	1,729	ATT / T—	576		26.8	-0.14	0.28	-1	L
ND4	9,097 – 10,437	1,341	ATG / TAG	446		26.0	-0.17	0.32	+31	L
ND4L	10,431 – 10,733	303	ATG / TAA	100		26.4	-0.23	0.45	-7	L
tRNA-Thr	10,736 – 10,798	63			TGT	20.6	0.04	0.08	+2	H
tRNA-Pro	10,799 – 10,863	65			TGG	18.5	0.06	0.50	0	L
ND6	10,866 – 11,372	507	ATT / TAA	168		25.8	-0.27	-0.36	+2	H
CYTB	11,372 – 12,506	1,135	ATG / T—	378		31.5	-0.15	-0.16	-1	H
tRNA-Ser[1]	12,507 – 12,570	64			TGA	20.3	0.14	-0.08	0	H
ND1	12,590 – 13,531	942	GTG / ATT	313		27.2	-0.25	0.29	+19	L
tRNA-Leu[1]	13,562 – 13,626	65			TAG	23.1	0.00	0.33	+30	L
16S rRNA	13,627 – 14,929	1,303				22.1	0.02	0.31	0	L
tRNA-Val	14,930 – 15,001	72			TAC	27.8	0.08	0.10	0	L
12S rRNA	15,002 – 15,818	817				22.3	0.03	0.30	0	L
All genes		14,737				27.4	-0.12	0.06		
Genes H-strand		7,773				29.4	-0.14	-0.14		
Genes L-strand		6,964				25.1	-0.10	0.31		
PCGs		11,174				28.6	-0.17	0.01		
rRNAs		2,120				22.2	0.02	0.30		
tRNAs		1,443				25.3	0.04	0.13		

[a] (A-T)/(A+T); [b] (G-C)/(G+C); [c] Intergenic nucleotides

Table 3. The number of codons and the relative synonymous codon usage (number/RSCU) in *Potamon fluviatile* mitochondrial PCGs.

Codon		Codon		Codon		Codon	
UUU(F)	290 / 1.70	UCU(S)	119 / 2.62	UAU(Y)	127 / 1.58	UGU(C)	26 / 1.44
UUC(F)	51 / 0.30	UCC(S)	21 / 0.46	UAC(Y)	34 / 0.42	UGC(C)	10 / 0.56
UUA(L)	390 / 4.04	UCA(S)	78 / 1.71	UAA(*)	8 / 1.80	UGA(W)	67 / 1.35
UUG(L)	30 / 0.31	UCG(S)	8 / 0.18	UAG(*)	3 / 0.70	UGG(W)	32 / 0.65
CUU(L)	93 / 0.96	CCU(P)	71 / 1.93	CAU(H)	55 / 1.39	CGU(R)	19 / 1.33
CUC(L)	16 / 0.17	CCC(P)	12 / 0.33	CAC(H)	24 / 0.61	CGC(R)	5 / 0.35
CUA(L)	45 / 0.47	CCA(P)	51 / 1.39	CAA(Q)	50 / 1.59	CGA(R)	29 / 2.04
CUG(L)	5 / 0.05	CCG(P)	13 / 0.35	CAG(Q)	13 / 0.41	CGG(R)	4 / 0.28
AUU(I)	315 / 1.80	ACU(T)	97 / 2.03	AAU(N)	113 / 1.60	AGU(S)	31 / 0.68
AUC(I)	34 / 0.20	ACC(T)	19 / 0.40	AAC(N)	28 / 0.40	AGC(S)	7 / 0.15
AUA(M)	204 / 3.47	ACA(T)	64 / 1.34	AAA(K)	85 / 1.81	AGA(S)	75 / 1.65
AUG(M)	28 / 0.48	ACG(T)	11 / 0.23	AAG(K)	9 / 0.19	AGG(S)	25 / 0.55
GUU(V)	81 / 1.49	GCU(A)	95 / 2.10	GAU(D)	52 / 1.49	GGU(G)	75 / 1.27
GUC(V)	7 / 0.13	GCC(A)	29 / 0.64	GAC(D)	18 / 0.51	GGC(G)	18 / 0.31
GUA(V)	121 / 2.23	GCA(A)	51 / 1.13	GAA(E)	52 / 1.39	GGA(G)	91 / 1.54
GUG(V)	9 / 0.15	GCG(A)	6 / 0.13	GAG(E)	23 / 0.61	GGG(G)	52 / 0.88

Discussion

The variation in the mitochondrial genome length of Potamidae species is mainly driven by variation in the sequence length of the relatively long non-coding regions, which are at times characterized by tandem repeat elements increasing difficulty in circularizing sequences unambiguously. On the other hand, coding genes vary very little in size from one species to another (Table 1), with the coding genes in *P. fluviatile* being within the expected range for Potamidae species (PCGs from 11,122 bp to 11,186 bp ; rRNA from 1,893 bp to 2,236 bp). This mitogenome exhibited 22 tRNA genes, that is the typical number of such genes in mitogenomes, though it is known that some mitogenomes of freshwater crab species, such as *Sinopotamon parvum*, *Tenuilapotamon latilum* and *Geothelphusa dehaeni* have undergone events such as gene duplication and possible tRNA remolding leading to a larger number of tRNAs (Zhang et al., 2020).

The gene order in the mitogenome of *P. fluviatile* did not show any deviation from the putative ancestral gene order identified as brancyuran mitogenome ground pattern in Zhang et al., (2020). While this pattern is likely to have occurred in the common ancestor the subfamily Potamiscinae as it is the most common gene order in this subfamily, yet a number of potamiscine species deviate from it by having different gene order rearrangements namely due to transposition, inversion transposition or duplication (Segawa & Aotsuka, 2005; Zhang et al., 2020). Identifying the ground patter in *P. fluviatile*, that is within the subfamily Potaminae, further confirms that this arrangement is an ancestral arrangement for the family Potamidae.

The phylogenetic analysis covered in this study further corroborated the work of Zhang et al., 2020, who indicated that the subfamily Potamiscinae is monophyletic. In the latter study this subfamily was found to be monophyletic when compared to the family Gecarcinucidae and other marine crabs as outgroup. The current study, through the data on *P. fluviatile*, we were able to compare the phylogenetic connection between subfamily Potaminae and the subfamily Potamiscinae. Here mitogenomic data confirmed that the subfamily Potaminae forms a distinct clade from its sister subfamily Potamiscinae, with the two sharing common ancestry which is supported by a bootstrap value of 100 (Figure 1). Together these two subfamilies form Potamidae (Ng et al., 2008), which is here represented as a monophyletic family distinct from Gecarcinucidae, that is another family of freshwater crabs. This confirms the outcome of previous molecular studies that used smaller DNA sequences for analyses (Klaus et al., 2009; Shih et al., 2009; Tsang et al., 2014).

Conclusion

In this study, we sequenced and annotated the first mitogenome for the subfamily Potaminae, as represented by *P. fluviatile*, adding valuable data to a better understanding of the phylogenetic and evolution patterns within Potamidae. Moreover, this work provides the required information to enhance the molecular identification of freshwater crabs and population studies assisting conservation efforts of this species that occurs in highly fragmented populations (Vella and Vella, 2020).

Ethical Statement

This study made use of a handling and nonlethal tissue sampling permit for conservation research from the local environment protection authority.

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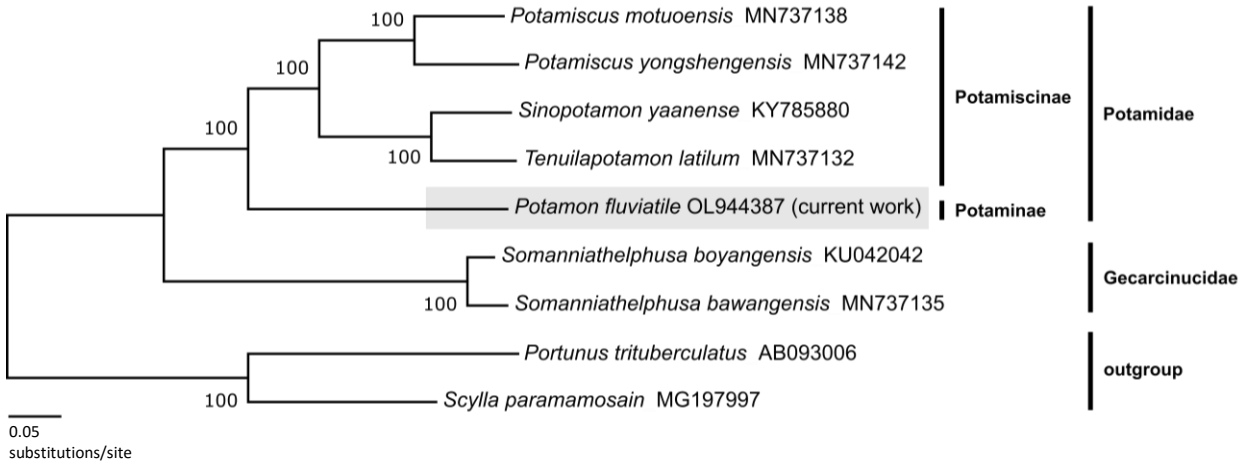

Figure 1. A maximum-likelihood tree using the 13 protein-coding and the two rRNA coding genes to show the phylogenetic relationship between *Potamon fluviatile* and other freshwater crabs of the family Potamidae and Gecarcinucidae, using two marine carbs as an outgroup (Jia et al., 2018; Yamauchi, Miya & Nishida, 2003; Yuhui et al., 2017; Zhong et al., 2017; Zhang et al., 2020). Bootstrap values are indicated at each node.

Author Contribution

Both AV and NV contributed towards the design of the research, sample collection, laboratory analyses, data analyses and manuscript writing. Both authors approved the final manuscript.

Conflict of Interest

The authors declare that there is no conflict of interest.

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