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Research Article

Complete Mitochondrial Genome Analyses of *Forcipomyia pulchrithorax* (Diptera: Ceratopogonidae): Genome Orientation and Phylogenetic Implications

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Abstract

Forcipomyia Meigen, 1818, is the second-largest genus of the family Ceratopogonidae, having medico veterinary and agricultural importance. A few mitogenomes of Ceratopogonidae have previously been available. Here, we aimed to characterize the whole mitogenome of *Forcipomyia pulchrithorax* Edwards, 1924, collected from Central Anatolia Region of the Türkiye using NGS to contribute to the genetic diversity within this family. The mitogenome of *F. pulchrithorax* (OR666457) consisted of a circular DNA molecule spanning 15,930 bp and containing 72.7% AT content. It includes 13 protein-coding genes (PCGs), 22 transfer RNA (tRNA) genes, 2 ribosomal RNA (rRNA) genes, and 1 control region. All PCGs exhibited typical ATN start codons and followed the conventional TAN stop codons, except COX1, COX2, COX3, ND1, and ND5, which ended with incomplete codons. The 22 tRNA genes demonstrated the ability to form a cloverleaf structure, except tRNA-Ser1, which lacked DHU arm, similar to most insect mitogenomes. The ML phylogenetic analyses clearly delineated the species of Culicomorpha into well supported monophyletic clades. *Forcipomyia pulchrithorax* was clustered with the Ceratopogonidae species constituting an outer taxon among the Culicomorpha. The comprehensive mitogenome of *F. pulchrithorax* will be valuable for future studies focused on the phylogenetic characterization and diversity of Ceratopogonidae within the Culicomorpha.

Keywords: Culicomorpha, Diptera, *Forcipomyia pulchrithorax*, Mitochondrial genome, Phylogeny

Introduction

The genus *Forcipomyia* Meigen, 1818 of Forcipomyiinae (Diptera: Ceratopogonidae) comprises medium-sized midges. The members of this subfamily have a considerable economic impact due to their role in pollinating cocoa and rubber trees [1].

Biting midges belonging to this genus have a worldwide distribution, inhabiting diverse environments such as paddy fields, bamboo forests, and marshlands. *Forcipomyia* is one of the most species-rich genera of the family Ceratopogonidae, with 1.186 described species in 36 subgenera [2]. Other insect orders (e.g., Phasmida, Orthoptera, Lepidoptera, Heteroptera, Odonata) often serve as hosts for adult female ectoparasites [3,4]. Along with the genera *Culicoides* and *Leptoconops* of the Ceratopogonidae, the females of the subgenus *Forcipomyia* also feed on vertebrate blood [3,5].

In insects, the mitochondrial genome is small, typically ranging from 15 to 20 kb, and encompasses 37 genes. These genes comprise 13 protein-coding genes (PCGs), 22 transfer RNA genes (tRNAs), and the large and small ribosomal RNA unit genes (rrnL and rrnS, respectively). Besides, the mitochondrial genome has a single, large non-coding region that contains controlling elements for replication and transcription, along with various spacer and overlapping regions [6-9]. Unlike the nuclear genome, mitogenomes are maternally inherited, occur in hundreds to thousands of copies per cell, and exhibit

minimal recombination in animals [10-12]. By comparing the mitochondrial gene arrangements of animals, scientists can infer long-term evolutionary relationships since rearrangements are unique and generally rare events that are unlikely to occur independently in separate evolutionary lineages [6].

Modern molecular tools have been successfully used to evaluate Ceratopogonidae's including the genus *Forcipomyia* through genetic markers such as partial or complete mitochondrial sequences [13-15]. Although Ceratopogonidae is one of the largest groups of Diptera insects, only a few complete mitogenomes have been characterized or published so far [14-17]. In the *Forcipomyia* genus, *F. makanensis*, which has a wide distribution in certain regions of China, is the only species with a published mitogenome characterization [10].

Considering the lack of mitogenome-based genomic information on the genus *Forcipomyia* of Ceratopogonidae, which has potential epidemiological interest and economic impact in many regions of the world, in this study, we performed for the first time the characterization of the mitochondrial genome of the species *F. pulchrithorax* Edwards, 1924, collected from the Central Anatolia Region of Türkiye. We reconstructed the phylogenetic relationship of the species within the families of Culicomorpha based on the concatenation of all 13 PCGs and two rRNA genes.

Material and Methods

Ethical Statement

This study does not require the approval of the Local Ethics Committee for Animal Experiments.

Collection of Specimens and DNA Extraction

Adult midges were sampled in August 2022 from a location in Sivas province of the Central Anatolia Region of Türkiye (Coordinates: 39.327151, 37.391583). The midges were captured using Onderstepoort-type 220 V blacklight traps with 8 W UV light tubes and stored in absolute ethanol for identification. Initial identification of the midges relied on their morphological characteristics using appropriate keys [18,19]. After morphological identification, the midges were preserved in absolute ethanol at -20°C. Genomic DNA (gDNA) was extracted from the whole body of the midges using a PureLink™ Genomic DNA Mini Kit (Thermo Scientific, Waltham, MA, USA) following the manufacturer's protocol. The obtained gDNA was quantified using a Qubit 3.0 Fluorometer (Thermo Scientific, Waltham, MA, USA) and stored at −20°C.

Long-range PCR Amplification of Mitogenome and Sequencing

To amplify the complete mitogenome of the specimens in two overlapping fragments, we first downloaded all full or partial mitochondrial sequences of Culicomorpha species available in GenBank and conserved regions were determined by multiple alignment of all sequences. Two sets of long-range PCR primers were designed from the corresponding gene regions using Primer3 v.2.3.7. [20], which was integrated with Geneious Prime software *(https://www.geneious.com)* (*Table 1*).

Long-range PCR was performed using LongAmp® Taq 2x Master Mix (New England Biolabs, Ipswich, MA, USA) under the following cycling parameters: 1 min at 94°C; 35 cycles of 30 s at 94°C, 30 s at 50°C (First fragment)/53°C (Second fragment), and 17 min (First fragment)/7 min (Second fragment) at 60.5°C; and 60.5°C for 10 min. Each amplification products (5 µL) electrophoretically resolved, purified, and quantified as described by Ciloglu et al.[21].

Preparation of Libraries, NGS, Annotation, and Analysis of Mitochondrial DNA

We diluted the PCR products with ddH_2O to 0.2 ng/µL of DNA concentration. Two diluted PCR products were pooled together in equimolar ratios. We used the Nextera XT DNA Library Prep Kit (Illumina, San Diego, USA) and the Nextera XT DNA Library Preparation Index Kit v2 Set A (Illumina, San Diego, USA) for the construction of the libraries following the protocols of the manufacturer. The amplicons of each library were quantified and diluted to approximately equal concentrations and were pooled. The pooled multiplexed libraries were sequenced on an Illumina MiSeq platform (Illumina, San Diego, USA) with the MiSeq Reagent Kit v2 (500 cycles).

FastQC [22] was used to assess the quality of the raw sequence data. We filtered out sequences shorter than 50

Table 1. *Primers used for the amplification of the mitogenome of F. pulchrithorax*

Organism	Primer set	Primer Name	Primer Sequence	Target Gene	TM Degree (°C)	Extension Time (min)	Size (bp)
Forcipomyia pulchrithorax	1	MidgeF1	TTATAATTGGRGGATTYGGWAATTG	COI	50	17	13.000
		MidgeF2	TTAAGTTACTTTAGGGATAACAGC	16SrRNA			
	2	MidgeF3	ATTACGCTGTTATCCCTAAAGTAAC	COI	53	7	5.165
		MidgeF4	GTTCAKCCRGTTCCDGCYCCAT	16SrRNA			

TM: melting temperature

bp, trimmed low-quality bases (Q-score <25) at the end of reads, and removed adaptors using the BBDuk plug-in of Geneious Prime to The paired-end clean reads (~400.000 reads) of *F. pulcrithorax* were assembled in Geneious Prime using the mitogenome of *F. makanensis* from GenBank (Accession number: MK000395) as a reference sequence [15]. The tool embedded in Geneious Prime, Map to Reference, was utilized with the Highest Sensitivity/Medium Geneious Mapper Algorithm and up to 25 iterations. To ensure coverage and completeness, we performed *de novo* assembly using GetOrganelle v1.7.7.0 [23]. Finally, the aliging of *de novo* contigs with those generated by mapping to the reference sequence of *F. makanensis* confirmed that the assembly method did not influence the final mitogenome sequences.

The mitochondrial genome of *F. pulcrithorax* was annotated using MITOS2 [24] and the invertebrate mitochondrial genetic code. tRNA genes were identified using tRNAscan-SE [25] and MITOS2. Gene borders were manually curated and checked using MITOS2. The tRNA visualization was performed with VARNA [26]. PCGs and rRNA genes were determined based on alignments with available Culicomorpha mitogenomes in GenBank. The graphical mitogenome circular map was drawn using Proksee Server [27]. RSCU, nucleotide, and translation statistics were calculated using Geneious Prime and MEGA 11 programs [28].

Phylogenetic Analyses

A total of 18 Culicomorpha species representing seven families and 15 genera were retrieved from GenBank to reconstruct the phylogenetic relationships of the *F. pulcrithorax* (*Table 2*). *Phlebotomus chinensis* was used as an outgroup taxon. The phylogeny data was based on the concatenated sequence alignments of nucleotide sequences of 13 protein-coding genes and two rRNA genes. Initially, the mitogenome sequences in the data set were aligned using MAFFT [34] in Geneious Prime. Poorly aligned sites were subsequently removed using the Gblocks server [35] with stringent criteria. The phylogenetic tree was constructed using the maximum likelihood (ML) method in MEGA 11 with the optimal nucleotide substitution model (GTR +I +G with 1000 replicates), determined by the jModel test v.0.1.1 [36].

Results

Mitogenome Organization

The complete mitogenome of *F. pulcrithorax* is 15.930 bp in length, including 13 PCGs, 22 tRNAs, and two rRNAs (rrnL and rrnS) (*Fig. 1*). The heavy strand (H-strand) encodes most of the genes (nine PCGs and 14 tRNAs) while, the light strand (L-strand) contains the remaining reverse complementary genes (four PCGs, eight tRNAs, and two rRNAs) as shown in *Table 3*. The AT content of the *F. pulcrithorax* mitogenome is 72.7% (*Table 3*).

Table 2. *Summary of taxonomic groups used in this study*

Family	Genus	Species	Whole Genome	Genbank Accession	References
Ceratopogonidae	*Forcipomyia*	*Forcipomyia pulchrithorax*	15.590	OR666457	This study
		Forcipomyia sp.	15.584	MK000395	Jiang [15]
	Culicoides	*Culicoides arakawae*	15.412	AB361004	Matsumoto et al.[14]
Dixidae	*Dixella*	*Dixella aestivalis*	16.465	KT878382	Briscoe et al.[29]
		Dixella sp.	15.574	KM245574	Kang et al.[30]
Culicidae	*Culex*	*Culex pipiens molestus*	16.323	MN389460	Unpublished
	Aedes	*Aedes koreicus*	15.843	MZ460582	Unpublished
	Anopheles	*Anopheles cruzii*	15.877	KJ701506	Unpublished
	Ochlerotatus	*Ochlerotatus vigilax*	16.445	KP721463	Hardy et al.[31]
Corethrellidae	*Corethrella*	*Corethrella condita*	14.520	MK281357	Zhang et al.[17]
Chaoboridae	*Chaoborus*	*Chaoborus sp.*	14.746	MK281356	Zhang et al.[17]
Simuliidae	*Simulium*	*Simulium variegatum*	15.575	KU252587	Unpublished
		Simulium noelleri	16.190	MT410847	Unpublished
Chironomidae	*Polypedilum*	*Polypedilum nubifer*	15896	MZ747090	Xiao et al.[32]
	Chironomus	*Chironomus flaviplumus*	15.739	MW770891	Unpublished
	Limnophyes	*Limnophyes minimus*	15.607	MZ041033	Unpublished
	Microchironomus	*Microchironomus tabarui*	15.667	MZ261913	Kong et al.[33]
Thaumaleidae	*Thaumalea*	*Thaumalea sp.*	14.610	MK281359	Zhang et al.[17]

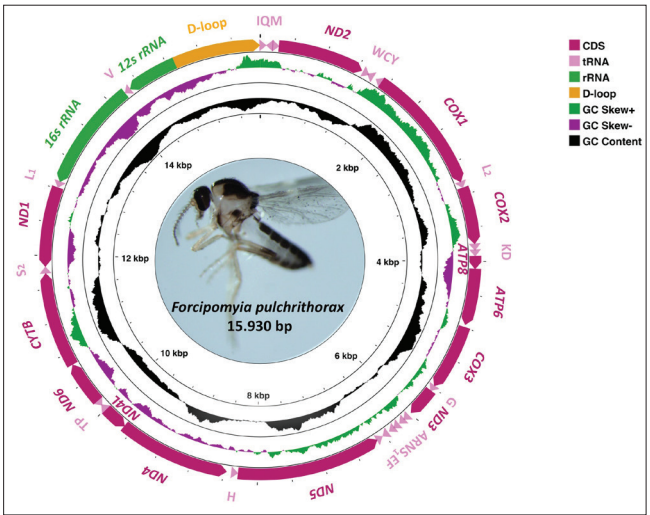

Fig 1. Circular mitogenome map of *F. pulcrithorax*. The internal values indicate the overall content of the nucleotide bases. The magenta, pink, and green blocks indicate PCGs, tRNAs, and rRNAs, respectively. Each tRNA is identified by a unique letter abbreviation. The genes located in the H (Forward) and L (Reverse) strands are shown in opposite directions

Protein-coding Genes (PCGs) and Codon Usage

The total length of 13 PCGs is 11.175 bp, accounting for 70.15% of the whole mitogenome length of *F. pulcrithorax*. All 13 PCGs of *F. pulcrithorax* initiate translation using ATN codons (ATG for *ND2*, *ND3*, *ND4*, *COX2*, *COX3*, *ATP6*, *ATP8*, and *CYTB*; ATA for *ND4L*; ATT for *COX1*, *ND1*, *ND5*, and *ND6*). For the stop codon, seven PCGs of *F. pulcrithorax* had the common mitochondrial stop codon TAA, while *ND4* was terminated with the stop codon TAG, *COX3* ended with "TA-", and *COX1*, *COX2*, *ND1*, and *ND5* ended with a single "T" *(Table 3)*.

The *F. pulcrithorax* mitogenome encodes 3.732 amino acids, excluding incomplete stop codons. This reflects the high A+T content observed throughout the genome, particularly in codon usage. In the *F. pulcrithorax* mitogenome *(Fig. 2)*, NNA and NNU codons (78.05%) are significantly more prevalent than NNC and NNG codons (21.95%). Among the 13 PCGs in *F. pulcrithorax*, Leu1 (403) is the most frequently used amino acid codon, followed by Thr (313). Cys (25) is the least frequently used amino acid codon *(Fig. 2)*.

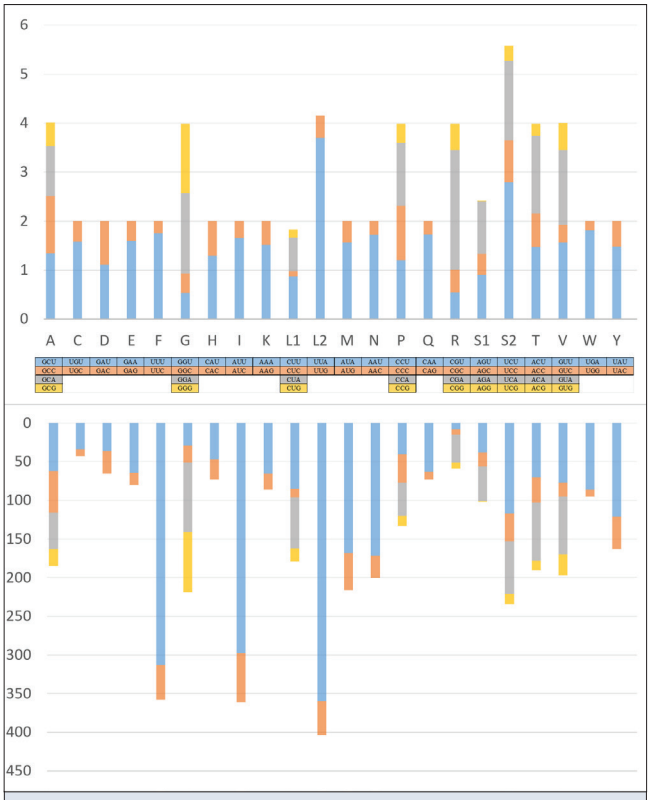

Fig 2. RSCU and codon usage of the *F. pulcrithorax* mitogenome. The graphics indicate (A): RSCU, (B): Codon usage counts. Amino acids were shown based on the IUPACIUB one-letter abbreviation. Codon families were given on the X-axis. The stop codons were not included

Transfer RNAs

The mitogenome of *F. pulcrithorax* contains all 22 tRNAs *(Fig. 3)*. The tRNAs exhibit a size range of 61 to 72 bp, with 13 tRNA genes located on the H-strand and the remaining nine tRNA genes on the L-strand *(Table 4)*. Most tRNA genes display classical clover-leaf secondary structures, except for trnS1(AGN), which possesses a simplified DHU arm *(Fig.3)*.

Phylogenetic Analyses

The phylogenetic analyses of the mitogenome concatenated PCGs and ribosomal genes separated the species in each family with an overall 36% mean genetic distance (10.6% to 53.4%) *(Table 5)*. The ML tree indicated the monophyly of all families in Culicomorpha with high bootstrap supports

Table 3. *Overall nucleotide composition in the mitogenome of F. pulcrithorax*

Forcipomyia pulchrithorax	**Size**	**%**	**%A**	**%T**	**%G**	**%C**	**%A+T**	**%G+C**
Total Length	15.930	100%	37.50%	35.20%	10.40%	16.90%	72.70%	27.30%
PCGs	11.175	70.32%	36.40%	33.70%	11.60%	18.30%	71.10%	29.90%
12s rRNA	614	3.85%	37.00%	38.90%	7.80%	16.30%	75.90%	24.10%
16s rRNA	1.320	8,28%	41.70%	41.00%	5.30%	12.00%	82.70%	17.30%
tRNAs	1.450	9.07%	37.70%	37.10%	10.90%	14.30%	74.80%	25.20%
Control Region (D-loop)	1.037	6.50%	44.10%	37.90%	6.30%	11.80%	82.00%	18.00%

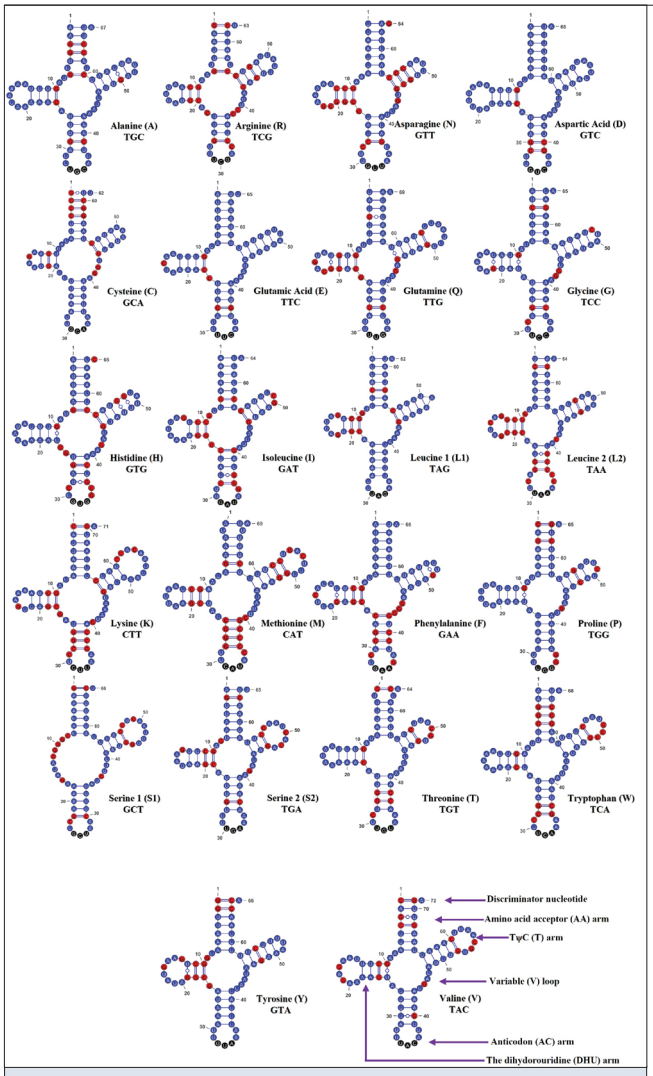

Fig 3. Predicted secondary cloverleaf structure for the tRNAs of *F. pulcrithorax*

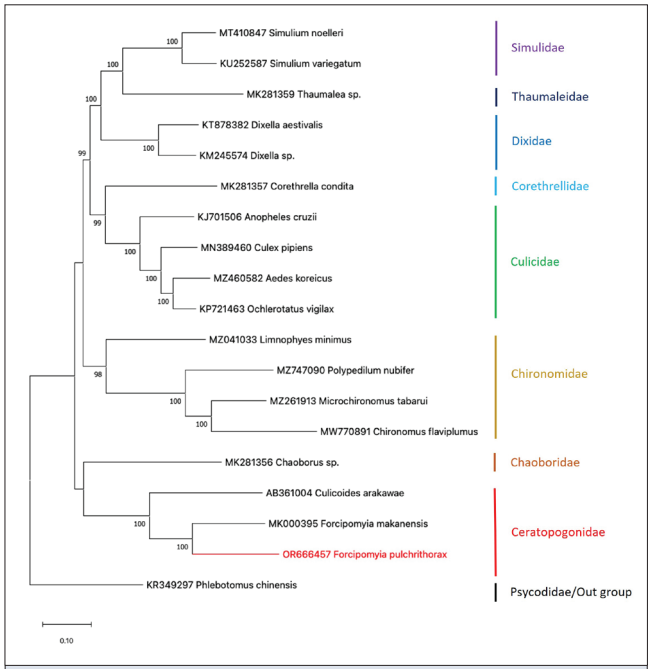

Fig 4. The reconstructed ML tree of mitogenome sequences of *F. pulcrithorax* (in red character), along with the representatives of other genera in the families of Culicomorpha is available in GenBank. The ML tree is based on the concatenated sequence alignments of nucleotide sequences of 13 protein-coding genes and two rRNA genes using GTR+I+G nucleotide substitution model. The bootstrap values over 70.0% are indicated at each node. *Phlebotomus chinensis* was employed as an outgroup taxa

(98.0% to 100.0%) *(Fig. 4). Forcipomyia pulcrithorax* was clustered with *F. makanensis*, and this clade was placed as a sister to *C. arakawae* with high bootstrap values (100.0%). In our analyses, Ceratopogonidae, including the newly characterized *F. pulcrithorax* constituted an outer taxon among the Culicomorpha. Chironomidae was recognized as a sister taxon to Culicidae + Dixidae + (Thaumaleidae + Simuliidae).

Discussion

The nucleotide composition of *F. pulcrithorax* is AT-biased, similar to the mitogenomes of several Culicomorpha species [15-17,29,30]. The AT content of *F. pulcrithorax* mitogenome was found to be 72.7% *(Table 3)*. The high AT content is widely attributed to the evolution of mitochondrial origin [37].

The whole mitogenome length of *F. pulcrithorax* is similar to *F. makanensis* [15] and relatively lower compared to *C. arakawae* [14] within the same family, Ceratopogonidae. Jiang et al.[15] identified all PCGs with the standard stop codon pattern "TAA," except *COX3*, which ended with an incomplete termination codon "TA-" in the mitogenome of *F. makanensis*. The COX3 gene of the *F. pulcrithorax* was also ended with incomplete stop codon "TA-" similar to *F. makanensis*. However further incomplete termination codon "T--" was also identified in the *COX1*, *COX2*, *ND1*, and *ND5* genes of *F. pulcrithorax*. Incomplete stop codons are also present in the PCGs of Culicomorpha species listed in *Table 3* and other common insect mitogenomes [14,15,17,29,33]. They are considered to produce functional stop codons in polyadenylation mechanisms and polycistronic transcription cleavage [38,39].

High A+T content was identified throughout the *F. pulcrithorax* mitogenome, particularly in PCGs. The relative synonymous codon usage (RSCU) matrix indicated the dominancy of NNA and NNU compared with the codons NNC and NNG. This type of RSCU is also frequently observed in other metazoan mitochondrial genomes [40-42].

The mitogenome of *F. pulcrithorax* contains all 22 tRNAs characteristic of dipteran mitogenomes. Most tRNA genes display classical clover-leaf secondary structures except for trnS1(AGN). This peculiarity of tRNASer is prevalent among insect and metazoan mitogenomes [8,43].

Table 4. *The mitogenome organization of F. pulcrithorax*

Gene	Direction	Minimum	Maximum	Size	Anticodon	Start Codon	Stop Codon	Intergenic Nucleotides
tRNA-Ile	H	1	64	64	GAT	-	-	13
tRNA-Gln	L	78	146	69	TTG	-	-	5
tRNA-Met	H	152	220	69	CAT	-	-	0
ND2 CDS	H	221	1252	1032	-	ATG	TAA	20
tRNA-Trp	H	1273	1340	68	TCA	-	-	-8
tRNA-Cys	L	1333	1394	62	GCA	-	-	53
tRNA-Tyr	L	1448	1513	66	GTA	-	-	34
COX1 CDS	H	1548	3045	1498	-	ATT	T--	0
tRNA-Leu	H	3046	3109	64	TAA	-	-	4
COX2 CDS	H	3114	3798	685	-	ATG	T--	0
tRNA-Lys	H	3799	3867	69	CTT	-	-	-1
tRNA-Asp	H	3869	3936	68	GTC	-	-	0
ATP8 CDS	H	3937	4095	159	-	ATG	TAA	-7
ATP6 CDS	H	4089	4766	678	-	ATG	TAA	24
COX3 CDS	H	4791	5578	788	-	ATG	TA-	0
tRNA-Gly	H	5579	5643	65	TCC	-	-	0
ND3 CDS	H	5644	5997	354	-	ATG	TAA	45
tRNA-Ala	H	6043	6109	67	TGC	-	-	3
tRNA-Arg	H	6113	6175	63	TCG	-	-	24
tRNA-Asn	H	6200	6263	64	GTT	--	-	0
tRNA-Ser	H	6264	6329	66	GCT	-	--	31
tRNA-Glu	H	6361	6425	65	TTC	-	-	15
tRNA-Phe	L	6441	6506	66	GAA	-	-	0
ND5 CDS	L	6507	8229	1723	-	ATT	T--	6
tRNA-His	L	8236	8300	65	GTG	-	-	60
ND4 CDS	L	8361	9701	1341	-	ATG	TAG	-7
ND4L CDS	L	9695	9997	297	-	ATA	TAA	4
tRNA-Thr	H	10002	10062	61	TGT	-	-	2
tRNA-Pro	L	10068	10132	65	TGG	-	-	1
ND6 CDS	H	10134	10655	522	-	ATT	TAA	-1
Cytb CDS	H	10655	11791	1137	-	ATG	TAA	4
tRNA-Ser	H	11796	11860	65	TGA	-	-	6
ND1 CDS	L	11867	12821	956	-	ATT	T--	4
tRNA-Leu	L	12826	12887	62	TAG	-	-	0
16S rRNA	L	12888	14207	1320	-	--	-	0
tRNA-Val	L	14208	14279	72	TAC	-	-	0
12S rRNA	L	14280	14893	614	-	-	-	0
Control Region	H	14894	15930	1037	-	-	-	0

Table 5. *Mean pairwise genetic distance matrix of the mitogenome, for the clades Culicomorpha species. Evolutionary analyses were conducted using the Kimura-2-parameter (K2P) distance model with MEGA version 11*

No	Species with Accession Number	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
1	OR666457 *Forcipomyia pulchrithorax*																	
2	MK000395 *Forcipomyia makanensis*	0.264																
3	AB361004 *Culicoides arakawae*	0.347	0.332															
4	MK281356 *Chaoborus* sp.	0.442	0.418	0.410														
5	MW770891 *Chironomus flaviplumus*	0.534	0.510	0.496	0.468													
6	MZ261913 *Microchironomus tabarui*	0.469	0.450	0.447	0.406	0.250												
7	MZ747090 *Polypedilum nubifer*	0.479	0.442	0.448	0.413	0.316	0.268											
8	MZ041033 *Limnophyes minimus*	0.436	0.389	0.391	0.362	0.401	0.357	0.363										
9	KP721463 *Ochlerotatus vigilax*	0.410	0.365	0.378	0.316	0.393	0.367	0.363	0.291									
10	MZ460582 *Aedes koreicus*	0.416	0.389	0.388	0.340	0.417	0.381	0.375	0.312	0.106								
11	MN389460 *Culex pipiens*	0.406	0.381	0.372	0.337	0.396	0.369	0.370	0.305	0.115	0.141							
12	KJ701506 *Anopheles cruzii*	0.404	0.373	0.371	0.330	0.411	0.374	0.379	0.305	0.166	0.190	0.178						
13	MK281357 *Corethrella condita*	0.428	0.392	0.411	0.359	0.437	0.388	0.407	0.337	0.269	0.295	0.294	0.278					
14	KM245574 *Dixell*a sp.	0.423	0.382	0.394	0.343	0.436	0.387	0.388	0.310	0.266	0.279	0.271	0.275	0.308				
15	KT878382 *Dixella aestivalis*	0.430	0.391	0.398	0.347	0.436	0.400	0.398	0.316	0.273	0.299	0.284	0.286	0.323	0.139			
16	MK281359 *Thaumalea* sp.	0.461	0.467	0.451	0.428	0.526	0.449	0.445	0.379	0.354	0.362	0.362	0.360	0.381	0.341	0.345		
17	KU252587 *Simulium variegatum*	0.431	0.417	0.416	0.377	0.454	0.402	0.407	0.330	0.292	0.305	0.302	0.306	0.341	0.299	0.303	0.343	
18	MT410847 *Simulium noelleri*	0.432	0.404	0.420	0.378	0.445	0.396	0.401	0.326	0.294	0.298	0.304	0.308	0.339	0.293	0.300	0.331	0.127

According to Henning's (1973) classification, Ceratopogonidae was placed within the superfamily Chironomoidea and identified as the sister taxon to Chironomidae [44]. Some subsequent studies [45,46] also supported this relationship. However, Ceratopogonidae was placed in the superfamily Simulioidea with Thaumaleidae and Simuliidae in Borkent's analysis based on six pupal and one adult synapomorphies [47]. Zhang et al.[17] also reported compatible findings with Borkent's analyses without strong Bayesian support for the sister-group relationship between Ceratopogonidae and Thaumaleidae + Simuliidae. In our analyses, Ceratopogonidae, including the newly characterized *F. pulcrithorax* constituted an outer taxon among the Culicomorpha. Chironomidae was recognized as a sister taxon to Culicidae + Dixidae + (Thaumaleidae + Simuliidae), which was congruent with the findings of the phylogenetic reconstruction of Miller et al.[48] and Zhang et al.[17]. On the other hand, the family Chironomidae, was also reported as a paraphyletic taxon by Zhang et al.[17] when using PCGs, rRNAs, and tRNAs in the phylogenetic analyses with different evolutionary models.

In conclusion, our study provides the first data on the mitogenome characterization and phylogenetic analyses of *F. pulcrithorax*, and the findings contribute to the taxonomy and phylogeny of Culicomorpha, which is considered a complex taxon. We also conclude that the mitogenome data of *F. pulchrithorax* can be used to identify genetic markers for species identification. Although Ceratopogonidae contains many described species, there are only three mitogenome data from the species of this family, including *F. pulchritorax* characterized in our study. Therefore, broader taxonomic sampling and mitogenome characterization are needed to better understand of the

phylogenetic and taxonomic evaluation of the members of Ceratopogonidae.

Availability of Data and Materials

The sequence of mitogenome was deposited in the GenBank under accession number of OR666457.

Ethical Statement

This study does not require the approval of the Local Ethics Committee for Animal Experiments.

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Conflict of Interest

The authors declared that there is no conflict of interest.

Author Contributions

Conceptualization, G.K.K, A.Y., and O.I; methodology, G.K.K., C.C.K., and A.M.T.; investigation, G.K.K. and S.T.; data curation, G.K.K. and A.M.T; writing-original draft preparation, G.K.K. and S.T.; writing-review and editing, G.K.K., A.Y., and O.I.; supervision, G.K.K. and A.Y. All authors have read and agreed to the published version of the manuscript.

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