COMPLETE MITOCHONDRIAL GENOME OF *TELMATOGETON* SP. (DIPTERA: CHIRONOMIDAE) FROM THE CASPIAN SEA BASIN

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Summary. The complete mitochondrial DNA (mtDNA) of *Telmatogeton* sp. (Diptera: Chironomidae: Telmatogetoninae) was sequenced using Illumina platform. The total length was 15652 bp which is one of the shortest mtDNA among chironomids. Circular mitochondrial genome keeps basic 13 protein-coding, 22 tRNA, 2 rRNA genes and control region which were annotated for *Telmatogeton* sp. and deposed in GenBank. Raw reads were unevenly distributed along the mitochondrial DNA, decreasing significantly around the control region. A phylogeny revealed new position of Telmatogetoninae on the chironomids tree as sister to Chironominae + Orthocladiinae but support for this node was low. The origin of *Telmatogeton* sp. in the inland Caspian Sea basin may results of natural habitat in Ponto-Caspian basins or invasion from the Black Sea in 20th century.

Key words: Chironomidae, *Telmatogeton*, mitochondrial genome, phylogenetics analysis, invasive species, Kazakhstan.

1

Резюме. Полная митохондриальная ДНК (мтДНК) *Telmatogeton* sp. (Diptera: Chironomidae: Telmatogetoninae) была прочитана с использованием платформы Illumina. Общая длина составила 15652 п.н., что делает ее одной из самых коротких мтДНК среди хирономид. Кольцевой митохондриальный геном имеет 13 белок-кодирующих генов, 22 тРНК, 2 рРНК и контрольная область, которые были аннотированы для *Telmatogeton* sp. и депонированы в GenBank. Сырые прочтения были неравномерно распределены вдоль митохондриальной ДНК, покрытие значительно снижалось в районе контрольной области. Филогения выявила новое положение Telmatogetoninae на дереве хирономид как сестринское для Chironominae + Orthocladiinae, но поддержка этого узла была низкой. Происхождение *Telmatogeton* sp. во внутреннем бассейне Каспийского моря, возможно, является результатом либо его исторического обитания в Понто-Каспийском бассейне или инвазии из Черного моря в ХХ веке.

INTRODUCTION

Telmatogetoninae is a highly specialized subfamily of chironomids, the larvae of which live in the littoral zones of the seas, but some species in Hawaii islands have secondarily evolved from the marine environment into freshwater lotic habitats (Newman, 1977). Subfamily includes two sister genera *Telmatogeton* Schiner and *Thalassomya* Schiner with divergence in the Cretaceous (Tang *et al*., 2022) and 39 described species (Ashe & Connor, 2009).

The specimen sequenced in this study (EAM940) morphologically belongs to *Telmatogeton japonicus* Tokunaga, 1933. According to Barcode of Life Data System (BOLD) to date *T. japonicus* is divided into 4 Barcode Index Numbers (BINs) three of which are found near the type habitat in Japan. On the other hand, none of the sequenced specimens were collected exactly in the type habitat in Karo, Tottori Prefecture, which not allow to assign one of four BINs to the 'true' *T. japonicus*. Morphological characteristics of larvae and adult males do not allow reliably distinguishing species from different BINs (personal communication from E.A. Makarchenko), which indicates that *T. japonicus* is cryptic species. In addition, at least two BINs are invasive and expanding its range using different types of marine structures (Coolen *et al*., 2020) and are currently widespread throughout the boreal zone. First species (BIN ACV4061) inhabits Sweden, Iceland and Japan (Hokkaido, Hakodate), and the second (BIN AAN6244), which was sequenced in this study inhabits USA (Atlantic coasts), Japan (Shirahama, Wakayama Prefecture) (Cornette *et al*., 2015) and Kazakhstan (inland brackish Caspian Sea, this study).

The monophyly of genera *Telmatogeton* and *Thalassomya* were well supported using a multi-locus approach (Tang *et al*., 2022). In turn, unlike most subfamilies,

Telmatogetoninae have an unstable position on chironomid tree using both morphological (Fig. 1A-D) and molecular data (Fig. 1E-H). It is noteworthy that high support for the Telmatogetoninae (Kutty *et al*., 2018, Tang *et al*., 2022, Semenchenko *et al*., 2024) node did not maintain a stable position (Fig. 1F-H).
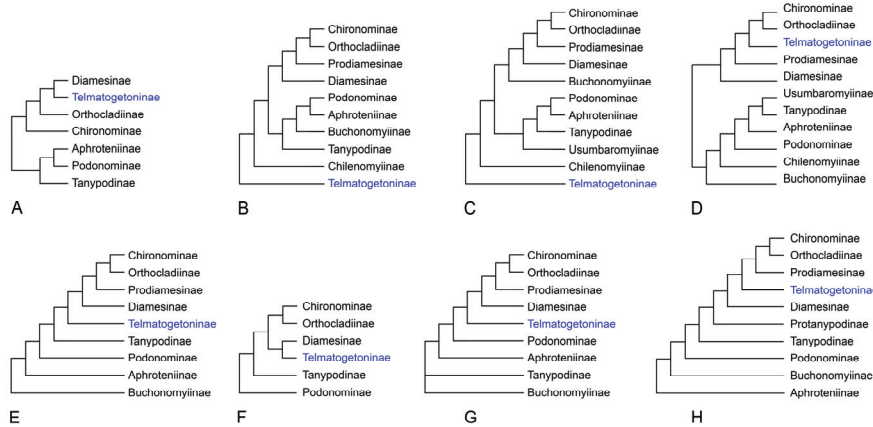

Fig. 1. Phylogeny of Chironomidae suggested by: (A) Brundin (1966), (B) Murray & Ashe (1981, 1985), (C) Sæther (1977), Brundin & Sæther (1978), Andersen & Sæther (1994), (D) Ache & Connor (2009), (E) Cranston *et al*. (2012), (f) Kutty *et al*. (2018), (G) Tang *et al*. (2022), (H) Semenchenko *et al*. (2024). Blue color indicates subfamily Telmatogetoninae.

In this study, we used Illumina Platform sequencing technology for paired-end sequencing to obtain the complete mitochondrial genome of *Telmatogeton* sp. This is the first complete mitochondrial sequence of subfamily Telmatogetoninae, which provide new insight into the phylogeny of this subfamily on the chironomid tree.

MATERIAL AND METHODS

The photograph (Fig. 2) was taken using an Olympus SZX16 stereomicrocope with an Olympus DP74 digital camera, and then stacked using Helicon Focus software. The final illustration was post-processed for contrast and brightness using Adobe®Photoshop® software.

Fig. 2. Larva of *Telmatogeton* sp. from Caspian sea. This image was taken by V.M. Loktionov at Federal Scientific Center of the East Asia Terrestrial Biodiversity, Russia.

A sequenced larva of *Telmatogeton* sp. was one of a broad sample of conspecific chironomids, including imago males. However, the adults of this species were too small in size, while the size of the larvae was sufficient to extract total DNA for NGS sequencing. Samples were collected on 09 September 2018 in Kazakhstan near Aktau city on a shore of Caspian Sea (50.871ºN, 44.056ºE) by Palatov D.M. and preserved in 95% ethanol. A specimen and extracted DNA was deposed at Federal Scientific Center of the East Asia Terrestrial Biodiversity (E.A. Makarhenko, *makarchenko@biosoil.ru*) under the voucher number EAM940.
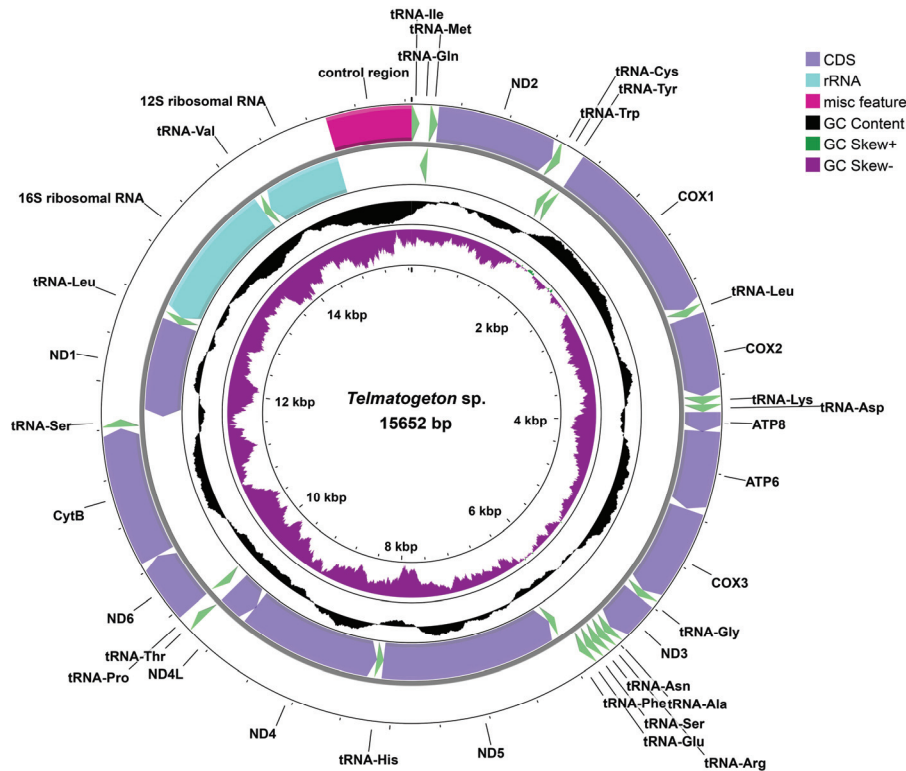

Fig. 3. Mitochondrial genome map for *Telmatogeton* sp. Plots of GC content and skew reflect GC content/skew scores. Positive and negative skew are indicated by purple and green color respectively.

Total DNA was extracted from whole body of larva using a DNeasy Blood & Tissue DNA kit (QIAGEN, Germany) according protocol and sent to 'Novogen' company for sequencing on the Illumina NovaSeq X Plus (Illumina, Hayward, CA). Adapter sequences and low-quality reads were trimmed from the raw data using Trimmomatic v0.38 (Bolger *et al.*, 2014) after that reads were assembled using SPAdes 3.15.5 (Bankevich *et al.*, 2012). The mitochondrial genome was annotated

using MITOS2 (http://mitos2.bioinf.uni-leipzig.de/index.py, Bernt *et al*., 2013) and annotations of mitochondrial genomes of other chironomids in the GenBank under accession number PP729117. We used CGView (Grant & Stothard, 2008) on web online server (https://proksee.ca/, Grant *et al*., 2023) to calculate GC content and make a mitochondrial map (Fig. 3). GetOrganelle (Jin *et al*., 2020) was used to calculate matching pairs across mitogenome (Fig. 4). The ribosomal 12S, 16S and 13 protein-coding genes (PCGs) were aligned with MAFFT 7 (Katoh *et al*., 2002) on web server MPI Bioinformatics toolkit (Zimmermann *et al*., 2018).
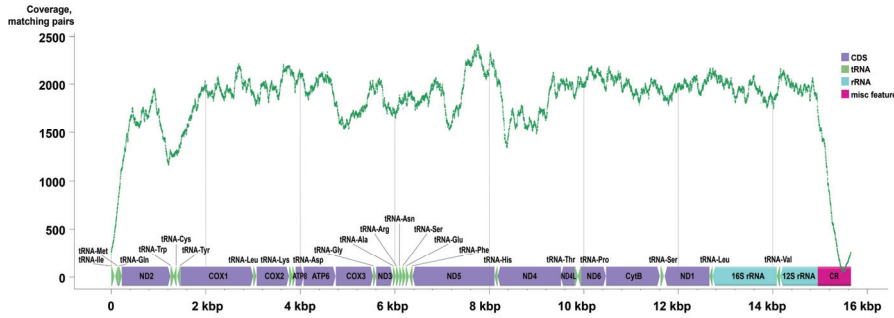

Fig. 4. The number of matching pairs across mitochondrial DNA showing the coverage-depth of the *Telmatogeton* sp.

To clarify the phylogenetic position of *Telmatogeton* sp. we obtained 26 complete mitochondrial sequences from the GenBank of chironomids belonging to 6 subfamilies. PartitionFinder 2.1.1 (Lanfear *et al*., 2017) is used to select the best-fit partitioning scheme and models separately for each codon position of each PCGs and ribosomal genes. A Bayesian Inferences (BI) analysis was performed with MrBayes v.3.2.7 (Ronquist *et al*., 2012) under the following conditions: 10 million generations with burn-in of 25% trees. Trace files were visually inspected in Tracer v. 1.7 (Rambaut *et al*., 2018). The Maximum Likelihood (ML) tree was reconstructed using IQ-Tree v.2.2.0 (Minh *et al*., 2020), with 1 million ultrafast bootstraps with PartitionFinder algorithm. Both trees were visualized in FigTree v. 1.4.4.

The data that support the findings of this study are openly available in GenBank at https://www.ncbi.nlm.nih.gov/nuccore/PP729117. The associated BioProject, SRA and BioSample numbers are PRJNA1120621, SRR29341651 and SAMN41705186 respectively

RESULTS AND DISCUSSION

The length of *Telmatogeton* sp. complete mitogenome was 15,652 bp and contains 13 protein-coding genes, 2 ribosomal (rRNA) genes, 22 transfer RNA (tRNA) genes and a control region (Fig. 3). Of these, 4 PCGs, 8 tRNA genes and both rRNA genes are encoded the light strand, and other genes are encoded on the heavy strand, that

corresponds to other chironomids (Zheng *et al*., 2021, Lin *et al*., 2022a, 2022b, Li *et al*., 2022, Qi *et al*., 2023). The base composition of the *Telmatogeton* sp. was 37.4% A, 35.7% T, 15.6% C and 11.3% G. Most of the PCGs initiation codon is ATG or ATT, except COI and ND1 genes starts with TTG, which is confirmed for Prodiamesinae and Orthocladiinae (Lin *et al*., 2022b). For the termination codons, all PCGs are terminated with the TAA stop codon, except COI that has incomplete stop codon T.

The number of matching pairs across mitogenome are shown on Fig. 4. The coverage depth is significantly decreased at the control region. Such decrease appears to be characteristic of most chironomid mitogenomes. This fact forces to increase the coverage using NGS sequencing or additionally amplify and sequence the control region by Sanger sequencing.

The Bayesian phylogeny revealed *Telmatogeton* sp. as sister to Chironominae + Orthocladiinae (Fig. 5). This position in new for Telmatogetoninae (Fig. 1), but support for this node was low by both BI and ML algorithms. On the other hand, the tree topology for the remaining subfamilies coincides with the results of previous studies using multilocus (Cranston *et al*., 2012, Tang *et al*., 2022, Semenchenko *et al*., 2024) or transcriptome (Kutty *et al*., 2018) datasets.
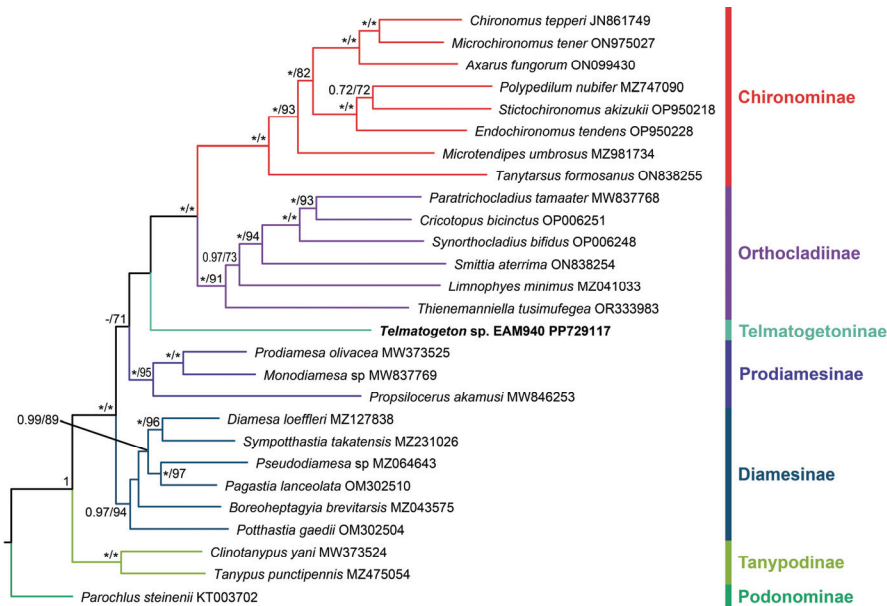

Fig. 5. Bayesian inference (BI) tree based on the mitochondrial 13 protein-coding genes, 12S and 16S ribosomal RNA of *Telmatogeton* sp. and some other available mitogenomes of Chironomids. Bayesian posterior probabilities (higher than 0.7) and Maximum Likelihood bootstrap values (higher than 70%) are given above tree nodes and asterisks (*) indicate full node support.

6

The origin of *Telmatogeton* sp. (BIN AAN6244) in the Caspian Sea basin remains unknown and puts forward two hypotheses, natural habitat and invasive colonization. The first hypothesis assumes that the Ponto-Caspian basins is the natural habitat for this species, and the connection between the Black, Azov and Caspian Seas existed until the late Pleistocene due to transgression (Yanina, 2014). In this case, the presence of the species in the USA and Japan is apparently a consequence of invasion. The second hypothesis suggests that the species is invasive to the Black Sea, and its colonization of the Caspian Sea basin may be associated with construction of the Volga-Don Canal since 1952 or ballast water tanks mounted on ships since 1980s (Shiganova *et al.*, 2023).

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