Complete mitochondrial genome sequence of the giant mud worm
Paraleonnates uschakovi Khlebovich & Wu, 1962 (Polychaeta: Nereididae)

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ABSTRACT
Complete mitochondrial genome sequence of the giant mud worm Paraleonnates uschakovi (Polychaeta: Nereididae) was determined in this study for the first time. The mitogenome of P. uschakovi is 15,540 bp in length. It has 13 protein-coding genes, two rRNA genes, 22 tRNA genes and a non-coding region. Mitogenome analysis of P. uschakovi showed inversion in the positions of three tRNAs compared to the mitogenome sequences of Perinereis albihitensis, P. nuntia and Platynereis dumerilii. The phylogenetic position of P. uschakovi compared to 15 selected polychaetes was investigated. P. uschakovi was grouped into the family of Nereididae. It is closely related to the clade containing Tylorrhynchus heterochaetus and Namalycastis abiuma.

The genus Paraleonnates (Khlebovich & Wu 1962) belongs to family Nereididae (Blainville 1818). It is comprised of three valid species: P. tenuipalpa (Pflugfelder 1933), P. uschakovi (Khlebovich & Wu 1962), and P. bolus (Hutchings & Reid 1991). Of the three species, P. uschakovi is widely distributed at Korean and Chinese coasts, and Thailand coast of Andaman Sea (Paik 1977; Wu et al. 1985; Hong et al. 2012). This species is known as a fishing bait for mullet in Korea. It is also a source of alkaline protease (Joo et al. 2001). Molecular phylogenetic studies have been conducted to solve the taxonomic problem of cryptic nereid species using mitochondrial partial genes such as COI and 16S (Park & Kim 2007; Tosuji & Sato 2008; Glasby et al. 2013). Complete mitochondrial genome sequence can provide more valuable evolutionary information compared to partial genes (Chen et al. 2016). So far, complete mitogenomes of five nereid species have been determined. For future molecular evolutionary studies of nereidid worms, more complete mitogenome information is needed. The objective of this study was to determine the complete mitogenome sequence of P. uschakovi.

The specimen was collected in muddy tidal flat of Ganghwa Island, Republic of Korea. Voucher specimen was deposited at National Institute of Biological Resources (NIBRIV0000539555).

A single live specimen of P. uschakovi was used to obtain pure mitochondrial genomic DNA. Total genomic DNA extraction, sequencing, and gene annotation methods described by Song et al. (2016) were used. Phylogenetic tree was constructed using MEGA6 (Tamura et al. 2013).

The complete mitogenome of P. uschakovi was 15,540 bp in length (GenBank accession no. KX462988), containing a total of 37 genes (13 protein-coding genes, two rRNA genes, and 22 tRNA genes) and a non-coding region of about 1kb. Gene order of P. uschakovi is identical to that of the following two nereidid species: Tylorrhynchus heterochaetus (Quatrefages 1866) and Namalycastis abiuma (Grube 1872). However, tRNA-Met, tRNA-Asp, ATP8, and tRNA-Tyr showed inversions in the other three nereid species: Perinereis albihitensis (Grube 1878), P. nuntia (Lamarck 1818), and Platynereis dumerilii (Audouin & Milne Edwards 1834). Thus, nereidid species are distinguishable with two different gene order groups so far. The gene order group including P. uschakovi has similar polychaetes ground pattern (tRNA-Asp, ATP8, tRNA-Tyr). The initiation codons of genes include ATT (cox1, cytb, nad2, nad5, nad6), ATA (cox2, nad3, nad4L), ATG (cox3, nad4, atp6, atp8), and ATC (nad1). Stop codons include TAG (cox1, nad6) and TAA (cox2, nad2, nad3, nad4, nad4L, atp6) excepting some genes (cox3, cytb, nad1, nad5, atp8) terminated with T.

To examine the phylogenetic position of P. uschakovi, maximum-likelihood analysis was performed using concatenated protein-coding genes from 15 selected polychaetes. The resulting tree showed that P. uschakovi was grouped into family Nereididae. It is closely related to the clade containing T. heterochaetus and N. abiuma with high bootstrap value (Figure 1).
The complete mitogenome information of *P. uschakovi* determined from this study will be useful for detailed phylogenetic and evolutionary analyses among nereidid species in the future.

**Disclosure statement**

The authors report no conflicts of interest. The authors alone are responsible for the content and the writing of the paper.

**Funding**

This work was supported by a grant (NIBR201601111) from the National Institute of Biological Resources (NIBR) funded by the Ministry of Environment (MOE), Republic of Korea. It was also supported by a grant (PJT200620, Genome analysis of marine organisms and development of functional applications) of the Marine Biotechnology Program funded by the Ministry of Oceans and Fisheries, Republic of Korea.

**References**

Audouin JV, Milne Edwards H. 1834. Recherches pour servir a l’histoire naturelle du littoral de la France, ou, Recueil de mémoires sur l’anatomie, la physiologie, la classification et les moeurs des animaux des nos côtes: ouvrage accompagné de planches faites d’après nature. Tome 2, Annélides. Tome 2, premiere part. Paris: Crochard; p. 290. plus 8 plates.

Blainville H. 1818. Mémoire sur la classe des Sétipodes, partie des Vers à sang rouge de M. Cuvier, et des Annélides de M. de Lamarck. Bulletin Des Sciences, Par La Société Philomatique De Paris. 1818:78–85.

Chen X, Li M, Liu H, Li B, Guo L, Meng Z, Lin H. 2016. Mitochondrial genome of the polychaete *Tylorrhynchus heterochaetus* (Phyllodocida, Nereididae). Mitochondrial DNA A DNA MappSeq Anal. 27:3372–3373.

Glasby CJ, Wei NWV, Gibb KS. 2013. Cryptic species of Nereididae (Annelida: Polychaeta) on Australian coral reefs. Inverbr System. 27:245–264.

Grube AE. 1872. Über die Gattung Lycastis und ein paar neue Arten derselben. Jahresbericht Der Schlesischen Gesellschaft Für Vaterländische Cultur. 48:47–48.

Grube AE. 1878. Annalata Semperiana. Beiträge zur Kenntniss der Annelidenfauna der Philippinen. Memoires De L’Academie Imperiale. Des Sciences De St.Petersbourg. Ser7. 25:1–300.

Hong JS, Choi BM, Kubo A, Sato M. 2012. Redescription of the giant mud worm *Paraleonnates uschakovi* Khlebovich and Wu, 1962 (Polychaeta: Nereididae) with special reference to the synonymy of *Periserrula leucophryna* Paik, 1977 and the difference from *Paraleonnates bolus* (Hutchings and Reid, 1991). Zootaxa. 3490:49–62.

Hutchings P, Reid A. 1991. The Nereididae (Polychaeta) from Australia-Leonnates, Platynereis and Solomononereis. Rec Austral Mus. 43:47–62.

Joo HS, Park GC, Kim KM, Paik SR, Chang CS. 2001. Novel alkaline protease from the polychaeta, *Periserrula leucophryna*: purification and characterization. Process Biochem. 36:893–900.

Khlebovich VV, Wu BL. 1962. Polychaetous worms of the Yellow Sea V: family Nereididae (Polychaeta Errantia). Acta Zool Sinica. 14:267–278. (In Chinese and Russian)

Lamarck JB. 1818. Histoire naturelle des Animaux sans Vertébrés, présentant les caractères généraux et particuliers de ces animaux, leur distribution, leurs classes, leurs familles, leurs genres, et la citation des principales espèces qui s’y rapportent; precedes d’une Introduction offrant la determination des caracteres essentiels de l’animal, sa distinction du vegetal et des autres corps naturels, enfin, l’Exposition des Principes fondamentaux de la Zoologie. Paris: Deterville; pp. 612.

Paik EI. 1977. Studies of polychaetous annelids worms of the family Nereididae (Polychaeta Errantia). Acta Zool Sinica. 14:272–278. (in Chinese and Russian)

Figure 1. Maximum-likelihood (ML) tree based on 15 mitogenome sequences including *Paraleonnates uschakovi* (present study). It was constructed using MEGA 6.0 software. Bootstrap replicates were performed 1000 times. Bootstrap values above 60% were indicated on the cladogram.
Song J-H, Kim S, Shin S, Min G-S. 2016. The complete mitochondrial genome of the mysid shrimp, Neomysis japonica (Crustacea, Malacostraca, Mysida). Mitochondrial DNA A DNA MappSeq Anal. 27:2781–2782.

Tamura K, Stecher G, Peterson D, Filipski A, Kumar S. 2013. MEGA6: molecular evolutionary genetics analysis version 6.0. Mol Biol Evol. 30:2725–2729.

Tosuji H, Sato M. 2008. Identification of three Asian Hediste species (Polychaeta: Nereididae) by PCR-RFLP analysis of the mitochondrial 16S rRNA gene. Plankton Benthos Res. 3:50–52.

Wu BL, Sun R, Yang D. 1985. (first published in 1981 in Chinese). The Nereidae (polychaetous annelids) of the Chinese coast. Berlin: China Ocean Press, Beijing and Springer-Verlag; p. 234.