Taylor & Francis
Taylor & Francis Group

MITOGENOME REPORT

🔓 OPEN ACCESS ✓ Check for updates

Complete mitochondrial genome of the hybrid flounder *Paralichthys olivaceus* (♀) × *Verasper variegatus* (♂)

HyeJin Kim[a] 🔟, YongHwi Kim[b] 🔟, Bong Han Yun[c] 🔟, Ho-Seop Han[a] 🔟, Ho Sung Lee[a] 🔟, In Gug Baek[a] 🔟 and In-Chul Bang[a] 🔟

ᵃDepartment of Biology, Soonchunhyang University, Asan, Republic of Korea; ᵇBio-laboratory, BioTNS Co., Ltd, Daejeon, Republic of Korea; ᶜInstitute of Korea Eco-Network, Daejeon, Republic of Korea

ABSTRACT

Two generations were hatched after artificially crossing *Paralichthys olivaceus* and *Verasper variegatus*, commercially valuable species of Pleuronectidae. Here, we report the complete mitochondrial genome and phylogenetic relationships of *P. olivaceus* (♀) × *V. variegatus* (♂). The mitogenome is 16,946 bp long and contains 13 protein-coding genes (PCGs), 22 tRNA genes, and 2 rRNA genes. Phylogenetically, it clustered with *P. olivaceus* within Pleuronectidae, providing a justification for the lack of information on flounder hybrids. Analysis of genetic information and genetic mapping studies of individuals with intermediate traits between the parents support previous research suggesting that it has economic value.

Introduction

The olive flounder, *Paralichthys olivaceus* Temminck & Schlegel, 1846 is a flatfish that is eaten in East Asia. It possesses large teeth, inhabits benthic environments, and exhibits leftward-shifted eyes (Bai and Lee 2010). In South Korea, 41,207 tons were harvested in 2017, establishing it as a valuable aquaculture species (FAO, 2018). The spotted halibut, *Verasper variegatus* Temminck & Schlegel, 1846, inhabits the East Asian coast and reaches approximately 60 cm in length. Although it offers superior nutritional value and flavor, its declining catch and low aquaculture production rate make it difficult to secure a stable supply (Ando et al. 1998; Nemoto 1999; Wada et al. 2004, 2010; Shimamura 2007). Through artificial insemination, we successfully hatched a hybrid of these two important species.

The structure and sequence of the compact, maternally inherited mitochondrial genome exhibit conservation among vertebrates, facilitating the study of phylogenetic relationships between species (Miya et al. 2003; Pardo et al. 2005). Many hybrids have been produced in attempts to create superior strains of fish for the purpose of fish cultivation. In this respect, the most interesting Pleuronectidae hybrid is the cross between *P. olivaceus*, which grows rapidly in cold water, and *V. variegatus*, which has good meat quality. Since hybrid traits are generally intermediate between those of the parents, this hybrid would be easy to grow and would have great potential. Consequently, this study aimed to determine the complete mitochondrial genome of *P. olivaceus* (♀) × *V. variegatus* (♂), enabling elucidation of its phylogenetic position.

Materials

In March 2023, larvae and adults of *P. olivaceus* (♀) × *V. variegatus* (♂), hatched at the Soonchunhyang University Marine Fisheries Research Institute (36°61′959″N 126°33′552″E, 236, Cheonsuman, Chungcheongnam, Republic of Korea), were immersed in 99.9% ethanol and deposited in a sample storage facility at Soonchunhyang University (Prof. I-C. Bang, incbang@sch.ac.kr), under voucher no. SUC26325 (Figure 1).

Methods

Genomic DNA (gDNA) was extracted from the whole body of *P. olivaceus* (♀) × *V. variegatus* (♂) larvae using a HiGene™ Genomic DNA Prep Kit (BIOFACT, Daejeon, Republic of Korea; Cat. No. GD264-060) in accordance with the manufacturer's protocol. A DNA library was prepared from the extracted gDNA using the MGIEasy DNA Library Prep Kit (MGI Tech, Shenzhen, China), and raw next-generation sequencing data were obtained through 150 bp paired-end reads on the MGISEQ-2000 platform (MGI Tech, Shenzhen, China). The raw data were trimmed using Cutadapt ver. 4.2 (Martin 2011), and the contig sequences were assembled using the CLC Genomics Workbench (ver. 20.04) (CLC, Aarhus, Denmark). The circular mitochondrial genome was contig sequenced using Geneious R (ver. 11.0.3) (https://www.geneious.com) (Kearse et al. 2012),

Figure 1. *P. Olivaceus* (♀) × *V. variegatus* (♂), SUC26325, 120 mm SL, Juvenile, Taean, Chungcheongnam, Korea, 10 April 2023. Photograph by Y.H. Kim, used with permission. The hybrid exhibits an oval body similar to its parent, with small black and white spots covering the entire body like *P. olivaceus*, and round black spots on the dorsal, pelvic, and caudal fins resembling *V. variegatus*.

and the final sequence was annotated using the MITOS web-based tool (http://mitos.bioinf.uni-leipzig.de) (Bernt et al. 2013). The complete mitogenome sequence was subsequently registered at NCBI with accession number OR353704.

The assembled raw data were aligned with ClustalW (Thompson et al. 1994) using BioEdit (ver. 7.7). Sequences were analyzed using jModelTest (ver. 2.1.10) (RRID:SCR_015244) (Darriba et al. 2012) and selected according to the Akaike Information Criterion (AIC) to place *P. olivaceus* (♀) × *V. variegatus* (♂) in a phylogenetic context. The maximum likelihood (ML) method was utilized to determine the most appropriate model (GTR+I + G).

The phylogeny was constructed using PHYML (ver. 3.0) (Guindon et al. 2010) and iTOL (ver. 6) (Letunic and Bork 2007). We referenced the phylogenetic list from a recent study of the flounder lineage (Chae et al. 2023) and incorporated three species from the maternal and paternal genera *Verasper* and *Paralichthys*. For precise intra-Osteichthyes
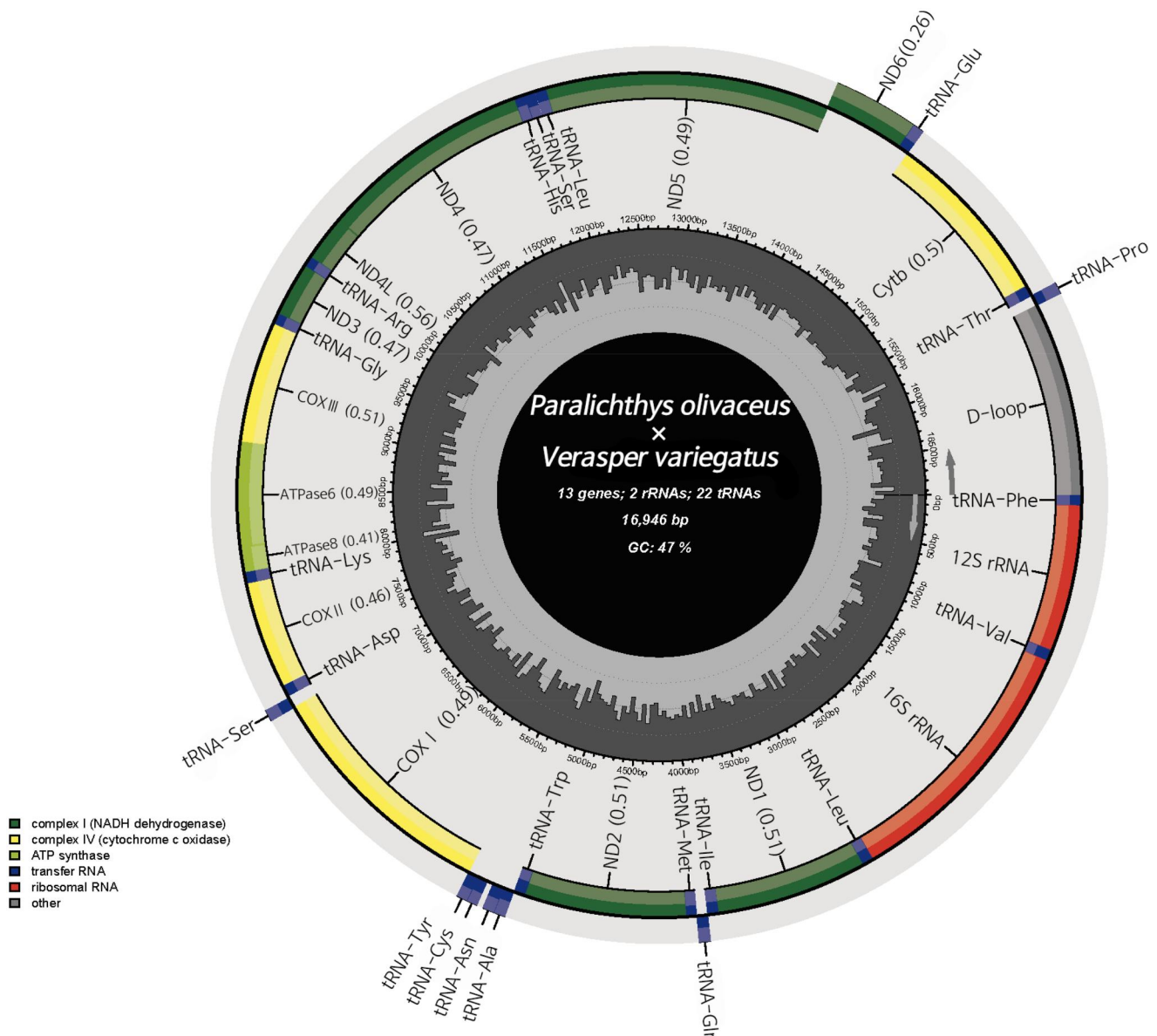
Figure 2. The mitochondrial genome map for *P. olivaceus* (♀) × *V. variegatus* (♂), produced on the MitoFish web server v.3.90, depicts 13 protein-coding genes, two ribosomal RNAs, 22 transfer RNAs, and a control region (GenBank acc. no.: OR353704). The mitochondrial gene order exhibits conservation among species.
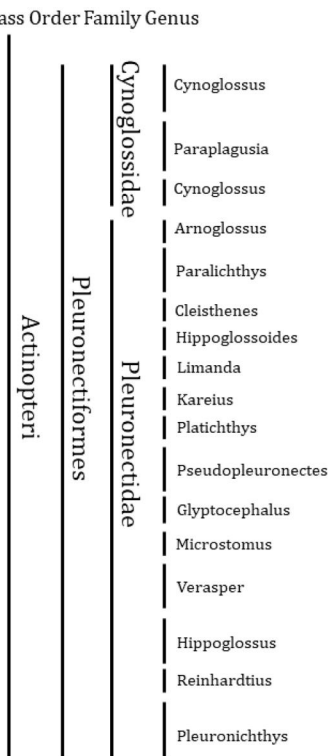
Figure 3. Phylogenetic tree of 13 pleuronectidae species and *P. olivaceus* (♀) × *V. variegatus* (♂) constructed using 13 mitochondrial PCGs and the maximum likelihood method. GenBank accession numbers precede the scientific names. The following sequences were used: *A. fulvescens* (MT667238.1; schroeter et al. 2020), *cynoglossus roulei* (MK574671.1; chen et al. 2019), *cynoglossus semilaevis* (EU366230.1; kong et al. 2009), *cynoglossus sinicus* (JQ348998.1; shi et al. 2012), *paraplagusia bilineata* (NC023227.1; fricke et al. 2024), *paraplagusia blochii* (JQ349002.1), *arnoglossus tenuis* (KP134337), *cleisthenes herzensteini* (KT223828.1), *glyptocephalus stelleri* (NC060723.1; xiao et al. 2010), *Hippoglossoides platessoides* (MN122825.1; margaryan et al. 2021), *Hippoglossus hippoglossus* (AM749122.1; Mjelle a., et al. 2008), *Hippoglossoides stenolepis* (AM749126.1; vinnikov et al. 2018), *kareius bicoloratus* (AP002951.1; miya et al. 2001), *limanda aspera* (NC028281.1; vinnikov et al. 2018), *microstomus achne* (OP066370.1; chae et al. 2022), *P. olivaceus* (NC002386.1; saitoh et al. 2000), *P. stellatus* (EF424428.1; vinnikov et al. 2018), *pleuronichthys cornutus* (JQ639071.1; shi et al. 2013), *pleuronichthys japonicus* (KY038655.1; song et al. 2017), *pseudopleuronectes herzensteini* (ON127848.1; chae et al. 2022), *pseudopleuronectes yokohamae* (KT878309.1; liu et al. 2017), *reinhardtius hippoglossoides* (AM749130.1; Mjelle a., et al. 2008), *verasper moseri*, and *V. variegatus* (NC008461.1, NC007939.1).

comparisons, sturgeon (*Acipenser fulvescens*) and available Cynoglossidae NCBI sequences were adopted as out-groups. The in-group comprised all Pleuronectidae genera registered in NCBI. Both genera belong to Pleuronectiformes, and 25 species were utilized (out-group: *Acipenser fulvescens* (MT667238), *Cynoglossus roulei* (MK574671), *Cynoglossus semilaevis* (EU366 230), *Cynoglossus sinicus* (JQ348998), *Paraplagusia bilineata* (NC023227), and *Paraplagusia blochii* (JQ349002); in-group: *Arnoglossus tenuis* (KP134337), *Cleisthenes herzensteini* (KT22 3828), *Glyptocephalus stelleri* (NC060723), *Hippoglossoides platessoides* (MN122825), *Hippoglossus hippoglossus* (AM749122), *Hippoglossus stenolepis* (AM749126), *Kareius bicoloratus* (AP002 951), *Limanda aspera*(NC028281), *Microstomus achne* (OP0663 70), *Paralichthys olivaceus* (NC002386), *Platichthys stellatus* (EF424428), *Pleuronichthys cornutus* (JQ639071), *Pleuronichthys japonicus* (KY038655), *Pseudopleuronectes herzensteini* (ON127 848), *Pseudopleuronectes yokohamae* (KT878309), *Reinhardtius hippoglossoides* (AM749130), *Verasper moseri* (NC008461), and *Verasper variegatus* (NC007939)).

Results

The total length of the final mitogenome of *P. olivaceus* (♀) × *V. variegatus* (♂) is 16,946 bp, encompassing 13 protein-coding genes (PCGs), 22 tRNA genes, and two rRNA genes.

All PCGs except *nad6* are coded on the positive strand (Figure 2). The total base composition is GC 46.99%, with adenine (A) 24.74%, cytosine (C) 30.37%, guanine (G) 16.62%, and thymine (T) 28.26%. The $A+T$ composition is 53%, comparable to the mitogenomes of other vertebrates (Saccone et al. 1999; Zhu et al. 2013). The 13 PCGs constitute 67.44% of the total length or 11,429 bp, and the control region spans 1,256 bp. The 12S rRNA (949 bp) is located between tRNA^{Phe} and tRNA^{Val}, whereas the 16S rRNA gene (1,714 bp) is located between tRNA^{Val} and tRNA^{Leu}. Excluding *COX1*, 12 PCGs began with the ATG start codon; *ND1*, *COX1*, *ATP8*, *NDL4*, and *ND5* ended with a complete TAA or TAG stop codon, whereas the remaining PCGs ended with T or A.

The mitogenome of *P. olivaceus* (♀) × *V. variegatus* (♂) contains 22 tRNA genes. Of these, tRNA^{Glu}, tRNA^{Pro}, tRNA^{Gln}, tRNA^{Ala}, tRNA^{Asn}, tRNA^{Cys}, tRNA^{Tyr}, and tRNA^{Ser} are on the negative strand; the remainder (tRNA^{Thr}, tRNA^{Phe}, tRNA^{Val}, tRNA^{Leu}, tRNA^{Ile}, tRNA^{Met}, tRNA^{Trp}, tRNA^{Asp}, tRNA^{Lys}, tRNA^{Gly}, tRNA^{Arg}, tRNA^{His}, tRNA^{Ser}, and tRNA^{Leu}) are on the positive strand and exhibit the typical tRNA structure.

Each species was classified as belonging to the family Pleuronectidae, family Cynoglossidae, or outgroup (*A. fulvescens*). *P. olivaceus* (♀) × *V. variegatus* (♂) clustered with *P. olivaceus* in Pleuronectidae and was analyzed as a distinct node in the phylogeny (Figure 3).

Discussion and conclusion

This study presents the first determination of the complete mitochondrial genome of a second-generation hybrid obtained through artificial crosses between *P. olivaceus* and *V. variegatus*, revealing its genetic characteristics. The mitogenome composition aligns with that of a typical vertebrate (Pereira 2000). In an analysis of several Pleuronectidae family genera, *P. olivaceus* (♀) × *V. variegatus* (♂) clustered with the maternal genus *Paralichthys* with high bootstrap values (1,000 BP). This observation aligns with other studies (Debes et al. 2013; Hou and Liu 2018; Li et al. 2018), suggesting that hybrids are significantly influenced by the maternal lineage. The examined hybrid likely possesses substantial economic value, based on previous research indicating growth rates similar to those of the olive flounder (Kim et al. 1995). In conclusion, the whole-genome study of this species presents a molecular phylogeny favorable for aquaculture cultivar development, and analysis of its genetic information in Pleuronectidae provides ideal material for genetic mapping and genomic studies of hybrids.

Acknowledgments

Hyejin Kim, YongHwi Kim, and In-Chul Bang conceived the original idea. Hyejin Kim led the experiments. Hyejin Kim wrote the manuscript with support from YongHwi Kim, Bong Han Yun, Ho Sung Lee, Ho-Seop Han, In Gug Baek, and In-Chul Bang. Bong Han Yun, Ho-Seop Han, Ho Sung Lee, and In Gug Baek analyzed the experimental data. All authors agreed to be accountable for all aspects of the work.

Authors' contributions

CRediT: **HyeJin Kim**: Conceptualization, Formal analysis, Software, Methology, Writing original draft; **YongHwi Kim**: Formal analysis, Software, Validation; **Bong Han Yun**: Conceptualization, Formal analysis, Methodology, Software, Writing – original draft; **Ho-Seop Han**: Investigation, Methodology, Writing – edit; **Ho Sung Lee**: Investigation, Methodology, Writing – edit; **In Gug Baek**: Investigation, Methodology, Writing – edit; **In-Chul Bang**: Conceptualization, Funding acquisition, Project administration, Resources, Supervision.

Disclosure statement

No potential competing interests (financial or non-financial) were reported by the authors and the Institute of Korea Eco-Network.

Ethical approval

The target species samples in this paper were cadavers, and according to the Animal Experiment Ethics of the Republic of Korea (standard operating guideline; IACUC – Institutional Animal Care and Use Committee, Book no. 11-1543061-000457-01, effective Dec. 2020) does not need any approval from an ethics committee. The data collection was conducted in accordance with the policies of the International Union for Conservation of Nature (IUCN) pertaining to research on species at risk of extinction (refer to the Guidelines for appropriate uses of IUCN Red List data). Additionally, the Convention on Biological Diversity and the Convention on the Trade in Endangered Species of Wild Fauna and Flora were also adhered to. The species used in this study are not endangered fish in the Republic of Korea and the sampling site is not located in any protected area.

ORCID

HyeJin Kim http://orcid.org/0000-0002-4109-1418
YongHwi Kim http://orcid.org/0000-0001-9901-5445
Bong Han Yun http://orcid.org/0000-0002-1885-5221
Ho-Seop Han http://orcid.org/0000-0001-9973-0457
Ho Sung Lee http://orcid.org/0009-0007-2874-2646
In Gug Baek http://orcid.org/0000-0001-7629-7232
In-Chul Bang http://orcid.org/0000-0003-4584-5384

Data availability statement

The genome sequence data that support the study findings are openly accessible in NCBI GenBank at https://www.ncbi.nlm.nih.gov/ under accession no. OR353704. The associated BioProject, SRA, and Bio-Sample numbers are PRJNA1049058, SRS19795251, and SAMN38681716, respectively.

Additional information

The English in this document has been checked by at least two professional editors, both native speakers of English. For a certificate, please see:

http://www.textcheck.com/certificate/5a4R0C

References

Thompson JD, Higgins DG, Gibson TJ. 1994. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Res. 22(22):4673–4680. doi:10.1093/nar/22.22.4673.

Ando M, Niho H, Tsukamasa Y, Makinodan Y. 1998. Valuation of cultured spotted halibut *Verasper variegatus* by comparing with cultured plaice *Paralichthys olivaceus*. Nippon Suisan Gakkaishi. 64(6):1027–1033. doi:10.2331/suisan.64.1027.

Bai SC, Lee S. 2010. Culture of olive flounder: korean perspective. In: Daniels HV, Watanabe WO, editors. Practical Flatfish Culture and Stock Enhancement (p. 156–166). Iowa: Wiley-Blackwell.

Bernt M, Donath A, Jühling F, Externbrink F, Florentz C, Fritzsch G, Pütz J, Middendorf M, Stadler PF. 2013. MITOS: improved *de novo* metazoan mitochondrial genome annotation. Mol Phylogenet Evol. 69(2):313–319. doi:10.1016/j.ympev.2012.08.023.

Chae JY, Kim J, Kang TW, Kim J, Lee HH, Kim MS. 2023. The complete mitochondrial genome sequence of Rikuzen flounder, *Dexistes rikuzenius* (Jordan & Starks, 1904). F1000Res. 12:935. doi:10.12688/f1000research.134705.1.

Darriba D, Taboada GL, Doallo R, Posada D. 2012. jModelTest 2: more models, new heuristics and parallel computing. Nat Methods. 9(8):772–772. doi:10.1038/nmeth.2109.

Debes PV, Fraser DJ, McBride MC, Hutchings JA. 2013. Multigenerational hybridisation and its consequences for maternal effects in Atlantic salmon. Heredity (Edinb). 111(3):238–247. doi:10.1038/hdy.2013.43.

Guindon S, Dufayard J-F, Lefort V, Anisimova M, Hordijk W, Gascuel O. 2010. New algorithms and methods to estimate maximum-likelihood phylogenies: assessing the performance of PhyML 3.0. Syst Biol. 59(3):307–321. doi:10.1093/sysbio/syq010.

Hou JL, Liu HJ. 2018. Gynogenesis and sex control in Japanese flounder. Sex Control Aquacul, 603–619. doi:10.1002/9781119127291.ch30.

Kearse M, Moir R, Wilson A, Stones-Havas S, Cheung M, Sturrock S, Buxton S, Cooper A, Markowitz S, Duran C, et al. 2012. Geneious basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. Bioinformatics. 28(12): 1647–1649. doi:10.1093/bioinformatics/bts199.

Kim KK, Bang IC, Kim Y, Kim DS. 1995. Survival and growth in larval stage of induced hybrid between olive flounder *Paralichthys olivaceus* and spotted flounder *Verasper variegatus*. J Aquacult. 8:69–76.

Letunic I, Bork P. 2007. Interactive Tree Of Life (iTOL): an online tool for phylogenetic tree display and annotation. Bioinformatics. 23(1):127–128. doi:10.1093/bioinformatics/btl529.

Li W, Liu J, Tan H, Luo L, Cui J, Hu J, Wang S, Liu Q, Hu F, Tang C, et al. 2018. Asymmetric expression patterns reveal a strong maternal effect and dosage compensation in polyploid hybrid fish. BMC Genomics. 19(1):517. doi:10.1186/s12864-018-4883-7.

Martin M. 2011. Cutadapt removes adapter sequences from high-throughput sequencing reads. EMBnet j. 17(1):10–12. doi:10.14806/ej.17.1.200.

Miya M, Takeshima H, Endo H, Ishiguro NB, Inoue JG, Mukai T, Satoh TP, Yamaguchi M, Kawaguchi A, Mabuchi K, et al. 2003. Major patterns of higher teleostean phylogenies: a new perspective based on complete mitochondrial DNA sequences. Mol Phylogenet Evol. 26(1):121–138. doi:10.1016/s1055-7903(02)00332-9.

Nemoto Y. 1999. Studies on spotted halibut (*Verasper variegatus* Temminck & Schlegel): I. Bull Fukushima Pref Fish Exp Stat. 8:5–16.

Pardo BG, Machordom A, Foresti F, Porto-Foresti F, Azevedo MFC, Bañon R, Sánchez L, Martínez P. 2005. Phylogenetic analysis of flatfish (Order Pleuronectiformes) based on mitochondrial 16s rDNA sequences. Sci Mar. 69(4):531–543. doi:10.3989/scimar.2005.69n4531.

Pereira SL. 2000. Mitochondrial genome organization and vertebrate phylogenetics. Genet Mol Biol. 23(4):745–752. doi:10.1590/S1415-47572000000400008.

Saccone C, De Giorgi C, Gissi C, Pesole G, Reyes A. 1999. Evolutionary genomics in Metazoa: the mitochondrial DNA as a model system. Gene. 238(1):195–209. doi:10.1016/S0378-1119(99)00270-X.

Shimamura S. 2007. The study of the spotted halibut, *Verasper variegatus*-II aspects of fishery utilization and life history in coastal waters of Fukushima Prefecture. Bull Fukushima Prefect Fish Exp Stn. 14:64–90.

Wada T, Aritaki M, Tanaka M. 2004. Effects of low-salinity on the growth and development of spotted halibut *Verasper variegatus* in the larva-juvenile transformation period with reference to pituitary prolactin and gill chloride cells responses. J Exp Mar Biol Ecol. 308(1):113–126. doi:10.1016/j.jembe.2004.02.015.

Wada T, Yamada T, Shimizu D, Aritaki M, Sudo H, Yamashita Y, Tanaka M. 2010. Successful stocking of a depleted species, spotted halibut *Verasper variegatus*, in Miyako Bay, Japan: evaluation from post-release surveys and landings. Mar Ecol Prog Ser. 407:241–253. doi:10.3354/meps08553.

Zhu Y, Chen Y, Cheng Q, Qiao H, Chen W. 2013. The complete mitochondrial genome sequence of *Schizothorax macropogon* (Cypriniformes: cyprinidae). Mitochondrial DNA. 24(3):237–239. doi:10.3109/19401736.2012.752478.