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An improved genome assembly and annotation of the Antarctic copepod *Tigriopus kingsejongensis* and comparison of fatty acid metabolism between *T. kingsejongensis* and the temperate copepod *T. japonicus*

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ABSTRACT

Copepods in the genus *Tigriopus* are widely distributed in the intertidal zone worldwide. To assess differences in fatty acid (FA) metabolism among congeneric species in this genus inhabiting polar and temperate environments, we analyzed and compared FA profiles of the Antarctic copepod *Tigriopus kingsejongensis* and the temperate copepod *T. japonicus*. Higher amounts of total FAs were found in the Antarctic copepod *T. kingsejongensis* than the temperate copepod *T. japonicus* under administration of the identical amount of *Tetraselmis suecica*. To determine the genomic basis for this, we identified fatty acid metabolism-related genes in an improved genome of *T. kingsejongensis*. The total length of the assembled genome was approximately 338 Mb with N50 = 1.473 Mb, 938 scaffolds, and a complete Benchmarking Universal Single-Copy Orthologs value of 95.8%. A total of 25,470 genes were annotated using newly established pipeline. We identified eight elongation of very long-chain fatty acid protein (*Elovl*) genes and nine fatty acid desaturase (*Fad*) genes in the genome of *T. kingsejongensis*. In addition, fatty acid profiling suggested that the duplicated Δ5/6 desaturase gene in *T. kingsejongensis* is likely to play an essential role in synthesis of different FAs in *T. kingsejongensis* to those in *T. japonicus*. However, further experimental research is required to validate our *in silico* findings. This study provides a better understanding of fatty acid metabolism in the Antarctic copepod *T. kingsejongensis*.

1. Introduction

Copepods dominate the zooplankton biomass in both freshwater and seawater habitats (Wells et al., 1984). In the aquatic environment, copepods play an essential role in carbon cycling between producers and higher trophic consumers (Theilacker and Kimball, 1984; Raisuddin et al., 2007). Among copepods, harpacticoid copepods in the genus *Tigriopus* have been a focus of study over the last few decades (Raisuddin et al., 2007). They dominate shallow supratidal rock pools and are widely distributed worldwide as they respond flexibly to changes in the aquatic environment. Among marine copepods, the Antarctic copepod *Tigriopus kingsejongensis* in the family Harpacticidae has been recently identified (Park et al., 2014) and the mitochondrial DNA of this species was sequenced for species identification barcoding (Hwang et al., 2019). This copepod is a promising model species for Antarctic marine ecotoxicology as its genome has been sequenced (Kang et al., 2017) and transcriptomic information is available (Kim et al., 2016). To date, the in vivo and in vitro effects of environmental stressors such as ultraviolet B radiation (Han et al., 2016), water-accommodated fractions of heavy oils (Han et al., 2017), and temperature changes (Han et al., 2018a; Han et al., 2018b) on *T. kingsejongensis* have been investigated.

Antarctic organisms are exposed to extreme environmental conditions and have evolved defense mechanisms to protect their bodies from these unfavorable conditions (Dahms et al., 2011). Fatty acids (FAs), as a primary nutrient source, play pivotal roles (*e.g.*, membrane construction, precursor generation, and temperature control) and function as second messengers in organisms (Pomorski et al., 2001; Maxfield, 2002; Mukherjee and Maxfield, 2004; van Meer et al., 2008; van Meer, 2010; Holthuis and Menon, 2014; Cheng et al., 2018). Temperature is an important factor that affects the fluidity of membranes, especially low temperatures (Carey and Hazel, 1989; Gibbs and Somero, 1990).

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Table 1
Studies on the elongation of very long-chain fatty acid protein and fatty acid desaturase genes in various invertebrates.

Gene name	Species	Subfamily	No. of genes	Phylogenetic analysis	Functional tests performed?	References
Elongation of very long-chain fatty acid protein	*Brachionus koreanus*	Elovl2/5	1	Done	None	Lee et al., 2019b
		Elovl3/6	3			
		Elovl9	5			
		Elovl10	1			
	Paracyclopina nana	N.D.	3	None	Done	Lee et al., 2016
	Chlamys nobilis	Elovl4	1	Done	Done	Liu et al., 2014
	Bombyx mori	Elovl1/7	11	Done	None	Zuo et al., 2018
		Elovl3/6	1			
		Elovl4	1			
	Octopus vulgaris	N.D.	1	Done	Done	Monroig et al., 2012
	Sepia officinalis	N.D.	1	Done	Done	Monroig et al., 2016
Fatty acid desaturase	*Spodoptera exigua*	Δ5	1	Done	None	Hasan et al., 2019
		Δ6	2			
		Δ9	4			
	Octopus vulgaris	Δ12	1	Done	Done	Garrido et al., 2019
		ω3	1			
		Δ9	1	None.	Done	Monroig et al., 2017
	Spodoptera litura	Δ11	1	Done	Done	Xia et al., 2019
	Spodoptera exigua	Δ11	1			
		Δ9	2			
	Nilaparvata lugens	Δ9	8	Done	Done	Zeng et al., 2019
		Δ4	1			
	Cherax quadricarinatus	Δ6	1	Done	None	Wu et al., 2018
	Sinonovacula constricta	Δ5	2	Done	Done	Ran et al., 2018
		Δ6				
	Helicoverpa armigera	Δ11	1	Done	None	Du et al., 2017
		Δ9	1			
		Δ5, 6, 14	2			
		Sphingolipid desaturase	1			
		Front-end desaturase	1			
	Riftia pachyptila	ω3	1	Done	Done	Liu et al., 2017
	Scylla paramamosain	Δ6	1	Done	None	Lin et al., 2017

Antarctic organisms are continuously exposed to near freezing temperatures (about −1.9 °C) (Bargagli, 2005); therefore, Antarctic organisms have an increased amount and length of unsaturated FAs (Lewis, 1962; Farkas and Herodek, 1964). Among various types of lipid-regulatory enzymes, elongases and fatty acid desaturases are essential for unsaturated FA synthesis; elongases lengthen the carbon chain of FAs (Cinti et al., 1992; Kohlwein et al., 2001), while desaturases modify the structure of FAs from C-C single bonds to C═C double bonds (Shanklin and Cahoon, 1998). Many elongase (elongation of very long-chain fatty acid protein; *Elovl*) and desaturase (fatty acid desaturase; *Fad*) genes have been reviewed (Monroig and Kabeya, 2018) and a studies on Elovl and Fad genes in invertebrates have been summarized in this study (Table 1). Nevertheless, despite the potential importance of fatty acids in the survival of Antarctic copepods, limited studies on their *Elovl* and *Fad* repertoires have been reported. It is vital to understand and verify the types of elongases and desaturases present in Antarctic organisms because these enzymatically modulated FA composition will affect the food web in the surrounding environment.

In this study, we improved the genome assembly of the Antarctic copepod *T. kingsejongensis* generated in a previous study (Kang et al., 2017). Based on the updated *T. kingsejongensis* genome, we investigated *Elovl* and *Fad* genes in Antarctic copepod *T. kingsejongensis* and compared the FA gene repertoire of this species with that of the temperate copepod *T. japonicus*.

2. Materials and methods

2.1. Culture and maintenance of Tigriopus kingsejongensis

Tigriopus kingsejongensis (kindly provided by Dr. Sanghee Kim, Korea Polar Research Institute, Incheon, South Korea) were maintained in 10 L tanks containing filtered (0.2 μm) artificial seawater (ASW) (TetraMarine Salt Pro, Tetra™, Cincinnati, OH, USA) adjusted to a salinity of 30 practical salt units at 14 °C under a 12:12 h (light:dark) photoperiod. Species identity was confirmed by morphometric analysis followed by molecular characterization of the cytochrome oxidase 1 mitochondrial gene as a universal barcode marker (Park et al., 2014; Hwang et al., 2019).

2.2. Analysis of fatty acid profiles

For fatty acid extraction, adult *T. kingsejongensis* (at 14 °C) and *T. japonicus* (at 25 °C) (approximately 200 individuals/100 mL per treatment) were exposed to the living microalga *T. suecica* (2.5×10^5 cells/mL). After 24 h of incubation, samples were harvested and freeze-dried for further analysis. Total lipids were extracted as described in a previous study (Folch et al., 1957) with minor modifications. Briefly, lipids were extracted with dichloromethane/methanol 2:1 (v/v) and nonadecanoic acid (C19:0) was added to the extracts as an internal standard. Extraction procedures were performed in triplicate with sonication. The lipid fraction was separated from the water-methanol phase and converted into fatty acid methyl esters (FAMEs) by saponification using 0.5 M KOH-methanol, followed by methylation with BF3-methanol. Concentrations and compositions of FAMEs were analyzed by gas chromatography (HP 7890A, Agilent Technologies, Santa Clara, CA, USA) with a flame ionization detector using a fused silica capillary column (HP-5MS, 30 m × 0.25 mm i.d., 0.25 μm film thickness). Helium was used as the carrier gas. Samples were injected in splitless mode at an initial oven temperature of 60 °C, which was then increased to 320 °C at a rate of 5 °C/min, and allowed to remain there for 10 min. FAs were identified from the retention times mass spectra from a gas chromatograph-mass spectrometer (HP-7820A; Agilent Technologies) equipped with a fused silica capillary column (DB-5, 60 m × 0.25 mm i.d., 0.25 μm film thickness). Derivatization with 4,4-dimethyloxazoline (DMOX) (Garrido and Medina, 1994; Spitzer, 1996) was conducted to check positions and numbers of double bonds within unsaturated FAs.

Briefly, FAMEs were converted into DMOX derivatives by the addition of 2-amino-2-methylpropanol. DMOX derivatives were analyzed from gas chromatograph-mass spectrometry and identified by their mass spectra. All experiments were performed in triplicate.

2.3. Genomic DNA extraction

To extract genomic DNA, *T. kingsejongensis* were fasted at least 3 days in a medium containing ampicillin (Sigma-Aldrich, St. Louis, MO, USA), streptomycin (Sigma-Aldrich), and tetracycline (Sigma-Aldrich) at a concentration of 50 mg/L each to avoid any contamination from the culture environment. Genomic DNA was isolated from adult specimen (approximately 200 individuals) and copepods were homogenized in three volumes of DNA extraction buffer (100 mM NaCl, 10 mM Tris-Cl, pH 8.0, 25 mM ethylenediamine-tetraacetic acid [EDTA], 0.5% sodium dodecyl sulfate, 100 μg/ml proteinase K, and 1 μg/ml RNase) using a Teflon homogenizer. The homogenized sample was incubated in a water bath at 55 °C overnight. Phenol/chloroform extraction followed by isopropanol precipitation with 0.2× volume of 10 M ammonium acetate was performed at 7500 g for 10 min. The pellet was rinsed with 70% ethanol, air-dried, and resuspended in TE buffer (10 mM TrisCl, 1 mM EDTA, pH 8.0). The quality of the isolated DNA was measured using a QIAxpert system (Qiagen, Hilden, Germany) and was visually assessed by electrophoresis on an agarose gel.

2.4. Whole-genome sequencing analysis and de novo genome assembly

For long-read sequencing, a SMRTbell library was constructed using the SMRTbell Express Template Preparation Kit. The library was sequenced as more than one kb on the PacBio Sequel platform (Pacific Biosciences, Menlo Park, CA, USA) with two cells. Long-read sequences were filtered by mapping them on a customized contaminant database within the NCBI REFGENE database (https://www.ncbi.nlm.nih.gov/refseq/about/prokaryotes/). Filtered data were assembled as *de novo* genome contigs using SMARTdenovo (https://github.com/ruanjue/smartdenovo) as shown in Suppl. Fig. 1. For accurate genome assembly, a 500 bp paired-end library (PE500) was constructed and sequenced on the Illumina HiSeq 2500 platform (Illumina, San Diego, CA, USA). PE500 library data were trimmed and filtered using Trimmomatic v0.33 (Bolger et al., 2014). Cleaned PE500 data were used to predict genome size with *K*-mer distribution to compare assembly. Furthermore, these data were used for error correction of a genome assembled from long-read sequences using Medaka v0.11.2 (https://github.com/nanoporetech/medaka) and Pilon v1.23 (https://github.com/broadinstitute/pilon/wiki). The completeness of the final genome was investigated by comparing it to the Arthropoda orthologous database using Benchmarking Universal Single-Copy Orthologs (BUSCO) v3.0 (Simão et al., 2015).

2.5. Genome annotation

The MAKER v.2.3.1 pipeline was used for *T. kingsejongensis* genome annotation (Holt and Yandell, 2011; Yandell and Ence, 2012). Transcriptome data and protein information of the closely related species *T. japonicus* (GenBank no. **GCHA00000000.1**) (Kim et al., 2016) and *T. californicus* (Barreto et al., 2018) in addition to other model species, including *Homo sapiens*, *Drosophila melanogaster*, *Daphnia pulex*, *Caenorhabditis elegans*, and *Mus musculus* were aligned to the assembled genome using Exonerate V2.2.0 (Slater and Birney, 2005). For manual curation, JBrowse was used to visualize gene prediction and evidence data. tRNAscan-SE v1.23 (http://lowelab.ucsc.edu/tRNAscan-SE/) and the Rfam v12.2 database were used to identify non-coding RNAs. Functional annotation of the predicted genes was performed using Blast2GO_cli v1.1.5 (https://www.blast2go.com/) with the program blastx against the non-redundant (NR) database of NCBI with an e-value of 1e-10.

2.6. Ortholog analysis among Tigriopus spp. genomes

To determine which genes were orthologous among *T. kingsejongensis*, *T. japonicus*, and *T. californicus*, we used the web-based tool OrthoVenn (Wang et al., 2015). Annotated data of three copepod species were uploaded and analyzed using platform (http://www.bioinfogenome.net/OrthoVenn/index.php). In addition to clustering orthologous genes, OrthoVenn provided Gene Ontology (GO) terms and GO enrichment by computing *P*-values for GO terms in overlapping clusters using a hypergeometric distribution.

2.7. In silico identification of elongation of very long-chain fatty acid protein and fatty acid desaturase genes and phylogenetic analysis

To identify *Elovl* and *Fad* genes, *in silico* analysis using genome and RNA-seq information of *T. kingsejongensis* and *T. japonicus* (GenBank no. **GCHA00000000**) was performed. Genes were subjected to BLAST analysis in the GenBank non-redundant (NR; including all GenBank, EMBL, DDBJ, and PDB sequences except EST, STS, GSS, and HTGS) amino acid sequence database (http://blast.ncbi.nlm.nih.gov/). All acquired contigs were mapped onto the genome to obtain the complete DNA sequence using Geneious (v.10.0.7; Biomatters Ltd., Auckland, New Zealand) (Kearse et al., 2012) and was used for the tandem duplication analysis. Annotation and nomenclature of all *Elovl* and *Fad* genes were done based on amino acid sequence similarities, while the conserved motifs of *Elovl* and *Fad* genes were analyzed based on Hashimoto et al. (2008) (Suppl. Figs. 3 and 4). To analyze evolutionary relationships of the Elovl and Fad genes of *T. kingsejongensis*, we performed phylogenetic analyses. Multiple alignments of translated amino acid sequences of genes from the two copepods *T. kingsejongensis* and *T. japonicus* with other species were first obtained using the ClustalW algorithm. To establish the best-fit substitution model for each gene, the model with the lowest score according to the Bayesian Information Criterion (BIC) (Schwarz, 1978) and Akaike Information Criterion (AIC) (Hurvich and Tsai, 1989; Posada and Buckley, 2004) was used in maximum likelihood (ML) analysis. Phylogenetic trees for both Elovl and Fad genes were constructed using MEGA software (ver.7.0) under the best-fit model (LG + G + I) (Center for Evolutionary Medicine and Informatics, Tempe, AZ, USA) (Kumar et al., 2016). The reliability of the tree topology was evaluated by bootstrap analysis (1000 replicates).

2.8. Statistical analysis

SPSS ver. 18.0 (SPSS Inc., Chicago, IL, USA) was used for statistical analysis. Data are expressed as means ± S.D. Significant differences between *T. kingsejongensis* and *T. japonicus* were analyzed using one-way analysis of variance (ANOVA) followed by Tukey's test. All assumptions for the ANOVA and Tukey test are checked and fulfilled. Differences with $P < 0.05$ were considered statistically significant.

3. Results and discussion

3.1. Analysis of fatty acid profiles

Full adult stage of *T. kingsejongensis* is achieved in four weeks after six nauplius and five copepodid stages. The overall body size is about 1.2 mm, which is approximately 1.5 times larger than that of the temperate copepod, *T. japonicus* (Fig. 1A). Concentrations of saturated fatty acids (SFAs), monounsaturated fatty acids (MUFAs), and polyunsaturated fatty acid (PUFAs) of *T. kingsejongensis* were higher than those measured in *T. japonicus* (Table 2). The PUFA/total fatty acid (TFA) ratio was similar in two species. However, the SFA/TFA ratio was higher in *T. japonicus*, while the MUFA/TFA ratio was higher in *T. kingsejongensis* (Fig. 1B and C). Fatty acid acts as a source of energy during over-wintering (Corsolini and Borghesi, 2017). For example, Antarctic phytoplankton store over 80% of fixed carbon in the form of

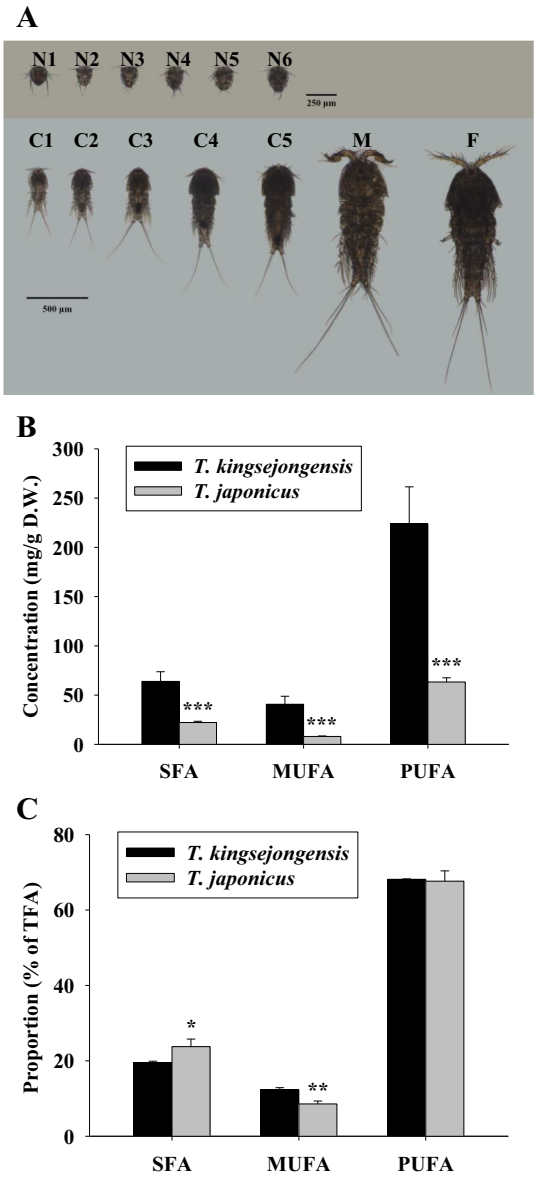

Fig. 1. (A) Different stages of development of the intertidal harpacticoid copepod, *Tigriopus kingsejongensis* maintained under standard culture condition (salinity 30 practical saline units [PSU], temperature 15 °C, 12-h light:dark cycle). Stages 1–6 are nauplius (N) stages, and the six stages in the second row (from 7 to 12) represent copepodite (C) stages. (B) Fatty acid concentration and (C) proportions of *Tigriopus kingsejongensis* and *Tigriopus japonicus*. Results represent the mean ± S.D. of three replicate samples. Asterisk (*) indicates significant difference between *Tigriopus kingsejongensis* and *Tigriopus japonicus* (*, $P < 0.05$, **, $P < 0.01$, *** $P < 0.001$).

lipids, whereas temperate phytoplankton store only 20% (Smith and Morris, 1980). Previous studies have reported that marine ectoderms and crustaceans living at lower temperatures had more unsaturated FAs than those living at higher temperatures (Lewis, 1962; Farkas and Herodek, 1964). This is likely related to membrane fluidity. An increase in unsaturated phospholipid content in membranes increases their fluidity (Cossins, 1994). At low temperatures, membranes are likely to be less fluid; therefore, a larger amount of unsaturated fatty acids is required to maintain normal membrane function at low temperatures (Cossins, 1994). The difference we observed in the MUFA/TFA ratio between the polar and temperature copepod species is likely a consequence of the evolutionary adaptation of the copepod *T. kingsejongensis* to extremely low temperatures. The high FA content and MUFA/TFA ratio of *T. kingsejongensis* are potential advantages of using *T. kingsejongensis* as a dietary source in marine fish hatcheries to enhance the overall content of fish lipids. However, it is necessary to compare the culture efficiencies of these two copepod species because their optimum culture temperatures and fertilities are different. In *T. kingsejongensis*, the survival rate was reduced beyond 18 °C (Han et al., 2018a). So it is impossible to accelerate their growth by raising temperature. Therefore, in the future, we will examine the effects of various temperatures to *T. kingsejongensis* to find out the best compromising temperature and lipid contents over the growth to apply in the aquaculture.

With regard to the composition of single FAs, 18:3n-3 was the most common FA in both species. FA concentration in *T. kingsejongensis* was generally higher than that of *T. japonicus*; however, the PUFA/TFA ratio was relatively similar (Table 2). In particular, we identified FAs present only in *T. kingsejongensis* (*i.e.*, 18:1n-7, 20:1n-7, 22:1n-11, and 22:1n-9). 22:1n-11 is known to be produced from wax esters in copepods (Lee et al., 1971; Pascal and Ackman, 1976; Falk-Petersen et al., 1990). Also, 22:1n-11 is a good indicator FA for understanding the trophic level within a food web, as 22:1n-11 has been widely detected in predatory fish and invertebrates (Ackman, 1980; Iverson, 1993; Dahl et al., 2000; Budge et al., 2002; Iverson et al., 2002; Cooper et al., 2005). We found different types of FAs in the Antarctic and temperate copepod species despite administration of the same amount of food (the green microalga *T. suecica*). This suggests differences in FA biosynthesis between these two *Tigriopus* species. Furthermore, we mapped the compositions of single FA to the unsaturated fatty acid biosynthesis pathway (Castro et al., 2016), and found that the long chain-omega-6 biosynthesis pathway is inactive in *T. kingsejongensis*, as 20:3n-6, 22:4n-6, and 22:5n-6 were not detected (Table 2 and Fig. 2). However, further analysis is required to confirm these findings. Additionally, 22:6n-3 (docosahexaenoic acid) is likely directly synthesized by Δ4 desaturase but not by the Sprecher pathway, as intermediate FAs were not detected (Sprecher, 2000). In general, FA content is modulated by temperature (Lewis, 1962; Farkas and Herodek, 1964; Smith and Morris, 1980). Also, in the cyclopoid copepod *Paracyclopina nana* (Lee et al., 2017), low-temperature exposure increased the FA content (especially in n-3 FAs), but the novel FA was not discovered at low-temperature exposure. However, in this study, despite the administration of the identical amount of *T. suecica*, differences in FA types were observed among the *Tigriopus* species. So, we hypothesized that the types of FA synthesizing genes would be different. Therefore, to confirm FA biosynthesis pathways in the genus *Tigriopus*, we identified the entire *Elovl* and *Fad* genes, which are responsible for modifying the chemical structure of FAs, in *T. kingsejongensis*.

3.2. De novo assembly and annotation of the genome of *T. kingsejongensis*

The final assembly of the Antarctic copepod *T. kingsejongensis* genome was 338,647,408 bp in length and consisted of 938 scaffolds (Tables 3, 4), which is the largest genome reported to date in the genus *Tigriopus* species. This scaffold number is lower than that reported in our previous study (Kang et al., 2017), indicating an improvement in the overall quality of genome assembly, with an enhanced final N50 value of 1,473,880 bp. The GC content was 47.3%, which is higher than that reported for *T. californicus* and *T. japonicus*. BUSCO value of *T. kingsejongensis* genome was 95.8%, higher than that of the previously published genome (70.9%) (Kang et al., 2017) (Tables 4, 5). A total of 35,739,996 coding sequence lengths were obtained from RNA-seq and *in silico* gene annotation (Table 6). After gene prediction based on the MAKER2 pipeline and manual curation by aligning the evidence data to the assembled genome scaffolds, 25,470 genes were annotated in *T. kingsejongensis* (Table 6; Suppl. file 1); this genome database can be accessed through JBrowse (http://rotifer.skku.edu:8080/Tk) (Suppl. Fig. 2). To better understand the relationship between genome structure and gene/protein function, comparative analyses of the orthologous

Table 2
Concentration and proportion (% of total fatty acid) of fatty acids in *Tigriopus kingsejongensis* and *Tigriopus japonicus*. Results represent the mean ± S.D. of three replicate samples. N.D.: not detected. SFA: saturated fatty acid, MUFA: monounsaturated fatty acid, PUFA: polyunsaturated fatty acid, TFA: total fatty acid.

	Concentration (mg/g dry weight)		Proportion (%)	
	T. kingsejongensis	*T. japonicus*	*T. kingsejongensis*	*T. japonicus*
14:0	1.07 ± 0.21	0.31 ± 0.09	0.32 ± 0.02	0.33 ± 0.09
15:0	1.16 ± 0.22	1.06 ± 0.22	0.35 ± 0.01	1.13 ± 0.21
16:3n-6	3.11 ± 0.53	N.D.	0.95 ± 0	N.D.
16:4n-3	17.26 ± 2.86	6.93 ± 0.72	5.26 ± 0.02	7.41 ± 0.59
16:2n-6	0.31 ± 0.06	N.D.	0.09 ± 0.01	N.D.
16:3n-3	2.61 ± 0.48	1.25 ± 0.24	0.8 ± 0.02	1.33 ± 0.23
16:2n-5	N.D.	0.27 ± 0.02	N.D.	0.29 ± 0.03
16:1n-9	3.55 ± 0.66	1.22 ± 0.24	1.08 ± 0.03	1.02 ± 0.23
16:1n-7	2.15 ± 1.9	0.95 ± 0.18	0.92 ± 0.01	1.31 ± 0.3
16:0	51.14 ± 8.74	15.81 ± 0.6	15.57 ± 0.04	16.94 ± 1.05
17:0	1.33 ± 0.25	0.59 ± 0.04	0.4 ± 0.01	0.63 ± 0.05
18:3n-6	2.69 ± 0.42	1.15 ± 0.13	0.82 ± 0.01	1.23 ± 0.11
18:4n-3	5.39 ± 0.86	2.46 ± 0.15	1.64 ± 0.04	2.64 ± 0.13
18:2n-6	28.9 ± 4.68	6.51 ± 1.22	8.81 ± 0.07	6.94 ± 1.14
18:3n-3	77.29 ± 12.56	19.58 ± 0.85	23.56 ± 0.15	20.95 ± 0.36
18:1n-9	18.75 ± 2.99	4.14 ± 0.12	5.72 ± 0.05	4.43 ± 0.08
18:1n-7	0.42 ± 0.06	N.D.	0.13 ± 0.01	N.D.
18:0	7.45 ± 1.27	3.76 ± 1.06	2.27 ± 0.08	4.04 ± 1.27
20:4n-3	7.31 ± 1.22	3.83 ± 0.19	2.23 ± 0.02	4.11 ± 0.32
20:5n-3	22.29 ± 3.93	6.47 ± 0.26	6.78 ± 0.05	6.92 ± 0.17
20:3n-3	3.33 ± 0.55	N.D.	1.01 ± 0.01	N.D.
20:4n-6	9.8 ± 1.68	1.15 ± 0.22	2.99 ± 0.1	1.23 ± 0.2
20:3n-6	N.D.	1.1 ± 0.06	N.D.	1.18 ± 0.08
20:2n-6	0.94 ± 0.16	0.45 ± 0.04	0.29 ± 0	0.48 ± 0.05
20:1n-9	13.64 ± 2.3	1.67 ± 0.19	4.15 ± 0.01	1.8 ± 0.26
20:1n-7	1.51 ± 0.25	N.D.	0.46 ± 0.01	N.D.
20:0	0.29 ± 0.05	0.08 ± 0.07	0.09 ± 0	0.13 ± 0.02
22:6n-3	33.97 ± 6.26	9.47 ± 1.24	10.33 ± 0.19	10.12 ± 1.07
22:5n-3	8 ± 1.36	1.74 ± 0.23	2.44 ± 0.03	1.86 ± 0.27
22:4n-3	0.56 ± 0.14	0.46 ± 0.16	0.17 ± 0.01	0.5 ± 0.18
22:1n-11	0.42 ± 0.08	N.D.	0.13 ± 0	N.D.
22:1n-9	0.27 ± 0.19	N.D.	0.08 ± 0.05	N.D.
22:0	1.27 ± 1.43	0.24 ± 0.06	0.43 ± 0.52	0.26 ± 0.06
24:0	0.17 ± 0.04	0.34 ± 0.05	0.05 ± 0.01	0.36 ± 0.04
22:5n-6	N.D.	0.43 ± 0.01	N.D.	0.46 ± 0.01
SFA	63.89 ± 9.84	22.18 ± 1.25	19.49 ± 0.42	± 23.77
MUFA	40.71 ± 8.14	7.99 ± 0.5	12.36 ± 0.53	± 8.57
PUFA	223.75 ± 37.69	63.26 ± 4.29	68.15 ± 0.11	± 67.66
TFA	328.34 ± 55.56	93.43 ± 2.59	100	100

clusters is extremely useful, as orthologous genes are clusters of genes that originated from a common ancestor prior to speciation (Fitch, 1970; Jensen, 2001). Thus, we analyzed orthologous genes in the genomes of *T. californicus* (Barreto et al., 2018), *T. japonicus* (Jeong et al., 2020), and *T. kingsejongensis* (Fig. 3). OrthoVenn revealed 14,029, 13,844, and 12,838 gene clusters in *T. kingsejongensis*, *T. japonicus*, and *T. californicus*, respectively, with 10,765 gene clusters shared among all three species. Within the gene clusters, 1765, 680, and 193 genes were specific to *T. kingsejongensis*, *T. japonicus*, and *T. californicus*, *respectively*. Although *T. kingsejongensis* has a much larger genome than those of *T. japonicus* (197 Mb) and *T. californicus* (191 Mb) (Table 4), the number of predicted genes was similar in each of the genomes (25,470 genes for *T. kingsejongensis* and 25,143 genes for *T. japonicus*), suggesting higher proportions of intergenic and/or repeated elements in *T. kingsejongensis* (Jeong et al., 2020). Datasets supporting the results in this article are available in NCBI under the accession number **PRJNA625855**. Comparative analyses of orthologous genes among these three copepods could also provide insight into their adaptations to different environments. In fact, the potential of organisms to adapt to changes in environmental conditions has previously been studied in rotifers using genomic signatures (Franch-Gras et al., 2018). Thus, the improved genome reported in this study could be used as an asset to better understand species-specific tolerances to environmental changes.

3.3. Phylogenetic analysis of Elovl and Fad genes

We identified all *Elovl* and *Fad* genes from the genome of *T. kingsejongensis*. These gene families were highly conserved between *T. kingsejongensis* and *T. japonicus* (Table 7, Fig. 4). In *T. kingsejongensis*, eight *Elovl* genes (*Elovl1/7a1*, *a2*, *b*, *c*, *d*, *Elovl3/6*, *Elovl4*, and *Elovl11*) were identified. Elongase (encoded by *Elovl*) lengthens the carbon chain of FA, and seven classes of Elovl genes (*Elovl1* to *7*) have been reported in animal taxa (Leonard et al., 2004; Jakobsson et al., 2006). However, recent studies on invertebrate species have reported novel *Elovl* classes. For example, two novel Elovl classes (Elovl A and B) were reported in the order Actinaria by transcriptome assembly analysis of *Actinia tenebrosa* [blue, brown, green, and red], *Telematactis* sp., and *Nemanthus annamensis* (Surm et al., 2018). Two novel Elovl classes (Elovl9 and 10) have also been identified in rotifers in the genus *Brachionus* (Lee et al., 2019b). Phylogenetic analysis revealed a novel Elovl class (Elovl11) in *Tigriopus*. Fatty acid desaturases (encoded by *Fad* genes) increase the degree of unsaturation of FAs. In *T. kingsejongensis,* eight *Fad* genes (*Δ4*, *Δ5/6a*, *b*, *c*, *d1*, *d2*, *e*, and *Δ9*) were identified, while in *T. japonicus*, different Fad genes (Δ5/6e2) were identified instead of Δ5/6d2. In a previous study (Kabeya et al., 2017), three *Fad* genes (*Pl-FadA*, *C1*, and *C2*) were found in the sea urchin *Paracentrotus lividus*, and *FadC1* and *C2* showed Δ8 desaturase activity. Our FA data indicated that *Tigriopus* species possibly have Δ8 desaturase activity (Fig. 2), therefore we included *P. lividus Fad* genes in our phylogenetic analysis. Our results

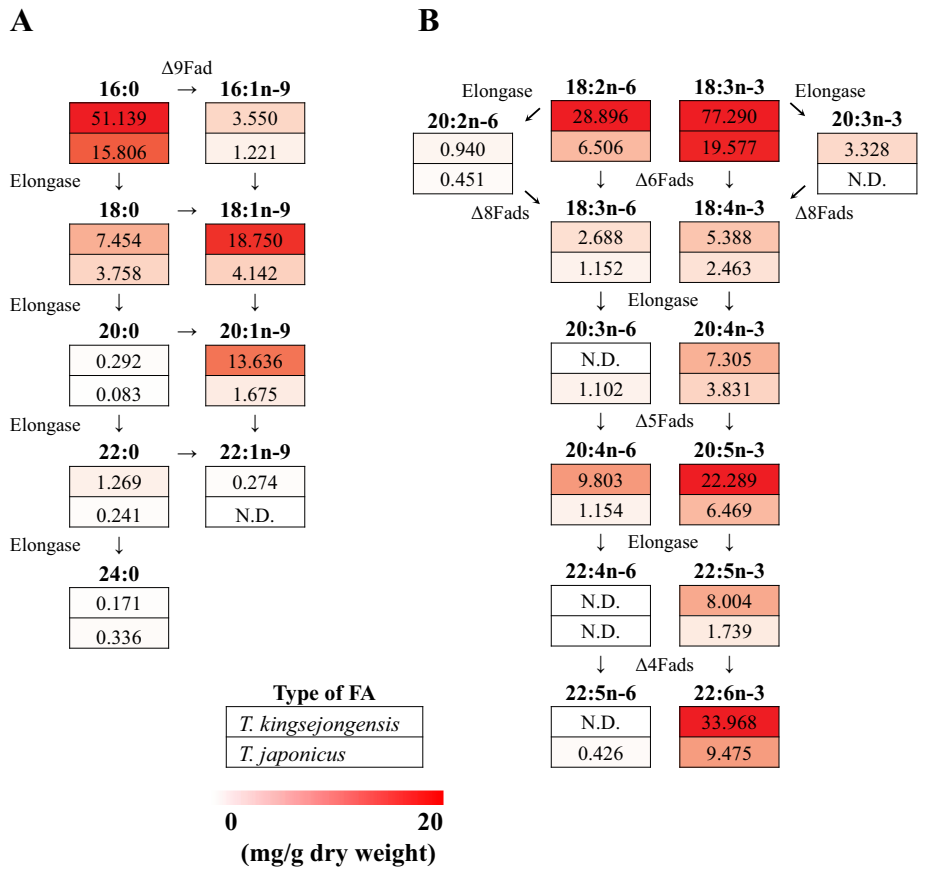

Fig. 2. (A) Biosynthetic pathways of monounsaturated fatty acids synthesis from saturated fatty acids. (B) Biosynthetic pathways of omega-3 and -6 fatty acids (modified from Castro et al., 2016). The concentrations of each FA compound were found within the boxes. N.D., not detected.

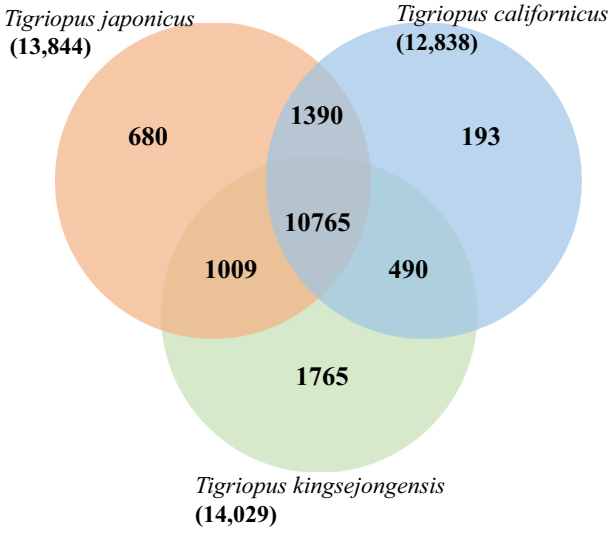

Fig. 3. Venn diagram of orthologous clusters from genomes of *Tigriopus japonicus*, *Tigriopus californicus*, and *Tigriopus kingsejongensis* using OrthoVenn. (Modified from Jeong et al., 2020).

indicated that the *Δ5/6 desaturase* gene likely has Δ8 desaturase activity. Previous studies have been reported that the mammalian gene *Fads2* has both Δ6 and 8 desaturase activity (Park et al., 2009; Stroud et al., 2009). Our results suggest that *Tigriopus* species may have Δ8 desaturase activity. To confirm our findings, we will analyze the fatty acid composition by measuring the activity of Fad genes in the near future.

Table 3
Summary of the sequencing libraries and reads generated for whole-genome assembly of *Tigriopus kingsejongensis*.

PacBio Sequel	
Number of SMRT cells	2
Polymerase read	
Total number of reads	1,399,771
Total number of bases	23,747,900,141
N50 (bp)	28,750
Mean read length (bp)	16,966
Subread	
Total number of reads	1,696,326
Total number of bases	23,735,932,513
N50 (bp)	23,742
Mean read length (bp)	13,993
Genome coverage (338.6 Mb)	70×
GenBank accession no.	**SRX8159652**
Illumina HiSeq 2500	
Raw reads	
Total number of reads	93,897,422
Total number of bases	23,521,304,211
Cleaned reads	
Total number of reads	65,943,964
Total number of bases	14,232,918,582
GenBank accession no.	**SRX8159653**

3.4. Tandem duplication of Tigriopus spp. Elovl and Fad genes

To investigate duplication patterns of *Elovl* and *Fad* genes in *Tigriopus*, we examined regions of tandemly duplicated *Elovl* and *Fad* genes. Among the *Elovl* genes, tandem duplication of *Elovl1/7a1*, *2*

Table 4
Statistics of genome assembly in *Tigriopus* species.
(Modified from Jeong et al., 2020).

Scaffold information	*T. kingsejongensis* (This study; GCA_012959195.1)	*T. kingsejongensis* (Kang et al., 2017; doi:10.5524/100249)	*T. californicus* (Barreto et al., 2018; GCA_007210705.1)	*T. japonicus* (Jeong et al., 2020; GCA_010645155.1)
Number of scaffolds	938	11,558	459	339
Total length of scaffolds (bp)	338,647,408	295,233,602	191,142,546	196,599,007
N50 (bp)	1,473,880	159,218	15,806,032	10,654,335
Largest scaffold (bp)	9,103,457	3,401,446	18,073,795	15,288,102
BUSCO complete copy (%)	95.8	70.9	94.5	96.3
Gap (%)	0.16	3.55	2.09	2.54
GC content (%)	47.3	44.4	41.31	39.17

Table 5
Completeness assessment of the assembled *Tigriopus kingsejongensis* genome.

	Arthropoda	
	%	No. of genes
Complete BUSCOs	95.8	1021
Complete and single-copy BUSCOs	92.7	988
Complete and duplicated BUSCOs	3.1	33
Fragmented BUSCOs	1.9	20
Missing BUSCOs	2.3	25
Total BUSCO groups searched		1066

Table 6
Gene annotation statistics for the assembled *Tigriopus kingsejongensis* genome.

Categories	Value
Number of genes	25,470
Total coding sequence length (bp)	35,739,996
Average gene length (bp)	3814
Largest gene length (bp)	114,816
Average CDS length (bp)	1403
Average intron length (bp)	847
GC content (%)	53.04

genes were identified in scaffolds 119 and 115 in *T. japonicus* and *T. kingsejongensis*, respectively (Fig. 5), in the opposite direction. Gene expansion of *Elovl* and *Fad* genes has been reported in previous studies. For example, in the silkworm *Bombyx mori* (Zuo et al., 2018), *Elovl1/7* genes were highly expanded, while in the rotifer *Brachionus* species (Lee et al., 2019a and b), *Elovl1/7* and *Elovl9* genes were identified, and there was expansion of *Δ5/6 desaturase* activity. Consistent with the aforementioned invertebrates, *Δ5/6 desaturase* genes (*Δ5/6a* to *e*) were also duplicated in the *Tigriopus* species we examined in a species-specific manner (*e.g.*, *Δ5/6d-1* and *-2* in *T. kingsejongensis*; *Δ5/6e-1* and *-2* in *T. japonicus*; *Δ5/6b-1* and *-2* in *T. californicus*) (Fig. 6). According to the phylogenetic tree, these genes duplicated after species differentiation, suggesting that the *desaturase* gene may have differentiated due to adaptation to the environment. Gene duplication is considered a key evolutionary process involved in the evolution of new functions (Moore and Purugganan, 2003). *Δ5/6* desaturases are key enzymes in endogenous desaturation (Martinelli et al., 2008; Lattka et al., 2010), and *T. kingsejongensis* has an extra *Δ5/6* desaturase than *T. japonicus*. These data suggest that the duplicated *Δ5/6d1* and *2* genes in *T. kingsejongensis* are involved in the synthesis of 22:1n-11 and/or 22:1n-9. The observed differences in *desaturase* duplication are likely closely related to the life history of species in the genus *Tigriopus*.

4. Conclusions

In this study, we compared and analyzed FA profiles of the Antarctic copepod *T. kingsejongensis* and the temperate copepod *T. japonicus*. *Tigriopus kingsejongensis* had higher concentrations of FAs compared to *T. japonicus*, in addition to unique FAs (22:1n-9 and 22:1n-11). To determine the basis for such differences, we identified all *Elovl* and *Fad* genes in the genome of *T. kingsejongensis* and compared them to those in *T. japonicus*. There was notable expansion of both *Elovl* and *Fad* gene families in the two *Tigriopus* species. Although there were no differences

Table 7
Characteristics of elongation of very long-chain fatty acid protein (*Elovl*) and fatty acid desaturase (*Fad*) genes identified in *Tigriopus kingsejongensis and Tigriopus japonicus*. Nucleotide and amino acid sequence are provided in Supplementary file 2.

Tigriopus kingsejongensis							*Tigriopus japonicus*						
Gene name	Scaffold	Start	End	Strand	Exon	Length	Gene name	Scaffold	Start	End	Strand	Exon	Length
Elovl1/7a1	115	409,610	408,582	−	2	960	*Elovl1/7a1*	119	290,895	291,759	+	2	804
Elovl1/7a2	115	407,161	405,349	−	2	927	*Elovl1/7a2*	119	292,320	293,664	+	2	816
Elovl1/7b	159	353,244	352,386	−	2	792	*Elovl1/7b*	111	76,713	74,127	−	2	789
Elovl1/7c	10	690,192	691,076	+	1	885	*Elovl1/7c*	14	1,659,459	1,658,638	−	1	822
Elovl1/7d	91	404,329	402,463	−	5	855	*Elovl1/7d*	10	1,428,556	1,426,415	−	5	858
Elovl3/6	31	74,784	76,311	+	4	843	*Elovl3/6*	2	2,330,062	2,327,201	−	4	861
Elovl4	16	1,385,838	1,386,683	+	1	846	*Elovl4*	163	120,157	118,235	−	2	942
Elovl11	86	31,164	34,387	+	4	540	*Elovl11*	2	2,519,428	2,521,170	+	4	714
D4	12	3,348,988	3,346,787	−	6	996	*D4*	53	391,360	394,601	+	6	1002
D5/6a	146	87,133	81,390	−	6	1287	*D5/6a*	48	545,924	553,143	+	6	1296
D5/6b	1	2,079,237	2,082,646	+	3	1323	*D5/6b*	79	87,227	83,952	−	3	1326
D5/6c	1	4,089,969	4,091,477	+	3	1293	*D5/6c*	12	1,997,864	2,000,444	+	3	1308
D5/6d1	25	638,948	641,715	+	4	1296	*D5/6d*	31	2,258,707	2,262,128	+	4	1320
D5/6d2	25	651,803	654,633	+	4	1293							
D5/6e1	25	607,645	615,280	+	4	1365	*D5/6e1*	31	2,249,394	2,252,637	+	4	1371
							D5/6e2	31	2,249,394	2,252,637	+	4	1434
D9	4	705,889	711,680	+	3	1035	*D9*	28	248,790	252,927	+	3	1068

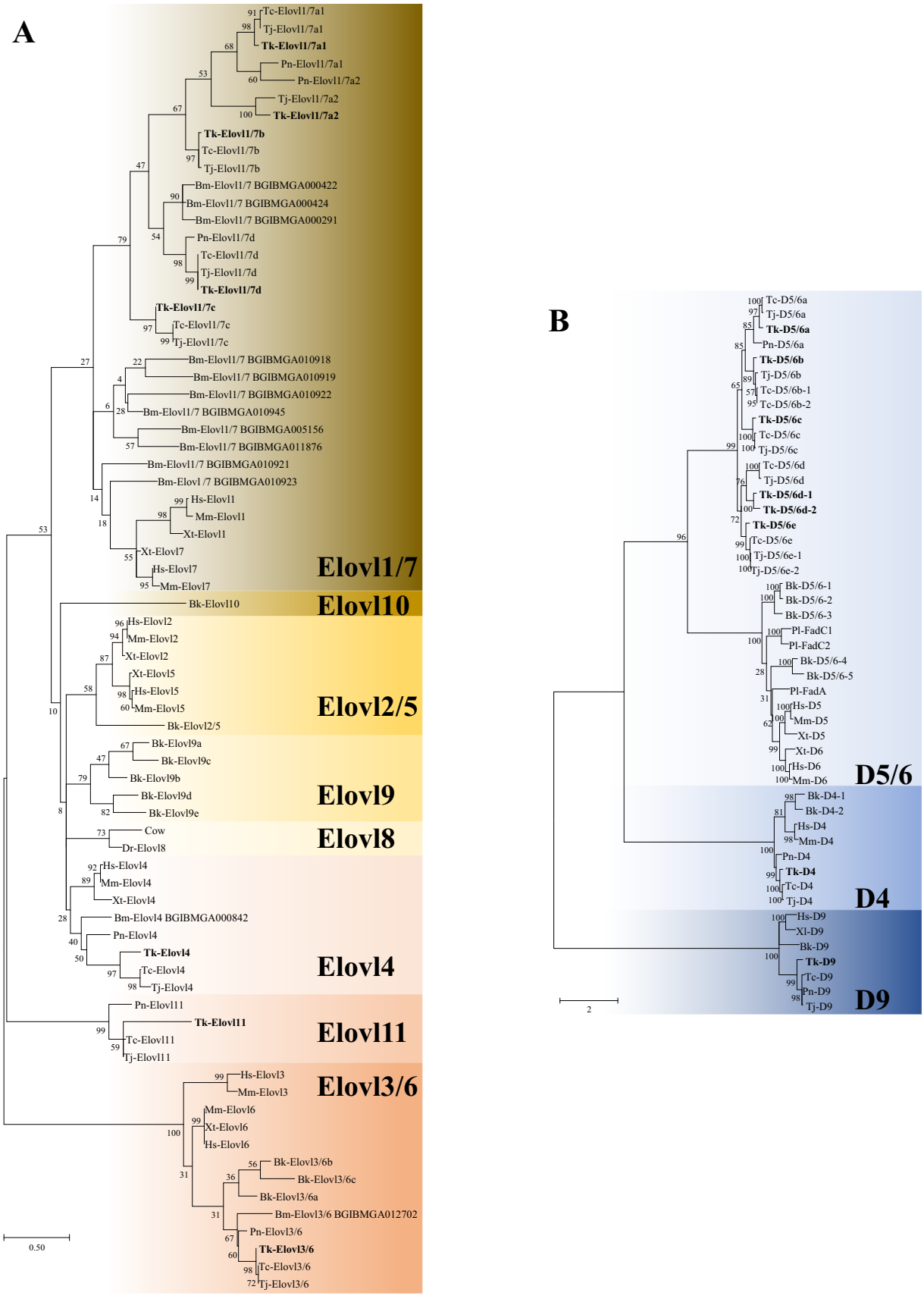

Fig. 4. Phylogenetic reconstruction of (A) elongation of very long-chain fatty acid proteins and (B) fatty acid desaturase proteins in *Tigriopus kingsejongensis* and other species using maximum likelihood. Numbers at nodes indicate bootstrap values.

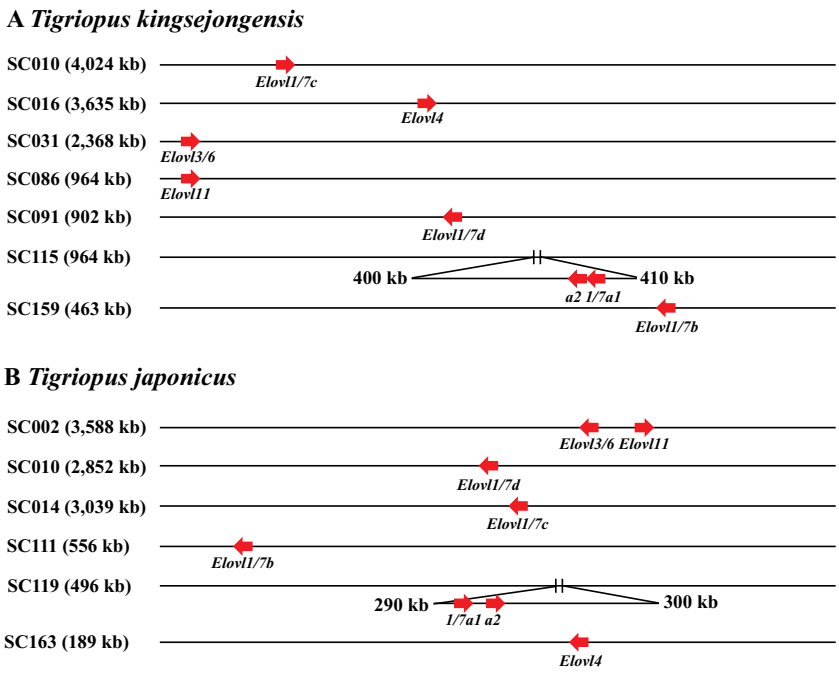

Fig. 5. Synteny analysis of elongation of very long-chain fatty acid protein genes in (A) *Tigriopus kingsejongensis* and (B) *Tigriopus japonicus*.

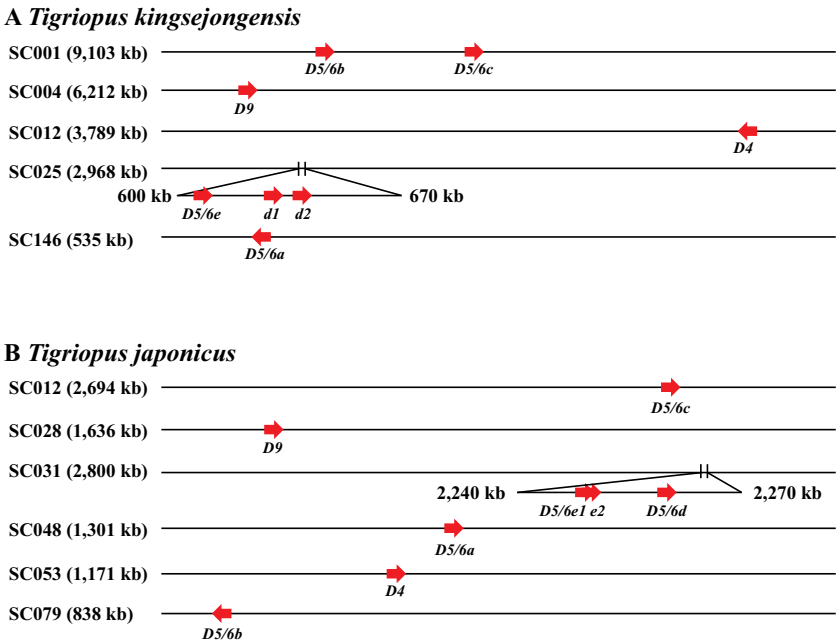

Fig. 6. Synteny analysis of fatty acid desaturase genes in (A) *Tigriopus kingsejongensis* and (B) *Tigriopus japonicus*.

in the number of *Elovl* and *Fad* genes between two species, there were differences in the repertoires of these genes. The *Δ5/6d-1* and *2 Fad* genes likely play a role in the synthesis of specific FAs. However, further research is needed in this area, as the functions of the proteins encoded by these genes have not yet been identified experimentally. In conclusion, we improved the whole-genome assembly of the Antarctic copepod *T. kingsejongensis* and identified all *Elovl* and *Fad* genes to gain a better understanding of fatty acid metabolism in the Antarctic copepod *T. kingsejongensis*.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cbd.2020.100703.

Declaration of competing interest

The authors have no conflict of interest in this paper.

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