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First Complete Mitochondrial Genome of a Tanaidacean Crustacean (*Arctotanais alascensis*)

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We present a complete mitochondrial genomic sequence for the tanaidacean *Arctotanais alascensis* (Richardson, 1899); this is the first complete mitogenome reported from the order Tanaidacea. The mitogenome is 13,988 bp long and contains 13 protein coding and two ribosomal RNA genes (as is typical for animal mitogenomes), and 21 of 22 transfer RNAs; we did not detect an isoleucine transfer RNA (trnI) gene. The gene order differed markedly from the hypothetical ground pattern for Pancrustacea; only four clusters (trnM + nad2; trnC + trnY + cox1 + trnL2 + cox2; trnD + atp8 + atp6 + cox3; trnH + nad4 + nad4l) ancestrally present were retained. In a malacostracan phylogenetic tree reconstructed from mitogenome data, basal relationships were marginally supported or incongruent with the traditional morphology-based classification and the latest phylogenetic reconstructions from large transcriptomic datasets. Relationships involving more recent divergences were better supported in our tree, suggesting that complete mitogenome sequences are more suitable for phylogenetic analyses within malacostracan orders, presumably including Tanaidacea.

Key words: high-throughput DNA sequencing, mitochondrion, phylogeny, Tanaididae, Tanaidoidea

INTRODUCTION

The animal mitochondrial genome (mitogenome) is typically a single, circular molecule 12–20 kb long and generally contains 13 protein coding, two ribosomal RNA (rRNA), and 22 transfer RNA (tRNA) genes (Boore, 1999; Kilpert and Podsiadlowski, 2006). As complete mitogenome sequences contain rich phylogenetic information and can be determined from specimens in various states of preservation (e.g., long preserved in ethanol, fixed in formalin, or mummified), they are increasingly widely used in phylogenetic (e.g., Uribe et al., 2016; Luchetti et al., 2019; Kim et al., 2020), taxonomic (e.g., Nakano et al., 2017), and phylogeographic studies (e.g., Bishop et al., 2018; Greig et al., 2018).

With approximately 17,000 described species, the superorder Peracarida is the most speciose crustacean subgroup (Appeltans et al., 2012). The current classification recognizes one extinct order (Pygocephalomorpha) and 12 extant orders (Amphipoda, Bochusacea, Cumacea, Ingolfiellida, Isopoda, Lophogastrida, Mictacea, Mysida, Spelaeogriphacea, Stygiomysida, Tanaidacea, and Thermosbaenacea) (Ahyong et al., 2011; Meland et al., 2015; Lowry and Myers, 2017). Although a few species attain lengths of over 300 mm (e.g., Clarke, 1961; Jamieson et al., 2013; McClain et al., 2015), most peracarids do not exceed 10 mm. Their generally small size, as well as limited commercial importance, may account for the paucity of molecular phylogenetic data compared to the closely related

Eucarida including crabs, prawns, shrimp, and krills. Complete mitogenome sequences have been determined for species only in the peracarid orders Amphipoda, Isopoda, and Mysida (Shen et al., 2015a, b).

The peracarid order Tanaidacea contains about 1500 described species that typically have body lengths of only a few millimeters (Kakui, 2016; Anderson, 2020). Tanaidaceans inhabit benthic brackish or marine habitats ranging in depth from the intertidal zone to the hadal zones at around 9000 m; as they often attain very high densities (e.g., 146,000 individuals/m²; Delille et al., 1985), they are ecologically important in various shallow- and deep-water ecosystems (Larsen et al., 2015).

Tanaidaceans have been underrepresented in molecular analyses. Kakui et al. (2011) reconstructed the higher-level phylogeny of the four major groups (Apseudoidea, Neotanaoidea, Paratanaoidea, and Tanaidoidea) based on sequences of the nuclear 18S rRNA gene, and two population-genetic studies have been conducted based on the mitochondrial cytochrome *c* oxidase subunit I (cox1) gene (Drumm and Kreiser, 2012; Kakui et al., 2020). Here we report the first complete mitochondrial genome from a tanaidacean, *Arctotanais alascensis* (Richardson, 1899) (Tanaidoidea: Tanaididae), and briefly discuss the utility of mitogenomic data in reconstructing malacostracan phylogeny.

MATERIALS AND METHODS

One individual of *Arctotanais alascensis*, 4.9 mm in body length, was collected from the intertidal zone at Oshoro, Hokkaido, Japan on 22 March 2016, kept alive in an aquarium until 20 May

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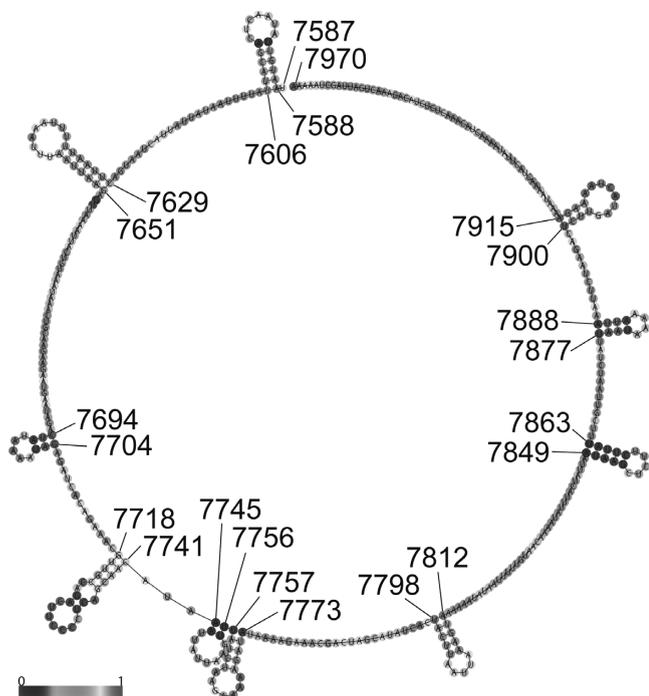


Fig. 2. Secondary structure of the putative control region in the mitogenome of *Arctotanaïs alascensis*, predicted with CentroidFold (inference engine = “McCaskill (BL)”, weight of base pairs = “2²”). Numbers represent positions in the 13,988-bp sequence.

program tranalign (<https://www.bioinformatics.nl/cgi-bin/emboss/tranalign>). Alignment-ambiguous sites were removed with Gblocks ver. 0.91b (Castresana, 2000) in NGPhylogeny.fr (Lemoine et al., 2019) under the “relaxed” parameters described in Talavera and Castresana (2007). Optimal substitution models were determined for different genes and codons (see Supplementary Table S2) under the corrected AIC (Akaike information criterion) option in PartitionFinder 2.1.1 (Lanfear et al., 2017), using a greedy algorithm (Lanfear et al., 2012). The data matrices for the individual genes were then concatenated into a single alignment 11,412 characters long in SequenceMatrix ver. 1.8 (Vaidya et al., 2011). A partitioned maximum-likelihood (ML) analysis was conducted in IQ-TREE ver. 1.6.12 (Nguyen et al., 2015; Chernomor et al., 2016); nodal support values were obtained from an ultrafast bootstrap analysis of 1000 pseudoreplicates under the “bnni” option (Hoang et al., 2018). The resulting ML tree was edited and drawn by using FigTree v1.4.4 (Rambaut, 2020).

RESULTS AND DISCUSSION

The mitogenome of *Arctotanaïs alascensis* was 13,988 bp long (accession number LC597489). Sequencing of PCR products obtained with primers ArcF/ArcR determined a 320 bp fragment corresponding to positions 13,678–13,988 and 1–9 in the 13,988 bp contig resulting from shotgun sequencing, confirming the complete, circular nature of the *A. alascensis* mitogenome.

We also obtained a second, longer (ca. 800 bp) fragment with primers ArcF/ArcR that we could not reliably sequence due to the presence of homopolymer regions, presumably resulting in polymerase slippage. This might indicate heteroplasmy (the presence of more than one type of mitogenome) in the sequenced specimen of *A. alascensis*, a condition previously reported for several other pancrusta-

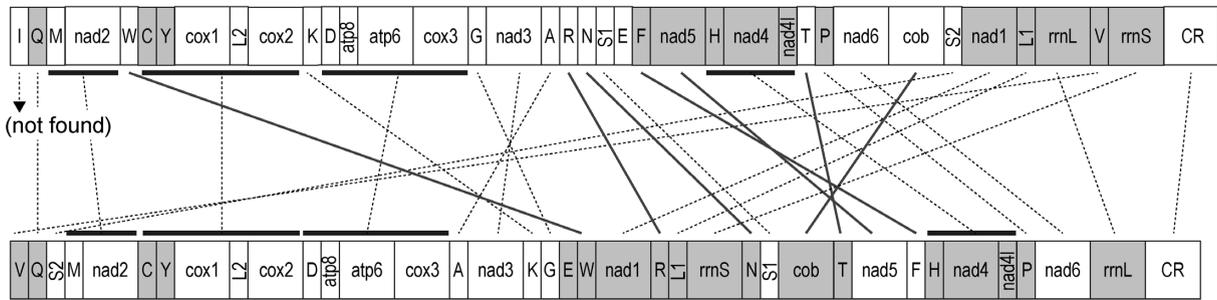
Table 1. Annotated genes in the mitochondrial genome of *Arctotanaïs alascensis* and their characteristic features. Negative values for the intergenic region (IGR) indicate an overlap between genes. CR denotes the putative control region.

Gene	Position		Length (bp)	Codon		Anticodon	Strand	IGR
	from	to		Start	Stop			
trnE	53	115	63			ttc	–	
trnW	115	177	63			tca	–	–1
nad1	198	1115	918	TAC	TAG		–	20
trnR	1110	1165	56			tcg	–	–6
trnL1	1166	1228	63			tag	–	0
rrnS	1230	1741	512				–	1
trnN	1866	1928	63			ggt	–	124
trnS1	1930	1982	53			tct	+	1
cob	1981	3024	1044	AAT	TAG		–	–2
trnT	3030	3095	66			tgt	–	5
nad5	3251	4750	1500	TTC	TAG		+	155
trnF	4749	4808	60			gaa	+	–2
trnH	4808	4868	61			gtg	–	–1
nad4	4859	6181	1323	TTT	TAG		–	–10
nad4l	6172	6477	306	CTC	TAG		–	–10
trnP	6469	6534	66			tgg	–	–9
nad6	6525	6995	471	CTA	TAA		+	–10
rrnL	7005	7586	582				–	9
CR	7587	7970	384				+	0
trnV	7971	8035	65			tac	–	0
trnQ	8037	8100	64			ttg	–	1
trnS2	8101	8161	61			tga	+	0
trnM	8162	8229	68			cat	+	0
nad2	8233	9192	960	TTC	TAA		+	3
trnC	9191	9243	53			gca	–	–2
trnY	9243	9305	63			gta	–	–1
cox1	9308	10870	1563	ATG	TAA		+	2
trnL2	10852	10914	63			taa	+	–19
cox2	10915	11580	666	ATA	TAA		+	0
trnD	11588	11638	51			gtc	+	7
atp8	11641	11790	150	ATG	TAA		+	2
atp6	11787	12452	666	ATG	TAA		+	–4
cox3	12513	13328	816	AAC	TAG		+	60
trnA	13292	13345	54			tgc	+	–37
nad3	13352	13687	336	CTA	TAA		+	6
trnK	13692	13756	65			ttt	+	4
trnG	13764	13825	62			tcc	+	7

cean species (e.g., Kuhn et al., 2008; Magnacca and Brown, 2010; Rodríguez-Pena et al., 2020). We cannot, however, rule out other possibilities, including a nuclear origin for the longer fragment.

The genomic annotation on the MITOS webserver identified 13 PCGs and two rRNAs, as is typical in animal mitogenomes, but among the 22 tRNAs identified, there was no tRNA for isoleucine (trnI) (Fig. 1; Table 1). The lack of one tRNA has been reported in some other peracarid crustaceans (e.g., Kilpert and Podsiadlowski, 2006; Bauzà-Ribot

Pancrustacean ground pattern



Arctotanis alascensis

Fig. 3. Comparison of gene order between *Arctotanis alascensis* and the hypothetical ancestral Pancrustacean (Boore et al., 1998). Solid and dashed lines connect corresponding genes and gene clusters with and without inversions, respectively. Gray shading denotes genes on the reverse (–) strand. CR, putative control region. Transfer RNA genes are labeled with their one-letter amino acid codes.

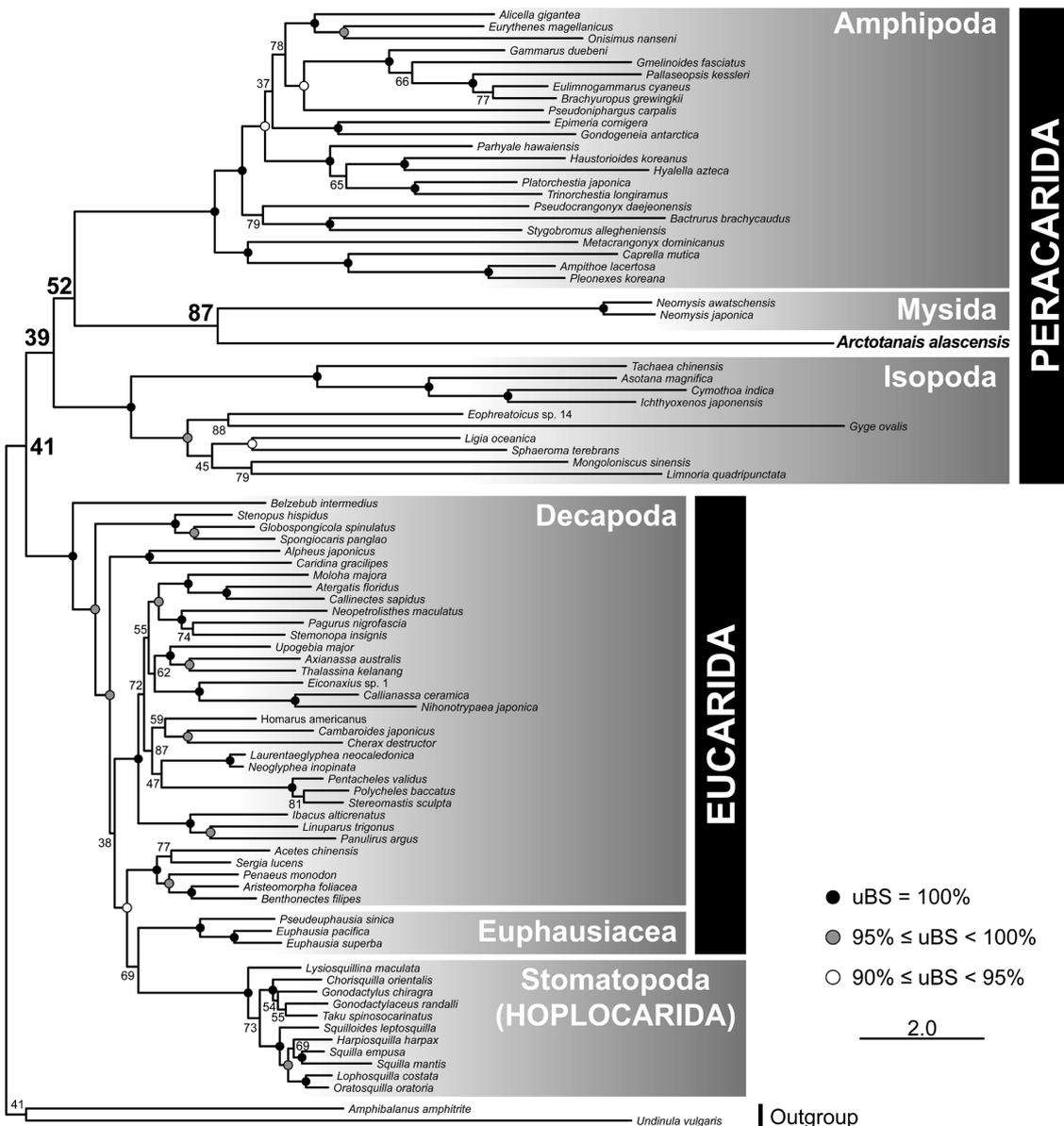


Fig. 4. Maximum-likelihood (ML) phylogeny for malacostracan crustaceans, based on mitogenome sequences (11,412 characters from 13 protein coding and two rRNA genes). Numbers near nodes are ultrafast bootstrap values (uBS).

et al., 2009). The putative control region (CR) was detected at positions 7587–7970, flanked by *rrnL* and *trnV* (Fig. 1). This was the longest (384 bp) continuous non-coding region in the *A. alascensis* mitogenome, characterized by high AT content (78.12%) and predicted to form multiple stem-loop structures (Fig. 2), similar to those in spiny lobsters (Qian et al., 2011).

The mitogenome of *A. alascensis* differed markedly in gene order from the hypothetical ground pattern for Pancrustacea (Boore et al., 1998), retaining only four ancestral clusters (*trnM* + *nad2*; *trnC* + *trnY* + *cox1* + *trnL2* + *cox2*; *trnD* + *atp8* + *atp6* + *cox3*; *trnH* + *nad4* + *nad4l*) (Fig. 3). Further studies on gene order in other tanaidaceans will help elucidate the evolution of the mitochondrial genome in Pancrustacea.

In the ML tree (Fig. 4), Amphipoda, Mysida, Isopoda, Euphausiacea, and Stomatopoda form robust clades, each with 100% ultrafast bootstrap support (uBS). Many of their internal nodes also received 100% uBS, suggesting that complete mitogenome sequences are most reliable for phylogenetic reconstruction within malacostracan orders, presumably including Tanaidacea. On the other hand, basal malacostracan relationships are only marginally supported or incongruent with the traditional morphology-based classification (Ahyong et al., 2011) and phylogenetic reconstructions based on large transcriptomic datasets (Schwentner et al., 2018). *Arctotanais alascensis* forms a moderately supported clade with Mysida (87% uBS) in Peracarida, whereas Schwentner et al. (2018) found Tanaidacea to be more closely related to Isopoda than to Mysida or Amphipoda, with near-maximum support values, a result that is congruent with the morphology-based phylogeny (e.g., Siewing, 1963).

Whether suitable for higher-level phylogeny or not, additional mitogenome sequences will greatly contribute to the understanding of internal relationships within the order Tanaidacea. Mitogenomic data also allow the design of PCR primers for taxon-specific markers for use in phylogeographic and taxonomic studies.

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COMPETING INTERESTS

The authors declare no competing interests.

AUTHOR CONTRIBUTIONS

KK conceived and designed the study, collected and identified the tanaidacean, did the molecular work and gene annotation, and constructed the graphical map of the mitogenome. YK assembled contigs from raw reads. KK and YK wrote the manuscript, and read and approved the final draft.

SUPPLEMENTARY MATERIALS

Supplementary materials for this article are available online. (URL: <https://doi.org/10.2108/zs200167>)

Supplementary Table S1. Species included in the phylogenetic analysis of malacostracan crustaceans.

Supplementary Table S2. Alignment lengths of two rRNA and 13 coding genes, and optimal substitution models.

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