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Genetype and phylogenomic position of the frilled shark *Chlamydoselachus anguineus* inferred from the mitochondrial genome

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ABSTRACT

The genetype of *Chlamydoselachus anguineus* from Australian waters is described using the whole mitochondrial genome obtained from Illumina NGS technology. Total length of the mitogenome is 17 313 bp, consisting of 2 rRNAs, 13 protein-coding genes, 22 tRNA genes and 2 non-coding regions thus updating the previously available mitogenome for this species. The phylogenomic reconstruction comprising all available species of Superorder Squalomorphi supports the inclusion of *C. anguineus* in a divergent clade inside Order Hexanchiformes. Phyletic relationships inferred from the whole mitochondrial genomes are in agreement with traditional taxonomy. The low divergence between *C. anguineus* genomes (>99.9% genetic identity) is consistent with a widespread population in the west Pacific Ocean.

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The frilled shark, *Chlamydoselachus anguineus* Garman, 1884, is one of the two species of deep water sharks in the Family Chlamydoselachidae, generally inhabiting the outer continental and insular shelves and continental slopes at depths between 120 m and 1500 m in the Atlantic and Pacific Oceans (Shirai 1996; Ebert et al. 2013). Seldom observed, the species is taken as bycatch in bottom trawling and longline fisheries within its distribution range, e.g. Suruga Bay, Japan (Paul & Fowler 2003). However, little is known regarding life history and potential impacts of non-targeted exploitation.

The genetype (genetic sequence from type specimens, Chakrabarty 2010) of *C. anguineus* is described here from a specimen caught off Lakes Entrance (Gippsland, Victoria, Australia) and deposited in the Griffith's Sea Shell Museum. Additionally, the evolutionary position and validity of the mitogenome are interrogated using a phylogenomic approach based on whole mitochondrial genomes.

Genetype: Frilled shark, *C. anguineus*, Paragenetype, Voucher: *e*Fish-1061, GenBank accession number KU159431, Female 160 cm total length, 700 m depth, 38°24′S 148°40′E, southeast Victoria, Australia.

A fin clip was obtained from a specimen caught as bycatch by bottom trawling on 5 January 2015. The genomic library for sequencing was developed with the TruSeq PCR-Free DNA sample preparation kit (Illumina Inc., San Diego, CA) and sequenced using the HiSeq 2000 (Illumina Inc., San Diego, CA) that supported the acquisition of 2×125 bp pair-end reads without polymerase chain reaction (PCR) amplifications. Resulting reads (26 873 205 sequences) were mapped against the closely related *Hexanchus nakamurai* (GenBank accession number AB560491), using Geneious 8.1.2 (Biomatters Ltd., Auckland, New Zealand). The 67 068 matching reads, with 100% coverage (min. 379, max. 763), were assembled producing a 17 313 bp complete mitogenome. Genes were annotated using the web-based tool MitoAnnotator (Iwasaki et al. 2013). The complete genomic DNA sequence library was lodged at the Genomic Database Repository, *e*Fish (BioVoucher: 2015-CAN-1061).

A dataset of available mitogenomes from closely related taxa in the Superorder Squalomorphi was constructed using available records from GenBank, in order to assess the level of inter-specific divergence and validity of the reported mitogenome following Vargas-Caro et al. (2015). The aligned dataset comprised 10 mitogenomes and 17 240 sites and included a mitogenome of *C. anguineus* from the type locality ('hologenetype'): Suruga Bay, Japan (Tanaka et al. 2013). The mitogenomic dataset was aligned using Geneious and trimmed with trimAl using the *automated1* option in the Phylemon web suite (Sánchez et al. 2011) in order to remove ambiguously aligned positions. A maximum likelihood (ML) tree was inferred under the GTR + GAMMA + I model implemented in PhyML (Guindon & Gascuel 2003). Node robustness was evaluated with 1000 bootstrap replicates. The resulting topology and branch length were used to validate the phylogenomic position and divergence of the reported genome with respect to other sharks within the Order Lamniformes.

The mitogenome had an strong A + T (65%) bias, as seen in the Order Hexanchiformes, and a nucleotide composition of A, 32.2%; C, 21.9%; G, 13.1%; and T, 32.8%. Gene order and structure of the *C. anguineus* mitogenome were consistent with other elasmobranchs, comprising 13 protein-coding regions, 22 tRNA genes, 12S and 16S ribosomal RNAs and 2

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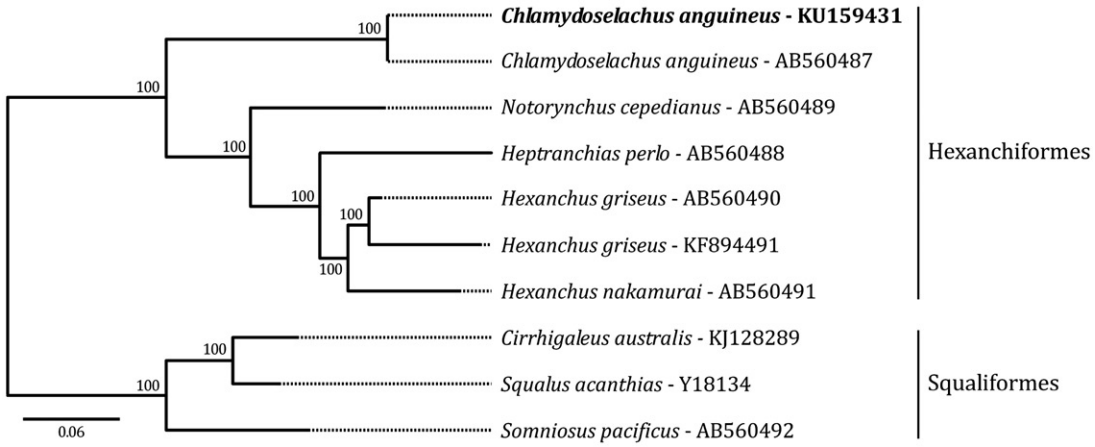

Figure 1. Phylogenomic relationships of *Chlamydoselachus anguineus* (in bold) inferred from whole mitochondrial genomes using closely related species of the Superorder Squalomorphi. Taxonomic subdivisions are indicated for each major clade. Bootstrap values obtained by 1000 steps are indicated for each node. GenBank accession numbers are provided following species names. Scale bar represents the expected substitution per site.

non-coding regions (control region and origin of L-strand replication). Most of the genes were encoded on the H-strand, except for *nadh*6 and eight tRNA genes that were encoded on the L-strand. The paragenetype mitochondrial genome was 99.92% identical with the hologenetype (Tanaka et al. 2013) and 14 single nucleotide polymorphisms (SNPs). Neutral (synonymous) mutations ($n = 7$) were observed in *cox*1 and *nadh*6 genes (1 SNP each), both ribosomal subunits 12S and 16S (1 and 2 SNPs, respectively) and the control region (2 SNPs). Non-synonymous mutations ($n = 7$) in the third-codon position were observed on the genes *cox*2, *nadh*2, *nadh*4 (1 SNP each) and nadh65 (4 SNPs). However, the paragenetype was shorter in length due to 1 bp indel at $tRNA^{Ile}$ (pos: 3 854). A putative tandem repeat reported in the control region of other hexanchoids by Tanaka et al. (2013) is absent in the *C. anguineus* genetype. This fragment, between 407 to 441 bp in length, may be a reflection of methodological bias associated with long-ranged PCR amplifications and shotgun sequencing as used by Tanaka et al. (2013).

The phylogenomic tree inferred from the mitogenomic dataset supports the inclusion of *C. anguineus* within the Order Hexanchiformes (Figure 1) in a monophyletic group (Family Chlamydoselachidae), in agreement with traditional taxonomy (Ebert & Compagno 2009) and phylogenetic analysis (Tanaka et al. 2013). This arrangement also supports the paraphyletic character of the Order Squaliformes with respect to Hexanchiformes. The level of divergence within the hexanchoids is higher (80–82% pairwise identity) than expected for two sister families (Chlamydoselachidae and Hexanchidae) within the same Order. The low nucleotide diversity and high mitogenome identity of *C. anguineus* is consistent with a west Pacific population; however, population structure should be examined if and when samples become available. The relative homogeneity and stability of deep water ecosystems may enhance the ability of individuals to cross biogeographic barriers known to differentiate many marine elasmobranch populations (Dudgeon et al. 2012). A relatively similar seascape over the outer continental shelf and slope in the eastern Pacific has been suggested in relation to the distribution of *Hydrolagus melanophasma* (Bustamante et al. 2012). However, more information is required to assess this hypothesis. Further research should examine the genetype of *Chlamydoselachus africana*, the last species within the Order Hexanchiformes for which genetic/genomic information is lacking.

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Disclosure statement

The authors declare no conflict of interest. The authors alone are responsible for the content and writing of the article.

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