**Mitogenome Assembly and Annotation**

We employed a strategy that uses a reference bait to select mitochondrial nanopore reads, assemble them into a single circular contig and polish it twice. To obtain the mitochondrial sequences, all passed ONT reads (with mean quality of ≥10), were mapped with Minimap2 v 2.24 (Li, 2018) against the circular complete mitochondrial genome of another specimen of *Octopus vulgaris* with accession number NC\_006353.1 and length 15,774 bp (Yokobori *et al.*, 2004) with option: ‘-ax map-ont’. We retained all reads with mapping quality = 13 (relatively unique) and at least 5,000 sequence matches to the mitochondrial genome reference; these included 15 reads accounting for 181,644 bp (12x coverage) with mean length 12,112 bp.

All the retained ONT reads were assembled with Flye v2.9 (Kolmogorov *et al.*, 2019) using the options: ‘flye --scaffold -i 2 -g 15744 --nano-raw --min-overlap 7000’. Note that two polishing iterations were run with the ONT reads on the final assembly with ‘-i 2’ and the assembly produced 1 circular contig of length 15,651 bp. Afterwards, the circular mitogenome was rotated and oriented as follows. First, we annotated the contig using mitos-2.1.3 (Bernt *et al.*, 2013) with parameters *-c 5 --linear --best -r refseq81m*. Second, we use the coordinates in the results.bed file to orient the mitogenome, so it starts with the conventional tRNA Phenyl-Alanine (*trnF*) (Formenti *et al.*, 2021).

To evaluate the assembly accuracy, we first aligned the selected ONT reads back to the assembly with Minimap2 and visualized the alignment with IGV v2.14.1 (Robinson *et al.*, 2023). Finally, the xcOctVulg1 mitogenome was aligned against the mitogenome of other species using DNAdiff v1.3 from mummer package v3.23 (Kurtz *et al.*, 2004). These species included the mitogenomes of another specimen of *O. vulgaris* (NC\_006353.1), *O. sinensis* (NC\_052881.1), *O. bimaculoides* (NC\_029723.1) and *A. fangsiao* (AB240156.1)**.**

**Mitogenome Results**

The mitogenome assembly of the *Octopus vulgaris* specimen (xcOctVulg1) has length 15,651 bp and contains 13 protein-coding, 23 ncRNA, 2 rRNA and 21 tRNA genes. The read alignments show a high support for the nucleotide sequence of the mitogenome except for 16 positions (Supplementary Figure IGV\_ONTvsMT\_1.png). However, these positions show that the polishing has introduced the base with higher coverage in the reads (Supplementary Figure IGV\_ONTvsMT\_2.png). Therefore, the sequence shows a high accuracy. When we align the current *O. vulgaris* mitogenome against other cephalopods, the percentages of identity (See Supplemental Table\_Dnadiff\_Mitogenomes.xlsx) are consistent with the phylogeny (Figure 2). Surprisingly, the japanese specimen identified as *O. vulgaris* shows a higher identity to *O. sinensis* (99.85%) than to our *O. vulgaris* specimen (96.79%) Thus, supporting that this sequence (NC\_006353.1) actually corresponds to a different *O. sinensis* specimen.

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