The second complete mitogenome of *Nemipterus virgatus* to dissect control region structure and phylogenetic problem of the superfamily Sparioidea (Teleostei, Perciformes)

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The golden threadfin bream *Nemipterus virgatus* (Houttuyn 1782) is one of the most widespread and abundant species of the family Nemipteridae (Teleostei, Perciformes), ranging from southern Japan to northwestern Australia and Arafura Sea (Russell 1990, p. 60). It is a benthic fish that usually inhabits muddy or sandy bottoms of the continental shelf at depths to 200 m, supporting an important commercial marine fishery in the southern part of the East China Sea and the northern South China Sea (Liu et al. 2016, p. 206). The previous studies of *N. virgatus* mainly reported on resource and fishery biology and little is known about its genetic background. Although the complete mitogenome of *N. virgatus* has been sequenced by Wu and Li (2016), its control region structure and the relationships of Nemipteridae and associated families were not analysed. Here we sequenced another complete mitogenome of *N. virgatus* to dissect control region structure and the phylogenetic problem of the superfamily Sparioidea.

One specimen of *N. virgatus* was obtained by longline fishing in October 2010 from Guangdong Leizhou Rare Marine Life National Nature Reserve, Beibu Gulf, the South China Sea (20°38’52”N, 109°43’28”E). It was preserved at 95% ethanol and deposited in Guangdong Ocean University (20101023059). The complete mitogenome of *N. virgatus* (GenBank accession number: KU933270) amplified by designing 15 primer pairs, and it was 17,073 bp in length with a slight bias towards A + T (GC ratio is 42.93%). It contained 13 protein-coding genes, 22 tRNA genes, and 2 rRNA genes, and 2 non-coding regions, with a similar gene order to other nemipterids (Li et al. 2016; Wu and Li 2016; Wu et al. 2016, 2017; Zhai et al. 2019). By comparing with previous *N. virgatus* mitogenome (KR701906; Wu and Li 2016), only 9 single nucleotide polymorphisms (SNPs) were identified, indicating the low level of genetic diversity in the species. Among them, one SNP was detected in each of 12SrRNA, 16SrRNA, ATPase6 and ND4 genes, but four SNPs were found in ND5 gene, suggesting that ND5 gene has higher variation than other mitochondrial protein-coding genes.

Four domains the termination associated sequence domain (TAS), the central conserved domain (CCD), the conserved sequence block domain (CSB) and tandem repeat sequence domain (TTD), were identified in the control region of *N. virgatus*. According to Guo et al. (2003) report, one extended terminal associated sequence (ETAS: TACATTACATGTA), were found in the TAS of *N. virgatus*. All the six conserved sequences of CCD, namely, CSB-F, CSB-E, CSB-D, CSB-C, CSB-B, and CSB-A, can be identified in *N. virgatus*. The first three are easily identified according to their general sequences described by Lee et al. (1995), while the latter three are recognised according to their core sequences: GCATAAGTT, ATGTA, and TTTTTAAACCA, respectively. The CSB1 (ATAATTAGAA TTCATGAGCATAA) in *N. virgatus* can be determined by its most conserved partial sequence among fishes, while the CSB2 and CSB3 were easily identified by containing two series of c-sequences at TA interval and riching in A and C characteristics, respectively. Nine tandem repeat sequences (TRS),
each of 28 bp in length, were detected in the TTD, which were three more than those previously reported by Wu and Li (2016). Such genetic variation supports that the control region could be developed molecular markers for studying the intraspecific micro-evolutionary of \textit{N. virgatus}.

To test the hypothesis that four families Nemipteridae, Sparidae, Centracanthidae, and Lethrinidae are classified as a monophyletic group of superfamily Sparoidea (Johnson 1980; Russell 1990), a total of 32 complete mitogenome sequences of the Percoidei were used for constructing bootstrapped neighbor-joining (NJ) phylogenetic tree using MEGA6.06 programme (Tamura et al. 2013). In NJ tree (Figure 1), six species of \textit{Nemipterus} and \textit{Scolopsis} were grouped monophyly of family Nemipteridae at the basal position of Percoidei, while the three associated families (Sparidae, Centracanthidae, Lethrinidae) were gathered into another clade with other 4 families. Obviously, this study does not support the hypothesis that the four families should be classified as a superfamily Sparoidea, although they have morphological similarities.

**Disclosure statement**

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