

# The complete mitochondrial genome of *Amphioctopus marginatus* (Cephalopoda: Octopodidae) and the exploration for the optimal DNA barcoding in Octopodidae

Yan Tang<sup>1,2</sup> · Xiaodong Zheng<sup>1,2</sup> · Yuanyuan Ma<sup>1,2</sup> · Rubin Cheng<sup>3</sup> · Qi Li<sup>1</sup>

Received: 2 May 2017 / Accepted: 23 May 2017  
© Springer Science+Business Media Dordrecht 2017

**Abstract** In this study, the complete mitochondrial genome of *Amphioctopus marginatus* (Cephalopoda: Octopodidae) was sequenced. The length of *A. marginatus* was 15,719 base pairs (bp) and the composition of genome is A (41.74%), T (33.53%), C (16.77%), and G (7.96%). The whole mitogenome was composed of 13 protein-coding genes (PCGs), 2 ribosomal RNA genes, 22 transfer RNA genes, and a major non-coding region. The overall A+T content of *A. marginatus* showed remarkable similarity to other Octopodidae species reported before, with the characteristic of extremely richer than G+C. The gene order of the protein-coding genes was identical to other octopodids, but the tRNA genes were rearranged, making the gene order unique. The phylogenetic analysis showed relationships between *A. marginatus* and *Amphioctopus aegina* is close. The new mtDNA data enriched the database, which was beneficial to refine the phylogenetic relationships within Octopodidae further. ND5 gene could become an optimal DNA barcoding in taxon identification of the family.

**Keywords** *Amphioctopus marginatus* · Mitochondrial genome · DNA barcoding · Octopodidae

**Electronic supplementary material** The online version of this article (doi:10.1007/s12686-017-0777-2) contains supplementary material, which is available to authorized users.

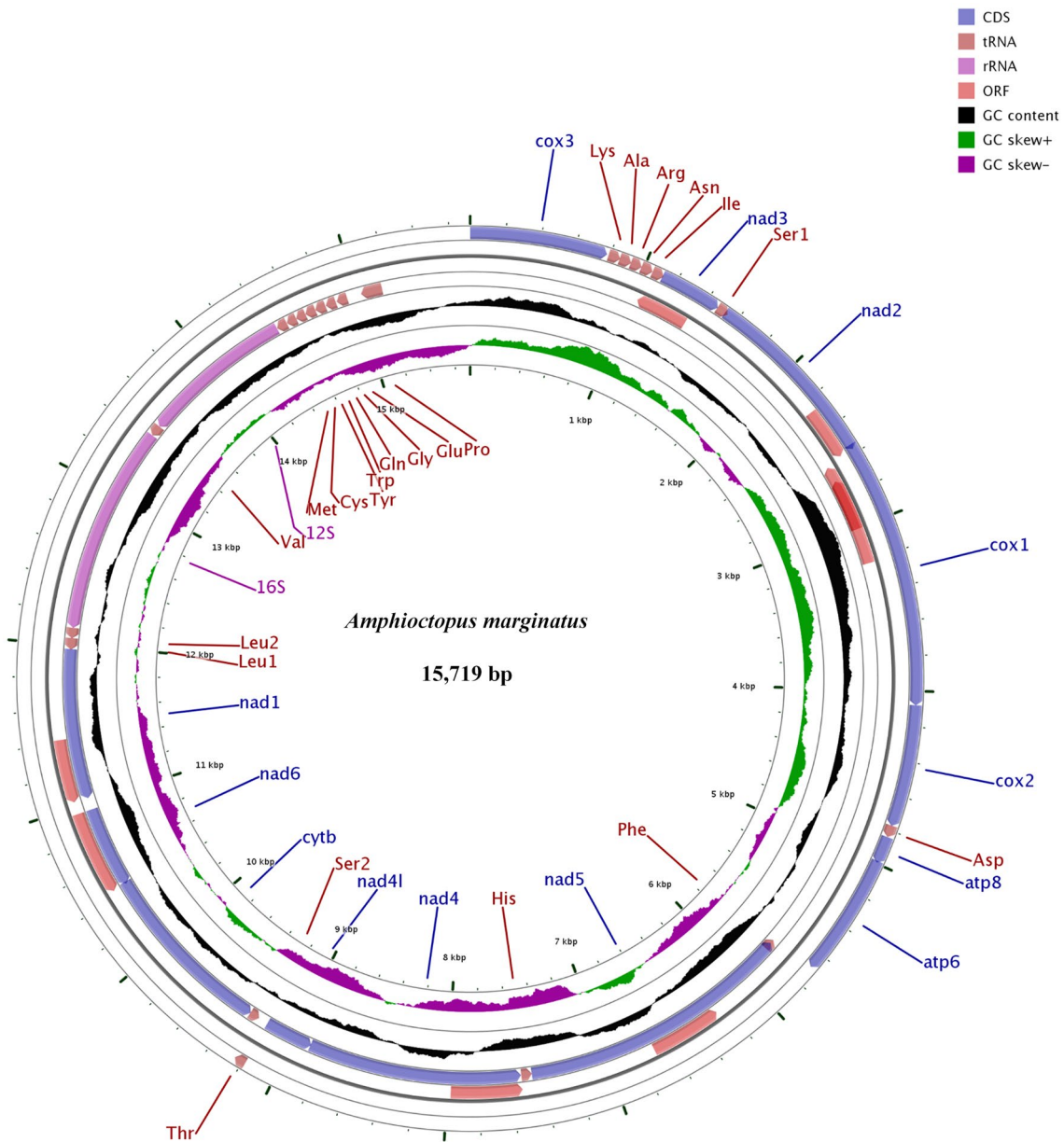
✉ Xiaodong Zheng  
xdzheng@ouc.edu.cn

- <sup>1</sup> Laboratory of Shellfish Genetics and Breeding, Fisheries College, Ocean University of China, Qingdao, China
- <sup>2</sup> Institute of Evolution & Marine Biodiversity, Ocean University of China, Qingdao, China
- <sup>3</sup> College of Pharmaceutical Science, Zhejiang Chinese Medical University, Hangzhou, China

*Amphioctopus marginatus* (Taki 1964) (Cephalopoda: Octopodidae) is a moderate sized octopus with a robust body. It is also known as the coconut octopus, frequently using coconut shells or seashells for shelter. *A. marginatus* generally lives in shallow waters on areas of sand, mud and rubble, and is found in tropical continental coastal waters of Indian and western Pacific Oceans (Norman 2000; Norman et al. 2013). *A. marginatus* is an important fishery species, due to its wide distributional range and high protein content. Recently, the natural resources diminished gradually with the multiple size of catch. Complete mitochondrial genomes provide a large set of genes when coded as amino acids, which might be suitable to clarify the relationships between some groups (Allcock et al. 2011). It is therefore useful to obtain the complete mitochondrial DNA of this species and to study its phylogenetic position in Octopodidae.

The sample was collected from Haikou Fishery Market, Hainan Province, China. Total genomic DNA of *A. marginatus* was extracted with the E.Z.N.A.<sup>TM</sup> Mollusk DNA Kit (OMEGA Bio-Tek Company, Norcross, GA). Partial primers were referenced by *Octopus conispadiceus* (Ma et al. 2014). Others were designed by those short sequences.

The complete mitochondrial DNA of *A. marginatus* (Genbank Assn. KY646153) was successfully sequenced, with the 15,719 bp long sequence and 13 protein-coding genes, 2 rRNA genes, 22 tRNA genes and non-coding regions (Fig. 1, Table 1). The content of A+T was 75.27%. The overall base composition of H strand was 41.74% A, 33.53% T, 7.96% G and 16.77% C. Seven of the thirteen proteins encoded by the H-strand. In this study, the most frequently used amino acids were Ile (12.71%) in *A. marginatus*, followed by Leu (11.49%), Ter (8.82%), Asn (8.59%), and Lys (8.44%). And we calculated the patterns of codon usage, AAA (lysine) as the most frequently used



**Fig. 1** Gene map of the mitochondrial genome of *Amphioctopus marginatus*. All the protein-coding genes, tRNA genes and rRNA genes are marked

codon was used 386 times for protein synthesis. There were 13 typical protein-coding genes (PCGs) in the mitogenome of *A. marginatus* in total (Table 1). Seven of the thirteen proteins were encoded by the H-strand, while the other six proteins are encoded by the L-strand (Fig. 1). The mitogenome of *A. marginatus* encoded 3757 amino acids with the exception of stop codons. Among all codon families, Ile, Leu (UUR), Phe, and Val occupied a predominant position. In addition, there were four nucleotide overlaps between adjacent PCGs, they were ND2 and COI, ATP8 and ATP6, ND4 and ND4L, Cob and ND6, respectively. There were

22 tRNA genes in the mt genome of *A. marginatus*, ranging from 64 to 73 bp (Table 1). Seven of twenty-two tRNAs are encoded by the H strand and 15 by the L-strand. And the GC content of the tRNA genes ranges from 12.3% to 29.4%. Notably, among the mt genome, trnP in *A. marginatus* was found to be at a unique position, which made the gene arrangement differ from other Octopodidae species. By comparison with all sizes of rrnL and rrnS genes among ten species in this study, the lengths of *A. marginatus* were similar to those of *Amphioctopus aegina* (Zhang et al. 2015), with the length of 1303 and 961 bp. These results

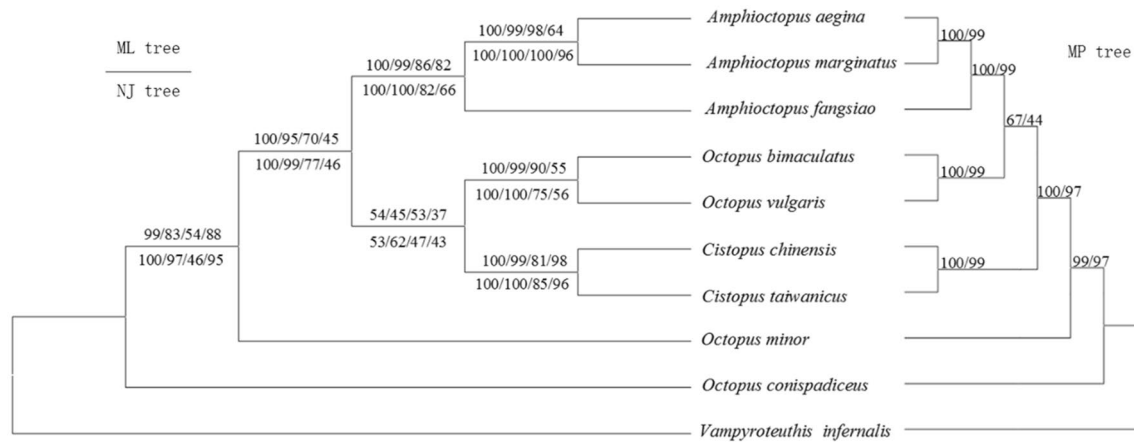
also proved the accuracy of the close relationship between *A. marginatus* and *A. aegina* from the phylogenetic trees. The A+T contents of the *rrnL* and *rrnS* for *A. marginatus* were 78.21 and 78.40%, respectively. The length of NCR in *A. marginatus* was 556 nucleotides, which was the shortest in ten species. And we detected a microsatellite-like (AT)<sub>11</sub> element at position of 15,689–15,710 in the NCR of *A. marginatus*.

In order to analyze the phylogenetic relationships among octopodids and search after the homogeneity between

mitochondrial DNA and DNA barcoding in the application of phylogenetic analysis. We constructed phylogenetic trees by maximum-likelihood, neighbor-joining and maximum-parsimony method (Tamura et al. 2013) based on the 13 concatenated proteins genes and each single mitochondrial gene potential to be barcoding, respectively (Fig. 2). On the whole, for the 13 concatenated proteins genes, the topological structures of ML tree and NJ tree were identical, with slight difference from MP tree. From the three kinds of trees, *O. conispadiceus* and *Octopus minor* were separated

**Table 1** Organization of the mitochondrial genome of *Amphioctopus marginatus*

Gene	Position	Size		Codon		Strand	Inter-genic region
		Nucleotides (bp)	Amino acids	Start	Stop		
<i>cox3</i>	1–780	780	259	ATG	TAA	+	556
<i>trnK (ttt)</i>	790–857	68				+	9
<i>trnA (tgc)</i>	855–923	69				+	–3
<i>trnR (tcg)</i>	922–986	65				+	–2
<i>trnN (ggt)</i>	986–1053	68				+	–1
<i>trnI (gat)</i>	1054–1120	67				+	0
<i>nad3</i>	1115–1471	357	118	ATA	TAA	+	–6
<i>trnS1(gct)</i>	1470–1538	69				+	–2
<i>nad2</i>	1521–2576	1056	351	ATA	TAA	+	–18
<i>cox1</i>	2548–4080	1533	510	ATG	TAA	+	–29
<i>cox2</i>	4080–4772	693	230	ATA	TAA	+	–1
<i>trnD (gtc)</i>	4771–4839	69				+	–2
<i>atp8</i>	4840–4995	156	51	ATG	TAA	+	0
<i>atp6</i>	4985–5689	705	234	ATA	TAG	+	–11
<i>trnF (gaa)</i>	5713–5782	70				–	23
<i>nad5</i>	5738–7474	1737	578	ATG	TAA	–	–45
<i>trnH (gtg)</i>	7474–7540	67				–	–1
<i>nad4</i>	7543–8886	1344	447	ATA	TAG	–	2
<i>nad4l</i>	8883–9188	306	101	ATA	TAA	–	–4
<i>trnT (tgt)</i>	9183–9248	66				+	–6
<i>trnS2(tga)</i>	9250–9313	64				–	1
<i>cob</i>	9312–10,457	1146	381	ATA	TAA	–	–2
<i>nad6</i>	10,444–10,956	513	170	ATG	TAG	–	–14
<i>nad1</i>	11,031–11,975	945	314	ATA	TAG	–	74
<i>trnL2(taa)</i>	11,972–12,044	73				–	–4
<i>trnL1(tag)</i>	12,042–12,109	68				–	–3
<i>rrnL</i>	12,109–13,425	1317				–	–1
<i>trnV (tac)</i>	13,426–13,496	71				–	0
<i>rrnS</i>	13,497–14,459	963				–	0
<i>trnM (cat)</i>	14,461–14,528	68				–	1
<i>trnC (gca)</i>	14,530–14,594	65				–	1
<i>trnY (gta)</i>	14,595–14,658	64				–	0
<i>trnW (tca)</i>	14,659–14,725	67				–	0
<i>trnQ (ttg)</i>	14,726–14,793	68				–	0
<i>trnG (tcc)</i>	14,797–14,862	66				–	3
<i>trnE (ttc)</i>	14,869–14,938	70				–	6
<i>trnP (tgg)</i>	15,092–15,163	72				–	153



**Fig. 2** Phylogenetic trees derived from Maximum Likelihood (ML), Neighbor Joining (NJ) and Maximum Parsimony (MP) based on partitioned nucleotide sequences of 13 mitochondrial protein-coding genes. For ML and NJ tree, each number on one side is performed by

mitogenome, ND5, ATP6 and Cob respectively. For MP tree, the first number at each node is the bootstrap probability based on 13 mitochondrial protein-coding genes and the second number is the bootstrap probability based on ND5 gene

at the earliest, the other octopodids were further divided into three small subclusters. Each small subcluster corresponds to taxonomical tribe, genus or subgenus (Fig. 2). The first subcluster included three *Amphioctopus* species, we can clearly find that *A. marginatus* is closest to *A. aegina*, with *Amphioctopus fangshiao* being the sister taxon. The second subcluster consisted of *Octopus bimaculatus* and *Octopus vulgaris*, and two *Cistopus* species formed into the third subcluster. The latter two were sister groups from ML tree and NJ tree, however, the MP tree indicated that the former two subclusters have closer relationships.

Compared with the topological structures of each of 13 PCGs from three trees, results revealed by 3 of 13 protein genes (Cob, ND5 and ATP6) were consistent. It was worth noting that these trees based on ND5 gene were held with strong support. We concluded that the mitochondrial ND5 gene encoding NADH dehydrogenase subunit 5 had the potential to be DNA barcodings for Octopodidae. And the results generally agreed with the traditional morphological classification.

**Acknowledgements** This study was supported by research grants from National Natural Science Foundation of China (Nos. 31172058;

31672257). We would like to thank Prof. Chung-Cheng Lu of Museum Victoria for critical revisions on the manuscript.

## References

- Allcock AL, Cooke IR, Strugnell JM (2011) What can the mitochondrial genome reveal about higher-level phylogeny of the molluscan class cephalopoda? *Zool J Linn Soc* 161:573–586
- Ma YY, Zheng XD, Cheng RB, Qi L (2014) The complete mitochondrial genome of *Octopus conispadiceus* (Sasaki, 1917) (Cephalopoda: Octopodidae). *Mitochondr DNA* 27:1058
- Norman MD (2000) Cephalopods: a world guide—Octopuses, Argonauts, Cuttlefish, Squid, Nautilus. Conchbooks, Hackenheim, pp 320
- Norman MD, Finn JK, Hochberg FG (2013) Family Octopodidae. In: Jereb P, Roper CFE, Norman MD, Finn JK (eds) Cephalopods of the world. An annotated and illustrated catalogue of cephalopod species know to date: Octopods and Vampire Squids. FAO species catalogue for fishery purposes. FAO, Rome, pp 75–76
- Tamura K, Stecher G, Peterson D, Filipski A, Kumar S (2013) MEGA6: molecular evolutionary genetics analysis version 6.0. *Mol Biol Evol* 30:2725–2729
- Zhang XY, Zheng XD, Ma YY, Li Q (2015) Complete mitochondrial genome and phylogenetic relationship analyses of *Amphioctopus aegina* (Gray, 1849) (Cephalopoda: Octopodidae). *Mitochondr DNA* 6:1