**ABSTRACT**

Picasso clownfish belong to the subfamily Amphiprioninae and are considered a variant of the genus *Amphiprion*. In this study, we first sequenced the complete mitochondrial genome of the Picasso clownfish by Illumina next-generation sequencing technology. The length of the whole mitogenome is 16,727 bp long, with a gene arrangement and composition similar to those of two other *Amphiprion* species (*Amphiprion ocellaris* and *Amphiprion percula*). The topological structure of the phylogenetic tree shows that the Picasso clownfish is more closely related to *A. percula* than it is to *A. ocellaris*, suggesting that the Picasso clownfish may be a variant of *A. percula*.

The subfamily Amphiprioninae (family Pomacentridae), which includes approximately 30 species (Allen 1991; Allen et al. 2008, 2010), is well known as the anemonefishes because these species form mutualistic symbiotic relationships with tropical sea anemones (Elliott et al. 1999). The Picasso clownfish, an anemonefish variety named for its irregular body stripes, still lacks a Latin name. In the present study, we determined the complete mitochondrial genome of the Picasso clownfish using next-generation Illumina sequencing technology and analyzed the phylogenetic relationships within the Amphiprioninae.

The Picasso clownfish were collected from Fangcun aquariums in Guangzhou (23°6′15″N, 113°26′29″E), Guangdong Province, China. The specimen was deposited at the Culture Collection of Fish at Fisheries Research Institute of Fujian, Sample Number:BJS17928. A 30–40 mg fin clip was preserved in 95% ethanol immediately and stored at −20°C until DNA extraction. Total genomic DNA was extracted using the DNeasy Tissue Kit (Qiagen, Germany) according to the manufacturer’s protocol. Adapter-modified DNA fragments were PCR-amplified using PE PCR primers. Libraries were sequenced using an Illumina HiSeq4000 at BGI-Shanghai, China, with 6 Gb of 2 × 150-bp paired-ends, which was constructed with two indexes using the Illumina TruSeq® DNA PCR-Free HT Kit. Finally, the complete mitochondrial genome sequence was submitted to GenBank with accession number SRR6363367.

The complete mitogenome (16,727 bp) of the Picasso clownfish, which was longer than those of the other two *Amphiprion* species (*Amphiprion ocellaris* and *Amphiprion percula*), contained 13 PCGs, 22 tRNA genes, two rRNA genes, and two non-coding regions. From the complete mitochondrial genome, the gene arrangement and translation direction were basically identical to those in *A. percula* and *A. ocellaris*. The BI and NJ trees based on 2 rRNAs and 13 PCGs showed similar topologies and branch support within Amphiprioninae, and the support values were robust with 90% bootstrap values (Figure 1). The anemonefishes in Amphiprioninae are separated into two clades. Clade I includes *Amphiprion bicinctus*, *Amphiprion clarkii*, *Amphiprion frenatus*, *Amphiprion polymnus*, *Amphiprion perideraion*, *Amphiprion sebae*, *Amphiprion ephippium* and *Amphiprion akallopisos* and corresponds well with a previous study based on morphological traits (Allen 1972, 1975). The support values for these clades were very high and stable for both analyses. In Clade II, we found that *Premnas biaculeatus*, *Aocellari*, *A. percula* and the Picasso clownfish clustered in one branch of the phylogenetic tree with high support values and that the Picasso clownfish was more closely related to *A. percula* than it was to *A. ocellaris*. Our study corroborates the relationship between *A. percula* and the Picasso clownfish, namely, we suggesting that the Picasso clownfish may be a variant of *A. percula*.
Disclosure statement

No potential conflict of interest was reported by the authors.

Authors’s contributions

HE Li-bin conceived and designed the study; HE Li-bin and WU Shui-qing collected the data; HE Li-bin and LUO Hui-yu performed the RNA-Seq analysis. HE Li-bin and ZHENG Le-yun wrote the manuscript. All authors read, edited, and approved the final manuscript for submission.

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Data availability statement

The data that support the findings of this study are openly available in GenBank database at https://submit.ncbi.nlm.nih.gov/subs/sra/SUB3297187/overview, reference number [SRR6363367].

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