Matching an Old Marine Paradigm: Limitless Connectivity in a Deep-Water Fish over a Large Distance

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Supplementary Material

Text S1

Molecular methods

DNA isolation

Before the DNA extraction, muscle tissues were treated adding 600 µL of ASL buffer (Qiagen) and about 50-100 µL of zirconium beads (Zirconia/Silica Beads, 0.1 mm). Samples were vortexed using the TissueLyser (Qiagen) for 1 min at 25 Hz followed by 1 min at 25 Hz and centrifuged at 14,000 g for 2 min. A total of 200 µL of supernatant was transferred in the S-block well for genomic DNA extraction. Total genomic DNA was obtained with the cador® Qiacube pathogen kit for QIAcube® HT System (Qiagen) and its quality was visualised on a 1.5% agarose gel electrophoresis. Failures in DNA extractions were followed by further attempts, using an improved protocol, in which approximately 0.02 g of tissue were homogenised in 100 µL of lysis buffer and the DNA IQ™ System-Database Protocol (Promega) was applied.

Nuclear markers and amplification conditions

A total of 22 microsatellite loci previously isolated and characterised from *Pagellus bogaraveo* [44,45] were amplified on the collected samples. Furthermore, we attempted the

cross-amplification of seven loci isolated and characterised from *Pagellus erythrinus* (Linnaeus, 1758) by Ramšak et al. [46]. In total, six different multilocus reactions were designed based on loci annealing temperature and expected amplicon size (Table S2). All multiplex PCR amplifications were performed using the Platinum® Multiplex PCR Master Mix (Thermofisher Scientific) in a total volume of 15 μL, with 7.5 μL of Multiplex master mix, 1.5 μL of enhancer and 1.1 μL of DNA. The amplification conditions included an initial denaturation at 95°C for 2 min, a denaturation at 95°C for 30 secs, an elongation at 60-62°C (depending on the mix) for 30 secs, a second denaturation at 95°C for 30 secs, an elongation at 68°C for 45 secs for a total of 32 cycles and a final extension step at 68°C for 30 secs. The separation of PCR products of individual samples was carried out on a 2.5% agarose gel electrophoresis. The fluorescently labelled DNA fragments carrying NED, FAM, PET, VIC and LIZ fluorochromes, were mixed with the GeneScan 500 LIZ (Applied Biosystems®) internal size standard and separated using the Applied Biosystems® 3130xl Genetic Analyzer. Technical replicates were included to estimate repeatability and error rates resulting from PCR amplification (assessing congruence of results between single locus and multiplex approaches) and fragment analysis. Replicates were issued from the same DNA source but were processed independently.

References

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