DNA extraction, amplification, and sequencing of Ophiothrix (Echinodermata: Ophiuroidea)

PLOS One

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dx.doi.org/10.17504/protocols.io.tz7ep9n

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ABSTRACT

DNA extraction, amplification, and sequencing, particularly for the brittel star *Ophiothrix.*

EXTERNAL LINK

https://doi.org/10.1371/journal.pone.0210331

THIS PROTOCOL ACCOMPANIES THE FOLLOWING PUBLICATION

Alitto RAdS, Amaral ACZ, Oliveira LDd, Serrano H, Seger KR, Guilherme PDB, Domenico MD, Christensen AB, Lourenço LB, Tavares M, Borges M (2019) Atlantic West *Ophiothrix* spp. in the scope of integrative taxonomy: Confirming the existence of *Ophiothrix trindadensis* Tommasi, 1970. PLoS ONE 14(1): e0210331. doi: 10.1371/journal.pone.0210331

PROTOCOL STATUS

Working

DNA extraction

1 Samples of tube feet from the arms or gonads were soaked in two changes of Tris-EDTA (TE) buffer for 30 minutes each. The samples were then macerated in 500 µl of TE buffer with a pestle. A volume of 300 µl of a 10 % Chelex 100 solution (BioRad) was added. The samples were then incubated at 55°C for 60 minutes, boiled for 8 minutes, cooled to room temperature, vortexed for 20 seconds followed by 60 seconds of centrifugation at 14,000 x*g*. The supernatant was decanted and kept refrigerated until use in polymerase chain reactions (PCR)

Amplification

2 A fragment of the mitochondrial 16S gene was PCR-amplified using the forward primer 16S Sofi F (5′-CAGTACTCTGACTGTGCAA-3′) and the reverse primer 16S Sofi R (5′-GGAAACTATGATCCAACATC-3′). A fragment of the mitochondrial cytochrome oxidase subunit 1 (COI) gene was amplified using the forward primer COIf-L (5′-CCTGCAGGAGGGGGAGAYCC-3′) and the reverse primer COIa-H (5′-TGTATAGGCGTCTGGATAGTC-3′).

PCR reactions were performed using PuReTaq Ready-To-Go™ PCR Beads for 25 µl reactions (GE Heathcare), complemented with 0.5 to 1 µl of 25mM MgCl2. Alternatively, PCR reactions were composed of 10 mM Tris-HCl, pH 8/1 mM KCl buffer, 1.5 mM MgCl2, and 200 µM each dNTP (Invitrogen). In both cases, 0.4 µM of each primer was used and template DNA concentration (ranging from 25.7 to 917.9 ng/µl) was tested.

Thermal cycling consisted of a single step at 94°C for 5 minutes, which was followed by 39 cycles (denaturation at 94°C for 45 seconds, annealing at 45.7°C to 51.3°C for 45 seconds, and extension at 72°C for 60 seconds) and a final extension at 72°C for 3 minutes on a thermal cycler (Eppendorf Mastercycler®).

Purification

3 PCR products were separated from excess primers and dNTP using a purification kit (Promega). Purified product was then used as a template for DNA sequencing.

Sequence

4 Reactions were done with the BigDye Terminator (Applied Biosystems) *Serviço de Sequenciamento de DNA – SSDNA IQUSP* with the same primers used in the amplification reaction.