# MOLECULAR ANALYSIS

New specimens of *C. dentatus* were collected from Lobito (Angola), Accra (Ghana) and Saint Louis (Senegal). Following collection, the specimens were fixed and preserved in 95% ethanol. For DNA extraction we used the genomic DNA isolation kit (Macherey-Nagel GmbH & Co. Germany), following the manufacturer protocol.

A segment of the mitochondrial cytochrome c oxidase subunit I (COI) was amplified using the universal barcode primers LCO1490 and HCO2198 (Folmer et al. 1994). The reaction mix, in a total volume of 25 µl contained: 10 ng of template DNA, 12.5 µl PCR mix (2X PCR HS Taq Mix Red; PCR Biosystem, London, UK) and 0.4 µM of each primer. The PCR profile was: Initial denaturation at 95°C for 1 min followed by 40 cycles of 15 sec at 95°C, 15 sec at 50°C, and 20 sec at 72°C with a final extension step of 3 min at 72°C.

A segment of the mitochondrial control region (D-loop) was amplified, using consensus primers designed by Y.T. (based on sequences from species of the Balanomorpha order):

CH-CR-F: CACCTTTGCAGCTTCAATGCAAAACTCTA

CH-CR-R: CGTATAACCGCGGCTGCTGGCACGCTATTTTCC

The reaction mix in a total volume of 25 µl was similar to that of the COI. The PCR profile was: Initial denaturation at 95°C for 3 min followed by 32 cycles of 30 sec at 95°C, 30 sec at 58°C, and 30 sec at 72°C with a final extension step of 3 min at 72°C.

A segment of the nuclear eukaryotic elongation factor 1α was amplified using the nEF1 F and nEF1 R primers (Wares et al. 2009). The reaction mix, in a total volume of 20 µl contained: 10 ng of template DNA, 10 µl PCR mix (2X PCR HS Taq Mix Red; PCR Biosystem, London, UK) and 0.4 µM of each primer. The PCR profile was: Initial denaturation at 95°C for 1 min followed by 32 cycles of 30 sec at 95°C, 30 sec at 57°C, and 30 sec at 72°C with a final extension step of 3 min at 72°C.

A segment of the nuclear Na-K-ATPase (NaKA) was amplified using the nNAKA F and nNAKA R primers (Wares et al. 2009). The reaction mix, in a total volume of 20 µl contained: 10 ng of template DNA, 10 µl PCR mix (2X PCR HS Taq Mix Red; PCR Biosystem, London, UK) and 0.4 µM of each primer. The PCR profile was: Initial denaturation at 95°C for 1 min followed by 32 cycles of 30 sec at 95°C, 30 sec at 57°C, and 30 sec at 72°C with a final extension step of 3 min at 72°C.

PCR products were visualized on 1.5% agarose gels and sequenced in both directions using the two PCR primer sets on an ABI 377 DNA Sequencer (Applied Biosystems, Foster City, CA, USA), following the manufacturer's instructions.

## REFERENCES

Folmer O, Black M, Hoeh W, Lutz R, Vrijenhoek R (1994). DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. Molecular marine biology and biotechnology 3:294–299.

Wares JP, Pankey MS, Pitombo F, Daglio LG, Achituv Y (2009). A "shallow phylogeny" of shallow barnacles (*Chthamalus*). PLoS ONE 4(5):e5567. https://doi.org/10.1371/journal.pone.0005567