# Genetic Stock identification of The African Hind *Cephalopholis taeniops* in the Cape Verde archipelago

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## INTRODUCTION

The stock concept is fundamental as a baseline information in fisheries management, whose applicable definition depends on the specific issues that want to be resolved. One of the most accepted definition is referred as an intraspecific group of randomly mating individuals with spatial and temporal integrity (Ihssen et al. 1981). Nevertheless, the genetic stock concept is based on a reproductively isolated unit, which is genetically different from others stocks (Ovenden 1990). Those two definitions share an important concept: random mating unit. Thereby, population genetic analyses allow detecting the spatial scale at which evolutionary processes explain significant departures of admixture to test biogeographic hypothesis that elucidate the extent of genetic drift effect that enhance reproductive isolation. The African Hind *Cephalopholis taeniops* is mainly distributed at the Eastern Atlantic, from Marroc to Angola, including Cape Verd, São Tomé and Principe islands (Pastor 2002, fishbase 2014, but see Brito et al. 2011 and Guidetti et al. 2010). This species is one of the most important commercial demersal species in the Cape Verde archipelago and the consequences of population reductions due to an intensive fishing effort and the unknown geographic distribution of the stocks (Pastor 2002) are the main concerns towards fishery's sustainability. Hence, the aim of the present study was to estimate island genetic diversity and to identify the genetic stocks across the archipelago to delimit discrete genetic units to contribute to an effective management in the region.

## GENETIC DIVERSITY RESULTS

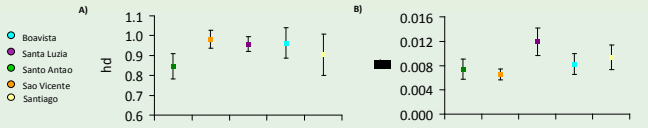


Figure 2. Island genetic diversity parameters of *Cephalopholis taeniops* at the Cape Verd archipelago. Haplotype diversity (a) and nucleotide diversity (b) and its standard deviations for Region Control of DNAmt were estimated.

Table I. Island genetic diversity parameters of *Cephalopholis taeniops* in the Cape Verd islands

| | CONTROL REGION | | | | COI | | | |
|---|---|---|---|---|---|---|---|---|
| **Islands** | **n** | **h** | $h_d \pm SD$ | $\pi \pm SD$ | **n** | **h** | $h_d \pm SD$ | $\pi \pm SD$ |
| **Santo Antão** | 17 | 7 | 0.846 ± 0.064 | 0.00741 ± 0.00164 | 20 | 5 | 0.742 ± 0.073 | 0.00148 ± 0.00025 |
| **São Vicente** | 11 | 10 | 0.982 ± 0.046 | 0.00658 ± 0.00090 | 14 | 4 | 0.495 ± 0.151 | 0.00084 ± 0.00030 |
| **Santa Luzia** | 19 | 15 | 0.959 ± 0.036 | 0.01194 ± 0.00225 | 12 | 4 | 0.561 ± 0.154 | 0.00148 ± 0.00063 |
| **Boavista** | 8 | 7 | 0.964 ± 0.077 | 0.00826 ± 0.00169 | 20 | 5 | 0.442 ± 0.133 | 0.00075 ± 0.00026 |
| **Santiago** | 7 | 5 | 0.905 ± 0.103 | 0.00934 ± 0.00199 | 0 | 0 | | |
| **Cape Verd Islands** | 62 | 34 | 0.927 ± 0.026 | 0.01088 ± 0.00116 | 66 | 9 | 0.572 ± 0.066 | 0.00113 ± 0.00019 |

A total of 9 haplotype sequences of 654 bp of **COI** were detected. The maximum and the minimum haplotype diversity values were registered at Santo Antão and Boavista islands, respectively. The maximum and minimum nucleotide diversity values were obtained at Santa Luzia and Santo Antão and Boavista islands, respectively. No geographic pattern of *hd* was detected across the sampled islands. (Table I, Fig. 2).
A total of 34 haplotype sequences of 420 bp of the **control region** were detected. The maximum and the minimum haplotype diversity values were registered at São Vicente and Santa Luzia islands, respectively. The maximum and minimum nucleotide diversity values were obtained at Santa Luzia and São Vicente islands, respectively. No geographic pattern of *hd* was detected across the sampled islands (Table I, Fig. 2).

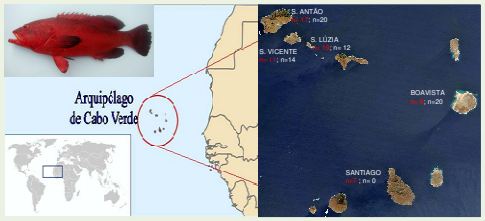


Figure 1. Study area and the location of the sampling secuences of *Cephalopholis taenipos* acorss the Cape Verd Islands. "n": sample size (red: CR; white: COI)

## METHODS

A total 62 samples of *Cephalopholis taeniops* were collected at 5 islands of the Cape Verd archipelago (Figure 1). After collection, samples were fixed in ethanol 96% and preserved at -20°C. DNA isolation was performed from a 30 mg of tissue following the E.Z.N.A. DNA Tissue kit (Omega Bio-Tek) protocol. The nucleotide sequences of the Cytochrome oxidase subunit I (COI) and the hypervariable Control Region (CR) of mitochondrial DNA were amplified through PCR. The COI was amplified using the forward Fish F2 and the reverse FishR2 primers (Ward et al. 2005). The COI PCR profile was: 95°C for 5 min, 36 cycles of 95°C for 40 s, 50°C for 50 s and 72°C for 60s, and a final extension of 72°C, 7 min. The CR was amplified using the L15998/CSBDH primers (Quinteiro 2010), and the PCR profile was: 95°C for 3 min, 36 cycles of 95°C for 40 s, 59°C for 50 s and 72°C for 50s, and a final extension of 72°C, 7 min. PCR products were purified using Exo-SAP-It (Affimetrix) protocol and fluorescently labelled nucleotide sequences were obtained using the BigDye 3.1 sequencing kit (Applied Biosystems). Finally, the extension products were purified using Byg Dye XTerminator sequencing kit (Applied Biosystems) and sequenced into an ABI3500 Genetic Analyzer (Applied Biosystems). The nuclotide sequences were analyzed and aligned using BioEdit 7.0.0 and ClustalX . Nucleotide ($\pi$) and haplotype ($h_d$) diversity were estimated using DnaSPv.5.10. In one hand, pairwise $F_{ST}$ values were estimated and tested, and in the other a hierarchical partitioning of genetic variation among and within islands was estimated by an AMOVA using the Arlequin 3.5 software. Then, a NJ distance-based unrooted tree was built using MEGA5. A Median-joining network of haplotypes was constructed using the Network v.4.6 software.

## GENETIC DIFFERENTIATION AND AMOVA RESULTS

Table II. Pairwise among islands $F_{ST}$ values DNAmt RC sequences of *Cephalopholis taeniops* in the Cape Verd archipelago.

| | Sta. Luzia | S. Vicente | Boavista | Santiago |
|---|---|---|---|---|
| S. Vicente | 0.0011 | | | |
| Boavista | -0.0149 | -0.0215 | | |
| Santiago | **0.0648*** | **0.0887*** | 0.0648 | |
| Sto. Antão | **0.0247*** | 0.0149 | 0.0122 | **0.0982**** |

* p < 0.05; ** p < 0.01

$F_{ST}$ values in Table II showed Boavista as the unique island with non significant values and sligth but significant genetic differentiation among those islands with bold $F_{ST}$ values. The AMOVA showed sligth but significant differentiation among island level analysis ($F_{ST}$ = 0.027, P < 0.05). Moreover, the 97% of the total variance of the nucleotide sequences data set was among the individuals within islands (Table III).

Table III. Hierarchical AMOVA of the DNAmt RC sequences of *Cephalopholis taeniops* in the Cape Verd islands.

| Source of variation | d.f. | SS | Variance components | % variation | Statistic | *P* -value |
|---|---|---|---|---|---|---|
| Among islands | 4 | 2.425 | 0.0128 | 2.74 | $F_{ST}$ = 0.027 | 0.041 |
| Within islands | 57 | 25.849 | 0.45349 | 97.26 | | |
| Total | 61 | 28.274 | 0.46629 | | | |

## MEDIAN-JOINING HAPLOTYPE NETWORK AND DISTANCE-BASED NEIGHBOUR-JOINING TREE RESULTS

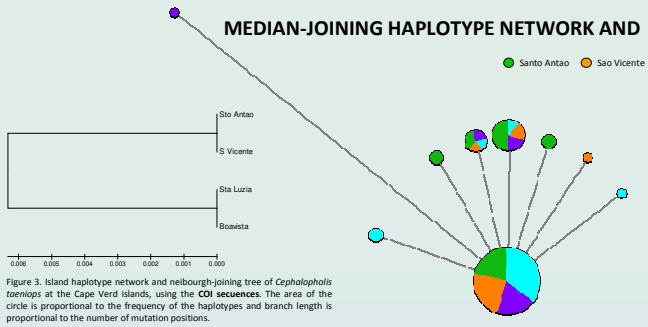


Figure 3. Island haplotype network and neibourgh-joining tree of *Cephalopholis taeniops* at the Cape Verd islands, using the **COI secuences**. The area of the circle is proportional to the frequency of the haplotypes and branch length is proportional to the number of mutation positions.

The **haplotype network of COI** showed a star pattern in which the highest haplotype frequency was detected in all sampled islands. The haplotypes with low frequency arose from this major haplotype through one single mutation with the exception of the most divergent haplotype which differs in 3 single mutations in Santa Luzia. Furthermore, two of those one single mutation derived haplotypes were detected at higher frequencies in all the islands (Fig 3). Unrooted $F_{ST}$ distance-based NJ tree showed two major divergent clusters of islands. The one containing Santo Antão and São Vicente neighbour islands and the other including Santa Luzia and Boavista. The typology of the tree showed a slight geographic pattern, almost among the most northwestern neighbour islands.

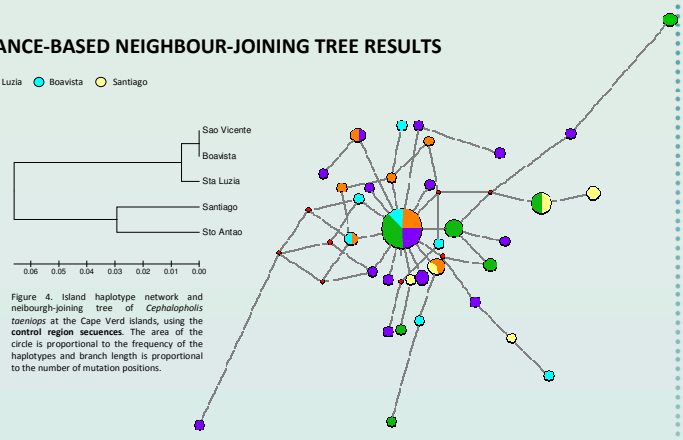


Figure 4. Island haplotype network and neibourgh-joining tree of *Cephalopholis taeniops* at the Cape Verd islands, using the **control region secuences**. The area of the circle is proportional to the frequency of the haplotypes and branch length is proportional to the number of mutation positions.

The haplotype network of CR showed more complex relationships among haplotypes due to the higher rate of evolution of this DNAmt region. In this case, the highest frequency haplotype was not detected Boavista Island and more than one single mutation characterized the derived haplotypes. Unrooted $F_{ST}$ distance-based NJ tree showed two major divergent clusters composed by: São Vicente, Boavista and Santa Luzia in one hand and the other by Santiago and Santo Antão (Fig. 4). As the length of the branches was directly related to the strength of genetic divergence among islands, the islands belonging to the first cluster were closer related than those ones of the second cluster. Finally, the typology of the tree did not show a geographic pattern.

## DISCUSION & CONCLUSION

The higher genetic diversity parameters of the DNAmt CR revealed the expected higher rate of evolution of this hypervariable region of the DNAmt at both island and archipelago scale. The low genetic differentiation of CR DNAmt among islands supported the non detection of neither genetic diversity nor NJ tree geographic pattern. Furthermore, the almost all proportion of genetic variation detected within islands and all of the above elucidated for CR DNAmt that gene flow was an important homogenizing evolutionary force which lead both within and among island evolution at the archipelago. Evenmore, the relationship among haplotypes of both COI and RC median joinning networks supported that island isolation was not enough to lead to a haplotype pattern at the archipelago. The latter could be explained as a result of an expected founder effect in oceanic islands, like Cape Verd, which were colonized by a small portion of the gene pool of the source continental populations. Finally, despite more sequences will be added to those analyzed here, these preliminary results suggested two complementary hypothesis that could elucidate the genetic stock/s of *Cephalopholis taeniops* at the Cape Verd. COI indicated one genetic stock in the area and the hypervariable but the more resolutive CR at the scale studied here pointed out that under archipelago level analysis, more than one genetic stock might be geographically delimited although it should be evaluated at finer scale and hence, more sequences need to be added to the analysis.

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