On the absence of genetic differentiation between morphotypes of the ballan wrasse *Labrus bergylta* (Labridae)

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Abstract

The ballan wrasse, *Labrus bergylta* (Labridae), is a protogynous hermaphrodite fish common in the north-eastern Atlantic from Norway to Morocco. It is a commercially important resource for local fisheries and is currently being used as cleaner fish to control sea lice in salmon

farms in northern Europe. Two distinct colour patterns have been recently reported in the literature: plain and spotted. These individuals follow strikingly different life history strategies raising the question of whether they represent one or two independent taxonomic units. Analyses of mitochondrial (18S, COI and control region) and nuclear (S7) markers revealed no genetic differences between these morphotypes. Alternative explanations for the origin and persistence of distinct morphotypes are discussed.

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Electronic supplementary material

The online version of this article (doi:10.1007/s00227-016-2860-8) contains supplementary material, which is available to authorized users.

Introduction

The ballan wrasse, *Labrus bergylta* Ascanius, 1767 (Labridae), is a protogynous hermaphrodite fish species common in rocky shores and kelp beds in the north-eastern Atlantic from Norway to Morocco, including the Macaronesian archipelagos of the Canaries, Madeira and Azores (Quignard and Pras 1986; Froese and Pauly 2012). It is a commercially important species to local fisheries in south-western Europe (Treasurer 1994; Villegas-Ríos et al. 2013a), and it is currently being used as a cleaner fish to control ectoparasites (Copepoda, Caligidae) in salmon farm facilities in northern Europe (Muncaster et al. 2010; Talbot et al. 2012). With the growing importance of the ballan wrasse as a cleaner fish, it became a target of intensive fishing in Norway. Additionally, due to the low abundance of individuals in their natural habitat, intensive culture of this labrid species is currently being developed (Skiftesvik et al. 2013). Although salmon farms use preferentially small specimens, the ballan wrasse is the largest labrid in the north-eastern Atlantic (Bañon et al. 2010), which may offer also new opportunities for larger hosts with larger ectoparasites.

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The systematic classification of the Labridae has attracted considerable attention due to the recent split of some taxa and the high intraspecific polymorphism, particularly in the genera *Labrus* and *Symphodus* (Almada et al. 2002; Hanel et al. 2002). Recently, Villegas-Ríos et al. (2013a, b) described the existence of two distinct colour patterns in *L. bergylta*: plain and spotted, with differences in many aspects of their life history strategy including the length–weight relationship, otolith and body growth, size and age structures, timing of sex change, reproductive investment and mortality rates. For example, spotted individuals show larger mean asymptotic sizes, lower mortality rates (Villegas-Ríos et al. 2013a) and lower reproductive investment compared to plain individuals (Villegas-Ríos et al. 2014). Both morphotypes are sympatric along the entire distribution area although the proportion and differentiation between them may change considerably among locations (pers. obs.). No microhabitat segregation or differences in behaviour have been reported so far (Villegas-Ríos 2013). Additionally, unlike most protogynous wrasses (Coulson et al. 2009; Alonso-Fernández et al. 2011), the distinct colour patterns within *L. bergylta* are not related to sexual dimorphism (Dipper and Pullin 1979; Villegas-Ríos et al. 2013a).

A fundamental question arises from these considerations: are these two morphotypes a single or two independent taxonomic entities? The present study is an attempt to answer this question using multiple genetic markers. This genetic assessment must precede the analysis of the population structure along its entire distribution area, and it is urgent due to its implication over future effects of individual translocations for aquaculture purposes and stock assessment studies.

Materials and methods

Specimen collection

A total of 181 samples were collected in four geographic locations throughout the distribution area of the species in the Atlantic north-east: Norway ($n = 57$), North Spain ($n = 89$), Continental Portugal ($n = 32$) and the Azores ($n = 3$; Table 1). Samples from Norway were collected with pots by local fishermen. Samples from Portugal (Continental and Azores) and Spain were collected by local fisherman or spear-fished by amateur divers. Individuals were classified as spotted or plain according to the

criteria described by Villegas-Ríos et al. (2013a). Caudal fin clips were collected, immediately immersed in 96 % ethanol and stored at 4 °C until use. Voucher tissues were deposited in ISPA (MARE—Marine and Environmental Sciences Centre) collections.

Table 1

Number of samples, morphotypes and sampling locations of *Labrus bergylta*, for each DNA marker

Morphotype	Region	Coordinates	Control region	COI	18S	S7
Plain	Norway	58°25'N/08°45'E	6	22	22	1
	North Spain	43°21'N/08°39'W	14	33	33	8
	Cont. Portugal	38°28'N/08°58'W	22	30	30	18
	Azores	38°33'N/28°46'W	1	1	1	1
Spotted	Norway	58°25'N/08°45'E	10	19	19	5
	North Spain	43°21'N/08°39'W	10	32	32	7
	Cont. Portugal	38°28'N/08°58'W	2	2	2	2
	Azores	38°33'N/28°46'W	2	2	2	2

DNA extraction, amplification and sequencing

DNA was extracted with the REDEExtract-N-Amp kit (Sigma-Aldrich, www.sigma.com) following the manufacturer's instructions or with proteinase K and standard phenol–chloroform method (Sambrook and Russell 2001). The quality and concentration of genomic DNA was tested by spectrophotometry using either a NanoDrop Spectrophotometer 2000c (Thermo Scientific, Wilmington, DE, USA) or a Qubit 2.0 Fluorometer dsDNA BR Assay Kit (Life Technologies, Thermo Fisher Scientific, MA), followed by agarose gel electrophoresis.

The following mitochondrial genes were sequenced: control region (CR) and cytochrome oxidase subunit I (COI). Additionally, the small-subunit (18S) ribosomal RNA gene and the first intron of the nuclear S7 ribosomal

protein gene (S7) were also sequenced. Whenever possible, each specimen was sequenced for the four selected DNA fragments. Primers and PCR conditions are presented in Online Resource 1.

PCR products were purified using MicroClean (Microzone; CR and S7), Illustra™ ExoStar™ 1-Step (VWR; COI) and QIAquick Gel Extraction Kit (Qiagen; 18S) following manufacturer instructions. The same primers were used for the sequencing reactions except for the 18S which encompassed an internal primer 528F (Elwood et al. 1985) due to the length of the 18S fragment (Online Resource 1). Sequencing was performed at STABVIDA (<http://www.stabvida.net/>) and KAUST BioSciences Core Laboratory (Thuwal, Saudi Arabia). Sequences were edited with Codon Code Aligner (Codon Code Corporation) and aligned with Clustal X 2.1 (Larkin et al. 2007).

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DNA analysis

ARLEQUIN software package V.3.5 (Excoffier and Lischer 2010) was used to estimate the genetic diversity and to compute pairwise FSTs. In the case of the S7 the analyses were also run in ARLEQUIN, after allowing the programme to reconstruct the genotypes present, using the ELB algorithm. Haplotype networks were built with the software TCS 1.21 (Clement et al. 2000) using the parsimony method of Templeton et al. (1992).

Results

Sequences have been deposited in the GenBank database under the following Accession numbers: KU306119–KU306185 (CR), [Accession numbers] (COI), [Accession numbers] (18S), KU306186–KU306230 (S7).

The 18S sequences with 1828 base pairs (bp) were identical for all the individuals analysed independently of the morphotype considered or its geographical origin.

The analysis of the COI sequences with 710 bp has revealed a single DNA polymorphism (SNP). This SNP did not reflect any divergence between morphotypes since both plain and spotted individuals presented the two alternative nucleotides.

The CR, with a total of 333 bp yielded 51 polymorphic sites, revealed 39

different haplotypes with six of them being shared by plain and spotted individuals. In general, diversity indices were high for both morphotypes (Table 2). No differences in pairwise FSTs ($F_{st} = 0.02$; $P = 0.14$) were found between morphotypes.

Table 2

Haplotype numbers and diversity indices for *Labrus bergylta*’s CR and S7

	CR					S7	
	<i>N</i>	<i>NH</i>	Haplotype diversity ± SD	Nucleotide diversity ± SD	Mean number of pairwise differences	<i>N</i>	<i>NH</i>
Plain	43	28	0.973 ± 0.011	0.028 ± 0.014	9.161 ± 4.297	58	17
Spotted	24	16	0.935 ± 0.039	0.037 ± 0.019	12.243 ± 5.731	32	19

N number of individuals, *NH* number of haplotypes (in S7, as calculated by ARLE ELB algorithm), *SD* standard deviation

The S7, with 534 bp yielded 12 polymorphic sites, was unable to discriminate between morphotypes either analysing pairwise FST’s ($F_{st} = 0.005$; $P = 0.34$) or considering each single polymorphism. Additionally, from the 22 haplotypes identified six were shared between plain and spotted individuals along the entire sampled area.

The CR and S7 haplotype networks (Fig. 1 a, b) fully support these results, with several haplotypes being shared by both morphotypes along the entire distribution area of this species, including remote locations such as the Archipelago of the Azores.

Fig. 1

a Haplotype networks for the CR and **b** S7 gene for *L. bergylta*. The ancestral haplotype estimated by TCS is identified by a *square*. *Circle area* is proportional to each haplotype frequency and each *circle* depicts the geographical origin of samples. The number of samples from plain and *spotted colour patterns* is represented by *green* (with a *dot*) and *red* (with a *cross*) *boxes*, respectively. *Notches* represent base pair differences between haplotypes

Discussion

The four mitochondrial and nuclear DNA fragments analysed in this study revealed no genetic differences between *L. bergylta*'s plain and spotted morphotypes, with shared haplotypes (15 % CR; 100 % COI; 100 % 18S and 27 % S7 first intron) along the entire sampling area. These markers

provide genetic information suitable for identifying cryptic species and very recent divergence events, especially CR data, which has been extensively used to detect phylogeographic structure in fish, including labrid species (e.g. Robalo et al. 2012; D'Arcy et al. 2013). Additionally, the combination CR + S7 has been used in several phylogeographic studies of north-eastern Atlantic fish species belonging to several families from different lineages (e.g. Blenniidae, Domingues et al. 2007a; Francisco et al. 2011; Sparidae, Domingues et al. 2007b; Cottidae, Almada et al. 2012). Nevertheless, we are aware that our sample coverage is not equivalent for the analysed markers; therefore, these results should be interpreted with caution.

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The existence of two distinct phenotypic forms throughout the distribution area of the species can be explained by different hypotheses. First, it could be the result of a divergent evolutionary process between sympatric populations of *L. bergylta* driven by assortative mating (prezygotic isolation) of the species. The combination of assortative mating and disruptive natural selection on a single trait, the so-called *magic trait* (Gavrilets 2004), can initiate speciation in marine environments, even in the absence of geographic barriers and high gene flow (Puebla et al. 2007; Barreto and McCartney 2008; Choat et al. 2012). Although the role of colour variation in speciation processes has not been well established yet, the potential for this phenotypic trait to drive diversification was demonstrated via assortative mating in marine and freshwater fish species (Puebla et al. 2007; Elmer et al. 2009; Puebla 2009). Second, it could also be due to a very recent allopatric divergence followed by a complete recolonization of the north-eastern Atlantic by both phenotypic forms. However, our results showing shared haplotypes in very distinct regions do not support cryptic speciation or recent divergence between both morphotypes. In fact our results suggest a geographic rather than a phenotypic signature with identical or similar haplotypes in different regions independently of their phenotypic form. Additional hypotheses to explain the differences between morphotypes include epigenetic regulation and environmental effects. In fact, phenotype evolution in fish, namely body colour pattern, may result from an epigenetic process associated with gene expression regulation (see Maan and Sefc 2013 for a review). On the other hand, different habitat and diet preferences are frequently related with differences in body colour patterns in fish (Sherwood and Grabowski

2010; Sefc et al. 2014). In the case of *L. bergylta*, although ad libitum scuba-diving observations point to some degree of segregation between breeding adults from both morphotypes, no microhabitat differences have been reported so far (Villegas-Ríos et al. 2013a and pers. obs.), and a comparative analysis of the diet of both morphotypes remains to be performed.

From a management perspective, fisheries preferentially target the largest and most valuable specimens. This implies that spotted individuals may be under stronger selective pressure because: (1) they attain larger mean sizes and larger market values since they are commercialized as a different species by local fisheries (Villegas-Ríos et al. 2013a and pers. obs.); (2) their larger sizes-at-age are attained at the cost of their lower reproductive output (Villegas-Ríos et al. 2013b); (3) unbalanced sex ratios due to male-biased overexploitation may occur since the ballan wrasse is a protogynous hermaphrodite fish. If this is true along the entire distribution area, and considering that this species is now being used as cleaner in salmon aquaculture, conditions are created to observe a rapid decline of the spotted compared to the plain morphotype, especially if the origin of these morphological differences remains unknown. Although there is still no information available supporting the genetic singularity of the morphotypes, we suggest that, as a precautionary measure, plain and spotted should be considered two independent management units.

Further studies are needed to address the life history differences between plain and spotted morphotypes. A set of 20 microsatellite loci was recently described for this species by Quintela et al. (2014) which will also allow for a detailed study of gene flow and recent population differentiation. Alternative markers, such as SNPs, could also give a more detailed picture if both neutral and non-neutral markers are considered. The results presented here point to the importance of expanding D'Arcy et al. (2013) phylogeographic analysis to the entire distribution area of this species to clarify the genetic distinctiveness of some remote locations (e.g. Azores). Although our results reveal no genetic isolation between both morphotypes, there is a suggestion that some degree of geographical structure may emerge when additional samples from more geographical areas are analysed in the future.

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Electronic supplementary material

Below is the link to the electronic supplementary material.

Online Resource 1

Primers and polymerase chain reaction conditions used for *Labrus bergylta* (PDF 363 kb)

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