Fisheries Research 199 (2018) 8–11

Contents lists available at ScienceDirect

Fisheries Research

journal homepage: www.elsevier.com/locate/fishres

Technical Note

A PCR-RFLP method for stock assignment in the endemic Macaronesian gastropod, *Patella candei* d'Orbigny, and its potential for future conservation strategies

Gilberto P. Carreira[a,*], Niall J. McKeown[b], Paul W. Shaw[b], João M.A. Gonçalves[c]

[a] Direção de Serviços de Biodiversidade e Política do Mar, Direção Regional dos Assuntos do Mar, SRMCT, Rua D. Pedro IV, n.° 29, 9900-011 Horta, Açores, Portugal
[b] Institute of Biological, Environmental and Rural Sciences (IBERS), Aberystwyth University, Penglais, Aberystwyth SY23 3FG, UK
[c] Departamento de Oceanografia e Pescas, Universidade dos Açores, Rua Professor Doutor Frederico Machado, 9900 Horta, Azores, Portugal

ARTICLE INFO

Handled by J Viñas

Keywords:
COI
Biodiversity
Stock assignment
Sustainable management

ABSTRACT

Patella candei d'Orbigny is an exploited limpet endemic to the archipelagos of the Azores, Madeira, Selvagens and Canaries (Macaronesia). Marked declines reported for the species likely reflect overexploitation and inadequacy of current management measures with biodiversity further threatened by illegal harvesting and anthropogenic movement of limpets among archipelagos. Previous phylogeographic analyses of mtDNA COI sequence variation revealed that the (i) Azores, (ii) Madeira and (iii) Canaries and Selvagens represent three reciprocally monophyletic genetic stocks. The objective of this research was to identify diagnostic DNA sequence polymorphisms among the stocks that could be assayed by PCR-RFLP, thus permitting cost effective high throughput stock assignment. PCR-RFLP analysis of a COI fragment with two restriction enzymes (*Taq*I and *Ava*I) was sufficient to define diagnostic stock genotypes for the three stocks and permitted unambiguous stock assignment of a large sample of individuals (n = 952). This PCR-RFLP approach can be readily extended to discriminate other *Patella* stocks, through judicious selection of restriction enzymes and similar protocols and offers great potential for eco-certification associated practises.

1. Introduction

Illegal, unreported and unregulated harvesting has had a major role in the overexploitation of global fish populations (Nielsen et al., 2012) as well as of other resources, such as limpets (Hawkins et al., 2000; Ferraz et al., 2001; Núnez et al., 2003).

In the Azores, two species of limpets *Patella candei* and *P. aspera*, inhabit the rocky shores and are actively exploited. *P. candei* is an endemic species, only found in the islands of Azores, Madeira, Selvagens and Canaries (Christiaens, 1973; Powell, 1973; Côrte-Real, 1992; Côrte-Real et al., 1996; Ridgway, 1994). In some areas, steadily decreasing abundances are reported, with *P. candei* being referred to as almost extirpated from the Canaries (Núnez et al., 2003). The most extreme population decreases have been observed in the Azores with pronounced oscillations in abundance described for both species since the 1980's, including a localised crash of populations from Pico, S. Jorge and Faial Islands occurring in 1983 that was linked to the so called limpet "disease" (*doença das lapas*). A catch injunction across the entire archipelago was subsequently enforced for the period between 1985 and 1992. After a consistent increase in abundance during this period, exploitation was then allowed to restart.

While there have been various management efforts employed in recent years, Ferraz et al. (2001) reported that the abundance of limpets across the eastern islands of São Miguel and Santa Maria has been steady decreasing since 1993 due to overexploitation. Decreasing abundance of limpets in the Azores and the enforcement of a closed catch season has increased the prices of limpets. A consequence of this has been an increase in illegal harvesting throughout the archipelago. Furthermore, importation of limpets (mainly *P. candei*) has been increasing from Madeira, but also importation of *P. aspera*, *P. vulgata* and *P. depressa* from the mainland (Portugal) has been occurring (personal observations).

Phylogeographic analysis of cytochrome oxidase I (COI) sequence variation revealed three reciprocally monophyletic geographically coherant clades consisting of (i) Azores (ii) Madeira and (iii) Selvagens and Canaries (Sá-Pinto et al., 2008; Carreira et al., 2017). The genetic differences were largely concordant with a previous suggestion by Christiaens (1973) that morphological diversity within *P. candei* reflected geographically distinct subspecies with populations of the Azores corresponding to *P.c. gomesi*, populations in Madeira to *P. c.*

* Corresponding author.
E-mail address: gilberto.mp.carreira@azores.gov.pt (G.P. Carreira).

https://doi.org/10.1016/j.fishres.2017.11.011
Received 1 August 2016; Received in revised form 13 October 2017; Accepted 10 November 2017
0165-7836/ © 2017 Elsevier B.V. All rights reserved.

ordinaria, and Selvagens to *P.c. candei*, which is currently still present in the Fuerteventura Island, in Canaries, along with the locally more abundant variety, *P.c. crenata*.

The ability to classify individuals to stocks would greatly aid management agents, in particular efforts to improve accuracy of stock harvest records (considerably compromised through false accounts of capture locations) and monitoring illegal translocations. While *P. candei* can be readily identified as distinct from its congener species, on account of its shell shape and foot colour, it is more difficult to morphologically classify *P. candei* individuals to the various subspecies/genetic stocks based on phenotypic characteristics, since accurate phenotypic based identification may be compromised by ontogenetic damage and/or operator experience (Ko et al., 2013). Genetic markers represent excellent tools for the classification of individuals to species/populations. As phylogeographic patterns have revealed the existence of fixed differences with cytochrome oxidase I mtDNA sequences among the Azores, Madeira and Canaries/Salvagens stocks, the objective of this research was to identify diagnostic DNA sequence differences that could be assayed by PCR-RFLP, permitting a cost effective and unambiguous method of genetic stock assignment.

2. Material and methods

COI sequences available on GenBank (provided by Sá-Pinto et al. (2008) and Carreira (2010)) were aligned using BioEdit (Hall, 1999). The alignment was used to identify restriction enzyme cleavage sites within amplicons using NEBcutter (Vincze et al., 2003). Patterns of cleavage site presence were compared across sequences to identify potential stock-specific diagnostic enzyme combinations for RFLP genotyping. Restriction digests were performed individually for enzymes following manufacturers (New England Biolabs) recommendations with products separated on 2% agarose gels and visualised by ethidium bromide staining. To validate the PCR-RFLP assay it was performed on a total of 952 *P. candei* specimens collected throughout the Azores, Madeira, Selvagens and Canary Islands (18 localities) (Table 1). Samples ranged from 24 to 78 specimens by locality (average sample size = 53) and were collected randomly across the intertidal rocky shore of the respective islands.Total genomic DNA was extracted from a small portion of foot muscle taken from each specimen (≈0.2 mg) using a standard CTAB (cetyl tri-methyl ammonium bromide) protocol with chloroform/isoamyl alcohol based separation of lipids. RFLP analysis was performed on a PCR amplicon produced using the universal primers LCO1490 and HCO2198 (Folmer et al., 1994). PCRs included an initial period of 95 °C for 5 min followed by 30 cycles of denaturation at 95 °C for 30 s, annealing at 45 °C for 60 s, and extension at 72 °C for 45 s, after which there was a final extension step at 72 °C for 3 min.

3. Results & discussion

Two enzymes were identified as permitting PCR-RFLP based diagnostic discrimination between three exploited stocks of *P. candei*: *Taq*I and *Ava*I (Table 2; Fig. 1). *Taq*I discriminates the Canary Islands (in which the stock of Selvagens is included) from the Azores and Madeira stocks while *Ava*I distinguished the Azores and Madeira lineages. Analysis of control specimens produced unambiguous genotypes with RFLP patterns conforming to expectations from sequences.

Several protocols have been developed for species/population/stock identification in recent years based on different technologies such as isoelectric focusing and starch gel electrophoresis (reviewed in Teletchea, 2009). DNA based methodologies are particularly promising as not only do they often confer accuracy but can be applied to a range of sample types (e.g. whole individuals, dried, processed tissue) and life history stages (McKeown et al., 2015). The integration of such methodologies has often been hampered by relatively high per sample costs and by the time and specialist skills needed (Lindeque et al., 2006). By omitting the need for sequencing, the PCR-RFLP method described here represents a simple and cost effective means for high throughput screening. Due to the universal PCR primers employed the PCR-RFLP method may also be extended to discriminate other *P. candei* stocks through judicious selection of cutting enzymes that may be identified from analysis of sequences on GenBank and/or by exploratory sequencing. Similar assays may also be developed for other Macaronesian limpets building upon the extensive genetic data available (Sá-Pinto et al., 2008; Carreira 2010).

This method confers a number of possibilities to assist management agents to enforce current and future conservation measures, many of which have been difficult to implement in the past (Hawkins et al., 2000). For example, in the short term, two direct applications of this new technique are suggested for *P. candei*. First, this method should be applied as part of routine monitoring scheme of the local markets operating in the Azores, Madeira and Canary Islands, in order to assess the extent of limpet trade between the three Macaronesian archipelagos. At present, there are no empirical data pertaining to this transport. In addition, an export market for this species is also located in the USA and Canada (Menezes, 1994), where there is a large community Portuguese

Table 1
Collection localities and sample sizes of *P. candei*.

Archipelago	Island	Locality	Longitude	Latitude	Sample size (n)
Azores	Corvo	*Porto da Areia*	31° 07′ 17,790” W	39° 40′ 21,710” N	78
	Flores	*Fajã Grande*	31° 15′ 45,230” W	39° 27′ 35,200” N	56
	Graciosa	*Costa do Carapacho*	27° 58′ 39,480” W	39° 00′ 39,250” N	52
	São Jorge	*Topo*	27° 45′ 11,160” W	38° 32′ 54,660” N	69
	Terceira	*Vila Nova*	27° 08′ 53,600” W	38° 47′ 13,560” N	47
	Faial	*Praia do Norte*	28° 45′ 31,170” W	38° 36′ 37,920” N	50
	Pico	*Porto do Cachorro*	28° 26′ 52,740” W	39° 00′ 39,250” N	49
	São Miguel	*Rabo de Peixe*	25° 36′ 17,670” W	37° 49′ 17,460” N	53
	Santa Maria	*Baía dos Anjos*	25° 09′ 36,520” W	37° 00′ 18,630” N	50
	Formigas Islets	Near the light house	24° 46′ 52,500” W	37° 16′ 12,300” N	24
Madeira	Madeira	*Reserva do Garajau*	16° 51′ 06,580” W	32° 38′ 15,650” N	67
	Porto Santo	Harbour	16° 18′ 49,870” W	33° 03′ 45,140” N	37
	Desertas	*Deserta Grande*	16° 30′ 13,320” W	32° 30′ 15,660” N	56
Selvagens	Selvagem Grande	*Baía das Cagarras*	15° 51′ 23,480” W	30° 08′ 58,810” N	62
Canary Islands	Gran Canaria	*Bañaderos*	15° 31′ 53,078” W	28° 09′ 13,346” N	58
	La Graciosa	*El Corral*	13° 29′ 25,368” W	29° 24′ 10,008” N	49
	Lanzarote	*Tymanfaya*	13° 48′ 52,075” W	29° 02′ 00,135” N	48
		Punta Pechiguera	13° 52′ 27,373” W	28° 51′ 23,097” N	47
Total					**952**

Download English Version:

https://daneshyari.com/en/article/8885526

Download Persian Version:

https://daneshyari.com/article/8885526

Daneshyari.com