Molecular taxonomy of South Australian sponges

by analyses of 28S rDNA, COI mtDNA and ITS2 rDNA sequences

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Aim

In this study, a combination of multilocus DNA markers (28S rDNA, COI mtDNA and ITS2 rDNA) was used for the first time with the aim to establish a reliable protocol for molecular taxonomic identification of 38 South Australian sponges.

Introduction

Sponges (Phylum Porifera) are sessile, benthic metazoans, which are highly diverse, ecologically important as filter feeders and of commercial importance to the pharmaceutical and biomaterials industry as producers of highly potent secondary metabolites [1]. More than 8,500 species are considered valid with most belonging to Class Demospongiae [2]. From a taxonomic and systematic point of view, Phylum Porifera is an important group of Metazoa but the species identification based on morphological characters is particularly difficult. The environment-induced morphological variability makes their unambiguous interpretation difficult and often results in homoplasies and erroneous classification [3]. A potential solution is provided by molecular taxonomic approaches such as DNA barcoding, which has been established as an aid to increase the speed of sponge identification [4] with varying degrees of success [5]. It is also used to study the sponge diversification patterns and phylogenetic relationships [6]. DNA barcoding traditionally utilizes one molecular identification marker - the mitochondrial cytochrome oxidase I (COI) gene. Recent approaches using a combination of sequencing information of two nuclear ribosomal rDNA gene attempt to overcome the disadvantage of using only a single DNA loci [7].

Materials

38 sponge specimens were collected from six different locations (Fig. 2) around South Australia.

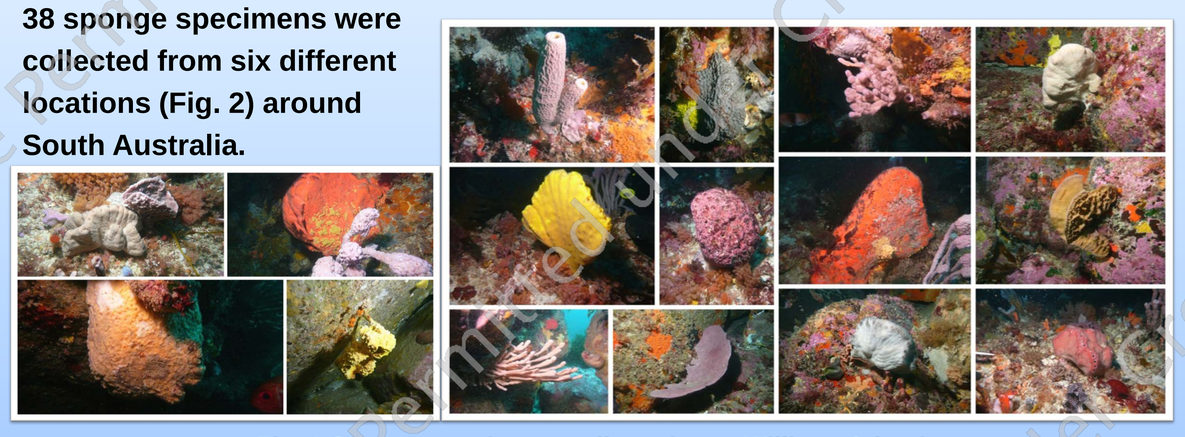

Fig. 1 Sponge specimens collected near Williams Island

Method

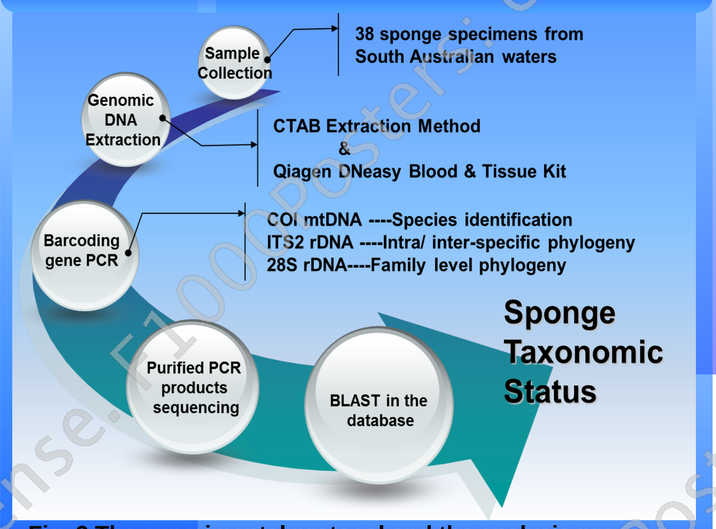

Fig. 3 The experimental protocol and the analysis process

Fig. 2 Sponge specimens collection locations in SA

Conclusion

The molecular taxonomy of 38 South Australian sponge specimens, representing 28 sponge species belonging to nine different orders Dendroceratida, Poecilosclerida, Hadromerida, Verongida, Astrophorida, Haplosclerida, Dictyoceratida, Halichondrida and Ceractinomorpha, were inferred from combined analyses of 28S rDNA, cytochrome oxidase subunit I (COI) mtDNA, and internal transcribed spacer 2 (ITS2) rDNA sequences. Among them there are 12 species with the cluster similarity lower than 97%. The selected three sets of primers are suitable and effective to amplify most of the three different target genes. All the BLAST results using three sequences match each other exactly at order level. Using all three group sequencing results, a combination of the COI mtDNA with 28S and ITS2 rDNA markers for sponge molecular taxonomy is a more reliable approach than using one DNA marker alone. This work is the first molecular study of South Australian sponges using a combination of three DNA markers for identification.

Results & Discussion

DNA extraction, PCR amplification and sequencing

Table 1.Sequencing Efficiency – the number of the sponge-associated products

	28S rDNA	COI mtDNA	ITS2 rDNA
Number of DNA Samples	38	38	38
PCR Product	27	34	17+20
Successful Sequencing	24	34	11
BLAST Result	23	31	8

High-quality DNA was obtained from all the 38 sponge specimens by using two extraction methods (Fig. 3). Using one selected primer set for each marker gene, most of the target genes could be amplified, with less successful in amplifying 28S rDNA (Table 1). The unsuccessful ones may require optimized primer combinations and PCR conditions. 89% of 28S rDNA, 100% of COI mtDNA and 65% (11/ 17) of ITS2 rDNA were sequenced successfully. Regarding ITS2 rDNA there are 20 more PCR products waiting for the sequencing results apart from the 11 (Table 1). Seven BLAST results show they do not belong to Phylum Porifera, which might result of the limited sponge gene in the database (Table 1).

Phylogenetic position of the sponge specimens

The three DNA markers focus on different taxonomic levels (Fig. 3). Phylogenetic trees showing the sponge taxonomic status were constructed according to the BLAST results (Fig. 4-6). Only 10 sponge species belonging to 10 different families could be inferred by 28S rDNA sequencing results. Combining 28S rDNA and COI mtDNA data, 16 additional species belonging to 9 different families were inferred. The ITS2 region is commonly used as a high resolution marker to study intra- and inter-specific evolution in sponges. Based on ITS2 rDNA sequencing, two more species belonging to two different families can be inferred.

Fig. 4 Neighbour-joining phylogenetic representation of 22 sponge specimens from South Australia and their closest NCBI (BLASTn) relatives based on 28S rDNA gene sequences analysis

Phelloderma sp. and *Psammoclema digitiferum* were used as outgroups.

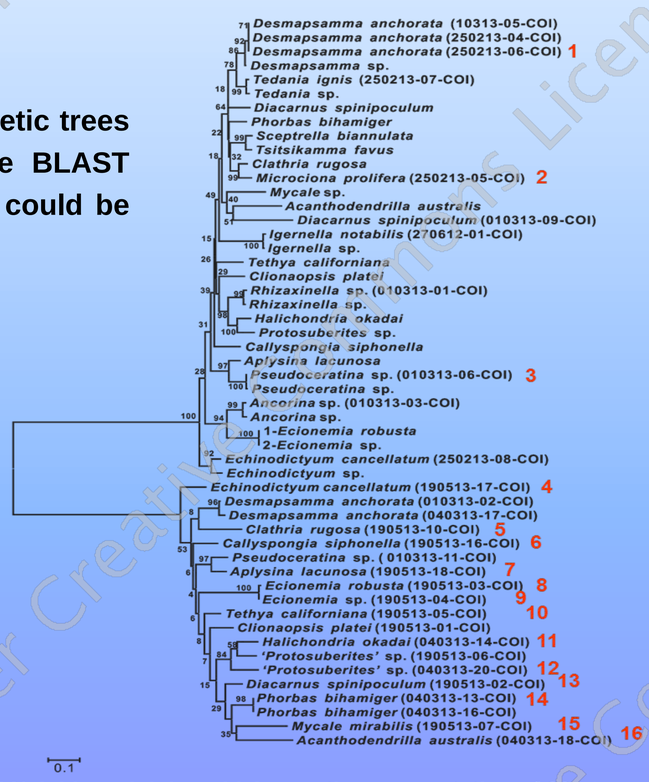

Fig. 5 Neighbour-joining phylogenetic representation of 30 sponge specimens from South Australia and their closest NCBI (BLASTn) relatives based on COI mtDNA gene sequences analysis

Tsitsikamma favus and *Sceptrella biannulata* were used as outgroups. The number at each branch points is the percentage supported by bootstrap.

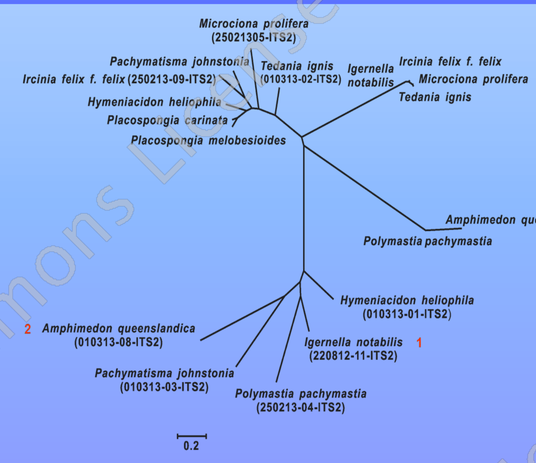

Fig. 6 Neighbour-joining phylogenetic representation of 8 sponge specimens from South Australia and their closest NCBI (BLASTn) relatives based on ITS2 rDNA gene sequences analysis

Placospongia carinata and *Placospongia melobesioides* were used as outgroups.

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