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Molecular biodiversity of Red Sea demosponges

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ABSTRACT

Sponges are important constituents of coral reef ecosystems, including those around the Arabian Peninsula. Despite their importance, our knowledge on demosponge diversity in this area is insufficient to recognize, for example, faunal changes caused by anthropogenic disturbances. We here report the first assessment of demosponge molecular biodiversity from Arabia, with focus on the Saudi Arabian Red Sea, based on mitochondrial and nuclear ribosomal molecular markers gathered in the framework of the Sponge Barcoding Project. We use a rapid molecular screening approach on Arabian demosponge collections and analyze results in comparison against published material in terms of biodiversity. We use a variable region of 28S rDNA, applied for the first time in the assessment of demosponge molecular diversity. Our data constitutes a solid foundation for a future more comprehensive understanding of sponge biodiversity of the Red Sea and adjacent waters.

1. Introduction

As anthropogenic impacts increasingly alter coral reefs (see e.g., Hughes et al., 2003; Hoegh-Guldberg et al., 2007), understanding the more natural state of these ecosystems is becoming urgent, so we have baseline conditions against which changes in biodiversity can be compared to aid conservation efforts. Sponges (Porifera) are one of the main filter-feeding guilds on coral reefs and major players in reef food webs (Perea-Blazquez et al., 2012; de Goeij et al., 2013). Little is known about sponge biodiversity in the Red Sea in comparison to the adjacent waters of Oman, or the more distant Seychelles, India and East Africa (Van Soest and Beglinger, 2008; Berumen et al., 2013). Our current knowledge of Red Sea Porifera is based largely on the works of Keller (1889, 1891), Row (1911) and Lévi (1958, 1965, 1966), as well as on contributions by several other authors (e.g., Topsent, 1892, 1906; Burton, 1952, 1959; Kelly Borges and Vacelet, 1995; Vacelet et al., 2001; Helmy et al., 2004; Ilan et al., 2004; Helmy and Van Soest, 2005; Gugel et al., 2011). Most studies have focused on the Gulfs of Suez and Aqaba, leaving large areas of the Red Sea largely underexplored (Berumen et al., 2013). Changes of the sponge species composition in the Red Sea have been suspected (Vacelet et al., 2001); therefore a comprehensive biodiversity assessment is overdue.

In recent years several expeditions have been conducted to fill in gaps in our knowledge of marine invertebrate biodiversity of the Saudi-Arabian Red Sea and adjacent areas. Species identification is generally the most challenging part of biodiversity surveys. Sponges are especially difficult to identify, because they have relatively few taxonomically-useful characters and exhibit high ecophenotypic plasticity (see e.g., Maldonado et al., 1999; Boury-Esnault, 2006; Lopez-Legentil et al., 2010). Classical (morphological) identification of sponge species requires experience and expertise in the regional fauna, substantial preparation of samples, and is therefore challenging to carry out accurately for rapid surveys and large collections. Recently molecular approaches have been initiated that aim for rapid and unambiguous identification of sponges (Wörheide and Erpenbeck, 2007; Wörheide et al., 2008b). Genetic identification is increasingly recognized as an especially effective, rapid, and reliable technique for delineating species and identifying specimens (see e.g. on Red Sea octocorals Haverkort-Yeh et al., 2013). High throughput extraction, PCR, and sequencing protocols facilitate the genetic study of large collections (Hajibabaei et al., 2007). Nevertheless some taxa pose technical challenges and require specialized protocols. Thus the establishment of high throughput extraction methods suitable for sponge tissue has

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facilitated the rapid molecular screening of sponge collections (Vargas et al., 2012).

The choice of a suitable marker is pivotal for molecular assessments of biodiversity. Classically, a region near the 5′ end of the cytochrome oxidase subunit 1 (CO1) has been suggested as a "universal barcoding region" for metazoans (Hebert et al., 2003), but slow evolutionary rates in demosponges reduce species-level resolution by this marker (e.g., Shearer et al., 2002), while high evolutionary rates for Calcarea prevent the application of universal primers (Voigt et al., 2012; Lavrov et al., 2013). Despite these shortcomings, CO1 has been successfully used for species discrimination in selected sponge lineages (see for examples López-Legentil and Pawlik, 2008; Ferrario et al., 2010; Pöppe et al., 2010) and is used for the Sponge Barcoding Database (www.spongebarcoding.org) to comply with the current Barcoding of Life standards. Other markers suggested for DNA barcoding of sponges, such as a region near 3′ end of CO1 (I3M11, Erpenbeck et al., 2006), were successfully applied on various sponge lineages, but are hampered by the need of nested PCR, which reduces amplification success (e.g., Erpenbeck et al., 2002; López-Legentil and Pawlik, 2008).

The nuclear large ribosomal subunit 'C-Region' (referred to as "28S" in the following) is also used for shallow-level phylogenetic study of sponges. This marker shows considerable phylogenetic signal on lower taxonomic levels (e.g., Chombard et al., 1998; Erpenbeck et al., 2007a; Cardenas et al., 2009; Schuster et al., 2015) and has also been successfully applied for the molecular taxonomy of Calcarea (Voigt and Wörheide, in press).

In this study we pursue a molecular survey of demosponge collections made in 2012–2013 along the length of the Saudi Arabian Red Sea, from the Gulf of Aqaba in the north to the Farasan Islands in the south. For reference, we also include specimens from Oman and Djibouti. We report on the establishment of a molecular biodiversity database of Arabian demosponges in the sponge barcoding project (www.spongebarcoding.org) based on CO1 and 28S markers and discuss the suitability of the 28S marker for molecular identification.

2. Material and methods

2.1. Demosponge samples

A total of 1014 samples were collected during three collecting trips for demosponges covering the northern, central and southern regions of Saudi Arabia's Red Sea (see Fig. 1). The northern and southern regions were sampled in the course of the Red Sea Biodiversity Survey's Phase 2 (2012, 431 samples from 34 stations at Farasan Islands, Al Qunfudah and Al Lith) and Phase 3 (2013, 377 samples from 25 stations at Al Wajh, Duba and Al Khuraybah), conducted by the King Abdulaziz University, Jeddah, Saudi Arabia, and the Senckenberg Nature Research Society, Frankfurt, Germany. Sampling in the central region (2013, 208 samples from 13 stations at Thuwal) was conducted in collaboration with the King Abdullah University of Science and Technology (KAUST). Samples were collected from depths of 1–30 m either by scuba diving, dredging or hand-picking in shallow water under rocks. All samples were photographed, directly preserved in 99% ethanol and subsampled for molecular work. Morphological vouchers are stored in the Senckenberg Museum Frankfurt, Germany (RSS-1), at KAUST, or at the Florida Museum of Natural History, University of Florida (UF). Ethanol of the subsamples for molecular work was exchanged after 24 h to avoid long-term storage in seawater-diluted EtOH. The subsamples for molecular work are registered in the Bavarian State Collection for Paleontology and Geology (see Supplementary Data 1).

2.2. Extraction, PCR and sequencing

DNA was extracted using the plate-based extraction method (Vargas et al., 2010) developed for the Sponge Barcoding Project (www.spongebarcoding.org). Fragments of the mitochondrial cytochrome oxidase subunit 1 (standard barcoding fragment) were amplified using the degenerated version of universal barcoding primers dgLCO1490 (GGT CAA CAA ATC ATA AAG AYA TYG G) and dgHCO2198 (TAA ACT TCA GGG TGA CCA AAR AAY CA) (Meyer et al., 2005). For the 28S fragment the primers 28S-C2-fwd (GAA AAG AAC TTT GRA RAG AGA GT) and 28S-D2-rev (TCC GTG TTT CAA GAC GGG) were used (Chombard et al., 1998). The 25 μL PCR mix consisted of 5 μL 5× green Go*Taq* ® PCR Buffer (Promega Corp, Madison, WI), 4 μL 25 mM $MgCl_2$ (Promega Corp, Madison, WI), 2 μL 10 mM dNTPs, 2 μL BSA (100 μg/ml), 1 μL each primer (5 μM), 7.8 μL water, 0.2 μL Go*Taq*® DNA polymerase (5 u/μl) (Promega Corp, Madison, WI) and 2 μL DNA template. The PCR regime comprised an initial denaturation phase of 94 °C for 3 min followed by 35 cycles of 30 s denaturation at 94 °C, 20 s annealing (45 °C for CO1; 51 °C for 28S), 60 s elongation at 72 °C each and a final elongation at 72 °C for 5 min. We employed a rapid PCR and sequencing screening strategy, which involved that successful PCR amplifications were

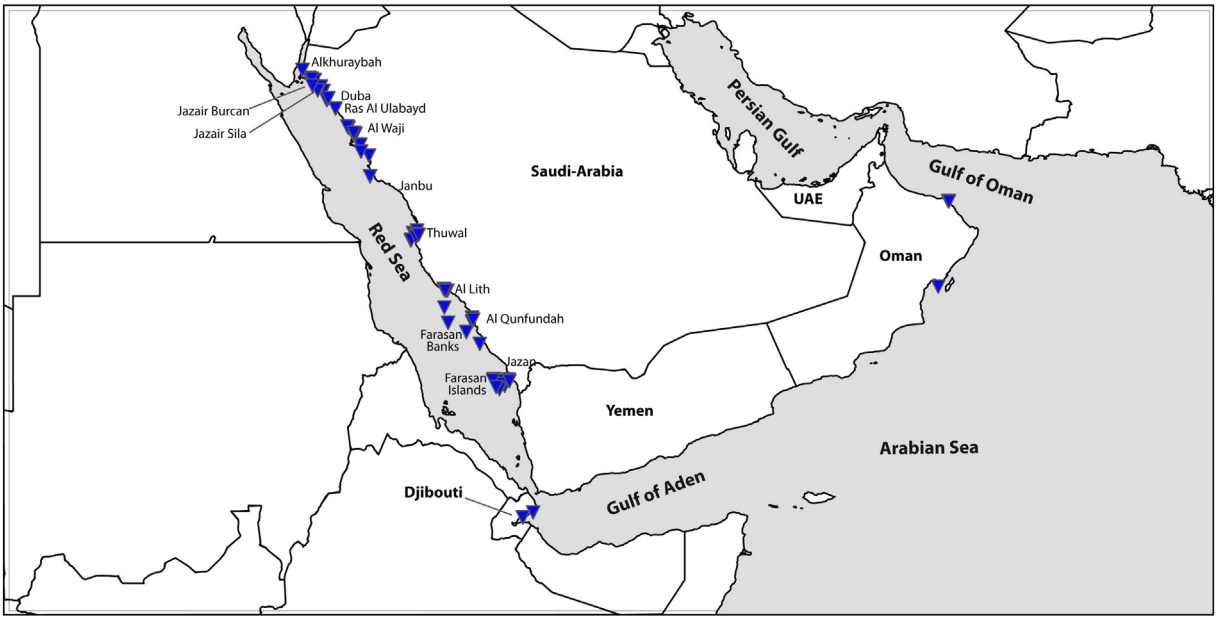

Fig. 1. Collection sites (triangles) of samples used in this study (Map created with Simplemappr Shorthouse, 2010).