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Identification of Two Species of the Family Sparidae for eDNA Sequences using a Representative 16SrRNA Reference Library for Omani Fish

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Abstract: Identification of fish species accurately is required to support efforts for conserving fishery biodiversity assessment and sustainable management. Recently, environmental DNA (eDNA) was suggested for regular monitoring of species for effective management of biodiversity. In Oman, molecular reference library representing fish species from the Omani coastline can support such potential approaches. In this communication, we used a local mtDNA-16SrRNA reference library representing Sparidae fish species from the Omani coastlines to identify two Sparidae eDNA sequences (zotu24 and zotu85, from Muscat and Dhofar, respectively) from *Acanthopagrus* and *Diplodus* genera reported by a recent eDNA study from Oman. The two species are found to be *Acanthopagrus berda* and *Diplodus cervinus* and their sampling sites matches the locations range of the reference specimens. The results indicate the utility and importance for DNA markers' reference library representing fish species from the Omani coastline to effectively sustain the marine resources using emerging technologies.

Keywords: *Sparidae, eDNA; 16SrRNA; Acanthopagrus berda; Diplodus* cervinus; Oman.

تحديد نوعين من عائلة السبارید لتسلسلين eDNA باستخدام مجموعة مرجعية لـ 16S rRNA تمثل الأسماك العمانية

وحيدة العامري وعلياء الأنصاري

الملخص: يعد تحديد أنواع الأسماك بدقة أمرًا ضروريًا لدعم الجهود المبذولة للحفاظ على تقييم التنوع البيولوجي لمصايد الأسماك والإدارة المستدامة. في الآونة الأخيرة، تم اقتراح الحمض النووي البيئي (eDNA) للمراقبة الدورية للأنواع من أجل الإدارة الفاعلة للتنوع البيولوجي. وفي عمان، يمكن للمجموعة المرجعية الجزيئية التي تمثل أنواع الأسماك من الساحل العماني أن تدعم مثل هذه الأساليب. ولأثبات ذلك، استخدمنا مجموعة مرجعية محلية لـ–mtDNA 16SrRNAتمثل أنواع أسماك السبارید (Sparidae) من السواحل العمانية لتحديد تسلسلين من الحمض النووي لجنس أكانثوسبارجس (Acanthopagrus ، zotu24) من مسقط و لدبلودس (Diplodus ، zotu85) من ظفار وجدت في دراسة eDNA حديثة من عمان ولم يتمكن من تعريف انواعها. تم تحديد النوعين على انهما أكانثوسبارجس بردا (*Acanthopagrus berda*) و دبلودس سرفينس *(Diplodus cervinus)* وتطابق مواقع أخذ العينات الخاصة بهما نطاق مواقع العينات المرجعية. تشير النتائج إلى فائدة وأهمية توفر مجموعة مرجعية لعلامات الحمض النووي التي تمثل أنواع الأسماك من الساحل العماني للحفاظ على الموارد البحرية بشكل فعال باستخدام التقنيات الناشئة.

الكلمات المفتاحية: سبارید، الحمض النووي البيئي، 16S rRNA، أكانثوسبارجس بردا، دبلودس سرفينس، عمان.

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1. Introduction

Sultanate of Oman coastline is 3165 kilometers long including three seas, namely the Arabian Gulf, the Sea of Oman and the Arabian Sea. It is one of the most diverse regions in marine organisms of the Arabian Peninsula. The fisheries sector represents a vital renewable resource for food security and an important economic sector, as it contributes to local production and provides job opportunities for citizens in the country [1]. However, the sector faces challenges, including unsustainable fishing practices with unrestricted access to new fishery technologies, and inadequate resource management [2]. The impact of fishing on the ecosystem, especially in the North Sea of Oman, highlights the need for effective management [3]. Identification of fish species accurately is required to support efforts for conserving fishery biodiversity, assessment and sustainable management [4].

Sparidae species hold significant commercial importance and feature prominently in both artisanal and industrial fisheries in Oman. Several studies have been conducted on its biology and the taxonomic composition and abundance of fish larvae in different periods across the Arabian Gulf, Oman Sea and the Arabian Sea in Oman. Chesalina et al investigated the Sparidae family, in the south-western part of the Sea of Oman and reported two Sparidae species, *Acanthopagrus bifasciatus* and *Argyrops spinifer* as the first and third in abundance, respectively [5]. The two species showed variations in seasonal abundance and distribution. However, Al-Abri et al. found that *Acanthopagrus latus* abundance in Muscat comes third and did not report other Sparidae species [6]. In contrast, Al-Abri et al. reported only *Pagellus affinis* (Boulenger, 1887) from the Sparidae family in Omani coastal waters of the Arabian Sea but none of the earlier reported species from the Sea of Oman of the Arabian Gulf [7]. This clearly illustrated the limitation of traditional larvae-based approach in documenting biodiversity as well as underscored the need for regular monitoring due to seasonal variations and distribution changes potentially due to climate change.

In contrast, molecular barcoding-based approaches have proven invaluable in resolving taxonomic uncertainties, helping in species identification and the phylogenetic relationships and therefore hold better potential for fishery biodiversity assessment and sustainable management. The mitochondrial DNA (mtDNA) based identification, known as DNA barcoding, have been developed and used for species identification. COI (cytochrome c oxidase subunit I) and 16S rRNA (16S ribosomal RNA) are common fish markers used in different applications [4].

Recently, environmental DNA (eDNA) metabarcoding, which characterizes multiple species from a single sample, have proven useful in monitoring aquatic environment through the DNA of organisms present for effective management of biodiversity [8, 9]. For successful eDNA approach, reference barcodes from correctly identified specimens is a prerequisite. This involves collecting specimens and identifying it to species level using traditional methods followed by generating DNA barcode sequences and registering them in an international database (such as GenBank). Mitochondrial DNA genes, such as 12S rRNA, Cytb (cytochrome b), 16S rRNA, and COI are usually chosen as the marker genes [8].

DiBattista et al. used eDNA to assess community composition of bony fishes by 16SrRNA, from 15 locations spanning the entire length of the Omani coast. Their results supported the known biogeographic break in fish communities between the north (Muscat and Musandam) and the south (Dhofar) of Oman. They identified the zero-radius operational taxonomic unit (zOTU) sequences by searching against the GenBank database using BLASTn at the National Centre for Biotechnology Information (NCBI) [10]. However, some of the genera sequences could not be assigned at the species-level. This was not possible due to the lack of representative 16SrRNA reference sequences from Omani fishes in the GenBank. Among the three Sparidae eDNA reported sequences (zotu24, zotu85 and zotu137), only one species was assigned (zotu137, *Acanthopagrus bifasciatus*).

We recently, generated a representative 16S rRNA library of sequences for Sparidae family species from Oman. The aim of the present study was to identify the two unidentified Sparidae eDNA sequences (Zero-radius Operational Taxonomic Units, zotus, 24 and 85 that belong to *Acanthopagrus* and *Diplodus* genera, respectively).

2. Materials and Methods

The unassigned eDNA Sparidae sequences and collection site/region (zotu24 from Muscat and zotu 85 from Dhofar) were retrieved from the original study's supplementary data (collapsed 16fish zotus) [8]. The sequences were identified based on sequence alignment with the two genera 16S rRNA reference sequences using Bioedit [11]. The reference sequences belong to Sparidae species specimens, which were originally identified based on morphological characteristics (Figure 1) and validated using 16S rRNA sequencing. The Sparidae family reference sequences from Oman submitted to GenBank (OR776953, OR776954, OR776957, OR776958, and OR776971-OR776972). Finaly, we checked if the identified species' collection sites match the origin of the reference specimens.

Figure 1. Images of reference specimens from Oman identified based on morphological features and 16S rRNA sequences. A. *Acanthopagrus berda* (Forsskål, 1775) specimen collected from Suhar (Sea of Oman). B. *Diplodus cervinus* (Lowe, 1838) specimen collected from Salalah (Arabian Sea).

3. Results and Discussion

The diverse commercial fishes of Oman, including species from the Sparidae family, are a key natural resource that should be taxonomically explored and documented at molecular resolution across the Arabian Gulf, the Sea of Oman, and the Arabian Sea in Oman.

Recently, eDNA metabarcoding approach was used to assess fish diversity along the coast of Oman. Three Sparidae family (sea bream) species were among the high-value demersal fishery that were detected in the study. Only one zotu sequence (zotu137) was assigned a species (*Acanthopagrus bifasciatus*) and the other two (zotu24 and zotu86) could not be identified due to the lack of species reference sequence representation from the region.

We have successfully identified zotu24 as *Acanthopagrus berda* and zotu85 as *Diplodus cervinus* with 100% identity for sequences more than 200bp long. The identification was based on reference sequences we generated for Sparidae species collected from Omani sites across the Arabian/Persian Gulf, the sea of Oman and the Arabian sea. The sequence alignment showed that Zotu24 matched *Acanthopagrus berda* (Forsskål, 1775) reference sequence for 206 bp with 100% identity (Figure 2, A) and zotu85 matched *Diplodus cervinus* (Lowe, 1838) reference sequence for 203 bp with 100% identity (Figure 2, B).

Notably, a short fragment of about 60 bp at the 5' end of the sequence could differentiate among species (Figure 3, A and B).

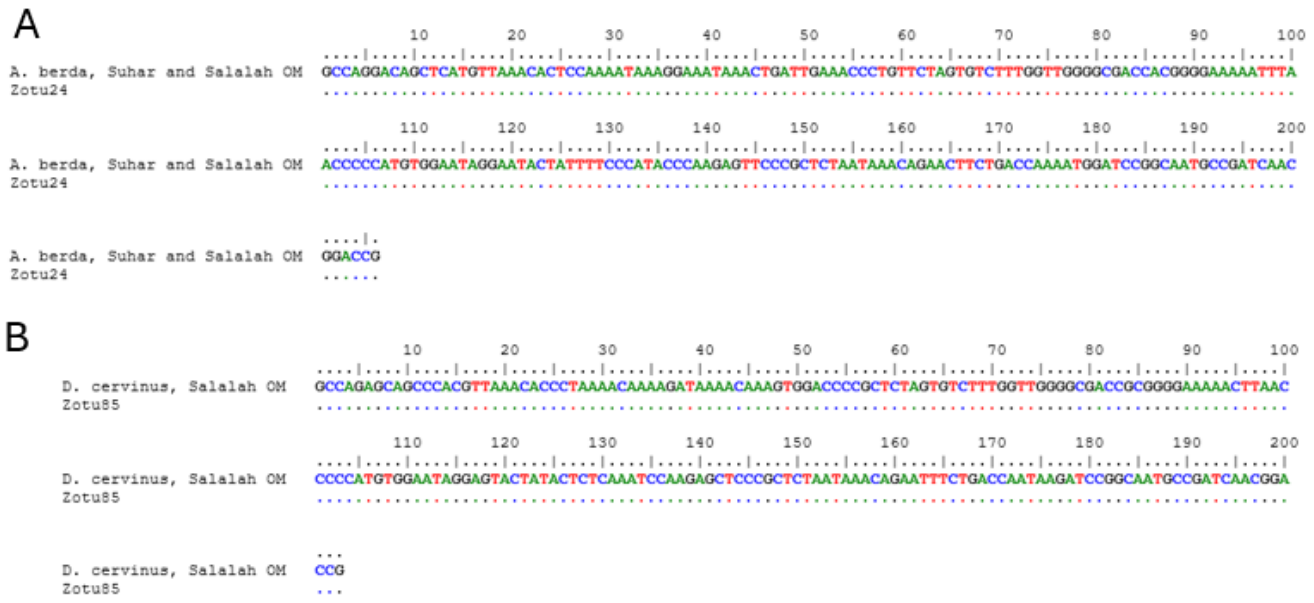

Figure 2. Alignments of the unassigned eDNA zotu sequences and the reference sequences. A. Zotu24 and *Acanthopagrus berda*. B. Zotu85 and *Diplodus cervinus*. Identical bases are represented with dots. The sequence layout is set to wrap at every 100 bases.

Notably, the two eDNA zotu sequences did not match other reference sequences for species from the same genera (Figure 3, A and B).

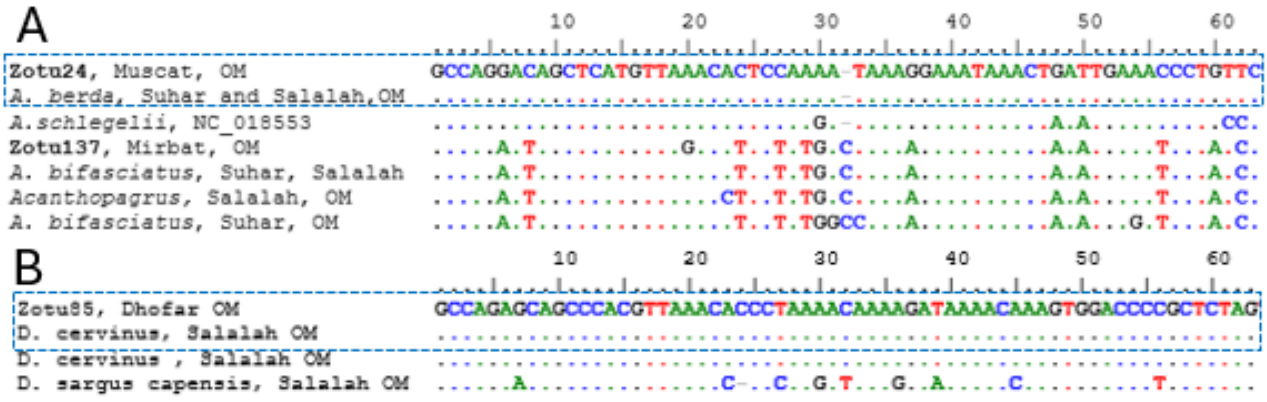

Figure 3. Partial sequence alignments of the unidentified zotu sequences with reference sequences representing different species from the same genera. A. Zotu24 and B. Zotu85. The zotu sequence and its matching sequence are enclosed by dashed line. The coloured bases indicate regions of nucleotides diversity among sequences. The identical bases are represented with dots.

Furthermore, we found that the sampling regions of the three Sparidae species from eDNA metabarcoding study [10] are the same as the reference samples origins (Figure 4).

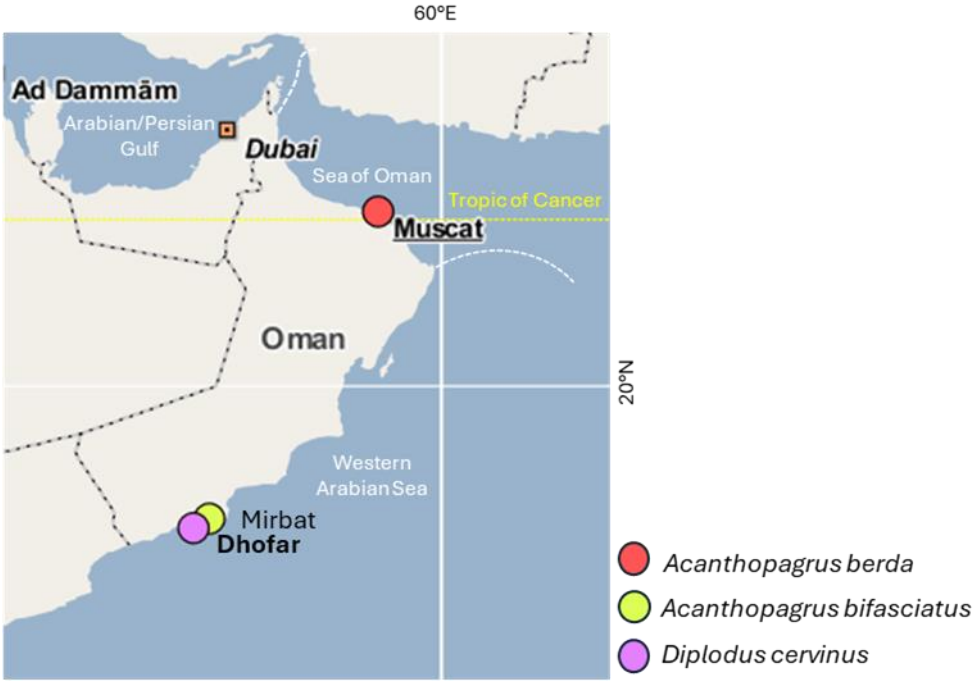

Figure 4. A map illustrating the locality of the three Sparidae species from the eDNA study, Acanthopagrus *berda*, *Acanthopagrus bifasciatus* and *Diplodus cervinus* along the Omani coastline.

Conclusions

In conclusion, this is the first study that has evaluated the utility of local 16S rRNA as representative references for the identification of fish species from Omani coasts, particularly Sparidae species. The local sequence data generated from Oman, much of which are lacking in GenBank, holds value for future molecular studies on fish diversity assessment. Thus, more DNA barcoding efforts should be supported.

Conflict of interest

The authors declare no conflict of interest

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