The deepest record of the octocoral *Acanthogorgia* from the Red Sea

Laura Macrina1,2*, Megan K. B. Nolan1,2, Tullia I. Terraneo2, Nicolas Oury1,2, Nico Augustin3, Froukje M. van der Zwan2,4 and Francesca Benzoni1,2*

1Marine Science Program, Biological and Environmental Science and Engineering Division, King Abdullah University of Science and Technology (KAUST), Thuwal, Saudi Arabia, 2KAUST Red Sea Research Center, King Abdullah University of Science and Technology, Thuwal, Saudi Arabia, 3GEOMAR Helmholtz Centre for Ocean Research, Kiel, Germany, 4Earth Science and Engineering Program, Physical Science and Engineering Division, King Abdullah University of Science and Technology (KAUST), Thuwal, Saudi Arabia

Octocorals (Cnidaria: Anthozoa) have a global distribution and form benthic assemblages along the depth gradient, from shallow to deep waters. They often occur below SCUBA diving limits, where they can become dominant habitat builders and aggregate different taxa. During a cruise in February 2023, one octocoral specimen was collected at 1453 m depth at Kebrit Deep, in the northern Saudi Arabian Red Sea axis, an area with extremely high temperature and salinity profiles at depth. Morphological analysis coupled with DNA barcoding using two mitochondrial markers (*COI* and *mtMuts*), revealed that the coral belongs to *Acanthogorgia*, a genus of azooxanthellate octocorals known to occur from 3 to 2300 m depths in cold, temperate and tropical waters. In the Red Sea, the genus was previously only known from shallower waters. Hence, we report the deepest record of the genus *Acanthogorgia* from the warm and saline Red Sea basin. This finding provides novel insights on deep-water octocoral diversity in the Red Sea, a still scantily explored area of the world, while emphasizing the need for further explorations at depth.

**KEYWORDS**
Octocorallia, deep sea, gorgonians, Marine Animal Forests, depth record

**1 Introduction**

Octocorals (Cnidaria: Anthozoa) occur throughout the world’s oceans and have been reported from cold, temperate and tropical waters (Fabricius and Alderslade, 2001). They are considered foundation species for marine animal forests (MAFs) (Orejas et al., 2022), supporting many marine organisms (Krieger and Wing, 2002; Cairns, 2007; Schweitzer and Stevens, 2019; Tsounis et al., 2020). Their three-dimensional colony structure provides...
Given the challenges in the identification of octocoral specimens in situ or solely based on macro-morphological characteristics (Fabricius and Alderslade, 2001), and the often unclear description of certain taxa (McFadden et al., 2010), DNA extraction and barcoding can aid the classification of these organisms (Bertin et al., 2001). Despite limited availability of species-level molecular data for Acanthogorgia in the literature, McFadden et al. (2006) found the genus to be paraphyletic based on the analysis of mitochondrial genes and comprising two distinct, and distantly related, clades. As such, the comparison of newly generated molecular data with previously published sequences allows to assign specimens to the correspondent genetic lineage.

In this study, we report the deepest record for the genus Acanthogorgia from the Red Sea based on recent sampling efforts in the basin. Additionally, we present the phylogenetic position of the collected specimen within the family Paramuriceidae as molecularly inferred through DNA barcoding using two mitochondrial loci, and a comparison with previously published representatives of this genus and of other 19 genera in the family.

2 Materials and methods

2.1 Sample collection

Deep-sea explorations of the seafloor occurred along the central axis of the Red Sea in February 2023 aboard the R/V Aageo, from the Hellenic Centre for Marine Research (HCMR, Greece). Sampling was carried out using a light work class remotely operated underwater vehicle (ROV) Max Rover Mark II, equipped with color CCD video cameras and a Hydrotek electro-hydraulic 5 function manipulator arm for sampling. Videos were georeferenced with a Trackpoint II USBL positioning system. Salinities and temperatures of the seawater were measured with a CTD (Conductivity, Temperature, Depth) sensor, attached to the ROV. During an ROV dive on the 11th of February 2023, octocoral colonies were observed and one was collected at 1453 m depth in the Kebrat Deep, northern Red Sea (22.4119°N, 37.6044°E) (Figure 1A) among several sponges (Figure 1B). The specimen was processed aboard the research vessel and the apical part of one colony branch was subsampled and fixed in 99% ethanol immediately after collection for subsequent molecular analyses. The rest of the colony was air-dried for subsequent morphological identification. In the Red Sea Research Center Laboratory at KAUST, the dry colony was photographed using a Nikon D7500 camera equipped with a Nikkor 18-55mm lens (Figure 1C). Images of the polyps and skeletal elements disposition and morphology were taken using a Leica M205A stereomicroscope with a Leica DMC 5004 camera (Figure 1D). Microscopical characterization of the sclerites was achieved using a Quattro S Environmental Scanning Electron Microscope (Thermo Fisher Scientific, Wilmington, USA) (Figure 1E) at KAUST Imaging Core Lab (Thuwal, Saudi Arabia). Sclerites preparation for SEM analyses was performed by subsampling a polyp and a small part of the axis from the dry colony and dissolving them in diluted sodium hypochlorite (NaClO) after rehydration in distilled water. Sclerites were then cleaned with distilled water and...
ethanol (EtOH) washes before being mounted on SEM stubs and coated with a 5-nm thick iridium layer through a Q150T S turbomolecular pumped coater (Quorum Technologies, Laughton, United Kingdom). The dry colony and ethanol-preserved sample are stored at the King Abdullah University of Science and Technology (KAUST, Saudi Arabia).

2.2 Morphological identification

The octocoral colony was analyzed and identified to genus level at KAUST based on traditional morphological characters used in octocoral taxonomy (Gray, 1857; Kükenenthal, 1908). This included the examination of macro-morphological characters, such as the colony shape and growth form (Fabricius and Alderslade, 2001), and microscopical observation of diagnostic features such as the morphology and arrangement of polyps and sclerites (Bayer et al., 1983; Grasshoff, 2000).

2.3 DNA extraction, PCR amplification, and phylogenetic analyses

Total DNA was extracted from approximately 1 cm of the ethanol-preserved specimen using a DNeasy® Blood and Tissue kit (Qiagen Inc., Hilden, Germany) and following the manufacturer’s protocol. Extracted DNA quantity and quality were assessed using a NanoDrop® 2000c spectrophotometer (Thermo Fisher Scientific, Wilmington, USA). A fragment (around 950 bp) of the Folmer region of the cytochrome oxidase I (COI) gene plus an adjacent intergenic region (igr1) was amplified using the primers COII8068F (McFadden et al., 2004) and COIOctR (France and Hoover, 2002), while around 830 bp of the mtMutS gene were amplified using the primers ND42599F (France and Hoover, 2001) and mut3458R (Sánchez et al., 2003). Polymerase chain reactions (PCRs) were performed in a final volume of 15 µL obtained with 1.2 µL of raw DNA, 1.5 µL of each primer, 3.3 µL of H2O and 7.5 µL of Multiplex PCR Master Mix (Qiagen, Hilden, Germany). The temperature profiles consisted of an initial step at 95°C for 15 min, 39 cycles of 95°C for 10 sec, 1 min at the annealing temperature (58°C for COI and 48°C for mtMutS) and 72°C for 1 min, and a final step at 72°C for 5 min. PCR products were purified with Illustra™ ExoProStar™ (Global Life Sciences Solutions Operations UK Ltd, Buckinghamshire, UK), following the manufacturer’s protocol, and directly sequenced in both forward and reverse directions using an ABI 3730xl DNA analyzer (Applied Biosystems, Massachusetts, USA) at KAUST BioSciences Core Laboratories (Thuwal, Saudi Arabia).

Forward and reverse sequences were assembled and edited using Geneious® v2023.0.1 (Biomatters Ltd., Auckland, New Zealand). Previously deposited sequences available on GenBank database (https://www.ncbi.nlm.nih.gov/genbank/) and representing specimens belonging to the genus Acanthogorgia and to other 19 genera within the family Paramuriceidae were aligned to the newly produced sequences using MAFFT v7.490 (Katoh and
through the E-INS-i settings. A sequence representing the species *Euplexaura rhipidalis* Studer, 1895 was included in the alignment and chosen as outgroup based on previously published phylogenies (McFadden et al., 2022). Alignments were manually inspected and edited using the software AliView v1.28 (Larsson, 2014). The newly produced sequences were deposited in GenBank database (https://www.ncbi.nlm.nih.gov/genbank/) (Accession numbers: OR588766 and OR588767). Evolutionary models were checked through jModelTest2 (Darriba et al., 2012) on CIPRES (Miller et al., 2010), which selected TVM+I for COI and HKY+G for the mtMutS alignment. Phylogenetic trees were then inferred using both Maximum Likelihood (ML) using RAxML HPC2 (Stamatakis, 2014) and Bayesian Inference (BI) through MrBayes 3.2.6 (Ronquist et al., 2012), on the CIPRES portal, using the default parameters with 1,000 bootstrap replicates and 10,000,000 generations with 2500 burn-in values, respectively.

3 Results

3.1 Environmental conditions

The specimen was collected from the north-western Kebrit Deep, one of the depressions that characterize the northern and central Red Sea (Pautot, 1983). Kebrit Deep hosts an ambient brine pool, notably characterized by extreme conditions, including high salinities (242 PSU), anoxic waters (0 mg O2 L−1), and low pH (5.5) (Eder et al., 2001; Schmidt et al., 2015; Vestheim and Kaartvedt, 2016). The octocoral colony reported here was collected from 1453 m depth, in proximity of the brine, which has its interface at ~1465 m depth (Vestheim & Kaartvedt, 2016) and above the ~1453 m depth, in proximity of the brine, which has its interface at ~1465 m depth (Vestheim & Kaartvedt, 2016) and above the <5 m thick halocline that marks the transition zone to the ocean bottom water. Seawater at this location had a salinity of 41.5 PSU and temperature of 22°C. The specimen was found on the hard substrate of a sulphide chimney, surrounded by, and growing on, sponges, with a diameter smaller than 5 cm (arrowheads in Figure 1B). Several other morphologically similar colonies were also observed at similar water depths along the brine shore.

3.2 Morphological results

The examined material, consisting of one dry specimen and a fragment of the same sample preserved in 99% EtOH, presents a bushy, fan-shaped, densely branched and fragile colony (Figure 1C). Branches are thin and do not present any anastomoses, polyps arise from a dark and thin axis (Figure 1C). Polyps are tall, trumpet-shaped, contractile, but non-retractile and arranged vertically and biserially on both sides of the branches (Figures 1C, D). The coenenchyma layer is contractile, but non-retractile and arranged vertically and biserially on both sides of the branches (Figures 1C, D). The coenenchyme layer is very thin and leaves the axis exposed. The surface of the polyps is covered in small spindles. Sclerites of the polyps are disposed on eight double rows on the body wall and are numerous, flat and bent or elongated. Polyps and tentacles are armed with long warty spindles which arise from the base and form a crown-shaped structure. All sclerites are colorless (Figure 1E).

3.3 Molecular results

PCRs amplifications and sequencing were successful for both COI and mtMutS regions. This allowed us to concatenate the results of the two loci sequenced in a final alignment of 1692 bp, of 98 sequences, consisting of the newly generated sequence and 97 previously published. Phylogenetic reconstructions based on both Maximum Likelihood and Bayesian Inference confirmed that the collected specimen belongs to the family Paramuriceidae and to the genus *Acanthogorgia* (Figure 2). The phylogenetic tree reported in Figure 2 also showed the occurrence of *Acanthogorgia* spp. sequences in two distinct, and not closely related, molecular lineages, highlighting the paraphyly of the genus according to analyses based on mitochondrial genes. The colony sequenced for this study appears to belong to the same lineage of previously published specimens identified as *Acanthogorgia aspera* Pourtalès, 1867, and phylogenetically distinct from the other lineage, which comprises species-level data for seven congeneric taxa, namely *Acanthogorgia angustiflora* Kükenthal & Gorzawsky, 1908, *Acanthogorgia armata* Verrill, 1878, *Acanthogorgia breviflora* Whitelegge, 1897, *Acanthogorgia hedlundii* Aurivillius, 1931, *Acanthogorgia radians* (Kükenthal & Gorzawsky, 1908), *Acanthogorgia spissa* Hiles, 1899, *Acanthogorgia spissa* Kükenthal, 1908 (Figure 2).

4 Discussion

According to morphological and phylogenetic analyses, the colony sampled at 1453 m depth at Kebrit Deep (Figure 1) belongs to the octocoral genus *Acanthogorgia* and represents the deepest record for the genus in the Red Sea and the deepest octocoral sampled in the basin.

While shallow-water coral reefs in the region have been relatively well studied over the past decade (Berumen et al., 2019b), the biodiversity of mesophotic and deep-sea coral assemblages has received less attention. Many studies focusing on octocorals from the Red Sea were based on historical material (e.g., Savigny, 1817; Kükenthal, 1913), and generally include shallow water (e.g., Thomson and Mcqueen, 1907; Verseveldt and Benayahu, 1978; Benayahu, 1990; van Ofwegen, 2016) or mesophotic specimens without clear depth indication (Benayahu et al., 2019). Although mesophotic octocorals were previously investigated in the Red Sea (Benayahu et al., 2017a; Benayahu et al., 2017b), such studies were restricted to the area of the Gulf of Aqaba, which likely reduced the levels of diversity that could be found when considering the whole basin and its depth gradient. Moreover, the majority of the octocoral specimens previously collected at depth in the Red Sea were only identified based on morphological characters (e.g., Grasshoff, 2000; Qurban et al., 2014), and molecular data matching the actual colonies are not available. Hence, the newly generated genetic data presented here contribute to a better characterization of the molecular diversity of the genus from the Red Sea basin and provide useful data for future studies addressing the depth and biogeographical distribution of the genus *Acanthogorgia*. 

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10.3389/fmars.2023.1305420
While morphological analyses allowed us to identify the collected specimen as an *Acanthogorgia* (Figure 1), it was not possible to reach a species-level classification based on the morphological characteristics presented in the literature (Grasshoff, 1999; McFadden et al., 2022). DNA amplification and sequencing allowed us to infer its phylogenetic position within the genus based on a comparison with previously published molecular data available in the literature and obtained from specimens sampled in other biogeographical areas (Figure 2; Supplementary Material 1). This highlighted that the collected specimen belongs to the same molecular lineage of octocorals identified as *A. aspera*, rather than the other lineage comprising the species *A. angustiflora*, *A. armata*, *A. breviflora*, *A. hedlundii*, *A. radians*, *A. spinosa* and *A. spissa* (Figure 2). Although the genus *Acanthogorgia* was previously reported to be paraphyletic through molecular analyses (McFadden et al., 2006), it was recently found to be monophyletic when performing phylogenomic analyses (McFadden et al., 2022). Therefore, given a lack of features that would allow a clear species distinction between the two lineages, care should be taken in the classification of *Acanthogorgia* specimens based on single-locus molecular markers. This mismatch between phylogenetic and phylogenomic data also highlights the need of further sampling and sequencing of this genus to better characterize and match its morphological and molecular diversity within the family Paramuriceidae (McFadden et al., 2006).

From an ecological point of view, the collection of an *Acanthogorgia* from an area such as the central axis of the Red Sea and at such depths emphasizes the remarkable ability of this taxon in spanning the depth gradient and withstanding a range of environmental conditions. Notably, Kebrit Deep is the only location in the Red Sea where *in situ* inactive hydrothermal sulphide chimneys have been found, occurring around the seawater-brine interface (Blum and Puchelt, 1991). Based on their chemistry, these
chimneys are thought to have precipitated from hydrothermal fluids with a temperature of 110-130°C, however, currently the system is extinct (Blum and Puchelt, 1991). The brine pool and chimney field form a distinct habitat, with a range of microbes previously reported from the brine itself (Merlino et al., 2018), and a deep-sea community composed of bivalves, gastropods, polychaetes, and sea anemones described from the inactive chimneys (Vestheim and Kaartvedt, 2016). Nevertheless, the colony reported here is the first octocoral collected from the Kebrit Deep. Additionally, reports of hydrothermal vent fields colonization by anthozoan taxa in other deep-sea areas are scarce (Zelnio et al., 2009). This finding in such a peculiar environment, strengthens the importance of identifying similar habitats in the deep Red Sea and characterizing the benthic communities therein residing and their diversity.

Taxa known to form MAFs in other ocean basins, such as black corals and hydrozoans, have previously been described from the deep Red Sea (Chimenti et al., 2022; Maggioni et al., 2022). However, these habitats have been understudied in the deep Red Sea, and only the occurrence of deep-water coral frameworks has been reported (Qurban et al., 2020). Therefore, the presence of Acanthogorgia sp. colonies below 1000 m provides new insights into the lower limits of colonization and the vertical zonation of habitat-forming species in the basin. The genus is known to contribute to MAFs (Rossi et al., 2017) along the depth gradient and in diverse environments elsewhere in the world (e.g., Cho and Hwang, 2018; Ramiro-Sánchez et al., 2019; Sánchez et al., 2019). For instance, A. armata shows high abundances on deep-sea coral rubble in the Mediterranean (Bo et al., 2023) and in the North Atlantic Ocean (Buhl-Mortensen and Buhl-Mortensen, 2004). This is the first time Acanthogorgia is reported from an area known to host inactive sulphide chimneys and close to a brine-seawater interface. This discovery also emphasizes a need to understand how these organisms can access food and resources in extreme environments. Finally, deep coral communities and MAFs are known to be threatened by anthropogenic activities (Aguilar et al., 2017). Hence, the finding of octocoral colonies in such an unexpected area increases our knowledge on their distribution and thus provides useful spatial information for management and conservation planning, especially considering the rates of endemism of marine organisms previously reported for the Red Sea (DiRattista et al., 2016).

The Red Sea central axis exploration efforts that led to the discovery reported in this study allowed us to document for the first time the presence of octocorals deeper than 1000 m in the basin. Given the observation of other benthic organisms in the surroundings (Figure 1B), this novel finding strengthens the importance of octocoral colonies in providing habitat and substrate in the aphotic zone. While octocoral assemblages and diversity change with the bathymetric range (Sánchez et al., 2021), little is still known about their occurrence and evolution along the depth gradient of the Saudi Arabian Red Sea. Therefore, further explorations and sampling of octocoral communities at depth are needed to better characterize their diversity and conserve these ecologically important organisms.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found in the article/Supplementary Material.

Ethics statement

The manuscript presents research on animals that do not require ethical approval for their study.

Author contributions

LM: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Validation, Visualization, Writing – original draft, Writing – review & editing. MN: Investigation, Writing – review & editing, Conceptualization, Data curation, Visualization. TT: Supervision, Validation, Writing – review & editing. NO: Supervision, Writing – review & editing, Validation. NA: Supervision, Writing – review & editing, Validation, Writing – original draft. FZ: Funding acquisition, Supervision, Writing – review & editing, Resources, Validation, Writing – original draft. FB: Funding acquisition, Resources, Supervision, Writing – review & editing, Validation, Project administration, Visualization, Writing – original draft.

Funding

The author(s) declare financial support was received for the research, authorship, and/or publication of this article. This study was supported by KAUST (Ship-time on the R/V Aegaeo and ROV use by Office of the VPR; baseline research funds BAS/1/1090-01-01 awarded to FB).

Acknowledgments

The authors are grateful to the captain and crew of R/V Aegaeo for their support at sea. We further appreciate the help of the scientific parties on board, particularly the HCMR scientists and the ROV team. We are grateful to KAUST Coastal and marine core labs for organizing the research expeditions and providing technical support on board. We would like to thank Professor Catherine S McFadden for suggestions on early molecular analyses for this manuscript.
Conflict of interest

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmars.2023.1305420/full#supplementary-material


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