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A taxonomic survey of Saudi Arabian Red Sea octocorals (Cnidaria: Alcyonacea)

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Abstract A preliminary survey of Saudi Arabian Alcyonacea is presented, which combines classical taxonomy, multilocus molecular barcodes, and in situ photographs. We explored 14 locations along the west coast of the Kingdom of Saudi Arabia to assess the regional taxonomic diversity of non-gorgonian alcyonaceans. We collected samples from a total of 74 colonies, distributed among four families: 18 colonies of Alcyoniidae, 14 of Nephtheidae, 9 of Tubiporidae, and 33 of Xeniidae. We sequenced the octocorals using multiple nuclear [ribosomal Internal Transcribed Spacers (ITS) and ATP Synthetase Subunit α (ATPSα)] and mitochondrial [MutS homolog (mtMutS) and Cytochrome C Oxidase subunit one (COI)] loci, providing molecular barcodes which will: (1) allow direct comparison of biodiversity from this location to others for which molecular data are available, and (2) facilitate future identifications of these taxa. Finally, this preliminary phylogeny of sampled taxa provides insights on the resolution of mitochondrial versus nuclear loci, and highlights octocoral taxa that require further taxonomic attention.

Keywords Octocorallia · Systematics · Phylogenetics · Taxonomy · Red Sea

Introduction

Octocorals of the order Alcyonacea are among the most common members of many tropical coral reef habitats, including the Red Sea (Benayahu and Loya 1981), the Indo-West Pacific (Fabricius 1997), and the Mediterranean Sea (e.g., Ros et al. 1985). They may also be conspicuous and ecologically important in many colder regions such as the North Atlantic (e.g., Migné and Davoult 1997), southern Africa (e.g., Benayahu 1993; Williams 1992), the Pacific Northwest coast of North America (McFadden and Hochberg 2003), and Antarctica (e.g., Slattery et al. 1995). For example, in the Indo-West Pacific, best known for the high diversity of scleractinian corals and reef-associated fishes, octocorals may occupy up to 25 % of primary space (Benayahu 1995; Fabricius 1997), and soft coral cover as high as 34.4 % has been reported at Sesoko Island, Japan (Loya et al. 2001). Some octocorals possess opportunistic life-history features such as rapid growth rates, high fecundity, and extensive asexual reproduction. This opportunistic life style, in combination with environmental changes that are currently impacting scleractinians, may lead to a shift from scleractinian-dominated coral reefs to ones in which much of the available space is occupied by octocorals (Fabricius 1995; Tilot et al. 2008). For example, Tilot et al. (2008) documented total coral cover on two South Sinai reefs (Egyptian Red Sea) in 2002, compared findings to a similar study in 1996 by Ormond, and found that the scleractinian cover had decreased by 5–25 % while the

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cover of octocorals had increased. Tilot et al. (2008) suggested that scleractinian cover at these sites likely decreased because members of the genus *Acropora* were affected by a crown-of-thorns starfish outbreak between 1996 and 1999 (De'ath and Moran 1998; Salem 1999). The decline in *Acropora* resulted in an increase in hard substrate available for settlement by octocorals of the family Xeniidae and the genera *Litophyton* (Nephtheidae), *Lobophytum* and *Sinularia* (Alcyoniidae), which were the primary taxa accounting for octocoral increase (Tilot et al. 2008).

There is a long history of octocoral collections from the Red Sea, with early publications such as Lamarck (1816), Ehrenberg (1834), Klunzinger (1877), and Kükenthal (1913). Numerous ecological studies on octocorals have also been conducted there (e.g., Benayahu and Loya 1981; Benayahu et al. 1989; Benayahu 1995; Zeevi-Ben-Yosef and Benayahu 2008). Many of these studies laid the foundation for similar studies worldwide (e.g., references in Benayahu 2002; Kahng et al. 2011). Despite the large size of the Saudi Arabian reef system (approximately 1,800 km of coastline), surveys of octocorals there remain scarce. Klunzinger (1877) and Kükenthal (1913) reported some octocorals from Saudi Arabia, but since these early studies no survey has been conducted on the octocoral fauna of this region of the Red Sea. Sheppard and Sheppard (1985, 1991) and Sheppard (1985) thoroughly examined the scleractinian fauna of the Saudi Arabian coast; however, their study did not include octocorals with the sole exception of the organ pipe coral, *Tubipora musica*.

With myriad global climate changes impacting coral reefs (Hughes et al. 2003; Hoegh-Guldberg et al. 2007), understanding the evolutionary relations among octocoral taxa, which are increasing in abundance on many reefs, becomes an issue of significant conservation relevance. Despite the capacity of classical taxonomy to provide reliable identifications for some octocoral taxa, the taxonomy and systematic relationships of octocorals remain subjects of considerable uncertainty (McFadden et al. 2010). This uncertainty is caused by a paucity of taxonomic studies on several octocoral families. Poor original descriptions, uncertainty concerning morphological features to be used for identification, possible polymorphism within species, and in some cases even overlapping morphology between species have raised the need for taxonomic revision of this sub-class. Several phylogenetic studies on Alcyonacea have found little correspondence between molecular clades and classical taxonomic groups, even at taxonomic levels as high as the sub-ordinal level (Berntson et al. 2001; Daly et al. 2007; McFadden et al. 2010). However, in other studies, correspondence between molecular clades and classical taxonomic groups is found at the genus level (Concepcion et al. 2008). For taxonomic convenience, Alcyonacea is often sub-divided into six sub-ordinal groups (Alcyoniina, Calcaxonia, Holaxonia, Protoalcyonaria, Scleraxonia, Stolonifera), representing different grades of colony form and skeletal composition (Fabricius and Alderslade 2001; Daly et al. 2007). However, it is widely acknowledged that these groups do not reflect phylogenetic relationships (Berntson et al. 2001; Fabricius and Alderslade 2001; McFadden et al. 2006a). For example, in a phylogenetic analysis of Octocorallia based on the mitochondrial genes *ND2* and *mtMutS*, McFadden et al. (2006a) found that topology tests rejected the monophyly of the sub-ordinal groups, Alcyoniina, Scleraxonia, and Stolonifera. This molecular and morphological discord has led to questions about the taxonomic value of some of the currently-used morphological characters (Fabricius and Alderslade 2001), and the evolutionary relationships inferred among families (McFadden et al. 2006a).

In this paper, we report on a study of octocorals, using a combination of classical morphological taxonomy, multilocus molecular barcodes [(from nuclear (*ITS* and *ATPS*α), and mitochondrial (*mtMutS* and *COI*) loci] and photography. The study included 13 genera within four families. Of the family Alcyoniidae (Lamouroux, 1812) we included three genera: (1) *Rhytisma* Alderslade, 2000, (2) *Sarcophyton* Lesson, 1834, and (3) *Sinularia* May, 1898. Within the family Nephtheidae (Gray, 1862), we included four genera: (1) *Dendronephthya* Kükenthal, 1905, (2) *Litophyton* Forskål, 1775, (3) *Paralemnalia* Kükenthal, 1913, and (4) *Stereonephthya* Kükenthal, 1905. The family Tubiporidae comprises only *Tubipora* (Linnaeus, 1758). Finally, the family Xeniidae (Wright and Studer 1889) included five genera: (1) *Anthelia* Lamarck, 1816, (2) *Heteroxenia* Kölliker, 1874, (3) *Ovabunda* Alderslade, 2001, (4) *Sympodium* Ehrenberg, 1834, and (5) *Xenia* Lamarck, 1816. Our goal was to provide a taxonomic survey of the Saudi Arabian octocorals, utilizing a combination of classical taxonomy, molecular data, and in situ photographs. Gorgonians were excluded due to limited collection time. The results of the survey provide a foundation for studies on the ecology and biodiversity of Saudi Arabian Alcyonacea, and will support conservation management decisions in this region. This survey also represents an important contribution to understanding regional (within the Red Sea) and broad-scale (Indo-West Pacific) octocoral biogeographic patterns. Our molecular data highlight several taxa for which further taxonomic study is warranted and will be useful given the increasing utilization of DNA barcoding as a means of facilitating genetic biodiversity surveys. Finally, the availability of molecular barcodes allows us to directly compare biodiversity of this location to others for which molecular data are available.

Materials and methods

Sample collection

Over the course of 8 days in April 2011, a total of 74 collections were made at 1–15 m depth from 14 reef sites in four

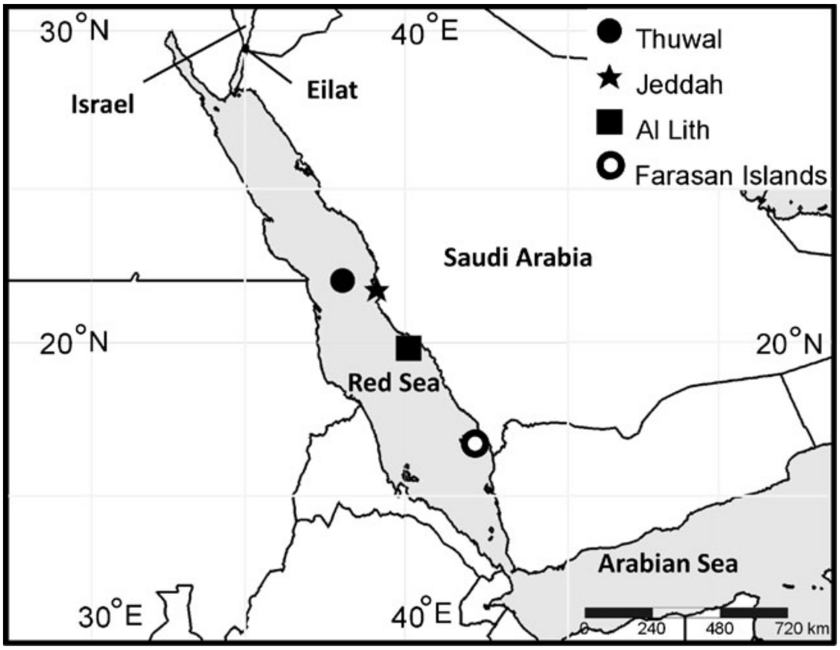


Fig. 1 Sites along the west coast of the Kingdom of Saudi Arabia where collections of octocorals were made: Jeddah (21°43′N, 39°06′E), Thuwal (22°15′N, 38°57′E), Al Lith (19°49′N′, 40°07′E), and the Farasan Islands (16°47′N, 42°11′E). (The map was designed with SimpleMappr; Shorthouse 2010)

main areas along the western coast of Saudi Arabia: Thuwal, Jeddah, Al Lith, and the Farasan Islands (arranged north to south) (Fig. 1; Table 1). We focused primarily on collecting all morphotypes of members of the family Xeniidae, but also collected other non-gorgonian alcyonacean octocoral morphotypes by the roving diver technique (Munro 2005; Hoeksema and Koh 2009). Depending on abundance of octocoral colonies, collections were made of up to four colonies for each (visible) morphotype, such as different color morphs. Each sampled colony was measured and a small piece (1–5 cm) was sampled using scissors or dive knife. Multiple photos and live videos (~1 min) were taken of each colony before and during disturbance (upon hand-touch) to record colony response as well as of the surrounding environment (Canon G11 camera with a Canon underwater housing). Morphological features and behavior of the colonies derived from the photographs aided the classical taxonomic identification (see below). Colony size and color, polyp retraction (process of invagination of the anthocodia within the upper part of the anthostele, see Fabricius and Alderslade 2001), tentacle contraction (process of deflation without invagination, see Fabricius and Alderslade 2001), stalk branching, and polyp pulsation (applicable to Xeniidae, see Reinicke 1997) were recorded for each colony (see Electronic Supplementary Material). In addition, the depth of collection and type of substrate (hard vs. sand) were recorded for each colony sampled. Paired tissue samples from each colony were stored in 95 % ethanol and saturated salt DMSO (SSD) buffer for molecular analyses (following Gaither et al. 2011), and an additional sample was stored in 70 % ethanol for morphological taxonomic study.

Classical taxonomy

Generic field identifications based on colony features were verified by subsequent expert taxonomic identification based on colony morphology, sclerites, presence of dimorphic polyps, and zooxanthellae (Fabricius and Alderslade 2001). Additionally, examination of xeniid octocorals included the number of pinnule rows on the polyp tentacles and the number of pinnules on the aboral side of the polyp tentacles (Reinicke 1997). For species identification, sclerites were obtained by dissolving small tissue samples in 10 % sodium hypochlorite, rinsed in distilled water and examined under a light microscope. Identifications were facilitated in part by comparisons with permanent sclerite preparations of type material kept at the Zoological Museum, Department of Zoology, Tel Aviv University, Israel (ZMTAU). Tentative field identifications of *Sarcophyton* colonies could not be confirmed due to lack of the base of the colony for those samples (see below: SA053, SA066, SA067, and SA077). All samples are deposited at the U.S. National Museum of Natural History, Washington, D.C. (USNM) (USNM 1201935–1202016) (Table 1).

DNA isolation and amplification

The mitochondrial markers *mtMutS* (previously named *msh1*, ~730 bp, $n=64$) and *COI* (~770 bp, $n=69$) were amplified and sequenced using published primers and protocols (McFadden et al. 2011). PCR products from the mitochondrial markers were purified and sent to the University of Washington's High-Throughout Genomics Center

Table 1 Specimen list with corresponding GenBank accession numbers (* means heterozygote colonies), corresponding USNM numbers (accession numbers at the Smithsonian Museum, Washington, D.C.), and corresponding sampling locations and coordinates. Only a single sample was collected for each individual listed here

	Family	Genus	Species	Site	Coordinates	Depth	USNM	*ATPSα 17*	*ITS*	*MtMutS*	*COI 71*
SA001	Xeniidae	*Ovabunda*	*ainex*	Thuwal, Palace North	22°18′N, 38°58′E	13 m	1201935	KC865037	KC864797	KC864858	KC864922
SA002	Xeniidae	*Ovabunda*	*gohari*	Thuwal, Shark Reef	22°25′N, 38°59′E	13 m	1201936	KC865042	KC864798	KC864859	KC864923
SA003	Xeniidae	*Xenia*	sp.	Thuwal, Shark Reef	22°25′N, 38°59′E	9 m	1201937	KC864994		KC864860	KC864924
SA004	Xeniidae	*Ovabunda*	*biseriata*	Thuwal, Palace North	22°18′N, 38°58′E	13 m	1201938	KC865013	KC864799	KC864861	KC864925
SA005	Xeniidae	*Ovabunda*	*arabica*	Thuwal, Palace North	22°18′N, 38°58′E	13 m	1201939	KC865041	KC864800	KC864862	KC864926
SA006	Xeniidae	*Xenia*	sp.	Thuwal, Shark Reef	22°25′N, 38°59′E	9 m	1201940	KC864995*	KC864801	KC864863	KC864927
SA007	Xeniidae	*Ovabunda*	*macrospiculata*	Thuwal, Palace South	22°15′N, 38°57′E	6 m	1201941	KC865029	KC864802	KC864864	KC864928
SA008	Xeniidae	*Xenia*	sp.	Thuwal, Shark Reef	22°25′N, 38°59′E	9 m	1201942		KC864803	KC864865	KC864929
SA009	Xeniidae	*Ovabunda*	*biseriata*	Thuwal, Shi'b Nazar	22°19′N, 38°51′E	12 m	1201943	KC865028*		KC864866	KC864930
SA010	Xeniidae	*Ovabunda*	*biseriata*	Thuwal, Palace North	22°18′N, 38°58′E	13 m	1201944	KC865040*	KC864804	KC864867	KC864931
SA011	Xeniidae	*Xenia*	*actuosa*	Al Lith, North Brown Reef	19°52′N, 40°06′E	7 m	1201945	KC864993	KC864805	KC864868	KC864932
SA013	Xeniidae	*Heteroxenia*	*fuscescens*	Al Lith, North Brown Reef	19°52′N, 40°06′E	9 m	1201947	KC865008	KC864806	KC864869	KC864933
SA014	Nephtheidae	*Litophyton*	sp.	Al Lith, South Brown Reef	19°49′N, 40°07′E	15 m	1201948			KC864870	KC864934
SA016	Alcyoniidae	*Rhytisma*	*fulvum fulvum*	Al Lith, South Brown Reef	19°49′N, 40°07′E	13 m	1201950		KC864807		KC864935
SA017	Alcyoniidae	*Rhytisma*	*fulvum fulvum*	Al Lith, North Brown Reef	19°52′N, 40°06′E	9 m	1201951		KC864808		
SA019	Tubiporidae	*Tubipora*	*musica*	Al Lith, South Brown Reef	19°49′N, 40°07′E	13 m	1201953			KC864871	KC864936
SA020	Xeniidae	*Ovabunda*	*ainex*	Al Lith, North Brown Reef	19°52′N, 40°06′E	8 m	1201954	KC865036	KC864809	KC864872	KC864937
SA021	Xeniidae	*Heteroxenia*	*fuscescens*	Thuwal, Palace South	22°15′N, 38°57′E	4 m	1201955	KC865010	KC864810	KC864873	KC864938
SA022	Xeniidae	*Ovabunda*	*ainex*	Thuwal, Shi'b Nazar	22°19′N, 38°51′E	8 m	1201956	KC865038	KC864811	KC864874	KC864939
SA023	Xeniidae	*Anthelia*	sp.	Thuwal, Shi'b Nazar	22°19′N, 38°51′E	10 m	1201957	KC865001	KC864812	KC864875	KC864940
SA024	Xeniidae	*Ovabunda*	*biseriata*	Thuwal, Shi'b Nazar	22°19′N, 38°51′E	8 m	1201958	KC865035	KC864813	KC864876	KC864941
SA026	Xeniidae	*Heteroxenia*	*fuscescens*	Thuwal, Palace South	22°15′N, 38°57′E	5 m	1201960	KC865009	KC864814	KC864877	KC864942
SA027	Xeniidae	*Anthelia*	sp.	Thuwal, Shi'b Nazar	22°19′N, 38°51′E	11 m	1201961	KC865000	KC864815	KC864878	KC864943

Table 1 (continued)

	Family	Genus	Species	Site	Coordinates	Depth	USNM	*ATPSα 17*	*ITS*	*MtMutS*	*COI 71*
SA028	Nephtheidae	*Litophyton*	sp.	Al Lith, North Brown Reef	19°52′N, 40°06′E	9 m	1201962	KC865011*	KC864816*	KC864879	KC864944
SA029	Tubiporidae	*Tubipora*	*musica*	Al Lith, North Brown Reef	19°52′N, 40°06′E	7 m	1201963		KC864817*	KC864880	KC864945
SA030	Tubiporidae	*Tubipora*	*musica*	Al Lith, Dora Reef	19°49′N, 39°53′E	6 m	1201964				
SA031	Tubiporidae	*Tubipora*	*musica*	Al Lith, Dora Reef	19°49′N, 39°53′E	12 m	1201965		KC864818*		KC864946
SA032	Xeniidae	*Anthelia*	sp.	Al Lith, Dora Reef	19°49′N, 39°53′E	10 m	1201966	KC864998	KC864819	KC864881	KC864947
SA034	Alcyoniidae	*Rhytisma*	*fulvum fulvum*	Al Lith, Dora Reef	19°49′N, 39°53′E	9 m	1201968	KC865044	KC864820	KC864882	KC864948
SA035	Xeniidae	*Sympodium*	*caeruleum*	Al Lith, Dora Reef	19°49′N, 39°53′E	6 m	1201969	KC865027			KC864949
SA036	Nephtheidae	*Dendronephthya*	sp.	Al Lith, Dora Reef	19°49′N, 39°53′E	9 m	1201970	KC865007	KC864821	KC864883	KC864950
SA037	Xeniidae	*Xenia*	sp.	Jeddah, Obhur Creek	21°43′N, 39°06′E	1 m	1201971	KC865033	KC864822	KC864884	KC864951
SA038	Nephtheidae	*Dendronephthya*	sp.	Al Lith, Dora Reef	19°49′N, 39°53′E	10 m	1201972	KC865006	KC864823	KC864885	KC864952
SA039	Tubiporidae	*Tubipora*	*musica*	Al Lith, Dora Reef	19°49′N, 39°53′E	12 m	1201973		KC864824*	KC864886	KC864953
SA040	Xeniidae	*Ovabunda*	*impulsatilla*	Al Lith, Dora Reef	19°49′N, 39°53′E	10 m	1201974		KC864825	KC864887	KC864954
SA041	Tubiporidae	*Tubipora*	*musica*	Al Lith, Dora Reef	19°49′N, 39°53′E	7 m	1201975	KC865025		KC864888	KC864955
SA042	Xeniidae	*Anthelia*	sp.	Al Lith, Marmar Reef	19°50′N, 39°56′E	6 m	1201976		KC864826	KC864889	KC864956
SA043	Alcyoniidae	*Rhytisma*	*fulvum fulvum*	Al Lith, Marmar Reef	19°50′N, 39°56′E	5 m	1201977		KC864827	KC864890	KC864957
SA044	Tubiporidae	*Tubipora*	*musica*	Al Lith, Dora Reef	19°49′N, 39°53′E	10 m	1201978	KC864996	KC864828*	KC864891	KC864958
SA045	Xeniidae	*Ovabunda*	*macrospiculata*	Al Lith, Marmar Reef	19°50′N, 39°56′E	5 m	1201979	KC865039	KC864829	KC864892	KC864959
SA046	Tubiporidae	*Tubipora*	*musica*	Al Lith, Dora Reef	19°49′N, 39°53′E	10 m	1201980			KC864893	KC864960
SA047	Xeniidae	*Anthelia*	sp.	Al Lith, Marmar Reef	19°50′N, 39°56′E	10 m	1201981	KC864999	KC864830*	KC864894	KC864961
SA048	Xeniidae	*Sympodium*	*caeruleum*	Al Lith, Marmar Reef	19°50′N, 39°56′E	5 m	1201982	KC865024			KC864962
SA049	Alcyoniidae	*Paralemnalia*	*eburnea*	Al Lith, Marmar Reef	19°50′N, 39°56′E	5 m	1201983		KC864831	KC864895	KC864963
SA050	Xeniidae	*Anthelia*	sp.	Al Lith, Marmar Reef	19°50′N, 39°56′E	6 m	1201984	KC864997	KC864832*	KC864896	KC864964
SA051	Alcyoniidae	*Sinularia*	sp.	Farasan Islands, Abulatt	16°47′N, 42°11′E	8 m	1201985	KC865022	KC864833		KC864965
SA052	Alcyoniidae	*Rhytisma*	*fulvum fulvum*	Farasan Islands, Abulatt	16°47′N, 42°11′E	8 m	1201986	KC865043*	KC864834*	KC864897	KC864966

Table 1 (continued)

	Family	Genus	Species	Site	Coordinates	Depth	USNM	*ATPSα 17*	*ITS*	*MtMutS*	*COI 71*
SA053	Alcyoniidae	*Sarcophyton*	sp.	Farasan Islands, Abulatt	16°47′N, 42°11′E	7 m	1201987		KC864835		
SA054	Alcyoniidae	*Sarcophyton*	*glaucum*	Farasan Islands, Abulatt	16°47′N, 42°11′E	10 m	1201988	KC865018*	KC864836*	KC864898	KC864967
SA055	Alcyoniidae	*Paralemnalia*	*thyrsoides*	Farasan Islands, Abulatt	16°47′N, 42°11′E	10 m	1201989	KC864991	KC864837	KC864899	KC864968
SA056	Alcyoniidae	*Sinularia*	*querciformis*	Farasan Islands, Abulatt	16°47′N, 42°11′E	11 m	1201990	KC865021	KC864838	KC864900	KC864969
SA057	Alcyoniidae	*Rhytisma*	*fulvum fulvum*	Farasan Islands, Abulatt	16°47′N, 42°11′E	9 m	1201991		KC864839	KC864901	KC864970
SA058	Alcyoniidae	*Sinularia*	*leptoclados*	Farasan Islands, Abulatt	16°47′N, 42°11′E	8 m	1201992	KC865020		KC864902	
SA059	Nephtheidae	*Litophyton*	sp.	Al Lith, Mulathu	19°44′N, 39°54′E	10 m	1201993	KC865012		KC864903	KC864971
SA060	Alcyoniidae	*Sarcophyton*	*auritum*	Al Lith, Mulathu	19°44′N, 39°54′E	7 m	1201994	KC865014	KC864840	KC864904	KC864972
SA061	Nephtheidae	*Dendronephthya*	sp.	Farasan Islands, Dahik	16°54′N, 52°07′E	9 m	1201995		KC864841		KC864973
SA062	Tubiporidae	*Tubipora*	*musica*	Farasan Islands, Dahik	16°54′N, 52°07′E	7 m	1201996			KC864905	KC864974
SA063	Nephtheidae	*Dendronephthya*	sp.	Farasan Islands, Dahik	16°54′N, 52°07′E	8 m	1201997	KC865002	KC864842	KC864906	KC864975
SA064	Nephtheidae	*Dendronephthya*	sp.	Farasan Islands, Dahik	16°54′N, 52°07′E	7 m	1201998	KC865003	KC864843	KC864907	KC864976
SA065	Nephtheidae	*Dendronephthya*	sp.	Farasan Islands, Dahik	16°54′N, 52°07′E	5 m	1201999	KC865005	KC864844	KC864908	KC864977
SA066	Alcyoniidae	*Sarcophyton*	*glaucum*	Farasan Islands, Dahik	16°54′N, 52°07′E	6 m	1202000	KC865017*	KC864845*		KC864978
SA067	Alcyoniidae	*Sarcophyton*	*auritum*	Farasan Islands, Dahik	16°54′N, 52°07′E	7 m	1202001	KC865015	KC864846	KC864909	KC864979
SA068	Nephtheidae	*Dendronephthya*	sp.	Farasan Islands, Abu Shariah	16°43′N, 42°15′E	6 m	1202002	KC865026		KC864910	
SA069	Nephtheidae	*Dendronephthya*	sp.	Farasan Islands, Abu Shariah	16°43′N, 42°15′E	6 m	1202003	KC865004	KC864847	KC864911	KC864980
SA071	Xeniidae	*Xenia*	*umbellata*	Jeddah, Obhur Creek	21°43′N, 39°06′E	8 m	1202005	KC865030	KC864848	KC864912	KC864981
SA072	Nephtheidae	*Paralemnalia*	*thyrsoides*	Jeddah, Obhur Creek	21°43′N, 39°06′E	8 m	1202006	KC864992	KC864849	KC864913	KC864982
SA074	Xeniidae	*Ovabunda*	*verseveldti*	Jeddah, Obhur Creek	21°43′N, 39°06′E	13 m	1202008		KC864850	KC864914	KC864983
SA075	Xeniidae	*Ovabunda*	*gohari*	Jeddah, Obhur Creek	21°43′N, 39°06′E	14 m	1202009	KC865034*	KC864851	KC864915	KC864984
SA076	Xeniidae	*Xenia*	sp.	Jeddah, Obhur Creek	21°43′N, 39°06′E	11 m	1202010	KC865032	KC864852	KC864916	KC864985
SA077	Alcyoniidae	*Sarcophyton*	*gemmatum*	Jeddah, Obhur Creek	21°43′N, 39°06′E	8 m	1202011	KC865016	KC864853	KC864917	KC864986

Table 1 (continued)

	Family	Genus	Species	Site	Coordinates	Depth	USNM	*ATPSα 17*	*ITS*	*MtMutS*	*COI 71*
SA081	Nephtheidae	*Stereonephthya*	sp.	Farasan Islands, East Farasan Island	16°44′N, 42°13′E	10 m	1202012		KC864854	KC864918	KC864987
SA083	Nephtheidae	*Stereonephthya*	sp.	Farasan Islands, Abulatt	16°47′N, 42°11′E	8 m	1202014	KC865023	KC864855	KC864919	KC864988
SA084	Alcyoniidae	*Sinularia*	*compressa*	Farasan Islands, Abulatt	16°47′N, 42°11′E	11 m	1202015	KC865019	KC864856	KC864920	KC864989
SA085	Xeniidae	*Xenia*	*umbellata*	Jeddah, Obhur Creek	21°43′N, 39°06′E	8 m	1202016	KC865031	KC864857	KC864921	KC864990
Total								53	61	64	69

[a] Heterozygote colonies

(Seattle, WA, USA) for sequencing following the methods in McFadden et al. (2011).

DNA for nuclear locus amplification was extracted following the coral DNA extraction protocol outlined in Concepcion et al. (2006, 2008). The nuclear marker *ATPSα* (~280 bp, n=53) was amplified using the published primers *ATPSα*f1 and *ATPSα*r1 (Jarman et al. 2002), and with the following PCR protocol: 94 °C for 2 min; 94 °C for 20 s, 57 °C for 60 s, 72 °C for 60 s (35 cycles); followed by a final extension of 72 °C for 6 min. The ITS region (~1,100 bp, n=61) was amplified using the published primers 1S-f and 2SS-r (annealing temperature: 50 °C; Wei et al. 2006), in addition to the newly designed primers ITSRHF 5′-TTGGCACCTGTCAGATGRKY-3′ and ITSRHR 5′-CACCCRTTTTRGGCTGCATT-3′ (45 °C). Primers were designed with Primer3 (Rozen and Skaletsky 2000), and PCR amplifications were performed on a Bio-Rad MyCycler™ with the following PCR protocol: 96 °C for 9 min; 96 °C for 10 s, 45 or 50 °C for 30 s, 70 °C for 4 min (33 cycles); followed by a final extension of 70 °C for 5 min (1 cycle). Each 25 μl PCR reaction contained 1.0 μl of template DNA (~5 ng μL), 12.5 μl BioMix Red 2× PCR reaction mix (Bioline), 0.325 μl of each primer (10 μM), 0.75 μl of BSA (10 mg ml), and 11.1 μl deionized sterile water. For some reactions, an additional 1 μl of DNA template was added to improve PCR product (final volume 26 μl). PCR products were visualized using 1.0 % agarose gels in 1× SB (sodium borate; Brody and Scott 2004) buffer stained with Gelstar® (Lonza). Amplification reactions for sequencing of nuclear genes were first treated with Exonuclease I and FastAP™ thermo sensitive alkaline phosphatase (Fermentas) using the following thermocycler profile: 37 °C for 60 min, 85 °C for 15 min. Treated PCR products were then cycle-sequenced using BigDye Terminators (Applied Biosystems) run on an ABI-3730XL DNA Analyzer at the Advanced Studies of Genomics, Proteomics and Bioinformatics (ASGPB) facility at UH Mānoa. Sequences are available from GenBank (accession numbers: KC864797 - KC865044).

Sequencing and phasing

For nuclear loci, Phase (Stephens et al. 2001) as implemented in DNAsp 5 (Librado and Rozas 2009) was used to resolve the alleles for each heterozygous colony, when alignment of direct-sequenced forward and reverse reads was possible. The PCR products of heterozygous colonies that could not be phased reliably from direct sequences were ligated into the pGEM®-T Easy cloning vector (Promega) and transformed into JM109 competent cells following the manufacturer's protocol. After blue/white colony selection, up to 12 colonies were screened by PCR to verify an insert of the correct size using the M13 vector primers. Initially, four colonies were sequenced with the original primers. Heterozygote alleles

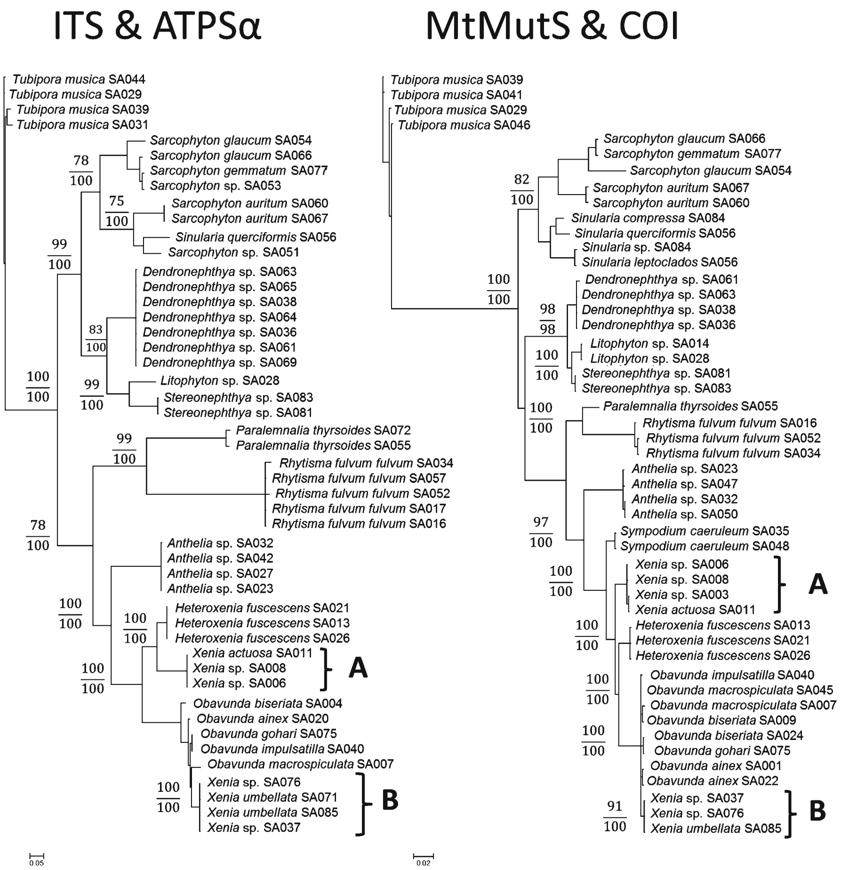
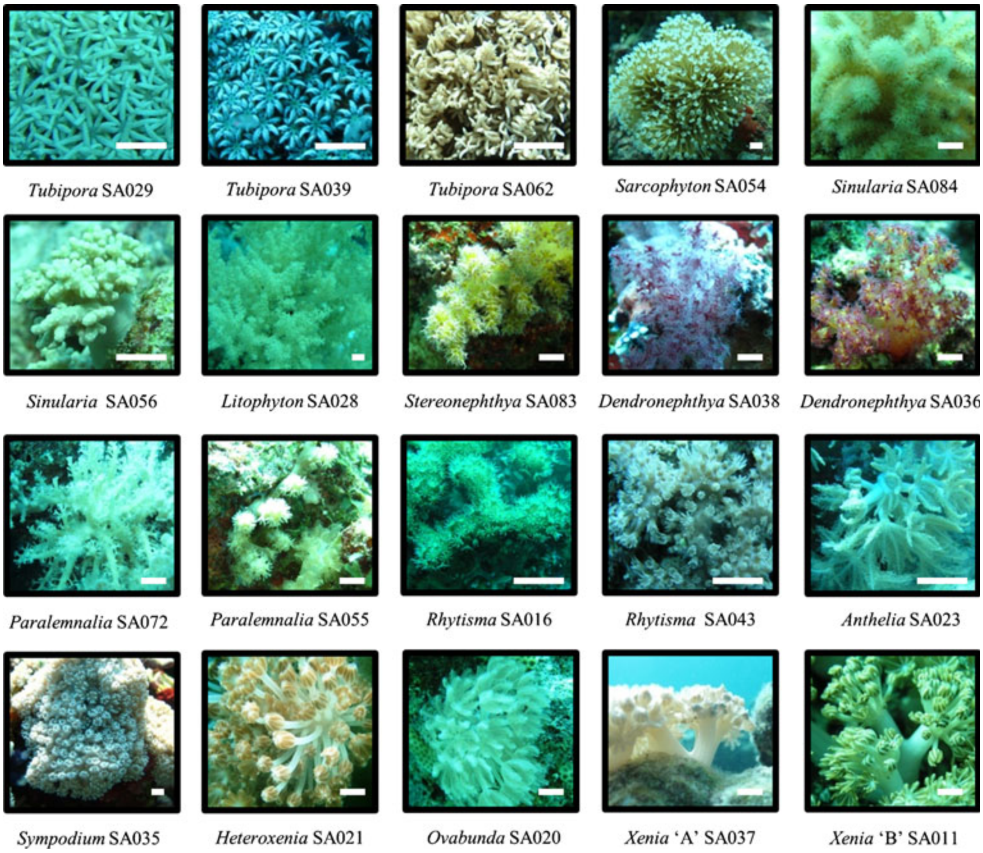


Fig. 2 Phylogenetic relationships among 13 genera in the anthozoan order Alcyonacea (subclass Octocorallia). *Left* combined phylogenetic tree of nuclear loci *ATPSα* and *ITS*. *Right* combined phylogenetic tree of mitochondrial loci *COI* and *mtMutS*. Values at nodes represent: *top* maximum likelihood bootstrap values, and *bottom* Bayesian Inference posterior probabilities. *Xenia* group A and B are marked



Tubipora SA029 *Tubipora* SA039 *Tubipora* SA062 *Sarcophyton* SA054 *Sinularia* SA084

Sinularia SA056 *Litophyton* SA028 *Stereonephthya* SA083 *Dendronephthya* SA038 *Dendronephthya* SA036

Paralemnalia SA072 *Paralemnalia* SA055 *Rhytisma* SA016 *Rhytisma* SA043 *Anthelia* SA023

Sympodium SA035 *Heteroxenia* SA021 *Ovabunda* SA020 *Xenia* 'A' SA037 *Xenia* 'B' SA011

Fig. 3 All non-gorgonian octocoral genera, and morphotypes within genera, that were surveyed along the west coast of the Kingdom of Saudi Arabia in Jeddah, Thuwal, Al Lith and the Farasan Islands. *Scale bars* 1 cm were accepted only if two or more copies of the same allele were found and could be matched with the direct sequence read from the original mixed template. If the first four colonies did not recover two copies of each allele, four additional colonies were sequenced until two copies of each allele were recovered.

Phylogenetic analyses

All mitochondrial sequences were inspected using Lasergene (DNAStar) and aligned using ClustalW 2.0.11 (Larkin et al. 2007) and MUSCLE 3.6 (Edgar 2004). All nuclear sequences were visually inspected using Geneious Pro 4.7.6 (Drummond et al. 2010) and aligned using both ClustalW and MUSCLE plugins in Geneious. Subsequently, phylogenetic relationships were reconstructed using Bayesian Inference (BI) (chain length=1.1×10^6, burn-in length=100,000) and maximum likelihood (ML) (10,000 bootstrap replicates) methods in Geneious Pro. BI trees were generated with MrBayes 2.0.2 (Huelsenbeck and Ronquist 2001), and ML trees were generated from PHYML 1.0 (Guindon and Gascuel 2003). Best substitution models were found with MrModeltest plugin in Geneious Pro. Because both nuclear and both mitochondrial loci fit the same best substitution model, no gene-specific models were selected in BI and ML analyses. In all analyses, the family Tubiporidae was used as outgroup based on earlier analyses (McFadden et al. 2006a).

Results

Classical taxonomy

Based on morphological taxonomic analyses, the survey yielded 28 species from the four families Alcyoniidae (n= 16), Nephtheidae (n=16), Tubiporidae (n=9), and Xeniidae (n=33). Based on our field observations, the outermost reefs in Al Lith had the highest number of species, and the southernmost sites in the Farasan Islands had the lowest number of species.

Several morphotypes were recognized during collections (Fig. 3). For example, we recognized three distinct morphotypes of *Tubipora musica* in the field based on varying polyp structure: (1) polyps with long curving tentacles displaying uniform width and short pinnules, (2) polyps with feathery shaped tentacles, no pinnules proximal to the mouth, and a decrease in pinnule length in the distal part of the tentacles, and (3) polyps with tentacles bearing short pinnules and a soft tissue appearance,waving with the current. We recognized two distinct morphotypes of *Sinularia* in the field based on varying colony structure: (1) short bulky bumpy stalks, and (2) longer finger-like stalks. Furthermore, two color morphs were recognized within the genus *Dendronephthya*: (1) dark red and white, and (2) orange and pink. Two morphotypes were recognized within *Paralemnalia*: (1) branching, and (2) encrusting with short extensions. Finally, we recognized two color morphs within *Rhytisma*: (1) green, and (2) gray.

Phylogenetic analyses

Among the four families collected, the mitochondrial marker *mtMutS* was successfully amplified from 67 colonies and the mitochondrial marker *COI* from 73 colonies (Table 1). The nuclear marker *ATPSα* was successfully amplified from 53 colonies, and the nuclear marker *ITS* from 51 colonies (Table 1).

COI and *mtMutS* provided very similar resolution, and members of different genera were clearly resolved, with the exception of *Ovabunda* and *Xenia* (Fig. 2). *COI* clearly separated 11 out of 13 genera, and *mtMutS* clearly separated 11 out of 12 genera. In both the *COI* and *mtMutS* phylogeny, *Xenia* had two lineages: one within the *Ovabunda* clade, and one as a sister group to *Heteroxenia*. *ATPSα* provided the least resolution among all markers tested, and 3 out of 13 genera were clearly resolved: *Paralemnalia, Rhytisma*, and *Ovabunda* (Fig. 2). *Xenia* colonies were divided into two lineages, one grouped close to *Ovabunda*, and the second part of a large clade containing all remaining genera, including *Heteroxenia*. *ITS* clearly separated 9 out of 12 genera,

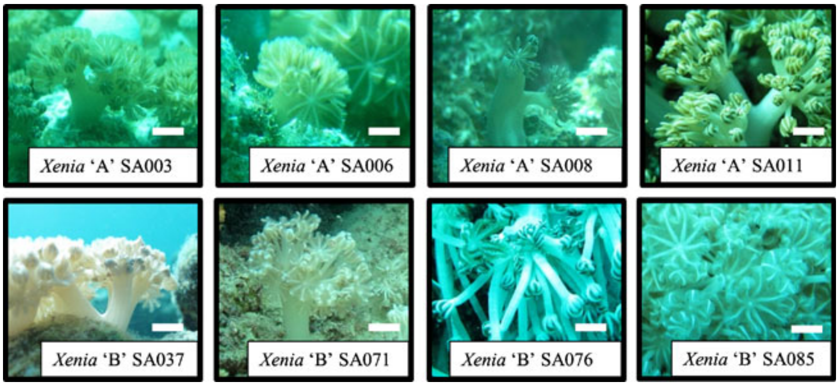


Fig. 4 Colony morphology differences between *Xenia* clade *A* (*top*) and *Xenia* clade *B* (*bottom*). *Scale bars* 1 cm

the exceptions being *Sarcophyton, Ovabunda*, and *Xenia* (Fig. 2).

Mitochondrial (*COI* + *mtMutS*) and nuclear (*ITS* + *ATPS*α) phylogenetic analyses were generally congruent (Fig. 2). In the nDNA phylogeny, but not in the mtDNA phylogeny, two *Sarcophyton* colonies grouped as sister taxa to *Sinularia* with 100 % ML bootstrap support. Furthermore, among all loci, a group of four *Xenia* colonies consistently grouped as a sister group to *Heteroxenia*, whereas all other *Xenia* species sampled grouped within *Ovabunda*. In all analyses, the family Tubiporidae was designated as the outgroup.

Within the genus *Sinularia*, *S. querciformis* revealed a different colony stucture than the other species (*S. leptoclados* and *S. compressa*) (Fig. 3) and all species were genetically different (Fig. 2). The two color morphs within *Dendronephthya* revealed no corresponding genetic differentiation. No *Dendronephthya* colonies were identified to species level, and no intrageneric phylogenetic groups were revealed to suggest cryptic diversity. Likewise, the different morphotypes within *Paralemnalia*, *Rhytisma f. fulvum*, and *Tubipora musica* did not correspond to different haplotypes in the preliminary phylogenetic analysis (Fig. 2).

Among the five Xeniidae genera, *Anthelia, Heteroxenia*, and *Sympodium* were consistently and clearly distinguished with both mitochondrial and nuclear loci, whereas *Ovabunda* and *Xenia* were not. Members of the Alcyoniidae and Nephtheidae consistently grouped in three clades in both mtDNA and nDNA phylogenies. One of these contained the Nephtheidae genera *Dendronephthya*, *Litophyton*, and *Stereonephthya*, the second contained the Alcyoniidae genera *Sarcophyton* and *Sinularia*, and the last was a mixed clade containing *Rhytisma* from Alcyoniidae and *Paralemnalia* from Nephtheidae (Fig. 2).

As an example of how our Saudi Arabian data can function to compare geographic biodiversity with other locations, we combined our *mtMutS* and *COI* dataset with a previous soft coral *mtMutS* and *COI* dataset from Eilat, Israel (McFadden et al. 2011). The majority of the mitochondrial gene haplotypes that were found among Saudi Arabian specimens were identical, or nearly so, to those from Eilat. The few exceptions with different haplotypes in Saudi Arabia were: one of the two distinct haplotypes of *Tubipora*; one of the two specimens identified as *Sarcophyton auritum*; and, among the Xeniidae, *Xenia* group ‘A’ (Figs. 2, 3, 4; discussed further below), *Anthelia* spp., and the *Ovabunda* group of SA007 and SA009.

Discussion

Here, we present our preliminary survey of Saudi Arabian Red Sea alcyonacean octocorals that combines classical taxonomy, molecular studies, and in situ photographs to document a total of 28 species in four families. Concordant with surveys of scleractinian corals (Sheppard 1985; Sheppard and Sheppard 1985, 1991), the lowest cover and diversity was observed at our southernmost site. However, we examined only a portion of the coast covered in the Sheppard’s survey.

In our study we found four families, 13 genera, and 28 species of non-gorgonian octocorals. By comparison, a recent octocoral survey of the Red Sea at Eilat, Israel (Gulf of Aqaba, northern Red Sea) included six families: Alcyoniidae, Briareidae, Nephtheidae, Nidaliidae, Tubiporidae, and Xeniidae, comprising 19 genera and 43 species (McFadden et al. 2011). The latter study included only part of the octocoral diversity known from that region. We attribute the greater diversity on Eilat reefs to the intense studies conducted here (Benayahu 1985; van Ofwegen 2002). A study in the Dahlak Archipelago (southern Red Sea) reported five families: Alcyoniidae, Nephtheidae, Nidaliidae, Tubiporidae, and Xeniidae, comprising 14 genera and 28 species (Benayahu et al. 2002), which is very similar to our finding along the Saudi Arabian coastline. In comparison to octocoral biodiversity surveys in the Red Sea, a survey in the southern Ryukyo Archipelago reported four octocoral families: Alcyoniidae, Briareidae, Clavulariidae and Tubiporidae, comprising 11 genera and 56 species (Benayahu 2002). Furthermore, in comparison to our study with specimens from 14 sites, Benayahu (2002) collected from 18 sites and Benayahu et al. 2002 collected from 17 sites. In a combined analysis of our dataset and a previously published octocoral dataset from Eilat (McFadden et al. 2011), we found that the majority of the mitochondrial gene haplotypes (*mtMutS* + *COI*) that were found among Saudi Arabian specimens were identical, or nearly so, to those from Eilat.

Phylogenetic analyses were generally consistent among nuclear and mitochondrial loci. Nine out of 13 genera that were included in this study were clearly and consistently distinct by both classical taxonomic and molecular systematic data, the exceptions being *Sarcophyton*, *Ovabunda*, and *Xenia*. It is important to note that sister group relationships reported here are tentative because of limited taxon sampling in this preliminary biodiversity survey of octocoral diversity. Regardless of the exact relationships, however, these studies are useful to highlight a number of taxa for additional study, such as the genus *Sarcophyton* which was recovered as monophyletic in mitochondrial analyses, but in the nuclear analyses, colonies of *Sarcophyton auritum* grouped as a sister group to the genus *Sinularia* (Fig. 2). A cryptic clade of *Lobophytum* and *Sarcophyton* species has previously been recognized (McFadden et al. 2006b), and the *Sarcophyton auritum* in the current study falls within that cryptic clade. However, the apparent sister relationship between *Sinularia* and the mixed clade of *Sarcophyton/Lobophytum* (Fig. 2) is not consistent with phylogenies reconstructed previously from both mtDNA and nDNA (McFadden et al. 2009; Brockman and McFadden 2012). We attribute this incongruence to limited taxon sampling in this Saudi Arabian octocoral survey.

Even with limited taxon sampling in this preliminary survey, we discovered clear polyphyly within the nominal genus *Xenia*. Four *Xenia* colonies group as a sister taxon to *Heteroxenia*, hereafter called *Xenia* group 'A', whereas the other *Xenia* colonies group as a sister taxon to *Ovabunda*, hereafter called *Xenia* group 'B' (Fig. 2; Stemmer et al. 2012). After discovering this cryptic diversity, reverse taxonomy (sensu Markmann and Tautz 2005) was applied; that is, we searched for supporting morphological characters that we may have previously overlooked, using phylogenetic patterns as a guide. Interestingly, we were able to detect differences in colony growth forms (Fig. 4), as well as skeletal features between the two *Xenia* groups. Colonies of *Xenia* clade 'A' are always small, the stem and tentacles are not very long, and the color pattern is white tentacles and polyps, and brown pinnules. The specimens of *Xenia* clade 'B' vary in appearance: some have the same colony morphological features as *Xenia* group 'A', but are much lighter in color (SA037, SA071), whereas others have different morphological features than *Xenia* group 'A': the stems and polyp tentacles are much longer, but the color pattern is the same as seen in colonies from *Xenia* group 'A' (SA076, SA085). Furthermore, *Xenia* colonies from group 'B' contain varying microscopic features: SA037, SA076 both contain two rows of pinnules, with 18–19 and 20–24 pinnules, respectively, at the outmost row, whereas SA71 and SA85 both contain three rows of pinnules and 19–26 and 21–27 pinnules, respectively, at the outermost row. Of *Xenia* group 'A', only specimen SA11 could be identified due to poor preservation. The colonies' tentacles have three to four pinnule rows and 22–26 pinnules at the outmost row. Despite the limited identification due to poor preservation, however, we found that all *Xenia* group 'B' colonies contain typical *Xenia* sclerites, whereas none of the *Xenia* colonies examined from group 'A' contain sclerites, thus indicating the importance of sclerites in taxonomic studies of this group. There are no morphological features that support a close relationship of Xenia group 'A' to *Heteroxenia*. Moreover, in a more extensively sampled analysis of *Xenia* including both samples from group 'A' and Xeniidae colonies from Indonesia, the colonies from group 'A' do not group as a sister taxon to *Heteroxenia*, but appear more related to an Indonesian species, *X. lillieae* (C.S. McFadden, unpublished data). Thus, the sister relationship observed in our preliminary analyses is tentative because of limited taxon sampling, but there is evidence of two polyphyletic groups in the nominal genus *Xenia* that merits further attention.

In many cases, variation in colony morphologies complicated the classification process. For example, within both species, *Rhytisma fulvum fulvum* and *Tubipora musica*, and in both genera, *Dendronephthya* and *Paralemnalia*, there was no correspondence between morphological differences among colonies and molecular haplotypes, indicating morphological polymorphism in these groups.

This survey of Saudi Arabian octocorals yields a number of questions for future study, and a wealth of specimens that can be used for future molecular and zoogeographic comparative analyses. Ten out of the 13 octocoral genera were clearly resolved with *ITS* sequence data, 11 out of 13 were clearly resolved using *mtMutS* and *COI*, but only 2 of the 13 were resolved using *ATPSα*. This finding confirms a relatively accurate understanding of the evolutionary relationships among morphological characters used to distinguish these octocoral taxa on generic level, with the exception of our discovery of polyphyly within the genus *Xenia*, which indicates cryptic diversity and merits additional taxonomic attention. Additionally, our sampling of four loci (two nDNA: *ITS*, *ATPSα*; and two mtDNA: *mtMutS*, *COI*) appears to strike the desirable balance between resolution, cost, and labor for future phylogenetic studies of alcyonacean corals, although *ATPSα* may be more useful at higher taxonomic levels. Further research is needed to determine whether Octocorallia mimic scleractinians in the diversity gradients across Saudi Arabia as reported by Sheppard and Sheppard (1985, 1991) and Sheppard (1985), but the trend is suggested by our observations. Overall, our study provides both a preliminary taxonomic survey of the region that includes classical taxonomy, multilocus molecular barcodes, and in situ photographs, and we provide an initial phylogenetic survey to guide future research efforts in the region and on octocoral relationships within the Alcyonacea.

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