INTRODUCTION

Soft corals (Cnidaria, Alcyonacea) are a major component of the sessile coral reef benthos and are highly diverse in tropical Indo-Pacific coral reefs (Dinesen 1983, Fabricius & Alderslade 2001), including the Red Sea (Benayahu & Loya 1977, 1981, Benayahu 1985, Reinicke 1997). Some soft corals of the families Xeniidae and Alcyoniidae contribute to the diet of coral reef fishes (Gohar 1940). Secondary metabolites of some soft corals have been shown to possess ecological functions including anti-predatory protection (La Barre et al. 1986), allelopathy (Sammaroo et al. 1983, 1985) and anti-fouling activity (Changyun et al. 2008, Limna Mol et al. 2010).

These chemical defenses may be as effective as biomineralized skeletons in that they protect hermatypic corals from predation by most reef fishes (Sammaroo & Coll 1992). For example, some Alcyoniidae species (e.g. Sinularia polydactyla, Rhytisma fulvum fulvum) were shown to possess secondary metabolites, which protected the soft corals against predation by carnivorous fish (Wylie & Paul 1989, Van Alstyne et al. 1994, Kelman et al. 1999). A survey by Coll et al.
(1982) showed a high prevalence of toxic species among the soft coral order Alcyonacea (>50% of the species) in the central Great Barrier Reef, suggesting that secondary metabolites, which are active against predators, are common in the Alcyonacea. In an extensive study by La Barre et al. (1986), it was found that the majority of soft coral taxa in the Great Barrier Reef possess defense mechanisms against fish predation, although toxicity and repellence are not always related to each other.

Chemical defense against predation may already be present in eggs, embryos or larvae of some soft corals (Coll et al. 1989, Kelman et al. 1999, Slattery et al. 1999, Lindquist 2002) indicating the importance of chemical anti-feeding defense throughout the life history of soft corals. In addition to their well-studied anti-feeding role, secondary metabolites of soft corals may also serve to combat fouling on the surface (Bhosale et al. 2002, Limna Mol et al. 2010) and may protect corals against viral infections (Ahmed et al. 2013). The conspicuous richness of chemical defenses in soft corals (Rocha et al. 2011) may therefore contribute to their remarkable invasion potential (Lages et al. 2006, Fleury et al. 2008).

In soft corals, chemical defense can be supplemented by mechanical defense such as mucus secretion (La Barre et al. 1986, Sammarco et al. 1987, Harvell & Fenical 1989) or elevated spicule concentration (Van Alstyne et al. 1992). Calcium carbonate spicules are common attributes in Octocorallia, as well as in Porifera, Echinodermata and Asciidacea (Kingsley 1984). The size and shape of the spicules are often species-specific and can be used as taxonomic tools (e.g. Bayer et al. 1983). In soft corals, they are assumed to mainly function as structural support for the polyps and colonies (Lewis & Von Wallis 1991, Van Alstyne et al. 1992, O’Neal & Pawlik 2002), however, they can also function as defensive structures. This was demonstrated for some soft coral species, where fishes rejected sclerites containing artificial food (Van Alstyne et al. 1992, 1994), but not for others (Kelman et al. 1999, O’Neal & Pawlik 2002). The anti-feeding defense by sclerites may be effective only in those parts of the colony where their concentration is particularly high (Puglisi et al. 2000). Where sclerites do play a defensive role, their shape, size and abundance determine their protective efficiency, traits which may differ throughout a coral colony (Sammarco et al. 1987, Van Alstyne et al. 1992, Koh et al. 2000).

The family Xeniiidae is composed of 34 species and is one of the most common and widely distributed octocoral families in the Red Sea (Reinicke 1997). It can cover up to 50% of the substrate in some shallow reef areas (~4 m depth), forming extensive carpets (Bennyahu & Loya 1981, Reinicke 1997). Xeniid ‘colonies’ consist of numerous conspecific individuals occurring side by side (Gohar 1940). The family differs from all other Octocorallia due to the soft, fleshy consistency of the colony and its non-retractile polyps (Ashworth 1899). Some xeniid species lack stinging nematocysts (Janes 2008), which might reduce their capacity for protection against predators (Vermeij 1978, Bakus 1981, McIwain & Jones 1997). Nevertheless, their competitiveness is high, presumably due to their motility as adults, their rapid asexual reproduction (Bennyahu & Loya 1981), and the widespread allelopathy against space competitors and hard coral recruitment (Sammarco et al. 1983, Atrigieno & Alino 1996). Secondary metabolites with antimicrobial (Kelman et al. 1998, 2006) and anti-fouling activity (König et al. 1989) also seem to be common in xeniid soft corals.

Indeed, xeniids, like many other soft coral taxa, are remarkably rich in bioactive secondary metabolites (König et al. 1989, El-Gamal et al. 2005). Some chemical compounds that have been isolated from xeniid species are considered to be useful candidates in the field of medicine, particularly against cancer cells. These include the compounds blumiolide A, B and C of the species Xenia blumi (El-Gamal et al. 2005), different umbellacins of the species X. umbellata (El-Gamal et al. 2006) and different xeniolide of the species X. blumi, X. novaebritanniae and X. umbellata (Bishara et al. 2006). To the best of our knowledge, their potential chemical defense against fish predation, which could contribute to their high abundance in the Red Sea, has not been investigated so far.

In this study, we investigated the chemical defense against fish predation of 2 particularly abundant xeniid species in the Red Sea, Ovabunda crenata and Heteroxenia ghardaqensis. We further studied whether or not chemical defense is enhanced by the presence of sclerites. To this purpose, artificial food was prepared and charged with crude extract of soft coral (1) at natural concentration and fed to the reef fish community in situ, and (2) at natural and reduced concentrations, with and without the addition of sclerites, and fed to the moon wrasse Thalassoma lunare in aquaria.

MATERIALS AND METHODS

Sample collection and identification

Soft coral samples were collected near the city of Jeddah, Saudi Arabia, in the central Red Sea. Coral
(hard and soft) cover in this area ranged from 36 to 61%, of which the family Xenidiaceae comprised 7.5 to 14% (determined by line intercept transects at 3 to 4 m depth). The 2 xenid soft coral species were collected by SCUBA diving in 3 to 6 m depth. *Oxabunda crenata* was collected at off-shore reefs (10 km from the coast) while *Heteroxenia ghardaqensis* was collected near-shore (50 m from the coast), where the respective species dominated the soft coral populations in the reefs. Five replicate samples of each species were collected at ~0.5 kg wet weight sample\(^{-1}\). The samples were brought to the laboratory, the volume was determined immediately by water displacement (live colonies), and their identity was verified under the microscope following the identification criteria of Reinicke (1997).

### Chemical extraction

Extraction was carried out in 2 steps in order to guarantee maximum metabolite extraction of a wide polarity spectrum. For the first extraction, fresh samples (whole colonies) were immersed in ethyl acetate for 24 h at room temperature (Lages et al. 2006). The gained crude extract was filtered through a paper filter and the solvent was removed with a rotary evaporator. The extracted coral tissue was stored in a freezer at −20°C until further processing. A second extraction followed, in which the frozen coral sample was freeze-dried, chopped into small pieces of about 0.5 cm\(^3\) and weighed (dry weight). The tissue was then immersed in a solvent consisting of a 1:1 (v/v) mixture of dichloromethane and methanol (DCM: MeOH) for 24 h at room temperature (Wylie & Paul 1989). This second crude extract was also filtered through filter paper, and the solvent was evaporated until dry. The first and second crude extract were combined, weighed and stored at ~20°C until assays were performed. The calculation of the natural concentration of crude extract was based on the volume of samples. The values were 32 mg ml\(^{-1}\) for *O. crenata* and 35 mg ml\(^{-1}\) for *H. ghardaqensis*, and were used as a reference for the preparation of the food pellets.

### Sclerite preparation

We prepared sclerite samples from *O. crenata* only, as *H. ghardaqensis* does not contain sclerites. In order to obtain pure samples, each colony of *O. crenata* was cut into small pieces and immersed in 12% sodium hypochlorite to dissolve the tissue and leave the sclerites. After 12 h, the supernatant was carefully decanted and fresh sodium hypochlorite was added. This process was repeated until the tissue was completely dissolved and the sclerites remained on the bottom of the tube. Sclerites were collected and rinsed 3 times with distilled water, dried in an oven at 80°C until completely dry, and weighed. The natural concentration of sclerites was calculated by dividing the dry weight of sclerites by the dry weight of the colony.

### Field assay

The frozen crude extract was re-dissolved in ethanol. Food pellets were produced following Pawlik & Fenical (1992) with some modifications: the basis of the food pellets was made by mixing and boiling 1.30 g phytagel (Sigma-Aldrich), 1.38 g of freeze-dried powdered squid and 30 ml distilled water. After the mixture cooled to ~40°C, the crude extract dissolved in ethanol (1.1 ml of *O. crenata* or 1.08 ml of *H. ghardaqensis*) was added at the natural concentration found in the soft coral tissue. The viscous mixture was poured into a plastic mould containing a piece of mosquito net with a mesh size of 1 mm\(^2\). After the matrix cooled down, the solidified gel was removed from the mould and cut into pieces of 3 different sizes: 1, 2 and 3 cm\(^2\). From each extract (n = 5), 3 pellets were made, resulting in a total of 15 pellets (replicate and sub-replicate) for each species.

The feeding assay was conducted at the same off-shore reef and at the same depth where the xenid samples had been collected. The procedure was similar to the method described by Van Alstyne et al. (1992, 1994), where pellets were individually weighed and fixed to a fishing line. Each size class (1, 2 or 3 cm\(^2\)) was represented as a pair with one pellet containing crude extract and the other pellet (of identical size) containing ethanol only. The distance between the pellets within a pair was 5 cm and the distance between pairs was 25 cm. A buoy at one end and a weight at the other end held the rope in a vertical position in the reef. The lowest pair was 1 m above the ground. The feeding activity of the reef fish was observed by SCUBA divers from a distance of ~3 m. The ropes were re-collected after one of either the control or treatment pellets on each rope were eaten completely by reef fishes. The pellets were re-weighed to determine percentage consumed.
Aquarium experiment

The food pellets for the aquarium experiment were made following Pawlik et al. (1995). The crude extract, dissolved in ethanol, was mixed with 0.3 g alginic acid and 0.5 g powdered squid. Distilled water was added to obtain a final volume of 10 ml. The mixture was stirred until it was homogeneous, and then loaded into a 10 ml syringe. The tip of the syringe was immersed into a 0.25 M CaCl_2 solution and the content of the syringe was slowly expelled into the CaCl_2 solution to form noodle-like food pellets. After several minutes, the solidified ‘noodles’ were rinsed with sea water and cut into pieces 2 to 5 mm long.

The effectiveness of the anti-feeding activity was tested with different concentrations of crude extract in the food pellets. This was done to determine the efficiency of secondary metabolites, which may vary in concentrations within the soft coral tissue seasonally, among populations, among organs and/or among life stages (Slattery et al. 1999, 2001). Thus, we produced pellets with 100, 50, 25, and 12.5% of the natural extract concentration. In order to assess the potential anti-feeding effect of the sclerites, sclerites were added to the food pellets (without extract) in their natural concentration (0.13 g sclerites g^{-1} soft coral dry weight). Additionally, sclerites were added to food pellets containing reduced concentrations of crude extract (25% of the natural concentration) in different concentrations (50, 100, and 200% of natural sclerite concentration) to determine the potential interactive effect of sclerites and secondary metabolites. We used 25% extract concentration so as not to mask any potential sclerite effect by a dominant chemical effect.

The feeding experiments in aquaria were carried out in Kiel, Germany, using the climate rooms of the GEOMAR institute. The moon wrasse *Thalassoma lunare* (purchased from Aqua Inspiration), was chosen because it is an abundant species in the central Red Sea and known to be a generalist feeder on a wide assortment of benthic invertebrates including soft corals (Randall 1983, Rotjan & Lewis 2008). Furthermore, this species has been used frequently for aquarium bioassays (Pawlik et al. 1987, Harvell et al. 1988, Kelman et al. 1999, Epifanio et al. 2007) due to its wide prey spectrum, its fast adaptation to aquarium conditions, and slow satiation (Pawlik et al. 1987). Each fish (n = 9) was placed in a separate aquarium filled with 40 l artificial sea water with 35 psu salinity, temperature 25°C and a 12 h light:12 h dark rhythm. The feed choice test was conducted by alternatingly feeding the fish control and treatment pellets loaded with extract and/or sclerites. In case the fish ignored the treatment pellet, another control pellet was offered in order to discriminate between the repellence of the treatment pellet and satiation. A pellet was considered rejected when it was ignored or spit out by the fish and the fish consumed a control pellet thereafter. The feeding tests were repeated with 10 control and 10 treatment pellets at once with each of the 9 fish, and the number of pellets consumed or rejected was recorded. Different treatments were tested on different days, with 3 to 5 d rest between each test. During resting time, fish were fed with artificial fish fodder.

Analyses

The feeding deterrence in the field assays was assessed using paired *t*-tests, by comparing the consumption rates on the pairwise deployed pellets containing or not containing extract. The consumption of pellets containing extracts from either of the 2 species was compared by *t*-test as well. Because the data of the extract-loaded pellets were used twice, the alpha-level was Bonferroni-corrected to α = 0.025. Only the data of the largest food pellets were used because the control pellets were not entirely consumed at the end of the experiment i.e. the fishes could choose between extract-loaded and extract-free pellets throughout deployment.

The learning capacity of fishes in the aquaria experiment, which were repeatedly (10×) fed an identical extract pellet (intermittently with a control pellet), was assessed as the % decrease of acceptance between successive offerings during a given test day (i.e. increasingly experienced fish) relative to the acceptance at the first offering of an extract-loaded pellet (i.e. naive fish). These slopes were calculated for the pellets containing 25% of the natural extract concentration, because with full concentration the acceptance in most cases reached zero too early to calculate reliable slopes, and at concentrations below 25%, repellence and learning were almost absent. Because the fish showed some learning capacity, only the acceptance or rejection of the first treatment pellet (i.e. the reaction of a naive fish) was used for the statistical assessment of the extract defense strength.

The discrimination between control and extract pellets was tested by Fisher’s exact test for the 2 soft coral species separately. Replication was done on the
extract side (n = 5 colonies extracted) and on the con-
sumer side (n = 9 individual fish tested). This pro-
cedure assessed the difference in proportion of con-
sumed relative to rejected pellets between pellets with versus without extracts for the 5 replicate
extracts per soft coral species offered to 9 fish. Analy-
theses were performed with the software Statistica v.8
(StatSoft).

RESULTS

In the field experiment, a mean (±SE) of 97 ± 2.5
and 92 ± 2.9% of the control pellets were eaten by
the reef fishes, while only 14.4% (±3.9) and 8.7%
(±3.2) of the extract containing pellets of Ovabunda
crenata and Heteroxenia ghardaqensis were eaten,
respectively (Fig. 1). The repellent effect was signifi-
cant for both species (p < 0.001; Table 1). The repel-
lenity between the food pellets containing extracts of
the 2 coral species at natural concentration did not differ (p = 0.53; Table 1). The main fish species
observed feeding on the pellets were Thalassoma
lunare, T. rueppellii, Pomacentrus sultureus, Suffla-
men albicaudatum, Oxycheilinus digramma, and
Cephalopholis argus. In addition, some allegedly
herbivorous fishes such as parrot fishes and surgeon
fishes occasionally fed on the pellets.

In the aquarium experiment, the fish quickly
adapted to the new condition and readily accepted
the control food pellets (without extract). For most
concentration levels of both crude extracts, most fish
individuals learned to recognize and avoid the deter-
rent pellets during the series of 10 subsequent
encounters with a given pellet type. For the 25%
natural concentrations, we assessed the slope of de-
creasing acceptance by increasingly experienced
fish. The average slopes of the learning curves of the 9 fish
i.e. increasing rejection with increasing experience, were −3.3
(±1.6 SE) and −8.6 (±2.7) for H. ghardaqensis and O. cre-
nata extracts, respectively. This meant that each time the fish
faced a further extract-loaded pellet they accepted it on aver-
age 3.3 to 8.6% less often than at the preceding encounter.
Between the 1st and the 10th encounter, the acceptance thus
decreased by 33 and 86% for H. ghardaqensis and O. crenata
extracts, respectively. The difference in learning speed of fish with regard to the 2 potential prey
species was, however, not significant (t = 1.6, df = 11,
p = 0.13).

At the first encounter between a pellet and a naive
moon wrasse, the control pellets were always eaten,
while the treatment pellets containing the natural
concentration of crude extracts were rejected to
different degrees. On average, only 21 ± 6.4% of
the pellets containing crude extract of O. crenata and
26 ± 8% of the pellets containing crude extract
of H. ghardaqensis were consumed by naive moon
wrasses (Fig. 2), which in both cases was significantly
less than the feeding on control pellets (Fisher’s exact
test, p < 0.001).

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<td>−15.677</td>
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Table 1. Results of t-tests comparing the consumption of Ovabunda crenata and
Heteroxenia ghardaqensis extract-loaded versus extract-free pellets, and the con-
sumption of extract-loaded pellets between species.
The deterrent activity decreased with decreasing crude extract concentration for both soft coral species (Fig. 2). This trend appeared to be slightly stronger for the *Heteroxenia ghardaqensis* extract compared to *Ovabunda crenata*, but both species were significantly distasteful even at the lowest tested concentration of crude extract (12.5%). Comparing only the treatment pellets of the 2 species (Fig. 2), *Ovabunda crenata* extracts appeared to be less repellant than *Heteroxenia ghardaqensis* extracts. However, this difference was significant at only the 25% natural concentration (Fisher’s exact test, *p* < 0.01).

Sclerites of *Ovabunda crenata* did not affect the feeding behavior of moon wrasses at any sclerite concentration (50, 100 or 200% of natural sclerites) when added to food pellets without coral extract or in combination with 25% of crude extract concentration (Fig. 3).

**DISCUSSION**

Our results show that the crude extracts from 2 highly abundant soft coral species in the Red Sea, *Ovabunda crenata* and *Heteroxenia ghardaqensis*, strongly deter reef fishes from feeding on their polyps. This protective effect was not only detected at natural concentrations but even at 4-fold reduced concentrations, highlighting the efficiency of the involved secondary metabolites. Consequently, these soft coral species are likely to be well-defended against fish consumption, even if the defense metabolite concentration fluctuates to some extent among individuals, populations, life history stages or seasons. This anti-feeding defense most likely contributes to the success and remarkable abundance of these soft coral species in the reefs along the Saudi Arabian Red Sea coast. Sclerites, in contrast, did not show any deterring effect against fish predation in *Ovabunda crenata*. A negative relationship between sclerite armament and chemical defense, suggestive of a defensive role of the sclerites, had previously been reported by Sammarco et al. (1987) for some soft coral taxa (*Sinularia, Lemnalia, Heteroxenia*).

The similarity of the results found in both the field and aquarium assays suggests that the secondary metabolites of xeniid soft corals are broadly distasteful repellents that are effective against predation by...
It is conceivable that the chemical repellence is complemented by other morphological or behavioral protective adaptations in these soft coral species. In both regards, the 2 species differ to a certain degree. Colonies of *O. crenata* reach a total height of 3 cm, while *H. ghardaqensis* colonies can reach 12 cm in height. *O. crenata* polyps do not show any pulsating activity, whereas the polyps of *H. ghardaqensis* feature continuous pulsation (Gohar 1940, Reinicke 1997). Whether these traits increase or decrease the species’ susceptibility to fish consumption is presently unknown. Furthermore, many xeniid species are known to release mucus upon mechanical stress (Gohar 1940, Ducklow & Mitchell 1979). If this mucus bears olfactory signals it might enhance the avoidance behavior of reef fishes. Another distinctive property with potential relevance to predation is that *O. crenata*, in contrast to *H. ghardaqensis*, possesses sclerites. These, however, did not affect fish feeding in the aquarium experiments, even at double the natural concentration. In contrast, the presence of calcareous sclerites in other prey species was reported to enhance the efficiency of chemical anti-feeding defenses by neutralizing the digestive enzymes in the stomachs of various consumers, including fishes (Hay & Kappel 1991, Van Alstyne et al. 1992). On the other hand, the soft coral *R. f. fulvum*, which contains sclerite concentrations of almost 80% of tissue dry weight, did not deter feeding (Kelman et al. 1999). Reasons for the lack of anti-feeding activity of the *O. crenata* sclerites may be (1) that the natural (13% of coral dry weight) and even the doubled sclerite concentration is too low to affect the predator’s enzymatic functionality, and/or (2) that the sclerite size and shape may be harmless to predator fishes (Van Alstyne et al. 1992). The latter reason is supported by results from Burns & Ilan (2003), who found that sponge spicules deterred fish only when larger than ~250 µm. The size of *O. crenata* sclerites in this study were below 50 µm in length and the sclerite morphologies were simple flat discs of round to oval shape (Reinicke 1997, Halász et al. 2014), and might, therefore, only play a role as structural support (Lewis & Von Wallis 1991, Van Alstyne et al. 1992). In *H. ghardaqensis*, structural support is provided by the mesoglea, which is particularly strong and well-developed compared to the mesoglea of other xeniid species (G. B. Reinicke pers. comm.).
In conclusion, the chemical defense of the 2 xeniid species clearly prevents fish-feeding, while the sclerites, where present, seem to serve only as structural support or have other functions unrelated to defense. The high anti-feeding efficiency of the metabolites most certainly contributes to the robustness, perseverance and considerable abundance of xeniid species in the Red Sea. The chemical repellency of the soft corals may be enhanced by the capacity of the fish to learn.

Data archive. Data sets to this article are available under http://doi.pangaea.de/10.1594/PANGAEA.841563

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