Trace Elements in *Corallium* spp. as Indicators for Origin and Habitat

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Abstract

Precious corals have been commercially exploited for many centuries around the world. The skeletons of these corals consist of calcium carbonate, and have been used as amulets or gemstones since ancient times. Different *Corallium* species of Coralidae family (e.g., *Corallium rubrum*, *Corallium elatus*, *Corallium konojoi*, and *Paracorellium japonicum*) were collected from different locations of the Mediterranean Sea (off Italy) and Pacific Ocean (off Japan and off Midway Island), and trace elements in their skeletons were analyzed. Results show that trace element concentrations in the skeletons of *Corallium* spp. were attributable to their habitat and origin. In particular, Mg/Ca and Ba/Ca ratios in the skeletons of *Corallium* spp. from the Mediterranean Sea and Japanese and the Midway Islands’ waters were found to be habitat-specific. This study also reveals that trace elements in the skeletons can be used as ecological indicator of the coral’s origin, and are expected to play an important part in the cultural study and sustainable management of precious corals. Findings of this study will also be of great relevance to the coral industry to authenticate and identify the habitat and origin of the corals.

Keywords: Precious coral, Coralidae, *Corallium* spp., Trace element, Habitat
1. Introduction

Precious corals are some of the most valuable living marine resources, and are harvested only in limited areas in the world. They belong to the functional group of deep corals and are important structure-forming organisms that provide shelter for other organisms and increase marine biodiversity (Tsounis et al., 2010). Precious corals are different from reef-forming corals in that their skeletons are closely-packed with high magnesium calcite, while the reef-building corals consist mostly of aragonite, and are porous because of its loosely-packed crystals.

Taxonomically, precious corals belong primarily to three orders of the class Anthozoa, and the most valuable species are red and pink corals of the genus *Corallium* and *Paracorallium* of the family Coralidae, which consist of 19 and 7 species, respectively (Tsounis et al., 2010). They are found mainly in the Mediterranean Sea and Pacific Ocean (Japanese waters and off Taiwan, off the Midway Islands and off the Hawaiian Islands) (Iwasaki and Suzuki, 2010). Some of the important precious corals include *Corallium rubrum*, *Paracorallium japonicum*, *Corallium elatius*, *Corallium konojoi* and *Corallium secundum* (Tsounis et al., 2010). The *Corallium* spp. are commonly known as deep-sea coral, and the red coral (*C. rubrum*) is produced in the Mediterranean Sea. The pink coral (*C. secundum*) is distributed in the seas around Hawaii and the Midway Islands, and also found in the waters close to the Midway Island (Grigg, 1993).
Japanese red coral (*P. japonicum*), pink coral (*C. elatius*) and white coral (*C. konojoi*) are distributed and harvested in waters near Japan (Iwasaki et al., 2009). *Paracorallium japonicum* is found at depths of 76-280 m on the rocky bottom in Sagami Bay (Pacific coast of Japan), in the waters from the Ogasawara Islands (Japan), and off the coast near the Goto Islands, Nagasaki (Japan) (Seki, 1991). *Corallium elatius* is distributed on the rocky bottom at a depth of 100-276 m in the waters near Wakayama (Pacific coast of Japan), from the Ogasawara Islands (Japan) to the northern South China Sea, and off the Goto Islands, Nagasaki, Japan (Iwasaki and Suzuki, 2010). *Corallium konojoi* is distributed on the rocky bottom at a depth of 76-276 m in the waters of Wakayama (Pacific coast of Japan), in the waters from the Ogasawara Islands (Japan) to the northern South China Sea, and off the Goto Islands, Nagasaki, Japan (Seki, 1991; Nonaka et al., 2004). *Corallium secundum* has been found to grow on flat exposed substrata whereas *C. regale* prefer encrusted uneven rocky bottom habitat in the Hawaiian Islands, and both species are absent from the shelf areas (<400 m depth) (Grigg, 1974).

Some *Corallium* species have a hard calcium skeleton of intense red and the others are pink and of pink (Iwasaki and Suzuki, 2010). Both spicules and skeletons of red coral (*C. rubrum*) are mainly made of calcium carbonate (CaCO₃) crystallized in the form of calcite, though small amounts of other trace elements such as magnesium (Mg), strontium (Sr), iron (Fe), aluminum (Al) and sulphur (S) are also found (Maté et al., 1986). Previously, Velimirov
and Bohm (1976) analyzed calcium (Ca) and Mg by atomic absorption spectroscopy and ethylenediaminetetraacetate (EDTA) titration with the aim of providing information on the mineral composition of gorgonians and the possible variations in different growth regions. They showed that CaCO₃, MgCO₃ and total mineral content increase markedly from branch to stem. Weinbauer and Velimirov (1995) determined Mg, Ca, and Sr in sclerites of four Mediterranean gorgonians and suggested that Mg/Ca and Sr/Ca ratios were very low (0.064-0.098 and 0.004-0.0025, respectively). They also revealed that calcium concentrations did not vary with geographical origin, while the variations of Mg/Ca and Sr/Ca ratios were related to water depth. Besides, there was a direct relationship between Mg concentration and temperature, and the Mg/Ca ratios increased significantly with the ambient water temperature (Weinbauer and Velimirov, 1995).

Reef-building coral is better understood, and concentrations of trace elements in its carbonate skeletons have been determined. The validity of their use as indicators of past environmental conditions, such as water temperatures, nutrients and pollution levels has been confirmed in earth and environmental science studies (Weber and Woodhead, 1970; Weber, 1973; Mitsuguchi et al., 1996; Mitsuguchi et al., 2001; Mitsuguchi et al., 2003). In contrast, studies on trace elements in precious coral have been focused mostly on Mediterranean red coral C. rubrum (Weinbauer and Velimirov, 1995; Weinbauer et al., 2000). Studies on trace elements in other precious corals, especially the Japanese white coral (C. konojoi), from
various locations is limited. Research on precious corals in Japanese waters has recently been started.

Precious corals have attracted worldwide attention as sparse biological resources, and Corallidae have been recently proposed for inclusion in Appendix II of the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) that regulates the international trade in endangered species by listing them in its appendices. The problem is that appendix II permits the export and/or import of corals from well managed stocks, while it prohibits that corals from unmanaged areas can pass the costumes borders. Therefore, appropriate scientific methods for the authentication of the uniqueness and origin of Corallidae are necessary to protect the coral resources and for international trade. This refers to both geographical and bathymetric origin, because shallow coral may be protected in one area, while deeper stocks may be harvested for commercial uses. In the present study, trace element concentrations in the skeletons of Corallium spp. of Corallidae family from different geographical locations (origins) were determined to investigate if the concentrations and distribution of trace elements were related to their origin and habitat. The identification of the origin of the corals via trace metal analysis will provide the opportunity to reveal smuggling of illegal corals with fake papers.

2. Materials and Methods
2.1. **Sampling site**

Samples were collected from Japanese waters, the Midway Islands’ waters and the Mediterranean Sea (off Italy) from fishermen, coral traders, and research institutes. A deep-sea coral (*Corallium* sp.) was collected by Marine Geological Research Vessel “Hakurei Maru” cruise GH85-1 conducted by the National Institute of Advanced Industrial Science and Technology. Two specimens of Japanese red coral (*P. japonicum*) were collected by a manned submersible “Hakuyo” from the Kochi Prefectural Deep Seawater Laboratory. The species and the locations from where the samples were collected are shown in Table 1.

Corals of the Mediterranean Sea and Japanese waters were sampled from a depth of < 150 m, while some samples of the Midway Islands were collected from a depth of 400-500 m and the other were from a depth of 900-1200 m. White coral (*C. konojoi*; Fig. 1) was collected from a depth of 100 m, off Cape Muroto, Kochi, Japan, in July 2004.

2.2. **Chemical analysis of skeleton composition**

Barium (Ba), Ca, Mg, and Sr concentrations were analyzed in skeleton of the corals. Each of the skeletons was ground in an agate mortar into a particle size of 5 mm in diameter and 0.1 g of it was taken into 10-mL polypropylene test tubes with three replications. The skeletons of precious corals were then cleaned following sequential methods of ultrasonic, oxidation, and reduction treatments (*Shen and Boyle, 1988*). At first the samples were treated
with ultrasonic waves in 1 mL of purified water and then in 1 mL of 0.2 M nitric acid for 10 min each. The samples were rinsed with purified water between the treatments. This procedure was repeated three times. After drying at room temperature, they were ground further in the agate mortar and were sieved through a 25-50 Teflon mesh screen of a polypropylene sieve. The samples were then collected from three different sieved samples in the same colony ($n = 3$).

After ultrasonic cleaning, the samples in 10-mL polypropylene test tubes were oxidized by addition of 1 mL solution prepared from 1:1 (v/v) 30% hydrogen peroxide ($\text{H}_2\text{O}_2$) and 0.2 M sodium hydroxide ($\text{NaOH}$). They were then placed in a steam and an ultrasonic bath for 2 min each for a total of 10 repeats. They were then sequentially treated with ultrasonic waves in 1 mL of 0.2 M nitric acid for 3 min, in 1 mL of purified water for 10 min and in 1 mL of 0.2 M nitric acid for 3 min, and then the oxidation treatment was applied once again. A reduction treatment was followed by oxidation treatment in which the samples were treated with 1 mL solution containing 97% hydrogen, concentrated ammonia, and 0.3 M citric acid in the ratio of 1:6:3. They were then placed in a hot (70 °C) and an ultrasonic bath for 2 min each for a total of 16 repeats. Finally the samples were cleaned by repeating oxidation treatments, treating ultrasonic waves (2-min) in 1 mL of 0.2 M nitric acid three times, and rinsing twice in 1 mL of 0.2 M nitric acid. Then the samples were dissolved in 1 mL of 2 M nitric acid.
The sample solutions were diluted to 1000 times with 0.5 M nitric acid, and the determination of trace element concentrations in the samples were carried out by inductively coupled plasma atomic emission spectroscopy (ICP-AES, Perkin Elmer, Optima 3300XL) in triplicates using a calibration curve method.

2.3. Analysis of inorganic elements with electron probe micro-analyzer (EPMA)

Each skeleton sample with dried organic tissues attached on the surface of white coral (C. konojoi) was embedded in polyester resin for EPMA. The skeletons were cut at 5 mm intervals perpendicular to the growth direction using a diamond saw, and one of the thin slices was ground to 100-200 μm with 600, 1000, and 2000 grits silicon carbide abrasive papers. The surface of the samples was then polished to a mirror finish using an alumina wrapping sheet (Marumoto Kogyo, Japan) with a particle diameter of 0.1 μm, and was coated with a 10-μm carbon film by evaporative deposition of carbon. EPMA was performed using an EPMA-8705 (Shimadzu Corporation, Japan). Two-dimensional images of elemental distribution were obtained by stage mapping the sample along the x and y axes with 29 μm raster spacing. Measurement parameters were set as follows: accelerating voltage at 15 keV, beam current at 0.3 μA, and a measurement time 0.04 sec. for Ca and Sr, and 0.22 sec. for Mg and Ba.
3. Results and Discussion

3.1. Trace element distribution in the skeletons of precious coral

Ca and Mg measurements in the cross-section of white coral (*C. konojoi*) skeleton reveal that Ca is distributed homogeneously (Fig. 1C), while Mg concentration is distributed concentrically forming growth rings (Fig. 1B). About 38 growth rings of Mg were observed in tiers on a 6 mm radius. If these growth rings are formed annually, the radial growth rate of this white coral will be 0.32 mm yr⁻¹. This rate agrees with the radial growth rate of the same coral obtained through infrared spectroscopy using synchrotron radiation as well as through ²¹⁰Pb dating (Hasegawa et al., 2010). As in *C. rubrum*, annual rings can also be observed by staining the organic matrix with toluidine blue. Vielzeuf et al. (2008) performed EPMA mapping on the skeleton of *C. rubrum* and found a negative correlation between Mg concentrations and the organic matter, except in the center part of the skeleton, indicating that Mg concentrations corresponded to annual rings. Thus, Mg growth rings can be considered as annual rings of precious coral.

Mg-rich layers grow in warm seasons, and the variations in Mg concentrations in the skeleton are very likely to be related to water temperatures. Weinbauer and Velimirov (1995) compared the Mg/Ca ratios in the skeletons of several *C. rubrum* species from different depths and found a positive correlation between the Mg/Ca ratios and water temperatures. Variations in Mg concentrations within an individual coral skeleton can be assessed as the
Mg/Ca molar ratio. The Mg/Ca ratio in white coral varies from 10 to 15% of the mean values, while that in *C. rubrum* obtained from EPMA study of Weinbauer et al. (2000) varied from 13 to 30%.

Other than these elements, Sr, Ba, I, and Mo concentrations have also been confirmed in Japanese red coral by two-dimensional images produced in a synchrotron-radiation X-ray Fluorescence (XRF) study (Hasegawa et al., 2010). These elements are distributed homogeneously across the cross-sections of the skeleton, and the variations of their concentrations are lower compared to Mg concentration. Weak correlation between Sr concentration and the number of growth rings on the skeleton of Japanese red coral obtained by XRF mapping analysis has been reported by Hasegawa et al. (2010). The present study also reveals a weak correlation between Sr concentration and growth rings on the skeleton of white coral obtained by EPMA analysis (Fig. 1E). The millimeter-scale variations of the Sr/Ca ratio do not correspond to those of growth rings, which is between 100 and 200 μm. On the other hand, the Sr/Ca ratio in the skeletons of *C. rubrum* has been reported to vary significantly in proportion to skeleton density (Weinbauer et al., 2000).

### 3.2. Trace element compositions reflecting the characteristics of coral habitats

The Mg/Ca and Ba/Ca ratios in the skeletons of *C. rubrum*, Japanese red, pink and white corals, and Midway corals are shown in Figure 2. Japanese red, pink and white corals co-habit
in sea-floors around Japan. The Mg/Ca and Ba/Ca ratios in these three corals collected from
the same area were within similar ranges without species-specific differences. On the other
hand, Mg/Ca and Ba/Ca ratios in corals from the Mediterranean Sea, Japanese waters and the
sea around the Midway Islands differed depending on their habitats (Fig. 2) except Sr/Ca ratio
(0.31-0.33 × 10^{-2} \text{ mol mol}^{-1}). Mg/Ca and Sr/Ca ratios in the skeletons of precious corals (C.
rubrum and Japanese red, pink and white corals) of present study determined by EPMA
analysis are well agreed with those in the skeletons of \textit{C. rubrum} measured by XRF
(Weinbauer and Velimirov, 1995). This study also showed that Ba/Ca ratio in the Midway
deep-sea corals is higher compared to that in other samples.

The Mg/Ca ratios in precious corals of the Mediterranean Sea and Japanese waters
ranged from 10-15 × 10^{-2} \text{ mol mol}^{-1}, while those in pink and white corals of the Midway
Islands were in the ranges of 9-11 × 10^{-2} \text{ mol mol}^{-1} and 8-9 × 10^{-2} \text{ mol mol}^{-1} for 400-500 m and
900-1200 m (deep-sea corals), respectively. Because Mg and Ca are the major salts in
seawater, and Mg/Ca ratio is almost constant in all parts of the ocean, it is extremely unlikely
that the observed variations in the Mg/Ca ratio were influenced by the Mg/Ca ratio in
seawater. In a previous study, Weinbauer and Velimirov (1995) observed that the Mg/Ca ratio
in the skeletons of \textit{C. rubrum} was usually related to ocean’s depth. They incorporated other
reports and estimated that the Mg/Ca ratio in \textit{C. rubrum} was directly proportional to water
temperature, increasing by 0.004-0.006 \text{ mol mol}^{-1} per 1 ^{\circ} \text{C}. Water temperatures near the
sediments, where the coral samples for the present study were collected (Table 1), were in the range of 13-24 °C in the Mediterranean Sea and Japanese waters, and 8-11 and 2-3 °C at a depth of 400-500 m and 900-1200 m in the Midway Islands, respectively. The difference in the water temperature could be predicted by the Mg/Ca ratios assuming that an increase of 0.004-0.006 mol mol⁻¹ Mg/Ca ratio per 1 °C was applicable to all precious corals in the subclass Octocorallia. The difference in temperature between the Mediterranean Sea/Japanese waters and the Midway shallow area is 2-16 °C, and between the Mediterranean Sea/Japanese waters and the Midway deep area is 10-22 °C, which can be calculated as 0.008-0.096 and 0.040-0.132 mol mol⁻¹ of Mg/Ca ratios, respectively. Because these figures are close to the difference in the Mg/Ca ratios in this study (Fig. 2), variations in the Mg/Ca ratio of precious corals are likely to be explained by difference in water temperature during coral formation.

Ba/Ca ratio in the Midway coral from a depth of 900-1200 m was significantly higher (10-15×10⁻⁶ mol mol⁻¹) than that of Midway coral from a depth of 400-500 m (5-8×10⁻⁶ mol mol⁻¹) and Japanese corals from a depth of 100-150 m (2-6×10⁻⁶ mol mol⁻¹). In contrast, the Ba/Ca ratios in C. rubrum showed a wider range (4-14×10⁻⁶ mol mol⁻¹). The proportions of Mg, Ca, and Sr concentrations, the major constituents of seawater, are almost identical to those in seawater, while Ba concentration increases with the depth of the ocean. For example, dissolved Ba concentrations of 30-40 mol kg⁻¹ at 50-200 m, 40-55 mol kg⁻¹ at 400-500 m, and 90-110 mol kg⁻¹ at 900-1200 m were reported from Corallium (what species) in the North...
Pacific (Chow and Goldberg, 1960; Boyle et al., 1976). These figures agree well with the Ba/Ca ratios in the skeletons of analyzed precious corals collected from the waters around Japan and around the Midway Islands.

Some overlaps of Mg/Ca and Ba/Ca ratios in corals from the Mediterranean and Japanese waters were observed in the present study. The data presented in Fig. 2 showed that the Mg/Ca and Ba/Ca ratios in only 5 samples of Japanese corals and 6 samples of the Mediterranean corals are in the range of 12-13x10^-2 and 4.6-6.0x10^-5 mol mol^-1, respectively, while 33 samples of the Japanese corals and 13 samples of the Mediterranean corals are distributed separately. One sample of the Japanese coral with 12.5x10^-2 mol mol^-1 of Mg/Ca and 6.6x10^-5 mol mol^-1 of Ba/Ca, and one sample of Mediterranean corals with 15.8x10^-2 mol mol^-1 of Mg/Ca and 3.9x10^-2 mol mol^-1 Ba/Ca might be exceptions. Thus, some degree of error was unavoidable in the same manner as other scientific methods for identification of origin and habitat of corals. Combined use of the information on color and appearance of coral samples would be helpful for reducing the error. In another study, we have detected other trace elements such as I, Mo, Sn, Mn, Zn, Cd and Br in skeletons of precious corals (Hasegawa et al., 2010), which would have the potential for additional indicators of geographical origin.

**Conclusion**
Apart from Mg concentrations that vary slightly with annual rings, trace elements in precious coral skeletons are distributed homogeneously. Our study reveals that the trace elements in skeletons of precious corals are habitat-specific rather than species-specific. The Mg/Ca and Ba/Ca ratios in skeletons of precious corals, particularly, are the indicators of their habitats and environments and, therefore, can be used to identify the harvested areas of coral products. X-ray fluorescent analysis is another useful method that can serve to identify the bathymetrical and geographic origin of coral (and other) products.

The proposal to list all species in the family Corallidae in Appendix II of the Convention on International Trade in Endangered Species of Wild Fauna and Flora was rejected in the Conferences of the Parties (CoP) 14 (FAO, 2007) and CoP 15 (FAO, 2010). The difficulties in the identification of coral products in trade have been highlighted in the debate about the feasibility to enforce such a listing effectively. Identification of raw coral to species level is easy to the coral specialists, and a taxonomic reference guide has been recently published by World Wildlife Fund Canada (WWF Canada) to help customs officials to identify raw coral to species level, however, it is likely that identification might not be possible when coral products such as jewelries of Corallium sp. and of other species that are resembled to Corallium sp. after dying (FAO, 2007; 2010). The findings of the present study would guide to a possible solution to this problem, and would contribute in developing a nondestructive analytical method, such as XRF, for the identification of raw corals as well as
coral products. Further research is necessary to develop a fully functional, cost-effective and readily applicable method.

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References


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proposals to amend appendices I and II of CITES concerning commercially-exploited aquatic species. Food and Agriculture Organization of the United Nations, Rome, pp. 133.


Table 1: Different types of precious corals collected for ICP-AES analysis. Samples were collected from Japanese waters, Midway Islands’ waters and Mediterranean Sea from fishermen, coral traders, and research institutes.

<table>
<thead>
<tr>
<th>Species</th>
<th>Common name</th>
<th>Collection sites</th>
<th>Habitat (m)</th>
<th>Number of Samples (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Corallium rubrum</em></td>
<td>Red coral</td>
<td>Off Italy, Mediterranean Sea</td>
<td>&lt;100</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Mediterranean Sea</td>
<td>&lt;100</td>
<td>15</td>
</tr>
<tr>
<td><em>Paracorallium japonicum</em></td>
<td>Japanese red coral</td>
<td>Goto Islands, Japan</td>
<td>140</td>
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</tr>
<tr>
<td></td>
<td></td>
<td>Amami Islands, Japan</td>
<td>100-150*</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Off Cape Muroto, Kochi, Japan</td>
<td>100-150*</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Off Cape Ashizuri, Kochi, Japan</td>
<td>100-150*</td>
<td>2</td>
</tr>
<tr>
<td><em>Corallium elatius</em></td>
<td>Pink coral</td>
<td>Ogasawara Islands, Japan</td>
<td>100-150*</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Amami Islands, Japan</td>
<td>100-150*</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Off Cape Muroto, Kochi, Japan</td>
<td>100-150*</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Goto Islands, Japan</td>
<td>100-150*</td>
<td>1</td>
</tr>
<tr>
<td><em>Corallium konojoi</em></td>
<td>White coral</td>
<td>Ogasawara Islands, Japan</td>
<td>100-150*</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Goto Islands, Japan</td>
<td>100-150*</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Off Cape Muroto, Kochi, Japan</td>
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<td>1</td>
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<td></td>
<td>Ogasawara Islands, Japan</td>
<td>1420-1620</td>
<td>1</td>
</tr>
<tr>
<td><em>Corallium spp.</em></td>
<td>Deep sea coral</td>
<td>Midway***</td>
<td>400-500 or 940-1100*</td>
<td>19</td>
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<td></td>
<td>Deep sea pink coral</td>
<td>Midway***</td>
<td>400-500 or 940-1100*</td>
<td>8</td>
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<tr>
<td></td>
<td>Deep sea white coral (garnet)</td>
<td>Midway***</td>
<td>400-500 or 940-1100*</td>
<td>2</td>
</tr>
</tbody>
</table>

* Estimated depth.
** Collected at St.4762 on GH85-1cruise conducted by the National Institute of Advanced Industrial Science and Technology.
*** Collected from northwestern (31°16'-36°47'N, 171°02'-175°55'E, 400-500 m) or northeastern (32°31'-32°58'N, 175°03'W-175°18'W, 940-1100 m) waters of Midway.
Fig. 1: Skeleton of Japanese white coral (*C. konojoi*). Photograph of white coral (A), mapping analysis of Mg (B), Ca (C), Ba (D), and Sr (E) in the skeleton of precious white coral by EPMA.
Fig. 2: Distribution of Mg and Ba in skeleton of precious corals collected from different depth and geographical locations indicating the origin of the corals. Values are mean ± SD.