

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

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22 **Abstract**

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

37

38

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

40

41 **1. Introduction**

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

49 Taxonomically, precious corals belong primarily to three orders of the class Anthozoa,
50 and the most valuable species are red and pink corals of the genus *Corallium* and
51 *Paracorallium* of the family Coralidae, which consist of 19 and 7 species, respectively
52 (Tsounis et al., 2010). They are found mainly in the Mediterranean Sea and Pacific Ocean
53 (Japanese waters and off Taiwan, off the Midway Islands and off the Hawaiian Islands)
54 (Iwasaki and Suzuki, 2010). Some of the important precious corals include *Corallium rubrum*,
55 *Paracorallium japonicum*, *Corallium elatius*, *Corallium konojoi* and *Corallium secundum*
56 (Tsounis et al., 2010). The *Corallium* spp. are commonly known as deep-sea coral, and the red
57 coral (*C. rubrum*) is produced in the Mediterranean Sea. The pink coral (*C. secundum*) is
58 distributed in the seas around Hawaii and the Midway Islands, and also found in the waters
59 close to the Midway Island (Grigg, 1993).

60 Japanese red coral (*P. japonicum*), pink coral (*C. elatius*) and white coral (*C. konojoi*)
61 are distributed and harvested in waters near Japan (Iwasaki et al., 2009). *Paracorallium*
62 *japonicum* is found at depths of 76-280 m on the rocky bottom in Sagami Bay (Pacific coast
63 of Japan), in the waters from the Ogasawara Islands (Japan), and off the coast near the Goto
64 Islands, Nagasaki (Japan) (Seki, 1991). *Corallium elatius* is distributed on the rocky bottom at
65 a depth of 100-276 m in the waters near Wakayama (Pacific coast of Japan), from the
66 Ogasawara Islands (Japan) to the northern South China Sea, and off the Goto Islands,
67 Nagasaki, Japan (Iwasaki and Suzuki, 2010). *Corallium konojoi* is distributed on the rocky
68 bottom at a depth of 76-276 m in the waters of Wakayama (Pacific coast of Japan), in the
69 waters from the Ogasawara Islands (Japan) to the northern South China Sea, and off the Goto
70 Islands, Nagasaki, Japan (Seki, 1991; Nonaka et al., 2004). *Corallium secundum* has been
71 found to grow on flat exposed substrata whereas *C. regale* prefer encrusted uneven rocky
72 bottom habitat in the Hawaiian Islands, and both species are absent from the shelf areas (<400
73 m depth) (Grigg, 1974).

74 Some *Corallium* species have a hard calcium skeleton of intense red and the others
75 are pink and of pink (Iwasaki and Suzuki, 2010). Both spicules and skeletons of red coral (*C.*
76 *rubrum*) are mainly made of calcium carbonate (CaCO₃) crystallized in the form of calcite,
77 though small amounts of other trace elements such as magnesium (Mg), strontium (Sr), iron
78 (Fe), aluminum (Al) and sulphur (S) are also found (Maté et al., 1986). Previously, Velimirov

79 and Bohm (1976) analyzed calcium (Ca) and Mg by atomic absorption spectroscopy and
80 ethylenediaminetetraacetate (EDTA) titration with the aim of providing information on the
81 mineral composition of gorgonians and the possible variations in different growth regions.
82 They showed that CaCO_3 , MgCO_3 and total mineral content increase markedly from branch to
83 stem. Weinbauer and Velimirov (1995) determined Mg, Ca, and Sr in sclerites of four
84 Mediterranean gorgonians and suggested that Mg/Ca and Sr/Ca ratios were very low
85 (0.064-0.098 and 0.004-0.0025, respectively). They also revealed that calcium concentrations
86 did not vary with geographical origin, while the variations of Mg/Ca and Sr/Ca ratios were
87 related to water depth. Besides, there was a direct relationship between Mg concentration and
88 temperature, and the Mg/Ca ratios increased significantly with the ambient water temperature
89 (Weinbauer and Velimirov, 1995).

90 Reef-building coral is better understood, and concentrations of trace elements in its
91 carbonate skeletons have been determined. The validity of their use as indicators of past
92 environmental conditions, such as water temperatures, nutrients and pollution levels has been
93 confirmed in earth and environmental science studies (Weber and Woodhead, 1970; Weber,
94 1973; Mitsuguchi et al., 1996; Mitsuguchi et al., 2001; Mitsuguchi et al., 2003). In contrast,
95 studies on trace elements in precious coral have been focused mostly on Mediterranean red
96 coral *C. rubrum* (Weinbauer and Velimirov, 1995; Weinbauer et al., 2000). Studies on trace
97 elements in other precious corals, especially the Japanese white coral (*C. konojoi*), from

98 various locations is limited. Research on precious corals in Japanese waters has recently been
99 started.

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

115

116 **2. Materials and Methods**

117 **2.1. Sampling site**

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

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

129

130 **2.2. Chemical analysis of skeleton composition**

131 Barium (Ba), Ca, Mg, and Sr concentrations were analyzed in skeleton of the corals.
132 Each of the skeletons was ground in an agate mortar into a particle size of 5 mm in diameter
133 and 0.1 g of it was taken into 10-mL polypropylene test tubes with three replications. The
134 skeletons of precious corals were then cleaned following sequential methods of ultrasonic,
135 oxidation, and reduction treatments ([Shen and Boyle, 1988](#)). At first the samples were treated

136 with ultrasonic waves in 1 mL of purified water and then in 1 mL of 0.2 M nitric acid for 10
137 min each. The samples were rinsed with purified water between the treatments. This
138 procedure was repeated three times. After drying at room temperature, they were ground
139 further in the agate mortar and were sieved through a 25-50 Teflon mesh screen of a
140 polypropylene sieve. The samples were then collected from three different sieved samples in
141 the same colony ($n = 3$).

142 After ultrasonic cleaning, the samples in 10-mL polypropylene test tubes were oxidized
143 by addition of 1 mL solution prepared from 1:1 (v/v) 30% hydrogen peroxide (H_2O_2) and 0.2
144 M sodium hydroxide (NaOH). They were then placed in a steam and an ultrasonic bath for 2
145 min each for a total of 10 repeats. They were then sequentially treated with ultrasonic waves
146 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
147 0.2 M nitric acid for 3 min, and then the oxidation treatment was applied once again. A
148 reduction treatment was followed by oxidation treatment in which the samples were treated
149 with 1 mL solution containing 97% hydrogen, concentrated ammonia, and 0.3 M citric acid in
150 the ratio of 1:6:3. They were then placed in a hot (70 °C) and an ultrasonic bath for 2 min
151 each for a total of 16 repeats. Finally the samples were cleaned by repeating oxidation
152 treatments, treating ultrasonic waves (2-min) in 1 mL of 0.2 M nitric acid three times, and
153 rinsing twice in 1 mL of 0.2 M nitric acid. Then the samples were dissolved in 1 mL of 2 M
154 nitric acid.

155 The sample solutions were diluted to 1000 times with 0.5 M nitric acid, and the
156 determination of trace element concentrations in the samples were carried out by inductively
157 coupled plasma atomic emission spectroscopy (ICP-AES, Perkin Elmer, Optima 3300XL) in
158 triplicates using a calibration curve method.

159

160 **2.3. Analysis of inorganic elements with electron probe micro-analyzer (EPMA)**

161 Each skeleton sample with dried organic tissues attached on the surface of white coral
162 (*C. konojoi*) was embedded in polyester resin for EPMA. The skeletons were cut at 5 mm
163 intervals perpendicular to the growth direction using a diamond saw, and one of the thin slices
164 was ground to 100-200 μm with 600, 1000, and 2000 grits silicon carbide abrasive papers.
165 The surface of the samples was then polished to a mirror finish using an alumina wrapping
166 sheet (Marumoto Kogyo, Japan) with a particle diameter of 0.1 μm , and was coated with a
167 10- μm carbon film by evaporative deposition of carbon. EPMA was performed using an
168 EPMA-8705 (Shimadzu Corporation, Japan). Two-dimensional images of elemental
169 distribution were obtained by stage mapping the sample along the x and y axes with 29 μm
170 raster spacing. Measurement parameters were set as follows: accelerating voltage at 15 keV,
171 beam current at 0.3 μA , and a measurement time 0.04 sec. for Ca and Sr, and 0.22 sec. for Mg
172 and Ba.

173

174 **3. Results and Discussion**

175 **3.1. Trace element distribution in the skeletons of precious coral**

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

188 Mg-rich layers grow in warm seasons, and the variations in Mg concentrations in the
189 skeleton are very likely to be related to water temperatures. Weinbauer and Velimirov (1995)
190 compared the Mg/Ca ratios in the skeletons of several *C. rubrum* species from different
191 depths and found a positive correlation between the Mg/Ca ratios and water temperatures.
192 Variations in Mg concentrations within an individual coral skeleton can be assessed as the

193 Mg/Ca molar ratio. The Mg/Ca ratio in white coral varies from 10 to 15% of the mean values,
194 while that in *C. rubrum* obtained from EPMA study of [Weinbauer et al. \(2000\)](#) varied from 13
195 to 30%.

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

208

209 **3.2. Trace element compositions reflecting the characteristics of coral habitats**

210 The Mg/Ca and Ba/Ca ratios in the skeletons of *C. rubrum*, Japanese red, pink and white
211 corals, and Midway corals are shown in [Figure 2](#). Japanese red, pink and white corals co-habit

212 in sea-floors around Japan. The Mg/Ca and Ba/Ca ratios in these three corals collected from
213 the same area were within similar ranges without species-specific differences. On the other
214 hand, Mg/Ca and Ba/Ca ratios in corals from the Mediterranean Sea, Japanese waters and the
215 sea around the Midway Islands differed depending on their habitats (Fig. 2) except Sr/Ca ratio
216 ($0.31\text{-}0.33 \times 10^{-2} \text{ mol mol}^{-1}$). Mg/Ca and Sr/Ca ratios in the skeletons of precious corals (*C.*
217 *rubrum* and Japanese red, pink and white corals) of present study determined by EPMA
218 analysis are well agreed with those in the skeletons of *C. rubrum* measured by XRF
219 (Weinbauer and Velimirov, 1995). This study also showed that Ba/Ca ratio in the Midway
220 deep-sea corals is higher compared to that in other samples.

221 The Mg/Ca ratios in precious corals of the Mediterranean Sea and Japanese waters
222 ranged from $10\text{-}15 \times 10^{-2} \text{ mol mol}^{-1}$, while those in pink and white corals of the Midway
223 Islands were in the ranges of $9\text{-}11 \times 10^{-2} \text{ mol mol}^{-1}$ and $8\text{-}9 \times 10^{-2} \text{ mol mol}^{-1}$ for 400-500 m and
224 900-1200 m (deep-sea corals), respectively. Because Mg and Ca are the major salts in
225 seawater, and Mg/Ca ratio is almost constant in all parts of the ocean, it is extremely unlikely
226 that the observed variations in the Mg/Ca ratio were influenced by the Mg/Ca ratio in
227 seawater. In a previous study, Weinbauer and Velimirov (1995) observed that the Mg/Ca ratio
228 in the skeletons of *C. rubrum* was usually related to ocean's depth. They incorporated other
229 reports and estimated that the Mg/Ca ratio in *C. rubrum* was directly proportional to water
230 temperature, increasing by $0.004\text{-}0.006 \text{ mol mol}^{-1}$ per $1 \text{ }^\circ\text{C}$. Water temperatures near the

231 sediments, where the coral samples for the present study were collected (Table 1), were in the
232 range of 13-24 °C in the Mediterranean Sea and Japanese waters, and 8-11 and 2-3 °C at a
233 depth of 400-500 m and 900-1200 m in the Midway Islands, respectively. The difference in
234 the water temperature could be predicted by the Mg/Ca ratios assuming that an increase of
235 0.004-0.006 mol mol⁻¹ Mg/Ca ratio per 1 °C was applicable to all precious corals in the
236 subclass Octocorallia. The difference in temperature between the Mediterranean Sea/Japanese
237 waters and the Midway shallow area is 2-16 °C, and between the Mediterranean Sea/Japanese
238 waters and the Midway deep area is 10-22 °C, which can be calculated as 0.008-0.096 and
239 0.040-0.132 mol mol⁻¹ of Mg/Ca ratios, respectively. Because these figures are close to the
240 difference in the Mg/Ca ratios in this study (Fig. 2), variations in the Mg/Ca ratio of precious
241 corals are likely to be explained by difference in water temperature during coral formation.

242 Ba/Ca ratio in the Midway coral from a depth of 900-1200 m was significantly higher
243 (10-15×10⁻⁶ mol mol⁻¹) than that of Midway coral from a depth of 400-500 m (5-8×10⁻⁶ mol
244 mol⁻¹) and Japanese corals from a depth of 100-150 m (2-6×10⁻⁶ mol mol⁻¹). In contrast, the
245 Ba/Ca ratios in *C. rubrum* showed a wider range (4-14×10⁻⁶ mol mol⁻¹). The proportions of
246 Mg, Ca, and Sr concentrations, the major constituents of seawater, are almost identical to
247 those in seawater, while Ba concentration increases with the depth of the ocean. For example,
248 dissolved Ba concentrations of 30-40 mol kg⁻¹ at 50-200 m, 40-55 mol kg⁻¹ at 400-500 m, and
249 90-110 mol kg⁻¹ at 900-1200 m were reported from *Corallium* (what species) in the North

250 Pacific (Chow and Goldberg, 1960; Boyle et al., 1976). These figures agree well with the
251 Ba/Ca ratios in the skeletons of analyzed precious corals collected from the waters around
252 Japan and around the Midway Islands.

253 Some overlaps of Mg/Ca and Ba/Ca ratios in corals from the Mediterranean and
254 Japanese waters were observed in the present study. The data presented in Fig. 2 showed that
255 the Mg/Ca and Ba/Ca ratios in only 5 samples of Japanese corals and 6 samples of the
256 Mediterranean corals are in the range of $12\text{-}13 \times 10^{-2}$ and $4.6\text{-}6.0 \times 10^{-5}$ mol mol⁻¹, respectively,
257 while 33 samples of the Japanese corals and 13 samples of the Mediterranean corals are
258 distributed separately. One sample of the Japanese coral with 12.5×10^{-2} mol mol⁻¹ of Mg/Ca
259 and 6.6×10^{-5} mol mol⁻¹ of Ba/Ca, and one sample of Mediterranean corals with 15.8×10^{-2} mol
260 mol⁻¹ of Mg/Ca and 3.9×10^{-2} mol mol⁻¹ Ba/Ca might be exceptions. Thus, some degree of
261 error was unavoidable in the same manner as other scientific methods for identification of
262 origin and habitat of corals. Combined use of the information on color and appearance of
263 coral samples would be helpful for reducing the error. In another study, we have detected
264 other trace elements such as I, Mo, Sn, Mn, Zn, Cd and Br in skeletons of precious corals
265 (Hasegawa et al., 2010), which would have the potential for additional indicators of
266 geographical origin.

267

268 **Conclusion**

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

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

288 coral products. Further research is necessary to develop a fully functional, cost-effective and
289 readily applicable method.

290

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302

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- 367

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.

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

* 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.

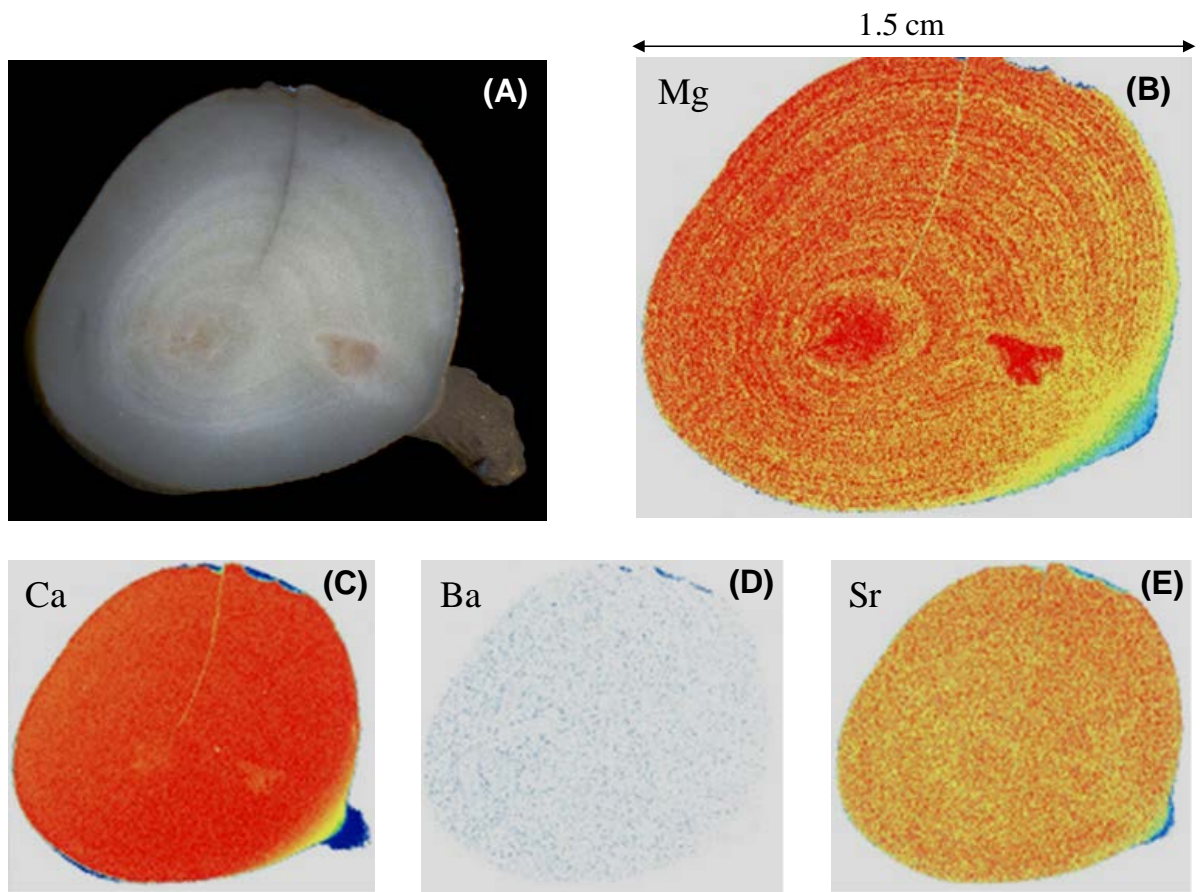


Fig. 1: Skeleton of Japanese white coral (*C. konojoi*). Photograph of white coral (A), mapping analysis of Mg (B), Ca (C), Ba (D), and Sr (E) in the skeleton of precious white coral by EPMA.

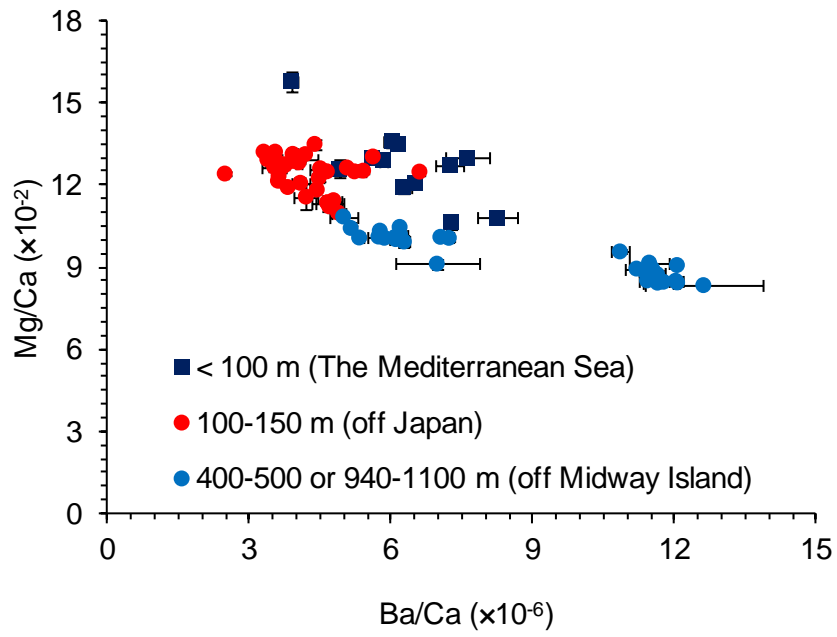


Fig. 2: Distribution of Mg and Ba in skeleton of precious corals collected from different depth and geographical locations indicating the origin of the corals. Values are mean \pm SD.