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Abstract: Precious corals from the Corallidae family (*Corallium*, *Hemicorallium*, and *Pleurocorallium* genera) are well known in the high-end jewelry industry due to their colorful and durable axial skeleton. They exist in various colors from white to pink to dark red. One highly appreciated shade is the light pink color, the so-called 'angel skin'. This color is most often associated with *Pleurocorallium elatius* and *Pleurocorallium secundum*, species listed in CITES Appendix III. However, this has been based on an assumption of their visual similarity and has never been underpinned by detailed morphologic or genetic data. In this study, we present the analysis of an 'angel skin' coral necklace of exceptional size and homogeneous color and quality. Visual observation and Raman spectroscopy confirmed that the necklace consists of genuine, untreated precious coral material. Following minimally destructive sampling, respectively, drilling 2.2, 2.4, and 2.4 milligrams of material from the existing drill-holes, three randomly selected beads from the necklace were subject to a routine genetic identification assay, which is based on sequencing a short, taxonomically informative mitochondrial region. This genetic analysis identified the coral material as not from *P. elatius* or *P. secundum* but from another *Pleurocorallium* species. We subsequently sequenced additional mitochondrial DNA fragments from one 'angel skin' coral bead and compared them against a well-represented, curated reference data set of *Pleurocorallium*, including the first-ever sequencing of *Pleurocorallium gotoense*, *Pleurocorallium johnsoni*, *Pleurocorallium* cf. *pusillum*, and *Pleurocorallium uchidai*. We concluded that the analyzed material of the 'angel skin' coral necklace belongs to the *Pleurocorallium norfolkicum* species complex but is not identical to any hitherto analyzed and published *Pleurocorallium* specimens. A comparison with further taxonomically unidentified precious coral colony fragments identified a single sample fished in Vietnam to be completely identical to the 'angel skin' coral bead in the studied DNA regions. Thus, by the analysis of a high-end jewel, we discovered a species new to the jewelry trade and potentially also unknown to science. This implies that the currently considered list of species present in the precious coral trade is incomplete.

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1. Introduction

The skeletal materials of several types of corals are used in jewelry, including bamboo corals, black corals, gold corals, and precious corals. Of these, the precious corals (Octocorallia: Coralliidae) represent the highest market values and importance due to their durable and shiny calcite skeletons with bright natural colors. The Coralliidae family contains three genera, i.e., Corallium, *Hemicorallium*, and *Pleurocorallium*, which all comprise species considered to be relevant in the jewelry trade. The commonly accepted species used in jewelry are *C. rubrum* and *C. japonicum* from the genus *Corallium*; *H. regale*, *H. laauense*, and *H. sulcatum* from *Hemicorallium*; *P. elatius*, *P. konojoi*, and *P. secundum* from *Pleurocorallium* [1]. However, Coralliidae corals are difficult to identify taxonomically to the species level, especially when the outer living tissue with the coral polyps and sclerites (cortex or coenenchyme) is removed and the skeleton is cut and polished. Therefore, the different types of precious corals in the trade are traditionally handled by trade names generally reflecting their color, such as 'oxblood', 'garnet', 'pure white', or 'white/pink'. The association of the trade names to biological species is in some cases not a simple task. For instance, the pink-colored 'miss coral' intensively collected around Taiwan in the 1970s to 1990s was only identified as *Hemicorallium sulcatum* (Kishinouye, 1903) decades later [2].

The identification of precious corals is crucial for their sustainable use and holds practical significance for manufacturers, traders, and owners, as four of the species important to the trade (*C. japonicum*, *P. elatius*, *P. konojoi*, and *P. secundum*) were listed by CITES (Appendix III) in 2008. To be able to conform to trade rules and identify the provenance and species of origin, Lendvay et al. [3] developed a method to distinguish CITES-listed and non-CITES-listed species based on genetic testing following minimally destructive sampling (drilling a few milligrams of material from the existing drill-holes or back sides of objects). This method is now in routine use at the Forensic Genetics Department of the Zurich Institute of Forensic Medicine, University of Zurich, Switzerland (ZIFM). Most of the coral jewelry submitted to the ZIFM by private or commercial clients for taxonomic identification has a red color (*C. rubrum*, *C. japonicum*, and *P. elatius*), which is in line with the fact that red (in different shades from dark orange and salmon to bright red to dark red) is the most traditional and most prevalent color of precious corals in the gem trade. According to the common trend, the other frequently found color varieties, white and pink, especially if uneven or spotted, are less commonly seen in jewelry. However, another, less frequently encountered color type, poetically referred to as 'angel skin' or 'boké' (a light pink color with different color intensities), is highly appreciated by jewelers and jewelry consumers [4–6]. Homogenously colored 'angel skin' precious corals are rare, which contributes to their high value. The precious corals of this color are generally associated with *P. elatius* [1,7] or occasionally also with *P. secundum* [8].

In March 2024, a client submitted an exceptional pink precious coral necklace consisting of 67 round coral beads that exhibited an 'angel skin' color for gemological testing to the Swiss Gemmological Institute SSEF (Basel, Switzerland). The analysis of the necklace included the genetic testing of the selected beads with the aim of identifying the taxonomic status of this 'angel skin' coral sample and inferring its geographic origin.

First, standard gemological tests were performed to confirm that the material is genuine, untreated precious coral. To test the hypothesis that the source material is *P. elatius* or *P. secundum*, a minimally destructive sampling method was applied to obtain powder material from three randomly selected beads. These samples were then sent to the ZIFM for

genetic testing. Following DNA extraction, the in-house developed and validated *Coral*-ID identification method of Lendvay et al. [3] was performed. The *Coral*-ID protocol uses the DNA sequence of a fragment of the mitochondrial *MutS* (mt*MutS*) gene to assign the coral material to six categories of Coralliidae precious corals. The *Coral*-ID method has been specifically developed to separate CITES-listed and non-CITES-listed Coralliidae precious corals. Within the genus *Pleurocorallium*, it can identify *P. secundum* (CITES-listed) and the *Pleurocorallium elatius* species complex (containing the CITES-listed *P. elatius* and *P. konojoi* and the non-CITES-listed *P. carusrubrum)*, while all other species, which are not considered important in the coral industry and not CITES listed, are grouped in the category "other *Pleurocorallium*". The identification process revealed that the sampled beads originate neither from *P. elatius* nor from *P. secundum*, but rather from another, undefined *Pleurocorallium* species not listed under CITES.

This unexpected result led us to perform a more in-depth genetic examination of one bead of the 'angel skin' coral necklace to clarify its taxonomic status and putative geographic origin. The *Coral*-ID method is optimal for a fast and robust initial identification of the producing species of coral jewelry. However, performing additional in-depth inferences of *Pleurocorallium* taxonomy is not feasible for two fundamental reasons: firstly, the *Coral*-ID marker is very short, which limits its usefulness for robust phylogenetic analyses; secondly, it is incomplete in terms of species sampling (Table 1). In particular, four *Pleurocorallium* species (*P. gotoense*, *P. johnsoni*, *P. pusillum*, and *P. uchidai*) have never been genetically analyzed. Therefore, we aimed to compare the DNA of the coral necklace with the most comprehensive set of *Pleurocorallium* reference DNA sequences available to date. In particular, we generated additional mitochondrial DNA sequence data for a bead from the necklace and compared this with a curated reference data set of *Pleurocorallium* samples [9]. To improve the existing *Pleurocorallium* reference data set, we obtained DNA sequence data of four *Pleurocorallium* species that have never been genetically studied. In addition, we screened a set of coral samples originating from a broad area in the North Pacific, in which a commercial precious coral fishery exists or existed. We were eager to see if we could find samples completely matching with the DNA of the 'angel skin' coral bead.

We conclude that the examined 'angel skin' coral bead originates from a *Pleurocorallium* species, but its DNA sequences do not completely match any previously analyzed coral specimens. However, we did find a single completely matching sample from a coral colony sourced from a commercial coral fishery of a previously not studied coral population, which suggests a putative geographic origin of the material of the 'angel skin' necklace. We discuss the implications of these results for the precious coral industry and trade. In addition, our established *Pleurocorallium* reference data set presents some novel taxonomic inferences of specific *Pleurocorallium* taxa.

2. Materials and Methods

2.1. Description of the 'Angel Skin' Coral Necklace

The necklace is a regularly graduated strand of 67 coral beads strung on a strand with an approximate length of 108 cm (Figure 1). The total weight of the necklace is 447.8 g, which includes the thread and tassels with two pearl imitations. The polished and drilled beads have a round shape with diameters ranging from ca. 10.55 mm to 26.75 mm.

Figure 1. Image of the 'angel skin' coral necklace. The necklace is highly impressive with an exceptional size of beads, a perfectly matching shape, and a subtle and attractive pink color.

2.2. Visual Inspection and Molecular Fingerprinting

The beads were inspected at 10×–50× magnification using an Eickhorst Gemmaster microscope with Zeiss optics (System Eickhorst, Hamburg, Germany) to confirm the presence of characteristic coral textures in these beads. As standard in gemological testing, a small subset of the samples, here, two beads—the 21st and 34th on the strand—were chosen for non-destructive molecular fingerprinting with Raman spectroscopy to test the authenticity of the material and detect the presence of any artificial color treatment of the coral beads. We used an InVia confocal Raman microscope (Renishaw, Wotton-under-Edge, UK) with a Peltier-cooled CCD detector (1024 × 256 pixels), resulting in a maximum spectral resolution of about 1.5 cm^{-1}. The system is coupled with a Leica stereo microscope and a set of objectives (5× to 50×). The Raman spectra were collected using an argon–ion laser emitting at 514 nm. The Raman shift was recorded in the range from 100 cm^{-1} to 2100 cm^{-1}. The laser beam was focused on the samples using a 50× long-range objective, resulting in a spot size of ca. 2 µm. The laser power was set at a standard of 35 mW (100% laser emission in our setup, measured on the sample surface). The Raman spectra of both samples were accumulated from 5 to 7 spectral scans at 15 s exposure time on the same analytical spot. All spectra were baseline corrected (Wire software, Renishaw) using a built-in cubic spline integration baseline function. The obtained spectrum was compared to the Raman spectra of Coralliidae precious corals from the scientific literature, i.e., [10–14].

2.3. Initial Genetic Testing Using the Coral-ID Method

Three beads were selected for sampling for genetic analysis at the Swiss Gemmological Institute, SSEF. The selected beads—Nos. 8, 25, and 40 on the strand—were randomly chosen from different parts of the graduated strand. Powder was drilled from the inside surface of the existing drill-holes with a manual electric drill device (Dremel, Mt Prospect, IL, USA) using a 0.9 mm diameter steel bit. The amount of the collected material was 2.4 mg (bead No. 8), 2.2 mg (bead No. 25), and 2.4 mg (bead No. 40). The drill bit was thoroughly cleaned prior to and between samples by consecutively soaking in 1% HCl, washing with an alkaline surfactant solution (5% neodisher lm3; Dr. Wiegert, Hamburg, Germany), then rinsing with distilled water and 96% ethanol. The powder was collected for each of the three samples in a microcentrifuge tube, sealed, and transferred to the ZIFM.

DNA was extracted from the three beads in an ISO17025-accredited trace DNA laboratory of the ZIFM. The DNA extraction was based on the complete decalcification and lysis of the skeletal powder, followed by a column purification using the method first used for precious coral skeletal material by Lendvay et al. [3,15], and recently further optimized for small sample amounts [9]. Specifically, the skeletal powder was completely decalcified during 24 h incubation at 56 °C in 1 mL of lysis buffer containing 0.45 M EDTA, 0.5% N-laurylsarcosyl, and 0.25 mg/mL of Proteinase-K. The lysate was concentrated to 50 µL on a 30 kDa Amicon Ultra filter (Merck, Darmstadt, Germany) and mixed with 1.3 × amount of TE buffer pH = 8, 0.1 × amount of 3 M Sodium–Acetate pH = 5.2 (Thermo Fisher) and 11.5 × amount of PB Buffer of the MinElute PCR Product Purification Kit (Qiagen, Hilden, Germany). The mixture was then purified with the MinElute kit according to the manufacturer's instructions and eluted in a 100 µL volume.

We performed PCR amplification and DNA sequencing of the *Coral*-ID identification marker using the DNA extracts of the three 'angel skin' coral beads according to Lendvay et al. [3]. Each of the three DNA extracts was tested in duplicate in independent reactions to verify the results.

2.4. Comparing the DNA of an 'Angel Skin' Coral Bead with an Extended Pleurocorallium Reference Data Set

The reference data set we used was based on 60 high-quality and taxonomically verified samples of 16 *Pleurocorallium* species assembled by Lendvay et al. [9]. The data set contains sequence information of three DNA fragments; the 260–523 base-pair (bp)-long mt*ND6-COI* intergenic spacer (*IGS*), a 400 bp long fragment of the DNA mismatch repair protein (mt*MutS*) gene, and a 127–128 bp long fragment of the large ribosomal RNA gene subunit (*LR*). The length of the concatenated *IGS*-mt*MutS-LR* sequences ranges between 788 and 1051 bp for individual samples. As outgroup taxon, the data set of the *Pleurocorallium* is accompanied by the *IGS*-mt*MutS-LR* sequences of a sample of *Corallium rubrum*, the type species of the Coralliidae.

The above-described *IGS*-mt*MutS-LR* data set of Lendvay et al. [9]. was complemented with the first-ever sequencing of *P. gotoense*, *P. johnsoni*, *P. pusillum*, and *P. uchidai*. We generated orthologous *IGS*-mt*MutS-LR* DNA sequence data for a sample of each of these species. Among these species, *P. johnsoni* (Gray1860) was the first one to be discovered and described. It was initially regarded as the type species of the genus *Hemicorallium*, but it was recently reassigned to the genus *Pleurocorallium* based on morphological characteristics. Tu et al. [16] aimed to include a sample of *P. johnsoni* in their phylogenetic study but were not successful in amplifying the potentially degraded DNA of their colony. *Pleurocorallium johnsoni* is found in the North-East Atlantic, a region where precious coral fishing is not common. The specimen analyzed in the current study originates from the Bay of Biscay, France, and is stored in the Smithsonian National Museum of Natural History (NMNH) with the lot number USNM 1010769.

Pleurocorallium gotoense and *P. uchidai* were described by Nonaka et al. [17]. The description of these species was carried out based on a morphological analysis of colonies originally identified as *P. elatius* and donated to the NMNH by Kamakichi Kishinouye—the most renowned expert of Pacific corals of his time—in 1904. These species are to date known solely from their holotypes with the lot numbers USNM 19,925 (*P. uchidai*) and USNM 19,928 (*P. gotoense*). The holotype colonies originate from Nagasaki Prefecture (*P. gotoense*) and the Tosa Province (*P. uchidai*) of Japan, from regions of paramount importance for the precious coral fishery.

Pleurocorallium pusillum was described from a colony originating from Tokyo Prefecture by Kishinouye [18], but this specimen has been lost. No other specimen of *P. pusillum* has been found for over a century, until the discovery of 11 colonies that resembled the

morphological description of *P. pusillum* and were therefore called *P.* cf. *pusillum* [19]. These samples were collected in 2009 and 2010 during the research conducted by the Fisheries Agency of Japan in the Emperor Seamounts. The Emperor Seamounts were the primary precious coral fishing area in the second half of the 20th century, and the east coast of Japan has been an important fishing ground for two centuries. Three *P*. cf. *pusillum* colonies, NSMT-Co1719, NSMT-Co1721, and NSMT-Co1726, from the study of Nonaka and Hayashibara [19], were further analyzed in the present study.

At the dry coral collection of the NMNH, respectively, 77, 5, and 13 mg fragments of coenenchyme were collected from the colonies of *P. gotoense*, *P. johnsoni*, and *P. uchidai*. DNA was extracted, amplified, and sequenced at the ZIMF as described by Lendvay et al. [9] for the coenenchymal samples. From the *P.* cf. *pusillum* colonies, DNA was extracted according to Takata et al. [20] and amplified and sequenced at the University of Tokyo. PCR amplification followed Lendvay et al. [9]. Sequencing at the ZIFM and the University of Tokyo was performed, respectively, on ABI (Applied Biosystems, Waltham, MA, USA) SeqStudio and ABI 3730 genetic analyzers.

We amplified and sequenced the *IGS*-mt*MutS-LR* fragments of an 'angel skin' coral bead as described above. Hence, the DNA data obtained from the 'angel skin' coral necklace became comparable with the extended, curated DNA reference data set of *Pleurocorallium* specimens. The DNA extracts of two beads of the 'angel skin' necklace were completely consumed for the tests with the *Coral*-ID assay; therefore, only one bead (No. 8 on the strand) could be used to carry out the additional genetic test. We aligned the DNA sequences in Geneious Prime 2024.0.7 (https://www.geneious.com), as described by Lendvay et al. [9], and a neighbor-joining phylogenetic tree was constructed with the Kimura 2-parameter method in MEGA7 [21] and 1000 bootstrap replicates. *Corallium rubrum* was used as the outgroup.

The phylogenetic study of precious corals by Ardila et al. [22] contains eight taxonomically unidentified *Pleurocorallium* specimens, which were assigned to three separate genetic lineages. These colonies were all collected from waters off New Zealand and Tasmania. Although the South Pacific has never been mentioned as an area of commercial precious coral fishery, we wished to compare the DNA sequences of these samples and those from the 'angel skin' bead. Ardila et al. [22] analyzed the DNA sequences of several mitochondrial DNA fragments, but only one of these—the *LR* region—was sequenced for the DNA from the 'angel skin' coral bead. We therefore compared the sequence similarities between these samples and the 'angel skin' coral bead at the *LR* region. For a comparison, we also report the *LR* sequence similarities between the 'angel skin' coral bead and each species in our extended *Pleurocorallium* reference data set.

2.5. Comparison of the DNA Sequences of the 'Angel Skin' Coral Necklace with Additional Samples

Samples obtained in scientific surveys generally have well-documented sampling locations and detailed taxonomic identifications. Scientific surveys, however, do not definitely reflect the true diversity of colonies ending up in the commercial trade. Precious corals found in the trade may have a poor documentation of origin (especially for older colonies), may be fragmented and decorticated, and therefore not suited for accurate morphological identification. We aimed to compare the DNA sequences of the 'angel skin' necklace with the sequences of an array of 18 samples. These were fished over decades from Midway and Hawaii (5 samples); Ogasawara, Emperor Seamount–Okinotorishima (no exact information on the location available, 7); Bering Sea (1); Vietnam (1); and various areas off Japan (4). Some of these colonies were allegedly collected as a bycatch of a longline fishery, while others were fished by the commercial precious coral fishery. No prior taxonomic identification was available for these samples due to their poor condition and

small size. Three colony fragments still contained fragments of the cortex, and from these, a piece of cortex was used to extract DNA as described by Lendvay et al. [9] for coenenchymal samples. From the remaining samples, skeletal material was scraped off using a scalpel, and DNA was extracted as detailed above for the samples from the 'angel skin' coral necklace at the ZIFM. In a first step, the *Coral*-ID assay was applied to determine the generic assignment of the species. Then, for the samples determined to be *Pleurocorallium*, the complete *IGS*-mt*MutS-LR* data set was generated independently in duplicate. Finally, the obtained *IGS*-mt*MutS-LR* sequences were aligned to the sequences of the 'angel skin' necklace in the software Geneious Prime 2024.0.7 with default settings, and the sequence similarities were observed to see if we could find a sample identical to the 'angel skin' coral necklace among the samples from the coral trade.

3. Results

3.1. Visual Inspection and Molecular Fingerprinting

The beads have a subtle and attractive pink color and a visible fine wavy to gently rippled pattern. Based on our experience, their surface pattern and color are typical for precious corals, in particular for the genus *Pleurocorallium*. The tentative species identity assumed based on morphology was *Pleurocorallium elatius*. The strongest peaks in the Raman spectrum at 1128 cm^{-1} and 1518 cm^{-1}, as well as the band at 1014 cm^{-1}, can all be attributed to unmethylated polyenic pigments (Figure 2). Additional bands at 279 cm^{-1}, 711 cm^{-1}, and 1086 cm^{-1} are characteristic of the presence of calcite. The Raman spectrum thus confirms the building material of the skeletal axis (calcite) and the known color pigments in Coralliidae precious corals. These results suggest that the necklace is of authentic, precious coral material without additional artificial color treatment.

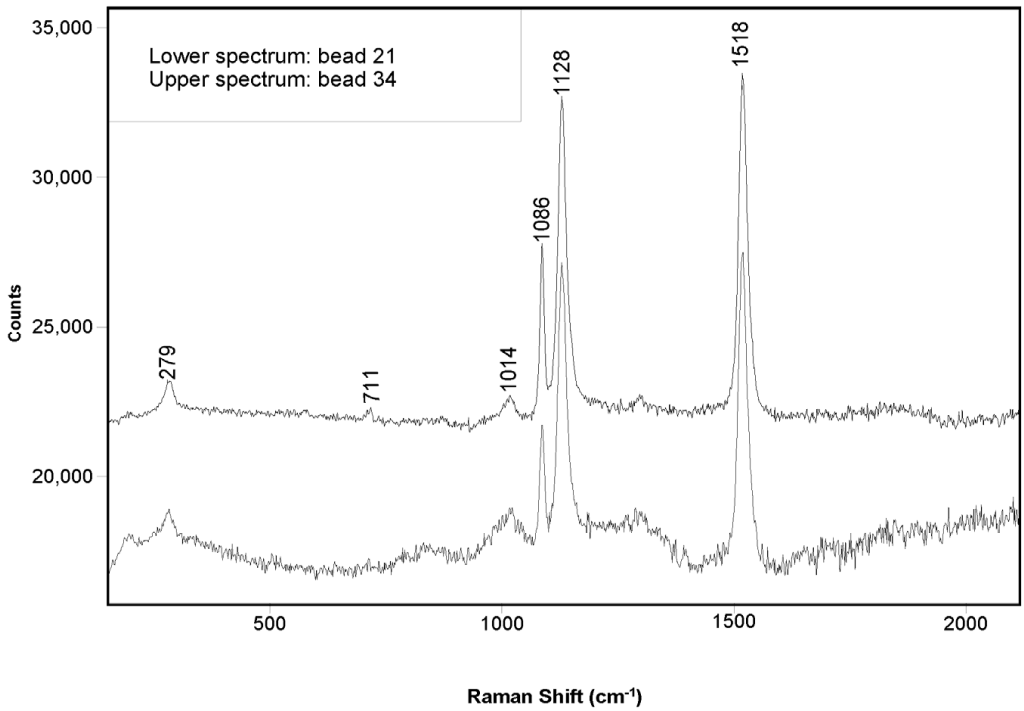

Figure 2. The Raman spectrum of two selected beads from the 'angel skin' necklace. The spectra fit with the expectations for untreated authentic Coralliidae precious corals.

3.2. Initial Genetic Testing Using the Coral-ID Method

DNA extracted from each of the three beads was analyzed using the *Coral*-ID assay twice independently to gain technical replicates for each bead. The replicates obtained

identical DNA sequences in each of the three DNA extracts, which confirms the authenticity and accuracy of the results. Moreover, the DNA sequences of each of the three DNA extracts were identical. The comparison with the reference data set and assignment to taxonomic categories resulted in the group "other *Pleurocorallium*", which contains all *Pleurocorallium* except those of *P. carusrubrum*, *P. konojoi*, *P. elatius*, and *P. secundum*. Alignment to the individual reference sequences of the "other *Pleurocorallium*" group revealed that the 'angel skin' coral necklace is identical in this 149 bp long gene fragment to a single precious coral colony studied to this date: the holotype of *Pleurocorallium bonsaiarborum*, which was collected in 2009 from the Loyalty Ridge, New Caledonia.

3.3. Comparing the DNA of an 'Angel Skin' Coral Bead with an Extended Pleurocorallium Reference Data Set

In an initial phylogenetic analysis, the sample of *P. johnsoni* was found to be an outgroup of all other *Pleurocorallium*. Therefore, the sequences of this sample were subject to an NCBI nucleotide BLAST search (blast.ncbi.nlm.nih.gov) on 15 March 2025. This revealed that—in both duplicates—the sequences of each of the three analyzed mitochondrial regions of the *P. johnsoni* colony are in fact *Hemicorallium*; thus, this sample was not included in further analyses.

The length of the concatenated *IGS*-mt*MutS*-*LR* DNA sequence of the 'angel skin' coral bead was 1050 bp long. The concatenated *IGS*-mt*MutS*-*LR* sequence alignment of the 'angel skin' coral bead and the extended *Pleurocorallium* reference data set (altogether 67 samples) was 1113 bp long (see Supporting Results S1 and S2). The topology of our phylogenetic tree is largely consistent with the one of Tu et al. [16], which is based on a more than three times longer stretch of mitochondrial DNA sequences; this confirms the reliability of our conclusions. The single discrepancy between the two trees is with regard to the position of *P. bonsaiarborum*, which is here basal to the *P. niveum*–*P. borneense*–*P. clavatum* clade, while in the analysis of Tu et al. [16], it groups together with *P. norfolkicum* and the undescribed species of the South Pacific.

Within this extended set of data, the DNA of the 'angel skin' coral bead was no longer identical to the specimen of *P. bonsaiarborum*: despite no differences in the mt*MutS* sequence, several polymorphic sites (including multiple indels) occurred in the *IGS* and *LR* regions between the 'angel skin' coral bead and *P. bonsaiarborum*. Instead, the 'angel skin' coral bead showed the highest similarity to the members of a clade consisting of *P. norfolkicum* as well as the undescribed species *P.* sp10, *P.* sp11, and *P.* sp12 sensu Tu et al. [16] with 93% bootstrap support value (Figure 3). These samples remained undescribed despite the results of their phylogenetic analysis, because their material was too small to allow their proper description as a novel species (T.-H. Tu, personal communication). The sequence similarities between the 'angel skin' and these species were the following: 99.3–99.4% to *P. norfolkicum*, 98.5–99.2% to *P.* sp10, 98.3% to *P.* sp11, and 98.3% to *P.* sp12. The comparison of the *LR* region with the undescribed *Pleurocorallium* specimens of Ardila et al. [22] revealed at least two mismatches in the 128 bp DNA fragment (Table 1). For the NCBI GenBank accession numbers and DNA sequence alignments of the newly obtained DNA sequences, refer to Supporting Results S1 and S2.

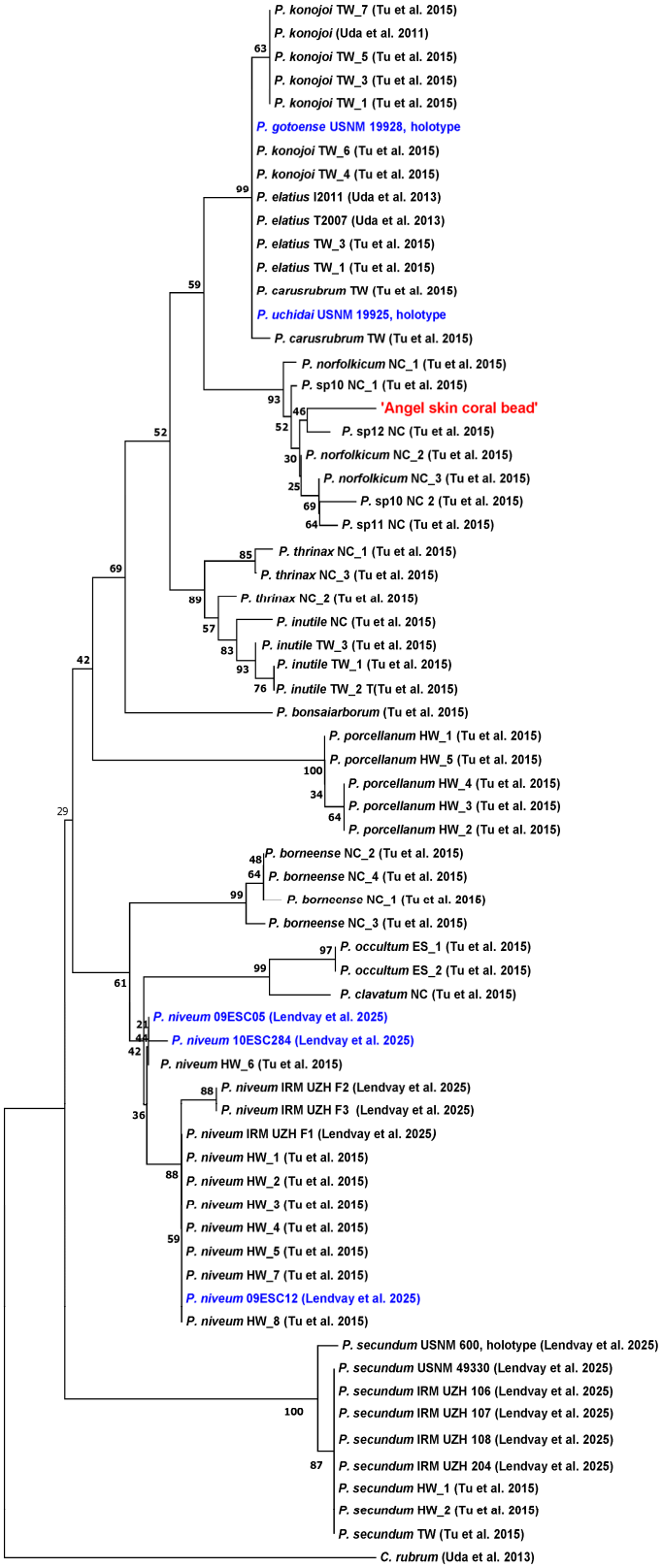

Figure 3. Neighbor-joining phylogenetic tree of an 'angel skin' coral bead and an extensive reference data set of *Pleurocorallium*. The 'angel skin' bead is highlighted in red. The samples of species sequenced for the first time in this study are highlighted in blue. The authors of the previously published samples are shown in brackets; for exact references and sequences please refer to Lendvay et al. [9]. Numbers on the nodes represent bootstrap support values.

Table 1. Dissimilarity of the DNA obtained from the 'angel skin' coral necklace from members of the *Pleurocorallium* genus based on sequence alignment of the concatenated *IGS*-mt*MutS*-*LR* regions (1113 base-pair long) and the *LR* region alone (129 base pairs).

Species	Species Author	Distribution Area	No. of Reference Samples	Base-Pair Differences from the 'Angel Skin' Coral Necklace	
				IGS-mt*MutS*-*LR*	Only *LR*
P. bonsaiarborum	Tu, Dai, and Jeng 2016	South-West Pacific	1	106	4
P. borneense	Bayer 1950	North-West Pacific	4	122–124	4
P. carusrubrum	Tu, Dai, and Jeng 2012	North-West Pacific	1	85	4
P. clavatum	Tu, Dai, and Jeng 2016	North-West Pacific	1	136	5
P. elatius	Ridley 1882	North-West Pacific	5	82–84	4
P. gotoense	Nonaka et al. 2012	North-West Pacific	1	83	4
P. inutile	Kishinouye 1902	North-West Pacific	4	275–313	3–4
P. johnsoni	Gray 1860	North-East Atlantic		n.a.	n.a.
P. konojoi	Kishinouye 1903	North-West Pacific	7	82–84	4
P. niveum	Bayer 1956	Central North Pacific	14	114–118	1–4
P. norfolkicum	Tu, Dai, and Jeng 2016	South-West Pacific	3	6–7	1–2
P. occultum	Tu, Altuna, and Jeng 2015	North-East Atlantic	2	127	4
P. porcellanum	Pasternak 1981	Central North Pacific	5	135–137	5
P. pusillum	Kishinouye 1903	North-West Pacific		n.a.	n.a.
P. secundum	Dana 1846	Central and North-West Pacific	9	129	7
P. thrinax	Bayer and Stefani, 1996	South-West Pacific	3	274–281	3–4
P. uchidai	Nonaka et al. 2012	North-West Pacific	1	83	4
P. sp10	sensu Tu, Dai, and Jeng 2015	South-West Pacific	2	8–16	1–2
P. sp11	sensu Tu, Dai, and Jeng 2015	South-West Pacific	1	18	2
P. sp12	sensu Tu, Dai, and Jeng 2015	South-West Pacific	1	18	1
Corallium sp1	sensu Ardila et al. 2012	South-West Pacific	4	n.a.	5
P. sp1	sensu Ardila et al. 2012	South-West Pacific	3	n.a.	2–4
P. sp2	sensu Ardila et al. 2012	South-West Pacific	1	n.a.	4

3.4. Comparison of the 'Angel Skin' Coral Necklace's DNA with Additional Samples

We performed the *Coral*-ID identification assay for 18 samples and compared them with the DNA of the 'angel skin' coral necklace. Twelve samples were revealed to be members of the genus *Hemicorallium*, while six of them were confirmed to be *Pleurocorallium*. From these samples, a single one, the branch from a colony fished in Vietnam (see photograph in Figure 4) had a completely identical sequence to the 'angel skin' coral necklace. The results of the remaining samples will be published elsewhere and are not detailed here.

Figure 4. The coral branch fished in Vietnam in 2008.

4. Discussion

In this study, we present the analysis of a highly impressive 'angel skin' coral necklace. Assembling a matching selection of natural, precious corals of such size and attractive color can be considered very rare and exceptional. Therefore, this strand possesses extraordinary characteristics that merit a detailed study.

The visual inspection and the nondestructive molecular fingerprinting could confirm that the 'angel skin' necklace is an authentic, precious coral material, and it has not been artificially color-treated. Commonly applied methods during gemological assessment could only provide tentative species identification. To provide a conclusive species identification, the owner of the necklace agreed to perform a minimally destructive sampling of three coral beads to conduct genetic tests. Testing each single bead would have been labor-intensive and costly; thus, three beads were chosen from different parts of the strand to provide general conclusions about the necklace. The material collected from the existing drill-holes caused no visual alterations of the beads, and the amount of material collected from the three coral beads, weighing 2.2–2.4 milligrams, can be considered exceptionally small, cf. [15]. The amount of 2.2 mg is, to date, the lowest-weighing sample that the ZIFM has successfully genotyped from a coral skeletal material. All our genetic tests rely on mitochondrial genomic regions, as these are most effective when working with low-template and fragmented DNA [23] and best characterized for taxonomic purposes in the Coralliidae precious corals. The mitochondrial DNA in precious corals is, however, highly conserved [24]. This, together with the very small amount of sampled material, led to a limited resolution of our genetic inferences, which were nevertheless robust enough to draw clear-cut conclusions.

As the first step in our genetic investigation of the 'angel skin' coral necklace, the identification tool named *Coral*-ID was applied, which is a sensitive and accurate method to identify the precious corals considered to be used in ornamental objects and jewelry [3]. The results of the *Coral*-ID assay rejected the possibility that the necklace is from the expected *P. elatius* or *P. secundum* and suggested that the three tested beads are from another, undetermined *Pleurocorallium* species. This intriguing result has led us to perform further genetic tests using the DNA extract from one bead.

4.1. Taxonomic and Geographic Origin Assignment of the 'Angel Skin' Coral Necklace

We compared DNA extracted from the material of the 'angel skin' coral necklace with data from the broadest possible collection of *Pleurocorallium* species and further data from

the scientific literature of undescribed species. Based on this set of data, we can conclude that the analyzed bead of the 'angel skin' coral necklace is from a *Pleurocorallium* precious coral species, which does not completely match the DNA of any hitherto described species. We note that two further cryptic lineages belonging to the genus *Pleurocorallium* were also discovered from Japan by Takata et al. (unpublished data); however, they are genetically considerably different from this 'angel skin' coral or other examined corals in this study based on *IGS*-mt*MutS-LR* sequences. According to our phylogenetic inference, this 'angel skin' coral necklace is on a phylogenetic branch containing colonies collected exclusively in the Coral Sea region of the South Pacific, more specifically, from around the Solomon Islands and New Caledonia. These colonies belong to *P. norfolkicum* and other putative species, whose taxonomic status is not yet fully clarified (i.e., *P*. sp. 10, *P*. sp. 11, and *P*. sp. 12). The members of this phylogenetic group share high sequence similarities and can be referred to as the *P. norfolkicum* species complex [16]. The members of the *P. norfolkicum* species complex were previously collected solely in the Coral Sea, which is over 5000 km away from the closest documented commercial precious coral fishing grounds, the Northern Philippines in the north-west direction and the Hawaii Islands in the north-east direction; [1,7]. Hence, it became clear that the 'angel skin' coral necklace is genetically distant from all currently known specimens from any precious coral fishing areas.

In addition to the *P. norfolkicum* species complex, other species complexes containing genetically similar but morphologically distinct morphospecies are also known in *Pleurocorallium* (i.e., the *P. elatius* and *P. occultum* species complexes [16]). The relatively well-studied *P. elatius* species complex comprises less genetic diversity than the *P. norfolkicum* species complex and contains five morphospecies that cannot be resolved by mitochondrial markers (see Figure 3 and [16]). Nevertheless, the two commercially important morphospecies, *P. elatius* and *P. konojoi*, could be differentiated based on genome-wide genetic data [25]. This result demonstrates that species complexes can contain valid, shallowly differentiated species. Only further studies will allow us to determine whether the *P. norfolkicum* species complex also contains distinct species and to infer if the 'angel skin' coral bead belongs to an undescribed coral species or if it is *P. norfolkicum*.

Despite the remaining taxonomic uncertainties, we went further to find the possible geographic origin of the material of the 'angel skin' coral necklace. We compared its DNA sequence data with DNA sequences of 18 samples from a broad range, including areas where precious coral reference samples are rare. A sample from such an area, Vietnam, showed 100% sequence similarity with the 'angel skin' coral necklace in the studied DNA regions. This small branch with fragments of the cortex and white axis was obtained at a coral auction in Japan in 2009, with the information that it was fished during the preceding year. A commercial precious coral fishery is known to have existed in Vietnam [1], but is poorly documented [26]. The very little information available about precious coral fishing in Vietnam is reflected by the fact that Vietnam has not reported any catch of precious corals to the Food and Agriculture Organization (FAO) of the United Nations [27]. According to Iwasaki [26], a manned submersible was used for coral fishing in the waters south of Ho Chi Minh in 2008, and the following year, 135 kg of precious corals were traded at a bid market in Japan. Traders involved in the Vietnamese coral fishery disclosed to us that a Soviet-built manned research submersible, TINRO-2, was employed in the precious coral collection operations, which has a maximal diving depth of 400 m. The species occurring in the coastal waters of Vietnam were thought to be *P. elatius* and *P. konojoi*; however, no taxonomic research has been conducted on the colonies living in these sea areas [26]. Our study demonstrates that another, undescribed *Pleurocorallium* species is also present in the South China Sea off the coast of Vietnam. Its description as a newly discovered species will only be possible if complete colonies become available, which allow detailed morphologic

and genetic analyses. As for determining the putative geographic origin of the material of the 'angel skin' coral necklace, we opt for staying conservative; the single reference sample with complete genetic identity restricts us to being very moderate in this question. Our opinion is therefore that the necklace originates from the North-West Pacific, potentially the South China Sea or the East China Sea.

Pleurocorallium pusillum is a species that has no known specimens (we identified the *P.* cf. *pusillum* colonies to be members of another taxon, see below) and likewise no genetic data. Its author, Kishinouye [18], described this species only very briefly; he stated that *P. pusillum* has an orangish to orangish-yellow skeletal axis. It is difficult to compare the color hues of the 'angel skin' coral necklace with this color description of only a few words, but based on it, we cannot exclude the possible identity of the 'angel skin' coral necklace as *P. pusillum*. *Pleurocorallium pusillum* was from a colony that has been lost, and no other specimens from this species have existed for over a century. The question of whether the 'angel skin' coral necklace may originate from *P. pusillum* cannot be settled until the discovery of precious coral colonies with complete morphological resemblance to *P. pusillum* from near its locus classicus.

4.2. Taxonomic Inferences of the Newly Analyzed Taxa

In the course of the current study, we analyzed the samples of species that have never been involved in genetic studies before. Although a byproduct in the sense of the ultimate goal of this study, we find it important to also briefly discuss the information learned about the taxonomy of these species.

Pleurocorallium gotoense and *P. uchidai* are both known only from a single holotype specimen. Before these colonies were described as novel species, they were both considered members of the species *P. elatius*. The results of this study confirm that *P. gotoense* and *P. uchidai* are part of the *P. elatius* species complex together with *P. carusrubrum*, *P. elatius*, and *P. konojoi*, and can be considered morphospecies. Within our *IGS*-mt*MutS-LR* data set, neither *P. gotoense* nor *P. uchidai* have a single idiosyncratic nucleotide character, which implies that with this data set (and the integral *Coral*-ID marker), any specimen from these species can only be identified as belonging to the *P. elatius* species complex.

The specimen on which the description of *Pleurocorallium pusillum* was based in 1903 is unaccounted for and potentially no longer exists, and there were no other coral colonies fitting its described characteristics until the study of Nonaka and Hayashibara [19]. In their study, these authors found 11 colonies from the Kanmu and Koko Seamounts in the Emperor Seamounts region that nearly completely matched the diagnosis of *P. pusillum* and, therefore, they named these colonies *P.* cf. *pusillum*. *Pleurocorallium pusillum* was described from Japan, and the discovery of conspecific colonies from an over 3000 km distance to the east would be unusual but not unprecedented; a similar disjunct distribution pattern was confirmed in *P. secundum* [9]. In our phylogenetic inference, the three *P.* cf. *pusillum* colonies were placed among samples of *P. niveum*. In particular, NSMT-Co1719 and NSMT-Co1726 showed the highest similarities (respectively, 99.7% and 99.6% sequence similarities) to sample *P. niveum* HW_6 from Lisianski Island, Northwestern Hawaiian Islands, while NSMT-Co1721 was most similar (99.6% sequence similarity) to *P. niveum* IRM-UZH 1 from Makapuu, Ohau, Southeastern Hawaii Islands. This result led us to review and discuss the morphologic similarities of the *P.* cf. *pusillum* specimens to *P. pusillum* and *P. niveum*. The latter two species are morphologically very similar to each other [28]. The main diagnostic feature differentiating the two species is the presence–absence of 6-radiates; *P. pusillum* lacks them while *P. niveum* is abundant in 6-radiates. In addition, *P. pusillum* was described to have an orangish to orangish-yellow axis, while *P. niveum*—as its name suggests—is traditionally thought to be pure white, both the cortex and the axis. The *P.* cf. *pusillum*

colonies from the Emperor Seamounts were found to have 6-radiates, a red to orange and yellowish cortex on the branchlets, with a white axis. Based on the color of the cortex, the snow-white *P. niveum* was not considered as a possible species during the identification of these 11 *Pleurocorallium* colonies by Nonaka and Hayashibara [19]. However, according to our experience, *P. niveum* can also have a colorful cortex (B. Lendvay, unpublished data). Furthermore, the newer identification keys also specify that the orange (*P. pusillum*) versus white color (*P. niveum*) pertains only to the axis (as in the key of Tu et al. [2]) or do not even mention the color as an identification key (as in the key of Tu et al. [29]). As a conclusion, based on the presence of 6-radiates, the white skeleton, and their genetic similarity with *P. niveum*, here, we revise the taxonomic identity of the *P.* cf. *pusillum* colonies of Nonaka and Hayashibara [19] and suggest that they are in fact *P. niveum*. This finding extends the known distribution of *P. niveum* along the Hawaii–Emperor Seamounts ridge with c. 1000 km north-westward.

The specimen we analyzed to produce reference data for *P. johnsoni*, USNM 1010769, turned out to be a *Hemicorallium*. This tiny specimen is the only one labeled as *P. johnsoni* in the NMNH, which holds one of the world's largest collections of corals. Other specimens attributed to this species are unfortunately also very small (on the scale of millimeters and a few centimeters) or exist as microscope slides [30]. We conclude that either the specimen we analyzed is mislabeled and is a member of a *Hemicorallium* species, or *P. johnsoni* in fact belongs to the genus *Hemicorallium*. Only further research involving a phylogenetic analysis of other specimens identified as *P. johnsoni* will be able to answer this question.

4.3. General Considerations for the International Coral Trade

What started as a routine analysis of a coral necklace has developed into a study that delivered unexpected results. Most importantly, this study demonstrates that there are more species present in high-end precious coral jewelry than the currently considered 10 species, cf. [1]. The discovery of a potentially undescribed species from a processed object clearly implies that our understanding of the diversity of precious corals in the trade is far from satisfactory. We suggest that fresh and well-documented samples of harvested corals be put aside for widespread taxonomic and phylogenetic analysis. At the same time, we anticipate the genetic testing of processed coral objects with special or irregular characteristics in an endeavor to discover the diversity of corals that have entered the trade.

The 'angel skin' coral can be considered a look-alike for the CITES-listed *Pleurocorallium elatius*. During the implementation of the CITES regulations, the trader has to prove that the traded item does not fall under CITES. In the case of precious corals, this means that even non-CITES-listed coral objects can be confiscated when they lack proof of their taxonomic identity, as shown by Lendvay et al. [3]. In order to avoid such situations, the Coral Commission of the World Jewelry Confederation (CIBJO) suggests non-CITES-listed precious corals be declared as CITES listed in several of its publications, e.g., [1,7,31]. While this biologically does not make much sense, it is a much better solution than the common process of laundering CITES-listed species by fraudulently declaring them as belonging to non-CITES-listed taxa.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/d17060395/s1, Supporting Results S1: NCBI GenBank accession numbers; Supporting Results S2: DNA sequence alignment in fasta format.

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References

1. CIBJO. *CIBJO Guides. Precious Coral*; Coral Commission of the World Jewellery Confederation: Milano, Italy, 2024.
2. Tu, T.-H.; Dai, C.-F.; Jeng, M.-S. Precious corals (Octocorallia: *Coralliidae*) from the Northern West Pacific region with descriptions of two new species. *Zootaxa* **2012**, *3395*, 1–17. [CrossRef]
3. Lendvay, B.; Cartier, L.E.; Costantini, F.; Iwasaki, N.; Everett, M.V.; Krzemnicki, M.S.; Kratzer, A.; Morf, N.V. *Coral*-ID: A forensically validated genetic test to identify precious coral material and its application to objects seized from illegal traffic. *Forensic Sci. Int. Genet.* **2022**, *58*, 102663. [CrossRef] [PubMed]
4. de Carvalho, R.G. Understanding Precious Coral Jewelry. In: Obsessed by Pearls, Assael. 2020. Available online: https://assael.com/blog/understanding-precious-corals (accessed on 4 May 2025).
5. Branstrator, B. 5 Things to Know About . . . Coral. National Jeweler. 2021. Available online: https://nationaljeweler.com/articles/9973-5-things-to-know-about-coral (accessed on 4 May 2025).
6. O'Steen, D. Defining Exceptional—The Beauty & Luxury of Angel Skin Coral. In: Obsessed by Pearls, Assael. 2018. Available online: https://assael.com/blog/defining-exceptional-the-beauty-luxury-of-angel-skin-coral (accessed on 4 May 2025).
7. CIBJO. *The Coral Book*; Coral Commission of The World Jewellery Confederation: Milano, Italy, 2020.
8. Cooper, E.W.T.; Torntore, S.J.; Leung, A.S.M.; Shadbolt, T.; Dawe, C. *Guide to the Identification of Precious and Semi-Precious Corals: In Commercial Trade*; TRAFFIC North America and WWF: Vancouver, BC, Canada, 2011.
9. Lendvay, B.; Morf, N.V.; Cartier, L.E.; Krzemnicki, M.S.; Nonaka, M. Trace-DNA from a century-old holotype specimen resolves taxonomic uncertainties: The case of the Hawaiian pink precious coral (*Pleurocorallium secundum*), a CITES-listed species used in jewelry. *Coral Reefs* **2025**, *in press*.
10. Bersani, D.; Lottici, P.P. Applications of Raman spectroscopy to gemology. *Anal. Bioanal. Chem.* **2010**, *397*, 2631–2646. [CrossRef]
11. Bergamonti, L.; Bersani, D.; Csermely, D.; Lottici, P.P. The nature of the pigments in corals and pearls: A contribution from Raman spectroscopy. *Spectrosc Let* **2011**, *44*, 453–458. [CrossRef]
12. Macchia, M.; Resta, V.; Quarta, G.; Calcagnile, L. Precious coral non-destructive characterization by Raman and XRF spectroscopy. *X-Ray Spectrom* **2016**, *45*, 281–287. [CrossRef]
13. Karampelas, S.; Fritsch, E.; Rondeau, B.; Andouche, A.; Métivier, B. Identification of the endangered pink-to-red *Stylaster* corals by Raman spectroscopy. *Gems Gemol.* **2009**, *45*, 48–52. [CrossRef]
14. Smith, C.P.; McClure, S.F.; Eaton-Magaña, S.; Kondo, D.M. Pink-to-red coral: A guide to determining origin of color. *Gems Gemol* **2007**, *43*, 4–15. [CrossRef]
15. Lendvay, B.; Cartier, L.E.; Gysi, M.; Meyer, J.B.; Krzemnicki, M.S.; Kratzer, A.; Morf, N.V. DNA fingerprinting: An effective tool for taxonomic identification of precious corals in jewelry. *Sci. Rep.* **2020**, *10*, 8287. [CrossRef]
16. Tu, T.-H.; Dai, C.-F.; Jeng, M.-S. Phylogeny and systematics of deep-sea precious corals (Anthozoa: Octocorallia: Coralliidae). *Mol. Phylogenet. Evol.* **2015**, *84*, 173–184. [CrossRef]
17. Nonaka, M.; Muzik, K.; Iwasaki, N. Descriptions of two new species and designation of three neotypes of Japanese Coralliidae from recently discovered specimens that were collected by Kishinouye, and the introduction of a statistical approach to sclerite abundance and size. *Zootaxa* **2012**, *3428*, 1–67. [CrossRef]
18. Kishinouye, K. Kosango. *Dobutsugaku Zasshi* **1903**, *15*, 372.
19. Nonaka, M.; Hayashibara, T. A report on Coralliidae (Cnidaria: Octocorallia) specimens collected from the Emperor Seamounts with descriptions of three new species. *Species Divers* **2021**, *26*, 297–342. [CrossRef]
20. Takata, K.; Iwase, F.; Iguchi, A.; Yuasa, H.; Taninaka, H.; Iwasaki, N.; Uda, K.; Suzuki, T.; Nonaka, M.; Kikuchi, T.; et al. Genome-wide SNP data revealed notable spatial genetic structure in the deep-sea precious coral *Corallium japonicum*. *Front. Mar. Sci.* **2021**, *8*, 667481. [CrossRef]

21. Kumar, S.; Stecher, G.; Tamura, K. MEGA7: Molecular Evolutionary Genetics Analysis version 7.0 for bigger datasets. *Mol. Biol. Evol.* **2016**, *33*, 1870–1874. [CrossRef]
22. Ardila, N.E.; Giribet, G.; Sánchez, J.A. A time-calibrated molecular phylogeny of the precious corals: Reconciling discrepancies in the taxonomic classification and insights into their evolutionary history. *BMC Evol. Biol.* **2012**, *12*, 246. [CrossRef]
23. Linacre, A.; Gusmão, L.; Hecht, W.; Hellmann, A.P.; Mayr, W.R.; Parson, W.; Prinz, M.; Schneider, P.M.; Morling, N. ISFG: Recommendations regarding the use of non-human (animal) DNA in forensic genetic investigations. *Forensic. Sci. Int. Genet.* **2011**, *5*, 501–505. [CrossRef] [PubMed]
24. Shearer, T.L.; Van Oppen, M.J.H.; Romano, S.L.; Wörheide, G. Slow mitochondrial DNA sequence evolution in the Anthozoa (Cnidaria). *Mol. Ecol.* **2002**, *11*, 2475–2487. [CrossRef]
25. Takata, K.; Taninaka, H.; Nonaka, M.; Iwase, F.; Kikuchi, T.; Suyama, Y.; Nagai, S.; Yasuda, N. Multiplexed ISSR genotyping by sequencing distinguishes two precious coral species (Anthozoa: Octocorallia: Coralliidae) that share a mitochondrial haplotype. *PeerJ* **2019**, *7*, e7769. [CrossRef]
26. Iwasaki, N. Asian regional report on the biology, fishery and trade of precious and semi-precious corals. In *Global Report on the Biology, Fishery Trade of Precious Corals*; FAO: Rome, Italy, 2019; pp. 191–254.
27. Shiraishi, H. *Seeing Red. Precious Coral Trade in East Asia*; TRAFFIC: Tokyo, Japan, 2018.
28. Bayer, F.M. Descriptions and redescriptions of the Hawaiian Octocorals collected by the US Fish Commission steamer "Albatross" (2. Gorgonacea: Scleraxonia). *Pac. Sci.* **1956**, *10*, 67–95.
29. Tu, T.-H.; Dai, C.-F.; Jeng, M.-S. Taxonomic revision of Coralliidae with descriptions of new species from New Caledonia and the Hawaiian Archipelago. *Mar. Biol. Res.* **2016**, *12*, 1003–1038. [CrossRef]
30. Tu, T.-H.; Altuna, A.; Jeng, M.-S. Coralliidae (Anthozoa: Octocorallia) from the INDEMARES 2010 expedition to North and Northwest Spain (Northeast Atlantic), with delimitation of a new species using both morphological and molecular approaches. *Zootaxa* **2015**, *3926*, 301–328. [CrossRef] [PubMed]
31. CIBJO. *Coral Guide for Customs. Classification & Identification of Coral Materials*; The World Jewellery Confederation: Milano, Italy, 2017.