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REPORT

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

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Abstract The holotype specimen holds the most authentic characteristics of a species; its features will serve as a foundation for the identification of individuals belonging to this species. The precious coral *Pleurocorallium secundum* was described in 1846 based on a colony from the Hawaiian Islands. This specimen has been preserved; however, it is decorticated and contains exclusively the axial skeleton, which has hindered its use for accurate species identification. Therefore, the species was redescribed in 1956 based on two lots collected in 1902. *Pleurocorallium secundum* was considered the most frequently fished precious coral species in the second half of the twentieth century with landings on the total scale of hundred-thousand kilograms, which was followed by its listing on CITES Appendix III. Recently, the conspecificity of the holotype and redescribed colonies was questioned, and specimens labeled in the scientific literature as *P. secundum* were discovered to be phylogenetically distant from each other. To clarify the identity of *P. secundum*, we took minimally destructive samples from the century-old holotype and fragments of redescribed colonies and applied techniques conforming to low copy number DNA analyses. DNA sequences of three mitochondrial regions were evaluated in a phylogenetic framework together with DNA sequences retrieved from freshly collected putative *P. secundum* specimens and sequences from the scientific literature. The results of this study clearly indicate that the holotype and the colony fragments used for the redescription of *P. secundum* represent the same species. Based on the specimens confirmed to be *P. secundum* with genetic evidence, the distribution area of *P. secundum* stretches from the Hawaiian Islands to the South China Sea. At the same time, our analysis uncovered both published and fresh specimens that are in fact not *P. secundum*. The latter includes the fished Makapuu coral bed in Hawaii, which used to be a significant coral fishing area. Based on the microscopic analysis of redescribed colony fragments, we complement the diagnosis of *P. secundum*. This, together with our genetic results will aid the identification of coral objects present in the international jewelry trade by providing authentic molecular barcoding markers and morphologic features for the identification of *P. secundum*.

Keywords Species identification · DNA barcodes · Coral taxonomy · Coralliidae · Octocoral

Introduction

Within the vastly diverse class of Octocorallia, precious corals (genera *Corallium*, *Hemicorallium* and *Pleurocorallium*, Coralliidae) are a distinctive group characterized by possessing an unjointed axial medulla of solid calcium carbonate with bright colors (Bayer 1956). This hard and colorful internal skeletal axis has made precious corals a highly valuable material for the jewelry industry for centuries (Cartier et al. 2018). Besides the long-known precious coral fishing grounds in the Mediterranean sea and the waters off the islands of Japan and Taiwan, precious corals have been

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discovered in the waters surrounding the Hawaiian Islands and the Emperor Seamounts in 1965 (Grigg 2002; Tsounis et al. 2010). This region has become the most important coral fishing area for decades (Bruckner 2009). The pink and white–pink spotted corals of the North Pacific originate from several *Hemicorallium* and *Pleurocorallium* species, among them the one claimed to be harvested in the largest quantities, the Hawaiian pink coral, *Pleurocorallium secundum* (Grigg 1993; Parrish et al. 2009, 2017; Nonaka & Hayashibara 2021). In the trade and jewelry industry, pink-colored Hawaiian corals are generally associated with this species and are known by several trade names, in particular Midway, pink, white/pink (bianco rosa), middo sango and rosato coral (Tsounis et al. 2010; Cooper et al. 2011; CIBJO 2017, 2020).

Pleurocorallium secundum was described by James D. Dana from the Hawaiian Islands with the former name *Corallium secundum* in 1846 as the second precious coral species in the Coralliidae family following the type species, *Corallium rubrum* (as *Madrepora rubra* Linnaeus, 1758) (Dana 1846). It was first mentioned as *P. secundum* by Gray (1867), and its assignment to the *Pleurocorallium* genus has been later supported by phylogenetic studies (Ardila et al. 2012; Tu et al. 2015). The *P. secundum* specimen illustrated in Dana's species description has been preserved in the Smithsonian National Museum of Natural History, Washington D.C. (NMNH) with the lot number USNM 600 and is recognized since as the holotype specimen (Fig. 1a, b; Bayer 1956; Tu et al. 2016). Dana described characteristics of the cortex; however, already on Dana's drawing, the specimen seems to completely lack the cortex as if it was meticulously removed and merely possess the axial medulla (Fig. 1c). For over a century, this specimen was the single colony known from the species. Then, in 1956 Fredrick Bayer identified *P. secundum* colonies from the material of the "Albatross" research vessel's Hawaiian expedition in 1902 (Bayer 1956). Moreover, he redescribed the species to amend its identification key to conform to other, later described morphologically similar species. Due to incompleteness of the holotype specimen, Bayer wrote the new diagnosis based on two samples of the "Albatross" material. The data record for one of these matches with those of lot USNM 49330 (Fig. 1d), which was considered *P. secundum*'s "neotype" by Nonaka et al. (2014). However, as Bayer did not designate these colonies as a neotype, in the following, we refer to USNM 49330 as "redescribed colony fragments." Nonaka et al. (2014) carefully studied Dana's holotype (USNM 600) and Bayer's redescribed colony fragments (USNM 49330). They concluded that due to Dana's vague species description and the incompleteness of the holotype specimen, there is "no objective evidence that USNM 600 and USNM 49330 may be or in fact are the same species, *C. secundum*." This implies that "if Bayer's specimen (USNM 49330) is a different species than Dana's specimen (USNM 600), then USNM 49330 needs to be described as a new species. In this case, almost all specimens heretofore identified as *C. secundum* would have to be reassigned to this new species."

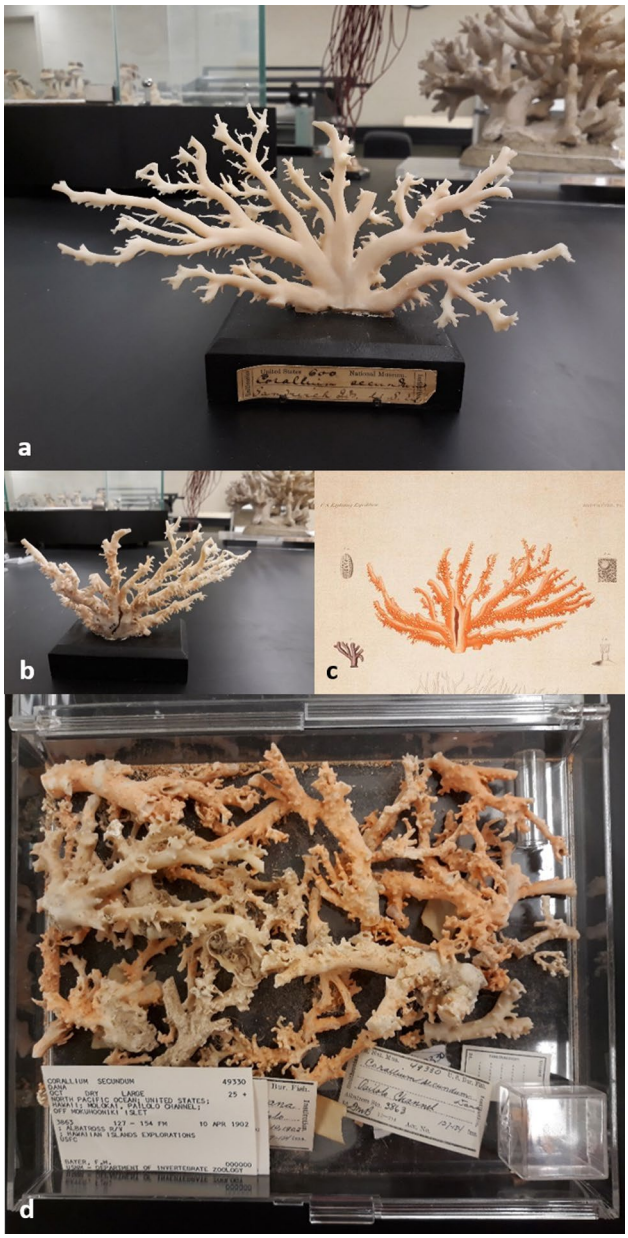

Fig. 1 The holotype and the redescribed colony fragments of *Pleurocorallium secundum* at the dry coral collection of the Smithsonian National Museum of Natural History. The holotype specimen is photographed from the labeled "front" side (**a**) and from an angle (**b**) similar from which Dana depicted the specimen in his original species description from 1846 (**c**). The lot of the redescribed colony fragments (**d**) contains multiple fragments collected in on April 10, 1902

Besides the upper outlined taxonomic uncertainty, there is an additional identification problem with specimens of *P. secundum*. Two phylogenetic studies, Ardila et al. (2012)

and Tu et al. (2015) analyzed altogether 14 *P. secundum* specimens of the USNM collection (Table 1). In both studies, these 14 specimens clustered in two clearly distinct genetic groups: 11 specimens clustered together with other Hawaiian specimens labeled as *Pleurocorallium niveum*. The remaining three specimens formed a well-supported genetic cluster and were considered *P. secundum*. These few samples served as reference samples for Lendvay et al. to characterize *P. secundum* for their genetic species identification assay called *Coral*-ID (2022). It was however noticed that based on this method, the single *P. secundum* complete mitochondrial genome on NCBI GenBank (accession number KC782347) from the study of Figueroa and Baco (2014) would classify as another *Pleurocorallium* species (B. Lendvay, unpublished data). To summarize the taxonomic uncertainties, (1) it has been questioned whether the decorticated holotype and the redescribed colony fragments indeed belong to the same species and (2) colonies that have been identified as *P. secundum* during the past decades based on morphology (of the redescribed colonies) were later identified to genetically belong to two distant phylogenetic groups; a monophyletic group then called *P. secundum*, and another group also containing all specimens of *P. niveum*. The phylogenetic relationship of the latter two groups, the redescribed colony fragments and the holotype remained unclear.

Pleurocorallium secundum was reportedly fished between the 1960s and 1990s around the Emperor seamounts and the Hawaiian Islands in quantities estimated to reach the scale of hundred-thousand kilograms (Cannas et al. 2019). The corals fished here entered the Japanese and Taiwanese trading hubs. At the time, heir characteristics and taxonomic identity were not studied and the available information about these harvests remains mainly anecdotal (Grigg 1993; Tsounis et al. 2010; Iwasaki 2018). Subsequently, *P. secundum*'s harvesting decreased dramatically due to the declining price of corals and resource depletion and its commercial harvest ceased completely in 2001 (Grigg 2002; Chang et al. 2013). However, the harvesting of *P. secundum* continued in the South China sea (around Taiwan) in small quantities (Shiraishi 2018). The high fishing-pressure placed on precious coral populations by the jewelry industry led UN parties to suggest international trade regulations on the entire Coralliidae family by placing them on the Appendix II of CITES; these proposals (by the USA in 2007 and by the USA and the EU in 2010; CoP14 Prop.21 2007; CoP15 Prop. 21 2010) were however rejected. Nevertheless, *P. secundum* has been placed on the CITES Appendix III together with three other precious coral species by China since July 2008. Listing on Appendix III can be performed by CITES party countries for species inhabiting their areas unilaterally without the need for common agreement of the parties. Although *P. secundum* is not known to inhabit sea-beds off mainland China, it is fished in the waters of the non-UN and -CITES member Taiwan, over which is the CITES member state China claims *de jure* control (Chang 2015). Due to the lack of a sound taxonomic identification of Chinese corals (fished off Taiwan) as *P. secundum*, the report published by the United Nations FAO in 2019 raised concerns over the legitimacy of the inclusion of *P. secundum* in CITES Appendix III by China (Cannas et al. 2019).

Given all the uncertainties surrounding the identity of *P. secundum*, we considered it timely to reevaluate its taxonomic status. Based on the genetic analysis of the holotype and redescribed colony fragments, fresh samples and published data from the literature, we aimed to answer the following specific questions: (i) Do the holotype and the redescribed colony fragments of *P. secundum* in fact belong to the same species? (ii) Is it possible to establish solid DNA barcodes and species-characteristic morphologic attributes for the identification of *P. secundum*? (iii) What is the geographical distribution of *P. secundum* and can we confirm that the corals considered *P. secundum* fished in Chinese waters really belong to this species?

Methods

Analyzed coral samples

We analyzed four types of *P. secundum* samples in this study: (i) the holotype, (ii) redescribed colony fragments, *(iii)* fresh samples of putatively *P. secundum* samples collected in two areas off the Southeastern Hawaiian Islands and *iv)* DNA sequence data of samples from the scientific literature—including data from *P. secundum* from Chinese waters collected by the Taiwanese precious coral fishery.

Holotype

Dana (1846) described *P. secundum* based on a single known specimen collected in the Sandwich Islands (Hawaiian Islands) by the U.S. Exploring Expedition, which visited the Hawaiian Islands in 1840–1841. Dana's original description contains an illustration of this idiosyncratic specimen; this completely matches with the specimen USNM 600 in the dry coral collection of the NMNH, which is regarded the holotype specimen of *P. secundum*. According to its lot description, the holotype specimen was collected at the French Frigate Shoals. The approximately 20 cm wide and 10 cm high coral colony is mounted on a wooden block. Interestingly, although the illustration by Dana (1846) depicts a coral colony decorticated already at that time, his description claimed that the dried crust still remained on the axis allowing the observation of the polyps. Two samples were collected from this specimen for genetic analyses in November 2022; the first sample, approximately 3 mg in

Table 1 All specimens that have been considered *Pleurocorallium secundum* before and/or after genetic analysis to this date

Original identification based on morphology	Museum lot number or collection identifier	Collection location	Collection depth (m)	Analyzed by	Previously published as	Identified in this study as *P. secundum*?	Point on the map (Fig. 3.)	Color on Fig. 2 & 3
P. secundum (holotype)	USNM 600	French Frigate Shoals, Hawaiian Islands	n.a	this study	n.a	the holotype	1	
P. secundum (redescribed colony fragments)	USNM 49330	Pailolo Channel, Hawaiian Islands	232–282	this study	n.a	Yes	2	
P. secundum	USNM 1010720	S of Kahoolawe, Hawaiian Islands	258	(Ardila et al. 2012; Tu et al. 2015),	*P. secundum*	Yes	3	
P. secundum	USNM 1010758	88 Fathom Pinnacle, Maui, Hawaiian Islands	240	(Herrera et al. 2010; Ardila et al. 2012; Tu et al. 2015)	*P. secundum*	Yes	4	
P. secundum	USNM 56188	Kailua-Kona, Hawaii Island, Hawaiian Islands	326–472	(Ardila et al. 2012)	*P. secundum*	Yes	5	
P. secundum	USNM 1072379	Bank 11, NW of Kure Island, Hawaiian Islands	564	(Tu et al. 2015)	*P. niveum*	No		
P. cf. secundum	USNM 1072415	Bank 8, NW of Lisianski Island, Hawaiian Islands	540	(Ardila et al. 2012)	*P. niveum*	No		
P. secundum	USNM 1072416	Bank 8, NW of Lisianski Island, Hawaiian Islands	544	(Ardila et al. 2012)	*P. niveum*	No		
P. secundum	USNM 1072438	Pioneer Bank, Hawaiian Islands	450	(Ardila et al. 2012)	*P. niveum*	No		
P. secundum	USNM 1072439	Pioneer Bank, Hawaiian Islands	444	(Ardila et al. 2012)	*P. niveum*	No		
P. secundum	USNM 1082650	Makapuu, Hawaiian Islands	431	(Tu et al. 2015)	*P. niveum*	No		
P. secundum	USNM 1082652	Brooks Banks, Hawaiian Islands	454	(Tu et al. 2015)	*P. niveum*	No		
P. secundum	USNM 1082653	Keahole Point, Hawaiian Islands	400	(Ardila et al. 2012)	*P. niveum*	No		
P. secundum	USNM 1082654	Cross seamount, Hawaiian Islands	409	(Ardila et al. 2012; Tu et al. 2015)	*P. niveum*	No		
P. secundum	USNM 1082655	Cross Seamount, Hawaiian Islands	414	(Ardila et al. 2012)	*P. niveum*	No		
P. secundum	USNM 56833	NW Of Nihoa Island, Hawaiian Islands	402–441	(Tu et al. 2015)	*P. niveum*	No		
P. tortuosum	USNM 1072376	Bank 10, NW of Kure Island, Hawaiian Islands	378	(Ardila et al. 2012)	*P. secundum*	Yes	6	
P. kishinouyei	USNM 1072407	Nero Seamount, SE of Kure Island, Hawaiian Islands	319	(Ardila et al. 2012)	*P. secundum*	Yes	7	
P. secundum	ASIZ80388	off Taiwan Island, South China sea	140	(Tu et al. 2015)	*P. secundum*	Yes	8	

Table 1 (continued)

Original identification based on morphology	Museum lot number or collection identifier	Collection location	Collection depth (m)	Analyzed by	Previously published as	Identified in this study as *P. secundum*?	Point on the map (Fig. 3.)	Color on Fig. 2 & 3
P. secundum	n.a	Pioneer Bank, Hawaiian Islands	n.a	(Figueroa & Baco 2014)	*P. secundum*	No		
"*P. secundum?*"	106	Kailua-Kona, Hawaii Island, Hawaiian Islands	273	(Lendvay et al. 2022), this study	*P. secundum*	Yes	5	
"*P. secundum?*"	107	Kailua-Kona, Hawaii Island, Hawaiian Islands	238	(Lendvay et al. 2022), this study	*P. secundum*	Yes	5	
"*P. secundum?*"	108	Kailua-Kona, Hawaii Island, Hawaiian Islands	221	(Lendvay et al. 2022), this study	*P. secundum*	Yes	5	
"*P. secundum?*"	204	Kailua-Kona, Hawaii Island, Hawaiian Islands	269	(Lendvay et al. 2022), this study	*P. secundum*	Yes	5	
P. cf. secundum	F1	Makapuu, Hawaiian Islands	414	(Lendvay et al. 2022), this study	*P. niveum*	No		
P. cf. secundum	F2	Makapuu, Hawaiian Islands	414	(Lendvay et al. 2022), this study	*P. niveum*	No		
P. cf. secundum	F3	Makapuu, Hawaiian Islands	414	(Lendvay et al. 2022), this study	*P. niveum*	No		

Colored squares represent the following: red: holotype; orange: the redescribed colony fragments; light blue and dark blue: respectively fresh samples and samples from the literature identified as *P. secundum*; light green and dark green: respectively fresh samples and samples from the literature originally identified as *P. secundum* but disproven here. Note that the *P. niveum* specimens marked with dark green squares were originally identified as *P. secundum* but already considered *P. niveum* by Tu et al. (2015), which is confirmed here

weight, was skeletal material scraped off the surface at the base of the specimen with a scalpel. The second sample contained formerly broken off skeletal buds with a total weight of approximately 5 mg.

Redescribed colony fragments

For the new diagnosis of *P. secundum*, Bayer (1956) relied on two species records. Based on the information of the sampling location, depth and date, these can be identified as the lots USNM 49330 and USNM 49471 of the NMNH; the samples were collected with a week difference at two nearby stations during the Albatross research vessel's Hawaiian Islands Exploration in the spring of 1902. While USNM 49471 is ethanol-stored, USNM 49330 has been preserved in the dry collection. The lot USNM 49330 contains over 25 branch fragments and considerable amount of fallen off coenenchymal debris. Its description states that it was collected at station 3863 off Mokuhooniki islet (Pailolo Channel) at depths between 127 and 154 fathoms on April 10, 1902. The lot does not contain any apparently heterogeneous coral material (i.e., multiple species). The single collection time and collection location also leads us to the assumption that the branch fragments originate from a single colony or at least from adjacent individuals within a population. We chose to perform genetic and morphologic analysis of this lot as it was already studied by Nonaka et al. (2014) and the large amount of coenenchymal debris allowed sampling for genetic testing with minimal destruction of this precious museum material. Furthermore, the DNA sequencing of bulk debris allowed us to confirm or reject the presence of different DNA haplotypes within the material of this lot. About 120 mg of bulk debris was collected for genetic testing. According to our experience, this is a considerable amount of material when performing DNA extraction from coenenchyme and we hoped it would give a good representation of the genetic material of this lot. Sampling was performed in November 2022.

Fresh samples

We analyzed seven fresh samples presumed to be *P. secundum* from two locations of the Hawaiian Islands. Three samples were collected in July 2013 at 414 m depth at the Makapuu coral bed located between Oahu and Molokai islands. These samples were later considered *P. niveum* by Lendvay et al. (2022). Four additional samples originated from the coast of Kailua-Kona, Hawaii Island and were collected in September 2016 at depths between 221 and 273 m. These samples were considered *P. secundum* by Lendvay et al. (2022) and were named "*P. secundum*?" in the publication by Conner et al. (2023). The fresh samples were collected using Pisces IV/V submersibles during Hawaii Undersea Research Laboratory (HURL) cruises, and stored and transported in ethanol solution.

Data from scientific literature

We searched all *Pleurocorallium* specimens that had DNA sequences available from three variable mitochondrial DNA regions; the mtND6-COI intergenic spacer (IGS), a fragment of the DNA mismatch repair protein (mtMutS) gene and a fragment of the large ribosomal RNA gene subunit (LR) on the NCBI GenBank platform. On November1, 2023, a total of 53 specimens were found and sequence data of the three genes were downloaded. These included 48 specimens analyzed by Tu et al. (2015), two specimens from Figueroa and Baco (2014) and Uda et al. (2013), respectively, and one specimen analyzed by Uda et al. (2011). These samples included four specimens labeled as *P. secundum*. The scientific literature was then reviewed in order to discover any other scientific works that analyzed these four specimens (based on DNA sequences of other genes).

Laboratory protocols

DNA was extracted from the skeletal material of the holotype specimen by applying a protocol specially optimized for DNA extraction from minute amounts of coral axis powder. The method is based on the complete decalcification and lysis of the skeletal powder followed by a column purification of the lysate and has previously proved to yield DNA from as low as 2.3 mg material drilled from polished coral twigs (Lendvay et al. 2020, 2022). 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 Proteinase-K. The lysate was concentrated to 50 µl on a 30 kDa Amicon Ultra filter (Merck) 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). The mixture was then purified with the MinElute kit according to the manufacturer's instructions and eluted in 100 µl volume.

The coenenchymal debris of the redescribed colony fragments and the coenenchymal tissue of the fresh samples were extracted using the QiaAmp DNA Mini kit (Qiagen) according to the manufacturer's protocol.

For all samples, we analyzed the DNA sequences of the three mitochondrial DNA regions, IGS, mtMutS and LR. Initial tests to amplify the IGS and mtMutS as single fragments using the primers of Tu et al. (2015) failed both in the case of the holotype and redescribed colony. This was not surprising given the minute amounts of material of century-old samples used for the DNA extraction. To amplify the potentially degraded DNA of the holotype and redescribed colony, for both IGS and mtMutS, we designed primers to amplify three overlapping short fragments of any *Pleurocorallium* species. The sequences of the newly designed IGS and mtMutS primers, PCR protocols, amplicon lengths and the positions of the amplified IGS and mtMutS fragments (as mapped on the *Pleurocorallium konojoi* mitochondrial reference genome NC_015406, Uda et al. 2011) are shown in Supporting information S1. The amplified short IGS and mtMutS fragments were sequenced by Sanger technology according to Lendvay et al. (2022). For the fresh samples that have less fragmented DNA, the IGS and mtMutS regions were amplified and sequenced as single fragments following Tu et al. (2015). The very short LR region was amplified for all samples using the same primers previously applied by Lendvay et al. (2020), see Supporting information S1. The LR amplicons were then converted to sequencing libraries using the KAPA HyperPrep PCR-free Kit (Roche) and run on a MiSeq instrument (Illumina).

DNA extracted from the holotype and redescribed colony fragments were PCR-amplified and sequenced twice to produce independent technical replicates in ISO17025-accredited trace-DNA laboratory rooms of the Institute of Forensic Medicine at the University of Zurich. The DNA of the fresh samples was amplified and sequenced in separate research-purpose laboratory rooms at the same facility.

DNA sequence analysis

For the IGS and mtMutS datasets, primer sequences were searched and trimmed from the raw DNA sequence data in Geneious Prime 2022.0.2 (www.geneious.com) with default settings. Then, in the case of the holotype and the redescribed colony fragments that were amplified as short overlapping DNA fragments, the short fragments were assembled to yield the complete analyzed IGS and mtMutS fragments with basic settings of the de novo assembly function in Geneious Prime. The LR sequence data were evaluated as described by Lendvay et al. (2020).

The DNA sequences obtained by the two technical replicates (for all three analyzed DNA regions: IGS, mtMutS and LR) performed for the DNA extracts of both holotype and redescribed colony fragments were aligned to each other with the default pairwise Geneious Alignment method in Geneious Prime to test for consistency of the replicate data. In the following, the DNA sequence data of the *P. secundum* holotype, the redescribed colony fragments, the fresh Hawaiian coral samples and the *Pleurocorallium* sequence data from the literature were aligned and trimmed together with the IGS, mtMutS and LR sequences of the *Corallium rubrum* mitochondrial reference genome (GenBank accession number AB700136, Uda et al. 2013), which was used as outgroup. Bayesian phylogenetic trees were created with partitioning the concatenated alignment of the three genes and run in MrBayes version 3.2.7 (Ronquist et al. 2012) with the settings used by Lendvay et al. (2020): mixed substitution model prior, 10^7 generations with sampling every 10^3 generations and 25% burn-in. Convergence of the two parallel analyses run by the software was ensured by the sufficiently low value (0.0047) of the average standard deviation of split frequencies. A majority rule consensus tree was constructed from the raw tree data in FigTree (Rambaut 2012). Based on their DNA sequence similarity and phylogenetic position compared to the holotype specimen, we reevaluated whether specimens formerly identified as *P. secundum* indeed belong to this species. We then pinned the location of the confirmed specimens on a map to estimate the approximate geographical distribution of *P. secundum*.

Morphologic analyses

Bayer (1956) provided description of the morphologic features of the redescribed colony fragments of lot USNM 49330. He did however not present the size and quantitative proportion of the sclerites, which have recently become standard for the taxonomic description of precious corals. Therefore, small amounts of coenenchyme were taken from four colony parts (tentacles, autozooid mounds, branch tips, and colony base; one coenenchyme sample per colony type) for scanning electron microscope (SEM) imaging of the sclerites. The sclerites were separated and cleaned using 5% sodium hypochlorite solution (household bleach), and details of them were observed with a Keyence VE-8800 instrument. Their length and widths were measured with SEM accessory software. The sclerites were classified according to standard taxonomic convention (Bayer 1956; Bayer et al. 1983). They were distinguished from each other by the number of their projections, for example 6 radiates, 7 radiates and 8 radiates. With more than eight projections, the sclerites were called multi-radiates. Sharp sclerites with undeveloped projections were called "rods." In addition, for this study, 6 radiates and 8 radiates were subdivided into the following two types; "symmetric" and "asymmetric." Asymmetric 6 radiates were intermediate between symmetric 6 radiates and double clubs. Double clubs were identified by clearly having "two handles." For the classification of these sclerites, see Fig. 2 in Nonaka and Hayashibara (2021).

Due to their current fragmented nature, we refrained from revising Bayer's description of the overall colony structure and branching patterns of lot USNM 49330. We however collected images of all colonies confirmed to be *P. secundum* based on phylogenetic analyses to describe the colony structures and branching patterns characteristic to *P. secundum*. The identity of these colonies was either confirmed directly by the current analysis (including colonies analyzed by Tu et al. 2015) or indirectly by imputing their identity through their phylogenetic clustering with colonies of Tu et al. (2015), as in the case of those analyzed by Ardila et al. (2012). In addition, we reviewed the taxonomic identification keys (Bayer 1956; Tu et al. 2016) and species descriptions (Nonaka et al. 2012; Tu et al. 2012; Nonaka and Hayashibara 2021) of the *Pleurocorallium* species of the Northern Pacific whose distribution ranges overlap with *P. secundum*'s occurrences and identify morphologic differences (both on colony-level morphological features and sclerites) to identify key diagnostic characteristics of *P. secundum*. For aiding the species identification of *P. secundum*, we also collected images of *P. niveum*, the morphologically most similar and most common species in the Hawaiian Islands and discuss colony-level differences between the two species.

Results

Two technical replicates were performed for each of the seven DNA fragments for both the holotype and the redescribed colony fragments. In all cases, these replicates resulted in identical DNA sequences, which testify the accuracy of the results. The DNA sequence data of the bulk debris of the redescribed colony fragments did not contain any indication of the presence of multiple haplotypes. Particularly, a single operational taxonomic unit was generated from the MiSeq data and no double peaks indicating single base changes or overlapping chromatograms indicating indels were visible in the Sanger sequence data. This implies that the lot containing many colony fragments collected at a single location on the single day indeed contains one precious coral species. The IGS, mtMutS and LR DNA sequences were respectively 487, 400 and 127 base-pair (bp) long in all newly sequenced samples. The NCBI GenBank accession numbers and the corresponding aligned sequences are presented in Supporting Information S2A.

The IGS and LR sequences were identical in the holotype and the redescribed colony fragments. The mtMutS region

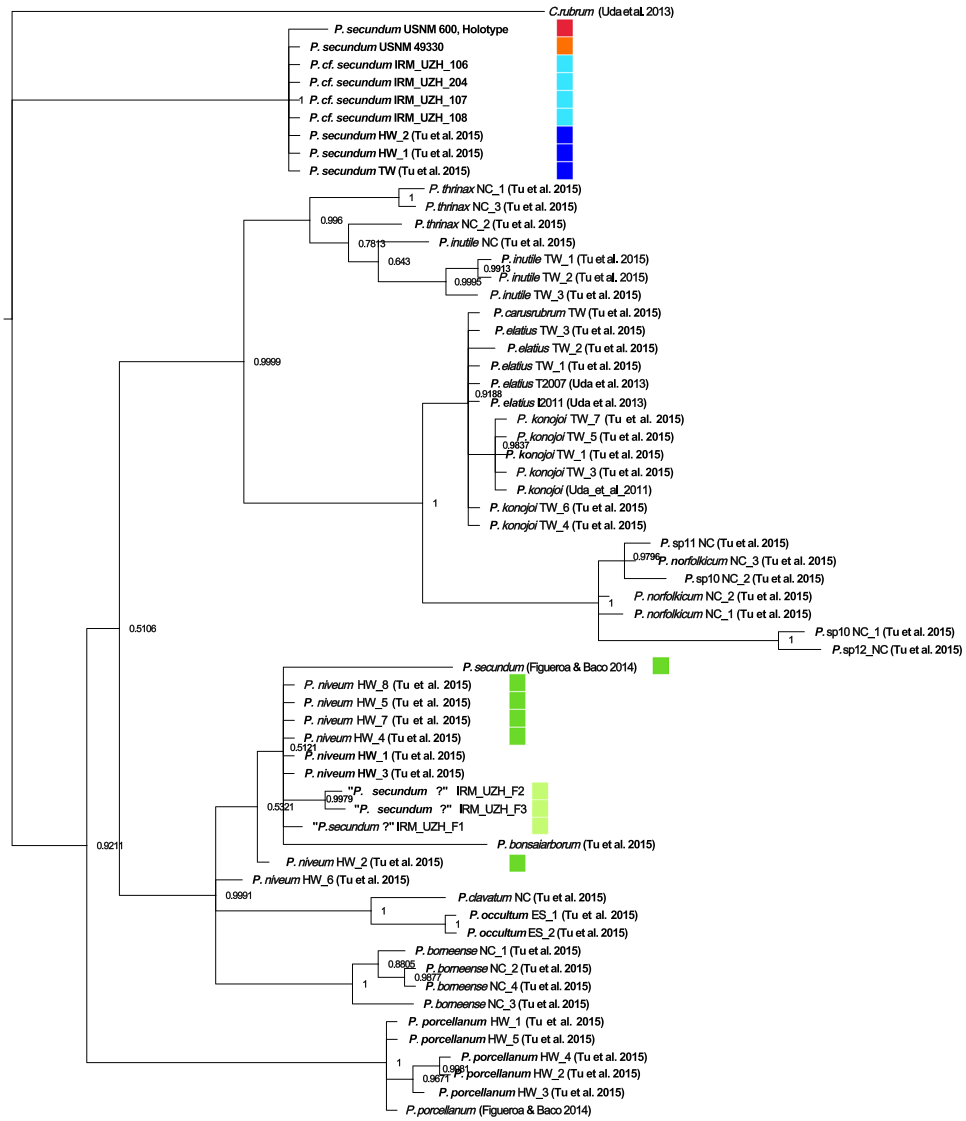

Fig. 2 Bayesian phylogenetic tree of the holotype and the redescribed colony fragments of *P. secundum* together with fresh samples and published *Pleurocorallium* data based on the concatenated dataset of three mitochondrial regions. Scientific names are followed by colony identifiers and literature references where applicable. Posterior probability values are displayed on each node. Colored squares represent the following: *red*: holotype; *orange*: the redescribed colony fragments; *light blue* and *dark blue*: respectively fresh samples and samples from the literature identified as *P. secundum*; light green and dark green: respectively fresh samples and samples from the literature originally identified as *P. secundum* but disproven here. Note that the *P. niveum* specimens marked with dark green squares were originally identified as *P. secundum* but already considered *P. niveum* by Tu et al. (2015), which is confirmed here

contained a single difference (T in the holotype, C in the redescribed colony fragments at the position of base 7221 in the *P. konojoi* mitochondrial reference genome); this third-codon base difference results in a same-sense mutation. The combined IGS-mtMutS-LR alignment was 1090 bp in total when including *C. rubrum* as outgroup, which introduced numerous indels in the alignment (see alignments in Supporting Information S2A and B).

The *P. secundum* holotype and the redescribed colony fragments created a highly supported phylogenetic branch (with posterior probability value of 1) together with the four fresh specimens *P. secundum* from Kailua-Kona, Hawaii Island and the three *P. secundum* specimens from the study of Tu et al. (2015; specimens marked HW_1, HW_2 and TW on Fig. 2). All samples clustering with the holotype specimen were identical with the redescribed colony fragments, sharing that single base difference compared to the holotype (99.9% sequence similarity compared to the *P. secundum* holotype). Any other analyzed *Pleurocorallium* sample comprised less than 97.3% sequence similarity compared to the *P. secundum* holotype. The fresh specimens from Makapuu as well as the *P. secundum* sample sequenced by Figueroa and Baco (2014) grouped together with specimens formerly found to be *P. niveum* from the Hawaiian Islands and *P. bonsaiarborum* from New Caledonia. We assessed whether the specimens previously considered *P. secundum* were correctly identified based on their phylogenetic position and genetic similarity to the *P. secundum* holotype (Table 1). We considered all specimens clustering together with the *P. secundum* holotype (with 99.9% sequence similarity) to be indeed *P. secundum* (marked orange, dark blue and light blue on Table 1 and Fig. 2). The collection location of these specimens was marked on a map using the same color code (Fig. 3). All other specimens were considered to originate

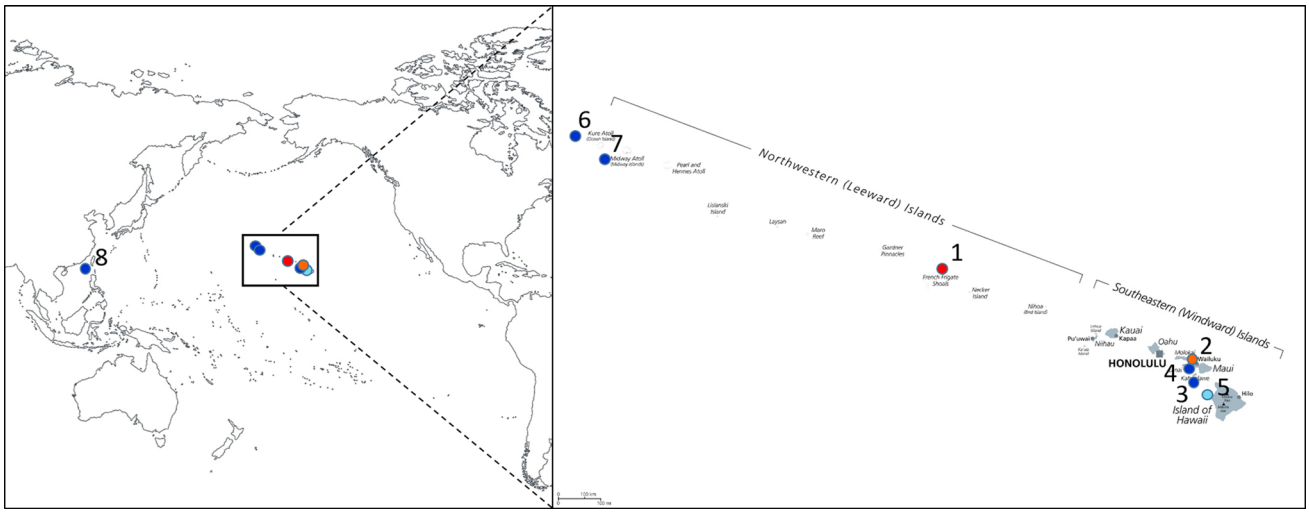

Fig. 3 The geographic distribution of *Pleurocorallium secundum* based on specimens confirmed by genetic analyses. Colors represent the following: *red*: holotype; *orange*: the redescribed colony fragments; *light blue*: fresh samples; *dark blue*: museum specimens. See Table 1 for information about the sample collection locations indicated by numbers (1–8)

from a different species. The "*Coral*-ID" fragment is monomorphic in all studied *P. secundum* samples. According to this 149 bp long fragment of the mtMutS gene, *P. secundum* is the most similar to *P. niveum* followed by *P. clavatum* and *P. porcellanum* (respectively 4, 5 and 5 fixed mismatches; Supporting Information S2C).

The SEM images and sizes of the redescribed colony fragments' (USNM49330) sclerites are shown on Fig. 4 and Table 2, respectively. The type of sclerites fit Bayer's description. Tentacles contained many 8 radiates, while spiny rods were absent. In the coenenchyme, double clubs were dominant, and 6, 7, 8 radiates were not common. However, in this study, unlike in Bayer's description, many asymmetric 8 radiates appeared. The colonies of *P. secundum* are characterized by orange–pink cortex, dense and rugged branching (Fig. 5). Acutely stemming prickle-twigs are common. *Pleurocorallium niveum* has apparently thick cortex and twig tips end in a cluster of autozooids and thus resembles "clavate form" (Bayer 1956; Supporting Information S2D).

Discussion

The genetic analysis of museum specimens has become routine in taxonomy and evolutionary biology (Raxworthy and Smith 2021). Type specimens would constitute the best reference material for species identification and the scientific value of DNA reference databases would be greatly enhanced if species were also represented by sequences of the respective type material, especially the holotype (Schäffer et al. 2017). However, the usage of holotypes in molecular systematics and taxonomy is scarce and frequently not even attempted, mostly due to their antiquity and preservation history (Erpenbeck et al. 2016). Nevertheless, holotype specimens have been used for elucidating taxonomic relations of various animal taxa, for example, sponges (Erpenbeck et al. 2016), fishes (Agne et al. 2022; Sullivan et al. 2022), amphibians (Rancilhac et al. 2020; Goutte et al. 2022), birds (Kirchman et al. 2010) or mammals (Castañeda-Rico et al. 2022; Nations et al. 2022) and even octocorals (Quattrini et al. 2024). On a single occasion, a precious coral species' type specimen was also genotyped (*Corallium medea* from the Bahamas, Ardila et al. 2012).

The genetic study of a holotype is especially beneficial if the morphological characteristics allowing sound taxonomic identification are lost, which is the case with the *P. secundum* holotype. At least since 1846, this specimen exists without its cortex – possessing the most important morphological features. This also means that the material generally used for genetic analysis of precious corals (polyps or less frequently cortex, e.g., France et al. 1996; Bayer and Cairns 2003; Aurelle et al. 2011; Ledoux et al. 2013) is lost. What remains is the skeletal axis, which is produced by a biomineralization process through the secretion of calcite crystals from an epithelial cell layer (Grillo et al. 1993; Perrin et al. 2015). Analogously to mollusk shells, the coral skeletal axis does not contain living cells and any trace-DNA molecules present in the skeletal axis are "trapped or absorbed" during the formation of the calcareous structure (Martin et al. 2021). Recent studies have however demonstrated that by using dedicated laboratory protocols it is feasible to perform genetic analysis even from as little as a few milligrams of precious coral axial medulla (Lendvay et al. 2020, 2022). The technical advancements in genetic analysis of minute amounts of coral skeletal material encouraged us to

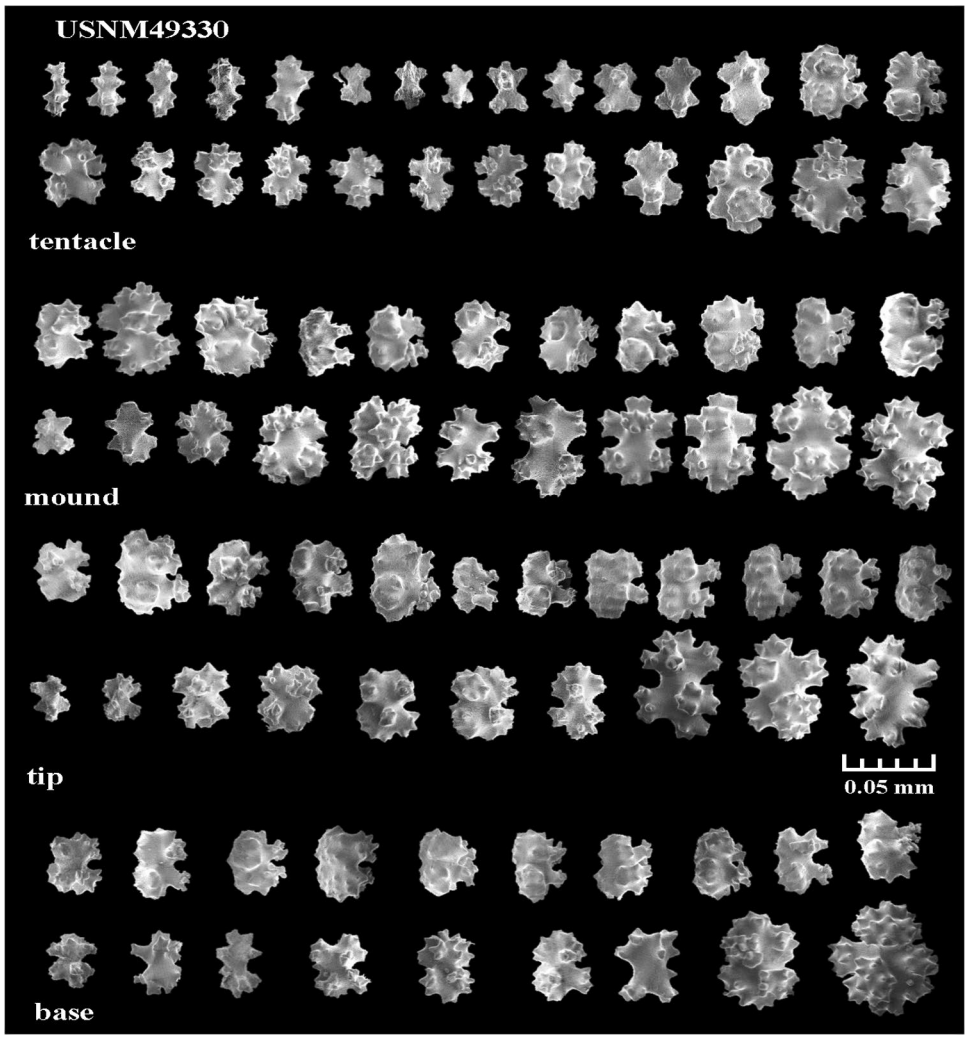

Fig. 4 Scanning electron microscopy images of the sclerites of the redescribed colony fragments of *Pleurocorallium secundum* (USNM49330)

ask permission to sample the holotype of *P. secundum* accompanied by its redescribed colony in order to analyze them in a phylogenetic context for resolving the taxonomic uncertainties surrounding the identity of *P. secundum*.

The mitochondrial genomes of octocorals are known to evolve at a low pace relative to other animals (Shearer et al. 2002). Nevertheless, the species-level phylogenetic and taxonomic studies in the Coralliidae family largely rely on mitochondrial DNA sequence data (i.e., Tu et al. 2015, 2016; Takata et al. 2019; Nonaka and Hayashibara 2021; Nonaka et al. 2023). The large amount of available reference data leads to mitochondrial DNA currently providing the best resource to distinguish and identify precious coral species with the exception of specific species complexes, which are not feasible to be resolved by mitochondrial DNA data, such as the *Pleurocorallium elatius* species complex in the *Pleurocorallium* genus (Tu et al. 2015; Takata et al. 2019). Two out of the three mitochondrial DNA regions used in this study were earlier suggested to be used for DNA barcoding due to their high variability (McFadden et al. 2011). The nearly identical DNA sequence data of the holotype and the redescribed colony fragments (0.1% dissimilarity) and their genetic gap from any other haplotypes (exceeding 2.7% dissimilarity) confirm that they indeed belong to the same species. This result indicates that the identification of coral specimens as *P. secundum* based on the identification key of Bayer (Bayer 1956) and the morphologic characters of the redescribed colony fragments (USNM 49330) is valid.

The confirmed conspecificity of the holotype and Bayer's redescribed colony allowed us to analyze the morphology of the latter in details and use this to identify key diagnostic characteristics for the identification of *P. secundum*. For this aim, we also consider the colony—structures and branching patterns of all *P. secundum* colonies whose identity have been confirmed by DNA sequence data (see Table 1).

Pleurocorallium secundum can be distinguished from the members of the *Pleurocorallium elatius* species complex (which contains the closely related species *P. carusrubrum, P. elatius, P. gotoense, P. konojoi, P. uchidai*, all from the NW-Pacific) and *Pleurocorallium inutile* (from the Hawaiian

Table 2 Summary of length and width measurements of sclerites of *Pleurocorallium secundum* USNM49330

Sclerite type	Tentacle				Autozooid mound				Branch tip				Colony base			
	Length	Width	N	(%)	Length	Width	N	(%)	Length	Width	N	(%)	Length	Width	N	(%)
Rods	36.5 ± 4.4	16.4 ± 3.4	18	12	–				–				–			
6 radiates (symmetrical)	37.2 ± 9.0	24.6 ± 7.5	27	19	42.1 ± 12.6	30.4 ± 9.9	11	9	36.4	26.6	8	11	35.9 ± 8.9	25.2 ± 6.8	14	10
6 radiates (asymmetrical)	52.7	34.7	5	7	49.4 ± 5.2	35.6 ± 3.9	27	22	48.3 ± 4.9	34.3 ± 4.2	41	55	48.1 ± 5.2	34.0 ± 3.6	39	27
7 radiates	41.2 ± 8.4	26.9 ± 5.6	24	17	40.6 ± 11.4	27.2 ± 8.7	17	14	48.1	31.8	6	8	44.8 ± 6.6	30.0 ± 5.1	15	10
8 radiates (symmetrical)	43.1 ± 9.7	26.8 ± 6.4	58	40	61.8 ± 11.3	38.5 ± 7.0	31	25	-				43.8	28.5	4	3
8 radiates (asymmetrical)	50.0	33.2	2	1	58.9	40.0	4	3	53.5	31.5	1	1	48.2	31.7	1	1
Multi-radiates	40.2	24.6	4	3	-				-				78.5	60.4	1	1
Double clubs	57.3	39.8	4	3	50.1 ± 4.9	36.3 ± 3.5	33	27	49.5 ± 4.2	35.3 ± 2.4	18	24	48.4 ± 4.3	34.6 ± 3.6	73	50

Measurements are reported as the average ± standard deviation (more than 10%), or only the average (less than 9%), in mm. Numbers with gray background indicate most abundant sclerite type. "-" means not found

Fig. 5 Images of all ten colonies besides the holotype and the redescribed colony fragments of *Pleurocorallium secundum* confirmed by genetic tests to this date. Colonies 106–204: samples collected by dives of the Hawaii Undersea Research Laboratory (images by Samuel Kahng), USNM colonies: collection of the Department of Invertebrate Zoology, Smithsonian National Museum of Natural History (images by Amanda Robinson) ASIZ colony: collection of the Biodiversity Research Museum, Academia Sinica (image by Meng-Min Hsueh). Each colony is shown besides a metric scale except for the ASIZ sample. For the collection locations, see Table 1

Islands) from their absence of eight-radiate sclerites, while *P. secundum* is abundant in eight radiates especially on the tentacles and around the autozooid mounds. *Pleurocorallium porcellanum* and *P. pusillum* can be distinguished by their absence of double clubs and six radiates, respectively, which are typical in *P. secundum*. *Pleurocorallium secundum* shares most characteristics with *P. niveum*, which explains the several *P. niveum* colonies incorrectly labeled as *P. secundum* (Table 1). A microscopic attribute separating these species is the clustered (*P. niveum*) *versus* even (*P. secundum*) distribution of cortical mounds, while on

the colony scale the dense branching and prickle-twigs are typical for *P. secundum* (see Fig. 5), but not for *P. niveum*. Besides, the "clavate form" twig tips of *P. niveum* are not characteristic to *P. secundum*. In contrast, the pure white color of *P. niveum* (expected according to its original species description, Bayer 1956), *versus* the pink—orange cortex of *P. secundum*, seems not to be a consistent trait as some *P. niveum* colonies also bear pink—orange coenenchyme, therefore the cortex color alone is not sufficient for precise distinction between *P. secundum* and *P. niveum*.

The small number of *P. secundum* lots/colonies with confirmed taxonomic identity and the fact that several of these are highly fragmented or exist as small branches prevents us from discovering variations in colony morphology that might be correlated with environmental factors or geographic differences. In particular, the single colony known from outside of the Hawaiian Islands—Midway atoll region (collection accession number ASIZ80388 at the Research Museum, Biodiversity Research Museum, Academia Sinica, Taipei), which originates from the South China sea is preserved as a tiny fragment. The only inference we can draw based on its morphology is that this does not contradict the expected characteristics of *P. secundum*.

The results of this study also provide a backbone for the genetic identification of *P. secundum* by determining DNA barcode sequences from the type specimens. These barcode sequences also give us the opportunity to review specimens used in previous studies and check whether they have been correctly identified a *P. secundum*. Our results confirm that the identification of colonies as *P. secundum* by Tu et al. (2015) was correct. As well, the corroborating results of specimens analyzed both by Tu et al. (2015) and Ardila et al. (2012) prove that the latter authors also identified *P. secundum* specimens correctly. Likewise, the genetic classification method of Lendvay et al. (2022), which was based on the data of Tu et al. (2015) correctly identifies *P. secundum* samples and separates them from any other coral species. We provide the confirmed *Coral*-ID barcoding DNA sequence of *P. secundum* in an alignment with other members of Coralliidae. This alignment contains species-specific nucleotides of *P. secundum* and is a ready-to-use resource for the genetic identification of *P. secundum*. In turn, the specimen available to create the complete *P. secundum* mitochondrial genome (NCBI GenBank accession number KC782347) is here identified not to be *P. secundum*. As this specimen from Pioneer Bank, Hawaiian Islands clusters together with Hawaiian *P. niveum* specimens, it is likely also a member of this species.

A significant result of this study is the species confirmation of the specimen from the South China Sea as *P. secundum* (number 8 on Fig. 3; P. secundum_TW, Tu et al. 2015). This specimen was collected by the Taiwanese precious coral fishery in February 2011 from the South China sea, southwest of Taiwan island. This means that the reported harvesting of *P. secundum* by Taiwanese coral fishermen (in small quantities and specifically off Lanyu Island, Shiraishi 2018; Cannas et al. 2019) during the 2010s is supported by genetic data from a nearby location. This result implies that the inclusion of *P. secundum* in CITES Appendix III by China was legitimate.

It is also worth to mention that the three fresh samples from Makapuu, Oahu (414 m depth) have proven not to be *P. secundum*. Besides the three specimens analyzed here, two additional specimens from Makapuu (USNM 1082650, 431 m depth, and USNM 56807, 366 m depth) have been genotyped and both turned out to be *P. niveum* (Tu et al. 2015). The precious coral bed off Makapuu (350–450 m depth) was discovered in 1966 and became the primary site of coral harvesting in the Southeastern Hawaiian Islands (Grigg 1993; Long & Baco 2014). Precious corals in this coral bed were previously ubiquitously considered *P. secundum* (Grigg 1988, 1993, 2002, 2010; Parrish 2007; Long and Baco 2014; Parrish et al. 2017). Our results raise the possibility that the fished Makapuu coral bed, at least partly, consists of a species other than *P. secundum*.

Based on the collection location of the specimens confirmed to be *P. secundum*, we can infer the approximate distribution area of the species (Fig. 3). This covers both the Hawaiian Islands and the disjunct observation in the South China Sea represented by a sole sample. The Hawaiian distribution of *P. secundum* includes the Northwestern and Southeastern Hawaiian Islands from Hawaii Island to at least the 2500 km distant Kure Island. The confirmed specimens were fished at 140 m and at between 230 and 380 m depths in the South China Sea and the Hawaiian Islands, respectively.

Conclusions

Identification of precious corals to the species level is often difficult even for experts. This is surely the case of coral skeletal axis fragments that are cut and polished for their use in jewelry. Lendvay et al. (2020) suggested minimally destructive genetic analysis as a promising tool for identification of corals from jewelry, and a protocol to distinguish CITES-listed and non-CITES-listed precious corals was developed by Lendvay et al. (2022). For representing *Pleurocorallium secundum*, this method relied on the mtMutS DNA sequence data of Tu et al. (2015), and the correctness of this data was confirmed by the present study.

According to the results of this study, there are altogether 12 existing coral colonies confirmed to be *Pleurocorallium secundum* worldwide. By confirming the accurate DNA barcoding reference data and providing additional morphological characters (sclerite measurements) not included in Bayer (1956), this study serves as a basis for the correct identification

of further *P. secundum* colonies and it supports the assessment of *P. secundum*'s prevalence in the precious coral trade.

Both studies of Lendvay et al. (2020) and (2022) identified jewelry objects that were observed to originate from *Pleurocorallium* species different from those that have been known to be present in the jewelry industry (*Pleurocorallium elatius*, *P. konojoi* and *P. secundum*). The current study also confirms that these are not from *P. secundum*. To investigate the taxonomy of these processed coral skeleton objects, reference data should be generated for the species of the genus that have no genetic data yet; *Pleurocorallium uchidai* and *P. gotoense* are both known exclusively from century-old holotypes from Japanese waters (Nonaka et al. 2012). A similar approach to the one used in the current study might be promising to test whether these species originating from coral fishing areas are the unknown taxa present in the coral jewelry trade.

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Declarations

Conflict of interest The authors have no competing interests to declare that they are relevant to the content of this article.

References

Agne S, Naylor GJ, Preick M, Yang L, Thiel R, Weigmann S et al (2022) Taxonomic identification of two poorly known lantern shark species based on mitochondrial DNA from wet-collection paratypes. Front Ecol Evol 10:910009

Ardila NE, Giribet G, Sánchez JA (2012) A time-calibrated molecular phylogeny of the precious corals: reconciling discrepancies in the taxonomic classification and insights into their evolutionary history. BMC Evol Biol 12:246

Aurelle D, Ledoux J-B, Rocher C, Borsa P, Chenuil A, Féral J-PJG (2011) Phylogeography of the red coral (Corallium rubrum): inferences on the evolutionary history of a temperate gorgonian. Genetica 139:855–869

Bayer FM (1956) Descriptions and redescriptions of the Hawaiian octocorals collected by the US Fish Commission steamer "Albatross" (2. Gorgonacea: Scleraxonia). Pac Sci 10:67–95

Bayer FM, Cairns SD (2003) A new genus of the scleraxonian family Coralliidae (Octocorallia: Gorgonacea). Proc Biol Soc Wash 116:222–228

Bayer, F.M., Grasshoff, M. & Verseveldt, J. (1983). *Illustrated trilingual glossary of morphological and anatomical terms applied to Octocorallia*. E. J. Brill / Dr. W. Backhuys, Leiden.

Bruckner AW (2009) Rate and extent of decline in *Corallium* (pink and red coral) populations: existing data meet the requirements for a CITES Appendix II listing. Mar Ecol Prog Ser 397:319–332

Cannas, R., Follesa, M., Cau, A., Cau, A. & Friedman, K. (2019). Global report on the biology, fishery and trade of precious corals. In: *FAO Fisheries and Aquaculture Circular No. 1184.* Rome.

Cartier, L.E., Krzemnicki, M.S., Lendvay, B. & Meyer, J.B. (2018). DNA Fingerprinting of Pearls, Corals and Ivory: A Brief Review of Applications in Gemmology. *Journal of Gemmology*, 36.

Castañeda-Rico S, Edwards CW, Hawkins MT, Maldonado JE (2022) Museomics and the holotype of a critically endangered cricetid rodent provide key evidence of an undescribed genus. Front Ecol Evol 10:930356

Chang S-K (2015) Precious corals become more precious in the northwestern pacific: Urgent need for integrated policy. Journal of Marine Policy 52:103–107

Chang S-K, Yang Y-C, Iwasaki N (2013) Whether to employ trade controls or fisheries management to conserve precious corals (Coralliidae) in the Northern Pacific Ocean. Mar Policy 39:144–153

CIBJO (2017). *Coral guide for customes. Classification & identification of coral materials.* The World Jewellery Confederation, Milano, Italy.

CIBJO (2020). *Coral Commission of The World Jewellery Confederation: The coral book.*, Milano, Italy.

Conner K, Sharma S, Uchiyama R, Tanaka K, Murakami-Sugihara N, Shirai K et al (2023) Raman analysis of octocoral carbonate ion structural disorder along a natural depth gradient, Kona coast, Hawai'i. Am Miner 108:999–1013

Cooper, E.W., Torntore, S.J., Leung, A.S., Shadbolt, T. & Dawe, C. (2011). *Guide to the identification of precious and semi-precious corals: In commercial trade*. TRAFFIC North America and WWF-Canada, Vancouver, Canada.

CoP14 Prop.21 (2007). Available at: https://cites.org/sites/default/files/eng/cop/14/prop/E14-P21.pdf.

CoP15 Prop. 21 (2010). Available at: https://cites.org/sites/default/files/eng/cop/15/prop/E-15-Prop-21.pdf.

Dana JD (1846) United States Exploring Expedition During the Years 1838–1842: Atlas Zoophytes. Lea and Blanchard, Philadelphia

Erpenbeck D, Ekins M, Enghuber N, Hooper JN, Lehnert H, Poliseno A et al (2016) Nothing in (sponge) biology makes sense–except when based on holotypes. J Mar Biol Assoc UK 96:305–311

Figueroa DF, Baco AR (2014) Complete mitochondrial genomes elucidate phylogenetic relationships of the deep-sea octocoral families Coralliidae and Paragorgiidae. Deep Sea Res Part II 99:83–91

France SC, Rosel PE, Ewann J (1996) DNA sequence variation of mitochondrial large-subunit rRNA. Molecular Marine Biology Biotechnology 5:15–28

Goutte S, Reyes-Velasco J, Kassie A, Boissinot S (2022) Genetic and morphometric analyses of historical type specimens clarify the

taxonomy of the Ethiopian Leptopelisgramineus species complex (Anura, Arthroleptidae). ZooKeys 1128:63
Gray, J. (1867). Additional note on Corallium johnsoni. In: *Proceedings of the Zoological Society of London*, pp. 125–127.
Grigg RW (1988) Recruitment limitation of a deep benthic hard-bottom octocoral population in the Hawaiian Islands. Mar Ecol Prog Ser 45:121–126
Grigg RW (1993) Precious coral fisheries of Hawaii and the US Pacific Islands. Mar Fish Rev 55:50–60
Grigg RW (2002) Precious corals in Hawaii: discovery of a new bed and revised management measures for existing beds. Mar Fish Rev 64:13–20
Grigg, R.W. (2010). *The Precious Corals. Fishery Management Plan of the Western Pacific Regional Fishery Management Council*.
Grillo M-C, Goldberg WM, Allemand D (1993) Skeleton and sclerite formation in the precious red coral *Corallium rubrum*. Mar Biol 117:119–128
Herrera S, Baco A, Sánchez JA (2010) Molecular systematics of the bubblegum coral genera (Paragorgiidae, Octocorallia) and description of a new deep-sea species. Mol Phylogenet Evol 55:123–135
Iwasaki N (2018) The academic pilgrimage to sustainable social development. Rissho University, Rissho, Japan
Kirchman JJ, Witt CC, McGuire JA, Graves GR (2010) DNA from a 100-year-old holotype confirms the validity of a potentially extinct hummingbird species. Biol Let 6:112–115
Ledoux J-B, Aurelle D, Féral J-P, Garrabou J (2013) Molecular forensics in the precious Mediterranean red coral, *Corallium rubrum*: testing DNA extraction and microsatellite genotyping using dried colonies. Conserv Genet Resour 5:327–330
Lendvay B, Cartier LE, Costantini F, Iwasaki N, Everett MV, Krzemnicki MS et al (2022) 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 58:102663
Lendvay B, Cartier LE, Gysi M, Meyer JB, Krzemnicki MS, Kratzer A et al (2020) DNA fingerprinting: an effective tool for taxonomic identification of precious corals in jewelry. Sci Rep 10:1–12
Long DJ, Baco AR (2014) Rapid change with depth in megabenthic structure-forming communities of the Makapu'u deep-sea coral bed. Deep Sea Res Part II 99:158–168
Martin KR, Waits LP, Parent CE (2021) Teaching an old shell new tricks: Extracting DNA from current, historical, and ancient mollusk shells. Bioscience 71:235–248
McFadden CS, Benayahu Y, Pante E, Thoma JN, Nevarez PA, France SC (2011) Limitations of mitochondrial gene barcoding in Octocorallia. Mol Ecol Resour 11:19–31
Nations, J.A., Giarla, T.C., Morni, M.A., Dee, J.W., Swanson, M.T., Hiller, A.E. et al (2022) Molecular data from the holotype of the enigmatic Bornean Black Shrew, Suncusater Medway, 1965 (Soricidae, Crocidurinae), place it in the genus Palawanosorex. ZooKeys 1137:17
Nonaka M, Hanahara N, Kakui K (2023) A new species of Coralliidae (Cnidaria: Octocorallia) collected from Eastern Japan. Species Diversity 28:231–243
Nonaka M, Hayashibara T (2021) A report on Coralliidae (Cnidaria: Octocorallia) specimens collected from the Emperor Seamounts with descriptions of three new species. Species Diversity 26:297–342
Nonaka M, Muzik K, Iwasaki N (2012) 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 3428:1–67
Nonaka, M., Takaoka, H. & Muzik, K. (2014). The taxonomic problem of the Hawaiian pink coral, Corallium secundum. In: *International Symposium on Pacific Precious Corals Final Report* Taipei, Taiwan, pp. 247–256.
Parrish FA (2007) Density and habitat of three deep-sea corals in the lower Hawaiian chain. Bull Mar Sci 81:185–194
Parrish, F.A., Baco-Taylor, A., Kelley, C. & Reiswig, H. (2017). State of Deep-Sea Coral and Sponge Ecosystems of the U.S. Pacific Islands Region. In: *The State of Deep-Sea Coral and Sponge Ecosystems of the United States Report* (eds. Hourigan, T, Etnoyer, P & Cairns, S). NOAA Technical Memorandum NMFS-OHC-4 Silver Spring, MD.
Parrish, F.A., Grigg, R.W., DeMello, J.K. & Montgomery, A.D. (2009). The status of *Corallium* spp. in Hawaii and the US Pacific: population status, trends and threats, fisheries, trade, management, enforcement and conservation. In: *Proceedings of the first international workshop on Corallium science, management, and trade* (ed. Roberts, ABG). NOAA Technical Memorandum NMFS-OPR-43 and CRCP-8 Silver Spring, MD, pp. 87–107.
Perrin J, Vielzeuf D, Ricolleau A, Dallaporta H, Valton S, Floquet N (2015) Block-by-block and layer-by-layer growth modes in coral skeletons. Am Miner 100:681–695
Quattrini AM, McCartin LJ, Easton EE, Horowitz J, Wirshing HH, Bowers H et al (2024) Skimming genomes for systematics and DNA barcodes of corals. Ecology Evolution 14:e11254
Rambaut, A. (2012). *FigTree v1.4. Available at:* http://tree.bio.ed.ac.uk/software/figtree.
Rancilhac L, Bruy T, Scherz MD, Pereira EA, Preick M, Straube N et al (2020) Target-enriched DNA sequencing from historical type material enables a partial revision of the Madagascar giant stream frogs (genus *Mantidactylus*). J Nat Hist 54:87–118
Raxworthy CJ, Smith BT (2021) Mining museums for historical DNA: advances and challenges in museomics. Trends Ecol Evol 36:1049–1060
Ronquist F, Teslenko M, Van Der Mark P, Ayres DL, Darling A, Höhna S et al (2012) MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. Syst Biol 61:539–542
Schäffer S, Zachos FE, Koblmüller S (2017) Opening the treasure chest: a DNA-barcoding primer set for most higher taxa of Central European birds and mammals from museum collections. PLoS ONE 12:e0174449
Shearer T, Van Oppen M, Romano S, Wörheide G (2002) Slow mitochondrial DNA sequence evolution in the Anthozoa (Cnidaria). Mol Ecol 11:2475–2487
Shiraishi, H. (2018). *Seeing red. Precious coral trade in East Asia*. TRAFFIC, Japan Office, Tokyo, Japan.
Sullivan JP, Hopkins CD, Pirro S, Peterson R, Chakona A, Mutizwa TI et al (2022) Mitogenome recovered from a 19th Century holotype by shotgun sequencing supplies a generic name for an orphaned clade of African weakly electric fishes (Osteoglossomorpha, Mormyridae). ZooKeys 1129:163
Takata K, Taninaka H, Nonaka M, Iwase F, Kikuchi T, Suyama Y et al (2019) Multiplexed ISSR genotyping by sequencing distinguishes two precious coral species (Anthozoa: Octocorallia: Coralliidae) that share a mitochondrial haplotype. PeerJ 7:e7769
Tsounis G, Rossi S, Grigg RW, Santangelo G, Bramanti L, Gili J-M (2010) The exploitation and conservation of precious corals. CRC Press, Boca Raton (USA), New York
Tu T-H, Dai C-F, Jeng M-S (2012) Precious corals (Octocorallia: Coralliidae) from the northern West Pacific region with descriptions of two New Species. Zootaxa 3395:1–17
Tu T-H, Dai C-F, Jeng M-S (2015) Phylogeny and systematics of deep-sea precious corals (Anthozoa: Octocorallia: Coralliidae). Mol Phylogenet Evol 84:173–184
Tu T-H, Dai C-F, Jeng M-S (2016) Taxonomic revision of Coralliidae with descriptions of new species from New Caledonia and the Hawaiian Archipelago. Mar Biol Res 12:1003–1038

Uda K, Komeda Y, Fujita T, Iwasaki N, Bavestrello G, Giovine M et al (2013) Complete mitochondrial genomes of the Japanese pink coral (*Corallium elatius*) and the Mediterranean red coral (*Corallium rubrum*): a reevaluation of the phylogeny of the family Coralliidae based on molecular data. Comp Biochem Physiol D: Genomics Proteomics 8:209–219

Uda K, Komeda Y, Koyama H, Koga K, Fujita T, Iwasaki N et al (2011) Complete mitochondrial genomes of two Japanese precious corals, *Paracorallium japonicum* and *Corallium konojoi* (Cnidaria, Octocorallia, Coralliidae): notable differences in gene arrangement. Gene 476:27–37