

Supporting Information

for the article titled

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

by the authors

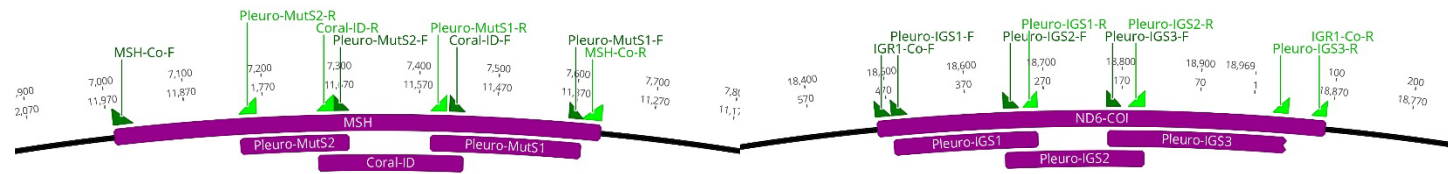
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S1 Supporting Methods

A) PCR primers used for amplifying the mitochondrial IGS and MutS DNA regions for the holotype and the redescribed colonies of *Pleurocorallium secundum*

Region	Primer name	Primer sequence	Amplicon length (bp, including primer sites)	Reference
IGS	Pleuro-IGS1-F	5'-ATC ACC ATA AAA CTA GCT CC-3'	186	this study
	Pleuro-IGS1-R	5'-GGC GCA TTC AAA AGT CC-3'		
	Pleuro-IGS2-F	5'-CCT CTA CAA CAG TTG ATA GG-3'	205	this study
	Pleuro-IGS2-R	5'-TCT CTA GTT AGG GTT TAC GC-3'		
	Pleuro-IGS3-F	5'-GAC TGG GCA GTT AGT GG-3'	231	this study
	Pleuro-IGS3-R	5'-TAT GAT TAG TAG AAA ATA GCC AGC-3'		
mtMutS	Pleuro-MutS1-F	5'-GAA CCA GAC TTA CTT TGG G-3'	195	this study
	Pleuro-MutS1-R	5'-CCA TCT ATC TGT TAT TCA GC-3'		
	<i>Coral-ID-F</i>	5'-TACGYTCATAAATTATHCCT-3'	189	Lendvay et al. (2022)
	<i>Coral-ID-R</i>	5'-AGATTTGCCATGGYACAGAA-3'		
LR	Pleuro-MutS2-F	5'-ATT GCT AGC CCT GAA TTT AC-3'	140	this study
	Pleuro-MutS2-R	5'-GAC GAT TTT CAT AAG GGC CAA-3'		
	LR-F	5'-AAG TAG TGT CAC TCC CAA ACA-3'	170	Lendvay et al. (2020)
	LR-R	5'-TGC AAA GAA GGA GAA CAA AAG G-3'		

B) The location of the PCR primers used in this study to amplify the IGS and mtMutS regions as mapped on the *Pleurocorallium konojoi* mitochondrial reference genome (NC_015406, (Uda *et al.* 2011)). Graphical output from Geneious Prime 2022.0.2 software



C) The applied PCR protocols

PCR reactions were performed in 25 μ l final volume containing 1 $\times$ Qiagen Multiplex PCR Master Mix (Qiagen) and 0.4 μ M of the forward and reverse primers, respectively and 5 μ l template DNA. Thermal cycling commenced with enzyme activation at 95 $^{\circ}$ C for 10 min followed by 50 cycles of denaturation 94 $^{\circ}$ C for 45 sec, 53 $^{\circ}$ C annealing for 1 min 30 sec and elongation at 72 $^{\circ}$ C for 45 sec, and a final elongation of 72 $^{\circ}$ C at 10 min was applied. PCR amplicons were purified with the AmPure XP bead system (Beckman Coulter) and quantified with Qubit fluorimeter device (Invitrogen).