

Supplementary Material

Unravelling the relationships among *Madrepora* Linnaeus, 1758, *Oculina* Lamark, 1816 and *Cladocora* Ehrenberg, 1834 (Cnidaria: Anthozoa: Scleractinia)

Anna M. Addamo^{A,B,C,F,*}, *Melinda S. Modrell*^A, *Marco Taviani*^{D,E} and *Annie Machordom*^A

^ADepartamento de Biodiversidad y Biología Evolutiva, Museo Nacional de Ciencias Naturales (MNCN-CSIC), E-28006 Madrid, Spain

^BEuropean Commission, Joint Research Centre (JRC), I-21027 Ispra, Italy

^CClimate Change Research Centre (CCRC), University of Insubria, I-21100 Varese, Italy

^DIstituto di Scienze Marine, Consiglio Nazionale delle Ricerche (ISMAR-CNR), I-40129 Bologna, Italy

^EStazione Zoologica Anton Dohrn, Villa Comunale, I-80121 Napoli, Italy

^FPresent address: Faculty of Biosciences and Aquaculture, Nord University, NO-8049 Bodø, Norway

*Correspondence to: Email: am.addamo@gmail.com

List of coral species used in this study

Table S1. Coral species used in the analyses (in alphabetical order).

Family code	Family	Genus	Species	Institute / Database	Contact	Voucher code / NCBI code	AMA code
ACR	Acroporidae	<i>Acropora</i>	<i>aspera</i>	NCBI		KF448532	
	Acroporidae	<i>Acropora</i>	<i>digitifera</i>	NCBI		KF448535	
	Acroporidae	<i>Acropora</i>	<i>divaricata</i>	NCBI		KF448537	
	Acroporidae	<i>Acropora</i>	<i>florida</i>	NCBI		KF448533	
	Acroporidae	<i>Acropora</i>	<i>hemprichii</i> §	UNIMIB	Francesca Benzoni	BA071	AMA-01
	Acroporidae	<i>Acropora</i>	<i>horrida</i>	NCBI		KF448530	
	Acroporidae	<i>Acropora</i>	<i>humilis</i>	NCBI		KF448528	
	Acroporidae	<i>Acropora</i>	<i>hyacinthus</i> §	UNIMIB	Francesca Benzoni	BA091	AMA-02
	Acroporidae	<i>Acropora</i>	<i>hyacinthus</i> §	NCBI		KF448531	
	Acroporidae	<i>Acropora</i>	<i>muricata</i>	NCBI		KF448529	
	Acroporidae	<i>Acropora</i>	<i>nasuta</i>	NCBI		KF448536	
	Acroporidae	<i>Acropora</i>	<i>robusta</i>	NCBI		KF448538	
	Acroporidae	<i>Acropora</i>	<i>tenuis</i>	NCBI		AF338425	
	Acroporidae	<i>Acropora</i>	<i>yongei</i>	NCBI		KF448534	
	Acroporidae	<i>Acropora</i>	<i>valida</i> §	UNIMIB	Francesca Benzoni	BA130	AMA-03
	Acroporidae	<i>Alveopora</i>	sp.	NCBI		KJ634271	
	Acroporidae	<i>Anacropora</i>	<i>matthaii</i>	NCBI		AY903295	
	Acroporidae	<i>Astreopora</i>	<i>explanata</i>	NCBI		KJ634269	
	Acroporidae	<i>Astreopora</i>	<i>myriophthalma</i>	NCBI		KJ634272	
	Acroporidae	<i>Isopora</i>	<i>palifera</i>	NCBI		KJ634270	
Acroporidae	<i>Isopora</i>	<i>togianensis</i>	NCBI		KJ634268		
Acroporidae	<i>Montipora</i>	<i>Cactus</i>	NCBI		AY903296		
AST	Astrangiidae	<i>Astrangia</i>	sp.	NCBI		DQ643832	
AGA	Agariciidae	<i>Agaricia</i>	<i>humilis</i>	NCBI		DQ643831	
	Agariciidae	<i>Pavona</i>	<i>clavus</i>	NCBI		DQ643836	
CAR	Caryophylliidae	<i>Caryophyllia</i> (<i>Caryophyllia</i>)	<i>calveri</i> §	CNR-ISMAR	Marco Taviani		AMA-282
	Caryophylliidae	<i>Caryophyllia</i> (<i>Caryophyllia</i>)	<i>diomedea</i> †	NMNH	Stephen Cairns	USNM 1072329	AMA-181
	Caryophylliidae	<i>Caryophyllia</i> (<i>Caryophyllia</i>)	<i>huinayensis</i> §	MNCN	Anna M. Addamo		AMA-58
	Caryophylliidae	<i>Caryophyllia</i> (<i>Caryophyllia</i>)	<i>smithii</i> §	US	Pablo José López-González		AMA-40
	Caryophylliidae	<i>Ceratotrochus</i>	<i>magnaghii</i>	NMNH	Stephen Cairns	USNM 98482	AMA-145
	Caryophylliidae	<i>Desmophyllum</i>	<i>dianthus</i> §	MNCN	Marco Taviani		DdROC163 (Italy)
	Caryophylliidae	<i>Desmophyllum</i>	<i>dianthus</i> §	MNCN	Anna M. Addamo		DdIJC432 (Chile)
	Caryophylliidae	<i>Desmophyllum</i>	<i>pertusum</i> §	CNR-ISMAR	Marco Taviani		AMA-52 (Italy)
	Caryophylliidae	<i>Desmophyllum</i>	<i>pertusum</i> §	MNCN	Anna M. Addamo		AMA-296 (Ireland)
	Caryophylliidae	<i>Desmophyllum</i>	<i>pertusum</i> §	NCBI		KC875349	
	Caryophylliidae	<i>Labyrinthocyathus</i>	<i>facetus</i>	NMNH	Stephen Cairns	USNM 1114920	AMA-191
	Caryophylliidae	<i>Paraconotrochus</i>	<i>antarcticus</i> §	US	Pablo José López-González		AMA-44
	Caryophylliidae	<i>Polycyathus</i>	<i>senegalensis</i> *	NMNH	Stephen Cairns	USNM 1026497	AMA-178
	Caryophylliidae	<i>Polycyathus</i>	sp.	NCBI		JF825140	
	Caryophylliidae	<i>Pourtalosmia</i>	<i>anthophyllites</i> §	CMAJA	Mari Carmen Arroyo		AMA-37
	Caryophylliidae	<i>Premocyathus</i>	<i>cornuformis</i> *	NMNH	Stephen Cairns	USNM 1010389	AMA-173
	Caryophylliidae	<i>Stephanocyathus</i> (<i>Odontocyathus</i>)	<i>coronatus</i>	NMNH	Stephen Cairns	USNM 1100225	AMA-187
	Caryophylliidae	<i>Stephanocyathus</i> (<i>Stephanocyathus</i>)	<i>diadema</i> †	NMNH	Stephen Cairns	USNM 100906	AMA-166
	Caryophylliidae	<i>Stephanocyathus</i> (<i>Stephanocyathus</i>)s	<i>regius</i>	NMNH	Stephen Cairns	USNM 98655	AMA-153
	Caryophylliidae	<i>Stephanocyathus</i> (<i>Stephanocyathus</i>)s	<i>moseleyanus</i> §	IEO	Alvaro Altuna	001-V03	AMA-269
	Caryophylliidae	<i>Tethocyathus</i>	<i>endesa</i> §	MNCN	Anna M. Addamo		AMA-59
	Caryophylliidae	<i>Trochocyathus</i>	<i>athoseptatus</i> †	NMNH	Stephen Cairns	USNM 1072318	AMA-180
	Caryophylliidae	<i>Vaughanella</i>	<i>concinna</i> §	IEO	Alvaro Altuna	038-DR15	AMA-266
Caryophylliidae	<i>Vaughanella</i>	<i>margaritata</i>	NMNH	Stephen Cairns	USNM 1008600	AMA-170	

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CLA	Cladocoridae	<i>Cladocora</i>	<i>arbuscula</i>	NCBI		AB117292	
	Cladocoridae	<i>Cladocora</i>	<i>caespitosa</i> §	MNCN	Annie Machordom		AMA-284 (A)
	Cladocoridae	<i>Cladocora</i>	<i>caespitosa</i> §	MNCN	Annie Machordom		AMA-286 (B)
DEN	Dendrophylliidae	<i>Balanophyllia</i> (<i>Balanophyllia</i>)	<i>europaea</i> §	MNCN	Anna M. Addamo		AMA-48
	Dendrophylliidae	<i>Balanophyllia</i> (<i>Balanophyllia</i>)	<i>floridana</i> *	NMNH	Stephen Cairns	USNM 68416	AMA-79
	Dendrophylliidae	<i>Balanophyllia</i> (<i>Balanophyllia</i>)	<i>regia</i> §	MNCN	Anna M. Addamo		AMA-47
	Dendrophylliidae	<i>Balanophyllia</i> (<i>Balanophyllia</i>)	<i>regia</i> §	NCBI		HG965314	
	Dendrophylliidae	<i>Cladopsammia</i>	<i>echinata</i> *	NMNH	Stephen Cairns	USNM 97628	AMA-141
	Dendrophylliidae	<i>Dendrophyllia</i>	<i>alcocki</i> *	NMNH	Stephen Cairns	USNM 1006512	AMA-168
	Dendrophylliidae	<i>Dendrophyllia</i>	<i>laboreli</i> §	JdA	Mari Carmen Arroyo		AMA-39
	Dendrophylliidae	<i>Dendrophyllia</i>	<i>johnsoni</i>	NMNH	Stephen Cairns	USNM 98444	AMA-143
	Dendrophylliidae	<i>Dendrophyllia</i>	<i>ramea</i> §	US	Pablo José López-González		AMA-45
	Dendrophylliidae	<i>Tubastraea</i>	<i>aurea</i> §	UNIMIB	Francesca Benzoni	Y755	AMA-07
	Dendrophylliidae	<i>Endopachys</i>	<i>grayi</i>	NMNH	Stephen Cairns	USNM 98983	AMA-161
	Dendrophylliidae	<i>Rhizopsammia</i>	<i>wettsteini</i> §	UNIMIB	Francesca Benzoni	Y756	AMA-08
	Dendrophylliidae	<i>Rhizopsammia</i>	<i>wettsteini</i> §	NCBI		HG965337	
	Dendrophylliidae	<i>Thecopsammia</i>	<i>socialis</i>	NMNH	Stephen Cairns	USNM 1114650	AMA-190
	Dendrophylliidae	<i>Tubastraea</i>	<i>micranthus</i> §	UNIMIB	Francesca Benzoni	Y757	AMA-09
EUP	Euphylliidae	<i>Fimbriaphyllia</i>	<i>ancora</i>	NCBI		JF825139; JQ968408; JQ968408	
	Euphylliidae	<i>Galaxea</i>	<i>fascicularis</i>	NCBI		AB086826	
FAV	Faviidae	<i>Colpophyllia</i>	<i>natans</i>	NCBI		DQ643833	
	Faviidae	<i>Mussa</i>	<i>angulosa</i>	NCBI		DQ643834	
FLA	Flabellidae	<i>Flabellum</i> (<i>Flabellum</i>)	<i>cinctutum</i> §*	IEO	Virginia Polonio	PATA02	AMA-61
	Flabellidae	<i>Flabellum</i> (<i>Flabellum</i>)	<i>curvatum</i> §	IEO	Virginia Polonio	PATA08	AMA-67
	Flabellidae	<i>Flabellum</i> (<i>Flabellum</i>)	<i>flexuosum</i>	NMNH	Stephen Cairns	USNM 82188	AMA-96
	Flabellidae	<i>Flabellum</i> (<i>Flabellum</i>)	<i>impensum</i> §	MNHN	Aude Andouche	MNHN-IK-2011-2437	AMA-238
	Flabellidae	<i>Flabellum</i> (<i>Flabellum</i>)	<i>knoxi</i> *	NMNH	Stephen Cairns	USNM 94332	AMA-109
	Flabellidae	<i>Flabellum</i> (<i>Flabellum</i>)	<i>lamellosum</i> §	MNHN	Aude Andouche	MNHN-IK-2011-2386	AMA-229
	Flabellidae	<i>Flabellum</i> (<i>Flabellum</i>)	<i>thouarsii</i> §	IEO	Virginia Polonio	PATA06	AMA-65
	Flabellidae	<i>Flabellum</i> (<i>Ulocyathus</i>)	<i>alabastrum</i>	NMNH	Stephen Cairns	USNM 1008601	AMA-171 (A)
	Flabellidae	<i>Flabellum</i> (<i>Ulocyathus</i>)	<i>alabastrum</i> §	IEO	Alvaro Altuna	025-DR15	AMA-262 (B)
	Flabellidae	<i>Flabellum</i> (<i>Ulocyathus</i>)	<i>apertum</i>	NMNH	Stephen Cairns	USNM 94298	AMA-107
	Flabellidae	<i>Flabellum</i> (<i>Ulocyathus</i>)	<i>lowekeyesi</i>	NMNH	Stephen Cairns	USNM 94318	AMA-108
	Flabellidae	<i>Javania</i>	<i>antarctica</i> §	IEO	Virginia Polonio	PATA03	AMA-62
	Flabellidae	<i>Javania</i>	<i>borealis</i>	NMNH	Stephen Cairns	USNM 1011112	AMA-174
	Flabellidae	<i>Javania</i>	<i>cailleti</i> §	IEO	Alvaro Altuna	026-DR15	AMA-270
	Flabellidae	<i>Javania</i>	<i>cailleti</i> §	NCBI		JQ611399	
	Flabellidae	<i>Javania</i>	<i>lamprotychum</i>	NMNH	Stephen Cairns	USNM 1071211	AMA-179
	Flabellidae	<i>Polymyces</i>	<i>wellsi</i>	NMNH	Stephen Cairns	USNM 1072331	AMA-182
FGY	Fungiacyathidae	<i>Fungiacyathus</i> (<i>Fungiacyathus</i>)	<i>stephanus</i> §	MNHN	Aude Andouche	MNHN-IK-2011-2388	AMA-230
	Fungiacyathidae	<i>Fungiacyathus</i> (<i>Fungiacyathus</i>)	<i>stephanus</i> §	NCBI		JF825138	
FUN	Fungiidae	<i>Heliofungia</i>	<i>actiniformis</i>	NCBI		DQ174720	
MER	Merulinidae	<i>Dipsastraea</i>	<i>mathaii</i> §†	UNIMIB	Francesca Benzoni	BA101	AMA-05
	Merulinidae	<i>Dipsastraea</i>	<i>pallida</i> §†	UNIMIB	Francesca Benzoni	BA061	AMA-04 (A)
	Merulinidae	<i>Dipsastraea</i>	<i>pallida</i> §	UNIMIB	Francesca Benzoni	BA119	AMA-06 (B)
	Merulinidae	<i>Favites</i>	<i>chinesis</i>	NCBI		AB094431	
	Merulinidae	<i>Orbicella</i>	<i>faveolata</i>	NCBI		AP008978	
	Merulinidae	<i>Platygyra</i>	<i>carnosa</i>	NCBI		JX911333	
MIC	Micrabaciidae	<i>Rhombopsammia</i>	<i>niphada</i>	NMNH	Stephen Cairns	USNM 96737	AMA-116
OCU	Bathyporidae	<i>Madrepora</i>	<i>oculata</i> §	CNR-ISMAR	Marco Taviani		AMA-51
	Bathyporidae	<i>Madrepora</i>	<i>oculata</i>	NCBI		JX236041	
	Oculinidae	<i>Oculina</i>	<i>diffusa</i>	NCBI		FJ966871	
	Oculinidae	<i>Oculina</i>	<i>patagonica</i> §	MNCN	Anna M. Addamo		AMA-50
	Oculinidae	<i>Oculina</i>	<i>patagonica</i>	NCBI		LN614380	

Family code	Family	Genus	Species	Institute / Database	Contact	Voucher code / NCBI code	AMA code
	Oculinidae	<i>Oculina</i>	<i>sp</i>	NCBI		LN614379; AY451365	
POC	Oculinidae	<i>Oculina</i>	<i>varicosa</i>	NCBI		FJ966875	
	Poritidae	<i>Goniopora</i>	<i>columna</i>	NCBI		JF825141	
	Pocilloporidae	<i>Madracis</i>	<i>myriaster</i> (formerly <i>miriabilis</i>)	NCBI		EU400212	
	Pocilloporidae	<i>Pocillopora</i>	<i>grandis</i> (formerly <i>eydouxi</i>)	NCBI		EF526303	
	Pocilloporidae	<i>Seriatopora</i>	<i>hystrix</i>	NCBI		EF633600; HM147127; M147130	
	Pocilloporidae	<i>Stylophora</i>	<i>pistillata</i>	NCBI		KC342191; AY360081; KC79165	
POR	Poritidae	<i>Porites</i>	<i>okinawensis</i>	NCBI		JF825142	
	Poritidae	<i>Porites</i>	<i>panamensis</i>	NCBI		KJ546638	
	Poritidae	<i>Porites</i>	<i>porites</i>	NCBI		DQ643837	
RHI	Rhizangiidae	<i>Siderastrea</i>	<i>radians</i>	NCBI		DQ643838	

Taxa in **bold** are the 7 species chosen for whole genome sequencing from which the 14 markers were developed and used for the phylogenetic analyses in this study. Taxa in **blue** are the species included in the combined trees (Fig. 1 and 2). Species collected in the field and preserved in ethanol for this study are denoted by a section symbol (§). Never-before-sequenced species are denoted by an asterisk (*). Species for which new *ITS* sequences were obtained (i.e. *ITS* sequence was not previously available for the species) are denoted by a dagger symbol (†). AMA codes are those assigned by the first author (Anna M. Addamo) also used in Campoy *et al.* 2020; Family codes are abbreviations for scleractinian families used in this study. NCBI codes are Accession numbers assigned to sequence records in the GenBank database. Voucher codes are those originally assigned by the museum or collection owner. Coral names have been cross-checked and aligned with the information available in WoRMS (last access on 29 December 2023). Abbreviations: CNR-ISMAR, Consiglio Nazionale di Ricerca – Istituto di Scienze Marine (Italy); IEO, Instituto Español de Oceanografía (Spain); JdA, Junta de Andalucía (Spain); MNCN, Museo Nacional de Ciencias Naturales (Spain); MNHN, Muséum national d'Histoire naturelle (France); NCBI, National Center for Biotechnology Information (USA); NMNH, Smithsonian National Museum of Natural History (USA); UNIMIB, Università degli Studi di Milano Bicocca (Italy); US, Universidad de Sevilla (Spain).

Description of the 14 markers developed from the genome screen

As a resource for this and future studies, seven temperate or cold-water coral species were selected for whole genome high-throughput sequencing to highlight the knowledge gap within the scleractinian phylogeny that currently exist for these under-sampled species: *Caryophyllia (Caryophyllia) smithii* Stokes & Broderip, 1828, *Dendrophyllia ramea* (Linnaeus, 1758), *Desmophyllum dianthus*, *Javania cailleti* (Duchassaing & Michelotti, 1864), *Madrepora oculata*, *Oculina patagonica* and *Paraconotrochus antarcticus* (Gardiner, 1929) (formerly *Gardineria antarctica* Gardiner, 1929).

Whole genome sequencing of seven coral species

The genomic libraries of the selected species were constructed at Genomics Research & Services (Parque Científico de Madrid, and Biomol-Informatics SL, Cantoblanco, Madrid, Spain). DNA was extracted as described above, except samples were adjusted to a final concentration of 25 ng μL^{-1} . For each specimen, 2.5 μg of gDNA were fragmented using a Bioruptor (Diagenode). DNA fragments ~400 base pairs (bp) in length were excised from agarose gels and purified, and TruSeq libraries (Illumina) were prepared according to the manufacturer's instructions. The seven DNA libraries were checked for size, concentration and integrity using a Bioanalyzer (Agilent), and DNA quantity was estimated by quantitative PCR (qPCR). An Illumina GAIIx sequencer was used for the shotgun sequencing with each species sequenced in separate lanes. Paired-end reads (2×100) were generated and the raw reads were filtered for quality (removal of adapters, artefacts, low-quality reads and duplicates) using the standard Illumina process and analysed using FastQC tool (S. Andrews, see <http://www.bioinformatics.babraham.ac.uk/projects/fastqc/>). Only reads with a Phred quality score > 30 for 95% of the nucleotide bases were kept for further analysis. An average of 66×10^6 reads per species were used for *de novo* assembly using the package SOAPdenovo (ver. 1.05, see <http://soap.genomics.org.cn/soapdenovo.html>; Luo *et al.* 2012) (Table S2) and oriented into scaffolds using Mugsy, an open-source multiple whole genome alignment tool (ver. 1.0, see <http://mugsy.sf.net>; Angiuoli and Salzberg 2010). To filter coral from non-coral reads (i.e. those of bacteria, archaea, fungi and other eukaryotes that may have been present on the coral specimen), Kmer thresholds were set during the assembly using SOAPdenovo and reads were mapped using the genome of *Acropora digitifera* (Dana, 1846) as a reference (Shinzato *et al.* 2011). The identification of orthologous groups and the genome annotation were performed using both OrthoMCL (ver. 1.4, see <https://orthomcl.org/orthomcl/app>; Li *et al.* 2003) and SWISS-PROT (ver. 2013_05, see <https://www.uniprot.org/>; Bairoch and Apweiler 1996).

For this study, the seven genomes were preferentially screened to select for homologous, orthologous, single copy protein-coding genes from the translated nucleotide sequences. For this, more than 1×10^6 scaffolds were manually filtered by length (set to ≥ 500 bp) and identity (set to $\geq 70\%$); also, a sequence consensus of multiple copies was considered. Multiple species alignments of the selected scaffolds were manually filtered by length (set

to ≥ 200 bp), strand representation and interspecific polymorphism using Gmaj (ver. 20181103, see <https://globin.bx.psu.edu/dist/gmaj/>; Blanchette *et al.* 2004). A total of 50 multiple alignments were obtained and then manually checked with Seaview (ver. 3.2, see <https://doua.prabi.fr/software/seaview3>; Gouy *et al.* 2010). Of these, 26 were selected after filtering for length (set to ≥ 400 bp), variable sites (set to $\geq 2\%$) and informative sites (set to $\geq 10\%$) as a potential source of markers. Degenerate primers, designed using CODEHOP (ver. 1.0, see <http://blocks.fhcrc.org/codehop.html>; Rose *et al.* 2003) and HYDEN (ver. 1.0, see <https://acgt.cs.tau.ac.il/hyden/>; Linhart and Shamir 2007), were manually checked to reduce degeneracy by using the ability of ‘mismatched’ base pairs to form a partial bond in primer-template interactions (Palumbi 1996). Corresponding amino acid translations of primers pairs were manually checked to maximise the efficiency of universal primer pairs by extending the match from 7 to 9 identical amino acids (Palumbi 1996). Of the 26 primers pairs initially tested, 14 resulted in successful PCR amplification and sequencing. Amplified products were sequenced and confirmed to not be potential chimeric sequences due to *de novo* assembly or multiple copies in the genome. Sequences were aligned using ClustalX (ver. 2.1, see <http://www.clustal.org/clustal2/>; Thompson *et al.* 1997) and the resulting alignments were checked manually using Se-Al (ver. 2.0a11, see <http://tree.bio.ed.ac.uk/software/seal/>). The 14 markers (4 mtDNA, 10 nDNA) developed from this screen were *16S* rDNA (different fragment from the common one), Actin, AMPt1, AMPt2, ATP6-NAD4, β -Actin, Creatine kinase, Heat shock-like, Helicase, NCAH-like, NAD3, NAD5, SIAH1 and UBB (Tables 1 and S3).

Table S2. Illumina GAllx information and number of scaffolds and multiple alignment per species.

Species	Average insertion size (bp)	Number of reads	Size (Mb)	Percentage cover	Number of scaffolds	Number of alignment
<i>Caryophyllia smithii</i>	513	57495676	57	13.57	221391	40894
<i>Dendrophyllia ramea</i>	470	76154662	76	18.10	277008	24513
<i>Desmophyllum dianthus</i>	430	64208496	64	15.24	194379	39916
<i>Javania cailleti</i>	541	96834006	99	23.57	191043	24637
<i>Madrepora oculata</i>	453	52968500	53	12.62	26214	10067
<i>Oculina patagonica</i>	511	56996784	57	13.57	269768	27105
<i>Paraconotrochus antarcticus</i>	524	56254806	56	13.33	142013	26151

Percentage cover is calculated considering the genome size of *Acropora digitifera* (420 Mbp) (Shinzato *et al.* 2011). Mb, megabases.

For this study, the seven genomes were preferentially screened to select for homologous, orthologous, single copy protein-coding genes from the translated nucleotide sequences. For this, more than 1×10^6 scaffolds were manually filtered by length (set to ≥ 500 bp) and identity (set to $\geq 70\%$); also, a sequence consensus of multiple copies was considered. Multiple species alignments of the selected scaffolds were manually filtered by length (set to ≥ 200 bp), strand representation and interspecific polymorphism using Gmaj (ver. 20181103; Blanchette *et al.* 2004). A total of 50 multiple alignments were obtained and then manually checked with Seaview (ver. 3.2; Gouy *et al.* 2010). Of these, 26 were selected after filtering for length (set to ≥ 400 bp), variable sites (set to $\geq 2\%$) and informative sites (set to $\geq 10\%$) as a potential source of markers. Degenerate primers, designed using CODEHOP

(ver. 1.0; Rose *et al.* 2003) and HYDEN (ver. 1.0; Linhart and Shamir 2007), were manually checked to reduce degeneracy by using the ability of ‘mismatched’ base pairs to form a partial bond in primer–template interactions (Palumbi 1996). Corresponding amino acid translations of primers pairs were manually checked to maximise the efficiency of universal primer pairs by extending the match from 7 to 9 identical amino acids (Palumbi 1996). Of the 26 primers pairs initially tested, 14 resulted in successful PCR amplification and sequencing. Amplified products were sequenced and confirmed to not be potential chimeric sequences due to *de novo* assembly or multiple copies in the genome. Sequences were aligned using ClustalX (ver. 2.1; Thompson *et al.* 1997) and the resulting alignments were checked manually using Se-Al (ver. 2.0a11). The 14 markers (4 mtDNA, 10 nDNA) developed from this screen were *16S* rDNA (different fragment from the common one), *Actin*, *AMPt1*, *AMPt2*, *ATP6-NAD4*, β -*Actin*, *Creatine kinase*, *Heat shock-like*, *Helicase*, *NCAH-like*, *NAD3*, *NAD5*, *SIAH1* and *UBB* (Tables 1 and S3).

Table S3. Description of the 14 markers developed in this study.

Number	Name (type)	Description
1	<i>16S rDNA</i> (mtDNA)	Mitochondrial large subunit ribosomal RNA is the only marker not derived from a protein-coding gene. This portion of <i>16S</i> differs from the one commonly used in phylogeny studies of Scleractinia, and it is located at ~1300 bp closer to the 5’ end.
2	<i>Actin</i> (ncDNA)	Actin is the most abundant protein in most eukaryotic cells. It is highly conserved and participates in more protein-protein interactions than any other known protein. Final alignment length: 303 bp.
3	<i>AMPt1</i> (ncDNA)	Adenosine monophosphate-protein transferase , a protein mediates the addition of adenosine 5'-monophosphate (AMP) to specific residues of target proteins. Final alignment length: 332 bp.
4	<i>AMPt2</i> (ncDNA)	Adenosine monophosphate-protein transferase a protein mediates the addition of adenosine 5'-monophosphate (AMP) to specific residues of target proteins. Final alignment length: 333 bp.
5	<i>ATP6NAD4</i> (mtDNA)	The sequence is formed by two fragments of the genes for ATP synthase subunit a and NADH-ubiquinone oxidoreductase chain 4 . The former is a multi-pass membrane protein with hydrogen ion transmembrane transporter activity (source Uniprot.org). The latter belongs the complex I subunit 4 family (for description of complex, see <i>NAD3</i> below). Final alignment length: 1223 bp.
6	β - <i>Actin</i> (ncDNA)	β-Actin gene (see description for <i>Actin</i>). Final alignment length: 383 bp.
7	<i>Creatine kinase</i> (ncDNA)	Creatine kinase belongs to the ATP:guanido phosphotransferase family. Final alignment length: 252 bp.
8	<i>Heat shock-like</i> (ncDNA)	Member of the Heat shock-like protein family involved in the general stress response to changes in a cell or organism’s state or activity. Final alignment length: 523 bp.
9	<i>Helicase</i> (ncDNA)	ATP-dependent RNA helicase belongs to the DEAD box helicase family, which catalyse the unwinding of double-stranded nucleic acids. Final alignment length: 466 bp.
10	<i>NAD3</i> (mtDNA)	NADH-ubiquinone oxidoreductase chain 3 protein, a core subunit of the mitochondrial membrane respiratory chain NADH dehydrogenase (Complex I). Final alignment length: 461 bp.
11	<i>NAD5</i> (mtDNA)	NADH-ubiquinone oxidoreductase chain 5 gene (see description for <i>NAD3</i>). Final alignment length: 779 bp.
12	<i>NCAH-like</i> (ncDNA)	Neurocalcin like protein , a calcium ion binding involved in regulation of cytosolic calcium ion concentration. Final alignment length: 528 bp.
13	<i>SIAH1</i> (ncDNA)	E3 ubiquitin-protein ligase , a protein mediating ubiquitination and subsequent proteasomal degradation of target proteins. Final alignment length: 601 bp.
14	<i>UBB</i> (ncDNA)	Polyubiquitin is derived from ubiquitin, a highly conserved 76-amino acid polypeptide found throughout the eukaryotic kingdom. Final alignment length: 730 bp.

mtDNA, mitochondrial DNA; ncDNA, nuclear DNA.

Supplementary results

Besides the results on the main species *C. caespitosa*, *M. oculata*, and *O. patagonica*, additional findings of this study are worth mentioning as they provide further information on some of the phylogenetic relationships of other Scleractinia (e.g. *Desmophyllum dianthus* v. *D. pertusum*, see Seiblitiz *et al.* 2022) or more molecular data for some groups (e.g. *Flabellum (Flabellum) cinctutum*, see Cairns and Polonio 2013).

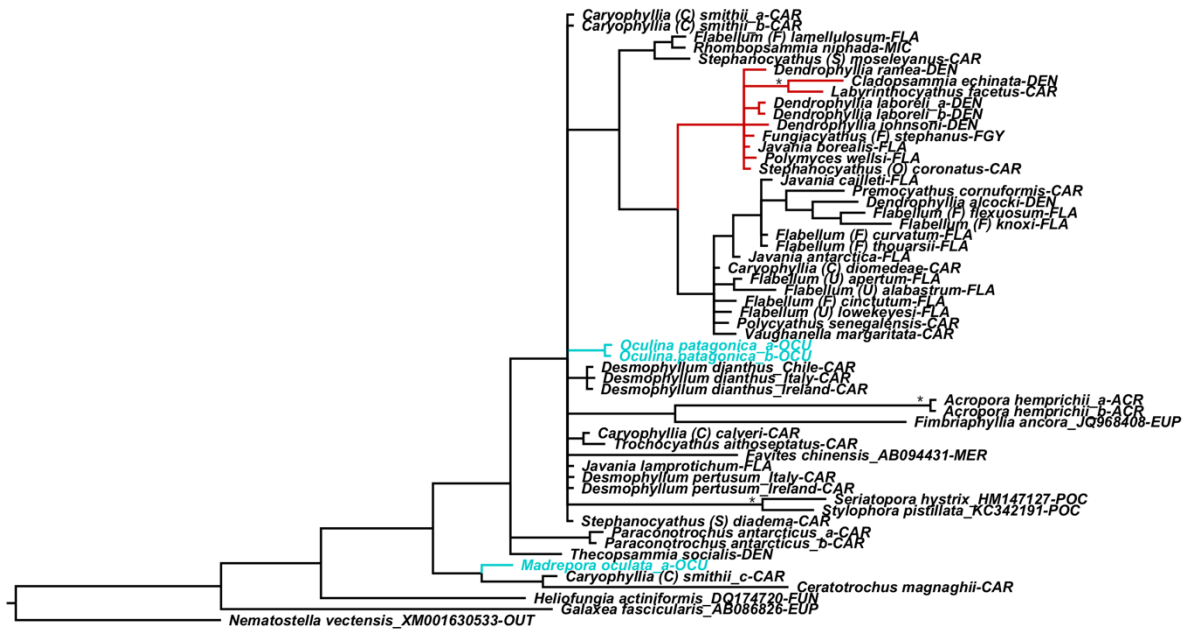
The phylogenetic analyses of the combined data sets and some of the individual loci provided additional support for a close relationship among certain Caryophylliidae taxa assigned to one of the three major families (Caryophylliidae, Dendrophylliidae or Flabellidae) whose taxonomic placement is still under discussion or that have not, until now, been included in molecular studies (e.g. see Arrigoni *et al.* 2014; Cairns 2016; Kitahara *et al.* 2016; Seiblitiz *et al.* 2022; Terraneo *et al.* 2022):

- caryophyllid species *D. dianthus* and *D. pertusum* (yellow clade in Fig. 1–3, and S1a–n), further support for the placement of *D. pertusum* as the second taxon within the genus *Desmophyllum*;
- *Caryophyllia (Caryophyllia) calveri* Duncan, 1873, *C. (C.) huinayensis* Cairns, Häussermann & Försterra, 2005, *C. (C.) smithii*, *Pourtalosmilia anthophyllites* (Ellis & Solander, 1786), *Stephanocyathus (Stephanocyathus) diadema* (Moseley, 1876) and *Trochocyathus aithoseptatus* Cairns, 1984 (green clade in Fig. 1–3 and S1b, c, e–g);
- *Caryophyllia (C.) diomedae* Marenzeller, 1904, *Polycyathus senegalensis* Chevalier, 1966, *Vaughanella margaritata* (Jourdan, 1895) and some flabellid species (pink clade in Fig. 2 and S1a–c, e, g–l, n);
- *Stephanocyathus (Odontocyathus) coronotaus* (Pourtalés, 1867), *Javania borealis* Cairns, 1994 (Flabellidae) and some dendrophyllid species currently assigned to three different groups (see Cairns, 2021) including *Dendrophyllia alcocki* (Wells, 1954) and *D. johnsoni* (Cairns, 1991) (group 3), *D. laboreli* Zibrowius & Brito, 1984 (group 2) and *D. ramea* (Linnaeus, 1758) (group 1) (red clade in Fig. 2, S1a–d, f, n);
- *Paraconotrochus antarcticus* (formerly *Gardineria antartica* and tentatively placed in the newly erected genus by Cairns 1989) and its relatively close relationship with caryophyllid species of the ‘robust’ clade–Vacatina suborder (see Fig. 1, 3, and S1a, d, i, l–n), supporting the hypothesised close relationship between *Conotrochus* and *Paraconotrochus* (Cairns 1989; Campoy *et al.* 2020) and the taxonomic re-evaluation with respect to *Gardineria*, which is considered the earliest diverging group of Scleractinia (Kitahara *et al.* 2010; Seiblitiz *et al.* 2022);
- dendrophyllid species *Tubastraea aurea* (Quoy & Gaimerd, 1833) and *Rhizopsammia wettsteini* (Quoy & Gaimerd, 1833) (blue clade in Fig. 1, 2, and S1b–d, f–g, n);
- finally, the seven species originally defined based on morphological characters and that were sequenced here for the first time are from different families and genera: *Balanophyllia (Balanophyllia) floridana* Poutalés, 1868, *Cladopsammia echinata* Cairns, 1984 and *Dendrophyllia alcocki* are currently assigned

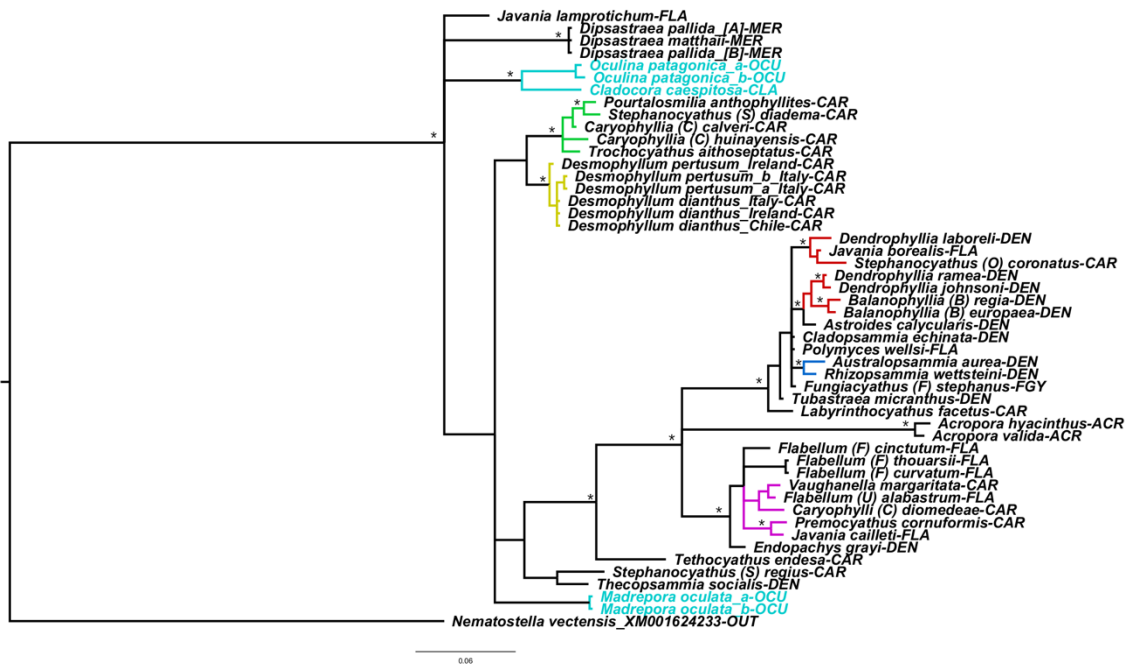
to the Dendrophylliidae, consistent with their assigned taxonomy based on morphology; *Polycyathus senegalensis* and *Premocyathus cornuformis* (Porutalés, 1868), to the Caryophylliidae; and *Flabellum (Flabellum) cinctutum* Cairns & Polonio, 2013 and *F. (F.) knoxi* Ralph & Squires, 1962, to the Flabellidae.

Although based on single markers, some of the reconstructions provide a starting point from which to consider the potential phylogenetic placement of these species. For instance, *B. (Balanophyllia) floridana*, *C. echinata* and *D. alcocki* all resolved as most closely related to other dendrophylliid species in the ‘complex’ clade–Refertina suborder of Scleractinia (Fig. S1b–d, g), consistent with their taxonomic assignment. By contrast, *P. senegalensis* and *P. cornuformis* appeared more related to flabelliid species in the ‘complex’ clade–Refertina suborder (Fig. S1a–d, f, n) than to caryophylliid species in the ‘robust’ clade–Vacatina suborder, suggesting that either their taxonomy or phylogenetic placement is doubtful. Likewise, *F. (F.) cinctutum* and *F. (F.) knoxi* resolved as more related to ‘robust’ clade–Vacatina suborder caryophylliid species instead of ‘complex’ clade–Refertina suborder flabelliid species as would have been expected by their taxonomic assignment.

The findings support the idea that Caryophylliidae should comprise less species than previously thought, as suggested by Seiblitiz *et al.* (2022). Also, the inclusion of as many extant species as possible in molecular phylogeny reconstructions are pivotal to determine the level of support for the monophyly of Dendrophyllidae, as suggested by Arrigoni *et al.* (2014) and Terraneo *et al.* (2022), or to investigate the evolutionary history of the family Flabellidae with taxa not yet represented in phylogenetic studies (see Cairns 2016; Campoy *et al.* 2020; Kitahara *et al.* 2016).

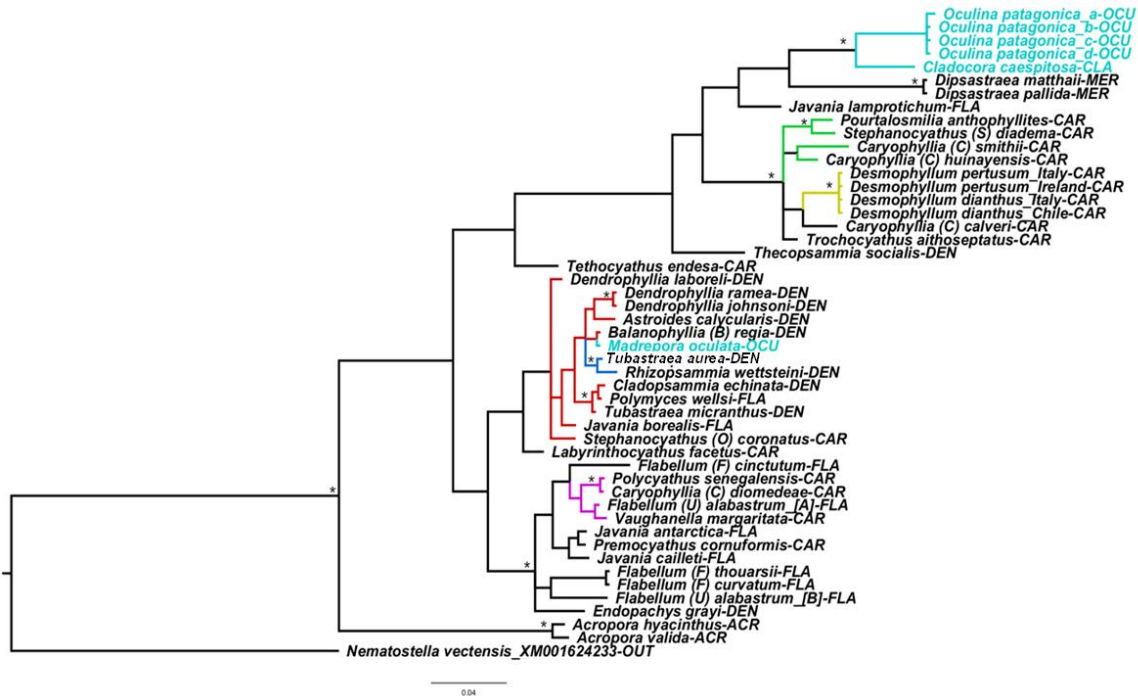


(a)

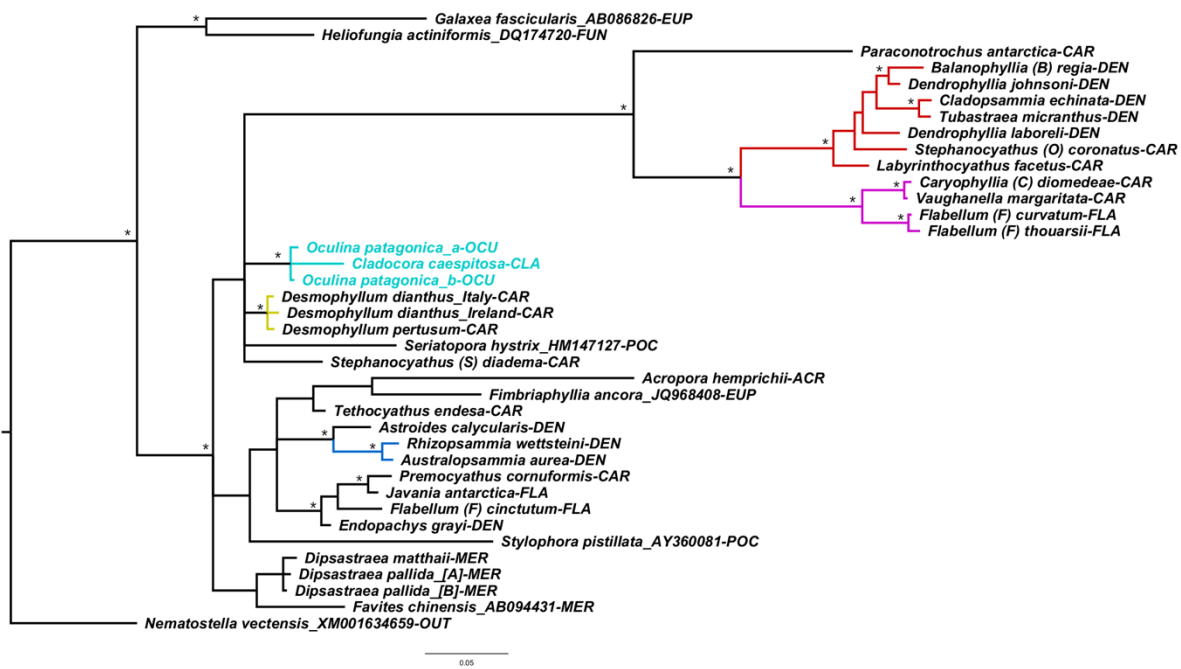


(b)

Figure S1. Phylogeny reconstruction of scleractinian relationships based on Bayesian and maximum likelihood analyses of the 14 studied markers. The first seven gene trees are of the markers included in the concatenated data set: (a) Actin, (b) AMPt1, (c) AMPt2, (d) β -Actin, (e) NAD3, (f) NAD5, (g) SIAH1, followed by the rest of the developed markers (h) ATP6-NAD4, (i) Creatine kinase, (j) Heat shock-like (k) Helicase, (l) NCAH-like, (m) UBB, (n) 16S rDNA. An asterisk (*) indicates a well-supported node (pp \geq 95; bootstrap > 70). *Cladocora caespitosa*, *Madrepora oculata*, and *Oculina patagonica* are highlighted in light blue. Clades showing other relationships worthy of further investigation are indicated in blue, pink, red, yellow and green. See Table 1 and Table S3 for marker information, and Supplementary Table S1 for species information. OUT, outgroup.

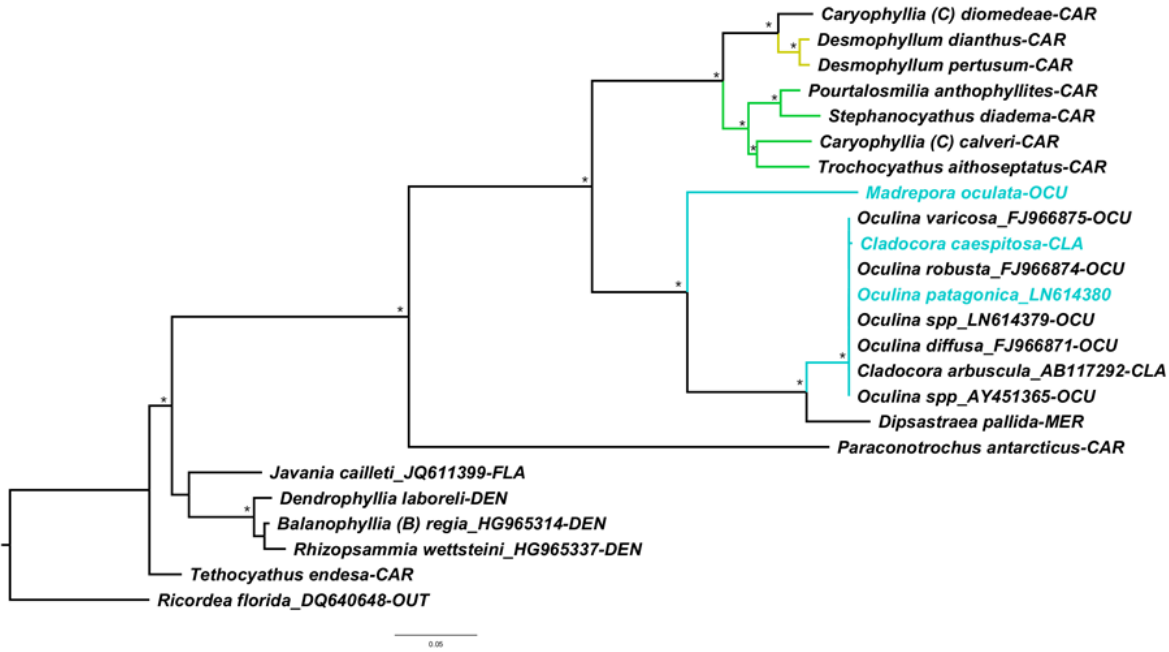


(c)

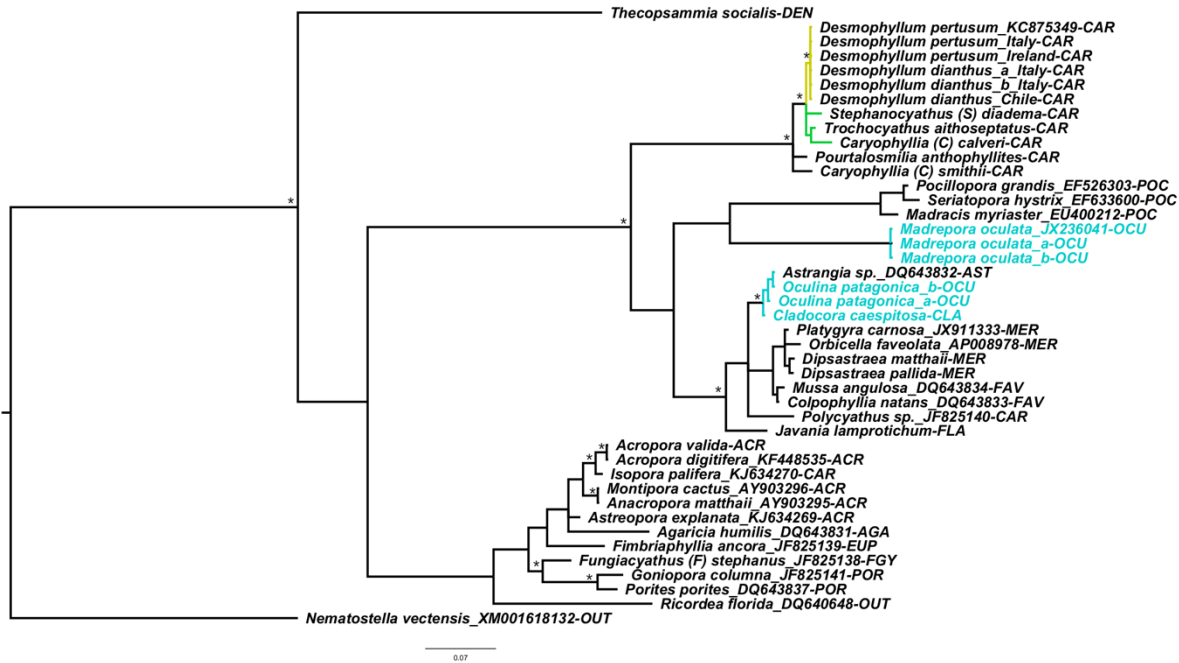


(d)

Figure S1. (Cont.)



(e)

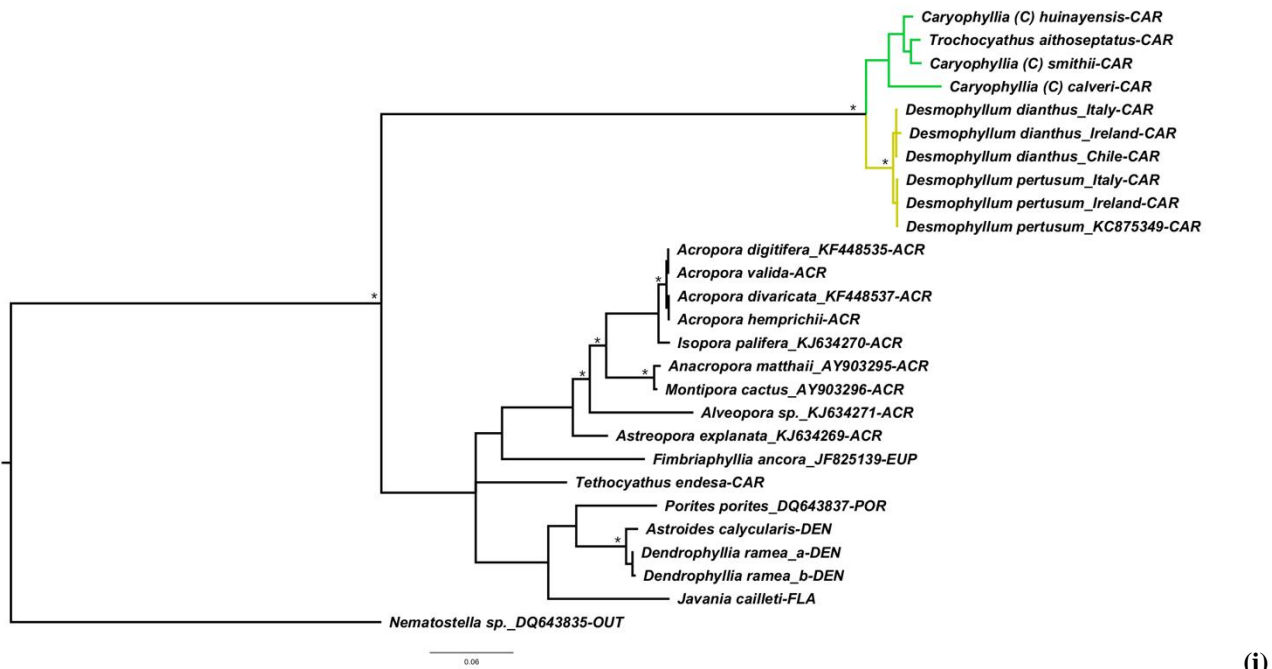


(f)

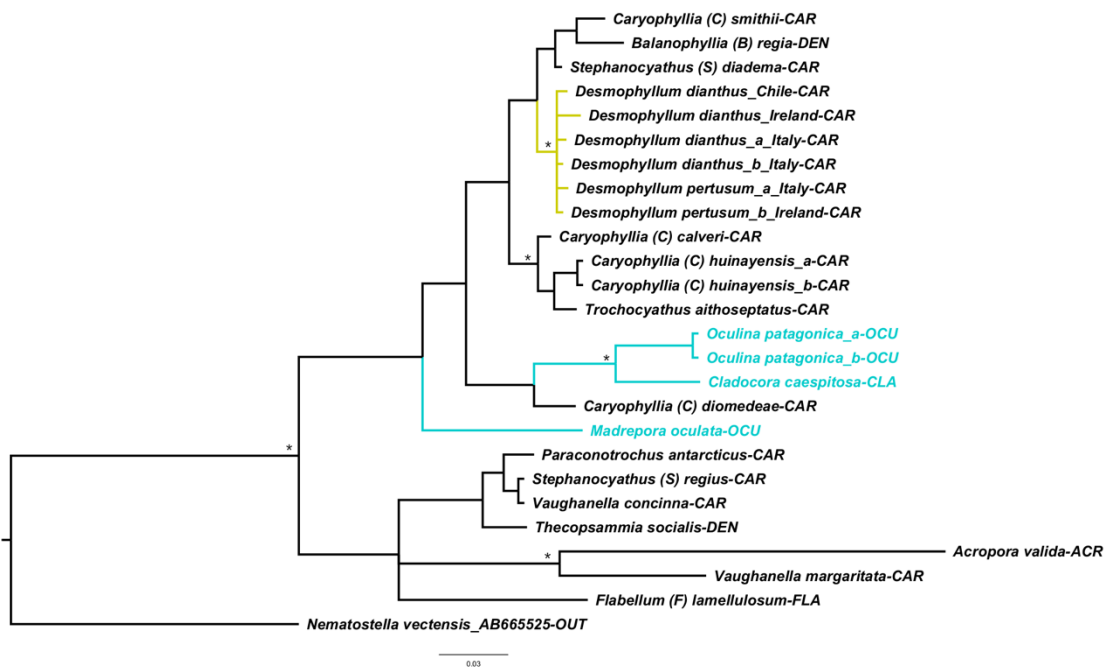
Figure S1. (Cont.)



Figure S1. (Cont.)

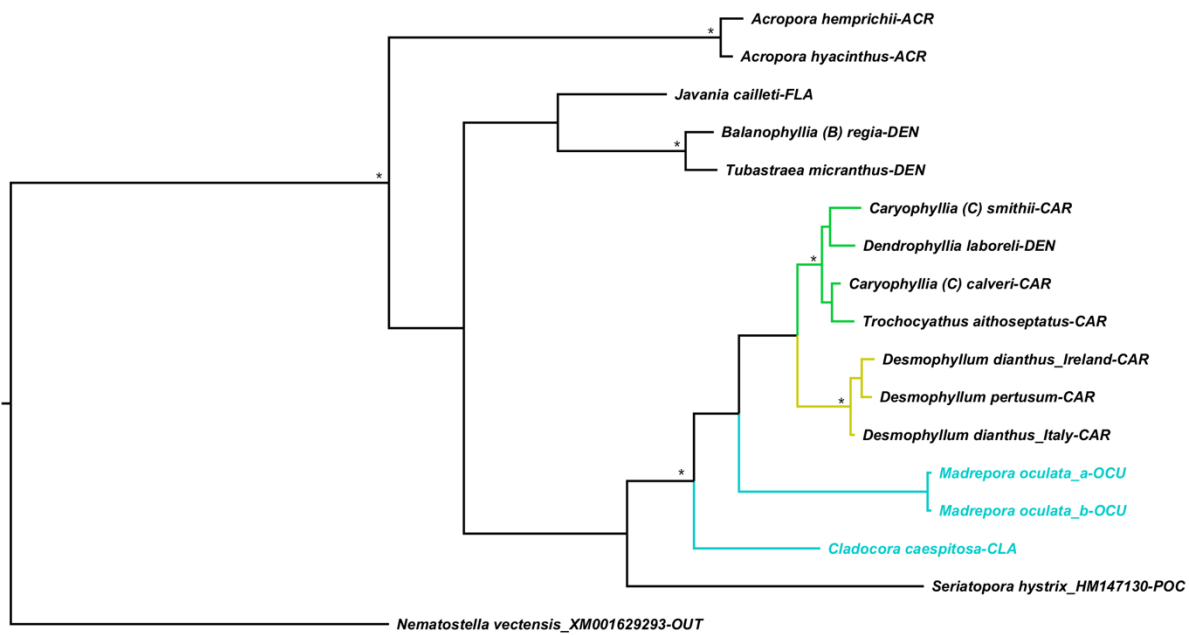


(i)

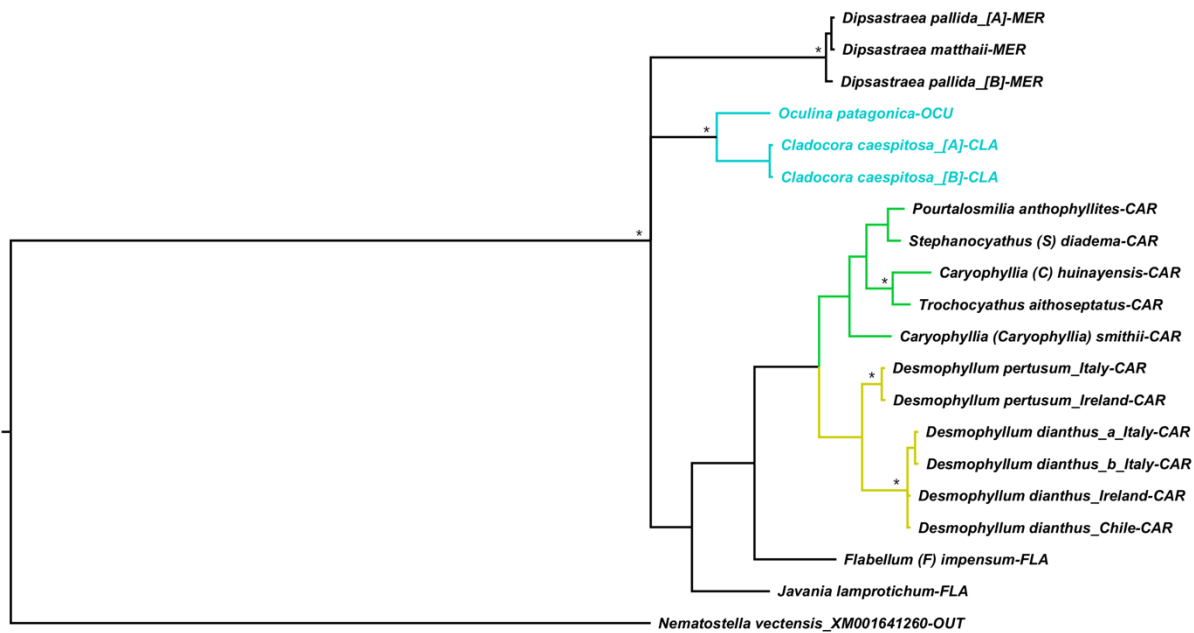


(j)

Figure S1. (Cont.)

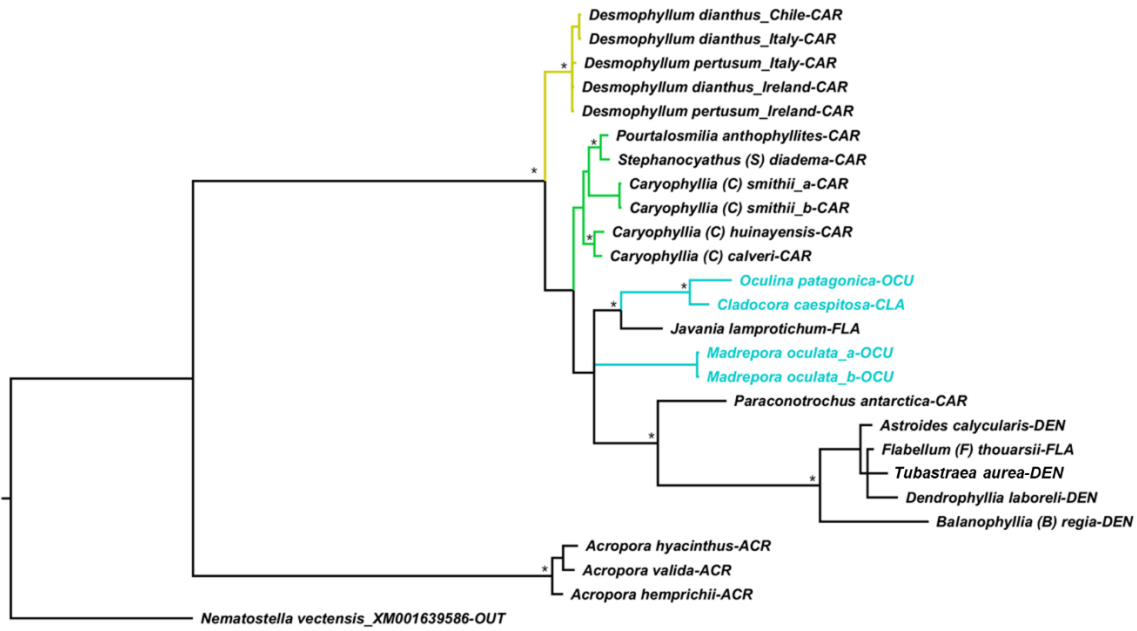


(k)

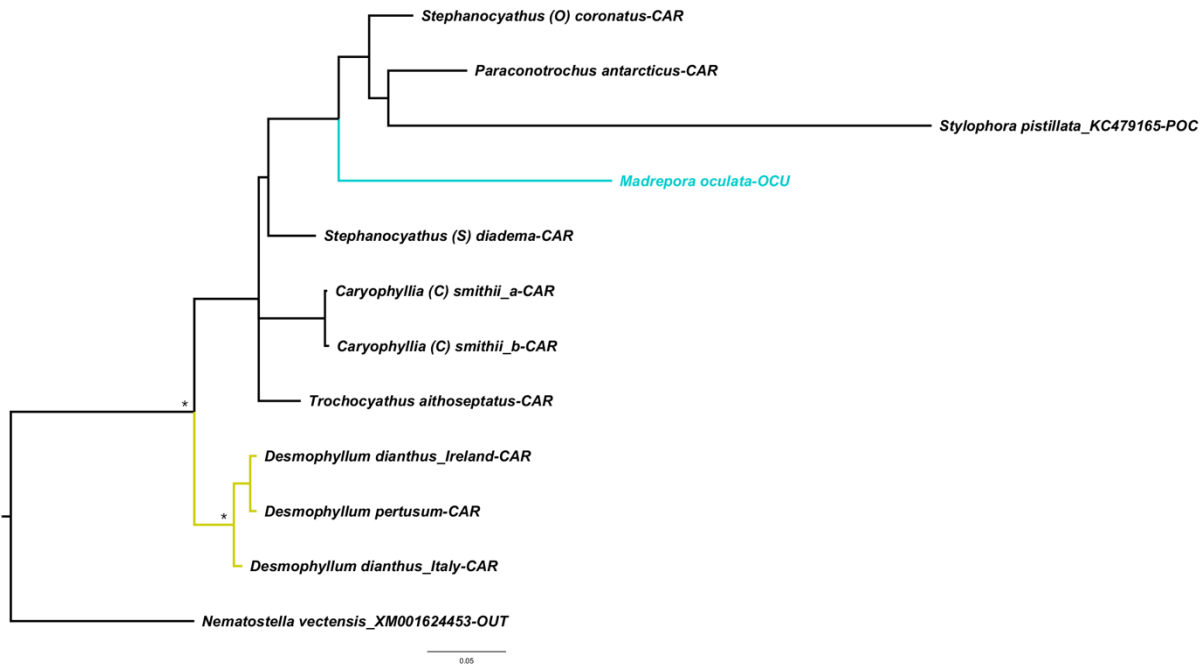


(l)

Figure S1. (Cont.)



(m)



(n)

Figure S1. (Cont.)



(9)

Figure S1. (Cont.)

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