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ORIGINAL ARTICLE

On the specific status of eastern Mediterranean *Dendrophyllia* corals (Cnidaria, Anthozoa): Genetic characterization and speciation scenarios

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Abstract

The deep-sea corals *Dendrophyllia ramea* and *Dendrophyllia cornigera* occur in Mediterranean and Atlantic waters. Both species are found in different environmental conditions, and they can colonize hard and soft substrates. These species then display an important ecological plasticity along with morphological plasticity. Nevertheless, there is a large knowledge gap on the genetic characteristics of the two species, including on the relationships between them and the possibility of cryptic species along their range. The recent discovery of *Dendrophyllia* populations off Cyprus in the eastern Mediterranean Sea raised new questions in this context. These corals were related to *D. ramea* but had some morphological

differences with other known populations of this species. Here, we study the specific status of *Dendrophyllia* corals from Cyprus on the basis of morphology and genetics. The genetic data are interpreted by comparison with the same analysis performed on two *Caryophyllia* species. Both morphological and genetic data confirm that corals found off Cyprus belong to the *D. ramea* species. We further tested the speciation scenario using transcriptome data: the results indicate an absence of current gene flow between *D. ramea* and *D. cornigera* and that the divergence occurred more than 3 million years ago. We discuss the possible historical and ecological factors which may have shaped speciation in these species.

KEYWORDS

Caryophyllia, corals, *Dendrophyllia*, genetics, morphology, species delimitation, transcriptome

1 | INTRODUCTION

Though less well-studied and publicized than their shallow-water counterparts, at least until recent years, deep-sea corals (DSC; i.e., coral species occurring below 50 m depth; Cairns, 2007) correspond to an important part of the diversity of Scleractinians, with 622 of the 7111 azooxanthellate scleractinian species occurring deeper than 50 m (Roberts et al., 2009). DSC develop complex three-dimensional structures, which can support an important biodiversity (Buhl-Mortensen et al., 2017; Henry & Roberts, 2017; Roberts et al., 2009; Rueda et al., 2019). The research on DSC in the Mediterranean has already a long history (Evans et al., 2019; Orejas & Jiménez, 2019). In the last years, many advances have been done regarding knowledge on their occurrence and distribution (Evans et al., 2019; Orejas & Jiménez, 2019; Taviani et al., 2017), associated biodiversity (Rueda et al., 2019), as well as some molecular studies (Altuna & Poliseno, 2019; Boavida, Becheler, Addamo, et al., 2019).

However, the study of the genetic diversity of DSC is still in its infancy, although fundamental, as the analysis of the genetic structure of coral populations gives important information on the connectivity and population structure, including the levels of clonal reproduction whenever applicable (Dahl et al., 2012). Further, population genomic data can be used to study the adaptation to local conditions such as depth or temperature (Pratlong et al., 2021). This last point has important consequences for the future evolution of coral populations in the context of climate change. For example, it has been proposed that deeper populations, less impacted by climate change than shallower ones, may contribute to the reseeding of shallow populations, the so-called deep refuge hypothesis (Bongaerts et al., 2017). Nevertheless, this is indeed a hypothesis, and such a reseeding scenario may not apply to all coral species (Bongaerts et al., 2017). The study of

genetic differentiation and adaptation to local environmental conditions is important for evolution-based management actions (Chen et al., 2022). However, genetic studies of connectivity and adaptation should rely on sound species delimitation (Pante et al., 2015). A correct species delimitation is also useful to better understand the evolution of deep ecosystems, for example, to test hypotheses on endemism (Castelin et al., 2010). The analysis of speciation scenarios can also be used to test occurrence of ongoing gene flow between species, with potential consequences for management and conservation (De Jode et al., 2022; Roux et al., 2016).

The questions of speciation processes and species delineation are particularly challenging and complex in anthozoans. The number of anthozoan species may have been underestimated in some groups because of a combination of morphological plasticity (Marti-Puig et al., 2014), morphological stasis (Bongaerts et al., 2021), or hybridization (Vollmer & Palumbi, 2004). Depending on the anthozoan taxa, mitochondrial DNA may provide adequate discrimination of the main specific units or not (Calderón et al., 2006; Erickson et al., 2021; Gélin et al., 2017). For example, in octocorals, the lack of variation of mitochondrial DNA limits its use for species delineation and barcoding (Aurelle et al., 2017; Calderón et al., 2006; McFadden et al., 2011). Conversely, in *Pocillopora* hexacorals, mitochondrial DNA can be useful for a first discrimination of species, to be complemented with independent and more variable molecular markers, such as microsatellites (Gélin et al., 2017) and single-nucleotide polymorphisms (SNPs; Oury et al., 2023).

In the Mediterranean Sea, studies of DSC mainly focused on the western basin (Evans et al., 2019; Orejas, Gori, et al., 2019). Among the Mediterranean DSCs, two species from the genus *Dendrophyllia*, *D. cornigera* and *D. ramea*, occur in the western and eastern basins (Castellan et al., 2019; Salomidi et al., 2010; Zibrowius, 1980). However, in the eastern Mediterranean,

the observations of live DSCs only referred to *D. cornigera* in the South of Crete (Salomidi et al., 2010), and *D. ramea* in Cyprus (Orejas et al., 2017; Orejas, Gori, et al., 2019; Orejas, Jiménez, et al., 2019), and Lebanon (Bigagli et al., 2018), along with some observations in the Ionian Sea (Angiolillo et al., 2022). The characterization of new DSC populations in the eastern Mediterranean can bring important information on the ecology and evolution of *Dendrophyllia* corals. Indeed, the occurrence of a species in such contrasted and distant locations (Atlantic, western and eastern Mediterranean) raises the question of the connectivity among populations for these two species, as well as their adaptation to local environments. Additionally, important genetic differentiation has been observed for several marine species between Atlantic and western Mediterranean populations and between eastern and western Mediterranean populations (e.g. Arnaud-Haond et al., 2007; Boavida, Becheler, Choquet, et al., 2019; Borsa et al., 1997; Coelho et al., 2023; El Ayari et al., 2019; Patarnello et al., 2007). Such phylogeographic breaks are important to understand the evolutionary history of species and therefore should be considered for DSC as well (Boavida, Becheler, Choquet, et al., 2019).

In 2015, the scientific cruise CYCLAMEN onboard the research vessel Aegeo, as part of the research project CYCLAMEN (TOTAL foundation), conducted the first studies of the distribution of *D. ramea* in a specific area off the eastern coast of Cyprus (Orejas, Gori, et al., 2019; Orejas, Jiménez, et al., 2019). There, the species was mainly observed between 125 and 155 m depth (Orejas, Gori, et al., 2019). The colonies were morphologically identified as a slender form of *D. ramea*, which contrasted with other more robust forms observed for this species in shallower waters off Portugal and Spain (Orejas et al., 2017; Orejas, Gori, et al., 2019). These morphological characteristics raised the question of the specific status of the *Dendrophyllia* specimens from Cyprus: do they correspond to a population of *D. ramea* differentiated from other known populations or maybe to a different species? Here, we address this question through the use of morphological and genetic (mitochondrial barcoding) data. We compared the samples from Cyprus from one side with *D. ramea* samples from other Mediterranean locations as well as from Atlantic areas and from the other side with *D. cornigera* samples from Atlantic and Mediterranean samples. We used another pair of congeneric Mediterranean scleractinians, *Caryophyllia inornata* and *C. smithii*, as a comparison to interpret the observed mitochondrial divergence in the *Dendrophyllia* genus. We completed these results with transcriptome sequencing in order to study the processes of speciation between the two *Dendrophyllia* species: this aims at understanding the history of divergence of the species and to test for current gene flow between them.

2 | MATERIALS AND METHODS

2.1 | Species distribution and sampling sites

Dendrophyllia cornigera (Lamarck, 1816) is known from the eastern and western Mediterranean Sea, from the Atlantic from the northern-most locations in Ireland, to the Archipelagos of Azores and Cabo Verde in the south (Castellan et al., 2019; Zibrowius, 1980; see Figure 1a as well). For mitochondrial sequencing, we used samples from the Lacaze-Duthiers Canyon near Banyuls (France) and from southern Portugal (Figure 1b). The transcriptome sequencing of *D. cornigera* was based on samples from the Balearic Islands (Figure 1b) kept alive in the aquaria facilities of the Monaco Scientific Center (CSM). We also sequenced the transcriptome of a *D. cornigera* colony from the Lacaze-Duthiers Canyon, which was kept alive at the biological station of Banyuls sur Mer (France). *Dendrophyllia ramea* (Linné, 1758) is known from the western Mediterranean, and from the eastern Atlantic, from Portugal to Cabo Verde (Salvati et al., 2021; Zibrowius, 1980; Figure 1a) and more recently from the eastern Mediterranean, in Greece, Cyprus, and Lebanon (Orejas et al., 2017; Orejas, Gori, et al., 2019; Orejas, Jiménez, et al., 2019; Salomidi et al., 2010). For mitochondrial sequencing, we used samples from Cyprus and southern Portugal (Figure 1b). The transcriptome sequencing of *D. ramea* was based on samples from Cyprus (Orejas et al., 2017; Orejas, Gori, et al., 2019; Orejas, Jiménez, et al., 2019), which were kept alive at the CSM (Table 1).

For a comparison on the inter-specific divergence of the mitochondrial DNA, we also sequenced samples of two *Caryophyllia* species: *Caryophyllia inornata* (Duncan, 1878), which is known in the whole Mediterranean Sea and in the eastern Atlantic, between the English Channel, Azores, and Canary Islands (Zibrowius, 1980), and *Caryophyllia smithii* Stokes & Broderip, 1828, which is present in the entire Mediterranean Sea and in the eastern Atlantic from mid-Norway and the Shetland islands, down to Azores and Congo (Zibrowius, 1980; Norwegian Biodiversity Information Centre; OBIS). For *C. inornata*, we used samples from three sites along the French Mediterranean coast (Figure S1), including the Drôme and Saint Dominique wrecks near Marseille (Table 1). For *C. smithii*, we used samples from the same wrecks as those of *C. inornata* (corresponding then to a sampling on the same site for the two species) and samples from the North Sea (Skagerrak; Sweden).

2.2 | Morphological analysis

Five *Dendrophyllia ramea* specimens were collected from different locations in the coral community off

Protaras (Cyprus; Table 1) using the Max Rover remotely operated vehicle (ROV) manipulator, on station 4, transect 2 (see Table S1 for details). Samples were obtained from entire colonies in most cases. The skeletal material of five selected colonies (15–20 cm maximum length) was cleansed from the organic material by immersion in diluted (5%) sodium hypochlorite, freshwater-rinsed, and air-dried for further inspection. Morphological characteristics were analysed utilizing available descriptions (Cairns, 2001; Cairns & Kitahara, 2012; Zibrowius, 1980).

2.3 | Analysis of mitochondrial sequences

For the barcoding approach, we sequenced part of the Cytochrome Oxydase I (COI) mitochondrial locus. The number and location of samples are indicated in Table 1. For the PCR amplification of COI, we used the primers COI_Caryophyllia_F AACAGCACATGCTTTTA TTATGAT and COI_Caryophyllia_R CCGGCTAAAAC AGGTAAAGATAA, designed on the basis of *Caryophyllia* COI sequences from GenBank. The PCR conditions for a
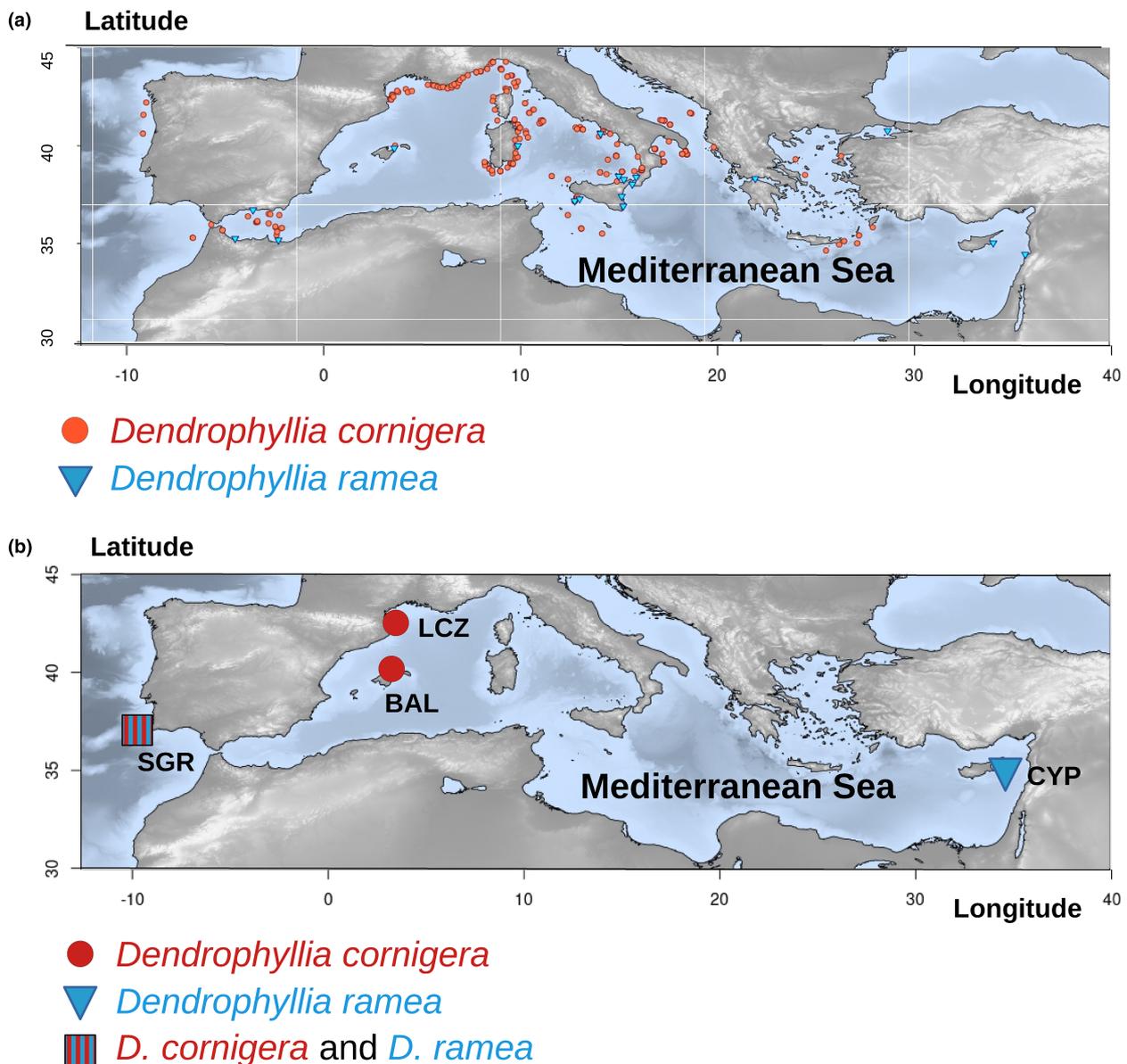

FIGURE 1 (a) Distribution of *D. ramea* and of *D. cornigera* in the Mediterranean Sea and neighbouring Atlantic. Based on Bigagli et al. (2018), Enrichetti et al. (2023), and Salvati et al. (2021). (b) Map of sampling sites for *Dendrophyllia* species. The codes refer to the sample list of Table 1. The dot with mixed colours indicates a sampling of the two species in sympatry. The maps have been created with the marmap R package (Pante & Simon-Bouhet, 2013). (a) Distribution. (b) Sampling.

TABLE 1 List and characteristics of samples used for genetic analyses.

Species	Region	Site	Code	Year	Depth m	N mit	N transc
Dendrophyllia cornigera	W Med	Balearic Islands, Menorca channel	BAL	2010	180–330	0	2
Dendrophyllia cornigera	W Med	Lacaze-Duthiers Canyon, Banyuls France	LCZ	NA	NA	3	1
Dendrophyllia cornigera	E Atl	Sagres, Portugal	SGR			1	0
Dendrophyllia ramea	E Med	Cyprus, Protaras	CYP	2015	150	1	2
Dendrophyllia ramea	E Atl	Sagres, Portugal	SGR			2	0
Caryophyllia inornata	W Med	Drôme wreck, Marseille France	DRO	2015	50	2	0
Caryophyllia inornata	W Med	Fréjus, France	FREJ	2015	20	2	0
Caryophyllia inornata	W Med	Maïre Island, Marseille France	MAI	2015	20	3	0
Caryophyllia inornata	W Med	Saint Dominique wreck, Marseille France	SDO	2015	30	0	3
Caryophyllia smithii	W Med	Drôme wreck, Marseille France	DRO	2015	50	2	0
Caryophyllia smithii	N Sea	East Søndre Søster, Sweden	ESS	2012	35–37	6	0
Caryophyllia smithii	W Med	Saint Dominique wreck, Marseille France	SDO	2015	30	0	3

Note: N mit and N transc correspond to the sample size for mitochondrial and transcriptome sequencing, respectively. The sampling location of *Dendrophyllia* in Cyprus (CYP) is described in Orejas, Gori, et al. (2019).

Abbreviations: Atl, Atlantic Ocean; Med, Mediterranean Sea; N Sea, North Sea; NA, unknown data for a sample held in the aquarium since several years when analysed.

25-µL final volume were as follows: Promega PCR buffer 1X, MgCl₂ 2.5 mM, 0.25 mM of each dNTP, 0.4 µM of each primer, Flexigotaq polymerase (Promega) 0.5 U, and 2.5 µL of DNA. The PCR program was as follows: 5 min at 94°C, 30 cycles with (1 min at 94°C, 1 min at 57°C, 1 min at 72°C), and a final extension of 10 min at 72°C. The PCR products were sent for sequencing at the Eurofins Genomics company.

The mitochondrial sequences have been analysed with UGENE v35 (Okonechnikov et al., 2012). The sequences from *Dendrophyllia* and *Caryophyllia* obtained here were used for a Blast search for similar sequences in GenBank. As our objective was not an in-depth study of phylogenetic relationships inside Caryophylliidae or Dendrophylliidae (see Kitahara et al., 2016; Quek et al., 2023 for more extended phylogenetic studies of Scleractinian corals), we only chose sequences close to those obtained here, from congeneric species or from related genera. Sequences were aligned with MUSCLE (Edgar, 2004) in UGENE. The phylogenetic reconstructions have been performed with the Maximum-Likelihood (ML) approach of IQ-TREE 2.1.1 (Nguyen et al., 2015). We used the ModelFinder option (Kalyaanamoorthy et al., 2017), and robustness was evaluated with 1000 ultrafast bootstraps (Hoang et al., 2018). The tree has been visualized with FigTree 1.4.4 (Rambaut, 2006) and was rooted at mid-point. The Genbank accession numbers of mitochondrial COI sequences obtained here or retrieved from GenBank are listed in Appendix S2.

2.4 | Analysis of the speciation scenario with transcriptome sequences

Total RNA has been extracted as in Haguenauer et al. (2013). RNAs were sent to the Genotoul platform for sequencing on one HiSeq 3000 lane (2 × 150 bp). The *D. ramea* and *D. cornigera* transcriptomes have been assembled with the *de novo* RNA-Seq Assembly Pipeline (DRAP; Cabau et al., 2017) with Oases (Schulz et al., 2012) and default parameters. We performed an individual assembly and a meta-assembly to be used as reference. The statistics describing the assembled transcriptomes are given in Table S2. The reads of each sample were mapped on the corresponding reference transcriptome with bwa mem option (Li & Durbin, 2009). The obtained sam files were converted in bam format and sorted with samtools 1.9 (Li et al., 2009). SNP genotypes were called with reads2snp with default parameters (Gayral et al., 2013; Tsagkogeorga et al., 2012). The resulting SNP dataset was first used to produce a network among samples: this tree was used to test if the different samples grouped well according to their specific status inferred from morphology and mitochondrial DNA whenever available. We used VCFtools (Danecek et al., 2011) to retain only variable positions and TASSEL 5.0 (Bradbury et al., 2007) to produce a distance matrix among samples based on the identity by state computed for the different positions. The resulting distance matrix was
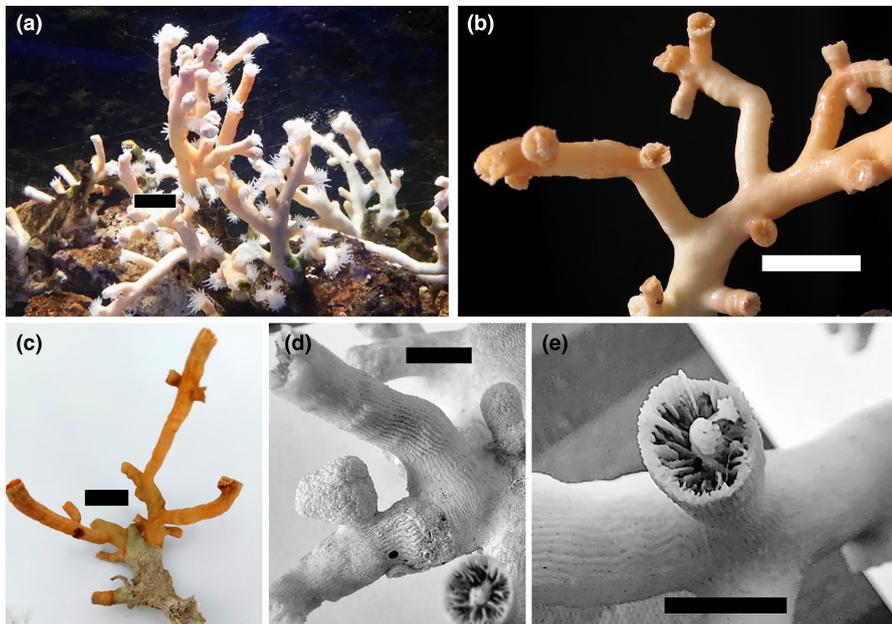
FIGURE 2 *Dendrophyllia ramea* colonies (130–150 m depth) from Protaras (Cyprus). Living colonies kept at the Protaras Ocean Aquarium (a). Typical slender form of colonies (b, c, d). Grooved surface of the corallum (d). Detail of the columella (e). For panels a–c, the scale varies in this perspective; sections near the scale bar are 20 mm. Scale bar 5 mm for panels (d, e).

used to produce a NeighborNet network with SplitsTree 5 (Huson & Bryant, 2006). This approach aimed at giving a crude representation of the distances among samples from *D. cornigera* and *D. ramea*. On the basis of these species categorizations, we studied speciation scenarios with the Demographic Inferences with Linked Selection (DILS) pipeline (Csilléry et al., 2012; Fraïsse et al., 2021; Pudlo et al., 2016). Briefly, DILS uses an Approximate Bayesian Computation (ABC) approach to analyse evolutionary scenarios in a hierarchical way (see figure 3 in Fraïsse et al., 2021). First, it will compare models with and without ongoing gene flow between the two species. Second, in case of ongoing gene flow, it will compare Isolation-Migration (IM) and Secondary Contact (SC) models. In case of current isolation, it will compare Strict Isolation (SI) and Ancestral Migration (AM) models. Third, for the models with current isolation, it will compare a model with homogeneous effective size among loci with a model with heterogeneous effective size among loci: this allows to model variation of drift among loci because of effects of linked selection. For the models with ongoing gene flow, DILS will compare the two models of effective sizes and a model with homogeneous gene flow with a model with heterogeneous gene flow among loci: this last comparison is used to test for the presence of barriers to gene flow in the genome. DILS was run online at http://dils.univ-lyon1.fr/. The parameters of the different analyses are detailed in Table S3. The transcriptome raw sequences have been deposited in the European Nucleotide Archive (ENA) at EMBL-EBI under accession number PRJEB63106 (https://www.ebi.ac.uk/ena/browser/view/PRJEB63106).

3 | RESULTS

3.1 | Morphological description

Based on gross morphological characteristics of the corallum, the Cyprus specimens were assigned to the *Dendrophyllia ramea* species (Figure 2): arborescent and robust growth form; colonies with large axial corallites from successive extratentacular budding; septal symmetry hexameral; septa arranged in a Pourtalès plan; six septal cycles; lateral short corallites usually arranged in opposite sides of the branches, almost in an alternating distribution, displaying a pronounced spongy/papillose columella; lateral calices branch off along the axis of the terminal calyx; surface of the corallum distinctively grooved. The living colonies displayed dark, light orange, to almost pink-coloured tissue; the identification of these samples as *D. ramea* was confirmed by Helmut Zibrowius (Personal commuications). The specimens analysed here and obtained with ROV were also morphologically similar to other *D. ramea* samples that have been collected as bycatch in other areas along the Cypriot coast (C. Jimenez, unpublished data).

3.2 | Phylogenetic analysis with mitochondrial markers

The phylogenetic tree based on COI sequences is presented in Figure 3. Regarding the two *Dendrophyllia* species analysed here, the mitochondrial COI sequences of *D. cornigera* and *D. ramea* were different, though with low divergence. No polymorphism was observed within each of these two species. These sequences were grouped

in a well-supported clade (clade A; 92% bootstrap) with sequences from other *Dendrophyllia* species, as well as species from other genera such as *Tubastrea* or *Madracis.* The phylogenetic relationships inside this clade A were not well-resolved. Importantly, these results agree with the identification of the recently discovered population of Cyprus as *D. ramea.* Regarding *Caryophyllia*, the COI mitochondrial locus also showed the distinction of *C. smithii* and *C. inornata*, without variation inside each species, whatever the geographical origin of the samples. The sequences of both species were grouped in the same clade (clade B; 88% bootstraps) along with sequences from other *Caryophyllia* and *Stenocyathus* species.

3.3 | Species divergence and scenario of speciation with transcriptomic data

The number of raw sequences per sample varied between 26×10^6 (for *D. ramea* MON_A) and 30.5×10^6 (for *D. cornigera* BAN_A; Table S2). The number of contigs obtained after assembly per sample varied between 53,943 and 56,581. The networks of distances among samples based on all variable positions are presented in Figure S2: the grouping of samples corresponds well to the morphological identification, with a clear distinction between *D. ramea* and *D. cornigera.*

The analysis of speciation scenarios was based on a final dataset with 1381 loci, with a mean F_{ST} among loci between species of 0.53 (Table S4). The analyses with constant and variable population sizes led to the same inferred scenario, with clear signals of current isolation (i.e., no gene flow) and heterogeneous effective size among loci (Table S4). In both cases, the data did not allow discriminating the scenarios of ancestral migration and strict isolation. The estimated divergence time was higher for the model with variable compared to constant population size. The estimated current effective size was higher for *D. cornigera* than for *D. ramea* with constant population size, but a reverse pattern was observed with variable population size (Table S4). When considering the median estimates, the gene flow during ancestral migration corresponded to 5% and 7% of the total divergence time for the models with constant and variable population size, respectively.

4 | DISCUSSION

4.1 | Species identity of *Dendrophyllia* corals from Cyprus

Morphological characteristics, especially at the skeleton level, have initially been the main characteristics used in the systematics of corals (Kitahara et al., 2016). The information supplied by morphology in coral systematics can be limited by several factors: intra-specific polymorphism, intra-colony variability, and morphological plasticity (Kitahara et al., 2016; Paz-García et al., 2015). The integration of genetic information is then useful for species delimitation in corals (Gélin et al., 2017).

Here, both morphological and genetic data agreed on the identification of Cyprus samples as *D. ramea.* From a genetic point of view, the COI mitochondrial sequence clearly linked the samples of Cyprus to *D. ramea*, with a grouping with other sequences from this species obtained here from Sagres (Atlantic). Conversely, other samples used here, and identified morphologically as *D. cornigera*, grouped separately from *D. ramea* and were identical to another *D. cornigera* sequence from GenBank. One can note that the divergence among *Dendrophyllia* species was quite low with COI. This low inter-specific divergence was also observed for the *Caryophyllia* species studied here. This relatively low divergence is consistent with the generally low rate of evolution of mitochondrial DNA observed in anthozoans (Calderón et al., 2006; Shearer et al., 2002), though with exceptions (Muthye et al., 2022).

The phylogeny obtained with mitochondrial sequences was not well-resolved, with several nodes lacking support, and several genera did not appear monophyletic. This lack of monophyly of genera in the Dendrophylliidae family has already been observed with two mitochondrial and one nuclear markers (Arrigoni et al., 2014). Nevertheless, our results also underline the need to go further than mitochondrial sequences to work on species delimitation in this family. Here, the transcriptome data clearly separated *D. ramea* and *D. cornigera* and agreed with the identification of Cyprus samples as *D. ramea.* Unfortunately, we did not get any transcriptome sequences for *D. ramea* samples from Portugal, which would be useful for a better understanding of the phylogeography of this species. This would be especially interesting to test for the divergence between Atlantic and Mediterranean populations observed in various marine species (Patarnello et al., 2007).

Regarding morphology, the colonies in the Cyprus population appeared slender when compared to other *D. ramea* populations. Despite this observation, the characteristics analysed here in Cyprus samples were different from those observed in *D. cornigera* and confirmed their identification as *D. ramea.* Specifically, in contrast to *D. ramea*, *D. cornigera* has five septal cycles with all septa of similar size; calices stem from the base, never arranged in series, and they are smaller and less numerous than in *D. ramea.*

The morphological differences observed between colonies of *D. ramea* from Cyprus and from other locations could be explained by two, non-mutually exclusive hypotheses. First, these morphological variations could be

driven by developmental plasticity in response to different environmental factors, such as sediment regimes and depth. The effect of sediment in the singular morphology of the specimens from Cyprus can be hypothesized due to the singular growth that the polyps of *D. ramea* display in the most distal part of the branches (see figure 23. 1c in Orejas, Jiménez, et al., 2019). To the best of our knowledge, the Cypriot *D. ramea* population (dozens if not hundreds of colonies) and the few colonies off Tarablus/Batroum in Lebanon are the deepest in the Mediterranean, and both are thriving on soft bottom and exhibit similar morphologies. A soft substrate might influence the growth pattern with a constraint for polyps not to be covered by sediments (Orejas, Gori, et al., 2019; Orejas, Jiménez, et al., 2019); such a morphological diversity according to the environment has also been proposed in the solitary species *C. smithii* (Zibrowius, 1971). Secondly, the morphological differences could also be determined by genetic differences in a context of the same or different related species. A more extended study of *D. ramea* populations in the Mediterranean, especially in the eastern basin, would be useful to go further on this question as well.

4.2 | Speciation scenarios in *Dendrophyllia*

The analysis of speciation scenario clearly supported a current isolation for the populations of *D. ramea* and *D. cornigera* studied here: though corresponding to geographically distant populations, this result points to a complete speciation with well-separated evolutionary lineages. It might also be noted that the F_{ST} estimated here for this comparison (around 0.53) is around the F_{ST} value (0.56) used by De Jode et al. (2022) and Roux et al. (2016) as the upper limit of their *grey zone* of speciation, above which most comparisons involved well-separated species. The only uncertainty regarding speciation scenarios here was between ancestral migration and strict isolation (i.e., no past gene flow): such uncertainty can be observed when the period of ancient gene flow is short (Fraïsse et al., 2021). Accordingly, with the scenario of ancestral migration, the length of the period of gene flow was short compared to the total divergence time. The median of the estimated divergence time was around 1.3–1.7 million generations depending on the model. This estimate depends on an unknown mutation rate. Without further information, we used the default prior mutation rate of DILS, i.e., 3×10^{-9}

per generation and per nucleotide, but this should be refined with a dedicated genomic study with calibration points. The generation time of *Dendrophyllia* species is not known. As a comparison, for another Mediterranean, but solitary, Dendrophyllidae coral: *Balanophyllia europaea*, sexual maturity occurs approximately when specimens reach the age of 3 years (Goffredo et al., 2002). If we use this value as a surrogate of generation time for the species analysed here, the divergence time between *D. ramea* and *D. cornigera* would be between 3.9 and 5.1 million years (Ma). This result is in line with other analyses of divergence time in Scleractinians: Johnston et al. (2017) estimated a maximum divergence time around 3 Ma for five *Pocillopora* species and 9 Ma for two *Seratiopora* species. Such estimates evidently depend on the species or populations used for comparison. It would then be interesting to analyse not only other populations from these two species but also other *Dendrophyllia* species to refine our scenarios of speciation in this genus.

The two competing speciation scenarios retained here then involve a relatively long time without gene flow. Both *Dendrophyllia* species have overlapping distribution range in the Mediterranean Sea and eastern Atlantic Ocean (Gori et al., 2014; Salvati et al., 2021; Zibrowius, 1980). *Dendrophyllia ramea* is present in shallower areas (20 to ca. 170 m depth) than *D. cornigera* (70 to more than 700 m depth) (Castellan et al., 2019), but with some possible overlap as well. Therefore, it seems possible that these two species may be currently in contact but do not hybridize because of reproductive incompatibilities (i.e., complete speciation). The evolution of reproductive isolation could have taken place thanks to past allopatric ranges (e.g., in different basins because of past fluctuations of sea level). In this context, the Messinian salinity crisis, between 6.3 and 5.3 Ma ago (according to estimates; Krijgsman et al., 1999; Manzi et al., 2013; Rouchy & Caruso, 2006), could have played a role in the first divergence steps of these two *Dendrophyllia* species. Differences in depth range could also be involved in speciation, as proposed for the evolution of the *Paramuricea* octocoral genus (Quattrini et al., 2022). The oldest known *Dendrophyllia* fossils have been found from early–middle Miocene deposits (Vertino et al., 2014). The more recent divergence time estimated here (during Pliocene) is then consistent with this datation.

Regarding the estimates of current effective population size (the size of an ideal population with the same rate of genetic change as the real one; Waples, 2002), we obtained contrasting results, with higher population size

FIGURE 3 Maximum-likelihood tree of COI sequences for *Dendrophyllia* species and *Caryophyllia* species. The numbers indicate bootstrap values in percent, only for nodes supported by more higher than 80%. bootstraps The different symbols highlight the sequences obtained here for *Dendrophyllia* and *Caryophyllia* corals. The GenBank accession numbers are given at the end of the names. The tree has been rooted at the mid-point. See Table 1 for information on samples and Appendix S2 for the list of accession numbers.
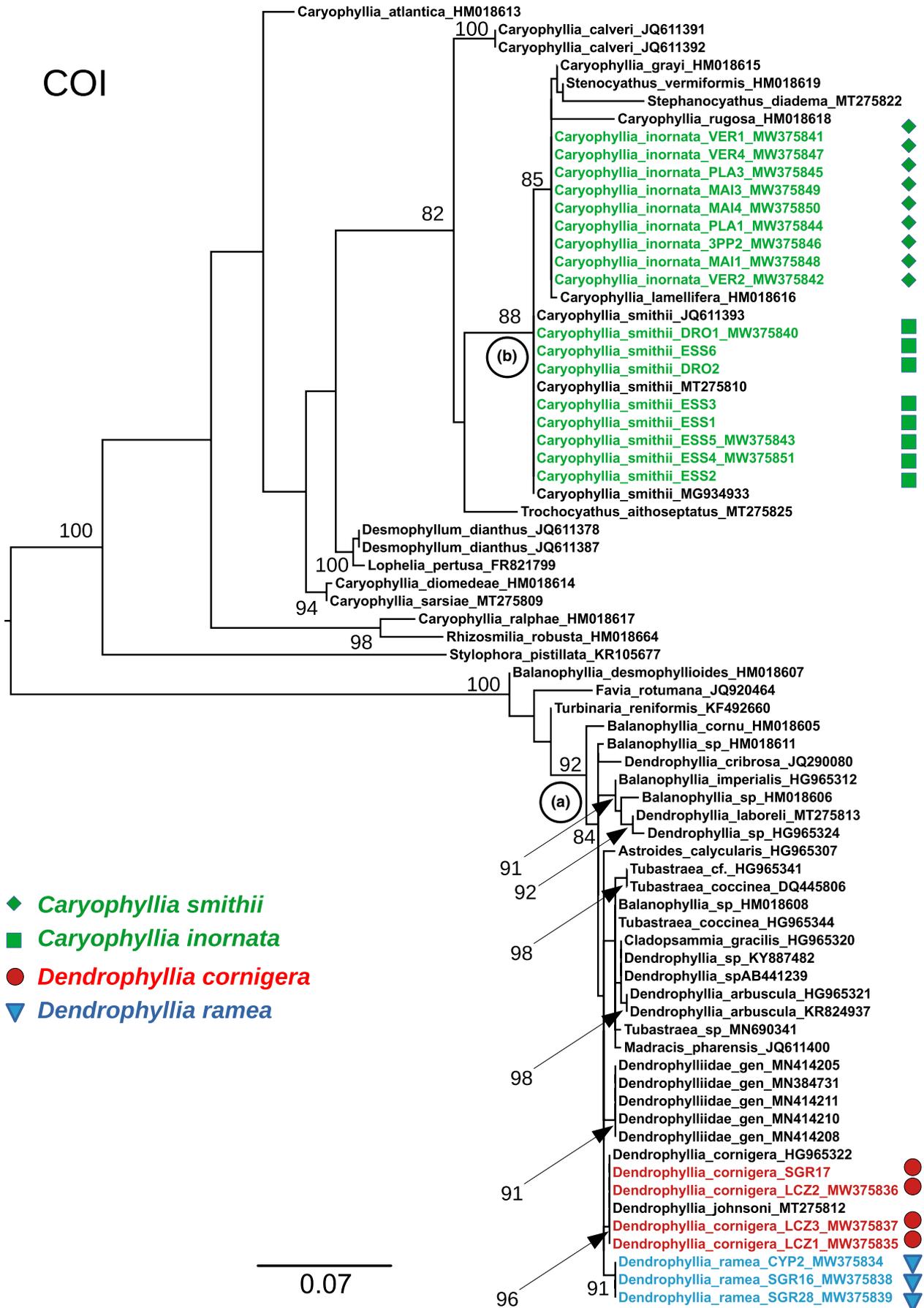
for *D. cornigera* or *D. ramea* depending on the inclusion or not of variable population size in the model. These differences in the estimate of effective population size could indicate different demographic histories for the two species. The main difference was observed for *D. cornigera*, with a lower estimate of current effective size when variable population size was allowed: it would be interesting to test different demographic scenarios in this species to analyse the origin of this effect. At a genomic level, both with ancestral migration and strict isolation, there was a clear signal towards models including heterogeneous effective size: this can be the consequence of background selection, that is, the elimination of deleterious mutations in the genome (Charlesworth et al., 1997; De Jode et al., 2022). As previously mentioned, our analysis of speciation scenarios involved allopatric samples from the western Mediterranean for *D. cornigera* and the sample from the eastern Mediterranean for *D. ramea*. It would then be interesting to extend this study to samples from the same area, to take into account potential phylogeographic breaks across the Mediterranean Sea (Borsa et al., 1997).

5 | CONCLUSION AND PERSPECTIVES

Our results clearly confirm that *Dendrophyllia* DSC from Cyprus indeed correspond to *D. ramea*. This population appears original by its morphology and with potential genetic differences with other Mediterranean *D. ramea* populations. The geographical location of these populations in the eastern Mediterranean probably makes them well-isolated from other Mediterranean ones. The presence of *Dendrophyllia* corals in this area could also point to particular adaptation to this local environment. Along with the ecological importance of DSC ecosystems, these particularities underline the need for protection of these populations. Further genomic comparisons with other Mediterranean populations will be helpful to study the evolutionary history of *Dendrophyllia* in Cyprus.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.