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Major loss of coralline algal diversity in response to ocean acidification

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Abstract

Calcified coralline algae are ecologically important in rocky habitats in the marine photic zone worldwide and there is growing concern that ocean acidification will severely impact them. Laboratory studies of these algae in simulated ocean acidification conditions have revealed wide variability in growth, photosynthesis and calcification responses, making it difficult to assess their future biodiversity, abundance and contribution to ecosystem function. Here, we apply molecular systematic tools to assess the impact of natural gradients in seawater carbonate chemistry on the biodiversity of coralline algae in the Mediterranean and the NW Pacific, link this to their evolutionary history and evaluate their potential future biodiversity and abundance. We found a decrease in the taxonomic diversity of coralline algae with increasing acidification with more than half of the species lost in high pCO_2 conditions. Sporolithales is the oldest order (Lower Cretaceous) and diversified when ocean chemistry favoured low Mg calcite deposition; it is less diverse today and was the most sensitive to ocean acidification. Corallinales were also reduced in cover and diversity but several species survived at high pCO_2; it is the most recent order of coralline algae and originated when ocean chemistry favoured aragonite and high Mg calcite deposition. The sharp decline in cover and thickness of coralline algal carbonate deposits at high pCO_2 highlighted their lower fitness in response to ocean acidification. Reductions in CO_2 emissions are needed to limit the risk of losing coralline algal diversity.

KEYWORDS
adaptation, biodiversity, climate change, ecosystem engineers, evolutionary history, macroalgae, *psb*A, seaweeds

1 | INTRODUCTION

When atmospheric CO_2 levels rise increasing amounts of this gas dissolve in seawater causing ocean acidification. This acidification can cause seawater carbonate saturation to fall below levels suitable for the biogenic construction of calcareous reefs (Albright et al., 2018), as confirmed by the fossil record from before and after ocean acidification events (Hönisch et al., 2012). For example, volcanic activity caused a quadrupling of atmospheric CO_2 levels 201 million years ago (Mya) which acidified the ocean and triggered

the extinction of around 80% of all living species on Earth. After ocean acidification events, weathering of rocks on land slowly increased the carbonate saturation state of the ocean, allowing calcareous organisms to diversify over timescales of millions of years. Volcanic CO_2 release 55.6 Mya is the closest geological analog to anthropogenic ocean acidification: Although it was slower than the present day rate of ocean acidification it led to major declines in the diversity and abundance of marine calcified organisms (Haynes & Hönisch, 2020).

Coralline algae are the only group of seaweeds that deposit calcite within their cell walls (Hurd et al., 2014), and thanks to these mineral cell walls they have an extensive fossil record (Aguirre et al., 2000). As calcium carbonate 'biofactories', they act as ecosystem engineers (Ballesteros, 2006; Nelson, 2009; Peña et al., 2021; Riosmena-Rodríguez et al., 2017) from the intertidal to deep water (down to 265 m) and from the tropics to the poles (Amado-Filho et al., 2012; Littler et al., 1985). They stimulate the settlement and metamorphosis of many invertebrates, including commercially important species such as lobsters, scallops, sea urchins and abalone (Huggett et al., 2006; Nelson, 2009). Loss of coralline algae simplifies coastal ecosystems (Harvey et al., 2021; Kroeker et al., 2013) and has a negative impact on ocean health and ecosystems services (Hall-Spencer & Harvey, 2019). An understanding of which coralline algae will be able to survive the current ocean acidification event is lacking, but needed given their critical role in coastal ecosystems.

Laboratory and field studies have shown that coralline algal recruitment, growth, skeletal strength and survival are generally negatively affected by increased CO_2 (McCoy & Kamenos, 2015; Smith et al., 2020). Observations along natural gradients of increasing CO_2 around shallow water volcanic seep systems show that many coralline algae are vulnerable to ocean acidification but a lack of taxonomic information in these observations is a key knowledge gap (Agostini et al., 2018; Fabricius et al., 2015; Kamenos et al., 2016; Martin et al., 2008; Porzio et al., 2011). Some coralline algae are resilient to ocean acidification which seems to be partly down to the conditions that they live in. For example, intertidal coralline algae can divert energy to fight ocean acidification conditions (Bradassi et al., 2013) and the intertidal often has rapid changes in water carbonate chemistry (Wootton et al., 2008). The rate of acidification is also important, as some coralline algae can tolerate gradual but not rapid change (Kamenos et al., 2013). Coralline algae are mostly long-lived organisms (Halfar et al., 2000, 2011); but some thin coralline algal crusts can grow and reproduce quickly and build resilience to ocean acidification conditions within a few generations (Cornwall et al., 2020).

Our key question is 'Does ocean acidification change the diversity of coralline algal communities?' and some clues lay in their fossil record. The Sporolithales, Hapalidiales, Corallinales and the Corallinapetrales are fully calcified orders of coralline algae (Jeong et al., 2021; Le Gall et al., 2010; Nelson et al., 2015; Peña et al., 2020). The Sporolithales is the oldest of these and first appeared ca. 137 Mya, in the Lower Cretaceous (Peña et al., 2020). In the Cretaceous, surface seawater carbonate saturation levels were high and marine life with calcareous shells and skeletons proliferated. This is when the Hapalidiales originated (ca. 116 Mya) followed by the Corallinales (ca. 112 Mya). A meteor strike that killed most dinosaurs 66 Mya caused ocean acidification which killed an estimated 67% of coralline algal species (Aguirre et al., 2000; Henehan et al., 2019). After this mass extinction event Sporolithales diversity remained low, whereas the Hapalidiales diversified (Aguirre et al., 2000) and then, during an increase in tropical coral reefs worldwide (ca. 28–12 Mya), the Corallinales became highly diverse with ca. 600 species alive today (Gabrielson et al., 2018; Guiry & Guiry, 2021; Peña et al., 2019; Rösler et al., 2016).

Research into the effects of ocean acidification on coralline algae has so far relied on the use of morphological characteristics for their identification, likely underestimating the impacts of ocean acidification on their diversity. Molecular systematics show that coralline algae have high levels of cryptic diversity globally (Gabrielson et al., 2018; Pardo et al., 2014; Pezzolesi et al., 2019) and are morphologically very variable and so are difficult to identify without DNA-based methods (Carro et al., 2014; Peña et al., 2021; Sissini et al., 2014). Here, we used molecular systematic tools to evaluate the impact of ocean acidification on the biodiversity of coralline algae along gradients of increasing seawater CO_2 in the Pacific and Mediterranean basins to assess the capacity of this important algal group to resist or adapt to changing ocean conditions.

2 | MATERIALS AND METHODS

2.1 | Study sites and carbonate chemistry

We used natural gradients in seawater carbonate chemistry at seabed CO_2 seeps off the volcanic coasts of Italy (Vulcano Island, Mediterranean Sea, 34°19′N, 139°12′E) and Japan (Shikine Island, North-Western Pacific, 38°25′N, 14°57′E). Physicochemical surveys have established these locations as natural analogues for the future effects of ocean acidification, as long as care is taken to avoid confounding factors (Agostini et al., 2015; Boatta et al., 2013). Monitoring has revealed locations where benthic organisms settle, grow and reproduce at higher pCO_2 levels than reference sites, but experience no differences in temperature, salinity, dissolved oxygen, total alkalinity, nutrients, wave exposure, current strength, substratum type or depth (NW Pacific: Agostini et al., 2018; Cattano et al., 2020; Harvey et al., 2019, 2021; Mediterranean: Aiuppa et al., 2021; Cornwall, Revill, et al., 2017; Harvey et al., 2016; Milazzo et al., 2014).

In the NW Pacific, seawater pH, temperature and salinity were measured *in situ* using a TOA-DKK multisensor (WQ-22C, TOA-DKK, Japan) and a durafet pH sensors (Seafet, Sea-Bird Scientific, Canada) during June and July 2017 (published in Agostini et al., 2018). Total alkalinity samples were collected at each site: 'Reference 1' ($n = 26$), 'Reference 2' ($n = 26$), 'Increased CO_2' ($n = 21$), 'High CO_2 1' ($n = 41$),

'High CO_2 2' (n = 4) and 'Very high CO_2' (n = 4), immediately filtered at 0.45 μm using disposable cellulose acetate filters (Dismic; Advantech) and stored at room temperature in the dark until measurement. Total alkalinity was measured using an auto-titrator (916 Ti-Touch; Metrohm) with HCl at 0.1 mol L^{-1}, and then calculated from the Gran function between pH 4.2 and 3.0. In the Mediterranean, carbonate chemistry was characterized on three visits in late May and June 2014 for four sites: (i) 'Reference', 'Increased CO_2', 'High CO_2' and 'Very high CO_2'. Temperature and salinity were measured *in situ* using a 556 MPS YSI. Seawater pH was measured using a meter (Orion Star A216 pH/RDO/DO) and pH electrode (Orion 8107BNUMD—Ross Ultra pH/AIC triode). Total alkalinity samples were collected at each site: 'Reference' (n = 3), 'Increased CO_2' (n = 3), 'High CO_2' (n = 2) and 'Very high CO_2' (n = 3). Total alkalinity was measured using an open-cell titration system (Metrohm 809 Titrando and 800 Dosino).

Carbonate chemistry parameters for both Shikine and Vulcano were calculated using the CO_2SYS software (Pierrot et al., 2006). Measured pH, total alkalinity, temperature and salinity were used as the input variables, alongside the disassociation constants from Mehrbach et al. (1973), as adjusted by Dickson and Millero (1987), KSO_4 using Dickson (1990), and total borate concentrations (Table 1).

2.2 | Sampling and data collection

In the Mediterranean, intertidal and subtidal bedrock at four CO_2 levels were surveyed and sampled in May 2014 (Table 1): (i) 'Reference' (mean pCO_2 385 ± 48 [SD] μatm); (ii) 'Increased CO_2' (mean pCO_2 467 ± 33 [SD] μatm); (iii) 'High CO_2' (mean pCO_2 735 ± 315 [SD] μatm); and (iv) 'Very high CO_2' (mean pCO_2 1012 ± 139 [SD] μatm). Intertidal bedrock was surveyed and sampled using 25 × 25 cm quadrats (n = 5 per site) thrown haphazardly. Coralline algal cover (%, from 0—absence—to 100%) was recorded using a 5 × 5 cm grid in the quadrats to assist these in situ estimates. Specimens were

TABLE 1 **Carbonate chemistry of reference and elevated pCO_2 sites in the NW Pacific (Shikine Island) and Mediterranean (Vulcano Island). The pH_T, salinity and total alkalinity (AT) were measured, others were calculated using the CO_2SYS program. Values presented as means, with standard deviation below**

pCO_2 (μatm)	Salinity (psu)	A_T (μmol kg^{-1})	pH_T	DIC (μmol kg^{-1})	HCO^{3-} (μmol kg^{-1})	CO_3^{2-} (μmol kg^{-1})	$\Omega_{calcite}$	$\Omega_{aragonite}$
NW Pacific								
Reference								
310.8	34.5	2264.8	8.14	1964.0	1742.3	211.6	5.08	3.30
46.0	0.42	15.8	0.05	34.3	55.0	22.2	0.53	0.34
Increased								
530.3	34.6	2258.1	7.94	2043.0	1868.7	157.9	3.80	2.48
123.1	0.06	17.2	0.08	37.9	58.5	23.3	0.56	0.36
High CO_2								
763.6	34.5	2269.6	7.81	2124.2	1983.2	116.3	2.79	1.81
241.6	0.35	19.8	0.09	34.5	45.6	18.4	0.44	0.28
Very high CO_2								
1796.3	34.7	2268.1	7.53	2212.0	2081.6	75.8	1.82	1.19
1285.2	0.74	20.5	0.24	82.9	82.3	33.3	0.80	0.52
Mediterranean								
Reference								
385.2	38.2	2500.8	8.08	2186.1	1948.9	224.8	5.24	3.41
47.5	0.05	16.5	0.04	27.7	44.1	17.9	0.42	0.27
Increased								
467.0	38.2	2456.7	8.01	2189.7	1981.8	193.0	4.50	2.93
32.8	0.04	69.0	0.03	13.7	21.4	8.6	0.20	0.13
High CO_2								
734.6	38.3	2518.7	7.87	2313.9	2132.8	157.6	3.67	2.39
314.6	0.10	2.4	0.15	72.1	104.8	42.8	1.00	0.65
Very high CO_2								
1012.5	38.2	2515.6	7.73	2385.6	2240.4	112.4	2.62	1.71
138.5	0.11	13.3	0.05	20.0	26.7	10.9	0.26	0.17

collected from the quadrats of all the different thallus morphologies present (using a chisel where necessary) and placed into labelled ziplock bags for subsequent laboratory investigation. In the subtidal survey conducted at 2–6-m depth, cover was not assessed but collections of specimens were made for molecular identification, as in the intertidal.

In the NW Pacific, intertidal bedrock and two subtidal benthic habitats (bedrock and rubble) were surveyed in July 2017 for coralline algal cover and sampled for molecular identification at four different levels of seawater CO_2 (Table 1): (i) 'Reference' (two sites, mean pCO_2 311 ± 46 [SD] µatm); (ii) 'Increased CO_2' (one site, mean pCO_2 530 ± 123 [SD] µatm); (iii) 'High CO_2' (two sites, mean pCO_2 764 ± 242 [SD] µatm); and (iv) 'Very high CO_2' (one site, mean pCO_2 1796 ± 1285 [SD] µatm). SCUBA diving was used for subtidal surveys in both benthic habitats (n = 6 per habitat per site) at a same depth range to the Mediterranean study site (2–6-m depth).

2.3 | Molecular identification of coralline algae

Specimens were rinsed in freshwater, air-dried and preserved in ziplock bags with silica gel. A fragment of each specimen was cleaned under a stereomicroscope, then a clean part was ground into powder for DNA extraction. Genomic DNA was extracted using a NucleoSpin® 96 Tissue kit (Macherey-Nagel, GmbH and Co. KG) following the manufacturer's protocol. The *psb*A locus was amplified using primer pairs: psbA-F1/psbA-R2 and psbA-F1/psbA-600R (Yoon et al., 2002), and eventually the primer pair psbA21-350F/psbA22-350R generated for coralline algae (Anglés d'Auriac et al., 2019). The mitochondrial COI-5P fragment was PCR-amplified for some Mediterranean specimens using the primer pair Gaz-F1/Gaz-R1 (Saunders, 2005). The thermal profile for *psb*A and COI-5P amplifications and PCR reactions followed Peña et al. (2015). PCR products were purified and sequenced by Genoscope (Bibliothèque du Vivant program, Centre National de Séquençage, France) and Eurofins (Eurofins Scientific, France). Sequences were assembled and aligned with the assistance of CodonCode Aligner® (CodonCode Corporation) and adjusted by eye using SeaView version 4 (Gouy et al., 2010). Sequences were submitted to the Barcode of Life Data Systems (project 'NGCOR', BOLD, http://www.boldsystems.org; Ratnasingham & Hebert, 2007) and GenBank (accession numbers are listed in Table S1). Calculation of genetic distance among *psb*A sequences (uncorrected p-distances) was calculated in MEGA v.6 (Tamura et al., 2013). Putative species boundaries were estimated using Poisson Tree Processes model (PTP, Zhang et al., 2013) on a phylogenetic tree inferred from Bayesian inference analysis of *psb*A sequences using MrBayes 3.2.1 (Ronquist & Huelsenbeck, 2003). Analyses were performed under a generalized time-reversible with invariant sites heterogeneity model (GTR+I+G, jModeltest 2.1.3, Darriba et al., 2012), with four Markov Chain Monte Carlo method for 10 million generations, and tree sampling every 1000 generations. Our species delimitation was contrasted with interspecific divergence—uncorrected *p*-distances—usually applied for *psb*A and COI-5P sequences generated for coralline red algae (e.g. Hind et al., 2016; Peña et al., 2015, 2021; Pezzolesi et al., 2017, 2019). For species identification, sequences were compared with publicly available sequences of coralline algae from GenBank.

2.4 | Carbonate biomass and complexity of biogenic habitat

Coralline algae collected from quadrats (n = 6) at each NW Pacific site (excluding the 'Very high CO_2' area as it lacked corallines) were dried at 60°C, then they were weighed and decalcified using HCl. After decalcification, samples were rinsed with distilled water, dried again and re-weighed. The $CaCO_3$ content in each site (as g 0.1 m^{-2}) was calculated based on the weight difference before and after decalcification, the estimated area occupied by each specimen within the quadrat (625 cm^2) and adjusted with the percentage cover estimated for coralline algae. A further set of samples of a coralline algal species that was common at the low tide mark was collected at low water from different levels of seawater pCO_2 and examined using micro-computed tomography (micro-CT scan, Skyscan 1172 system). Tomography was used to illustrate changes in thickness and $CaCO_3$ content of coralline algae. Parameters used were: 70 kV, 142 µA, 13.57-µm pixel size, 180° rotational angle and a rotation step of 0.4°. A 0.5-mm-thick aluminium filter was used. X-ray projection images obtained during scanning were reconstructed with the software NRecon (Bruker). Sections were processed with software programs CTAn and DataViewer (Bruker).

2.5 | Statistical analyses

Differences in coralline algae cover recorded at increasing levels of seawater CO_2 were assessed using one-way ANOVA tests, or by Welch and Brown–Forsythe tests if the requirement of equality of variance (Levene test) was not met. Site comparisons were analysed by post hoc Tukey's HSD tests. Statistical analyses were conducted by IBM® SPSS® Statistics v. 24 (IBM Corporation, license University of A Coruña).

3 | RESULTS

3.1 | Carbonate chemistry

The salinity of our NW Pacific sites was 34.5–34.7 with an alkalinity range of 2258–2269 µmol kg^{-1} compared with 38.2–38.3 and 2456–2518 µmol kg^{-1} in the Mediterranean (Table 1). These are typical values for these regions. Reference conditions in the NW Pacific had relatively low values of around 310 µatm pCO_2 compared with 385 µatm in the Mediterranean. In both regions, increasing pCO_2 levels resulted in significant seawater acidification down from ca. pH 8.1 at

reference sites to 7.53 at our 'Very high CO_2' Pacific site (1796 µatm pCO_2) and 7.73 at our 'Very high CO_2' Mediterranean site (1012 µatm pCO_2) and both of these locations had low mean aragonite seawater saturation levels with periods of aragonite undersaturation (Table 1).

3.2 | Fall in coralline algal diversity at increasing levels of CO_2

We generated 223 sequences (211 *psb*A and 12 COI-5P) for 221 specimens collected (166 in the NW Pacific, 55 in the Mediterranean). The largest dataset (*psb*A) had 189 haplotype sequences with 348–891 base pairs, with 369 variable sites. The phylogenetic tree inferred from our *psb*A data estimated 57–90 putative species using the maximum likelihood approach and 66–70 putative species using the bayesian approach. Based on these estimates and the known range of infraspecific pairwise distances recorded in coralline algae (up to 2.92% for the highly diverse *Lithophyllum byssoides* (Lamarck) Foslie, Pezzolesi et al., 2017), we estimated that 41 coralline algal species were collected at our NW Pacific sites and 19 were collected at our Mediterranean sites (Figure 1; Tables S1 and S2) with the largest infraspecific divergences found among clades within the Mediterranean, *Neogoniolithon brassica-florida* and *Neogoniolithon* sp.1 (1.9%–2.1%). These belonged to the three orders: Sporolithales, Hapalidiales and Corallinales, with the latter being the most diverse (40 spp.). No shared taxa were found between our NW Pacific and Mediterranean collections, and sequences generated for most of the taxa did not return a species match in GenBank.

Both ocean basins had a sharp decline in coralline species diversity with increasing CO_2, in both intertidal and subtidal habitats (Figure 2; Table S2); 38 species found at 'Reference' sites were not observed in any of the elevated CO_2 sites. Sporolithales were only detected at the NW Pacific Reference sites. Seven species of geniculate coralline algae, in four genera (*Corallina*, *Ellisolandia*, *Jania* and *Amphiroa*), were found at reference sites but only two species (*Corallina* cf. *crassissima* and *Amphiroa* sp.) occurred at elevated CO_2. In the Mediterranean the coralligenous habitat forming species *L. stictiforme* only occurred at the 'Reference' site and epiphytic corallines (*Lithophyllum* cf. *pustulatum*, *Lithophyllum* sp. 8, *L.* sp. 9, Corallinales sp.) were also lost as CO_2 levels increased above 'Reference' levels.

Although coralline algal diversity declined with increasing ocean acidification, 15 of the coralline taxa found in 'Reference' sites (nine in the NW Pacific, six in the Mediterranean) were also found living at elevated CO_2 sites (Figure 1; Table S2). In particular, ten species were able to survive in the 'High CO_2' areas although none were found at the 'Very high CO_2' sites. Except for two taxa of the order

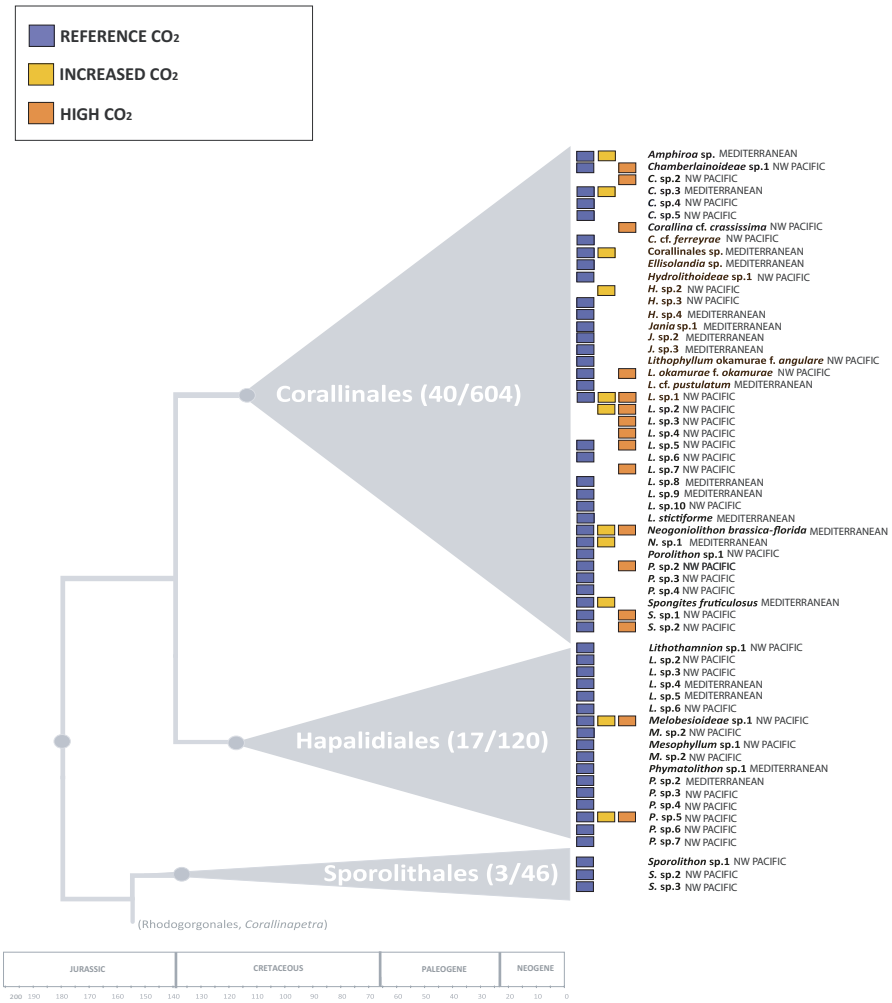

FIGURE 1 Species of Corallinales, Hapalidiales and Sporolithales coralline algae recorded along CO_2 gradients in the NW Pacific and Mediterranean. For each order, the number of species recorded and the current number of species estimated from Algaebase (Guiry & Guiry, 2021) are shown in brackets. Colours show the different levels of ocean acidification ('Reference', 'Increased CO_2' and 'High CO_2') that the taxa were able to survive. Time calibration of the three calcified orders based on Peña et al. (2020)

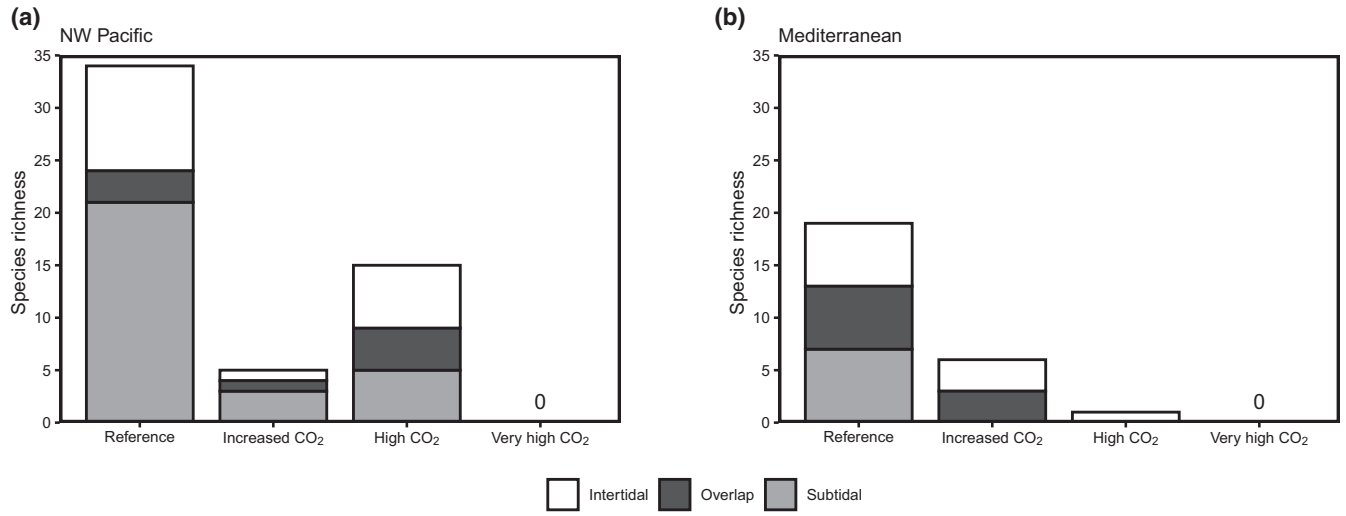

FIGURE 2 Coralline algal species richness recorded at different levels of CO_2 in the NW Pacific (a) and the Mediterranean (b). Species richness recorded per CO_2 level is shown as well as the number of species found in subtidal, intertidal and both shore levels (overlap). '0' indicates the absence of coralline algae at the given site

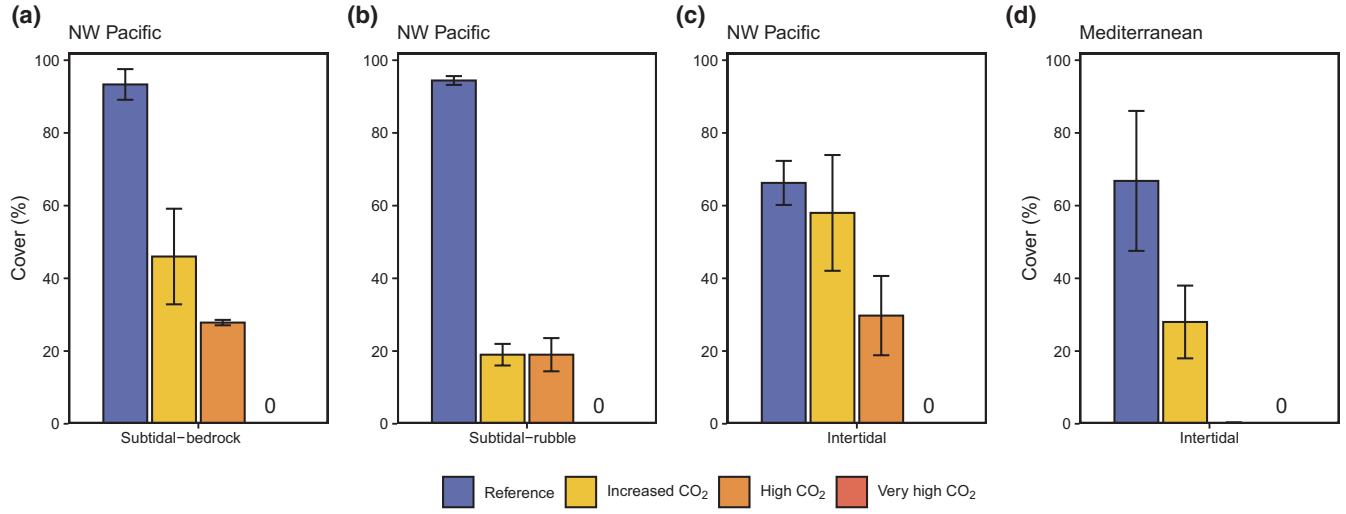

FIGURE 3 Coralline algal cover (%, mean ± SE, $n = 6$) on subtidal bedrock and rubble and intertidal bedrock in the NW Pacific (a–c) and on intertidal bedrock in the Mediterranean (d) exposed to different levels of CO_2 dissolved in seawater. '0' indicates the absence of coralline algae at the given site

Hapalidiales (Melobesioideae sp.1 and *Phymatolithon* sp.5), the remaining species found across the CO_2 gradient belonged to the order Corallinales. By contrast, seven species collected at 'Increased' and 'High CO_2'sites were not recorded in the 'Reference' sites; all these species corresponded to the order Corallinales, and four of them belonged to the same genus (*Lithophyllum*).

3.3 | Coralline algal cover, complexity of biogenic habitat and carbonate biomass

In both regions, the cover of coralline algae—expressed in %—decreased significantly as CO_2 levels rose (Figure 3; Table S3). Significant differences were detected between 'Reference' sites and the 'High CO_2' and 'Very high CO_2'sites in the intertidal (66 ± 13% vs. 30 ± 24% and 0% in the NW Pacific, and 67 ± 43 vs. 0% in the Mediterranean) as well as subtidally (93 ± 9% vs. 28 ± 2% and 0%, respectively in the NW Pacific).

At community scale, in both regions, as coralline algae declined, non-calcified macroalgae were more abundant among which we can list the reds *Grateloupia elata* (Okamura) S. Kawaguchi & H.W. Wang and crustose *Peyssonnelia* spp. and *Hildenbrandia* spp., the browns *Cystoseira* spp. *sensu lato*, *Padina pavonica* (Linnaeus) Thivy and *Taonia atomaria* (Woodward) J. Agardh, and the green *Caulerpa* spp., as well as large diatom colonies in the 'Very high CO_2' NW Pacific site (Figure 4). In addition to the reduction in coralline cover, the complexity of biogenic habitat created by coralline algae decreased with increasing CO_2. Intertidal samples of *Phymatolithon* sp.

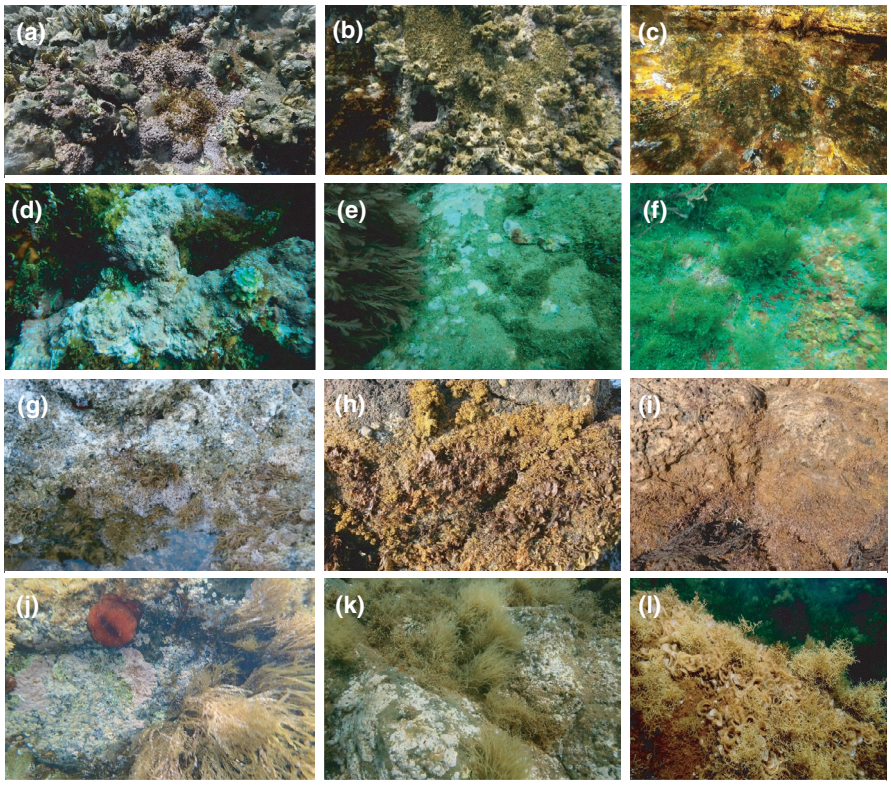

FIGURE 4 Representative pictures of coralline algal decline at 'Reference', 'Increased CO_2' and 'High CO_2' levels in the NW Pacific and the Mediterranean. Japan intertidal (a–c) and subtidal (d–f); Italy intertidal (g–i) and subtidal (j–l)

5 (Figure 5) had thick crusts (up to 2 cm) with a diverse and abundant associated calcified faunal community in 'Reference' site conditions, but as CO_2 levels increased the cover of this intertidal coralline alga decreased, forming only very thin crusts (<0.2 cm in the 'High CO_2') with minimal associated calcified fauna and becoming completely absent at 'Very high CO_2'.

In the NW Pacific site, the coralline algal calcium carbonate per unit area showed a profound decline with increasing CO_2 (Figure 6). The calcium carbonate per unit area in the 'High CO_2' sites (7.96–8.73 ± 4.01–7.97 g $CaCO_3$ 0.1 m^{-2}) was <2% of that found at 'Reference' sites (505.77–700.48 ± 173.44–275.49 g $CaCO_3$ 0.1 m^{-2}). Subtidal rubble was the most impacted habitat with low amounts of coralline algal $CaCO_3$ at the 'Increased CO_2' and 'High CO_2' sites.

4 | DISCUSSION

This is the first molecular systematic study of the effects of ocean acidification on coralline algae and shows that this approach is essential for an accurate understanding of how the diversity of coralline algae is affected by CO_2 emissions (Twist et al., 2020). Highly diverse coralline algal assemblages were simplified under elevated levels of CO_2. Corallinales, the most recently evolved order of the coralline algae (Peña et al., 2020), was the most diverse of the orders we recorded and the most resilient to acidification, with 35% of the species present at high CO_2. By contrast, only ca. 12% of the Hapalidiales were found in the high CO_2 sites, and the Sporolithales disappeared with increased CO_2. We expected Sporolithales and Hapalidiales to be more resistant to ocean acidification since they first appeared in a period of Earth's history when seawater CO_2 levels were high (600–1500 ppm) whereas Corallinales originated in waters with relatively low CO_2 (400 ppm; Bergstrom et al., 2020; Hansen et al., 2013; Hönisch et al., 2012; Royer et al., 2004). A mesocosm study of six species (Bergstrom et al., 2020) has found that the Sporolithales and Hapalidiales species generally had a greater capacity for CO_2 use than the Corallinales species and related these differences with ocean pCO_2 conditions that prevailed when each group originated. An experimental study on *Sporolithon* has shown that recruits were more sensitive to elevated temperature/pCO_2 than adult plants (Page & Diaz-Pulido, 2020) which may explain the absence of any of these taxa at our increased CO_2 sites. Laboratory work showing the vulnerability of *Sporolithon durum* to ocean acidification and the resilience of *Neogoniolithon* (Comeau et al., 2018; Cornwall, Comeau, et al., 2017) and *Chamberlainium* sp. (Kim et al., 2020) confirm our field results.

A lack of accurate identification of the coralline species using molecular tools makes comparison of our results with previous studies problematic. At Mediterranean CO_2 seeps, Porzio et al. (2011) observed the replacement of thick coralline algal communities by thin crusts of *Hydrolithon cruciatum*—order Corallinales—at low pH sites. On settlement tiles at volcanic CO_2 seeps, the most pH tolerant taxa were found to be thin crusts of *Lithoporella melobesioides*, *Dawsoniolithon conicum* and *Porolithon onkodes* (order Corallinales) in Papua New Guinea (Fabricius et al., 2015), and *Lithophyllum*, *Titanoderma* (Corallinales) and *Phymatolithon* (Hapalidiales) in Italy (Kamenos et al., 2016). In addition, except for a few experimental studies (e.g. Bergstrom et al., 2020; Comeau et al., 2018; Cornwall, Comeau, et al., 2017) that encompassed several coralline taxa

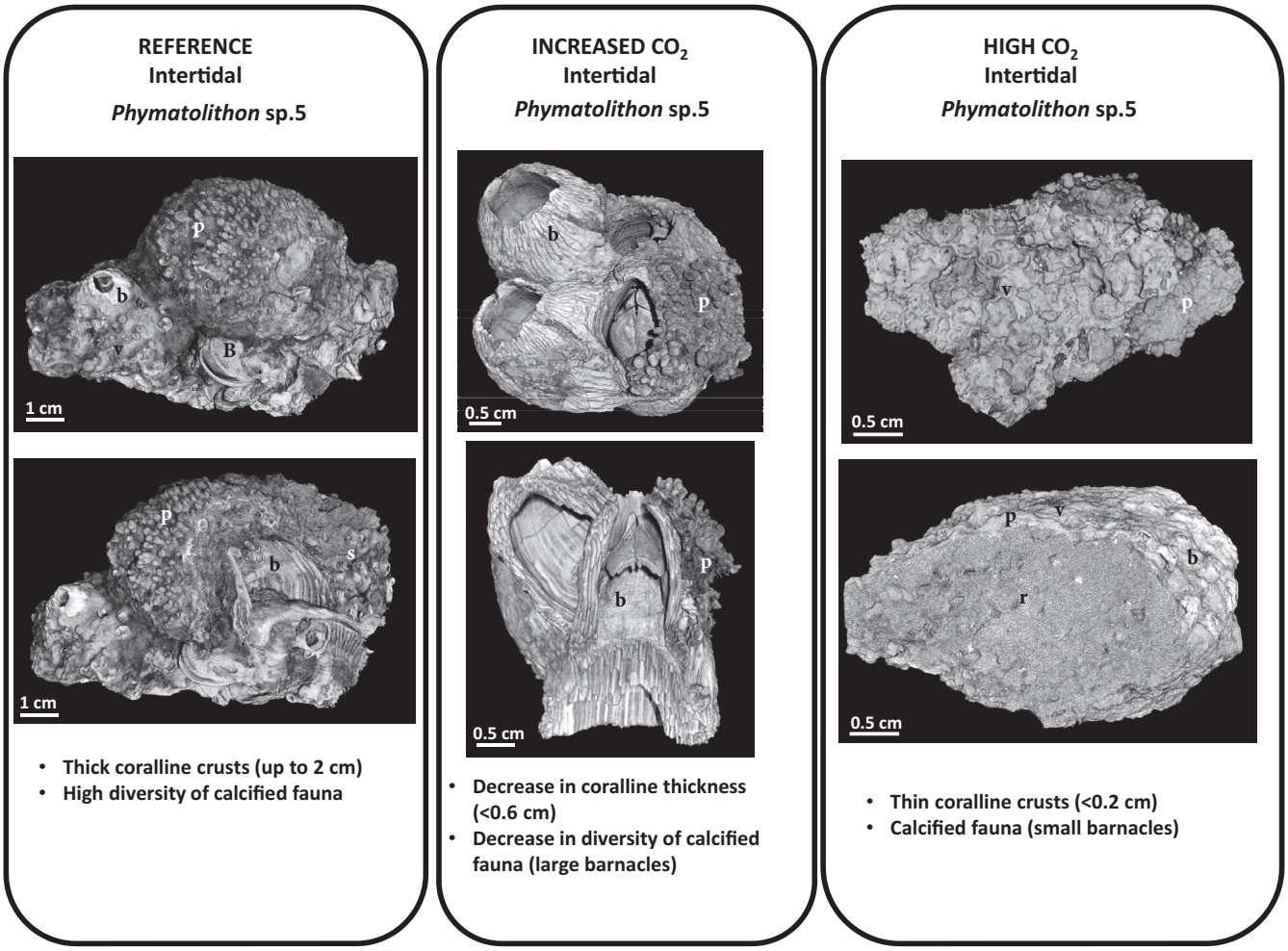

FIGURE 5 Reconstructed 3D images of intertidal samples of *Phymatolithon* sp. 5 and calcified fauna concretions along a NW Pacific CO_2 gradient. Images obtained by micro-computed tomography (surface view and vertical section). b, barnacle crustacean; B, bivalve mollusc and on the bottom right image r (rock); p, *Phymatolithon* sp. 5; s, serpulid polychate; v, vermetid mollusc

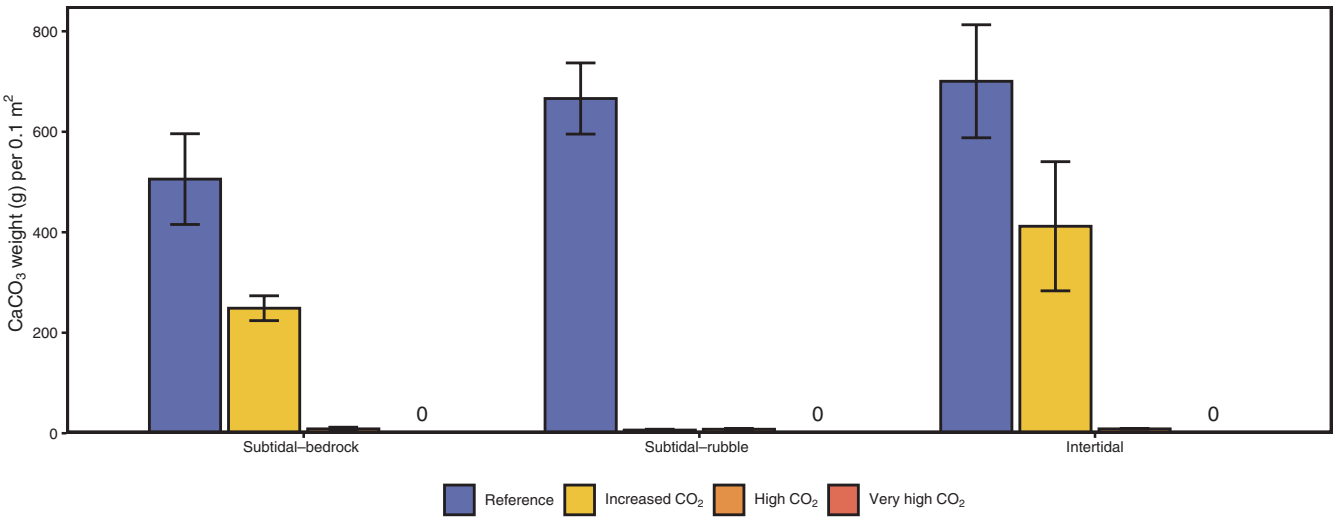

FIGURE 6 Amount of coralline algal calcium carbonate (g, 0.1 m^{-2}, mean ± SE, $n = 6$) along a NW Pacific CO_2 gradient on subtidal bedrock, subtidal rubble and intertidal bedrock. '0' indicates the absence of coralline algae at the given site

belonging to different orders, most of the literature is focused on the physiological responses of a single species, and it often pertains to the order Corallinales because of their ecological role as reef builders (e.g. *Hydrolithon*, *Porolithon*, *Lithophyllum*; Comeau et al., 2013; Martin et al., 2013; Scherner et al., 2016; Semesi et al., 2009). In response to ocean acidification, both acclimation and adaptation can be crucial to the survival and prevalence of organisms (Sunday et al., 2014). Despite long-term exposure to increased pCO_2, no evidence of acclimatization of the reef coralline *Lithophyllum kotschyanum* has been shown (Comeau et al., 2019) whereas Cornwall et al. (2020) observed that *Hydrolithon reinboldii* (now *H. boergesenii*) gained tolerance to ocean acidification over several generations. Our results show that 15 coralline species, most of them from the order Corallinales, can survive high pCO_2 conditions; in particular, the genus *Lithophyllum* which is one of the most diverse genera (130 species, Guiry & Guiry, 2021). Higher genetic diversity may give them an advantage over the less-resistant taxa, particularly Hapalidiales and Sporolithales species. On the other hand, mechanistic reasons related with their skeletal structure could be also involved. Kamenos et al. (2016) observed changes to a less reactive polymorph of $CaCO_3$ in small crustose corallines growing under high pCO_2 in volcanic CO_2 vents at Ischia (Italy). In addition, the reduction of calcium carbonate production may particularly affect thick species (McCoy & Ragazzola, 2014) and it is also related with alterations in the species dominance (McCoy et al., 2016; McCoy & Pfister, 2014).

As in many other studies along natural gradients in pCO_2 we observed large reductions in the cover of the coralline algae as carbonate saturation state declined. The thickness and amount of $CaCO_3$ per unit area also declined abruptly in intertidal and subtidal rubble samples with increasing seawater pCO_2. Samples from subtidal bedrock had a similar amount of $CaCO_3$ per unit area at high pCO_2 due to the presence of *Peyssonnelia* algal crusts. These observed declines in coralline algal thickness and cover are likely due to a combination of disruption to spore settlement and growth, increased energetic burden of skeletal maintenance, increased dissolution, and high sensitivity of the unpigmented algal tissues plus disruption (Bradassi et al., 2013; Diaz-Pulido et al., 2012; Kato et al., 2014; McCoy & Kamenos, 2015; Ordoñez et al., 2017; Porzio et al., 2018). It was conspicuous in the field, and well illustrated using tomography, that a thickly encrusting species (*Phymatolithon* sp.5) dominated the low intertidal fringe of our study region of Japan but showed severe reductions in cover and thickness with increased pCO_2.

In the Mediterranean CO_2 vents of Ischia (Italy), Porzio et al. (2011) recorded a decrease in the diversity and abundance of coralline algae. This loss of habitat complexity provided by coralline algae has many ecological implications. For instance, decreased size and thickness increases coralline algal susceptibility to grazing (Johnson & Carpenter, 2012; McCoy & Kamenos, 2018) and reduces their ability to compete for space with other seaweeds (Kroeker et al., 2013; Linares et al., 2015). The knock-on effects of reduced coralline algal diversity and dominance by species with a thin growth form can include: reduced reef accretion in tropical and temperate environments (Adey, 1978; Ballesteros, 2006; Fine et al., 2017; Goreau, 1963), reduced habitat provisioning for associated fauna and endolithic organisms (e.g. Tribollet & Payri, 2001), and alterations in epiphytic microbial biofilms (Huggett et al., 2018) that disrupt settlement and larval recruitment of marine invertebrates (e.g. coral, sea urchins, Doropoulos et al., 2012; Gómez-Lemos et al., 2018; Rahmani & Ueharai, 2001; Steller & Cáceres-Martínez, 2009). Considering adverse effects related to the interaction with other stressors, like coastal pollution (Schubert et al., 2019), predatory fishery (Fragkopoulou et al., 2021) and the importance of their ecosystems to socio-environmental and economic well-being (Moura et al., 2021), besides reducing CO_2 emissions, further effort should look to improve ocean health as a whole (Laffoley et al., 2020), fostering the creation of no-take marine protected areas with focus on these reef builders to enhance their resilience and survival (Sissini et al., 2020).

Here we also showed with molecular systematic tools that coralline algal assemblages can be highly diverse on hard substrata in shallow waters. Our study, conducted in the North-Western Pacific ocean basin and the Mediterranean Sea, detected 60 coralline algal species belonging to the orders Sporolithales, Hapalidiales and Corallinales, with the latter being the most diverse. This represents ca. 14% of the known species of Hapalidiales and ca. 7% of the known species of Corallinales and Sporolithales (Guiry & Guiry, 2021). The Japanese site was much more diverse than the Italian one (41 vs. 19 species) due a naturally more diverse flora in Japan combined to the fact that we sampled a variety of habitats (bedrock and rubble) but only bedrock in Italy. Most (87%) of the species detected in the present study (52 out of 60) did not return a relevant match with the publicly available GenBank dataset (containing ca. 3780 *psb*A sequences). This highlights the substantial diversity of coralline algae (Gabrielson et al., 2018; Pezzolesi et al., 2019) and emphasizes a clear need for molecular systematics tools, particularly in global biodiversity hotspots such as Japan (Tittensor et al., 2010) which remains poorly studied using this approach (except for Kato & Baba, 2019; Kato et al., 2011, 2013).

5 | CONCLUSION

We show, for the first time, that ocean acidification can cause a decline in the biodiversity of coralline algae. We reveal an exceedingly high level of cryptic diversity in the Japanese coralline algal flora and show that the effects of ocean acidification on coralline algal diversity worldwide have previously been underestimated. It is now clear that identification using molecular systematics tools significantly advances insights into the responses of marine communities to global change. Shallow-water CO_2 seep systems in two widely separated biogeographic regions revealed consistent long-term, multigenerational assemblage shifts in the coralline algae. A decrease of coralline algal species diversity was accompanied by a major loss in seabed cover and we quantify, for the first time, the extent to which ocean acidification reduces algal carbonate accretion. The order Corallinales, in particular the genus *Lithophyllum*, was by far the most diverse group of taxa able to survive ocean acidification. The ability

of coralline algal species to tolerate rising CO_2 levels is underpinned by what these species experience in terms of environmental variability today, as well as their evolutionary history. Ocean acidification reduces coralline algal habitat complexity and is projected to adversely affect ecosystem services, and so reductions in CO_2 emissions are needed to reduce risks to coastal ocean function.

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AUTHOR CONTRIBUTION

Viviana Peña: original concept, Pacific and Mediterranean fieldwork, DNA sequencing and morpho-anatomical study, writing; Ben P. Harvey: carbonate chemistry data and Pacific fieldwork, writing, figures; Sylvain Agostini: carbonate chemistry data and Pacific fieldwork, writing; Lucia Porzio: writing; Marco Milazzo: carbonate chemistry data and Mediterranean fieldwork, writing; Paulo Horta: writing; Line Le Gall: DNA sequencing, writing; Jason M. Hall-Spencer: original concept, Pacific and Mediterranean fieldwork, writing.

DATA AVAILABILITY STATEMENT

The data that supports the findings of this study are available in the Supporting Information of this article.

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SUPPORTING INFORMATION

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