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Symbiotic associations of the deepest recorded photosynthetic scleractinian coral (172 m depth)

Héloïse Rouzé¹ · Pierre E. Galand² · Mónica Medina³ · Pim Bongaerts⁴ · Michel Pichon⁵ · Gonzalo Pérez-Rosales¹ · Gergely Torda⁶ · Aurelie Moya^{6,7} · Under The Pole Consortium · Jean-Baptiste Raina⁸ · Laetitia Hédouin¹

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Abstract

The symbiosis between scleractinian corals and photosynthetic algae from the family Symbiodiniaceae underpins the health and productivity of tropical coral reef ecosystems. While this photosymbiotic association has been extensively studied in shallow waters (<30 m depth), we do not know how deeper corals, inhabiting large and vastly underexplored mesophotic coral ecosystems, modulate their symbiotic associations to grow in environments that receive less than 1% of surface irradiance. Here we report on the deepest photosymbiotic scleractinian corals collected to date (172 m depth), and use amplicon sequencing to identify the associated symbiotic communities. The corals, identified as *Leptoseris hawaiiensis*, were confirmed to host Symbiodiniaceae, predominantly of the genus *Cladocopium*, a single species of endolithic algae from the genus *Ostreobium*, and diverse communities of prokaryotes. Our results expand the reported depth range of photosynthetic scleractinian corals (0–172 m depth), and provide new insights on their symbiotic associations at the lower depth extremes of tropical coral reefs.

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✉ Héloïse Rouzé
heloise.rouze@gmail.com

- ¹ PSL Research University, EPHE-UPVD-CNRS, USR 3278 CRIOBE, LabEx CORAIL, BP 1013 Papetoai, 98729 Moorea, French Polynesia
- ² Sorbonne Université, CNRS, Laboratoire d'Ecogéochimie des Environnements Benthiques (LECOB), Observatoire Océanologique de Banyuls, Banyuls-sur-Mer, France
- ³ Department of Biology, Pennsylvania State University, 208 Mueller Lab, University Park, State College, PA 16802, USA
- ⁴ California Academy of Sciences, San Francisco, CA 94118, USA
- ⁵ Museum of Tropical Queensland, Townsville, QLD 4810, Australia
- ⁶ ARC Centre of Excellence for Coral Reef Studies, James Cook University, Townsville, QLD 4811, Australia
- ⁷ Department of Biology, University of Konstanz, Universitätsstraße 10, 78464 Konstanz, Germany
- ⁸ Climate Change Cluster, University of Technology Sydney, Ultimo, NSW 2007, Australia

The ecological success of scleractinian corals, the engineers of one of the most productive and diverse ecosystems on Earth, relies on a myriad of symbiotic associations with microorganisms [1]. Among these symbioses, the association between the coral host and unicellular algae from the family Symbiodiniaceae is central to coral health and powers the metabolically expensive process of calcification [2]. The coral host provides limited inorganic nutrients, while Symbiodiniaceae share essential organic compounds derived from their photosynthetic activity [3]. This light-dependent association has mainly been studied in shallow waters (<30 m) because of technical limitations imposed by traditional scientific scuba diving. However, photosynthetic scleractinian corals have been observed in the mesophotic reef slope down to 150–165 m depth [4, 5].

As depth increases, the waveband of solar radiation used by most algae for photosynthesis (from 400–700 nm) becomes attenuated in both intensity and width. Even in clear tropical waters, the irradiance levels below 120 m depth can be less than 1% of surface values, and the light spectrum is shifted toward the blue and blue–green wavelengths (~475 nm) (e.g. [4]). These light limitations pose a major constraint for the productivity of benthic organisms that rely on photosynthetic symbionts [6],

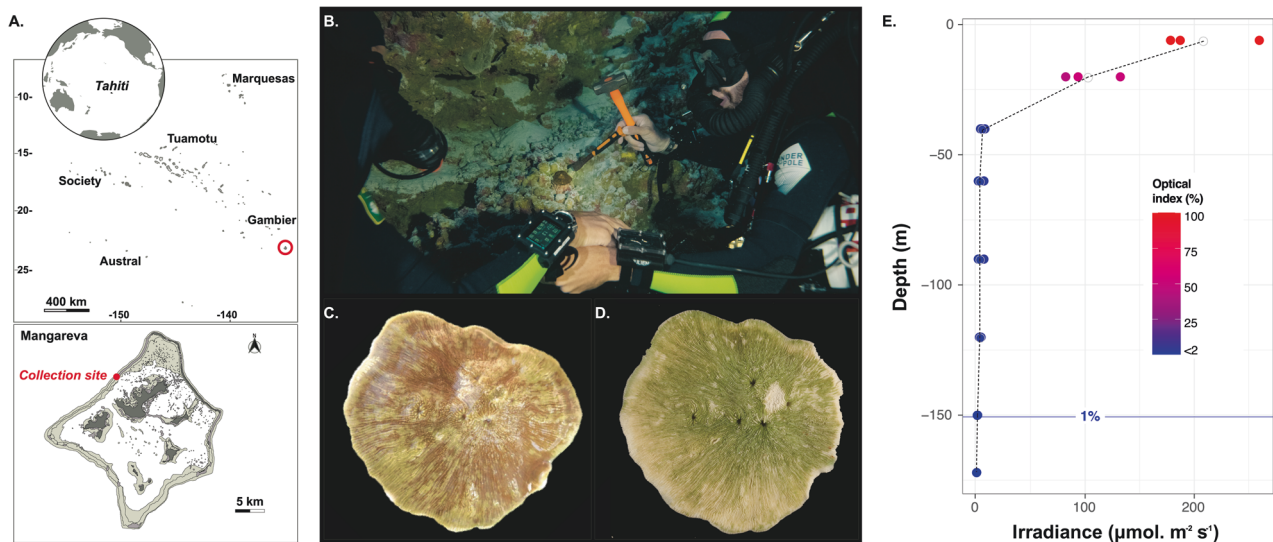


Fig. 1 Sampling location of the deepest photosymbiotic scleractinian coral recorded to date. **A** Map of the Gambier archipelago, French Polynesia. Pictures of *Leptoseris hawaiiensis* collected at 172 m depth in the Gambier archipelago (**B**) during the in situ sampling (screenshot of video © UTP III), (**C**) after reaching the surface and (**D**) after bleaching for taxonomic identification with the green color indicating the presence of endolithic algae. **E** Variation of the optical

index of irradiance (in PAR) along the coral reef depth gradient from 6 to 120 m depth (predictions for 150 and 172 m depths) at Mangareva. For each depth, the three values represent a mean value for 3 days of measurements recorded every 5 min with a PAR logger (DEFI2-L Advantech) at three different time periods of the day (9 h30–10 h00, 12 h30–13 h00 and 15 h30–16 h00).

including reef-building corals (scleractinians). While the scleractinian coral species *Leptoseris hawaiiensis* has been reported to occur as deep as 153 m in Hawaii and 165 m at Johnston atoll (reviewed in [4]), no live specimens were collected at these extreme depths. The fact that Symbiodiniaceae have been found at much greater depth in association with Antipatharians (396 m) [7], raises the possibility that they might also be present in scleractinian corals deeper than 165 m. Previous studies have genetically confirmed and identified endosymbiotic Symbiodiniaceae in *Leptoseris* down to 70 m on the Great Barrier Reef [8] and down to 125 m depth in Hawaii [9–11]. A specific host-Symbiodiniaceae association was reported between deep *L. hawaiiensis* and a *Cladocopium* from the ancestral C1 radiation [9–11], which represents a diverse group of Symbiodiniaceae commonly found in association with scleractinians on shallow coral reefs [8, 9, 12, 13]. To better understand how scleractinian corals can survive so far away from their presumed light optimum, it is critical to determine if these deep specimens (1) maintain their association with photosynthetic algae and/or (2) if their survival in the deepest mesophotic coral ecosystems requires a shift in their microbial communities, including Symbiodiniaceae and other microorganisms such as endolithic algae and bacteria.

Here we report on the observation and collection of the deepest scleractinian corals in association with Symbiodiniaceae and other photosymbionts. Technical divers using closed-circuit rebreathers recovered three *L. hawaiiensis* colonies from the Gambier archipelago (French Polynesia,

Fig. 1A) at 154, 168, and 172 m depth ($n = 2$ subsamples for each depth; Fig. 1B–D). Irradiance measured at 120 m depth was <2% of that recorded at 6 m depth and irradiance at 172 m was predicted to be <1% (Fig. 1E and S1). ITS2 sequencing revealed Symbiodiniaceae presence in all three lower mesophotic colonies sampled, with nearly all of the retrieved amplicon sequence variants (ASVs; with most of these representing intragenomic sequence variants) classified as *Cladocopium* (Fig. 2). The most common ITS2 ASV representative sequence associated with these *Leptoseris* hosts (S-01, Fig. 2 and S2; 50–57% of total ASVs in each sample) was C1 (GeoSymbio and SymPortal databases; see supplementary methods). This represents one of the most common groups of Symbiodiniaceae, and it has previously been reported in *Leptoseris* [9, 10, 14], as well as other host species at depths ranging from the surface to 125 m [8, 10, 11, 13–15]. As a complementary approach, ITS2 profiles predicted by SymPortal were used as proxy for Symbiodiniaceae genotypes ([16]; see supplementary methods and data files S1–S4). These predicted ITS2 profiles were largely consistent among replicates but confirmed a different profile for the colony at 172 m depth compared to those at 154 and 168 m depth (Fig. S2). Nonetheless, the Symbiodiniaceae communities shared three ASVs that exactly matched C89 (S-02: 5% at 172 m vs. 17–19% at 154–168 m) and two different C variants (both S-05 and S-07: 7% at 172 m vs. ~2% at 154–168 m) in public databases (Fig. S3; GeoSymbio, SymPortal or Genbank). Of the 26 ASVs identified across all samples, one sequence originated

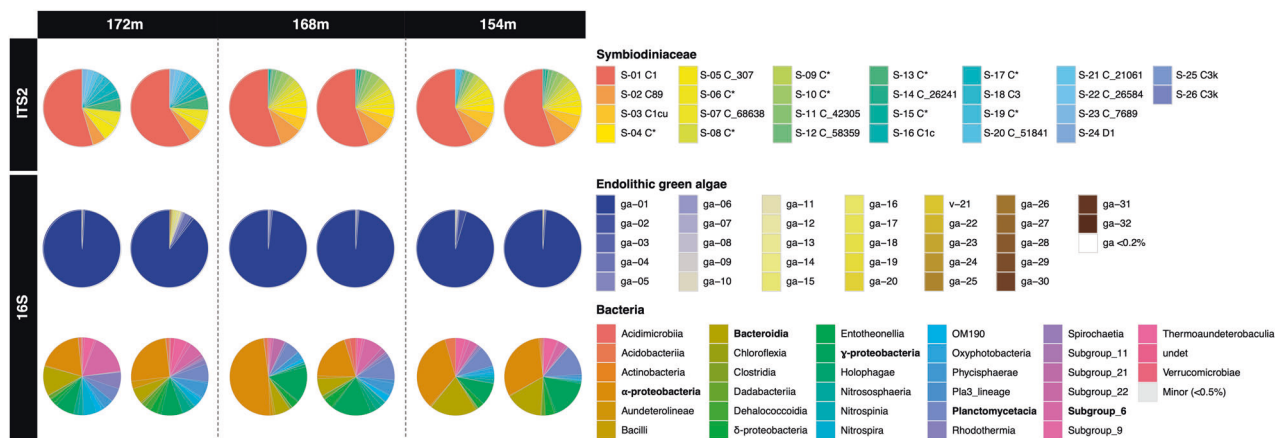


Fig. 2 Microbial communities harbored by the three deep colonies. Composition of the microbial community in *Leptoseris hawaiiensis* collected at 172, 168, and 154 m. At each depth, two subsamples were analyzed for each colony. The ITS2 marker shows the relative proportion of different Symbiodiniaceae ASVs (with GeoSymbio and

SymPortal v.2020-05-19_07-23-40 affiliations). The 16S rDNA marker shows the relative proportions of different ASVs for endolithic algae chloroplast composition and bacteria classes. Asterisk represents sequences with no exact match in the SymPortal database for Symbiodiniaceae.

from *Durusdinium* (S-24 D1 with GeoSymbio and SymPortal databases). This sequence is found in multiple heat-tolerant *Durusdinium* species including the enigmatic, cosmopolitan [17], host generalist *D. trenchii* [18]. However, whether or not the Symbiodiniaceae sampled here is *D. trenchii* or indeed thermally tolerant cannot be confirmed without further genetic and phenotypic data. Low abundance ASVs were observed at all three depths (172 m: 8 ASVs, 154 and 168 m: 10 ASVs, Fig. S3), including nine ASV sequences (Fig. 2) that have not been reported previously in the GeoSymbio [13] and SymPortal (access date: 2020-05-19_07-23-40) [16] databases (Fig. S3). Comparison of the overall Symbiodiniaceae SymPortal predicted ITS2 profiles (Fig. S2) did not confidently identify matches with previously encountered profiles (predominantly from shallow reef environments), indicating that they might be specific to this species and/or mesophotic environment. Given the extreme paucity of light at these depths, we hypothesize that lower mesophotic *L. hawaiiensis* may use different strategies to photoacclimate. Morphologically, the coral species were characterized by a thin flat skeleton (Fig. 1B–D), which is optimal for light harvesting and reducing skeletal carbonate deposition [19]. *Leptoseris hawaiiensis* has also been shown to display depth-associated physiological specialization and trophic plasticity (acquiring energy from different food sources) [9], and an unusual light-harvesting system, which enlarges the spectrum of wavelengths for photosynthesis by transforming the short, blue-shifted wavelength with their autofluorescent pigments [19].

To identify other microorganisms associated with our lower mesophotic scleractinian colonies, we targeted the 16S rRNA gene (V4–V5 region; see supplementary methods). Sequencing data revealed the presence of green algal chloroplast sequences belonging to the genus *Ostreobium*

(Fig. 2). This endolithic alga was abundant in the deep coral colonies as suggested by the marked green color observed below the living tissues (Fig. 1C) and within the skeleton after removing the soft tissues in bleach (Fig. 1D). We identified a single *Ostreobium* species (ASV ga-01), belonging to clade 2, that was dominant in all the colonies (Fig. 2 and S4), and has been previously reported across the depth gradient in scleractinian corals and octocorals worldwide [20, 21]. The nature of the interaction between corals and *Ostreobium* has been debated. Evidence supports a mutualistic association under extreme conditions such as coral stress (inducing bleaching) [22] or drastically reduced light exposure [23]. Under the low light conditions of the deep mesophotic fore reef slope, *Ostreobium* might complement Symbiodiniaceae's function by providing photosynthates to the host. These endolithic algae are adapted to photosynthesize in near-darkness with increased numbers of light-harvesting xanthophyll pigments that can use shorter wavelengths compared to other green algae and optimize light capture (e.g. [24]).

Bacteria associated with the lower mesophotic scleractinian colonies had an observed richness ranging from 106 to 211 ASVs per sample (Fig. S5). These bacteria mainly belonged to the classes Alpha- (19–49%) and Gamma-proteobacteria (8–17%), Bacteroidia (6–20%) and subgroup-6 of Acidobacteria (1–17%) (Fig. 2), which are known to associate with corals [25]. In total, we detected 843 different bacterial ASVs, among which 67–89% were unique to one colony or even unique to one subsample (Fig. S6 and Table S1). Our data suggest that the coral hosts displayed individual microbial signatures with some common ASVs shared between subsamples of the same colony (Fig. S6). However, this result might have been affected by the low-sequencing depth of the microbiome following the removal of

the *Ostreobium* reads. Our results corroborate previous reports describing the high intra-specific variability of coral-associated bacterial communities at different spatial scales (e.g. [25, 26]), which might be driven by biological traits, such as the age [27] or diets of the colonies [28].

This study reports a new depth record for scleractinian corals associated with symbiotic algae at 172 m. Similar to conspecifics previously sampled in mesophotic environments between 115 and 125 m depth [10], the deepest *L. hawaiiensis* reported here associated with symbiotic-microalgae belonging to the highly diverse C1 lineage. The deep colonies were also characterized by the presence and abundance of a single species of endolithic alga from the genus *Ostreobium* (clade 2). These filamentous green algae adapted to thrive in extreme low light conditions [24] might highly contribute to the survival of *L. hawaiiensis* at depth through photosynthates translocation [29]. In addition, bacterial communities were diverse, with intraspecific differences in community composition. Our findings provide new insights into the symbioses of scleractinian corals at depth, through the conservation of their associated photosymbiotic algae, raising important questions about the nature and mechanisms involved in the interactions between host and Symbiodiniaceae and/or *Ostreobium* (e.g. evolutionary theory of symbiosis [30]). Future studies should establish the contribution of photosynthetic symbionts to the energy budget of mesophotic corals. Understanding the biology of ecosystem engineers, such as tropical reef corals, living at the edge of their habitat range is important to determine the plasticity of these organisms and their ability to withstand environmental pressure.

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Under The Pole Consortium G. Bardout⁹, E. Périé-Bardout⁹, E. Marivint⁹, G. Lagarrigue⁹, J. Leblond⁹, F. Gazzola⁹, S. Pujolle⁹, N. Molon⁹, A. Mittau⁹, J. Fauchet⁹, N. Paulme⁹, R. Pete⁹, K. Peyrusse⁹, A. Ferucci⁹, A. Magnan⁹, M. Horlaville⁹, C. Breton⁹, M. Gouin⁹, T. Markocic⁹, I. Jubert⁹, P. Herrmann⁹.

⁹Under The Pole, 29900 Concarneau, France

Author contributions All authors conceived and designed this study; LH and HR coordinated the study; HR, GP-R, MP, UTP III

consortium and LH collected field data; MP identified coral species; HR carried out analysis and graphics and drafted the manuscript; HR, PG, MM, JBR, PB, and LH wrote the manuscript. All authors commented on the manuscript and gave their approval to submit.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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