Molecular Taxonomy and Diversity of *Symbiodinium* Spp. Based on 28S rDNA Sequences Within 15 Coral Species in Daao Bay, Shenzhen

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**Abstract:** The identity and diversity *Symbiodinium* spp. of scleractinian corals are valuable to understand the response of reef ecosystems to environment change. The molecular taxonomy and genetic diversity of *Symbiodinium* of scleractinian coral were studied by specific amplification and sequencing of 28S rDNA, which belong to 5 families, 9 genera and 15 species in Daao Bay, Shenzhen. The 28S rDNA sequence of *Symbiodinium* from 45 samples of scleractinian corals was obtained by direct PCR sequencing. The molecular phylogenetic tree was constructed by using the method of Neighbor-Joining (NJ). The *Symbiodinium* of reef coral in Daao sea area of Shenzhen could be divided into two groups of *Symbiodinium*. Among them, 11 species belong to group D and 4 species belong to group C, and the average contents of G+C base and A+T base of 28 s rDNA gene of reef coral *Symbiodinium* in this area are similar. The results showed that the gene evolution rate of 28 s rDNA was fast, which was suitable for the identification of coral reef coral *Symbiodinium* community. The ability of light utilization and resistance of reef coral *Symbiodinium* in Daao sea area were strong, but the diversity is low. It suggests that the symbiotic system of reef coral *Symbiodinium* is fragile and poor resistance to external environmental pressure in this area. This single *Symbiodinium* with the scleractinian coral strategy may be an adaptation to the environment stress in this area.

1. Introduction
Scleractinian corals live in tropical and subtropical seas, an oligotrophic alkaline environment, characterized by high calcium and carbonate saturation states, which are habitats that support very high biodiversity [1, 2]. At least 19% of all reefs worldwide have been permanently lost, and of those remaining, over 60% are at immediate risk from direct human activities [3]. Coral reefs, for instance, are increasingly under pressure due to coastal development and resource use [4]. They live in a symbiotic relationship with the dinoflagellate *Symbiodinium* sp. (zooxanthellae) and due to light limitation in deep water, corals are more successful in shallow water [5]. The endosymbiotic relationship provides the foundation of coral reef ecosystems by providing the energy to construct the three-dimensional framework of coral reefs [6]. The knowledge to taxonomy and diversity of *Symbiodinium* is important, which could acquire more information about recruitment and growth across environmental of the coral. Morphological classification is difficult for *Symbiodinium* spp., and the molecular DNA barcoding take a novel method to taxonomy for it. Fabricius investigated the genetic identity and diversity of *Symbiodinium* at three reefs with contrasting histories of bleaching mortality, water temperature and
shading, in the Republic of Palau [7]. Hauf used ITS2 sequence of the *Symbiodinium* reveal that the offshore reef samples displayed higher numbers of transitions of *Symbiodinium* subclade types between seasons in the Lower Florida Keys, while inshore fragments demonstrated more stability and may explain previously measured thermotolerance [8]. Leydet surveyed the *Symbiodinium* communities associated with Oculina corals in the western North Atlantic and the Mediterranean using one clade-level marker found that Oculina corals harbor different *Symbiodinium* communities across their geographical range [9]. Scleractinian corals live in Shenzhen is suffering high mortality rates due to coral bleaching. The present study used 28S rDNA sequences of *Symbiodinium* studied *Symbiodinium* community characteristics which could understand the functional diversity and acclimatization potential of the coral host well.

2. **Materials and Methods**

2.1. **Research Area**

The research was conducted off the Daao Bay coast, Shenzhen in November 2017, which was used for collection of coral samples for genetic analyses (114°28′02.10″ E, 22°33′06.21″ N ~114°27′58.52″ E, 22°33′10.59″ N) (Fig. 1). The survey water bottom sea temperature was 22.5°C, transparency was 2.5 m and salinity was 32.2‰. With handheld GPS Positioning System (78s, Garmin, American) determines the exact position in this investigation. Sampling sea water depth was range of 3-10 meters.

![Figure 1. Scleractinian coral sampling stations](image)

2.2. **Sample Collection for DNA Analysis**

Sampling was achieved by chipping off a 3 cm piece of coral from fragments using a hammer and chisel by SCUBA diver. Tissues of scleractinian corals were collected from coral fragments, which was removed from the skeleton using a water pick. The homogenate was poured into centrifuge tubes and pelleted (500 × g for 10 minutes), and the supernatant decanted, washed with *Symbiodinium* isolation buffer, and pelleted (500 × g for 10 minutes) a second time. The tissues were flash-frozen in liquid nitrogen and stored at -80°C in the laboratory for study. The samples of scleractinian coral belong to 5 families, 9 genera and 15 species, which were 3 repeat of each species.

| Family      | Genus       | Species            |
|------------|-------------|--------------------|
| Acroporidae| Acropora    | Acropora robusta   |
|            |             | Acropora digitifera|
|            |             | Acropora pruinosa  |
| Poritidae  | Porites     | Porites lutea      |

![Table.1 15 kinds of scleractinian corals samples of Shenzhen Daao bay](image)
2.3. DNA Extraction and Sequencing
Samples of Symbiodinium stored at -80 °C were macerated into a fine powder using a mortar and pestle pre-chilled in liquid nitrogen. Upon collection of the tissue pellet, total DNA was extracted using a plant DNA extraction kit (QIAGEN Plant Mini Kit, QIAGEN, German) according to manufacturer’s instructions [8].

For polymerase chain reactions (PCR) of the 28S rDNA, using the whole genome as the template, the primers were forward primer (GCC GAC CCG CTG AAT TCA AGC AT A T) and reverse primer (TGT GGC AYG TGA CGC GCA AGC TAA G). The reaction system was 50μL, containing Taq Master Mix 25 μL, ddH2O 17 μL, DNA template 4 μL, forward primer 2 μL and reverse primer 2 μL. The reaction conditions referred to the denaturation temperature of the primers and PCR Mix specification, and were set as follows: 5min pre-denaturation at 94°C, denaturation at 94°C for 30s, 53°C annealing for 1 min, 72°C extended 2min, (30 cycles) and extended at 72°C for 10 min. After the reaction, the 5 μL PCR products were detected by electrophoresis in the buffer system of 1% agarose gel with 0.5 times TBE. All Symbiodinium PCR solution were taken 30 μL respectively and sent to Beijing Qingke Biotechnology Co., Ltd to complete bidirectional sequencing.

2.4. Data Analysis
The accuracy and completeness of the sequence were confirmed by NCBI alignment. The sequence was spliced by DNAmann software (DNAMAN 6.0.3.99; Lynnon, 2005), the base composition of different sequences was analyzed by MEGA6 (MEGA 6.0.2.74; Arizona State University, 2011), and the current molecular classification method was used. A phylogenetic tree was constructed by comparing the 28S rDNA gene sequences of 15 species of coral Symbiodinium from Daao Bay, Shenzhen; with the sequences of different strains of Symbiodinium obtained from GenBank, the molecular diversity of symbiodinium in this sea area was analyzed. The faunal division of 15 species of coral Symbiodinium in Daao Bay of Shenzhen was carried out. Comparison of two Symbiodinium of the Gymnodinium genus (AF060901, AF060909) as external sequences. Constructing phylogenetic tree based on Neighbor-Joining. The confidence level of each branch was evaluated by the 1000 bootstrap resampling method.

3. Results

3.1 Analysis of base composition of 28S rDNA sequence
The length of the 28S rDNA gene amplified from 15 species of Symbiodinium in Daao Bay, Shenzhen, was 515-553 bp (mean 532 bp), and the average content of C base was the highest (26.09%) in this experimental; the average content of G base was the lowest (23.17%). The average contents of A base and T base were 24.67% and 25.95%, respectively; and the average contents of G+C base and A+T base were approach, which were 49.27% and 50.73%, respectively.

| Agariciidae | Pavona | Porites lobata |
|------------|--------|---------------|
|            | Pavona minutum   | Pavona decussata |

| Merulinidae | Hydrophora | Hydrophora exesa |
|------------|------------|-----------------|
| Pavona     | Pavona     | Pavona minuta   |

| Favidae | Plesiastrea | Plesiastrea versipora |
|---------|-------------|----------------------|
| Goniastrea | Goniastrea | Goniastrea pectinata |
| Favites | Favites abdita | Favites micropentagona |
| Platygyra | Platygyra carnosa |
Table 2. Average nucleotide frequencies of 28S rDNA sequences of 15 species of *Symbiodinium* (%)

| Number | *Symbiodinium*                        | A   | G   | T   | C   | G+C |
|--------|--------------------------------------|-----|-----|-----|-----|-----|
| 1      | *Symbiodinium* sp. Acropora robusta  | 24.03 | 19.77 | 27.13 | 28.88 | 48.64 |
| 2      | *Symbiodinium* sp. Acropora digitifera| 26.58 | 27.85 | 24.05 | 21.52 | 49.37 |
| 3      | *Symbiodinium* sp. Acropora pruinosa  | 27.13 | 28.88 | 24.22 | 19.77 | 48.64 |
| 4      | *Symbiodinium* sp. *Porites* lutea    | 23.70 | 20.56 | 26.48 | 28.89 | 49.44 |
| 5      | *Symbiodinium* sp. *Porites* lobata   | 26.37 | 28.94 | 23.44 | 21.25 | 50.18 |
| 6      | *Symbiodinium* sp. *Pavona* minuta   | 23.05 | 21.00 | 27.32 | 28.44 | 49.44 |
| 7      | *Symbiodinium* sp. *Pavona* decussata| 23.50 | 21.31 | 26.41 | 28.60 | 49.91 |
| 8      | *Symbiodinium* sp. *Hydnophora* exesa| 23.75 | 20.27 | 27.03 | 28.96 | 49.23 |
| 9      | *Symbiodinium* sp. *Plesiastrea* versipora| 26.39 | 29.06 | 23.33 | 21.03 | 50.10 |
| 10     | *Symbiodinium* sp. *Cyphastrea* serailia| 24.03 | 20.13 | 26.94 | 28.68 | 48.84 |
| 11     | *Symbiodinium* sp. *Goniastrea* pectinata| 23.88 | 19.81 | 26.99 | 29.13 | 48.93 |
| 12     | *Symbiodinium* sp. *Favites* abdita   | 23.67 | 20.64 | 27.08 | 28.60 | 49.24 |
| 13     | *Symbiodinium* sp. *Favites* micropentagona| 23.72 | 20.68 | 26.94 | 28.46 | 49.15 |
| 14     | *Symbiodinium* sp. *Favites* pentagona| 23.84 | 20.52 | 27.36 | 28.28 | 48.80 |
| 15     | *Symbiodinium* sp. *Platygyra* carnosa| 26.36 | 28.18 | 24.55 | 20.91 | 49.09 |
| Mean   |                                      | 24.67 | 23.17 | 25.95 | 26.09 | 49.27 |

3.2 Homology comparison and phylogenetic tree construction based on 28S rDNA sequence

The 28S rDNA sequence of *Symbiodinium* from Daao Bay of Shenzhen was selected on the sequenced to BLAST on the NCBI database. A phylogenetic NJ tree was constructed by selecting the 28S rDNA gene fragment of *Symbiodinium* Clade-C and Clade-D which is most similar to the sample gene; and two *Gymnodinium* genus *Symbiodinium* were selected as external sequence comparison.

The results of NJ tree based on 28S sequence (Fig. 2) showed that all samples were divided into three groups, the first clustered group including *Symbiodinium* sp. *Acropora* robustaus and *Symbiodinium* sp. *Porites* lutea et al., 10 species of *Symbiodinium* totally. Which were within Clade-D. The second group including *Symbiodinium* sp. *Acropora* digitifera and *Symbiodinium* sp. *Acropora* pruinosa et al., 5 species of *Symbiodinium* totally. Which were within Clade-C. The third group was exogenous sequence. The results showed that 66.67% of scleractinian coral species were symbiotic within Clade-D group *Symbiodinium* and 33.33% of stone scleractinian coral species were symbiotic with Clade-C group in this vessel.
Figure 2. 50% majority-rule consensus of the 1000 bootstrap NJ tree resulting using the P-distance model

4. Discussions

In exchange for protection, CO$_2$ and substrates for cellular synthesis, the *Symbiodinium* supply their hosts with essential metabolites [10, 11]. Corals obtained *Symbiodinium* from two models, which were vertical (Direct progeny of corals isolation of *Symbiodinium* from the parent) and horizontal model (ThCe offspring of corals obtain *Symbiodinium* from the outside environment) [12]. Variation at the nuclear ribosomal internal spacer regions has often been used to approximate species-level diversity in *Symbiodinium*. However, delineations between *Symbiodinium* groups at finer scales are often blurred and inconsistent due to the application of different makers and techniques, different naming systems and sometimes unknown origins of variation that could be inter- vs. intragenomic [13]. Research in *Symbiodinium* diversity has allowed the identification of specific or generalist host associations [14].

The problem of fully interpreting srDNA variation in natural samples of *Symbiodinium* is challenging [15]. The present study showed that the 28S rDNA of *Symbiodinium* could distinguish the species-level diversity well in this sea area. The results showed that the 28S rDNA of *Symbiodinium* showed AT base bias in the studied area. It is consistent with the researches by Madeleine and Yamashita [16, 17]. Hugall thinks that high A+T content accelerates variation of amino acid sequence [18].

The genus *Symbiodinium* is currently classified into nine genetic clades (A–I) [19]. Studies on reef coral *Symbiodinium* have shown that the *Symbiodinium* of A, B and F Clade are more present in higher latitudes, while Clade-C *Symbiodinium* are more widely distributed in tropical waters [20, 21]. The water depth of Clade-D *Symbiodinium* are widely distributed, which may occur in the deeper sea area, intertidal zone and coastal coral reef area with environmental stress [22]. *Symbiodinium* subclade type may be an important driver of holobiont stress tolerance [8, 23]. Furthermore, the clade-D *Symbiodinium* are more common in marginal environments where other *Symbiodinium* are not suitable for survival. In addition, studies have shown that scleractinian corals might release *Symbiodinium* when suffered environmental stress and replace it with Clade-D *Symbiodinium* [24]. It may be a strategy for the holobiont which successful colonisation (recruitment and growth) across environmental gradients.
requires that both the symbiotic microalgae (Symbiodinium spp.) and the host coral optimise available resources, while retaining the physiological plasticity needed to survive under different conditions [25]. Previous researches on coral transplantation and blanching recovered observed also showed that genus Clade-D symbiodinium are the dominant in inshore [15, 26]. And the coral assemblages from inshore reefs demonstrate higher thermostolerance than their offshore conspecifics [27]. The genus Clade-C symbiodinium can provide more efficient photosynthesis for corals, but their tolerance to environment may decreased [28]. The present results showed that 10 species were found to be symbiotic with Clade-D symbiodinium and 5 species symbiotic with Clade-C symbiodinium in this area, which both were the higher resistance and adaptability species to this sea environment. It suggested that the environmental pressure on the reef coral and Symbiodinium system in this waters should not be ignored.

It was widely assumed that one species of coral associates with only one species of Symbiodinium. However, Symbiodinium are diverse and it is now recognized that some species of corals associate with multiple species of Symbiodinium [15]. The adaptation mechanism of Symbiodinium is the switch and shuffle. In the process of coral growth, the new Symbiodinium species were obtained by corals due to the change of environment and other factors. The shuffle shows that the species and proportion of the Symbiodinium change correspondingly when corals respond to the changes of external environment. In this study, some different species scleractinian corals belonging to the same genus are symbiotic with different Symbiodinium species, which may be the result of respond to the environmental changes of scleractinian corals [29]. The mechanism of the switch and shuffle of Symbiodinium indicates that the stone coral itself can be symbiotic with different or different species of Symbiodinium at the same time or at different times, which indicates that the coral itself should have symbiotic polymorphism. But all samples have contained only one genotype of Symbiodinium in this study [15], which are all vertical transmitters. Forming symbiosis with the best acclimatized symbiont, instead of with a diverse group of symbionts with different physiological performances, either sequentially or simultaneously, may be a strategy used by this area [30].

Corals have survived global changes since the first scleractinian coral-algal symbioses appeared during the Triassic, 225 million years ago [31]. While molecular biology have much to offer, the synergistic effects of multidisciplinary approaches, including such other fields as geochemistry, biochemistry, paleoclimatology, invertebrate physiology, stratigraphy and paleontology will improve our knowledge about the scleractinian coral-algal symbioses correspond with the environment changes. DNA sequences data are easily obtained, at reasonable cost, from many samples of Symbiodinium, which allows ecological data to inform taxonomic decisions. It could be assist the corals to cope with stressful conditions.

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