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# ARTICLE

# Six Thallus Surface Types of Coralline Algae with Descriptions of Two New Records of *Amphiroa beauvoisii and Neogoniolithon setchellii* in Sanya reef, China

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#### ABSTRACT

Coralline algae are globally distributed calcifying species and play critical ecological roles to marine ecosystems by contributing significantly to their structural complexity and diversity. Thallus surface types of historical samples in Sanya coral reef reserve were studied based on the scanning electron microscope (SEM) method. Our results show six thallus surface types within the study area: *Corallina*-type, *Jania*-type, *Leptophytum*-type, *Phymatolithon*-type, *Pneophyllum*-type, and *Spongites*-type. The *Phymatolithon*-type is the dominant surface type in Sanya reefs. Two new record species in the region are described: *Amphiroa beauvoisii* and *Neogoniolithon setchellii*. Although thallus surface types provide useful diagnostics characters for distinguishing coralline algae at tribe or subfamily level, species identification needs to refer to the reproductive features. This is the first surface study of coralline algae in the South China Sea. This result provides the baseline data needed for the monitoring and management of reef-building organisms of coral reef in China.

## 1. Introduction

oralline algae (orders Corallinales, Hapalidiales, and Sporolithales) comprise numerous of living calcifying red algae that distributed globally from shallow to deep-water marine habitats, and they contribute important ecological roles in the marine ecosystem <sup>[2,5,10,17,19,23,31,38]</sup>. Despite they have been well studied across the ecological sciences as important organisms in the context of global environmental change <sup>[1,26,27,30,36,42]</sup>, basic

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questions should be paid attention to their systematics and biodiversity.

Two main morphological types of coralline algae are geniculate taxa and nongeniculate taxa, the former growing with crustose bases, alternating calcified and noncalcified erect genicula; the latter lack genicula and developing predominately as crusts or unattached rhodoliths <sup>[10,31]</sup>. It is known that the gross morphology of coralline algae is strongly affected by various environmental conditions of which can result in their identification difficult, and then they could divide into several form-functional groups <sup>[38]</sup>. Coralline algae species can be characterized by the anatomical characteristics of the hypoothallus, perithallus and trichocytes (size, shape, and arrangement), and also the other vegetative and reproductive features <sup>[45]</sup> together with the growth forms <sup>[47]</sup> can be used for species identification. Except for the morphological features, molecular tools have been well used in the coralline algae species identify [6,11,29,43,24].

Thallus surface features associated with epithallial cells and trichocytes provide useful diagnostic characters for the species identification of coralline algae <sup>[7,8,12,28,34]</sup>, and they also emphasized the systematic value of surface types by using the scanning electron microscopy (SEM) study. Furthermore, the surface of different coralline algae species associated with distinct bacterial communities that served as important settlement substrata for a variety of marine invertebrate larvae <sup>[[18,20,37]</sup>. However, the surface feature of coralline algae species had never been studied in China.

The purpose of this study is to examine the surface feature of coralline algae species collected in Sanya National Coral Reef Reserve using scanning electron microscopy (SEM) method. We chose the coralline species that had previously been reported in the area <sup>[22]</sup>. We compare the coralline species surface types and two new species records in this area in order to enrich the reef-building organisms in the South China Sea.

#### 2. Material and Methods

#### 2.1 Description of Study Area

Materials for this study were obtained during monitoring campaign within the Sanya national coral reef reserve, Hainan Island (109°20'~109°30'E, 18°11'~18°18'N), southern China. It is an extensive coral reef area with abundant rocky and sandy shores, and many kinds of tropical habitats such as coral reefs and mangroves. The reserve area mainly covered three separate regions: (1) Xidao and Dongdao Islands region, (2) Luhuitou, Xiaodonghai and Dadonghai coastal region, and (3) Xipai, Dongpai, and Yezhudao Islands region (Figure 1).



Figure 1. Sampling sites for coralline algae species within Sanya National Coral Reef Reserve, Hainan, China

#### **2.2 Species Sampling**

Within the Sanya fringing reef, samples of coralline algae were collected at 2~9m depth with a hammer and chisel by SCUBA divers. The spatial distribution of these species in this area was described in a previous study<sup>[22]</sup>. Specimens for stereomicroscope examining and molecular analyses were preserved in 95% alcohol, and for scanning electron microscopy (SEM) examining were fixed in 5% glutaraldehyde. The biological survey was carried out from Oct. 2009 to Nov. 2014, and a total of 226 samples of coralline algae was collected.

#### 2.3 Morphological Analyses and Identification

For the coralline algae identification, vegetative morphological features were considered, including the color of the thallus in living condition, growth forms, edge, thickness and organization and position of thallus, cell connections, epithallial and perithallial cells, trichocytes, reproductive structures, and conceptacle shape. Specimens were first examined with a Leica stereomicroscope to record the anatomical characteristics of the hypothallus, perithallus and trichocytes (size, shape, and arrangement), and then separated them into different form-functional groups following the classification of <sup>[38]</sup>. The samples were described according to the vegetative and reproductive features <sup>[45]</sup>, and their growth forms were divided as encrusting and branched, following the categories of <sup>[47]</sup>. Molecular tools were also applied to identify some species that morphology vary greatly, according to the previous study methods [11,39,43].

Each sample was assigned a species name according to the descriptions and keys from a number of sources <sup>[3,4,14,25,41,45,47,48,49]</sup>, as well as specialized literature on the taxonomy of the group were consulted. All the specimens were stored in the Marine Biodiversity Collection of South China Sea, Chinese Academy of Sciences.

## 2.4 SEM Analysis

Specimens for SEM examining, fragments of material were rinsed in distilled water, air-dried, fractured and mounted on stubs with silver conducting paint, and then coated with gold. Specimens were examined in a Hita-chi S-3400N scanning electron microscope (SEM) at an accelerating voltage of 20 kV, following the methods of previous studies <sup>[3,12,34]</sup>.

## 3. Results

### 3.1 Surface types

Based on the SEM analysis, there were 6 thallus surface types of coralline algae in all the species examined (Figure2, Table 1). The surface pattern characterizations were in general agreement with the previous reports respectively<sup>[7,12,44,34]</sup>. The 24 species of 12 genera within the 226 samples had been published in a previous study <sup>[22]</sup>. In terms of all the genera in Sanya coral reef, each genus has one surface type expect for *Lithophyllum* (Table 1). Genera *Lithophyllum* has 2 surface types that are *Leptophytum*-type and *Phymatolithon*-type. The dominant surface type is *Phymatolithon*-type (*Mesophyllum simulans, Porolithon onkodes, M. mesomorphum, Hydrolithon boergesenii*), followed by *Corallina*-type (*Amphiroa ephedraea, Neogoniolithon fosliei*) and *Pneophyllum*-type (*Pneophyllum conicum*).

The characteristics of the 6 thallus surface types are as following:

(1) *Corallina*-type surface (C-type): epithallial concavities had relatively rounded, non-elongate outlines, and the site of hair emergence was visible at the thallus surface only as a raised pore surrounded by symmetrical epithallial concavities.

(2) *Jania*-type surface (J-type): the thallus surface concavities with shallow, longitudinally elongate and polygonal cell outlines were surrounded by calcareous ridges; trichocyte bases appeared as raised areas with excentric pores.

(3) *Leptophytum*-type surface (L-type): presence on the thallus surface of at least some flat-topped, polygonal cells with small central holes and surrounded by thin calcareous ridges.

(4) *Phymatolithon*-type surface (Ph-type): The thallus surface has flat cell surfaces each with a minute central hole and thickened calcareous ridges surrounding central concavities.

(5) *Pneophyllum*-type surface (Pn-type): Thallus surface consisted of regularly arranged cells, epithallus cells

are concavely elliptical, and usually produce a small, round trichocyte at the corner of the cells.

(6) *Spongites*-type surface (S-type): The epithallus cells are barrel-shaped, slightly protruding on the thallus surface, or with thickened calcareous ridges.



Figure 2. Six thallus surface types: a. *Corallina*-type surface (C-type); b. *Jania*-type surface (J-type); c. *Leptophytum*-type surface (L-type); d. *Phymatolithon*-type surface (Ph-type); e. *Pneophyllum*-type surface (Pn-type); f. *Spongites*-type surface (S-type)

Table 1. Thallus surface types and the genus involved in		
Sanya coral reef reserve		

No.	Thallus surface types	Genus
1	Corallina-type	Amphiroa; Neogoniolithon
2	Jania-type	Lithoporella; Mastophora
3	Leptophytum-type	Lithophyllum
4	Phymatolithon-type	Lithophyllum; Porolithon; Hydrolithon; Lithothamnion; Mesophyllum; Sporolithon
5	Pneophyllum-type	Pneophyllum
6	Spongites-type	Spongites

#### 3.2 Species Observations

According to the 24 species founded in this research area, 2 of them were the new records in Hainan, China, that were *Amphiroa beauvoisii* and *Neogoniolithon setchellii* (Figure 3, 4). The systematics is as the following:

Ordor Corallinales P.C. Silva & H.W. Johansen, 1986 Family Lithophyllaceae Athanasiadis, 2016 Genera *Amphiroa* J.V. Lamouroux, 1812 Family Spongitaceae Kützing, 1843 Genera *Neogoniolithon* Setchell & L.R. Mason, 1943 *Amphiroa beauvoisii* J.V. Lamouroux <sup>[21]</sup>



**Figure 3.** a. image of *Amphiroa beauvoisii*; b. *Corallina*-type thallus surface and the scattered trichocyte; c. secondary pit connections between the adjacency filaments (arrow), intergenicula is composed of a medulla of long cells alternating with short cells; d. uniporate tetrasporangial conceptacle and tetraspores in the conceptacle chamber

Description: The species characteristic is similar to the description of Harvey et al <sup>[15,16]</sup>. The fronds are calcified forming erect clumps, up to 2-8cm in height with regularly dichotomous branching, and color varied from rose to gray-purple; attached to the substrate by a calcified, nongeniculate base; Branches of plants lacking fascicles of five or more branches arising from a single geniculum (Figure 3a). Most intergenicula compressed to flattened and occasionally more terete near the base. Genicula is composed only of decalcified core-region cells. The thallus surface type is *Corallina*-type (Figure 3b). The trichocytes are round with slightly swollen base, located at the junctions of several cells and they are smaller than the surrounding concavities. Cells of adjacent filaments linked by secondary pit connections (Figure 3c). Core region filament cells are mostly aligned in 9-12µm in diameter and two to three arching tiers of longer cells (46-83µm long) followed by a single arching tier of shorter cells (6-38µm long).

Uniporate tetrasporangial conceptacles formed in peripheral regions of intergenicula and scattered over the surface of intergenicula. Top of tetrasporangial conceptacle pore canals surrounded by a ring of large block-shaped cells (Figure 3d). Tetrasporangia arising peripherally to a central columella; conceptacle chamber floor more or less flat or sometimes with a central hump. Each mature sporangium 26-59mm in diameter and 72-79mm long, containing four zonately arranged tetraspores. Bisporangia not seen.

Holotype and locality: CN, unnumbered, designed by

Harvey et al. <sup>[16]</sup>: AUSTRALIA, Victoria, Port Fairy <sup>[16]</sup>; General Herbarium: AD-A46537, Queensland <sup>[15]</sup>.

Etymology: Named for honoring the French botanist Ambroise Marie François Joseph Palisot, Baron de Beauvois (1752-1820).

Habitat and distribution: It is a marine species. The species occurs epilithic on rocks, intertidal and in pools and to depth of 15-17m in Australia <sup>[15,16]</sup>. In this study, the plans were collected from the Sanya coral reef area, China, in depth of 2-9m.

*A. beauvoisii* was firstly recorded in Portugal coasts <sup>[21]</sup>, and probably a widespread species, occurs in most tropical and subtropical regions, Mediterranean Sea, Southwestern Indian Ocean, South Africa, according to Norris and Johansen <sup>[32]</sup>. It has been reported in other places from Europe, Atlantic Islands, Western Atlantic, North America, Central America, South America, Caribbean Islands, Africa, Asia, South-west Asia, South-east Asia, Australia and New Zealand, Pacific Islands, Antarctic and the sub-antarctic islands <sup>[13]</sup>.

Gene sequences: The 18S ribosomal RNA, 23S ribosomal RNA, cytochrome oxidase subunit 1 gene (COI) and cytochrome c oxidase subunit 1 (COX1) gene sequences of *A. beauvoisii* have been deposited in GenBank with the accession number GQ227511.1, KF367784.1, KF367761.1, MG030753.1, LC071726.1, LC071727.1, LC071728.1, LC071729.1.

Heterotypic Synonym: They were listed in Harvey et al. <sup>[16]</sup>, requiring further reassessment. Other heterotypic synonyms listed by Guiry and Guiry <sup>[13]</sup>.

Neogoniolithon setchellii (Foslie) W.H. Adey [1]



Figure 4. a. image of dried thallus of *Neogoniolithon setchellii*. b. *Corallina*-type thallus surface and the trichocyte. c. cell fusion connections between the adjacency filaments (arrowhead) and the trichocyte (arrow). d. surface view of thallus showing uniporate tetrasporangial conceptacle and remains of tetrasporangia

Description: Thalli nongeniculate, encrusting with irregular shape and fragile, and color varied from pale red to grav-purple: Plants attached to the substratum by cell adhesion, overlapping and loosely bound together, 85-780µm thick (Figure 4a). The thallus surface type is Corallina-type (Figure 4b). Thallus pseudoparenchymatous, cells of adjacent filaments connected commonly by fusions, secondary pit-connections absent; Trichocytes occur singly and scattered in the dorsal region, 16-20µm wide and 39-42µm long (Figure 4c). Thallus organization dorsiventral, and construction monomerous. Ventral region (medulla) composed of 6-8 layers of filaments with cells oriented more or less parallel to the thallus surface; cells oriented in a coaxial, and in 85-100µm thick; medullary cells 10-13µm wide and 13-26µm long. Dorsal regions (perithallus) composed of multilayer of irregular cells that become oriented perpendicularly to the thallus surface. Cortical cells ovoid, 7-10µm in diameter and 10-12µm long.

Tetrasporangial conceptacles uniporate, protruding, scattered and apiculate, their chambers  $580-780\mu m$  in diameter and  $230-360\mu m$  high, pore canals lined by cells oriented more or less parallel to the thallus surface, each chamber containing tetrasporangia across floor and periphery, and lacking a central columella (Figure 4d, d-1).

Holotype and locality: TRH, A11-616, designed by Woelkerling et al. <sup>[46]</sup>: U.S.A, San Pedro, in tide pools; General Herbarium: TRH A11-616, California <sup>[46]</sup>.

Habitat and distribution: It is a marine species. The species occurs encrusting on hard substratumin in tide pools, San Pedro, U.S.A <sup>[46]</sup>. In this study, the plans were collected from the Sanya coral reef area, China, in depth of 2-6m.

Homotypic Synonym(s): *Lithothamnion setchellii* Foslie 1897.

Heterotypic Synonym(s): *Hydrolithon setchellii* (Foslie) Setchell & L.R. Mason 1943; *Neogoniolithon neopacificum* M. Baba ex B. Xia 2013.

Gene sequences: There no explicit gene sequences of *N*. *setchellii* in GenBank with the accession number at present.

#### 4. Discussion

Although the Sanya coral reefs are the most typical tropical fringing reef in Southern China, except for the reef corals have been studied extensively, other calcifying organisms in these reefs had been largely ignored. An initial objective of the present study was to determine the coralline species surface features, as the prior studies have noted the systematic value of that. This study is the first attempt to document the species surface features and notes on the two new records of the previously published coralline species <sup>[22]</sup> based SEM examining in Sanya coral reefs. The results of SEM analysis showed that 6 thallus surface types within the 12 genera species.

This study supports the views from previous observations that thallus surface features provide useful diagnostic characters for some coralline algae species [7,8,12,28,34], and the great advantage of employing thallus surface patterns as a character is it can eliminate the need of reproductive structures to distinguish some species <sup>[7,8,12,34]</sup>. However, the patterns of surface concavities and other characteristics do not always constitute independent characters (e.g. the positions and shapes of trichocyte bases) in some genera or species <sup>[34]</sup>, and some thallus surfaces are dynamic with extensive sloughing and regeneration of epithallial cells <sup>[28,35]</sup>. Therefore, our results in agreement with the conclusions that surface types can be a useful diagnostic character at tribe or subfamily level [8,34], additionally, if a species has more than one surface type or different species have the same surface pattern, species identification has to refer to the reproductive features <sup>[7]</sup> or other effective tools.

In the past, only a few studies report some coralline algae species in this area<sup>[40,41,48]</sup>. Lei et al <sup>[22]</sup> reported the distribution pattern of 24 coralline species in Sanya reefs. Here, we describe only the two new record species by focusing on the SEM features. Tseng [41] report a synonym of A. beauvoisii (A. zonata) distributed in Huanghai Sea and East China Coasts, and then found it in Shandong, Zhejiang, and Taiwan, China<sup>[9,48]</sup>. The species has not been found in Sanya coral reefs and rocky shores around Hainan Island before 2018 [22]. Top of tetrasporangial conceptacle pore canals surrounded by a ring of large block-shaped cells in species A. beauvoisii can delimit this species within the genus distributed in Sanya coral reef reserve. Xia [48] and Ding et al [9] reported N. setchellii distributed in Fujian, China, but the original materials are not exactly clear. According to the type species of genus Neogoniolithon (N. fosliei) described by Penrose [33] and Mateo-Cid et al [29], a suite of vegetative and reproductive characters can segregate the currently named species N. setchellii, including (1) unbranched thalli, (2) with thallus surface type of Corallina-type, (3) monomerous construction, in 85-780µm thick, (4) trichocytes occur singly and scattered in dorsal region, 16-20µm wide and 39-42µm long, (5) mature tetrasporangial conceptacles chambers 580-780µm in diameter and 230-360µm high, (6) 12-17 cell layers comprising tetrasporangial conceptacle roof, and (7) cells that line tetrasporangial pore canal oriented more or less parallel to the thallus surface.

# 5. Conclusions

Based on the limited sampling that we have done within the Sanya national coral reef reserve, 6 thallus surface types have been found in the present 24 species. Two new record species in this area, *A. beauvoisii* and *N. setchellii* are described herein according to their SEM features. The applications of surface type in species identification need to be confirmed by reproductive features (e.g. tetrasporangial conceptacles), sequencing type or topotype material.

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## **Authors' Contributions**

LXM, HH conceived the paper and input regarding the manuscript. JL, SYF, ZYY, ZGW, YJH, LJS did the field investigation in Sanya reef and data collection. ZYY, ZGW. All authors read and approved the final manuscript.

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# Availability of Data and Materials

Species list of this study are available in in a previous study<sup>[22]</sup>.

# **Ethics Approval and Consent to Participate**

Not applicable.

# **Consent for Publication**

Not applicable.

# **Competing Interests**

The authors declare that they have no competing interests.

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