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Marine green alga *Cladophora aokii* Yamada and its epiphytes from Yinggehai, Hainan, China: morphological taxonomy, formation and analysis of its environmental adaption

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Abstract
Based on specimens collected in Yinggehai, Hainan, China from 2013 to 2016, a stable epiphytic taxon is found on the surface of the individual of marine green alga *Cladophora aokii* Yamada. According to the morphological characteristics, the taxonomy of *Cl. aokii* and its epiphytes is carried out. There are some epiphytes attached on *Cl. aokii* Yamada including *Cl. fascicularis* (Mertens ex C. Agardh) Kützing, *Chaetomorpha pachynema* (Montagne) Kützing, *Ceramium camouii* Dawson, *Licmophora abbreviata* Agardh, *Lyngbya* sp. and *Chattonella* sp.. The formation of the individual of *Cl. aokii* is dissected and explained, which can help to analyze the adaption in details among this species, its epiphytes and native marine environment. The results reveal the marine macro-epiphytic taxonomy in Hainan, China, and preliminarily explain the adaptive relationship between macroalgae and environment.

Key words: Hainan, Yinggehai, Cladophoraceae, morphological taxonomy, growth pattern, environmental adaption

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1 Introduction

The marine green algae of family Cladophoraceae have importantly potential economic/environmental values. They can be served as the natural food of marine animals *Gammarus aequicauda* and *Haminoea callidegenita* (Zhang et al., 2014), and effective scavenger of the nutrients such as N, P, benzenes and heavy metals (Fan et al., 2010; Cao et al., 2010; Cong et al., 2014). They can also be used for sewage treatment (Xie et al., 2016), preparing feed yeast for fermentation (Yang et al., 2013a) and production of bioethanol (Yang et al., 2013b). In aquaculture, the green algae can not only bring some economic value (Xie et al., 2008; Wang, 2015) but detriment. The bad water quality is caused by the corruption and decomposition of the plants (Gao et al., 2013). Their proliferation by increasing biomass also brings economic losses to the aquaculture industry (Lapointe et al., 2011).

Family Cladophoraceae belongs to Chlorophyta, Cladophorales. Its marine species can be mainly divided into three genera, i.e., *Chaetomorpha*, *Cladophora* and *Rhizoclonium*. They were found to have rich distribution in the coasts of China. In China, *Chaetomorpha area* was first reported by Cotton in 1915 at Weihai, Shandong (Teng, 2011). At present, more than 52 species can be found in the coasts of China seas (Ding, 2013). However, some basic data of some species are still absent at the identification and characteristic description. It is necessary to collect data and carry out investigation thoroughly.

During our classification to these samples, a special growth pattern and epiphytic feature are found to marine green alga *Cladophora aokii* Yamada, the species distributed at Yinggehai, Hainan, China. It will be of great significance to clarify marine environmental adaptation between the macroalgae and their habitats.

2 Materials and methods

2.1 *Materials*

The samples were collected at Yinggehai, Hainan from December 2013 to December 2016. They are preserved as following: (1) dried specimen for appearance observation and permanent preservation; (2) liquid samples in 8% formalin seawater for microscopic observation; and (3) fast dried samples by quick dehydration of the allochroic silicagel for subsequent molecular identification.

2.2 *Methods*

2.2.1 *Observation and measurement of appearance features*

Under the stereoscopic microscope (Nikon, SMZ25, Japan), some morphological features of the samples were observed as the overall profile, texture, color, branch and so on. The data for identification were measured as plant height, rhizoid and main branch length and so on. The characteristic pictures were taken

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and kept.

2.2.2 *Microscopic observation and measurement*

The morphological characteristics were observed to the holdfast, cells at the basal part, main branch, branch and terminal branchlet under the positive fluorescence microscope (Lycra, DM5000, Germany). The data for identification were measured as length, width and wall thickness of cell, and the characteristic pictures were taken and kept.

2.2.3 *Morphological identification*

The species was identified by the comparison between the experimental data and recorded ones from the literatures.

3 Results

3.1 *Morphological observation*

By the comparison and analysis to the samples collected from December 2013 to December 2016, a stable epiphytic issue was found based on *Cl. aokii* Yamada at the Yinggehai, Hainan. Some algal species were identified as following.

3.1.1 *Cl. aokii Yamada (Fig. 1)*

Yamada, 1925: 85, Fig. 3; Okamura, 1936: 56; Tseng, 1936b: 150, Figs 13, 14; Sakai, 1964: 54, Fig. 25; Tseng, 1983: 258, Pl. 128, Fig. 3; Yoshida, 1998: 61, Figs 1–6A; Ding, 2013: 97.

Description: Thalli are dull greyish-green, rigid, 4–6 cm high, and composed of many counter-clockwise intertwined individuals, which are 8–17 mm high. It holds rhizoidal holdfast including primary and adventitious rhizoids. The rhizoids can produce branch with cells, which are absent of constricted side wall. The primary rhizoids are born on the basal part of the plants, adventitious rhizoids are born at the lower side of branch cells. The rhizoidal cells are 30–80 μm in diameter, to about 100 μm near to principal filament, 2–5 mm in length. The branches are dichotomous, trichotomous or fascicular. The cells of the main branch and branches are clavate, slightly swollen at the divergent point, 0.5–1 mm in length and 80–200 μm in diameter. The branchlets are blunt at the distal part.

Voucher specimen number: 201312170031, 201411240014 and 20161202003.

Habitat: rocky and sandy pools in the intertidal zone.

3.1.2 *Cl. fascicularis (Mertens ex C. Agardh) Kützing (Fig. 2)*

Kützing, 1843: 268; 1849: 393; De Toni, 1889: 316; Tseng and Chang, 1964: 1; Sakai, 1964: 25, Pl. 14:1, Fig. 8; Tseng, 1983: 258, Pl. 128, Fig. 4; Yoshida, 1998: 62; Ding, 2013: 108.

Conferva fascicularis Mertens ex C. Agardh, 1924: 114

Cl. mauritiana Kützing, 1849: 399; De Toni, 1889: 328

Cl. utriculosa sensu Yendo, 1914: 265; Okamura, 1936: 57

Description: Thalli are pale green with spots, upright, about 10 cm in height, and forming entangled tufts. It holds the rhizoidal holdfast with branches of 4–4.5 mm in length. The branches are generated from basal cells of the fronds. The branches are dichotomous or trichotomous, slender and entangled. The branchlets at the upper part of the thalli are densely fascicular, multiforked branching, with slightly swollen cells near to the divergent point. The terminal branchlets bend inward in sickle shapes, 1.5–2 mm in length. The terminal branches are composed of 2–5 single unilateral branchlets, which present the pectinate structure. The cells of the main branches, branches and branchlets are 1 000–2 000 μm, 1 000–1 500 μm and 100–400 μm in length, and 100–200 μm, 100–200 μm and 50–120 μm in breadth, respectively. The heteromorphous branchlets are developed at the apex of the thalli. The cells of the heteromorphous branchlets are 400–500 μm in length and 200–260 μm in diameter, with bud cells opposite bilaterally or lateral at the apical part. The bud cells are 150–300 μm in length, 50–60 μm at the upper part, 70–80 μm at the middle part, 70–80 μm at the lower part in breadth, respectively, and dense green in color.

Voucher specimen number: 201312170031, 201411240014 and 20161202003.

Habitat: The plants attach to the thalli of *Cl. aokii.*

3.1.3 *Ch. pachynema (Montagne) Kützing (Fig. 3)*

Kützing, 1847: 166; Yoshida, 1998: 57; Ding, 2013: 89.

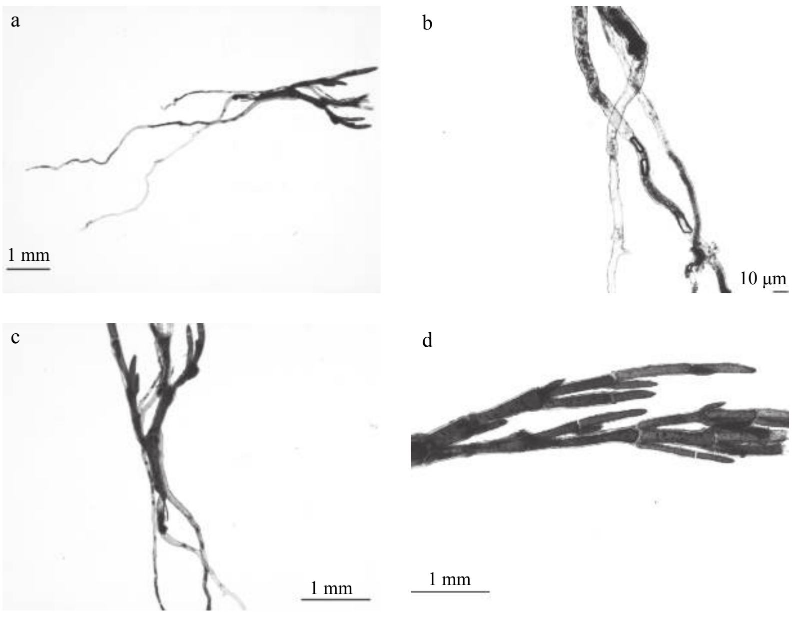

Fig. 1. Morphologic characteristics of *Cl. aokii*. a. The habit sketch of the frond, b. the basal rhizoids and the lower part of the frond, c. the adventitious rhizoids, and d. the branchlet and branches.

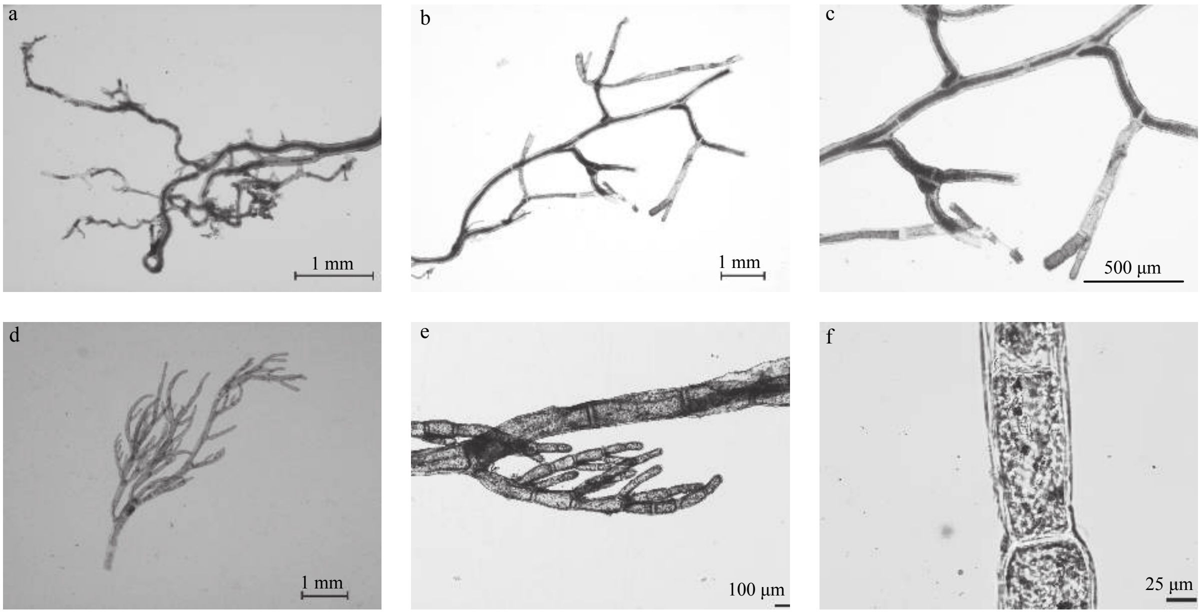

Fig. 2. Morphologic characteristics of *Cl. fascicularis*. a. The rhizoidal holdfast, b. the main branch and branches, c. the branch cells, d. the branchlets, e. the characteristics branchlet, and f. the black spot on the cell surface.

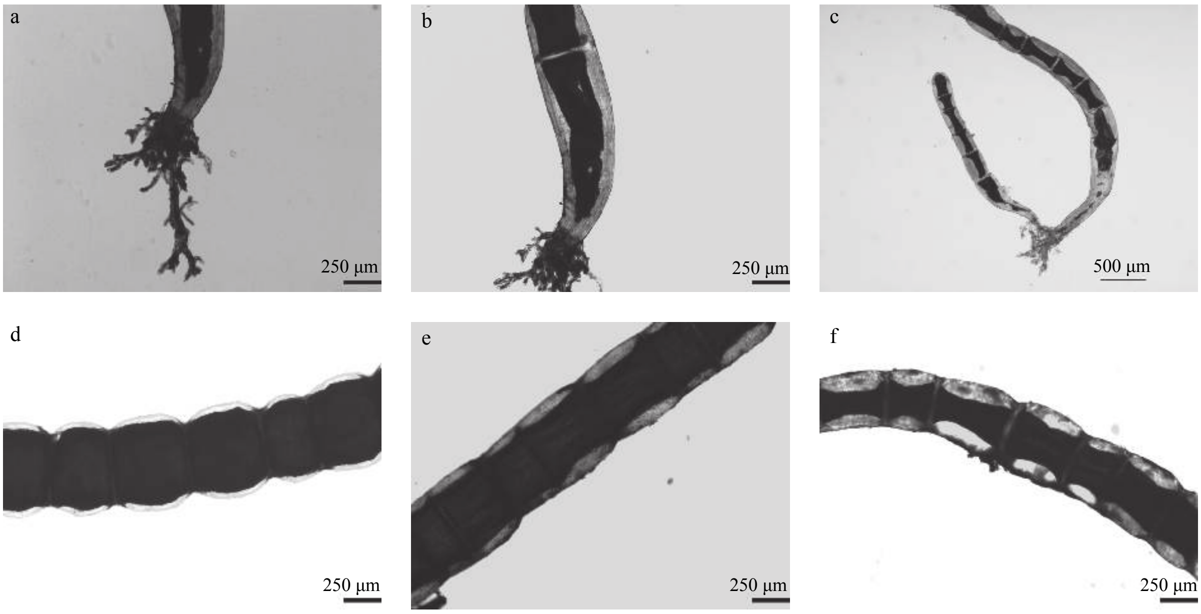

Fig. 3. Morphologic characteristics of *Ch. pachynema*. a. The rhizoidal holdfast, b. the basal cell, c. the younger plant, d. the cells at the upper part, e. the cells at the middle part, and f. the cells at the lower part.

Conferva pachynema Montagne in Webb et Berthelot, 1841: 184.

Chaetomorpha pachynema Montagne in Kützing, 1849: 379; Børgesen, 1925: 41, Figs 11, 12; Tseng, 1936a: 17; Yamada, 1941: 195, Fig. 1.

Description: Thalli are dark-green tufts, hard texture, uniform thickness, 4–10 cm high. The cells of the basal part are not spirally curled, and they lack circular constrictions on the lateral walls. They hold the rhizoidal holdfasts, which are born laterally on the basal cells and branching. The younger plants erectly grow from the rhizoidal laterals. The basal cells of the thalli are inverted cone and slightly curved, 1.3–1.9 mm in length, 406–460 μm at the upper part and 107–270 μm at the lower part in breadth, L(length)/B(breadth) 3.2–4.1. At the lower part of thalli, the cells are rectangular with slightly constricted lateral walls, 307–496 μm in length and 362–465 μm in breadth, L/B 0.84–1.06, 67–92 μm in thickness of the cell wall. At the middle part, the cells are rectangular with slightly constricted lateral walls, 290–409 μm in length and 508–591 μm in breadth, L/B 0.6–0.7, 40–72 μm in thickness of the cell wall. At the upper part, the cells are moniliform, 397–469 μm in length and 509–526 μm in breadth, L/B 0.78–0.89, 31–42 μm in thickness of the cell wall.

Voucher specimen number: 201312170031, 201411240014 and 20161202003.

Habitat: The plants attach to the thalli of *Cl. aokii*.

3.1.4 *Ceramium camouii* Dawson (Fig. 4a)

Dawson, 1944: 319, Pl. 51, Figs 2, 3; 1950: 129; Børgesen, 1953: 41–42, Fig. 20; Zheng et al., 2001: 34.

Description: The thalli are light red, laterally branched, about 500 μm in height, with slender hairs. The apex of branch is blunt and slightly curved. The longitudinal axis of centrical cells can be distinguished into node and internode. The node is surrounded by outward swollen cortex, diameter 60–70 μm, composed of the rows of small cells. The length of the internode becomes short from bottom to top gradually, diameter 50–60 μm.

Voucher specimen number: 20161202003.

Habitat: The plants attach to the thalli of *Cl. aokii.*

3.1.5 *Licmophora abbreviata* Agardh (Fig. 4c)

Agardh, 1831: 42; Cupp, 1943: 177, f. 127; Kokubo (Rucheng Hua translation), 1960: 294, f. 311; Van Landingham, 1967–1975: 2103; Jin et al., 1965: 189. f. 177A–D; 1982: 183; 1992: 194; Cheng et al., 2012: 31.

Description: The thalli are blue-green, fan-shaped circular plate, with a very short gelatinous stipe. The shell plane is clavate, not constricted at the apex and gradually narrowed, 40–45 μm in length, 10–15 μm at the upper part in width and 4–6 μm at the lower part in width. The cells contain a number of oval blue-green chromatophores.

Voucher specimen number: 20161202003.

Habitat: The thalli attach to the thalli of *Cl. aokii* and *Cl. fascicularis.*

3.1.6 *Lyngbya* sp. (Fig. 4b)

Tseng, 1983: 26–28.

Description: The thalli are purple, clusters and not branched, erect, flexible in texture, 1–2.5 mm in height, 27–30 μm in diameter, thickness uniform, uneven fine hairs distributed bilaterally. The outer fine hairs are 10–15 μm long. Colloidal sheathes are colorless and transparent, clearly visible, 1.5–4.5 μm in diameter. At the middle of thalli, the transparent reproductive belts result in the discontinuous distribution of cells. The cells of thalli are rectangular, 20–29 μm in breadth, 1–3 μm in length and absent of constricted cell lateral walls. The apical cells are rectangular without thickening or cap-like structure.

Voucher specimen number: 20161202003.

Habitat: The thalli attach to the plants of *Cl. aokii.*

3.1.7 *Chattonella* sp. (Fig. 4d)

Law and Lee, 2013: 24–26.

Description: The thallus is a single cell, light green, spherical, 50–61 μm in diameter. At the outer layer of the cell, the large and transparent mucus bubble can be visible, and light green globular chloroplast at the middle part.

Voucher specimen number: 20161202003.

Habitat: The thallus attaches to the plants of *Cl. aokii.*

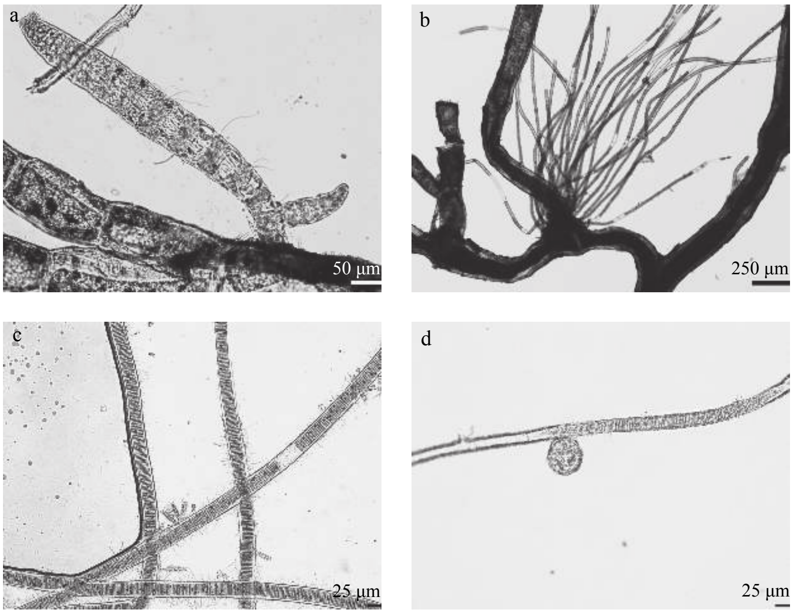

Fig. 4. morphological characteristics of several epiphytic algae. a. *Ceramium camouii*, b. *Lyngbya* sp., c. *Licmophora abbreviata*, and d. *Chattonella* sp.

3.2 *The plant's formation of Cl. aokii*

Through the dissection and observation of 30 samples, it was found that the adult individual of *Cl. aokii* is not a true plant thallus, but of hierarchical combination by the latter. It can be called subplant, which is developed into a completed plant system including the rhizoid and erect branches. A common plant is organized hierarchically through the basal part adhering of some corresponding branches of the different subplant on the anatomy. Then a common plant with 4–6 cm high actually is composed of some subplants with 8–17 mm high. The order of their development is presumed as following:

(1) The earliest subplant attaches to the rock on the bottom or inner side of the intertidal rock pool by longer primary rhizoids. Subsequently, many branches and branchlets are born from the subplant. The basal cells of the branches and branchlets produce downward adventitious rhizoids (secondary rhizoid).

(2) Adventitious rhizoids elongate downward and are entangled or pierce into the adjacent branches or adventitious rhizoids. The initial formation of common plant is structured. Through the entangled and pierced formation, it increases the stability of the plant structure.

(3) Considered as the targets to the branches or branchlets of

parent subplant, the adventitious rhizoids of the baby subplant generally entangles anticlockwise themselves surrounded in order to dense attachment.

(4) During the growth period, the individual of *Cl. aokii* continuously produces new branches, branchlets and adventitious (indefinite) rhizoids. The adventitious rhizoids and branchlets wrap around each other, and stabilize the dense structures at the upper part of plant. It results up to 4–6 cm in height.

This distinguished growth pattern of *Cl. aokii* may be related to its dense branches and adventitious rhizoids, and the feature of the long adventitious rhizoid can be produced by any contacted point, in addition to its own factors and related to long-term environmental adaptation. At the same time, it is also found that the dense and stable thalli structure, whose varied space formed by entangled or pierced of subplants and coarse surface of branches and branchlets, offers good substrate for attachment of other epiphytes. These stable epiphytes include *Cl. fascicularis*, *Ch. pachynema*, *Ceramium camouii*, *Lyngbya* sp., *Chattonella* sp. and some taxa of microalgae unidentified.

4 Discussion

Yinggehai is located in the southwest of Hainan Island (Cao, 2014), 18°30′N, 108°41′E, across the Beibu Bay facing to Vietnam. The annual temperature is 19–30°C (Wang, 2002). The coast is bedrock headland bay. In winter, there are usually big waves and strong wind (Wang, 2002). According to the data from the China Meteorological Network records and the Department of Marine and Fisheries of Hainan Province, from November 2016 to January 2017, there are 12 d of typhoons over 3 levels, 3 d over 6 levels, 35 d marine waves over 2 m high and up to 3.5 m high.

During some field investigations, we found a stably epiphytic phenomenon based on the thalli of *Cl. aokii* as the substrate in the intertidal rock pools with the surface covered by mud and sand. The adult thalli of the *Cl. aokii* are kept 4–6 cm in height. It can be speculated as following:

(1) The origin of appearance and height of *Cl. aokii*

As mentioned above, the individual of *Cl. aokii* is composed of some subplants adhering to each other. In an optional situation, the appearance and height of the individual should be unlimitedly expanded under this special individual formation of *Cl. aokii*. Actually, it is inconsistent with the results obtained from field investigation. We consider that there are two reasons for the specific height of *Cl. aokii* at the Yinggehai area. One is environmental factors. The coast of Yinggehai is rock bay. In winter, the big waves and strong wind are rather hostile environment for the growth of macroalgae. It is obvious that excessive heights of the plants are not advantage to settle stably in the rocky coasts and also does not adapt to the climate characteristics of the waves and wind. In order to adapt to the environment, the individual of *Cl. aokii* usually settles in the bottom and lateral wall of the intertidal rock pools in order to decrease environmental stress and increase anchorage. Another is restriction of the growth cycle. After several field investigations, it is concluded that *Cl. aokii* in Yinggehai appears in mid to late November to early January. The limited growth season results in its specific appearance height.

(2) The forming causes of multiple epiphytic algae on the individual of *Cl. aokii*

There are a variety of epiphytic algae on the individual of *Cl. aokii* at the Yinggehai area. The main epiphytic algae are *Cl. fascicularis* (Mertens ex C. Agardh) Kützing, *Ch. pachynema* (Montagne) Kützing and so on. We consider that the formation of the epiphytes is related to some factors. Firstly, it contacts with their own habits. Based on the literature (Ding et al., 2013), these two species have the epiphytic habits and easily attach to other algal species. Some published experiments on epiphytic habit of algae also proved that these two species are more likely to be attached to artificial materials such as nylon ropes, but not easily attached to smooth surface such as silver sand (Shi et al., 2016; Wang et al., 2008). Because of the special growth mode of *Cl. aokii*, it makes its structure similar to nylon rope. Comparing to other substrates at the coast of Yinggehai, the surface of the individual of *Cl. aokii* becomes the best one. Secondly, the living environment is also an important factor chosen by *Cl. aokii*. It is a more suitable environment for the residence and growth of marine benthic algae as well. By a specific growth pattern, it offers to rich space for the settlement of other epiphytes. On the morphological structure of *Cl. aokii*, the subplants weave each other to the stable individual construction in addition to the primary rhizoid to rock substrate. The coarse surface in texture increases the attachment rate for the reproductive cell of other species. Finally, environmental factors contribute to an important position. The climate conditions at Yinggehai area are not suitable for the residence of epiphytic algae. Obviously, in order to adapt to the harsh winter environment of Yinggehai, it is inevitable result for these epiphytes to find a better substrate than coast rock. As a result, *Cl. aokii* becomes the best substrate for other epiphytes and forms a unique epiphytic phenomenon in the Yinggehai area.

(3) The successive occurrence of *Cl. aokii*, *Cl. fascicularis* and *Ch. pachynema*

Based on the observation to the settlement positions of a large number of samples, we infer that sequence of occurrence of the three algae is *Cl. aokii*>*Ch. pachynema*>*Cl. fascicularis*. *Cladophora aokii* is the first to be born. It settles and grows in the bottom and lateral wall of the intertidal rock pools. It provides the stable conditions for other epiphytes. *Chaetomorpha pachynema* is following the former to be born. It generally settles on the lower part of thalli of *Cl. aokii. Cladophora fascicularis* settles on the upper part of thalli of *Cl. aokii*. It could be later developed than *Ch. pachynema.*

The specific attachment between *Cl. aokii* and its epiphytes connects the algal morphological features, growth patterns and environmental adaptation together. It reflects adaptation between the occurrence of algae in the water body and its marine surrounding environment, and can preliminarily explain origins on some morphological characteristics. It also provides some cites for clarifying the relationship between algal morphological features and environmental factors.

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