EPIPHYTIC MICROBIOTA OF KAI *Cladophora* sp. FROM NAN RIVER, NAN PROVINCE, THAILAND

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จุลชีวภาพอิงอาศัยของไก *Cladophora* sp. จากแม่น้ำน่าน จังหวัดน่าน ประเทศไทย

น.ส.กาญจนา เรือนผาม

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต
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Thesis Title	EPIPHYTIC MICROBIOTA OF KAI *Cladophora* sp. FROM NAN RIVER, NAN PROVINCE, THAILAND
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Field of Study	Botany
Thesis Advisor	Assistant Professor ANCHITTHA SATJARAK, Ph.D.
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กาญจนา เรือนผาม : จุลชีวภาพอิงอาศัยของไก *Cladophora* sp. จากแม่น้ำน่าน จังหวัดน่าน ประเทศไทย. (EPIPHYTIC MICROBIOTA OF KAI *Cladophora* sp. FROM NAN RIVER, NAN PROVINCE, THAILAND) อ.ที่ปรึกษาหลัก : ผศ. ดร.อัญชิษฐา สัจจารักษ์, อ.ที่ปรึกษาร่วม : ศ. ดร.ลินดา อี. แกรม

ไก หรือ สาหร่ายไก คือ สาหร่ายสีเขียวสกุล *Cladophora* ที่กระจายพันธุ์จำนวนมากในภาคเหนือของประเทศไทย นิยมใช้เป็นอาหารและยา ในธรรมชาตินั้นสาหร่าย *Cladophora* ทำหน้าที่เสมือนนิเวศวิศวกร (ecological engineer) โดยปรับแต่งและรักษาสภาพของแหล่งที่อยู่อาศัยด้วยการผลิตออกซิเจนและให้ที่อยู่อาศัยแก่สิ่งมีชีวิตทั้งโพรแคริโอตและยูแคริโอตที่อาศัยอยู่บนพื้นผิวของสาหร่ายซึ่งเรียกว่าจุลชีวภาพอิงอาศัย (epiphytic microbiota) และอาจมีบทบาทสำคัญต่อระบบนิเวศเช่นกัน การศึกษาจุลชีวภาพอิงอาศัยในสาหร่าย *Cladophora* ในอดีตมีเพียงการศึกษาในสหรัฐอเมริกาเท่านั้น เพื่อเพิ่มองค์ความรู้จุลชีวภาพอิงอาศัยในสาหร่าย *Cladophora* นั้นการศึกษานี้จึงมีวัตถุประสงค์ในการศึกษาจุลชีวภาพอิงอาศัยในสาหร่าย *Cladophora* จากแม่น้ำน่าน จังหวัดน่าน ประเทศไทย โดยใช้ amplicon-based metagenomic analysis จากการศึกษาพบยูแคริโอตจำนวน 575 สกุลและแบคทีเรียจำนวน 698 สกุล โดยมีแบคทีเรีย 227 สกุลปรากฏในทุกพื้นที่ที่เก็บตัวอย่างในการศึกษา แบคทีเรียที่มีความหลากหลายเหล่านี้มีหน้าที่เกี่ยวข้องในกระบวนการต่าง ๆ เช่น การย่อยสลายเซลลูโลส การสังเคราะห์วิตามินบี 12 และการตรึงไนโตรเจน ทั้งนี้จากการเปรียบเทียบข้อมูลจากการศึกษานี้และการศึกษาก่อนหน้าพบแบคทีเรียร่วม 17 สกุลที่ปรากฏในทั้งการศึกษานี้และการศึกษาในสหรัฐอเมริกา จากการอนุมานบทบาทของแบคทีเรียจากชื่อสกุลพบว่าแบคทีเรียที่พบร่วมกันเหล่านี้อาจมีบทบาทสำคัญต่อในกระบวนการต่าง ๆ เช่น การสังเคราะห์วิตามินบี 12 (*Flavobacterium* และ *Pseudomonas*) การหลั่ง extracellular polymeric substance (*Terrimonas*) และ ดีไนตริฟิเคชัน (*Acidovorax* และ *Methylotenera*) ผลการศึกษานี้ช่วยสร้างความเข้าใจต่อองค์ประกอบทางอนุกรมวิธาน (taxonomic composition) และการอนุมานหน้าที่ทางนิเวศ (putative ecological function) ของจุลชีวภาพอิงอาศัยในสาหร่าย *Cladophora* sp.

สาขาวิชา พฤกษศาสตร์
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ลายมือชื่อนิสิต ....
ลายมือชื่อ อ.ที่ปรึกษาหลัก ....
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Karnjana Ruen-pham : EPIPHYTIC MICROBIOTA OF KAI *Cladophora* sp. FROM NAN RIVER, NAN PROVINCE, THAILAND. Advisor: Asst. Prof. ANCHITTHA SATJARAK, Ph.D. Co-advisor: Prof. Linda E. Graham, Ph.D.

Cladophora is a cosmopolitan green algal genus in northern Thailand, commonly known as Kai or Sa Rai Kai, which the local people use for culinary and medicinal purposes. In nature, *Cladophora* acts as an ecological engineer in the ecosystem by creating, modifying, and maintaining the habitat by providing oxygen and microhabitats for several organisms, including prokaryotes and eukaryotes, namely epiphytic microbiota. These epiphytic microbiota living on the algal host's surface might exhibit crucial roles in the ecosystem. However, most understandings of the *Cladophora* microbiota were from the USA. Therefore, this study aims to investigate the *Cladophora* microbiota collected from Nan River, Nan Province, Thailand, by using amplicon-based metagenomic analysis. Results revealed a total of 575 eukaryotic genera and 698 bacterial genera, which were present variedly in the study sites. Among the identified bacterial taxa, 227 genera were shared taxa, taxa commonly present in all sites. Some were highly abundant taxa, which might be involved in various processes, e.g., cellulose degradation, vitamin B_{12} biosynthesis, and nitrogen fixation. Among the shared taxa, 17 genera were core taxa, taxa present in *Cladophora* microbiota of this and other previous studies. Some were bioinformatically inferred to play crucial roles in freshwater *Cladophora* microbiome, e.g., vitamin B_{12} biosynthesis (*Flavobacterium* and *Pseudomonas*), extracellular polymeric substance secretion (*Terrimonas*), and denitrification (*Acidovorax* and *Methylotenera*). These findings allow us to understand more of the taxonomic composition and the putative ecological functions of *Cladophora* sp. microbiome.

Field of Study:	Botany	Student's Signature ....
Academic Year:	2021	Advisor's Signature ....
		Co-advisor's Signature ....

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CHAPTER I
INTRODUCTION

1.1 Background and rationales

Cladophora is a cosmopolitan green algal genus widely distributed in diverse environments across arctic and tropical regions. The algal genus was established in 1843 by Friedrich Traugott Kützing (Munir et al., 2019). The unique characteristics are dichotomous branching filaments and reticulate plastid. In the filament, the apical or intercalary cells divide the lateral branches below the septums between cells, resulting in a high-surface-area of *Cladophora*. The attachment organ that holds the algal body to the substrate is called rhizoid or holdfast. This organ includes branched filaments that grow from basal cells or other cells in the basal region. If not attached to the substrate, *Cladophora* freely floats on the water surface like a mat or tuft (Dodds & Gudder, 1992; Michalak & Messyasz, 2021; Setchell & Gardner, 1920; van den Hoek, 1963; Zulkifly et al., 2013).

In Thailand, *Cladophora* is widely present in most of the regions of the country, if not all (e.g., Laungsuwon & Chulalaksananukul, 2014; Peerapornpisal et al., 2005; 2006; Satjarak et al., 2021; Surayot et al., 2016; Thiamdao et al., 2012; Tsutsui et al., 2015; Yarnpakdee et al., 2021). However, the study of *Cladophora* has long been focused on the alga present in the Nan River, where it abundantly appears as turfs attached to substrate underwater or freely floated on the water surface in the dry season during November and March (e.g., Buntha et al., 2020; Peerapornpisal et al., 2005; 2006; Thiamdao et al., 2012). This green alga is known as Kai or Sa Rai Kai and is traditionally used for medicine and food as side dishes, e.g., seasoned ground paste known as Kai Yee and seasoned crisps known as Kai Phan (Peerapornpisal et al., 2005).

In terms of ecology, *Cladophora* acts as an ecological engineer, an organism that creates, modifies, and maintains the habitat by providing oxygen and microhabitats for several organisms, including prokaryotes and eukaryotes, namely epiphytic microbiota (Michalak & Messyasz, 2021; Zulkifly et al., 2012). To investigate the taxonomic diversity of these epiphytic microbiota, classical approaches, such as morphological observation under light microscope and cultured-dependent methods, have been employed. However it has been estimated that only approximately 1% of the diversity can be cultivated in the laboratory, and thus the majority of the taxa were not known (Hugenholtz & Tyson, 2008; Riesenfeld et al., 2004). The advancement of sequencing technology, e.g., next-generation sequencing technology, thus plays an important role in overcoming these limitations. By means of metagenomic sequencing, the techniques capture the DNA of the microbiota, which allows us to infer the taxonomic diversity by comparing the sequenced DNA data to the appropriate taxonomic references.

Currently, there are two main approaches for metagenomic studies – shotgun and amplicon methods. Shotgun method captures all the DNA present in the sample and thus is usually high-priced. Another method is the amplicon method, which selectively captures the regions of interest, typically hypervariable regions of marker genes that can be used for taxonomic classification. Therefore, this amplicon method is more economical and is suitable for studies focusing on the taxonomic diversity of the microbiota (Levy et al., 2018; Ranjan et al., 2016).

At the current time, the epiphytic microbiota of *Cladophora* has been studied in only a few places, i.e., Lake Michigan and Lake Mendota, USA (Braus et al., 2017; Chun et al., 2017; Graham et al., 2015; Zulkifly et al., 2012) and the epiphytic microbiota of *Cladophora* in Thailand has not been explored. Therefore, this work aims to study the taxonomic diversity of epiphytic microbiota of Kai *Cladophora* sp. from Nan River, Nan Province, Thailand, using amplicon-based metagenomic analysis. The results from this study will help us to understand the taxonomic composition and putative ecological functions of Kai *Cladophora* sp. microbiota.

1.2 Objective

This study aims to study the taxonomic diversity of epiphytic microbiota of Kai *Cladophora* sp. from Nan River, Nan Province, Thailand.

CHAPTER II

LITERATURE REVIEW

2.1 Genus *Cladophora* Kützing

Genus *Cladophora* Kützing (1843) is a cosmopolitan green alga widely distributed in freshwater, marine, and brackish shoreline habitats across arctic and tropical regions (Cambridge et al., 1990; Hayakawa et al., 2012; van den Hoek, 1979; Whitton, 1970). The unique morphological characteristics of genus *Cladophora* are filamentous, monosiphonous, branched, and multinucleated. Each cell contains parietal or reticulate chloroplast with numerous pyrenoids. Either or both apical and intercalary cells usually divide the lateral branch just below the septum between cells by forming an oblique cross wall, resulting in dichotomous branching. Asexual reproduction occurs via fragmentation, production of the four-flagellated zoospores from zoosporangia, and production of akinete (thick-wall resting cells) when subjected to unfavorable conditions. For sexual reproduction, fertilization of the two-flagellated isogametes forms the zygote. The attachment organ that holds the algal body to the substrate is called rhizoid or holdfast, which grows from basal cells or other cells in the basal region. Various substrates have been recorded, e.g., rock, bedrock, boulder, cobble, gravel, wood dock, plants, and shells of *Dreissena* species. When not attached to those substrates, this alga freely floats on the water surface as a mat, packed patch, or tuft (Dodds & Gudder, 1992; Michalak & Messyasz, 2021; Setchell & Gardner, 1920; van den Hoek, 1963; Zulkifly et al., 2013). Over the past 200 years, 600 species have been classified as genus *Cladophora*, whereas 400 species were re-classified as synonyms. Nowadays, 198 species are taxonomically accepted (Guiry & Guiry, 2022; van den Hoek, 1963, 1979).

2.2 *Cladophora* sp. from Nan River

In northern Thailand, the Nan River is one of the main tributaries of the Chao Phraya River that flows from the northern part of the country toward the central plain, where it joins with the three other main tributaries, Ping River, Wang River, and Yom River, in Nakhon Sawan Province (Figure 2.1). The Nan River flows through Nan, Uttaradit, Phitsanulok, Phichit, and Nakhon Sawan Provinces, where Nan Province accounts for one-third of the course of the river (Nusit et al., 2019; Trisurat et al., 2019).

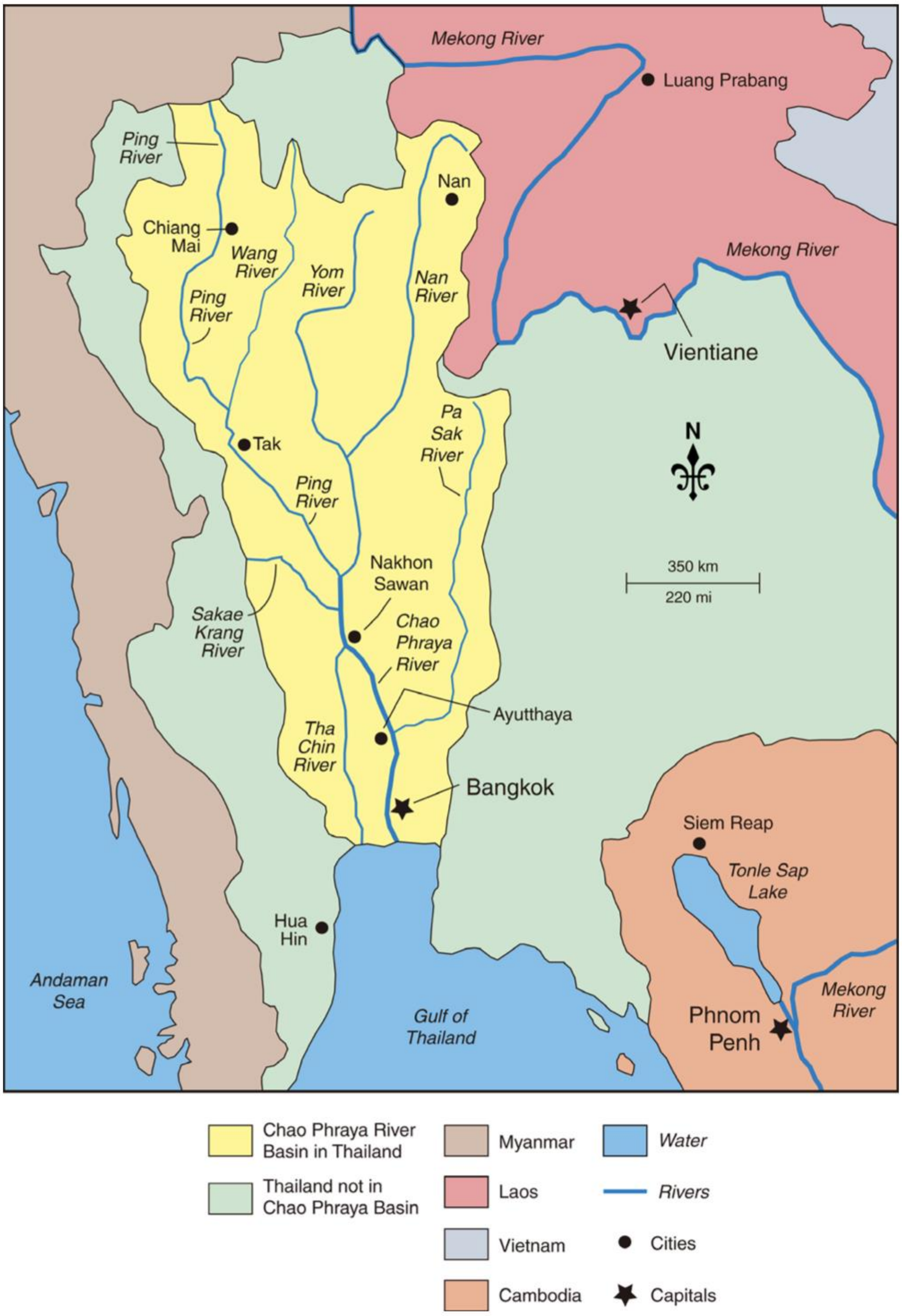


Figure 2.1 Rivers and tributaries in Thailand.

Map created by Mic Greenberg and taken from Olson and Kreznor (2021).

Cladophora bloom present as green turfs attached to substrates underwater or freely floated on the water surface in Nan River, Nan Province, in the dry season during November and March (Buntha et al., 2020; Peerapornpisal et al., 2005; Thiamdao et al., 2012). The alga is locally known as Kai or Sa Rai Kai, which the local people use for culinary purposes in various cuisines, e.g., Kai with curry paste, ground and seasoned Kai known as Kai Yee, crispy and seasoned Kai known as Kai Phan, Kai noodles, Kai cracker biscuits, and Kai butter cakes.

The toxicological evaluation showed that *Cladophora* growing in clean water is safe to consume. Analysis showed that the alga is highly nutritious. It is a good source for carbohydrates, pigments, fatty acids, phenolic compounds, and vitamins (Prazukin et al., 2020). Local people also use Kai for medicinal purposes, e.g., rejuvenation, induction of appetite, soothing stomach ulcers, and expediting recovery from several common disorders (Fahprathanchai et al., 2006; Peerapornpisal et al., 2005). However, the practice is area-specific and is much less popular in the newer generations.

The presence of the algal bloom and its usage by local people initiated various scientific research questions. Studies reported that extraction from *C. glomerata* exhibited anti-gastric ulcer, anti-inflammatory, analgesic, hypotensive, anticancer, antidiabetic, and antioxidant activities. *C. glomerata*, thus, could be a potential source of 4.16 materials used as therapeutic agents (Khuantrairong & Traichaiyaporn, 2009; Laungsuwon & Chulalaksananukul, 2013; Peerapornpisal et al., 2006; Srimaroeng et al., 2015; Suanmali et al., 2017). The high cellulose content in the *Cladophora* cell wall could be a material for bioethanol production (Boonprab et al., 2018). Also, these turfs of *C. glomerata* aided in wastewater treatment by removing nutrients, such as phosphorus (Khuantrairong & Traichaiyaporn, 2011, 2012).

In contrast to the presence of various applications of the alga, these studies based the algal taxonomy on only one taxonomic study done by Thiamdao et al. (2012). In the study, they explored the diversity of the *Cladophora* species present in the Nan River and suggested that only one species of *Cladophora*, which was *C. glomerata*, was present in the Nan river basin. However, their maximum likelihood estimation suggested that the amplified DNA region used in their analysis exhibited high variability in the DNA sequences of the resolved clades. In this study, the *C. glomerata* samples collected from Nan were not resolved as a single clade but were separately placed within clades consisting of *C. glomerata* collected from other river basins. They also suggested that the algal sequences showed a high variability when compared among samples. However, the sequences were not deposited in the public repository, and thus further tests could not be performed. For this reason, sampling and annotating the algal host by performing phylogenetic analysis of marker genes employed in this study would provide crucial information for the study of genus *Cladophora* in Thailand.

2.3 Metagenomic analysis

To study the composition of the microbiota, the classical approach, i.e., culturing technique, has mainly been used in the laboratory. Although the technique is beneficial as researchers obtain all the surviving isolated microbial taxa, it has been estimated that only approximately 1% of the microbial diversity can be cultivated in the laboratory when using standard culturing techniques (Amann et al., 1995). Thus, most of the microorganisms in the environmental samples were not discovered, and a more advanced technique was needed to investigate the unknown species. Consequently, metagenomic sequencing was introduced. This technique captures DNA of microbiota in the environmental samples, which allows us to infer the diversity by comparing the sequenced DNA data to the appropriate taxonomic references.

Formerly, the traditional metagenomic approach was cultivation-dependent and used to identify genes of interest or novel microorganisms. The first step of this method is to extract all microbial DNA from the environmental samples, representing the collection of DNA of all environmental microbes. Then, the DNA libraries are constructed using restriction enzymes or mechanical shearing. The DNA fragments are then inserted into an appropriate vector system depending on library size. The DNA vectors will later be transformed into suitable hosts as recombinant vectors. Then, this set of hosts will be further screened using functional and sequence-based approaches with different random vectors. Therefore, this traditional metagenomic approach requires culture-dependent techniques and cannot capture the entire microbial community, namely, microbiota residing in the environmental samples (Daniel, 2005; Hugenholtz & Tyson, 2008; Mande et al., 2012; Riesenfeld et al., 2004).

The advancement of sequencing technology, especially next-generation sequencing technology, thus plays an essential role in overcoming the limitations of culture-dependent techniques. This next-generation sequencing technology helps to obtain the information of the uncultured epiphytic microbiota by two main approaches – shotgun and amplicon methods. The shotgun method captures all the DNA present in the sample by sequencing all the extracted metagenomic DNA and thus provides us both the taxonomic diversity and functional diversity of the microbiota. However, it requires a laborious analysis of extensive, complex, and complicated information and is comparatively high-priced. Another method is the amplicon method, which selectively captures the regions of interest, typically the hypervariable regions of marker genes, by utilizing the universal primers and polymerase chain reactions. The amplicons of the marker genes are then sequenced and used for taxonomic classification by comparing against the appropriate databases. Examples of the widely used universal gene markers are regions of small subunit ribosomal ribonucleic acid (SSU; 16S rRNA and 18S rRNA genes), large subunit ribosomal ribonucleic acid (LSU; 23S rRNA and 23S rRNA genes), and the internal transcribed spacer (ITS) region. By sequencing only the selected regions, i.e., the amplicon product, this amplicon method thus is more economical and is suitable for studies focusing on the taxonomic diversity of the microbiota (De Filippis et al., 2017; Levy et al., 2018; Ranjan et al., 2016; Sharpton, 2014).

2.4 Previous studies of *Cladophora* metagenomic analysis

Previously, the epiphytic microbiota of *Cladophora* has been studied in only a few places, i.e., studies from Lake Michigan and Lake Mendota, USA. The study of taxonomic diversity of the epiphytic microbiota of *Cladophora* using metagenomic approach was firstly done in 2011, which was reported by Zulkifly et al. (2012). In the study, they used 16S amplicon analysis to investigate the microbiota of *Cladophora* present in Lake Mendota. In the study, they found 99 unique bacterial genera belonging to classes Betaproteobacteria, Alphaproteobacteria, and Gammaproteobacteria. Then, in the following year, additional information of the *Cladophora* microbiota from Lake Mendota was obtained by using the shotgun method and was published by Graham et al. (2015). All together, the amplicon and the shotgun metagenomic studies bioinformatically suggested the presence of various bacteria, protists, fungi, and metazoans that might provide key biogeochemical and ecological functions, including cobalamin (vitamin B_{12}) biosynthesis, methane oxidation, cellulose degradation, and nitrogen fixation.

Naturally, *Cladophora* requires vitamin B_{12} for survival (Hoflmann, 1990), but this alga cannot produce the vitamin itself. The source of this vitamin for *Cladophora* was not known until Zulkifly et al. (2012) revealed the presence of vitamin B_{12}-producing bacterial genera *Flavobacterium*, *Cetobacterum*, and *Plesiomonas shigelloides* in the *Cladophora* microbiome. Later, this finding was supported by the presence of enzymes in aerobic and anaerobic vitamin B_{12} synthesis pathways in the shotgun analysis by Graham et al. (2015). The presence of these vitamin B_{12}-producers within the microbiome fosters not only the *Cladophora* host but also other epiphytic vitamin B_{12}-requiring microbes, e.g., methanotrophs (Graham et al., 2015).

The algal habitat for these two studies was Lake Mendota, a freshwater habitat known to house methanogens, bacteria that produce methane, one of the strongest greenhouse gases. To reduce the amount of methane, a bacterial group namely methanotroph is of interest because these methanotrophs can oxidize methane, resulting in carbon dioxide and oxygen. Results from Zulkifly et al. (2012) showed that the aerobic methanotrophs – *Methylotenera*, *Methylibium*, and *Methylobacter* were present in the *Cladophora* microbiome. The presence of these methanotrophs contributed to two main functions in the microbiome. Firstly, they reduce the amount of methane in the habitats by oxidizing methane using oxygen gas released as the by-product from photosynthesis of the *Cladophora* host. In the meantime, by using the oxygen gas released by the alga, the methanotrophs also reduce the risks of photorespiration in the alga.

Another biochemical function of the epiphytic microbes is cellulose degradation. One good source of cellulose is the algal cell wall, which contains a high cellulose content. Results from Zulkifly et al. (2012) suggested the presence of cellulose-degrading bacteria, e.g., *Byssoorax* and *Sorangium*. In congruence, Graham et al. (2015) revealed additional cellulose-degrading bacteria, fungi, and protists, and their 25 putative cellulose-degrading enzymes (cellulases). Overall, the massive growth of *Cladophora* (representing the carbon source of the habitat) and the presence of cellulase sequences suggested that the alga-microbe interaction plays an essential role in the carbon cycling process within the microbiome. Lastly, nitrogen fixation was recognized by the presence of nitrogenase (*nif*H) genes and nitrogen-fixing bacteria – *Dechloramonas*, *Blastobacter*, and *Devosia* (Zulkifly et al., 2012).

The work published by Zulkifly et al. (2012) and Graham et al. (2015) initiated Braus et al. (2017) to investigate the spatiotemporal dynamics of the bacterial microbiota of *Cladophora* present in Lake Mendota. In the study of Braus et al. (2017), they used the universal primers to target the hypervariable region of 16S rRNA gene to capture the bacterial taxonomic diversity of the algal microbiome in three different sites of the lake during six different times in 2014. Results showed that the taxa present within the *Cladophora* microbiota were similar to that of Zulkifly et al. (2012) and Graham et al. (2015). However, the diversity and the abundance of bacteria were different when compared among collecting sites and times of the year.

In addition to Lake Mendota, another place where *Cladophora* abundantly grows is Lake Michigan. Studies show that the alga collected from Lake Michigan also harboured various kinds of microbes that also displayed dynamics of diversity and abundance (Byappanahalli et al., 2019; Chun et al., 2017). These microbes were hypothesized to be involved in many biochemical activities. Among these, nitrogen fixation was their main interest as one of the studies performed shotgun method to capture the taxonomic diversity and additionally performed amplicon method to capture the region of nitrogenase (*nifH*) gene. Results showed that not only the non-photosynthetic bacteria living in the algal microbiota that can fix nitrogen molecules but also organisms belonging to other lineages, such as cyanobacteria and archaea (Byappanahalli et al., 2019).

CHAPTER III
MATERIALS AND METHODS

This work was divided into two parts, including fieldwork and laboratory work. For the fieldwork, fresh algal samples were collected from Nan River, Nan Province, Thailand. Then the preserved algal samples were later processed in the laboratory at the Plants of Thailand Research Unit, Department of Botany, Faculty of Science, Chulalongkorn University.

3.1 Materials

3.1.1 Sample collection

- Whirl-Pak® (Nasco, USA)
- DNA/RNA Shield™
- Nitrile disposable gloves
- Cooler box

3.1.2 Identification of the *Cladophora* host

- Compound microscope: ECLIPSE E100 microscope (Nikon Corporation, Japan)
- PCR machine: PTC-100™ Programmable Thermal Controller (MJ Research Inc, USA)
- Green PCR Master Mix Direct-Load (biotechrabbit, Germany)
- Micropipette: Pipette One Touch™ Single-channel (Biosigma, Italy)
- Primers (Macrogen, Inc., South Korea)

  SB1F (5’–GATTAAGCCATGCATGTSTARG–3’)
  SB1R (5’–CGTYCGTTACCGGAATCAACCTGAC–3’)
  p23SrV_f1 (5’–GGACAGAAAGACCCTATGA– ‘3)
  p23SrV_r1 (5’–TCAGCCTGTTATCCCTAGAG– ‘3)
  C1 (5’–ACCCGCTGAATTTAAGCATAT– ‘3)
  D2 (5’–TCCGTGTTTCAAGACGG– ‘3)
  CladoITS-9F (5’–CCGCCCGTCGCTCCTACCGATTGGGTGTG– ‘3)
  CladoITS-7R (5’–TCCCTTTTCGCTCGCCGTTACTA– ‘3)

3.1.3 Study of the diversity of epiphytic microbiota of Kai *Cladophora* sp.

- Compound microscope: ECLIPSE E100 microscope (Nikon Corporation, Japan)
- DNA/RNA Shield™
- Quick-DNA™ Fecal/Soil Microbe Kits (Zymo Research, USA)
- Nanodrop Spectrophotometers (Nanodrop Technologies, USA)

3.2 Methods

3.2.1 Literature review

The related literature was searched through an online database. In addition, the four physical parameters of Nan River, including Water Quality Index (WQI), Dissolved Oxygen (DO), Biological Oxygen Demand (BOD), and ammonia nitrogen content, were obtained from the Regional Environmental Office 2 (Lampang) (http://www.reo02.mnre.go.th/th/index; accessed on 27 August 2021). These physical parameters were collected from four collecting sites as follows (Figure 3.1).

1) Site NR1 located at Thung Chang district (19°25'37.1"N 100°52'50.7"E)
2) Site NR2 located at Tha Wang Pha district (19°07'31.2"N 100°48'38.5"E)
3) Site NR4 located at Mueang Nan district (18°46'32.0"N 100°46'44.8"E)
4) Site NR6 located at Wiang Sa district (18°34'13.2"N 100°45'17.5"E)

Moreover, the total water level and rainfall of Nan River were obtained from the telemetering system of the Electricity Generating Authority of Thailand (EGAT) (https://watertele.egat.co.th). These parameters were collected from three collecting sites as follows (Figure 3.1).

1) Site NR3 located at Tha Wang Pha district (19°00'54.3"N 100°46'51.1"E)
2) Site NR5 located at Mueang Nan district (18°46'27.8"N 100°46'39.1"E)
3) Site NR7 located at Wiang Sa district (18°31'24.2"N 100°46'33.8"E)

3.2.3 Sample collection

Sample collections were done during the late growing season in March 2020. Five collecting sites were selected along Nan River, Nan Province, Thailand as follows (Figure 3.1).

1) Site CKD located at Chiang Klang district (19°13'34.0"E 100°49'22.8"N)
2) Site PUA located at Pua district (19°09'37.4"E 100°48'39.0"N)
3) Site TD1 located at Tha Wang Pha district (19°07'00.8"E 100°48'11.9"N)
4) Site TD2 located at Tha Wang Pha district (19°02'18.8"E 100°46'56.2"N)
5) Site MND located at Mueang Nan district (18°58'52.1"E 100°46'36.3"N)

Four replicates of the algal samples were collected from each site. To prevent contamination in the fieldwork, disposable nitrile gloves were worn during the sample collection. The fresh algal samples were collected and submerged in their environmental water in the sterile Whirl-Pak® sampling bags (Figure 3.2). Additionally, some of the algal filaments from each replicate were carefully separately stored in microcentrifuge tubes by submerging in DNA/RNA Shield™ for later DNA extractions. Then, all samples were kept in the cooler box during transportation.

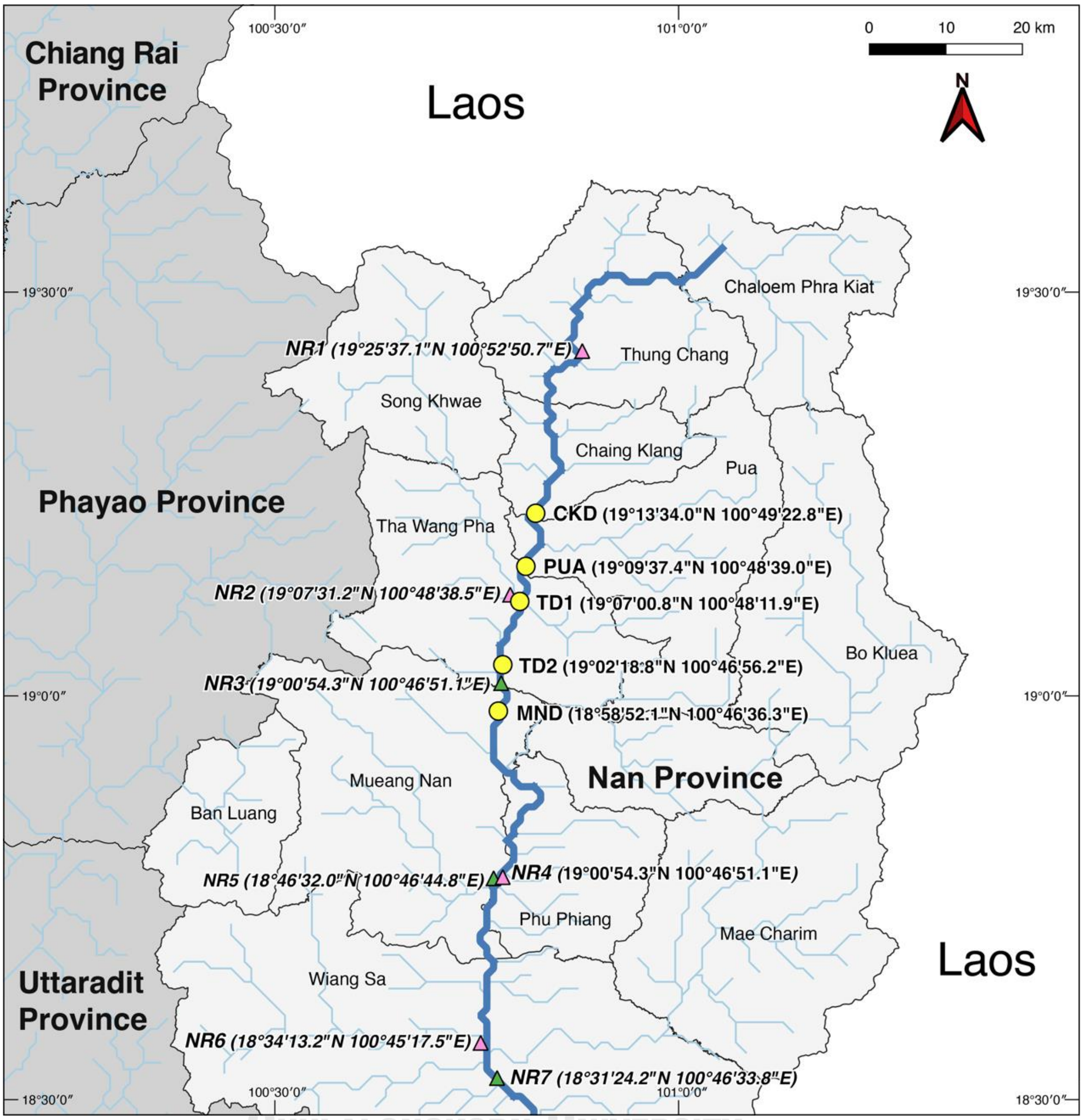


Figure 3.1 Algal sampling sites and locations of reported water quality along the Nan River, Nan Province, Thailand.

The river network was derived from HydroSHEDS (Lehner et al., 2008). The thick blue line represents the Nan River of Nan Province, where the water flows from the northern to the southern part of Nan Province. Thinner blue lines represent other smaller rivers. Yellow circles represent algal sampling sites. Triangles represent sites with reported water physical quality, where pink triangles were locales reported by the Regional Environmental Office 2 (Lampang), and green triangles were locales reported by the telemetering system of the Electricity Generating Authority of Thailand.

Figure 3.2 Four replicates of fresh algal samples collected from a sampling site stored in sterile Whirl-Pak® sampling bags.

3.2.3 Identification of the *Cladophora* host

Morphological identification of the Cladophora *host*

To identify the *Cladophora* host, the algal samples were transferred to sterile Petri dishes. Some of the algal filaments were picked using sterile forceps and washed three times to remove loosely attached materials. The washed samples were then observed under an ECLIPSE E100 compound light microscope and identified based on their morphology to the genus level using "How to know the freshwater algae" (Prescott, 1964). Only the samples identified as *Cladophora* were further studied, and the non-*Cladophora* samples were discarded.

DNA extraction and amplification of marker genes

Samples morphologically identified as genus *Cladophora* were chosen for the downstream analysis. The corresponding samples stored in DNA/RNA Shield™ were washed three times using DNA/RNA Shield™ to remove loosely attached materials. The algal filaments were then morphologically identified for reassurance. Then, the total genomic DNA was extracted using Quick-DNA™ Fecal/Soil Microbe Kits.

This study used four conserved regions as marker genes, including 18S rRNA gene, 23S rRNA gene, 28S rRNA gene, and ITS1–5.8S–ITS2 (ITS) region. Each conserved region was amplified using Green PCR Master Mix Direct-Load and primer pair (Table 3.1) in a PTC-100™ PCR machine with suitable thermal cycles as follows.

18S rRNA gene was amplified using primers SB1F and SB1R (Thiamdao et al., 2012) with an initial denaturation step at 94°C for 4 min, followed by 35 cycles of 30 sec at 95°C, 30 sec at 56°C, and 1 min at 72°C, with a final extension at 72°C for 6 min.

23S rRNA gene was amplified using primers p23SrV_f1 and p23SrV_r1 (Sherwood & Presting, 2007) with an initial denaturation step at 94°C for 4 min, followed by 35 cycles of 20 sec at 94°C, 30 sec at 55°C, and 30 sec at 72°C, with a final extension at 72°C for 10 min.

28S rRNA gene was amplified using primers C1 and D2 (Zhu et al., 2018) with an initial denaturation step at 94°C for 5 min, followed by 31 cycles of 30 sec at 94°C, 30 sec at 57°C, and 30 sec at 72°C, with a final extension at 72°C for 5 min.

ITS region was amplified using primers CladoITS-9F and CladoITS-7R (Zhu et al., 2018) with an initial denaturation step at 94°C for 1 min, followed by 30 cycles of 10 sec at 98°C, 30 sec at 65°C, and 2 min at 68°C, with a final extension at 72°C for 10 min.

Table 3.1 Primer sets for *Cladophora* marker genes amplification used in this study.

Marker gene	**Primer name**	**Primer sequence**	**Referenc**
18S rRNA	SB1F	F: 5’–GATTAAGCCATGCATGTSTARG–3’	Thiamdao et al., 2012
	SB1R	R: 5’–CGTYCGTTACCGGAATCAACCTGAC–3’	
23S rRNA	p23SrV_f1	F: 5’–GGACAGAAAGACCCTATGA–3’	Sherwood & Presting, 2007)
	p23SrV_r1	R: 5’–TCAGCCTGTTATCCCTAGAG–3’	
28S rRNA	C1	F: 5’–ACCCGCTGAATTTAAGCATAT–3’	Zhu et al., 2018
	D2	R: 5’–TCCGTGTTTCAAGACGG–3’	
ITS region	CladoITS-9F	F:5’–CCGCCCGTCGCTCCTACCGATTGGGTGTG–3’	Zhu et al., 2018
	CladoITS-7R	R: 5’–TCCCTTTTCGCTCGCCGTTACTA–3’	

The amplified PCR products were then sequenced using Sanger sequencing technology done by Macrogen, Inc. (Seoul, South Korea). To check the quality of amplified sequences, the raw reads were trimmed using 4Peaks v 1.8 software (http://nucleobytes.com/index.php/4peaks) to remove the nucleotide positions whose Phred quality scores were less than 25. Then, the trimmed sequences were compared to the NCBI non-redundant nucleotide database (accessed on 20 April 2021) using BLASTN (Altschul et al., 1990). Only sequences that returned the BLASTN search results as *Cladophora* were used for downstream phylogenetic analysis.

Phylogenetic estimation of Cladophora *host using 18S, 23S, and 28S rDNAs, and ITS reads sequenced by Sanger sequencing*

To estimate the relationship of the *Cladophora* host collected in this study, *Cladophora* 18S rDNA, 23SrDNA, 28S rDNA, and ITS region sequences were acquired from NCBI GenBank (accessed and retrieved on 20 April 2021 [Table B.1–4]). The nomenclatures of the downloaded sequences were re-evaluated by searching in AlgaeBase (http://www.algaebase.org/; accessed on 30 April 2021) to make sure that the sequence names were updated to the current taxonomic classification.

Sequences belonging to each corresponding conserved DNA region were aligned using MAFFT alignment v 7.490 (Katoh et al., 2009). Then, the substitutional model of each alignment matrix was assessed using jModelTest2 v 2.1.6 (Darriba et al., 2012) before performing maximum likelihood analysis using RAxML-NG (Kozlov et al., 2019) via the web-server available at https://raxml-ng.vital-it.ch. The substitution models used in this study included TIM2+G for 18S rDNA, TPM1uf+G for 23S rDNA, HKY+G for 28S rDNA, and TIM3+G for ITS. Additional Bayesian framework for all marker genes was performed using MrBayes v 3.2.7a (Ronquist et al., 2012) available on the CIPRES XSEDE Portal (Miller et al., 2012). Four independent chains were run for 1,000,000 cycles, and consensus topologies were calculated after 25,000 burn-in cycles. Then, the trees were visualized using FigTree v. 1.4.4 (Rambaut, 2018).

Additional phylogenetic estimation of Cladophora *sp. using amplicons from the universal 18S rDNA primers and Illumina Miseq sequencing*

The presence of ambiguity in the sequenced amplicons and the limited number of reference sequences made it impossible to compare the taxonomy of the *Cladophora* host across the sampling sites. Therefore, to investigate whether the collected *Cladophora* samples were from the same algal population, the sequences of the 18S rDNA regions amplified by the universal 18S rDNA primers in the metagenomic analysis were compared and used for phylogenetic estimation, as described in the previous step.

3.2.4 Study of the diversity of epiphytic microbiota of Kai *Cladophora* sp.

3.2.4.1 Morphological identification

Similarly, only samples from *Cladophora*-dominant sites were observed for the microscopic organisms under the compound light microscope. At this step, the observed microbes were identified to genus level using suitable identification keys based on the lineages of the organisms. The keys included “Key to some common freshwater protozoa” (Johnson, 1956), “An annotated key to the identification of commonly occurring and dominant genera of algae observed in the phytoplankton of the United States” (Greeson, 1982), “Freshwater algae in Northwest Washington” (Matthews, 2016), and “Diatom taxonomy and identification keys” (Blanco, 2020).

3.2.4.2 Taxonomic identification using amplicon-based metagenomic analysis

DNA extraction, marker gene amplification, and sequencing

The metagenomic DNA was extracted using Quick-DNA™ Fecal/Soil Microbe Kits. The quality and quantity of the extracted DNA were assessed using Nanodrop Spectrophotometers. Then, the taxonomic identification of the microbiota was assessed using the amplicon metagenomic approach. In this study, the amplicons of 16S rRNA, 18S rRNA, and ITS marker genes were amplified by the universal primer sets (Table 3.2) and thermal cycles as follows.

16S rRNA region V3–V4 was amplified using primers 341F and 805R (Klindworth et al., 2013) with an initial denaturation step at 94°C for 3 min, followed by 25 cycles of 20 sec at 98°C, 30 sec at 55°C, and 30 sec at 72°C, with a final extension at 72°C for 5 min.

18S rRNA region V4 was amplified using primers Reuk454FWD1 and V4r (Bradley et al., 2016) with an initial denaturation step at 98°C for 2 min, followed by 28 cycles of 20 sec at 98°C, 30 sec at 50°C, and 30 sec at 72°C, with a final extension at 72°C for 1 min.

ITS region was amplified using primers ITS-1F and ITS-2R (White et al., 1990) with an initial denaturation step at 94°C for 3 min, followed by 25 cycles of 20 sec at 98°C, 30 sec at 60°C, and 30 sec at 72°C, with a final extension at 72°C for 5 min.

Table 3.2 Primer sets for 16S rRNA, 18S rRNA, and ITS amplicon analyses.

Marker gene	**Primer name**	**Primer sequence**	**Reference**
16S rRNA region V3–V4	341F	F: 5’–CCTACGGGNGGCWGCAG–3’	Klindworth et al., 2013
	805R	R: 5’–GACTACHVGGGTATCTAATCC–3’	
18S rRNA region V4	Reuk454FWD1	F: 5’–CCAGCASCYGCGGTAATTCC–3’	Bradley et al., 2016
	V4r	R: 5’–CCTTCYGCAGGTTCACCTA–3’	
ITS region	ITS-1F	F: 5’–CTTGGTCATTTAGAGGAAGTAA–3’	White et al., 1990
	ITS-2R	R: 5’–GCTGCGTTCTTCATCGATGC–3’	

The 16S, 18S, and ITS amplicons were amplified, purified, indexed, and 250 bp paired-end sequenced using an Illumina MiSeq at Omics Sciences and Bioinformatics Center (Chulalongkorn University, Bangkok, Thailand). The raw reads are available under NCBI BioProject PRJNA761577 and BioSamples SAMN21356006 (CKD), SAMN21356007 (PUA), and SAMN21356008 (TD1).

Read propressing

The 250 paired-end raw reads were accessed for quality using FastQC (Andrews, 2010), as shown in figure C.1A. Then, the low-quality nucleotides were removed by using Trimmomatic v. 0.39 (Bolger et al., 2014) with the parameter

SLIDINGWINDOW:4:30, where the software scanned the nucleotide reads as a four-base wide sliding window and trimmed the sequence by removing the nucleotide positions starting at the window where the average quality within dropped below 30 of the Phred quality score. Then, FastQC was used again to re-check the quality of the trimmed reads (Figure C.1B).

Taxonomic classification

To annotate the taxa present in the *Cladophora* microbiota, the sequenced amplicons were compared to their appropriate reference databases. 16S and 18S amplicons were compared against the SILVA ribosomal RNA gene database release 138.1 (Quast et al., 2013) using SILVA next-generation sequencing (SILVAngs) analysis platform. In this automatic software pipeline, the input reads were aligned using SILVA INcremental Aligner (SINA) (Pruesse et al., 2012). In this step, only the 16S and 18S amplicons that were highly similar to the reference sequences were retained. Reads that did not meet the alignment criteria were all discarded from the data set. The discarded reads included reads shorter than 50 aligned nucleotides, reads with ambiguities greater than 2%, reads with homopolymer greater than 2%, and reads with low aligned quality reads (aligned reads with identity value under 50% and alignment score under 40).

The reads that passed this alignment step were clustered into Operational Taxonomic Units (OTUs) by using cd-hit-est (Li & Godzik, 2006) with the parameters accurate mode, ignoring overhangs, and assigning identity criteria of 1.00 and 0.98. After this step, the highly similar reads were then grouped together as an OTU, and only a single sequence from each OTU was used for taxonomic classification by performing BLASTN against the non-redundant SILVA SSU Ref database release 138.1 (Quast et al., 2013) with standard settings.

In metagenomic analysis, due to the conservation of the DNA markers used in the analysis, the PCR amplicons might be formed from two or more biological sequences joined together, known as chimeric reads. Therefore, this study checked for the presence of these chimeric reads by searching the annotated amplicons against the SILVA release 138.1 SSU by using VSEARCH v. 2.8.3.0 (Rognes et al., 2016) implemented in Galaxy v. 1.39.5.1 (Afgan et al., 2018). Then, the annotated OTUs that arose from these chimeric amplicons were discarded from the analysis. Finally, only the reads with good BLASTN results with a percentage of "(% sequence identity + % alignment coverage)/2" greater than or equal to 93 were taxonomically classified into taxa. If not, the OTUs were defined as "No relatives".

Amplicons of ITS were similarly searched using BLASTN against their appropriate database, the UNITE fungal database v. 7.2 (Nilsson et al., 2019), using parameter E-value = 1E-10, and the chimeric reads were similarly removed by searching against UCHIME reference datasets v. 7.1 (Nilsson et al., 2015).

3.2.4.3 Ecological functional inference

To infer the putative function of the present microbiota, concepts described in Zulkifly et al. (2012), where the functional inference could be estimated at the genus level of the microbiota. Therefore, in this study, the genus names of the annotated OTUs were used as the queries for literature searches.

3.2.4.4 Diversity of *Cladophora* sp. microbiota

The annotated *Cladophora* microbiota collected from different locales were then assessed for their diversity. In this study, the alpha diversity was assessed to estimate the diversity within one collecting site by using alpha diversity indices, including Shannon's index, Simpson's index, Chao1, and richness. The Shannon's and Simpson's indices were calculated by using function diversity implemented in the vegan package v. 2.5-7 (Oksanen et al., 2020). The Chao1 and richness were calculated using function estimateR implemented in the same vegan package. To evaluate whether the values of alpha diversity indices were statistically different or not, One-way ANOVA ($P < 0.05$) and Tukey's HSD were performed using function aov implemented in stats package v. 4.0.0 (R Core Team, 2021) and function HSD.test implemented in agricolae package (de Mendiburu, 2021), respectively. These alpha indices and Tukey's HSD results were then visualized in box plots using package ggplot2 (Wickham, 2016). Beta diversity was assessed to estimate the diversity between collecting sites using Bray-Curtis dissimilarity. This Bray-Curtis dissimilarity was calculated using function vegdist implemented in vegan package (Oksanen et al., 2020) and visualized in PCoA using function cmdscale complemented in stats package (R Core Team, 2021) and package ggplot2 (Wickham, 2016).

3.2.5 Comparative analysis of *Cladophora* microbiota

To compare the microbiota of the *Cladophora* sp. collected from Nan River in this study to those collected from *Cladophora* spp. in different locales, which were Lake Michigan and Lake Mendota, USA. The read archives of those studies (Table 3.3) were acquired and re-analyzed using the methods described above. This step of re-analyzing the archived data from previous studies was crucial as the newer version of the reference might help in capturing the taxa that were not identifiable at the time.

Table 3.3 Sequence read archives of previous *Cladophora* studies.

BioProject	**Sequencing technology**	**Sampling site**	**Reference**
PRJNA360140	16S amplicon sequencing	Lake Mendota, Madison, USA	Braus et al., 2017
PRJNA326542	16S amplicon sequencing	Lake Michigan, Chicago, USA	Chun et al., 2017
PRJNA267502	Shotgun metagenomic sequencing	Lake Mendota, Madison, USA	Graham et al., 2015

CHAPTER IV

RESULTS

4.1 Identification of the *Cladophora* host

In this study, the algal samples were collected from five collecting sites, four replicates per site, along Nan River, Nan Province, Thailand, in March 2020 (Figure 3.1). During the sampling time, the water levels at the collecting sites were about ankle to knee height (Figure 4.1), and the *Cladophora* masses appeared as turf attached to rocks, pebbles, or other substrates, such as tree branches (Figure 4.2).

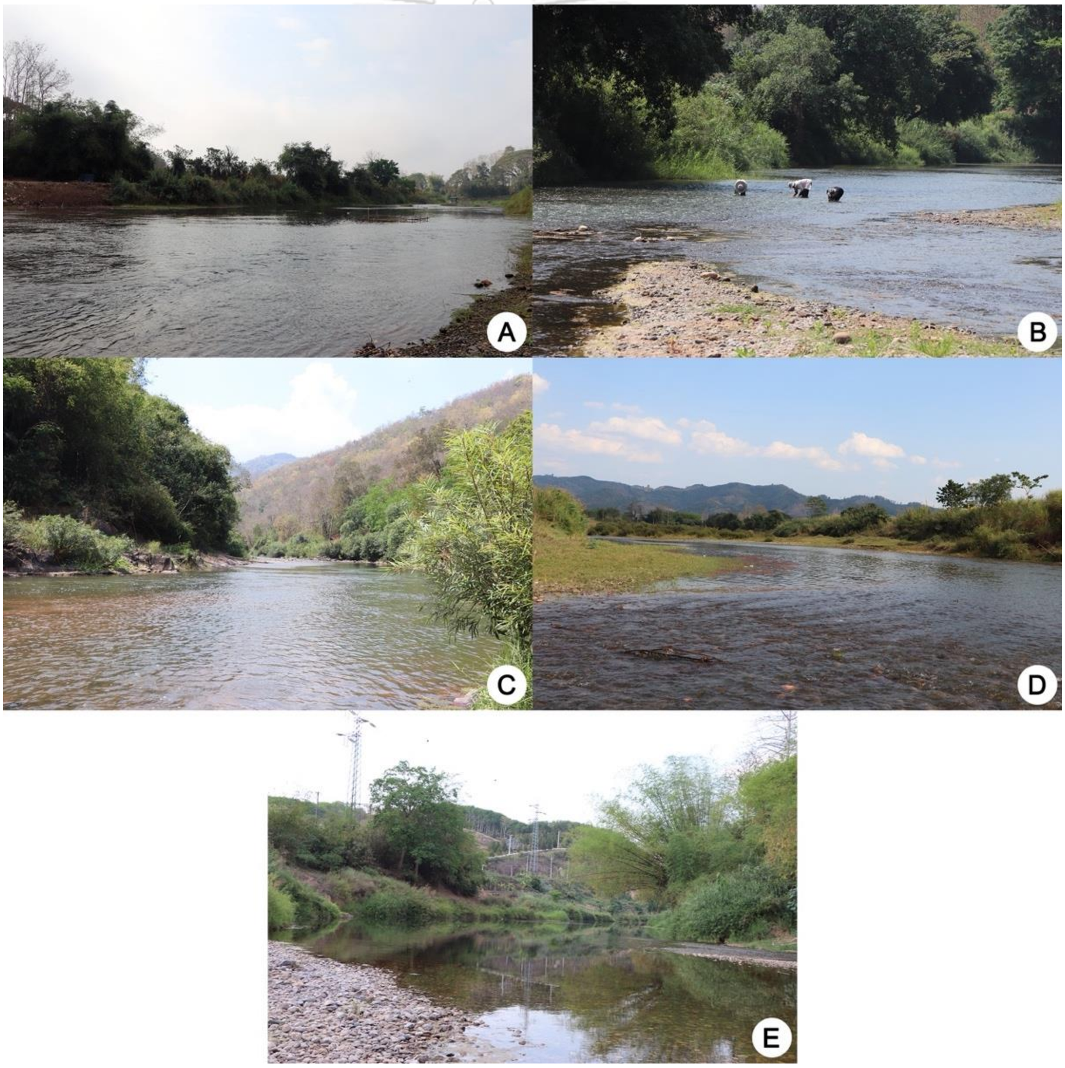


Figure 4.1 Views of collecting sites of Nan River, Nan Province, Thailand. A. Site CKD, B. Site PUA. C. Site TD1, D. Site TD2, and E. Site MND.

Figure 4.2 Habitats of algal samples collected from Nan River, Nan province.
A–C. Algae attached to rocks and pebbles. D. Algae attached to rocks and tree branches.

In addition to collecting the algal samples, for each replicate, the water samples were also measured for pH, ammonium, nitrate, and total phosphorus contents. Unfortunately, the water analysis was not possible. To solve this problem, the secondary data on physical parameters were acquired from Regional Environmental Office 2 (Lampang), and the information of water level and total rainfall were acquired from the telemetering system of the Electricity Generating Authority of Thailand (Figures 4.3–4).

In general, the total water level and rainfall were congruent, except for the rainy season of site NR3. During the sampling time (March 2020), when the alga was in bloom, the total water level and rainfall were averagely lower than that of other seasons (Figure 4.3). Similarly, the physical parameters were generally similar across sites (although statistical tests were not possible). By observing the graphs plotted by available information, during February and May 2020, the WQI ranged from 56 (poor) to 95 (excellent), the DO ranged from 6.1 to 8.1 mg/L, the BOD ranged from 0.47 to 2.1 mg/L, and the ammonia content ranged from 0 to 0.25 mg/L (Figure 4.4).

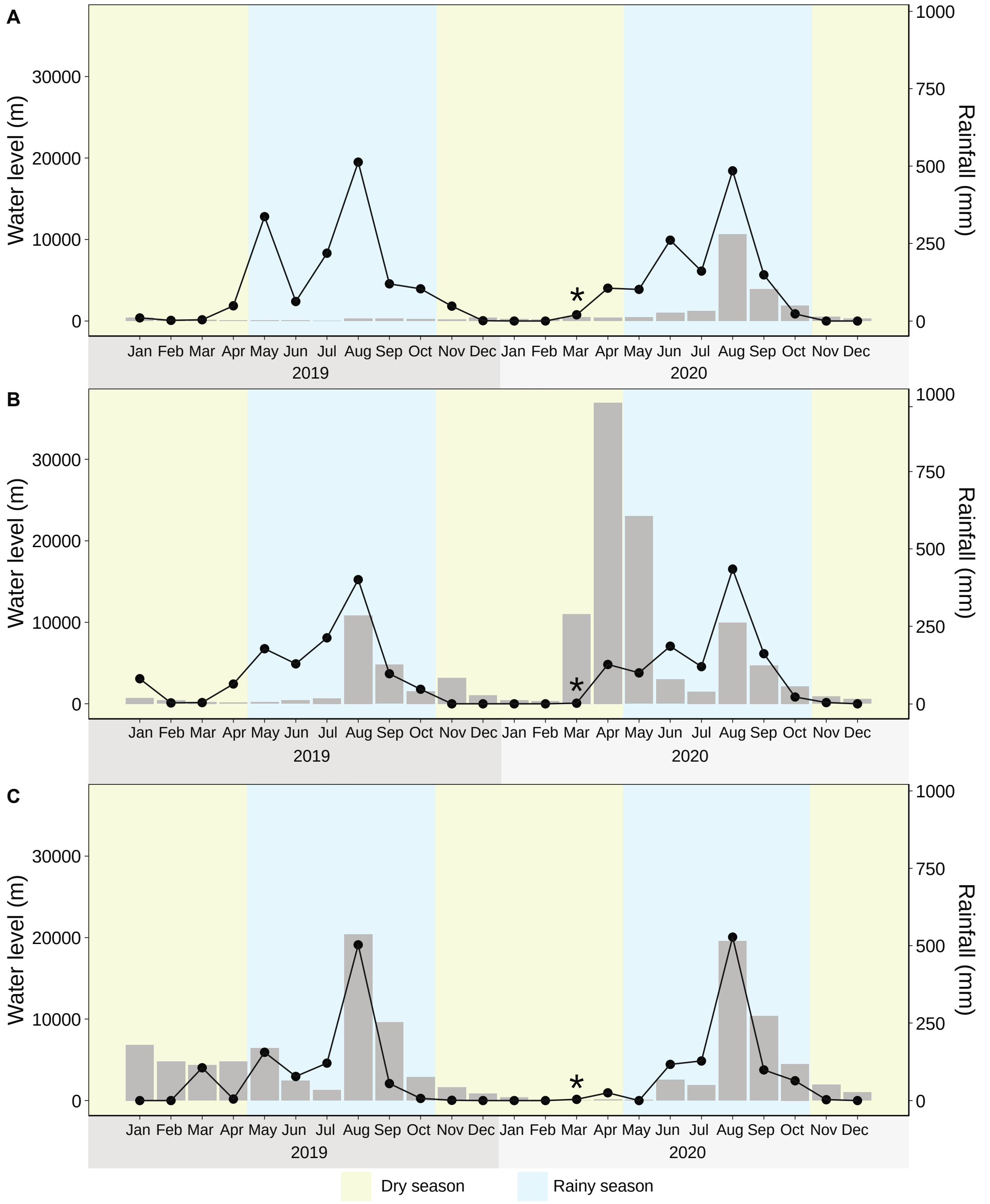


Figure 4.3 Total water level and rainfall of Nan River during 2019–2020.

A. Site NR3, B. Site NR5, and C. Site NR7. The locations sites NR3, 5, and 7 shown in figure 3.1. Bar graphs indicate water level and line graphs indicate total rainfall. Pale yellow background indicates the dry season (November–April), and light blue background indicates the rainy season (May–October). Asterisks indicate the estimated sampling time (March 2020).

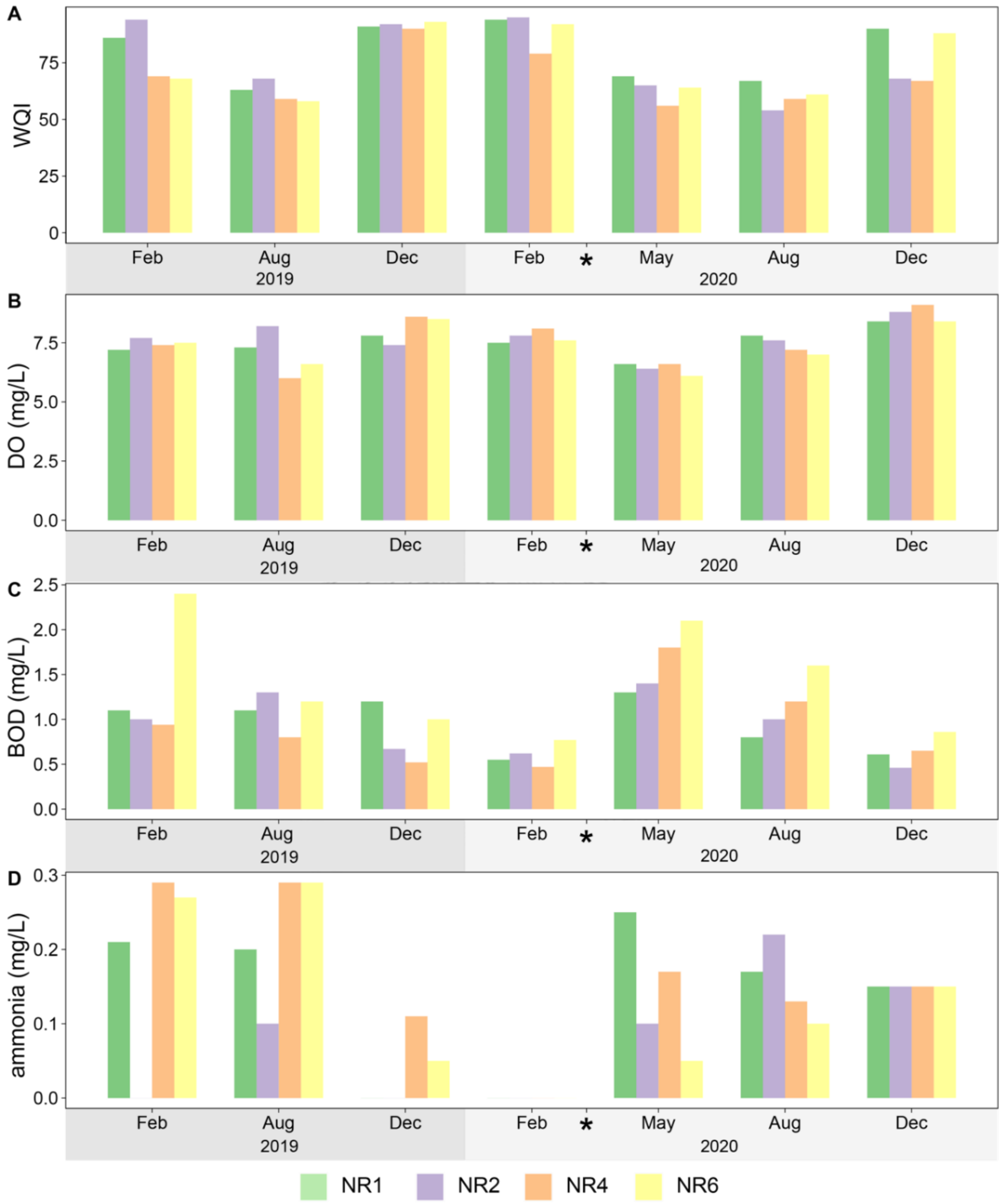


Figure 4.4 Physical parameters of Nan River collected at seven-time points from February 2019 to December 2020.

A. Water quality index (WQI), B. Dissolved oxygen (DO), C. Biological oxygen demand (BOD), and D. Ammonia nitrogen content. WQI values were calculated from five parameters: DO, BOD, total coliform bacteria, fecal coliform bacteria, and ammonia nitrogen content. Rating scales of WQI were ranked into five classes: excellent (91–100), good (71–90), fair (61–70), poor (31–60), and very poor

(0–30). Each color indicates different water sampling sites. Asterisks indicate the estimated sampling time (March 2020).

Morphological identification of the Cladophora *host*

The dominant algal host of collected samples was identified to the genus level using “How to know the freshwater Algae” (Prescott, 1964). According to the algal morphology of having conspicuous branching filament, reticulate chloroplast, and thick cell wall, the samples from sites CKA, PUA, and TD1 were *Cladophora*-dominant samples (Figures 4.5A–B). On the other hand, the algal samples collected from sites TD2 and MND were *Spirogyra*-dominant (Figures 4.5C–D), as they were unbranched filaments with spiral chloroplast and mucilaginous sheath. Therefore, only *Cladophora*-dominant samples from sites CKD, PUA, and TD1 were used for this study.

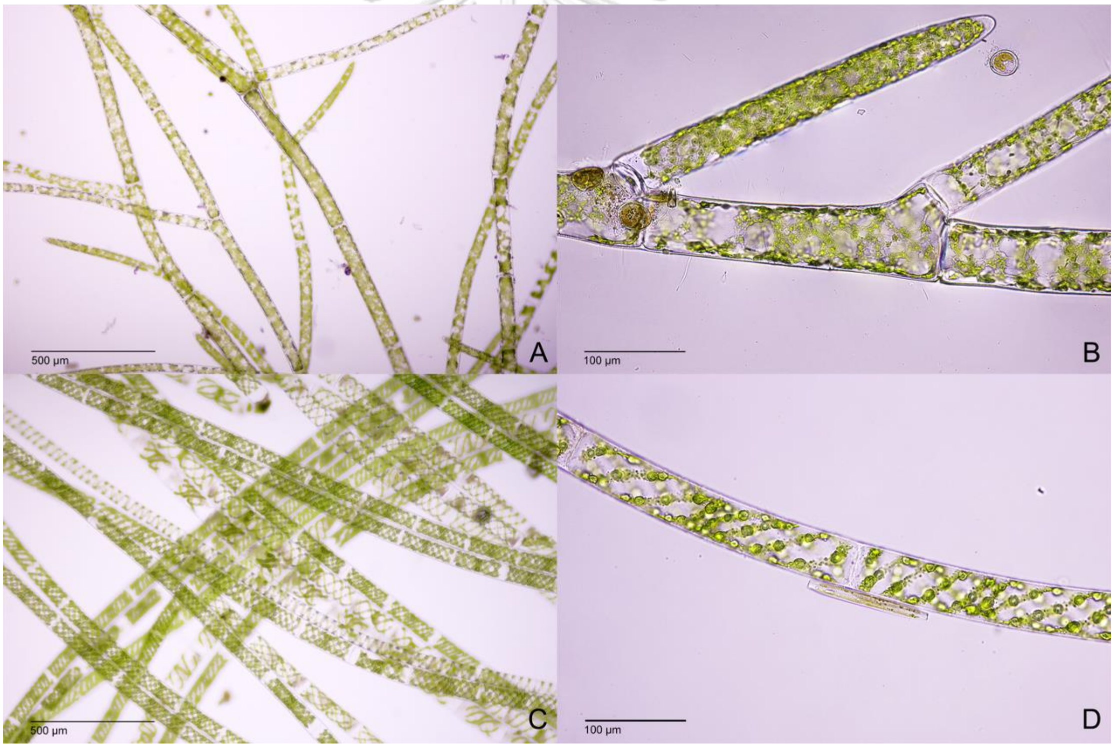


Figure 4.5 Micrographs of algal samples collected from Nan River, Nan Province. A–B. *Cladophora*-dominant samples from sites CKD, PUA, and TD1. C–D. *Spirogyra*-dominant samples from sites TD2 and MND.

Phylogenetic estimation

DNA extraction and amplification of marker genes

The regions of 18S rRNA, 23S rRNA, 28S rRNA, and ITS were successfully amplified from the extracted DNAs of *Cladophora*-dominant samples. These amplified PCR products were visualized using gel electrophoresis (Figure 4.6) and Sanger sequenced from both forward (5’ terminal) and reverse (3’ terminal) ends of the amplicons (Table 4.1). Then, only the trimmed reads with BLASTN hits annotated as *Cladophora* sequences were used for phylogenetic estimation (Tables A1–4).

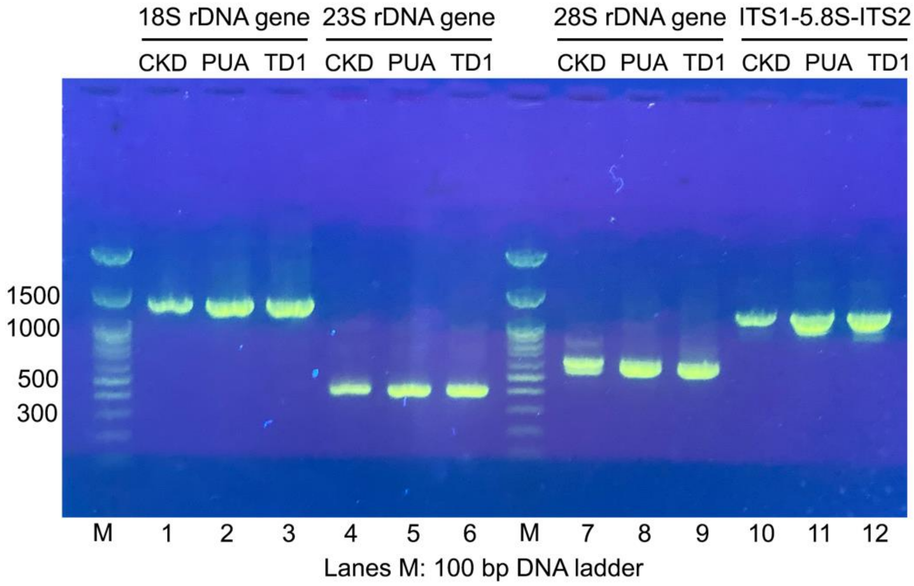


Figure 4.6 Agarose gel electrophoresis of PCR amplified products from primers listed in table 3.1.

Lanes M are 100 bp DNA ladders. Lanes 1–3 are PCR amplified products of the 18S rDNA gene. Lanes 4–6 are PCR amplified products of the 23S rDNA gene. Lanes 7–9 are PCR amplified products of the 28S rDNA gene. Lane 10–12 are PCR amplified products of the ITS1-5.8S-ITS2 region. Lanes 1, 4, 7, and 10 are amplified products of CKD. Lanes 2, 5, 8, and 11 are amplified products of PUA. Lanes 3, 6, 9, and 12 are amplified products of TD1.

Table 4.1 Raw reads of 18S, 23S, 28S rDNAs and ITS amplicons obtained from Sanger sequencing technology.

Letters separated by hyphen of sequence name indicate marker gene, collecting site, and sequencing direction. F indicates the direction starting from the 5’ terminal (forward primer). R indicates the direction starting from the 3’ terminal (reverse primer). * indicates sequences that returned BLASTN search results as *Cladophora.*

No.	**Sequence name**	**Sequence**
1	18S-CKD-F*	TCAGGGTTAGCTACATATGGCTCGGTTCATCCGTTATAGATTATCTTCGATAGTCGCATCCTACCCGGTACCGCAATAACACTAGAGCTAATACGTGCGTAAATCCCGACTTTTGGAAGGGACGTATTTATTAGATTTATGGCCAGCCGGGCTTGCCCGACCAGCGGTGAATCATGGTAACTTGACGGATTGCATGGCCTCCGTGCCGGCGACGTGTCATTCAAGTTTCTGCCCCATCAGGTTTCGACTGTAGTGTATTGGACTACAGTGCCTATAACGGGTAGCGGAGGATTAGGGTTCGATTCCGGAGAGGGAGCCTGAGAAACGGCTACCACATCCGAGGAAGGCAGCAGGCGCGCAAATTACCCAATCCCAACTCAGGGAGGTAGTGACAAGAAATAGCAATGCGGGGCTTCTTGCTCTGCAATTGGAATGAGAACAATTTAAATCACTTAACGAGTATCAATTGGAGGGCAAGTCTGGTGCCAGCAGCCGCGGTAATTCCAGCTCCAATAGCGTATATGTAAGTTGTTGCGATTAAAAAGCCCGTAGCTGAACCTCGGGCAACCCTGACGGTCCGCGTTTCGTGAGTACTGTCGTGGGTTGTCTTTCTGGCAAGACCGTATCTCGGCGTTAACTCGCCGGGAACGGCGCTTGCCAAGGTTACTTTGAGTAAAATAGAGTGTTCAAAGCAAGCTCGCGCTCTGAATACACTAGCATGGGATAACACGACATGACCTATGCTCTATCTGGTTGGCCTGTAGACATGTAGATAATGGCCAATACGGAACGCTCAGGGGCATTCGTATTTCGCTGTCAGAGGTGAAATTCTCGCATTTGCGAAAATAGGAACAATTGCGAAACCATTTGCCAACGACGCTTTCATTAACTCAAGGCTCAAAACTCCGGGGGATCGAATCACAATCAGAATCCCGCCGTAACCCTCGACCAGAAACGATGCCCATTAGGGTTTGGAGGGACGCATCCAAGTAGGCCTACCCCTGGAAACCTTCCGCCGAAAGCCAAAAATTTACAGGCCTTTCCGGGGGAAGTTTAACCTCCCAAGACCTAAAAATTTAAAGGGAATTTGGGGGGAACCCCCC
2	18S-CKD-R*	AGGACATGAGCTATGACCCGCCATGCACCACCATCCGCAACATCTTTAAAGAGCTATCAATCTGTAAATCCGAATCTTGTCTGGACCTGGTAAGTTTTCCCGTGTTGAGTCAAATTAAGCCGCAGGCTCCACGCCTGGTGGTGCCGTTCCGTCAATTCCTTTAAGTTTCAGCCTTGCGACCATACTCCCCCCGAAGCCCATAAATTTTGCTTTCGCGGAAGGTGCTGGAGGAGGCATATTGGATCCGCCCTCCAATCCCTAATTGGCATCGTTTATGGTCGAGACTACGACGGTATCTAATCGTCTTCGATCCCCCGACTTTCGTCCTTGACTAATGAAAGCGTCCTTGGCAAATGCTTTCGCAGTAGTTCGTCTTTCGCAAATCCAAGAATTTCACCTCTGACAGCGAAATACGAATGCCCCCGACCGTCCCTATTAGCCATTACCAACAGGTCTACAGGCCAACAAGATAGACCATCGGTCATGTCGTGTTATCCCATGCTAGTGTATTCAGAGCGCGAGCTTGCTTTGAACACTCTATTTTACTCAAAGTAACCTTGGCAAGCGCCGTTCCCGGCGAGTTAACGCCGAGATACGGTCTTGCCAGAAAGACAACCCACGACAGTACTCACGAAACGCGGACCGTCAGGGTTGCCCGAGGTTCAGCTACGGGCTTTTTAATCGCAACAACTTACATATACGCTATTGGAGCTGGAATTACCGCGGCTGCTGGCACCAGACTTGCCCTCCAATTGATACTCGTTAAGTGATTTAGATTGTTCTCATTCCAATTGCAGAGCAAGAAGCCCCGCATTGCTATTTCTTGTCACTACCTCCCTGAGTTGAGATTGGGTAATTTGCGCGCCTGCTGCCTTCCTTGGATGTGGTAGCCGTTTCTCAGGCTCCCTCTCCGGAATCAAACCCTAATCCTCCGCTACCCGTTATAGGCACTGTAGTCCAATACACTACGGTCGAAACCTGATGGGGCAGAAACTTGAATGACACGTCGCCGGCACGAAGCCATGCAATCCGTCAAGTTACCATGATTCACCGCTGGTCGGGCAAGCCCG
3	18S-PUA-F*	CCAGTAACCCGGTCGCTCCAATGGCTCGGTAATCAGTTATAGTTTATTTGATAGTGCATCCTACTCGGATAACCGTAGTAACACTAGAGCTAATACGTGCGTAAATCCCGACTTTTGGAAGGGACGTATTTATTAGATTTATGGCCAGCCGGGCTTGCCCGACCAGCGGTGAATCATGGTAACTTGACGGATTGCATGGCCTCCGTGCCGGCGACGTGTCATTCAAGTTTCTGCCCCATCAGGTTTCGACTGTAGTGTATTGGACTACAGTGCCTATAACGGGTAGCGGAGGATTAGGGTTCGATTCCGGAGAGGGAGCCTGAGAAACGGCTACCACATCCGAGGAAGGCAGCAGGCGCGCAAATTACCCAATCCCAACTCAGGGAGGTAGTGACAAGAAATAGCAATGCGGGGCTTCTTGCTCTGCAATTGGAATGAGAACAATTTAAATCACTTAACGAGTATCAATTGGAGGGCAAGTCTGGTGCCAGCAGCCGCGGTAATTCCAGCTCCAATAGCGTATATGTAAGTTGTTGCGATTAAAAAGCCCGTAGCTGAACCTCGGGCAACCCTGACGGTCCGCGTTTCGTGAGTACTGTCGTGCGTTGTCTTTCTGGCAAGACCGTATCTCGGCGTTAACTCGCCGGGAACGGCGCTTGCCAAGGTTACTTTGAGTAAAAATAGAGTGTTCAAAGCAAGCTCGCGCTCTGAATACACTAGCATGCGGATAACACGACATGACCTATGGTCTATCTTGGTTGGCCCTGTAAGACCTGTTGGTAATGGCTAAATACGGGACGGTCAGGGAGCATTCCTTATCTTTGCTGTCATCAGGTGAAATTCCTCGCATGTTGCTGAAGAGACGGAACTACTGGCGACAACCAGTTTGCCCAACGAAGGCTTGTCATTAGCCCATGGACCAAGAGTCCGTGGGATCGAAATCACAATCCAGAATACCCGCCGGTAGCCCCTCGAACCAAGAAACCGGATGGCCCCATTAAGGGATTTGGGAGGGGAGCATCCCAAATAGGGCCCCACCCCCTTGGACAACCTCTTTCCCCGCCGAAAAAGCCAAAAAATTTGATCGGGGGCCCTTTCGGGGGGGGAAGGTTTTGGGCCCCCCAAATGCCCCCCCAAATCTTCCTG

No.	Sequence name	Sequence
4	18S-PUA-R*	GGGCAATGACTGTTTCGACATGCACCACCATCCAAAAAATCTTTAAAGAGCTATCAATCTGTAAATCCTAATCTTGTCTGGACCTGGTAAGTTTTCCCGTGTTGAGTCAAATTAAGCCGCAGGCTCCACGCCTGGTGGTGCCGTTCCGTCAATTCCTTTAAGTTTCAGCCTTGCGACCATACTCCCCCCGAAGCCCATAAATTTTGCTTTCGCGGAAGGTGCTGGAGGAGGCATATTGGATCCGCCCTCCAATCCCTAATTGGCATCGTTTATGGTCGAGACTACGACGGTATCTAATCGTCTTCGATCCCCCGACTTTCGTCCTTGACTAATGAAAGCGTCCTTGGCAAATGCTTTCGCAGTAGTTCGTCTTTCGCAAATCCAAGAATTTCACCTCTGACAGCGAAATACGAATGCCCCCGACCGTCCCTATTAGCCATTACCAACAGGTCTACAGGCCAACAAGATAGACCATCGGTCATGTCGTGTTATCCCATGCTAGTGTATTCAGAGCGCGAGCTTGCTTTGAACACTCTATTTTACTCAAAGTAACCTTGGCAAGCGCCGTTCCCGGCGAGTTAACGCCGAGATACGGTCTTGCCAGAAAGACAACCCACGACAGTACTCACGAAACGCGGACCGTCAGGGTTGCCCGAGGTTCAGCTACGGGCTTTTTAATCGCAACAACTTACATATACGCTATTGGAGCTGGAATTACCGCGGCTGCTGGCACCAGACTTGCCCTCCAATTGATACTCGTTAAGTGATTTAGATTGTTCTCATTCCAATTGCAGAGCAAGAAGCCCCGCATTGCTATTTCTTGTCACTACCTCCCTGAGTTGAGATTGGGTAATTTGCGCGCCTGCTGCCTTCCTTGGATGTGGTAGCCGTTTCTCAGGCTCCCTCTCCGGAATCGAACCCTAATCCTCCGCTACCCGTTATAGGCACTGTAGTCCAATACACTACAGTCGAAACCTGATGGGGCAGAAACTTGAATGAAACGTCGCCGGGCACGAAGGCCATGCAATCCGGTCAAGTTACCATGATTCACCGCTTGTTCGGCA
5	18S-TD1-F*	CCGGGTAACTAGACATGGCTCGGTAATCAGTTATAGTTTATTTGATAGTGCATCCTACTCGGATAACCGTAGTAACACTAGAGCTAATACGTGCGTAAATCCCGACTTTTGGAAGGGACGTATTTATTAGATTTATGGCCAGCCGGGCTTGCCCGACCAGCGGTGAATCATGGTAACTTGACGGATTGCATGGCCTCCGTGCCGGCGACGTGTCATTCAAGTTTCTGCCCCATCAAGTTTCGACTGTAGTGTATTGTACTACAGTGCCTATAACAGACAGCGAAGGATTATGGTTCGATTCCAGAGAGGGAGCCTGAGAAACGGCTACCACATCCGACGAAGGCAGCAGGCGCGCAAATAACCCGATCCCAACTCATGGAGGTAGTGACAAGATATAGCAATGCGGGGCTTCTTGCTCTGCAATTGGAATGAGAACAATTTAAATCACTTAACGAGTATCAATTGGAGGGCAAGTCTGGTGCCAGCTGCCGCGGTCATTCCTGCTCCGATAGCGTACATGTAAGTAGTTGCGATTATAACGCCCGTAGCTCAACCTCGGGCAACCCTCACGGTACGCGTTTCGTGAGTACTGTCGTGCGTTGTCTATCTCGCAAGACCGTACTCTCGACGTTGCACTACGCCTCGGAACTGCCCTAGCTACGGTTACTTTGCAGTTTAAATCGAGTGTTTCTAAGCAAGCTCCCCGCTCTAAACTACACTTTACCGGCGGATAACCGGGACCTGAACTTAGCGCTCCAATCTGGTACGCCGTGTAAGACATGTTAGGTAGTGCCCAATCCGGGTACGCTCAGCAGGCATTCCGCATTTTTGCTTATCACAGGTGAAATTCTTCGCATTTGTCGAGAATAGGAAAATATTGGCGAAACCCTGTTGGCCTACGTAGGCATTTCGTTAACCCATTGCACAAAAGTTCCCGGGGAACCGAAAACAAAAACCAGAATTCCCCCCCTAACCCCTTAACCAGGCACCGATTCCCAATAAAGGGATTTGGAAAGGAACGGCTTGCCAGGGGGCCCAACCCTTTGGACACCCTTCCCTGCCAACCGCCCAAAAATTTCCCCGGGGCCTC
6	18S-TD1-R*	TCCGTGAGCTAGTACCAGGCCATGCACCACCATCCAAAAATCTTTAAAGAGCTATCAATCTGTAAATCCTAATCTTGTCTGGACCTGGTAAGTTTTCCCGTGTTGAGTCAAATTAAGCCGCAGGCTCCACGCCTGGTGGTGCCGTTCCGTCAATTCCTTTAAGTTTCAGCCTTGCGACCATACTCCCCCCGAAGCCCATAAATTTTGCTTTCGCGGAAGGTGCTGGAGGAGGCATATTGGATCCGCCCTCCAATCCCTAATTGGCATCGTTTATGGTCGAGACTACGACGGTATCTAATCGTCTTCGATCCCCCGACTTTCGTCCTTGACTAATGAAAGCGTCCTTGGCAAATGCTTTCGCAGTAGTTCGTCTTTCGCAAATCCAAGAATTTCACCTCTGACAGCGAAATACGAATGCCGCCGACCGTCCCTATAAGCCATTACCAACAGGTCTACAGGCCAACAAGATAGACCATCGGTCATGTCGTGTTATCCCATGCTAGTGTATTCAGAGCGCGAGCTTGCTTTGAACACTCTATTTTACTCACAGTAACCTTGGCAAGCGCCGTTCCCGGCGAGTTAACGCCGAGATACGGTCTTGCCAGAAAGACAACCCACGACAGTACTCACGAAACGCGGACCGTCAGGGTTGCCCGAGGTTCAGCTACGGGCTTTTTAATCGCAACAACTTACATATACGCTATTGAAGCTGGAATTACCGCGGCTGCTGGCACCAGACTTGCCCTCCAATTGATACTCGTAAAGTGATTTAGATTGTTCTCATTCCAATTGCAGAGCAAGAAGCCCCGCATTGCTATTTCTGGTCACTACCTCCCTGAATTGGGGATTAGGGTAATTTGCGCCGCCGCCTGCCTTCCTGGGATGTGGTAACCCGTTTTCTCAAGGCTCCCTCTTCCGGAATCGAAACCCAAATCCTCCCGCTAACCCGTTTATAGGCCCTGGAAGTCCCATTAACCTTACGTCTGAAAACCTGGAGGGGGCAAAAAACCTTGAAAAGACCCTCCCCCGGGCCAGGAAAGCCTGGGCTTTTCCGGTAAATTTACCCTAAATTTCCCCCGTTTTTTGGGGAAAAACCCGGGGGGGCCAAAAATTTTAAAAAAA
7	23S-CKD-F	GAGGTCGTAGCTTATCTGGCGAGGTACGTAGAGGCTATGATCATATTCCTTTCGGCTTGTCTGTAGCCTCTCTAACATACACTCTTGTTATGCTCGATTCTGACTTCGGCCATTATCAGGTACGTACAGTGCCTCGTGGACAGTTTGCAGGCGCGGCGCCTCCAAACTGTCACGGAGGCACGGTCAGGTTCCCTCATGATGGGCGGAAATTAGACTTTGAGTTTAAAGGCAGAGGGAAGCTTGACTGGGATACCGATAGTCGAGCAAGGACGAAAGTCGGCCTCAGTGATCCGACGCTCTGAATGCAAAGGCCGTCTCTCCAGCGGAAAAAATTCTCTAGGTAATTTGGTCCAAAA
8	23S-CKD-R	CATTCCCTCGGCACGTCGGATCACTAAGAGCCGACTTTCGTCTCTGCTCGACTTGTCAGTCTCGAGTCAAGCTCCCTTATGCCTTTACACTCGACGGCTGATTTTCCGACCAGTCTGAGGGAACCTTTGCGCGCCTCCGTTACCTTTTAGGAGGCGACCGCCCCAGTCAAACTGCCCACCTGAAACTGTCCCCTAACCGGATTATGGGGCAAAGTTAGAAATCTCAAGCTACCCGAAGGGGTGTCTCACGGATGGCTCCCATATACCCGGAGAAAGAAGATTCAAAGCCTCCCACCTATCCTGCGCAAATGAAGACCAAAGCCAATTTCAAGGTACAGAAAAGTTCCTAGGGTTTTTTTTTCCAGAAA

No.	Sequence name	Sequence
9	23S-PUA-F*	ACGCTTCAGTTTATGCGCAGTATAGGTGGAGGCTATGATAATCTTTTTGCGGATGTTTGATCCTCTCTGACATACCACTCCCATTACGCTAGATTTCTAACTTTGCATCATTATCAGGACACCGTACAGCGCCTCGCGGACAGTTTGACTGGCGAGCTCGCCTCTCAAACGGTCACGGAGGCACAGTACGGTTTCCTCAGAACGGGTCGAAATCGTATCTCGAGTGTAAAGGCATAGGGAAGCTTGACTGTGAGACCTACTAGTCGAGCAGGGACGAAAGTCGGTCCTAGTGATCTGACGGTGCTGAGTGGAAAGGCCGTCACTCAACGGATAAAGTTACTCTAGGATTTTTTGCTTCAAAA
10	23S-PUA-R*	CCACTTTGATGAGGCGTTCCCTCAGCACCGTCAGATCACTAAGACCGACTTTCGTCCCTGCTCGACTTGTAGGTCTCACAGTCAAGCTTCCTTATGCCTTTACACTCTAGATACGATTTCTACCCGTTCAGAGGAAACCTTTGTGCGCCTCCGTTACCGTTTGGGAGGCGACCGCCCCAGTCAAACTGCCCGCCTGAAACTGTTCTTTGACCAGATAATGATGCAAAGTTAGAAATCTAACATTATAAGAGTGGTATCTCACTGAGGGCTCAAACCTTTCCGCAAAAAGATTTTCATAGCCTCCCACCTATACTGCGCATATAAAGTCCAATTTCAATCTCAAGTTACAGTAAAGCTTCATAGGGTATTTCCTGGTCCAAAAAACCAACTCACAATATTTTCACATATATACAGCAAAAATCACAGGCCCCCCAACCGCCCTATTAGCCATTTAAACATAGGTTCCTAGGGACAAATTAAAAAGACCAAGCGCTGATGTGGCTCTGTCCCCCTTGTGAAGTGTATCGAAAAGCGGAGATCTTTGTCTTTGTTATCGTCACTTTTTTTGGAGCTCAAAATGCACGAACGATTTGCAGAAAAAGCGAGCCTGTCTACTGGCTGACGTATGGTAATAACCCGACAGAATAAATTGTCCGGTTGTGGTGGTTGGGACGGAGAAAGGAAAAGAACCAGGGAAACCCCAAATCAAATCTCATCGTCGAAACCGCTAAAAGAACTCGGAACGGGGGGCCCTGGGGGACTTCCATGGGTGGGTTCTTCGGGGTTAATCGCGAAAAAAGGAGGAATATTTGAAACCCCCCTATGAGGCGGGTTGATTGGGCATGAAATAAATATTATTATATAATGTGTTGGGGGTACGCAAGGAGGAGCAAGAAAACCCCTTATGAGATAGCCACCCTTTTACAAAGGCCCCCCGTCCCCTCCTACCGGTAAGGGATTGTTGTGAGGGAGAGAAACGCCCCTCTCTTTTTGGAGGGCACACAACAATATTGTGTAAGAAGCACCCCATGTGATGAGGCGAGGAGCGCCTGATGGGGCCCGCCCTGGGGTGAGGGAAAAACACACATCGTGGAGAGAAAACGGTCTGTTTTGCGAGGCCTCCTCTTTGTGCTATCCGCAGACAGAAATATTTGTAGGGGAAAGTTAATGTGGGCTCCCGAGACCTATCCTTAAGTGGGTGTTTTGGGAAAGAGGTGTTTATAAAAAAGCTAGCTCTTTGTGGGTAATTTATCCATGCATCAAAATTTCTCTCTCCCCCCCACAACTGTGTCTTATGGCGGGGAGAAAGTAAGGAGAGAGCGCCCCAGGCGGGGGAAAAAAGGTTCCTCCCCCGCCGGATCCTGCTCGATTTTTGGGGGGCTAGAATTTGTGTTTTCATACAATATTGTGGGGTCCCCCCAAGAGGGAAAAAAATACTTTTCCGCCCCCCCGTGACAGAGGGGTATGTGGGGGAAAATAATTTTTAGGGGGGAGGGGGTTGACAAAATTATTGTTGTGGATGACGTCCTCCGCTCCCCTCTTGGTGGTATGGGGGAAAAGTGTTATTTGTTGTGTTTATCGGGGCCATATGTGGGTAGGGGGGGGATGGAAGGTGGCCGCGCGGAGGAAGGTTCTCTTCTCGCGAGGGGGGGGGTGGCGTCCCGCAGGGATAACGCCTCCCCGGAGGAAAAAATACCATATGAGGAAAAAAGACTCTGACGCGTAGAAGATTACTCTGTTGTATGTACGGGGCGTCTATCCCGACCTGGGGGTGTGATCAACAATGAATTGGGAGAACAACACTTGGTGGGAGAGGGGGTGCTGCGTAAGAAAAATTGATAACCCCGGGTCATAAAAAACGAATGGGTGCCGCGGAGAAAAAAAGTCTACCTAGTGAGGAAATGGACGGGTGGCTGGGTGTGCCGGGGGAAGAATGTCACCGATTTTATTGAGGGGAAGAGTACGTGATTAGAACACGGTAGGGATGCTAAACAGAGAATCCTCTGTGCCGGCGGCGGCGCGGGCATGTAGATATGGGGACCCGCACGAATGGAGGATAGTGATATATTTACCACCGTGTGTCTGTCACCACAGAAAGTGTTGTTGGTCGTCGCCAAGAGAAGTAGAGAAACATCTTCCTCAATAAGGCGGGCGGAGGTGATGTGGGGCGCTTTCGTGGTGTGTGGAGAGAAAGGATGCCCGCCCGTAGGG
11	23S-TD1-F*	CGTAAATCGTGATGAGGCGGTCCCTACATGCGGTCATATCGTGAAGACCTACTTTCGTCCTTGTTCGACTTGTAGGTCTCACAGTCAAGCTTCCTTATGCCTTTACACTCTAGATACAATTTCTACCCGTTCAGAGGAAACCTTTGTGCCCCTCCGTTACCGTTTGGGAGCCACCCCCCCAAGTCAACTGGCCCCCTGAACTGTTCCTTGACCAGATAAAGAAGGTGGTTAGAAATATTATTTTAAGAGTTGTATCATCTGATGGACCTCCGTGAGGGAAAAAGTTTCATAGCGTTCTCGGACCGGGCATGCGGGTGCACGTCAAAAGAGGGACGGTAAAGCTTTGCGGGTTGTTCTGTTCACAATCCGGGAATTCAGTTAGGAGCGAATGCGGGGGTTCCGAAGGCCGTTAGGGATAAAAAAAGCTAAGGACAAGAAGAAGGACGAGGGTAATGTCGGGTTATCAGGTGGAGTGTATTAAGAGAGCGAGGGTGTGAGAGGCTTATATGATTAAAGCAGTCCTCAAAGCGGCGTTTCCGTGAGAAAGCGGAGATGTGTCTGGAGAGGAAAATGCAGACATATCTTGAAAGCGGCCGCCGGTGGTTTTAGGATGGCTAGGGTCTTTTATTAAGGAATTTACAATTTAGATTGGGTTAAATGATTGGGTGTGGACAAGAATAGCTTTGAATGATAGGGGGTGGTGAGAAAATAGTTTCATTCAAATGCATGGA
12	23S-TD1-R*	TTTTCCCTCAGCACCGTCAGATCACTAAGACCGACTTTCGTCCCTGCTCGACTTGTAGGTCTCACAGTCAAGCTTCCTTATGCCTTTACACTTCTAGATACGATTTTCCACCCCGTTCAGAGGAAACCTTTTGTGCGCCTCCGTTACCGTTTTGGGAGGCGACCGCCCCCAGTCAAACTGCCCGCCTGACACTGTTCTTTGACCAGATAATGATGCAAAGTTAGAAATCTAACATTATAAGAGTGGTATCTCACTGATGGCTCACACCTTTCCGCAGAAAGATTTTCATAGCCTCCCACCTATCCAGCGCATATCAAGTCCAATTTCAATCTCAAGTGACAGTAAAGCTTCATAGGCCTTTTTTGGTCCCCGAA

No.	Sequence name	Sequences
13	28S-CKD-F*	TCCGTTAGTAACGGCGAGCGAAGCGGGAAGAGCCCAGCATGTAAATCTCCGGTTTCCGGCTAATTGTATTCTGGAGTAACAACTCCTGGTGCCGTGCCGGCCCAAGCATCTTGGAATAAATGGCCTCGAAAGGTGAGAGCCCCGTAGGCCGGCACGAGCTCCCACCCTGAGTGCTCACGCATAGTCGGGTTGTTTGGGATTGCAGCCCCAATTGGGTGGTAAACCCCATCTAATGCTAAATACCTGTGGGAGACCGATAGCCAAAAAGTACCTCGAGGGAACGATGATGAGACCGCTGGCAAGCGAGTGAAACAGTGCATGACATTGTATCGAGGGAAGGGATGGCCTGGCGTATTGCCCGCGGGTATACACTGCTCTCTGAGCTCTTGAAGACACCGTGAGCTGGCTGGCGCCGACGCCTGCTCTTGCAAGACCCCAAACTAAGGTGTTTCGGCTCTTCACGTGCTGTGTTGAGGTAGATCGCGCTTTGTATTAAACTGCGCCGACATAAGCATCTGCTGAGCCCGTCCATCTACTCTCTGAAAACCGGATA
14	28S-CKD-R*	TTGAACATACGAGACGATCAACCTCATCACCCGCGCTTGAAGAGCCAACACCTTAGTTTGGGGATTTGCAAGCGCAGGTGTCGGCACCATCCTGCTCAAGGTGTATTCTAGAGCTTTTAGAGCACTGTATACCCTCGGGCAATACGCCCCGCCATCCCTTCCCACTATACTTTTCATGCACTCTTTCACTCGCTTGCCAGCGGTCTTTTCATCGTTACCTCGAGGTACTTTTTTGCTATCGGTCTCCCACAGGTATTTAGCCATATATGGGGTTTACCACACAGTTGGGGCTGCATTCCCGAACAACCCTACCCTGCGTGAGCACTCCGTGTGGGAGCTCGTGCCGGCCTACGGGGCTCTCAACCTCTGAGGCTATCTATTCCAAGATGCTTGGGCCGGCTCGGCACCAGGAGTTGCTACTCCAGACAACTAATCGCCGGATACCGGATATTTACATGCTGGCTTCATCGTCATTTCGCTCGCCGGTACTAAAGGATCCCCGGTTGAGTTTCTTTTCCTCCGCTTATTGATATGCTTATGCAAACCGGGTAA
15	28S-PUA-F	AGATCCCTTTCCTCAGTAACGGCGAGTGAAGCGGGATGAGCCCACCATGTGATCTGGACTTGAAGTTTTTGTCGTTTAAGTGTGATCTGTGGGGTGGGTGTTCTGTGAGCCAGGTTTGTTACATGGACAAGGAAGGTGAGAACGGTGAAACTCTCGATGAGCTCCCACTCAAGCCCCTCTCGCAAAATCGGGTTGTTTGGGAATGCAGCCCCTATTGGGTGGTAAACCCCATCTTAGGCTAAATATTTGTGGGAGACCGATATCCGCAACGTACCTTGAGGGAACAGATAAAAAGAGAGCTTGCAAGAGAGTGAAAGAATACCTGAAATTGTTGAAAAGGAAGCGATGGAATGCCGTGTAGAGCGAGGGCATACTTCCCTCTCCGATAGCTTGGAGACGCACTGTGCCTAACGGCGGCTACGCTTGCTTGTACTGACCGCATCACTCGAGCGTATTTATCTGTCTCGCGGTTTTCTGGCGACCGCTTTTTTTTCCACAGCGGACAAAAGGTAAACCAGCTGGAGGCCGGTGCATCTCCCCGTCATGTCCCCCGGACACTTTTACGGACCGGTCTAGAACCCCGGATATA
16	28S-PUA-R	TAGGGGCAGCTACCACATATGTGGCATCCAGTCCATCCAACCCAATCGGGCAGACGCTAGCCACAGCGCCCCAAGCCGCAAGCGGAGGGGGAAGTACGACCTTGCTTGACACTGGTTTCCTTCGCTTCCGTTTCAGTATTTCAGGTACTCTTTAACTCTCTTTTCAAAGTCCTTTGCATCTTTCCCTCACGGTATTTGTGCGCTGTCAGTCTTCCATTCATAGTTACCTCTAGGTGGAATTTAGCTACCACTTTCAGCTGCAATCCCAAACAACTAGACGGTTCGACACGGGCTCGATGGCCAGGAAAACGGGAATATCACCCTCTCGGCTGCACTCTTACGAGAAACTTGGCCGGGCAAAATGAATGCCCACCTCCACAGACCACAATTCAAAGACGCAAAATATCCGGCGGCGATTCAGACTGTAAGCTCAAACGCTTCACTCGCCCATACAGAGAAATCCTGGTTGGTTCTTTTCCTCCGCTGAATTATTACCTAGGATCGCAGGTTAGATTCTTTGCTCCGGGATTGATATGCTTAATTCCCCGGGTACCTTACGGAATGCTAAATCCGGGAGTATC
17	28S-TD1-F*	GGCTGTCAGTAACGGCGACTGAAGCGGGATAGAGCTCACAATGTAGAATCTCGCGCTTGATATTTTTTGGCGTTGAGTTGTAGGTCCGCGGACGTGCGTGTTCATTGTGCCGGCTCGAATTACATGGACACGGAGGGTGAAAACGCTGATACTCCCCTTAACCTCCCCCTCGAACCGCTCACGCAGAGTCGGGTTGTTTGGGATTGCAGCCCCAATTGGGTGGTAAACTCCATCTTAGGCTAAATATCGGTGGGAGACCGATAGCCCAAAAGTACCTTGAGGGAAAGATGAAAAGGACGCTTGAAAGAGAGTGAAAGAGTACCTGAAATTGCTTAAAGGGAAGGGATGGACTGGAGTATTGCCCGCGGGTATACTTTCCCCCTCCCCTCTCTGGGAGCGGCCTGTGCCTGCCGGCTGCCACGCCTGGGCGTACAGACCGCTACCTCATGGTGTTTGGCTTGCCCCGCCGGTGTTCAGGTTGAACGGCTCTTTTTTCCCCTCTGAAAACCGGGAAACCTCTGAATCCTGTCCTCTACCCCTCTGAAACCGGAAAA
18	28S-TD1-R	TTGATGGTGGGAAGGAATAACCGACCTGTGGAATCCGCTCCATCACCCCCAACCTGGCAGACGCTACCCACAGCGCCCCAAGCCGCAAGCGGAGGGGGAAGTATGACCTTGCTTGACACGGGTTTCCTTCGCTTCCGTTTCAGCAATTTCAGGTACTCTTTAACTCTCTTTTCAAAGTCCTTTGCATCTTTCCCTCACGGTATTTGTGGGCTGTCGGTCTCCCATTCATATTTACCCCTAGATACTTTTTACCACCCACTTTAAGCTGCAATCCCAAACAACTCGACTCTTTACACACGGGCTCGATGGCGAGGAAAACGGGAGTATCACCCTCTCGGCTGTCCTTTTCCAAGAAACCTGGCCGGGCACAAGGAATGCCCACCTCCACAGACAACAATTCAACGACGCAAAATATCGGCGCGCCGATTCACACTTGCTAGCTCATCACGCTTCACTCGCCCTTACTGGAGATAATACATGCTTGGTTTCTTTTCCTCTCGCTTAATTATTACGCACAGATCCCCCCGGGGTGATATTCTTTTCACTCCGCTTATTGATATGCTTAAGCCACCCGGGTAAAA

No.	Sequence name	Sequences
19	ITS-CKD-F*	GCACGGGATTGGTAGCCTATTCCAGTAAGGGAGGCTCCTGCAGAAGTTCACTGAACCCTCTCATCTAGAGTAAGGAGAAGTCGTAACAAGGTCTCCGTAGGTGAACCTGCGGAGGGATCCATAGCATTCGTAAACCAACTTGTCGGTCAAAAATAAAAGCGAGGGGGGACGCCCTCTCGAACGACCCTCCTTGGCTAGGGCTGGCCGCCCGAGCGGCGCCAGACACAGGCCTCACTGCCACGGTACCGTGGTGCCCTGCAACCCCCGGGAGAACGTTGTCCCATTGGGGGCGCGCGGGGACCCAAGCACCCGGTACGGACTTGCGCTTAAAACCGACACCCCCCTTGCGGGTGGGCTCGGGCTTACGGGACGGGAAAGTGGGGTTCAAAAAGACGCTCAGCTACAAAGTGGTCCAGCTCGAGGGCTTGGGGGGTAGCGGGGGTAGCCAAATTCGATTATTCCAAGAATTCTTACATTCATACTAACAATCGCCCTTTGTGTGGTGTCTTGAGCGATTTGGGGGCCAAGAAATCCATCGGAAAAGAGTTTCTGTTCGGTTGGATTTGCTTGGCTCCGCCATAGACTAAGAGCGCAGCCAAGCGCGGAATCTAATGAGAATTGTAGAATTACGGTGAATAAGCGAATTTGTTGATCCCACATTGCGCCCAAGCCTTCGGGATTGACCATGTCGGCCTTTGCCTTCGTTTCAAATGGCTTGCCGTCGGCGGAGCCTGGCCGCTCCTCATGGAGTGGTGTGGGTTTAAGCCGTGACCTCTGGCACGTCGCCTATGCCTGGGCGGCCCGCGACAGCAGCACTTTCCTGGCACTGGGTCAGCGGAAGCCTGCGGCATCTCGTGGCACAGATCCGCTTCTAGCGCCCCTCCGGCGTACATCCGCTGCGGCTATGGTGGTCGTTCGAACCAAGACCGCCACACCTTCCTTTTTTTTCTTACCGTATATGTTCCTTTACTAATTCCAGGGATGCGTCCAAGGTTCATCTGCTGGAAACCTTGTCTCCGACTCACACCTACTCGAGCTGAGATGAGTCAGGGTACTCTCAGACTCTCGCTTACGATAGCTGACGATAGAACTTCAGCAT
20	ITS-CKD-R*	CGTTATCGTCCGCTTATTGCATATAGCGTTAAGCTTCAGCGGGATAACCCTGCCTAAACTCAGGTCGAATGGTGTGTTGTCGCTGCCAGGTGTCCAGCCGGAAGAAGCTTGCGCGCGCTCCCTGCAAATACACATGCACCTTGTGCACGCACACGCATGCAGGTGTGCGCTGTGGGTTGGAGGAGCCTCCAGCCGCAGGGACGAACCCCCGAGCCCATGGGACCCATATGTCACTGCAGATGACCGTGCTGCCCAAGACCCGTGCCGAGAACGTTGTCCCATTTGGGGCGCGCGGTATCGGCGCACGTGCCGGAAATCTTGCGCTTAAAACCGAGCCCTCCTTCGCGGGCGGCTAGGGTTCCGGACGGAAAGCGATTTCAAAAAACGCTCAGCTACACATGCGTCAACTCGAGGGCTTGGGCGCTATGTGTGGTCCCAAATTCCGCTAATCCCAGAACTCCTACAATCATACTAACAATCGCCCTGTGCTTGTTTCTTGAGCGACTTGGGAGCCAAGAAATCCATTGTAAAGAAATTCTTTCGATGGATTTCTTGGCGCGACATACGCTAAGAGCACACCAAAGCGCGATTCTGATGAGAATTGCAGAAGATCTTGTATAAGCGTAGTTTGAACCCCATCGCGCTCAAGCGTTAGGGATGAGCACGTCGGATCACCTCGTTGAGGTCCCTCGTCGCGGGGACGCCGCCCACCGATGGGTGGATGGGTTCAGCCGTAACCCTACAGGCGCCTAGCTGGCGCGCCAGGGACATTTCTGCCTGCTTGCGGGACACGTATCTGCGCAAGGCGCTACGGGCCCCGGGGTCTCCTCGCTGGTGTCGTCGACAGCTCACCCTCTTTTTCTACTATGGTCTTATATTCAGGACCCCCACTTCTCGCGAACTGTCGACTCACTCTCACTAGAGTGCGGACTCTCAGACCTCCTACGAAGGAGAAAAATTCGTCCCACGGG
21	ITS-PUA-F*	CTATTGGCTAGCCTTTCCGTAAGCGGAGGCTCCTGAGAAGTTCACTGAACCCTCTCATCTAGAGGAAGGAGAAGTCGTAACAAGGTCTCCGTAGGTGAACCTGCGGAGGGATCCATAGCATTCGTAAACAAACTTGTCGGTCAAAAATACAAGCGAGGGGGGACGCCCTCTCCACCGACCCTCCTTGGCTAGGGCTGGCCGCCCGAGCGGCGCCGGACCCAGGCCTCACTGCCACGGTACCGTGCTGCCCTGGACCCCCGGGAGAACGTTGTCCGATTGGGGGCGCGCGGTACCCAAGCACCCGGTACGGGCTTGCGGCCGGACCGACCCAACCCAAGCGGGCGGCTCGGCCTCCGGACGGAAGCTGGGTCCTCCCAACACGCAGTACCCAGTGGTCCATTCGAGGGTAGAGCGCTAGCTGTGTACCCCAACTCCACTAATCAAGTCTCCTACACCATCCTAACAATCGCCTGTGTTGTGTCTTGAGCGTTTGAACCAAGAATCCTTGAAAGAGCTCTGTAGATGGATTGTTGGCGCAATCGTAAAGACAGCAAGGCGTTCTATGGATTGAGAGTCGTGATACGATTTGACCCTTGCCCAGCTTAGCTGACTGCGCTACTCTGAGTCCTGCTCCTGACTGCGTCGTGAGTGGGTCACCTACCTCGGCCCAGCTGGCGCGGAGCCTCTGCTGTACGGCTCGACTGGAATCGTCACGCTGGTCTCTCGTGGCCCACAACCCCTCTTTTTTCATTCTTCATCAGACCCCATCCCGACTGCCTCACTATATGTCGTCCCATCCAAAGAG
22	ITS-PUA-R*	TGCTGCTTATTGATATAGCTTAAGTTCAGCGGGTAACCCTGCCTAAACTCAGGTCGAATGGTGTGATGCCCAACCAGGTGTCCCGCCGAAGCAGCTTGCGAGGGCACCCCGCAAACACACATGCACACACACGCGTGCACACGCATGCGGGTGTGCGCTGTGGGTTGAAGGGGCATCAAGCCGCACCGACGAACCAGCAGGGCCGATGGTAAAGCATATGTGCCAGGAGATGCACCATGCTGACCATGAGCAGTGCCGAAAAAGGCTGCTGCCGCGCGGGCGCGCGGTATCGGCGACGTGCCGGAGATCATGCCTTAAAACCGAGCCCTCCTTCGCGAGCGGCTAGGGTCACGGACGGCAAGCCATTTGTAACAACGCTCAGGCACACATGCTTCCGCTCGAAGGCTTGAGCGCTATGTGTGTACCAAAATTCGATGATTCACAGAACTCTGACAATCATACTACCAATCGCGCTTTGCTGCTGTCTTCATCGATGTGCGAGCCAAGAAATCCAATGTACAGAGATTCTGTCGGATGGATTTGCTTGGCTGTGCTATACGCTCAAAGCACACACACAGCGCGATTCGTGAGTGATGAGTTGTAGTAGTATCGTAGTATCAGCGTAGTTGTTGGCATCGCAACAGTCTGCGCTCATGCGGTCTCACGGACTGGACATCGTCGGTCCTCTAGCTTTGCGTTGGCAGATCGCTCGTCGTCGCGTGACGCCTGACCGCTCCTGCATGGAGTGGCAGTGCGGTTTCAGCCGCTGAGTCTCGTAGCAGGGTCGCTTGAGTCCTGGCGCGGCGCGCGTCGAGGCACGACGTTTCTGCGCACGTGGTTAGCAGTGACGACTAGCTGGCACCTGTCAGCAAAAGACCTGCTAACTAGGGCGCTGTGGGTGCATGCCCTACGCTTGATGCCCTCTCAAACACAAAGCGGTCACACTCCTTCTATTTCTGACGGACTAGGTCGTTTACTATGGCAGGGAGCTCGAAAGTTTCTCTGACGGAAACCTGGTACGCACTTCACTTTCTGACATAGGATAGGGTGCAGGGTAAACTTCCGAGATCTACCCTTTACGAAGGCTACGATCGAACCTT

No.	Sequence name	Sequences
23	ITS-TD1-F*	CGGGGTCATGTTCGGATTGGTAGCCTATTCCGTAAGGTAGGTTCCTGAGAAGTTCACTGAACCCTCTCATCTAGAGGAAGGAGAAGTCGTAACAAGGTCTCCGTAGGTGAACCTGCGGAGGGATCCATAGCATTCGTAAAGAAACTTGTCGGTCAAAAATAAAAGCGAGGGGGGACTCCCTCTCCAACGACCCTCCTTGGCTAGGGCTGAACGCCCGAGCGGCATGGTACACAGGCCTTGCTGCCACAGTACCGTGGTGCCCTGCAACCCCCGGGAGAAAGGTGGCCCATTTGGGGCGCCCGTGTACCCAAGCACCCGGTACAGATCTTGGGCTTAGAACCAAGCCCTCCTTCAGGAGCGGCTCGGCTCCCGGACGGAAACTGATTTGAAAAAACGCTGAGGCAGACATGGTCAAGCCCGAAGGCTTGGGCGGTATGTGCGTTCCAAAACTCCATGAATCCCAGAACTCTGACATTCACCCTACCAATCACGCTGTGCTGCGTTCTTCATCGATGTGGGAGCCAAGAAATCCATTGTAAAGAGTTTCTGTTCGGTGGATTTCTTGGCTCCGCTATACGCTAAAAGCACACACAAAGGTTGATTCTGATGATAATTGTAGAGTTTGTAGTATCAGCGTATTTTTGCTCCCACACTGCCCTCATGCCCTACGAATGGACCATGTCTGCGCTGCTTGGTTTTAAATGGCTTGTCCCGCCGGAGGCTTGCCCATCCGCTTGGATTGTGTGGGTTAAGCCGTGATCCCGTGGCACGTCGCTTGAGACATGGGCGGCGCAAGTGAGACCACTTTCTGGCACTGCTTGCAGGACGCATGCGGTATCTCGTGGCACAGACGCGGGTACTGGGGGGCCCTCGGGGGTTAGTGCGGTGCGGCATTGCAGGCTTCGTCAAACAAGCGCATCACACCTCGCATTTTGATTTTTTTACGTACAATGTTTATTTACTGAATGCTATGAGCCTCGCAAGCTTCATCTGCTGAACCTGTTACGGACATTCTACTACTCGACATGAGATGAGGTCAGGATACTTCGCTAGAGCTCTACGCTTATCGATAGGCGTACGAATACGAACATATCAGCGAT
24	ITS-TD1-R*	CTTTTCGTCCGCTTATTGATATGCTTAAGTTCAGCGGGTAACCCTGCCTAAACTCAGGTCGAATGGTGTGTTGCGCAGCCAGGTGTCCAGCCGAAGCAGCTTGCGCGCGCACCCCGCACACACACATGCACACACACGCACGCACACGCATGCAGGTGTGCGCTGTGGGTTGGAGGGGCAGCAAGCCGCACCGACGAACCAGCAGGGCCCATGGTAAAGCATATGTGCCAGGAGATGCAGCATGCTTACCATGAGCAGTGCCGGAAAGGCTGCTGCCGCGCGCCGGCCATGTATAGGCGACGTGCCGGAGATCATGGCTTAAAACCATGCCCTCCTTCACGAGCGGCTAGGGTCACGGACGGCAAGCCATTTGAAACGACGCTCAGGCAGACATGCTCAAGCTCGAGGGCTTGAGCGCAATGTGCGTTCCAAAATTCGATGATTCACGGAATTCTGCAATTCATACTACCTATCGCGCTGTGCTGCGGTCTTCATCGATGTGGGAGCCAAGAAATCCATTGTACAGAGATTCTGTCGGTTGGATTTGCTTGGCGTGCTATACGCTCAAGAGCACACACACAGCGTTGATTCGTGAGTGATGAGTTGTAGTAGATCGTAGTATAGCGTAGCTGTTGGCACCGACAAGCTACCCCTCATGCGTCTCACGAGATGGGACCATCGTCGTTCGTTGCTTTGGTTGGAAGATCGCCTCGTCCGTGGCGTGACGCCTGGCCCATCCTGCATGGAGTGGTGTCGGTTCAAGCCGCAGGTCTCCTAGCCGGTCGCTTGAGTTCTGCGCGGCCCCGAATGAGAGACGTTCTCGCCGGTGGTTAGCAGGACGCTGCGGCACCTTGGCAGTCAAGACTCTGGTACTGGGGGCCCCTGTGCGTCATCCCGTACGCATGATGCTCCCTGTCGGAACAGGCGTCCACACCTCCTTTTTATTTTCTGACGTACTAGTTCGTTTACTATTGCAAGGAGCCCTCCAAGCTTCTATTCTGACGGAACCTTGTCAGACTTCACTATCTACTAGAGTTAGGTCAGGTATCTCCAACTCCCTTACGTAGCTAGATCGAACTTCCGATCCTCTAGTCGCGAGG

BLASTN analysis of all Sanger sequenced reads suggested that only sequences of 18S rDNA (No. 1–6), 23S rDNA (No. 9–12), 28S rDNA (No. 13, 14, and 17), and ITS region (No. 19–24), as listed in table 4.1, were annotated as *Cladophora* (see more details in Tables A.1–4). Therefore, only these sequences were used for phylogenetic-based *Cladophora* host identification.

Phylogenetic estimation of Cladophora *host using 18S, 23S, and 28S rDNAs, and ITS reads sequenced by Sanger sequencing*

Maximum likelihood tree constructed by using amplicons of 18S, 23S, and 28S rDNAs, and ITS region placed the amplicons within clades of *Cladophora* species (Figures 4.7–10). The 18S rDNA tree showed that the algal samples from this study were placed in the clade of *Cladophora* spp. with various other species and *C. glomerata* collected from Thailand (Figure 4.7, JQ071987.1–JQ072004.1, table B.1). For 23S rDNA, only the amplicons from PUA and TD1 were *Cladophora* (Table 4.1), where the maximum likelihood tree topology suggested that these taxa were closely related to the unclassified *Cladophora* collected from Northern California (Figure 4.8, MG021094.1, table B.2). For 28S rDNA, the reads from CKD and TD1 were *Cladophora* (Table 4.1), where the maximum likelihood tree suggested that the CKD reads were closely related to an unclassified *Cladophora* collected from China (Figure 4.9, KU904748.1, table B.3) and *C. columbiana* (Figure 4.9, LT607285.1 and LT607285.1, table B.3), whereas TD1 read was closely related to *C. vagabunda* (Figure 4.9, KX281896.1, table B.3) from North Carolina, USA. For ITS region, all reads from the three sites were closely related to *Cladophora* (Table 4.1), where the maximum likelihood tree suggested that they were closely related to *C. glomerata* (Figure 4.10, OM478590.1, LC KC914578.1, LC536858.1, LC536864.1, and LC482128.1, table B.4) and the unclassified *Cladophora* clade (Figure 4.10, KU185997.1, KU904777.1, and 25 species in collapsed branch, table B.4).

Figure 4.7 Maximum likelihood trees inferred from 18S rDNA of *Cladophora* and other members of Cladophoraceae.

Rhizoclonium pachydermum was used as an outgroup. Values at nodes indicate maximum likelihood bootstrap values (left) and Bayesian posterior probability values (right). Stars represent sequences obtained from marker-gene primers and Sanger sequencing. F indicates the Sanger sequencing direction starting from the 5’ terminal of the amplicon. R indicates the Sanger sequencing direction starting from the 3’ terminal of the amplicon. Circles represent the 18S amplicon read obtained from universal primers and Illumina Miseq sequencing identified as *Cladophora*. NCBI accession numbers are labelled in parentheses. Roman numerals indicate the number of sequences included in each species. Branches with two diagonal lines were shortened to one-fifth of their original length. The scale bar represents the estimated number of nucleotide substitutions per site.

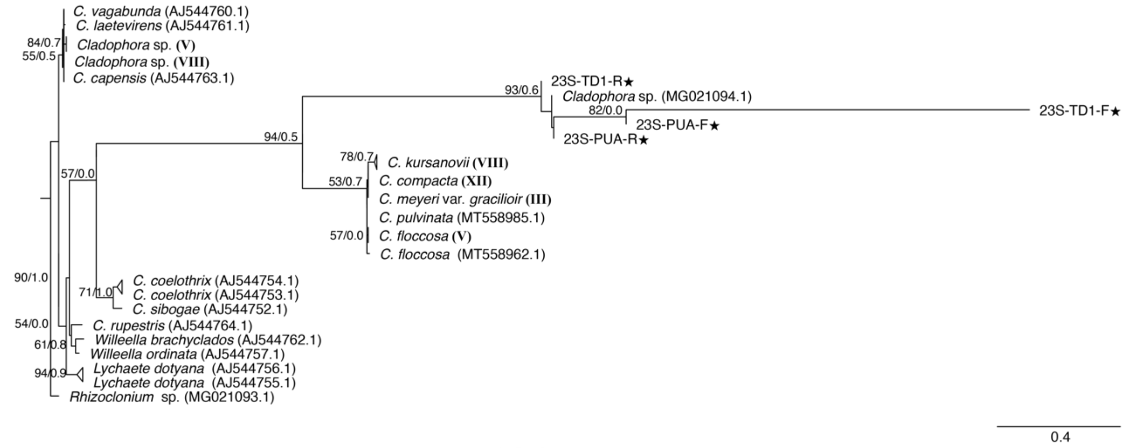


Figure 4.8 Maximum likelihood trees inferred from 23S rDNA of *Cladophora* and other members of Cladophoraceae.

Rhizoclonium sp. was used as an outgroup. Values at nodes indicate maximum likelihood bootstrap values (left) and Bayesian posterior probability values (right). Stars represent sequences obtained from marker-gene primers and Sanger sequencing. F indicates the Sanger sequencing direction starting from the 5’ terminal of the amplicon. R indicates the Sanger sequencing direction starting from the 3’ terminal of the amplicon. NCBI accession numbers are labelled in parentheses. Roman numerals indicate the number of sequences included in each species. The scale bar represents the estimated number of nucleotide substitutions per site.

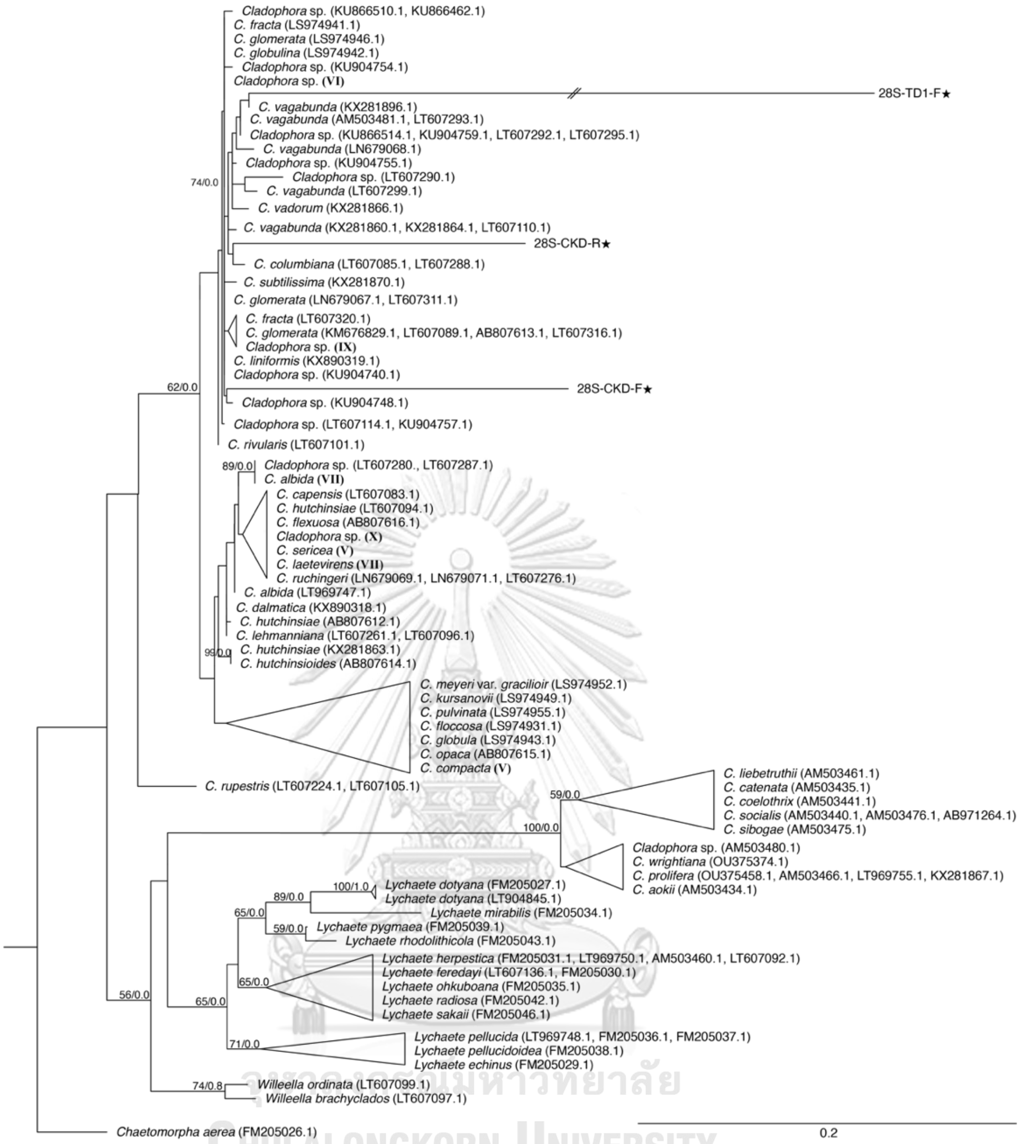


Figure 4.9 Maximum likelihood trees inferred from 28S rRNA of *Cladophora* and other members of Cladophoraceae.

Chaetomorpha aerea was used as an outgroup. Values at nodes indicate maximum likelihood bootstrap values (left) and Bayesian posterior probability values (right). Stars represent sequences obtained from marker-gene primers and Sanger sequencing. F indicates the Sanger sequencing direction starting from the 5’ terminal of the amplicon. R indicates the Sanger sequencing direction starting from the 3’ terminal of the amplicon. NCBI accession numbers are labelled in parentheses. Roman numerals indicate the number of sequences included in each species. Branches with two diagonal lines were shortened to one-fifth of their original length. The scale bar represents the estimated number of nucleotide substitutions per site.

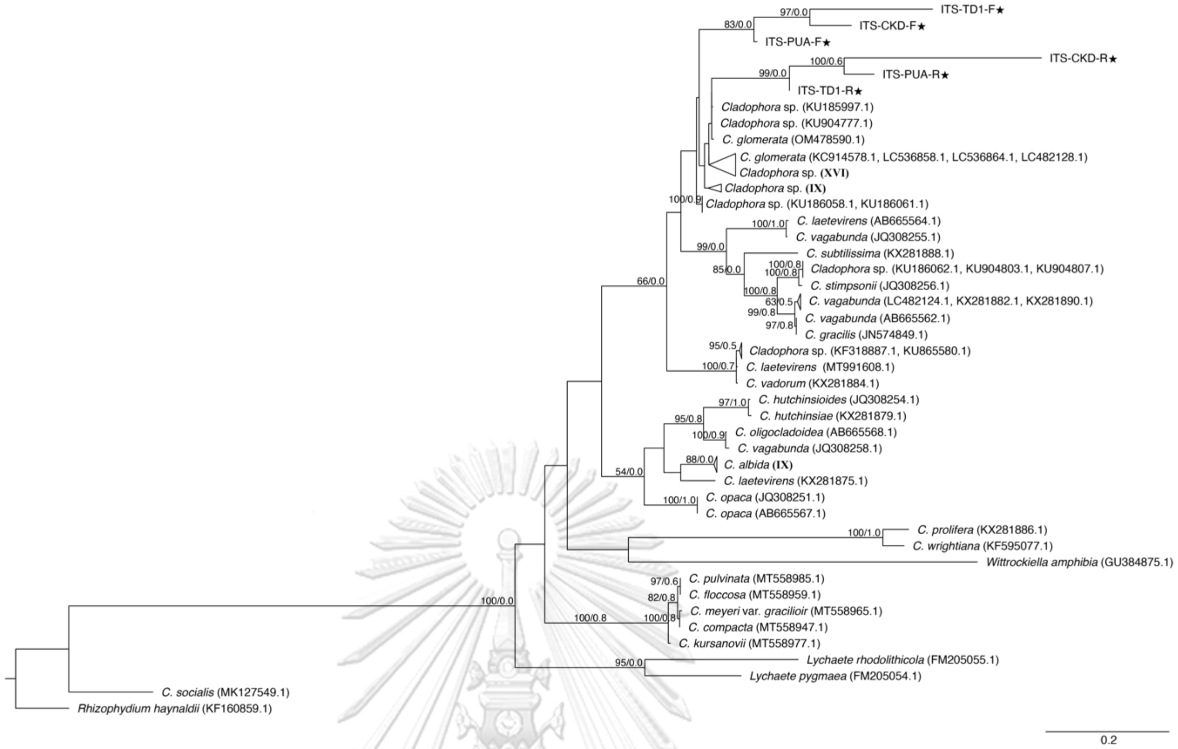


Figure 4.10 Maximum likelihood trees inferred from ITS rRNA of *Cladophora* and other members of Cladophoraceae.
Rhizophydium haynaldii was used as an outgroup. Values at nodes indicate maximum likelihood bootstrap values (left) and Bayesian posterior probability values (right). Stars represent sequences obtained from marker-gene primers and Sanger sequencing. F indicates the Sanger sequencing direction starting from the 5’ terminal of the amplicon. R indicates the Sanger sequencing direction starting from the 3’ terminal of the amplicon. NCBI accession numbers are labelled in parentheses. Roman numerals indicate the number of sequences included in each species.

Additional phylogenetic estimation of Cladophora *sp. using amplicons from universal 18S rDNA primers and Illumina Miseq sequencing*

To overcome the problems of ambiguity present in the amplified sequences, the *Cladophora* 18S rDNA reads obtained from the metagenomic analysis were additionally used to construct the maximum likelihood tree (Table 4.2). The alignment of the data matrix showed that the 18S rDNA reads from all collecting sites were identical (Figure A.25). Results from maximum likelihood tree topology suggested that the *Cladophora* collected from this study was placed in the clade of *Cladophora* spp., together with the 18S rDNA amplified from primers listed in table 3.1.

Table 4.2 The raw reads of 18S rDNA amplified by the universal 18S rDNA primers and sequenced by Illumina Miseq technology from site CKD, representing the identical *Cladophora* reads obtained from the *Cladophora* host of this study.

Sequence name	**Sequence**
18S-CKD-*Cladophora*	GCAGTTAAAAAGCTCGTAGCTGAACCTCGGGCAACCCTGACGGTCCGCGTTTCGTGAGTACTGTCGTGGGTTGTCTTTCTGGCAAGACCGTATCTCGGCGTTAACTCGCCGGGAACGGCGCTTGCCAAGGTTACTTTGAGTAAAATAGAGTGTTCAAAGCAAGCTCGCGCTCTGAATACACTAGCATGGGATAACACGACATGACCGATGGTCTATCTTGTTGGCCTGTAGACCTGTTGGTAATGGCTAAT

4.2 Study of the diversity of epiphytic microbiota of Kai *Cladophora* sp.

4.2.1 Morphological identification

Under the best circumstances, the epiphytic microbiota of *Cladophora* microbiome were observed under the light compound microscope and captured. For all the *Cladophora* samples, the prokaryotes and eukaryotes were classified into different taxonomic groups. These included cyanobacteria – *Chamaesiphon* (Figure 4.11A) and *Oscillatoria* (Figure 4.11B), a protozoa *Vorticella* (Figure 4.12), and stramenopiles – *Cocconeis* (Figure 4.13A), *Gomphonema* (Figure 4.13B), *Navicula* (Figure 4.13C), *Pinnularia* (Figure 4.13D), *Synedra* (Figure 4.13E), and *Synura* (Figure 4.13F). In greater detail, *Cocconeis, Gomphonema,* and *Synedra* appeared attached to the *Cladophora* cell surface, while *Navicula, Pinnularia,* and *Synura* were present as both attachments and free-floating within the algal biosphere.

Figure 4.11 Micrographs of cyanobacterial members of *Cladophora* microbiota.
A. Black arrowhead points *Chamaesiphon* attached to surface of *Cladophora* host.
B. *Oscillatoria* in *Cladophora* dominant sample.

Figure 4.12 Micrograph of protozoal member of *Cladophora* microbiota.

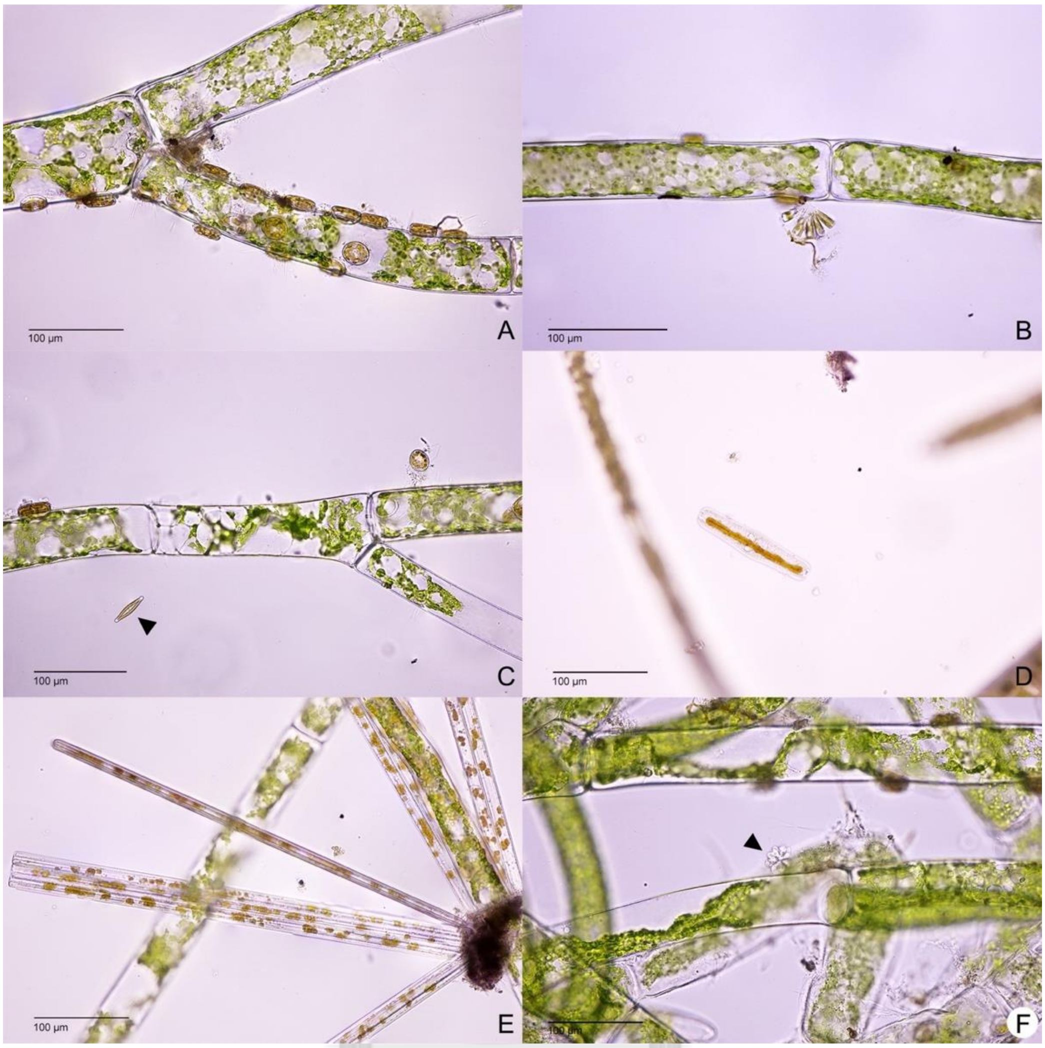


Figure 4.13 Micrographs of stramenopile-members of *Cladophora* microbiota.

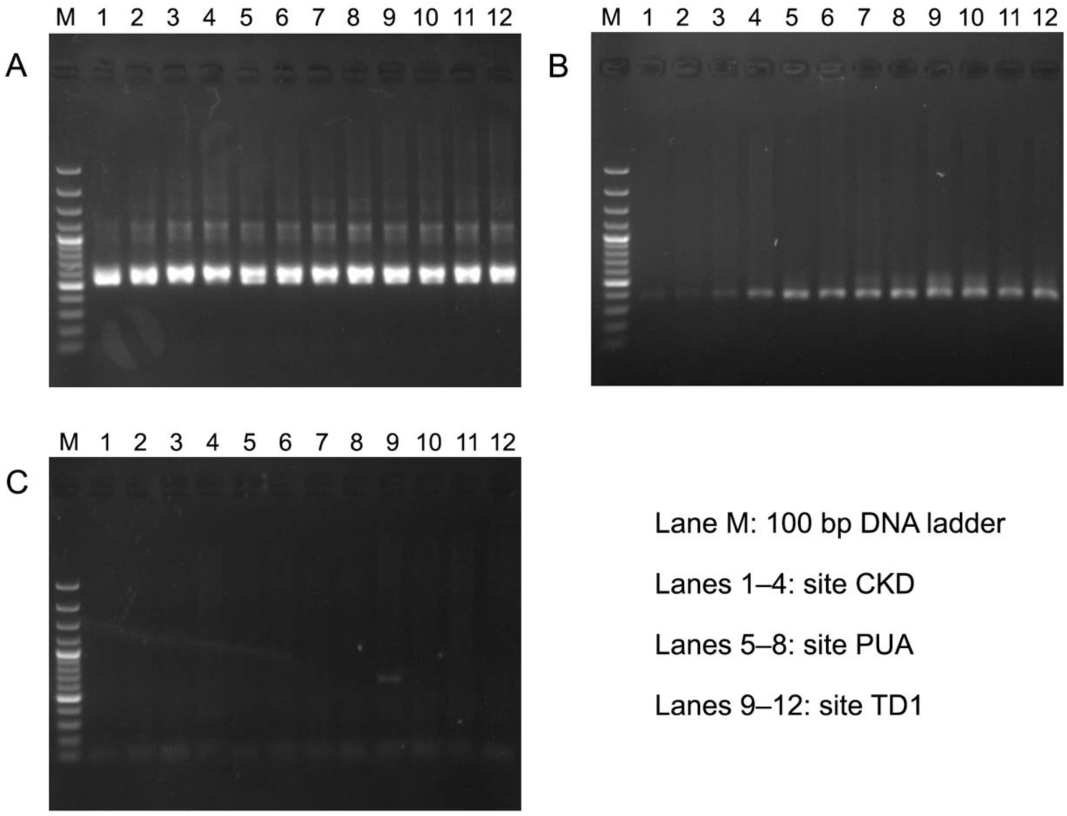
A. *Cocconeis*, B. *Gomphonema*, and E. *Synedra* attached to the surface of the *Cladophora* host. C. Black arrowhead-pointed *Navicula*, D. *Pinnularia*, and F. black arrowhead-pointed *Synura* in *Cladophora* dominant samples.

4.2.2 Taxonomic identification using amplicon-based metagenomic analysis

DNA extraction, marker gene amplification, and sequencing

The metagenomic DNAs of *Cladophora*-dominant samples (CKD, PUA, and TDI) were successfully extracted and amplified for the 16S rRNA, 18S rRNA, and ITS (Figure 4.14A-C), where the amplicons were then paired-end sequenced using an Illumina MiSeq, 250 bp, by Omics Sciences and Bioinformatics Center (See more details in table C.1).

The extracted DNAs from three *Cladophora* dominant sites, each with four replicates, totaling twelve extracted DNAs, were amplified for 16S rRNA, 18S rRNA, and ITS regions using the primers listed in table 3.2. Agarose gel electrophoresis showed that all replicates of 16S rDNA (Figure 4.14A) and 18S rDNA (Figure 4.14B) amplicons were successfully amplified, while only one replicate of ITS regions (Figure 4.14C) from site TD1 was successfully amplified. These amplicons were paired-end sequenced using an Illumina MiSeq, 250 bp, by Omics Sciences and Bioinformatics Center.



Lane M: 100 bp DNA ladder

Lanes 1–4: site CKD

Lanes 5–8: site PUA

Lanes 9–12: site TD1

Figure 4.14 Agarose gel electrophoresis of amplicons.
A. 16S rDNA amplicon, B. 18S rDNA amplicon, C. ITS amplicon. Lanes 1–4 are four replicates from site CKD, lanes 5–8 are four replicates from site PUA, lanes 9–12 are four replicates from site TD1, and lanes M are 100 bp DNA ladders. All replicates of 16S rDNA (A) and 18S rDNA (B) amplicons were successfully amplified, while only one replicate of ITS amplicon (C; lane 9) from site TD1 was successfully amplified.

Taxonomic classification

In this study, three universal primer sets were used to capture the hypervariable regions of 16S rDNA, 18S rDNA, and ITS for taxonomic classification. By utilizing these regions, the organisms present in the *Cladophora* microbiota could be categorized into three groups which are bacteria, eukaryotes, and fungi.

Bacterial taxa

The identified bacteria included 45 distinct phyla, plus some unidentified taxa (Figure 4.15; see more details in table C.2). The five most abundant phyla in all study sites were Proteobacteria, Bacteroidota, Firmicutes, Verrucomicrobia, and Planctomycetota. For site CKD, the five most abundant phyla were Proteobacteria (40.04%), Bacteroidota (21.07%), Firmicutes (10.59%), Verrucomicrobia (5.30%), and Planctomycetota (4.80%). For site PUA, the five most abundant phyla were Proteobacteria (29.29%), Bacteroidota (22.50%), Firmicutes (10.86%), Planctomycetota (6.88%), and Cyanobacteria (6.20%). For site TD1, the five most abundant phyla were Proteobacteria (31.77%), Bacteroidota (24.37%), Firmicutes (6.67%), Verrucomicrobia (6.22%), and Cyanobacteria (4.98%).

At the genus level, as shown in figure 4.16 and table C.3, the members of these 45 bacterial phyla were classified into 689 distinct genera. For site CKD, the five most abundant genera were Proteobacteria *Aeromonas* (5.58%), Bacteroidota *Paludibacter* (4.40%), Bacteroidota *Flavobacterium* (2.67%), Proteobacteria *Acinetobacter* (2.65%), and Campylobacterota *Pseudarcobacter* (1.87%). For site PUA, the five most abundant genera were Bacteroidota *Paludibacter* (5.62%), Proteobacteria *Aeromonas* (2.95%), Bacteroidota *Flavobacterium* (2.56%), Verrucomicrobia *Luteolibacter* (1.33%), and Planctomycetota *Fimbriiglobus* (1.17%). For site TD1, the five most abundant genera were Bacteroidota *Paludibacter* (6.57%), Bacteroidota *Flavobacterium* (1.86%), Proteobacteria *Propionivibrio* (1.75%), Proteobacteria *Hydrogenophaga* (1.36%), and Verrucomicrobia *Luteolibacter* (1.20%) (Figure 4.15).

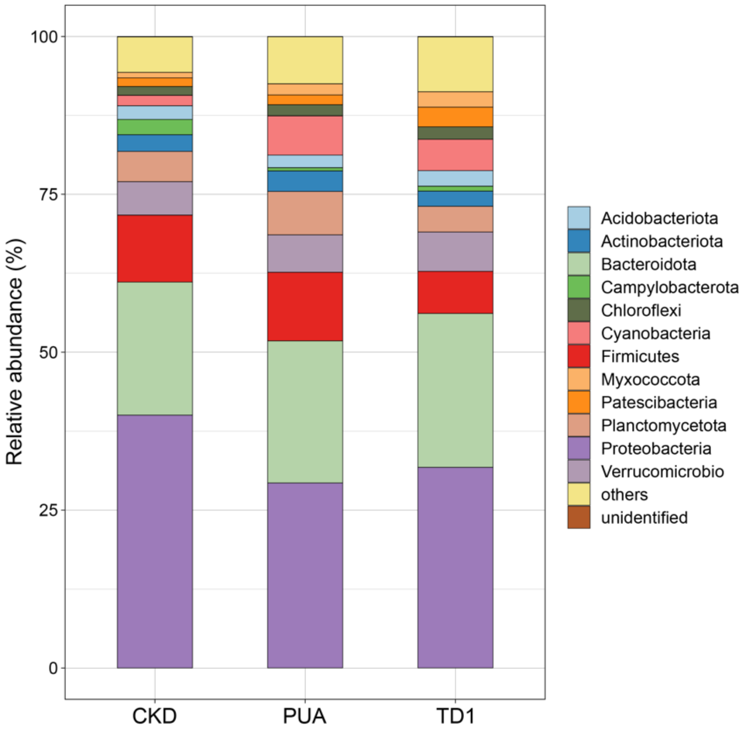


Figure 4.15 Stacked bar chart of relative abundance of bacterial phyla based on 16S amplicon analysis.

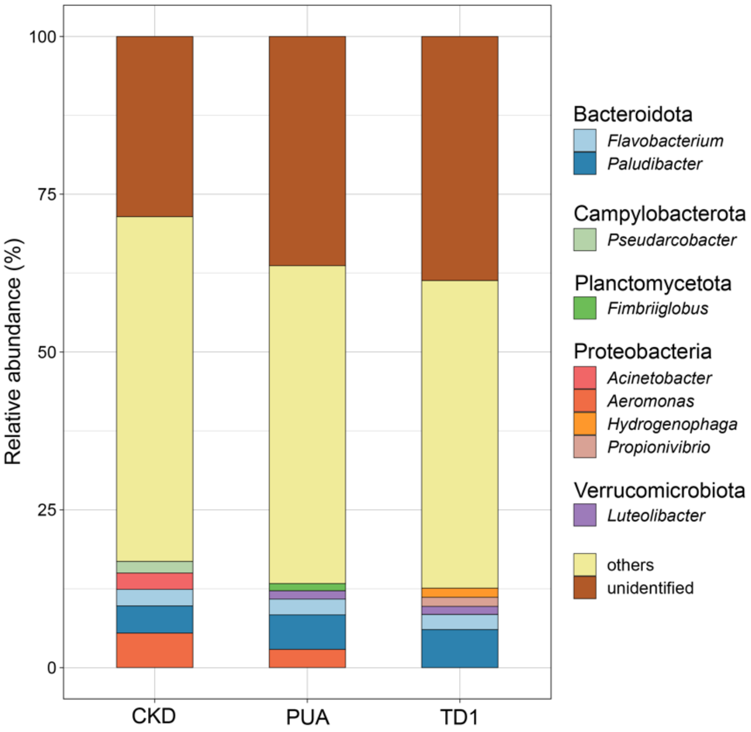


Figure 4.16 Stacked bar chart of relative abundance of the five most abundant genera and others based on 16S amplicon analysis.

The annotated 689 bacterial genera present in all replicates were classified into three groups. The first group included 163 bacterial genera present in all replicates at only one sampling site, named “unique taxa”. The second group included 346 bacterial genera present in all replicates of more than one sampling site, named “shared taxa”. The third group included 180 genera, including the bacterial taxa present in only some replicates.

The unique and shared bacterial taxa present in the *Cladophora* microbiota were the main focus of this study. As shown in the Venn diagram (Figure 4.17), 46, 60, and 57 unique taxa were observed in CKD, PUA, and TD1, respectively. For the shared taxa, 269, 240, 291, and 227 taxa were present in CKD–PUA, CKD–TD1, PUA–TD1, and CKD–PUA–TD1, respectively. For the 227 taxa that were shared across all sites, 42 taxa appeared to be more abundant at CKD than at other locales. These taxa with high abundance at CKD, written here as the relative abundance present at CKD, PUA, and TD1, included Proteobacteria *Aeromonas* (5.58%, 2.95%, and 0.57%), Bacteroidota *Flavobacterium* (2.67%, 2.56%, and 1.86%), Proteobacteria *Acinetobacter* (2.65%, 0.32%, and 0.16%), Campylobacterota *Pseudarcobacter* (1.87%, 0.16%, and 0.08%), Firmicutes *Clostridium sensu stricto* 12 (1.77%, 0.77%, and 0.25%), Bacteroidota *Bacteroides* (1.66%, 0.25%, and 0.05%), Proteobacteria *Vogesella* (1.23%, 0.87%, and 0.25%), Bacteroidota *Acetobacteroides* (1.18%, 1.15%, and 0.81%), and Bacteroidota *Cloacibacterium* (0.69%, 0.44%, and 0.11%). On the other hand, 50 taxa appeared to be more abundant at TD1 than at other locales. These taxa with high abundance at TD1, written here as TD1, PUA, and CKD, included Bacteroidota *Paludibacter* (4.40%, 5.62%, and 6.57%), Proteobacteria *Hydrogenophaga* (0.45%, 0.74%, and 1.36%), Firmicutes *Fusibacter* (0.34%, 0.63%, and 1.42%), Proteobacteria *Phreatobacter* (0.24%, 0.61%, and 0.67%), and Proteobacteria *Leptothrix* (0.24%, 0.37%, and 0.86%).

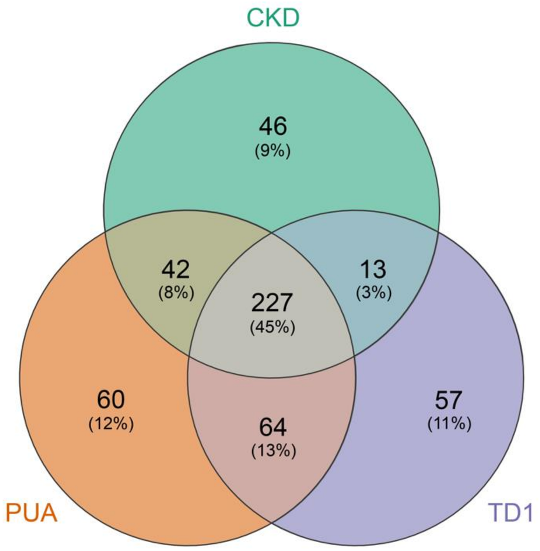


Figure 4.17 Venn diagram of 509 bacterial genera obtained from 16S rDNA amplicon analysis.

Eukaryotic taxa

The identified eukaryotes included six supergroups plus some unidentified taxa (figure 4.18 and table C.4). The five most abundant supergroups in all study sites were Amorphea, Stramenopiles, Archaeplastida, Rhizaria, and Alveolata. For site CKD, the five most abundant supergroups were Amorphea (46.19%), Stramenopiles (27.10%), Archaeplastida (17.33%), Rhizaria (5.55%), and Alveolata (3.66%). For site PUA, the five most abundant supergroups were Stramenopiles (45.84%), Amorphea (28.95%), Archaeplastida (15.71%), Rhizaria (5.33%), and Alveolata (4.01%) For site TD1, the five most abundant supergroups were Stramenopiles (46.04%), Amorphea (27.83%), Archaeplastida (12.55%), Rhizaria (7.02%), and Alveolata (6.37%).

At the genus level, as shown in figure 4.19 and table C.5, the members of these six supergroups were classified into 575 distinct genera. For site CKD, the five most abundant genera were Stramenopiles *Cocconeis* (4.86%), *Ochromonas* (3.64%), *Pythium* (3.26%), Amorphea *Sorodiplophrys* (3.07%), and *Paramicrosporidium* (2.45%). For site PUA, the five most abundant genera were Stramenopiles *Cocconeis* (27.43%), Archaeplastida *Spirogyra* (4.27%), Stramenopiles *Ochromonas* (4.19%), Amorphea *Sorodiplophrys* (2.70%), and Stramenopiles *Poteriospumella* (1.16%). For site TD1, the five most abundant genera were Stramenopiles *Cocconeis* (21.10%), *Gomphonema* (4.59%), Amorphea *Sorodiplophrys* (4.09%), Archaeplastida *Spirogyra* (3.40%), and Stramenopiles *Ochromonas* (3.06%).

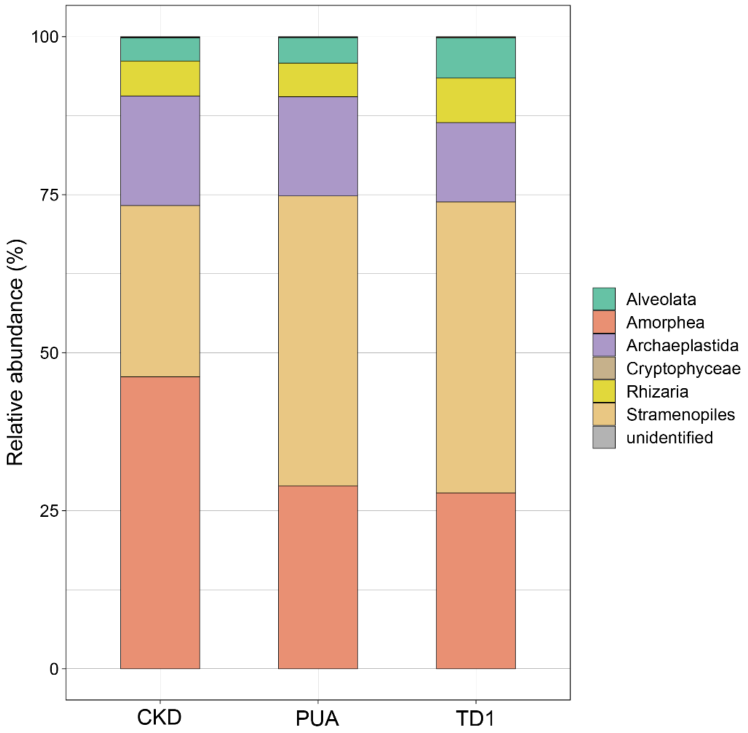


Figure 4.18 Stacked bar chart of relative abundance of eukaryotic supergroups based on 18S rDNA amplicon analysis.

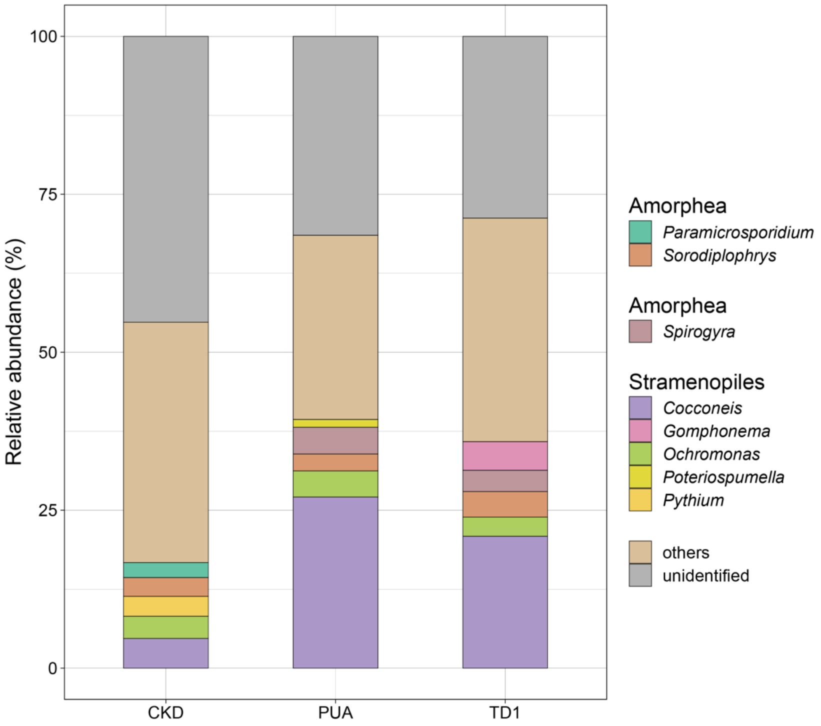


Figure 4.19 Stacked bar chart of relative abundance of the five most abundant genera and others based on 18S rDNA amplicon analysis.

Among the 575 eukaryotic genera, only 83 genera were "unique taxa", taxa present in all four replicates of only one sampling site. In addition, 96 genera appeared as "shared taxa", the taxa commonly present in more than one study site. The unique taxa present in CKD, PUA, and TD1 were 26, 9, and 48 taxa, respectively. For the shared taxa, CKD–PUA, CKD–TD1, PUA–TD1, and CKD–PUA–TD1 shared 58, 60, 80, and 51 taxa, respectively (Figure 4.20).

For the 51 taxa that were shared across all sites, 14 taxa appeared to be more abundant at CKD than at other locales. These taxa with high abundant at CKD, written here as the relative abundance present at CKD, PUA, and TD1, included Amorphea *Paramicrosporidium* (2.45%, 1.05%, and 0.18%), Stramenopiles *Poteriospumella* (1.42%, 1.26%, and 0.44%), Archaeplastida *Planktosphaeria* (0.97%, 0.60%, and 0.21%), and Archaeplastida *Deasonia* (0.70%, 0.44%, and 0.08%). On the other hand, five taxa appeared to be more abundant at TD1 than at other locales. These taxa with high abundance TD1, written here as the relative abundance present at TD1, PUA, and CKD, included Stramenopiles *Cymbella* (0.34%, 0.66%, and 1.12%) and Rhizaria *Cercomonas* (0.14%, 0.21%, 0.56%).

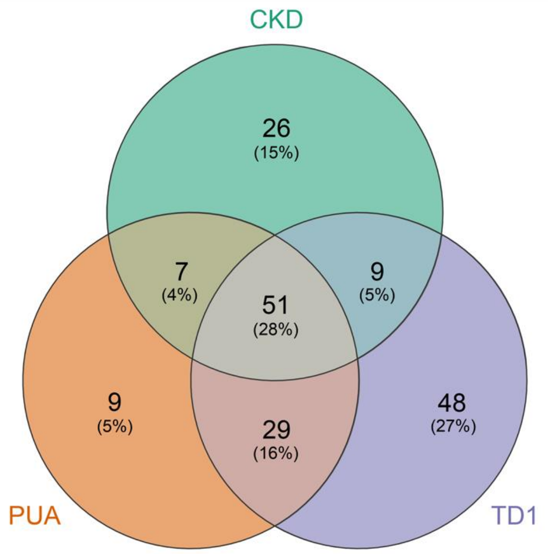


Figure 4.20 Venn diagram of 179 eukaryotic genera obtained from 18S rDNA amplicon analysis.

Eukaryotic taxa identified by ITS amplicon

Only one replicate of ITS amplicons (Figure 4.14C) from site TD1 was successfully amplified and sequenced. The identifiable fungi included eight fungal phyla, including Ascomycota (53.96%), Chytridiomycota (29.95%), Basidiomycota (9.22%), Rozellomycota (0.81%), Glomeromycota (0.26%), Entomophthoromycota (0.12%), Mortierellomycota (0.06%), and Mucoromycota (0.04%), as shown in figure 4.21A. At the genus level, the members of these eight phyla were classified into 133 distinct genera (Table C.6). The seven most abundant genera were Chytridiomycota *Avachytrium* (18.57%) and *Entophlyctis* (10.95%), Ascomycota *Cladosporium* (3.61%) and *Capnobotryella* (2.54%), Basidiomycota *Hannaella* (2.40%), Ascomycota *Glutinoglossum* (2.33%), and Basidiomycota *Sporobolomy* (2.29%), as shown in figure 4.21B.

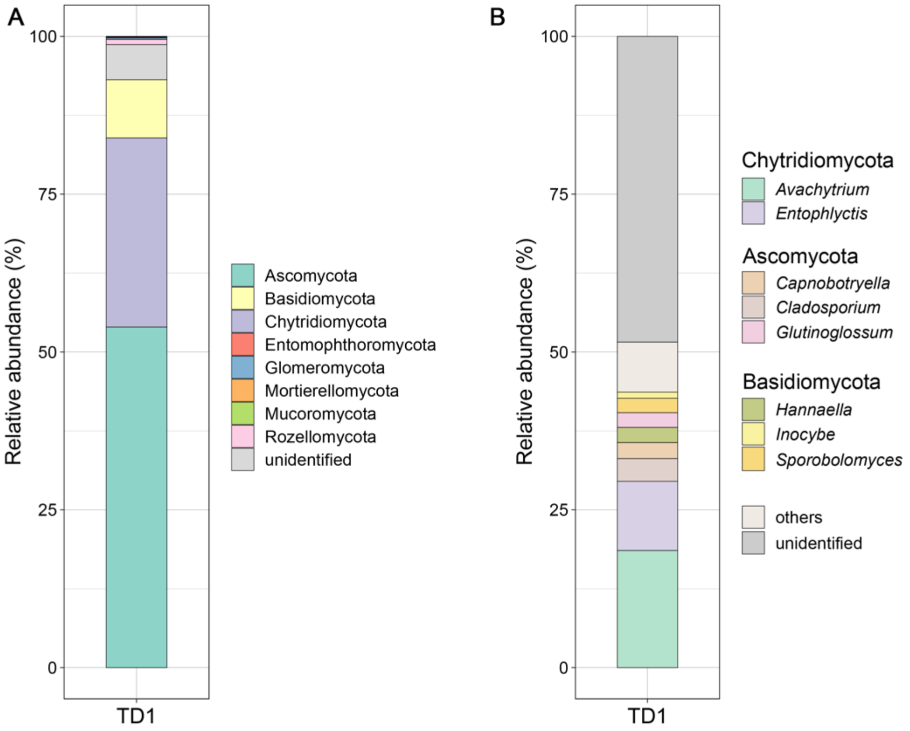


Figure 4.21 Stacked bar chart of relative abundance of fungal taxa based on the ITS amplicon analysis.

A. phylum level and B. genus level.

Fungal taxa present in 18S rDNA vs ITS amplicon analyses

18S rDNA and ITS amplicon analyses showed the presence of fungal genera (Figures 4.14A and 4.14C). In this study, only one replicate of the amplicon from TD1 was successfully sequenced. Therefore, to compare the taxonomic results obtained from 18S rDNA and ITS analyses, the identified fungal taxa present in at least one replicate of 18S rDNA were compared to that of TD1 ITS. Results showed that 185 and 133 fungal genera were obtained from 18S rDNA and ITS of three sampling sites, respectively. Among these, 82 fungal genera were commonly found by using both conserved regions (Figure 4.22).

When comparing the 18S rDNA results of identified fungal genera across sites, results showed that 51, 4, and 14 fungal genera were uniquely present at CKD, PUA, and TD1, respectively (Table C.7). CKD shared 43 genera with PUA and 61 genera with TD1, PUA shared 37 genera with TD1, and 36 genera were commonly present in all sites, as shown in figure 4.22 and table C.7. When adding the ITS results at TD1 to the comparison, results showed four additional genera were shared between TD1 and PUA, which were Ascomycota *Phaffomyces*, Basidiomycota *Tilletiopsis*, *Trametes*, and *Tropicoporus*. In addition, four genera were shared for all sites. These included Basidiomycota *Symmetrospora*, Ascomycota *Parastagonospora*, *Mycosisymbrium*, and *Neotestudina*.

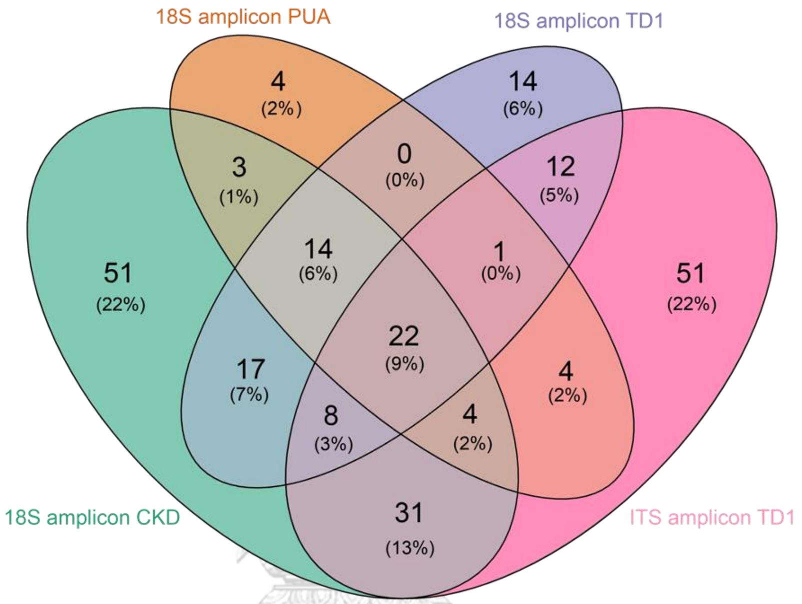


Figure 4.22 Venn diagram of 236 fungal genera obtained from 18S rDNA and ITS amplicons analyses.

4.2.3 Ecological functional inference

Bacterial ecological functions

To demonstrate the ecological roles of annotated *Cladophora* microbiota, literatures were searched for the putative ecological functions of the shared taxa. The functions were categorized into two main groups: nitrogen cycling and other nutrient cyclings (Tables 4.3–4).

For nitrogen cycling, the functions were divided into eleven ecological functions, written with the example of the taxa with relative high abundance, including heterotrophic nitrification and aerobic denitrification (*Aeromonas* and *Acinetobacter*), dissimilatory reduction of nitrate to ammonium (*Aeromonas*, *Lacunisphaera*, and *Pelosinus*), ammonium oxidation (*Pirellula*), nitrate reduction (*Vogesella*), denitrifying phosphorus-accumulation, nitrite reduction, and nitrogen fixation (*Dechloromonas*). For other nutrient cyclings, there were bacteria involved in amino acid fermentation (*Acidaminobacter*), cellulose degradation (*Bacteroides* and *Paludibacter*), chitin degradation (*Fimbriiglobus*), degradation of aromatic compound (*Hydrogenophaga*), fermentative hydrogen production (*Acetobacteroides* and *Clostridium sensu stricto* 12), polyphosphate accumulation (*Acinetobacter* and *Propionivibrio*), and vitamin B_{12} biosynthesis (*Flavobacterium*).

Fungal ecological functions

The ecological functions of fungi present in this study included decomposition, plant-fungal interaction, predation, mutualism, and parasitism in algae, amoebae, and other fungi. Among the taxa, some showed high relative abundances when compared to others. These included *Capnobotryella* (lichen-forming fungus), *Entophlyctis* (parasite in algae), *Paramicrosporidium* (parasite in amoebae), and *Hannaella* (phylloplane fungus), as shown in table 4.5.

Table 4.3 Putative ecological functions involving in nitrogen cycling.
* indicates bacteria with high relative abundance.

Ecological function	**Bacterial genera**	**Reference**
Denitrification	*Acidovorax*	Heylen et al., 2008
	Arenimonas	Feng et al., 2020
	Chromobacterium	Bazylinski et al., 1986
	CL500-29 marine group	Chen et al., 2020b
	Comamonas	Zhang et al., 2016
	Devosia	Tanikawa et al., 2018
	Hyphomicrobium	Martineau et al., 2015
	Leptothrix	Feng et al., 2020
	Methylotenera	Kalyuhznaya et al., 2009
	Chloroflexi bacterium OLB13	Zhang et al., 2021
	Opitutus	Tanikawa et al., 2018
	Paracoccus	Feng et al., 2020
	Pseudomonas	Tanikawa et al., 2018
	Rhodobacter	Tanikawa et al., 2018
	Planctomycetes SM1A02	Qi et al., 2020
	Thauera	Liu et al., 2013

Ecological function	**Bacterial genera**	**Reference**
Heterotrophic nitrification and aerobic denitrification	*Acinetobacter**	Chen et al., 2019
	*Aeromonas**	Chen et al., 2014a
	Bacillus	Yang et al., 2011
	Chryseobacterium	Kundu et al., 2014
	Comamonas	Chen & Ni, 2011
	Klebsiella	Padhi et al., 2013
	Massilia	Qiao et al., 2020
	Paracoccus	Yang et al., 2008
	Pseudomonas	Yang et al., 2019
	Rhizobium	Qiao et al., 2020
Denitrifying phosphorus-accumulation	*Candidatus* Accumulibacter	Huang et al., 2020
	*Dechloromonas**	Huang et al., 2020
Dissimilatory reduction of nitrate to ammonium	*Aeromonas**	Zhao et al., 2020
	Geobacter	Zhao et al., 2020
	*Lacunisphaera**	Zhao et al., 2020
	*Pelosinus**	Beller et al., 2013
Ammonium oxidation	*Blastopirellula*	Khramenkov et al., 2013
	Brevifollis	Muwawa et al., 2021
	Ellin6067	Podlesnaya et al., 2020
	Gemmata	Tian et al., 2015
	mle1-7	Podlesnaya et al., 2020
	oc32	Podlesnaya et al., 2020
	*Pirellula**	Tian et al., 2015
	Planctomycetes SM1A02	Vico et al., 2021
Nitrate reduction	*Noviherbaspirillum*	Wu et al., 2021
	Rhizobacter	Goto & Kuwata, 1988
	Sulfurospirillum	Hubert & Voordouw, 2007
	Thauera	Hubert & Voordouw, 2007
	*Vogesella**	Rameshkumar et al., 2016
Nitrite oxidation	*Candidatus* Nitrotoga	Ishii et al., 2020

Ecological function	**Bacterial genera**	**Reference**
Nitrite reduction	*Arenimonas*	Huang et al., 2020
	Azoarcus	Huang et al., 2020
	*Dechloromonas**	McIlroy et al., 2016
	Haliangium	McIlroy et al., 2016
	Rhodoferax	Hougardy & Klemme, 1995
	Sulfuritalea	McIlroy et al., 2016
Nitrogen fixation	*Anaeromyxobacter*	Masuda et al., 2020
	Azospira	Bae et al., 2007
	*Dechloromonas**	Salinero et al., 2009
	Devosia	Rivas et al., 2002
	Mesorhizobium	Laranjo et al., 2014
	Methylocystis	Takeda, 1988
	Nordella	Yu et al., 2019
	Pelomonas	Xie & Yokota, 2005
	Rhizobium	Sheu et al., 2015
	Shinella	Taulé et al., 2016
Nitrous oxide reduction	*Gemmatimonas*	Park et al., 2017

Table 4.4 Putative ecological functions involving in other nutrient cyclings.
* indicates bacteria with high relative abundance.

Ecological function	Bacterial genera	Reference
Iron cycling		
Fe (II) oxidation	*Curvibacter*	Gülay et al., 2018
	Leptothrix	Peng et al., 2021
	Noviherbaspirillum	Wu et al., 2021
	Sideroxydans	Liu et al., 2012
	Undibacterium	Gülay et al., 2018
Fe (III) reduction	*Acidibacter*	Falagan & Johnson, 2014
	Geobacter	Peng et al., 2021
	Rhodoferax	Peng et al., 2021
Manganese cycling		
Mn (II) oxidation	*Pedomicrobium*	Ridge et al., 2007
	Sideroxydans	Liu et al., 2012
Mn (IV) reduction	*Geobacter*	Peng et al., 2021
	Rhodoferax	Peng et al., 2021
Phosphorous cycling		
Polyphosphate accumulation	*Acinetobacter**	Seviour et al., 2003
	Arcicella	Chai et al., 2017
	Limnohabitans	Chai et al., 2017
	*Propionivibrio**	Li et al., 2019a
Sulfur cycling		
Sulfate reduction	*Desulfobulbus*	Taylor & Parkes, 1983
	Desulfomicrobium	Copeland et al., 2009
	Desulfovibrio	Karnachuk et al., 2021
Sulfur oxidation	*Limnobacter*	Chen et al., 2016
	Meiothermus	Sun et al., 2018
Sulfur reduction	*Fusibacter*	Fadhlaoui et al., 2015
Uranium cycling		
U (IV) reduction	*Anaeromyxobacter*	North et al., 2004
	Geobacter	North et al., 2004

Ecological function	**Bacterial genera**	**Reference**
Degradation		
Alkane degradation	*Aquabacterium*	Masuda et al., 2014
	Tropicimonas	Harwati et al., 2009
Cellulose degradation	*Aquitalea*	Woo et al., 2014
	*Bacteroides**	Hatamoto et al., 2014
	Caulobacter	Song et al., 2013
	Cellvibrio	DeBoy et al., 2008
	Cloacibacterium	Cui et al., 2019
	Cytophaga	Xie et al., 2007
	Exiguobacterium	Cui et al., 2019
	Ilumatobacter	Cai et al., 2018
	*Paludibacter**	Cui et al., 2019
	Roseimarinus	Co & Hug, 2021
	Ruminiclostridium	Ravachol et al., 2016
Chitin degradation	*Chitinibacter*	Gao et al., 2015
	Chitinimonas	Joung et al., 2014
	*Fimbriiglobus**	Ravin et al., 2018
	Massilia	Chen et al., 2020a
	Planctomyces sp. SH-PL14	Kulichevskaya et al., 2019
Degradation of aromatic compounds	*Acinetobacter*	Vedler et al., 2013
	Azoarcus	Peng et al., 2021
	*Hydrogenophaga**	Fan et al., 2019
	Hyphomicrobium	Kim et al., 2020
	Leptothrix	Peng et al., 2021
	Limnobacter	Vedler et al., 2013
	Methylibium	Kane et al., 2007
	Ottowia	Kim et al., 2020
	Pseudomonas	Vedler et al., 2013
	Sulfuritalea	Kim et al., 2020
	Thauera	Mechichi et al., 2002
Degradation of biodegradable plastics	*Sphingopyxis*	Verma et al., 2020
Volatile fatty acid degradation	*Ohtaekwangia*	Shu et al., 2015
Other organic pollutant degradation	*Rheinheimera*	Kumar & Chandra, 2020

Ecological function	Bacterial genera	Reference
Vitamin biosynthesis		
Cobalamin (vitamin B_{12}) biosynthesis	*Bacillus*	Raux et al., 1998
	Candidatus Udaeobacter	Brewer et al., 2016
	*Flavobacterium**	Tekedar et al., 2017
	Mycobacterium	Zhang et al., 2021
	Porphyrobacter	Krohn-Molt et al., 2017
	Porphyromonas	Roper et al., 2000
	Pseudomonas	Cameron et al., 1989
Phototrophy		
Cyanobacterial phototrophy	*Chamaesiphon*	Kurmayer et al., 2018
	Cyanobium	Komárek et al., 1999
Bacterial phototrophy	*Chloroflexus*	Herter et al., 2002
Anoxygenic phototrophy	NOR5/OM60 clade	Yan et al., 2009
	Rhodobacter	Yutin & Beja, 2005
	Rhodoferax	Jung et al., 2004
	Tabrizicola	Tarhriz et al., 2019
Photoheterotrophy	*Rubrivivax*	Li & Fang, 2008
Chemotrophy		
Aerobic chemoheterotrophy	*Armatimonas*	Tamaki et al., 2011
	*Fimbriiglobus**	Kulichevskaya et al., 2017
	Flavisolibacter	Yoon & Im, 2007
	Hirschia	Schlesner et al., 1990
	Lewinella	Khan et al., 2007
	Phaeodactylibacter	Chen et al., 2014b
Aerobic chemoorganotrophy	*Ahniella*	Watanabe et al., 2020
	Albidovulum	Albuquerque et al., 2002
	Bryobacter	Kulichevskaya et al., 2010
	Chryseobacterium	Vandamme et al., 1994
	Haloferula	Yoon et al., 2008
	Hyphomonas	Weiner et al., 2000
	Ideonella	Tanasupawat et al., 2016
	Larkinella	Vancanneyt et al., 2006
	Lysobacter	Christensen & Cook, 1978
	Novosphingobium	Takeuchi et al., 2001
	Polaromonas	Irgens et al., 1996

Ecological function	**Bacterial genera**	**Reference**
Aerobic chemoorganotrophy	*Runella*	Larkin & Williams, 1978
	Stenotrophobacter	Pascual et al., 2015
	Truepera	Albuquerque et al., 2005
	Zavarzinella	Kulichevskaya et al., 2009
Anaerobic chemoorganotrophy	*Anaeromusa* and *Anaeroarcus*	Strömpl, 2015
	Phascolarctobacterium	Del Dot et al., 1993
	Saccharofermentans	Chen et al., 2010
	Sporomusa	Möller et al., 1984
Carbohydrate fermentation	*Alistipes*	Song et al., 2006
	Ferruginibacter	Li et al., 2019b
	Prevotella 9	Bai et al., 2021
	Treponema	Abt et al., 2013
	Vallitalea	Sun et al., 2019
Animo acid fermentation	*Acidaminobacter**	Stams & Hansen, 1984
	Anaerovorax	Matthies et al., 2000
Fermentative hydrogen production	*Acetobacteroides**	Su et al., 2014
	Clostridium sensu stricto 1	Yang & Wang, 2019
	Clostridium sensu stricto 12*	Lu et al., 2020
	Cytophaga xylanolytica	Haack & Breznak, 2004
Other		
Antifungal effect	*Duganella*	Haack et al., 2016
Predator	*Bdellovibrio*	Starr & Baigent, 1966
	Herpetosiphon	Kiss et al., 2011
Methane oxidation	*Gemmobacter*	Takeda, 1988
	OM43 clade	Esson et al., 2016
	Methylocystis	Takeda, 1988
	Methylovulum	Esson et al., 2016
Extracellular polymeric substance (EPS) secretion	*Terrimonas*	Zhao et al., 2019
Biosorption of heavy metals	*Sphaerotilus*	Esposito et al., 2001

Table 4.5 Putative ecological functions of fungal genera present in the *Cladophora* microbiota.

[a] Found in both 18S rDNA and ITS amplicon analyses of TD1 sample. [b] Found in only ITS amplicon analysis. * indicates fungal genera with high relative abundance.

Ecological function	**Bacterial genera**	**Reference**
Decomposing cycling		
Saprotrophy	*Acremonium*[a]	Fernandez-Trujillo et al., 1997
	Alternaria[a]	Woudenberg et al., 2013
	Arthrinium	Crous & Groenewald, 2013
	Aspergillus[a]	de Vries & Visser, 2001
	Avachytrium[b]	Gleason et al., 2008
	Capnobotryella[*,b]	Sert et al., 2007
	Chaetospermum	Rungjindamai et al., 2008
	Chytriomyces	Reisert & Fuller, 1962
	Cladosporium[a]	Bensch et al., 2012
	Emericellopsis	Zuccaro et al., 2004
	Entophlyctis[b]	Longcore, 1995
	Fusarium[a]	Fracchia et al., 2000
	Galactomyces	Varnaitė et al., 2011
	Gibellulopsis[a]	Giraldo et al., 2019
	Glutinoglossum[b]	Fedosova et al., 2018
	Hannaella[*,a]	Landell et al., 2014
	Helicascus	Zhang et al., 2014
	Inocybe[b]	White et al., 1993
	Lentithecium	Calabon et al., 2021
	Mortierella[a]	Li et al., 2018
	Nowakowskiella	Marano et al., 2011
	Ochroconis[a]	Machouart et al., 2014
	Piromyces	Ali et al., 1995
	Pyrenochaeta[a]	Khan et al., 2011
	Sporobolomyces[a]	Wang & Bai, 2004
	Wiesneriomyces	Suetrong et al., 2014

Ecological function	**Bacterial genera**	**Reference**
Plant-fungal interaction		
Ectomycorrhizal fungi	*Inocybe*[b]	White et al., 1993
Endophytic fungi	*Acremonium*[a]	White et al., 1993
	Alternaria[a]	Woudenberg et al., 2013
	Arthrinium	Crous & Groenewald, 2013
	Cladosporium[a]	Bensch et al., 2012
Phylloplane fungi	*Cladosporium*[a]	Bensch et al., 2012
	Geotrichum	Pereira et al., 2002
	*Hannaella**[,a]	Landell et al., 2014
	Occultifur[a]	Srisuk et al., 2019
	Pichia	Limtong & Kaewwichian, 2015
	Rhodotorula[a]	Into et al., 2020
	Sporobolomyces[a]	Limtong & Kaewwichian, 2015
	Vishniacozyma[a]	Into et al., 2020
Plant growth-promoting fungi	*Mortierella*[a]	Ozimek & Hanaka, 2021
Plant pathogen	*Alternaria*[a]	Woudenberg et al., 2013
	Arthrinium	Crous & Groenewald, 2013
	Cladosporium[a]	Bensch et al., 2012
	Fusarium[a]	Ma et al., 2013
	Gibellulopsis[a]	Giraldo et al., 2019
	Sporisorium[a]	Que et al., 2014
Mutualism		
Lichen-forming fungi	*Capnobotryella**[,b]	Sert et al., 2007
Predation		
Amoebophagous fungi	*Stylopage*	Corsaro et al., 2018
Nematophagous fungi	*Stylopage*	Persmark et al., 1995
Parasitism		
Endoparasitic chytrid	*Rozella*	Corsaro et al., 2014
Hyperparasites on other fungi	*Cladosporium*[a]	Bensch et al., 2012

Ecological function	Bacterial genera	Reference
Parasites of algae	*Entophlyctis**,[b]	Shin et al., 2001
	Phlyctochytrium	Letcher & Powell, 2005
	Rhizophydium	Gromov et al., 1999
Parasites of amoebae	*Acaulopage*	Hirotani-Akabane & Saikawa, 2010
	Cochlonema	Hirotani-Akabane & Saikawa, 2010
	*Paramicrosporidium**	Corsaro et al., 2014
Parasitoids of algae	*Aphelidium*	Karpov et al., 2014
	Paraphelidium	Karpov et al., 2019

4.2.4 Diversity of *Cladophora* sp. microbiota

Prokaryotic taxa

To investigate the diversity level of bacteria present in each site, Shannon’s index, Simpson’s index, Chao1, and richness or number of identified taxa were employed. At the phylum level, as shown in figure 4.23, Shannon’s indices of CKD, PUA, and TD1 were 2.00, 2.24, and 2.23, respectively, where the values for PUA and TD1 were statistically higher than that of CKD ($p < 0.001$). Simpson’s indices of CKD, PUA, and TD1 were 0.77, 0.83, and 0.82, respectively, where the values for PUA and TD1 were statistically higher than that of CKD ($p < 0.005$). Additionally, estimated Chao1 and richness were equal for CKD, PUA, and TD1, which were 31, 36, and 37, respectively, in which the value from TD1 was statistically higher than that of CKD ($p < 0.01$). Results of Shannon’s index, Simpson’s index, Chao1, and richness overall suggested that the bacterial phyla present at sites PUA and TD1 were more diverse than that of CKD.

At the genus level, as shown in figure 4.24, Shannon’s indices of sites CKD, PUA, and TD1 were 4.91, 5.11, and 5.12, respectively. Simpson’s indices of all sites were 0.98. Chao1 for CKA, PUA, and TD1 were 488, 497, and 496, respectively, and the richness was 461, 474, and 470, respectively. All alpha diversity indices of bacteria at the genus level were not statistically different, suggesting that the diversity of bacterial genera of all locales was not different.

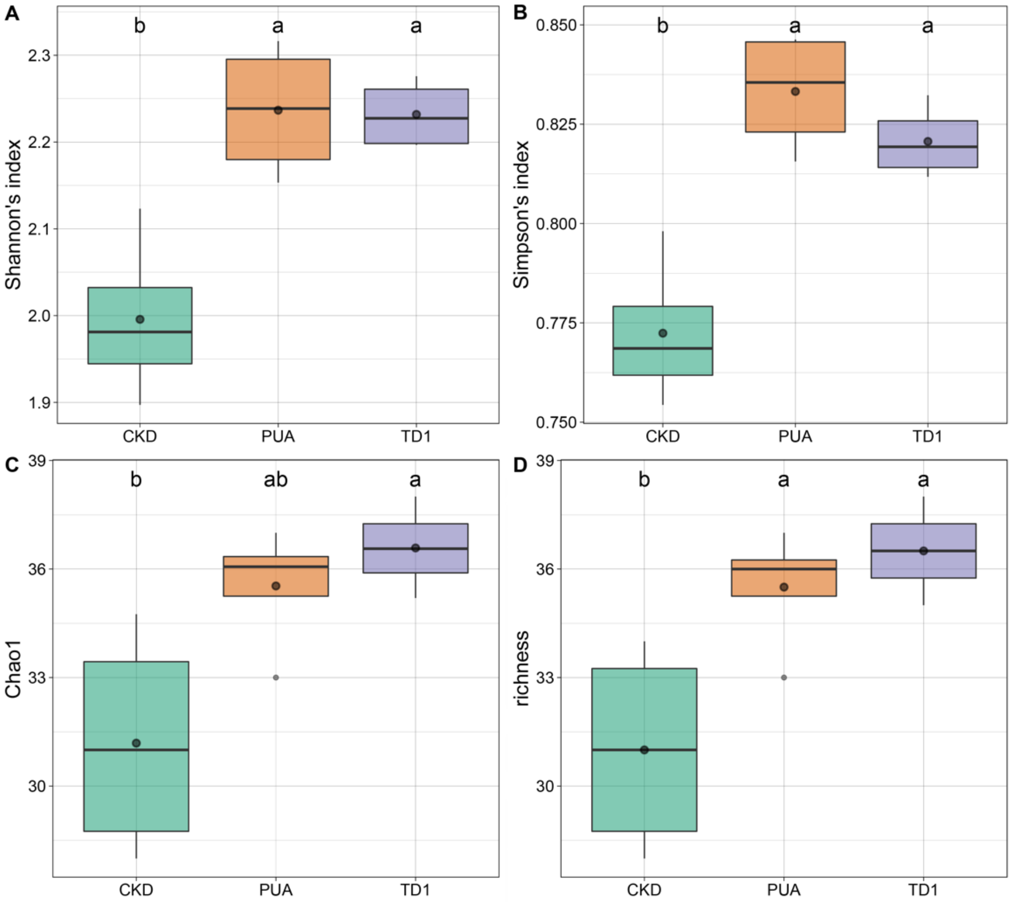


Figure 4.23 Box plots of bacterial phyla alpha diversity indices.

A. Shannon's index, B. Simpson's index, C. Chao1, and D. richness. Different lowercase letters indicate statistically significant differences using Tukey's HSD at 0.05 significance level.

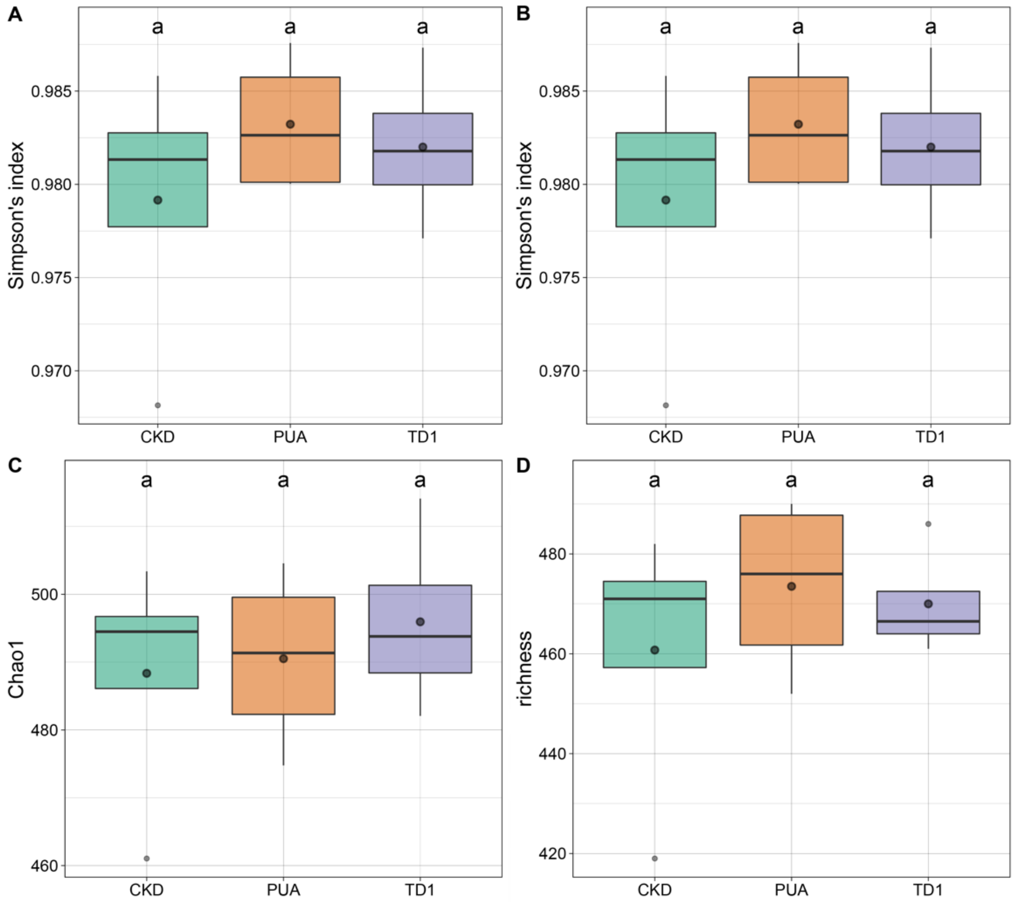


Figure 4.24 Box plots of bacterial genera alpha diversity indices.

A. Shannon’s index, B. Simpson’s index, C. Chao1, and D. richness. Different lowercase letters indicate statistically significant differences using Tukey’s HSD at 0.05 significance level.

Eukaryotic taxa

At the genus level, Shannon’s indices of CKD, PUA, and TD1 were 4.34, 3.23, and 3.71, respectively, where the value for CKD was statistically higher than that of PUA ($p < 0.01$). Simpson’s indices of sites CKD, PUA, and TD1 were 0.96, 0.82, and 0.90, respectively, where the values for CKD and TD1 were statistically higher than that of PUA. Chao1 of CKA, PUA, and TD1 were 290, 210, and 264, respectively, and the richness were 263, 190, and 237, respectively. The estimated values of Chao1 and richness from the three sampling sites were not statistically different (Figure 4.25).

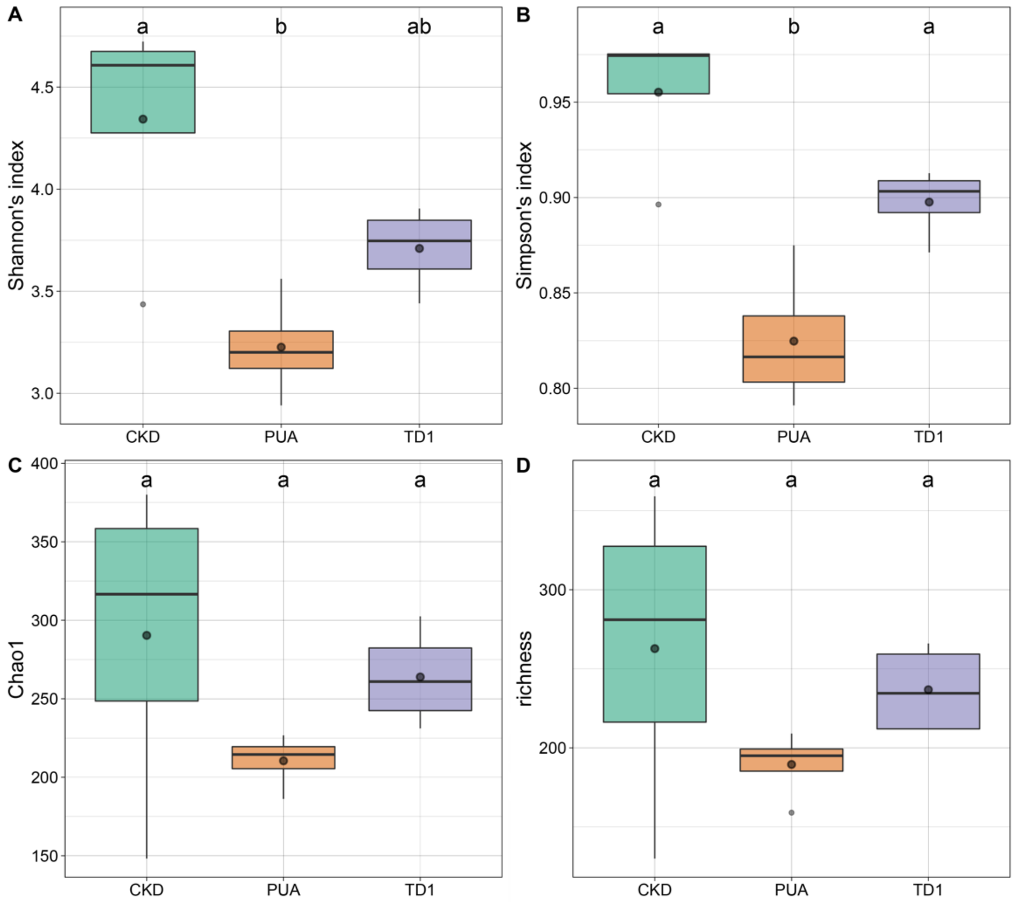


Figure 4.25 Box plots of eukaryotic genera alpha diversity indices.

A. Shannon’s index, B. Simpson’s index, C. Chao1, and D. richness. Different lowercase letters indicate statistically significant differences using Tukey’s HSD at 0.05 significance level.

To compare the diversity of the bacterial genera and eukaryotic genera between sampling sites, principal coordinate analysis based on Bray-Curtis dissimilarity was employed. For bacteria, at the genus level, as shown in figure 4.26A, the variance explained for PCo1 and PCo2 were 45.31% and 22.42% (67.73% in total). R of ANOSIM was 0.852 as $p < 0.001$ and p value of permANOVA was 0.001. For eukaryotes, at the genus level, as shown in figure 4.26B, the variance explained for PCo1 and PCo2 were 47.86% and 22.17% (70.03% in total). R of ANOSIM was 0.630 as $p < 0.001$ and p value of permANOVA was 0.001. Ellipsoids represent a 95% confidence interval enclosing each site. These suggested that the diversity of bacteria and eukaryotes present in the four replicates of each study site had smaller dissimilarity than that of other sites. Also, as appeared in figure 4.26, the clusters of bacterial genera ordinated further apart than those of eukaryotic genera.

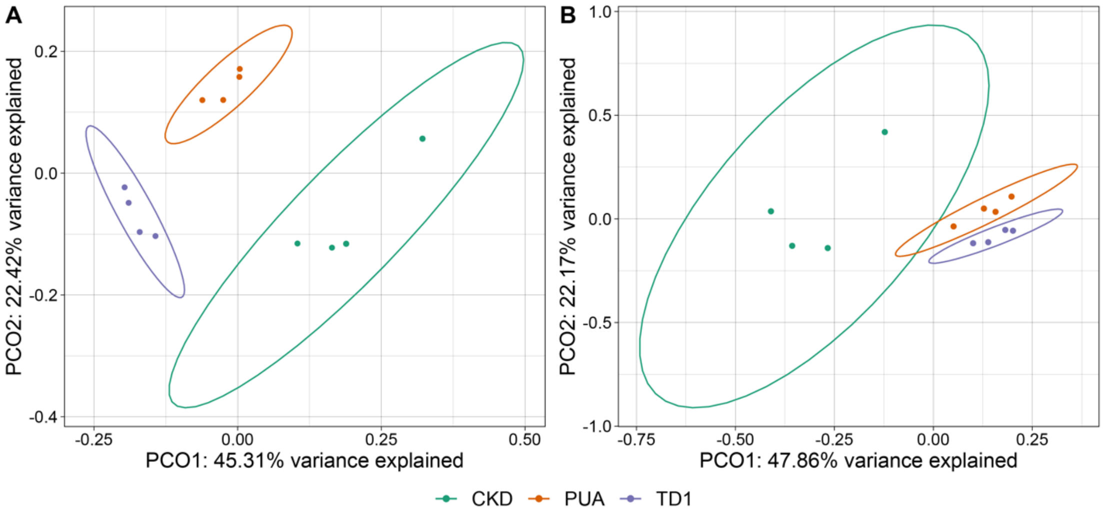


Figure 4.26 Principal coordinate analysis based on Bray-Curtis dissimilarity of *Cladophora* microbiota.

A. bacterial genera and B. eukaryotic genera. Ellipsoids represent a 95% confidence interval enclosing each site.

4.3 Comparative analysis of *Cladophora* microbiota

This study investigated the microbiota of *Cladophora* present in three sites in the course of the Nan River, Thailand. To expand the knowledge of the *Cladophora* shared prokaryotic and eukaryotic microbiota, the shared taxa of CKD, PUA, and TD1 identified in this study were compared to microbiota studied worldwide and identified as the “core taxa”, which were the taxa shared in all the studies. After comparing the results from this study to that of Lake Mendota reported by Braus et al. (2017), and Lake Michigan reported by Chun et al. (2017) and Graham et al. (2015). Among the identified taxa, 17 bacterial genera were present in all the studies. These bacteria included Bacteroidota *Flavobacterium*, *Terrimonas*, *Chryseobacterium*, Deinococcus-Thermus *Deinococcus*, Proteobacteria *Pseudorhodobacter*, *Pseudomonas*, *Hyphomicrobium*, *Rhodobacter*, *Altererythrobacter*, *Novosphingobium*, *Sphingopyxis*, *Acidovorax*, *Aquabacterium*, *Hydrogenophaga*, *Methylotenera*, *Acinetobacter*, and

Bdellovibrio (Table C.8). Some were inferred to play crucial roles in freshwater *Cladophora* microbiome (Tables 4.3–4), e.g., vitamin B_{12} biosynthesis (*Flavobacterium* and *Pseudomonas*), extracellular polymeric substance secretion (*Terrimonas*), aerobic chemoorganotrophy (*Chryseobacterium* and *Novosphingobium*), anoxygenic phototrophy (*Rhodobacter*), polyphosphate accumulation (*Acinetobacter*), denitrification (*Acidovorax* and *Methylotenera*), degradation of alkane (*Aquabacterium* and *Hyphomicrobium*), and aromatic compounds (*Hydrogenophaga*).

CHAPTER V
DISCUSSION

5.1 Identification of the *Cladophora* host

According to morphological identification, the algal samples from sites CKD, PUA, and TD1 were *Cladophora*-dominant samples. The presence of *Cladophora* in the locales was congruent with reports from previous studies (Laungsuwon & Chulalaksananukul, 2014; Peerapornpisal et al., 2005; 2006; Surayot et al., 2016; Yarnpakdee et al., 2021), where the researchers found *C. glomerata*, locally called "Kai", in water bodies in Pua and Tha Wang Pha districts, Nan province. On the other hand, algal samples from sites TD2 and MND were *Spirogyra*-dominant samples and were discarded from the analysis. This presence of *Spirogyra*-rich algal turf was also congruent with the previous study by Peerapornpisal et al. (2005), where they found *Spirogyra*, commonly called "Tao" or "Tao Nam", present in Pua, Tha Wang Pha, and Mueang Nan districts. Anyhow, this study focused on the microbiota of *Cladophora*. Therefore, only the algal samples collected from CKD, PUA, and TD1 were used in this study.

All the collected *Cladophora* samples exhibited similar morphology and appeared as thalli attached to similar substrates – rocks and pebbles. To identify the alga to the species level, phylogenetic relationships using gene markers were estimated. Maximum likelihood trees of 18S rDNA, 23S rDNA, and ITS sequences suggested that *Cladophora* sequences amplified from genes-specific primers placed the samples in clades consisting of *Cladophora* species. However, sequences from each replication differed and the bootstrap values for those observed clades were low. Therefore, the species of *Cladophora* samples could not be inferred from the amplicons amplified from gene-marker-specific primers. The presence of the different PCR products arising among replicates might be from the PCR steps, where the primers were not sufficiently specific and bound to DNA from various organisms other than the *Cladophora* sp. and thus give us a combination of various sequences within a band observed by gel electrophoresis. Additionally, the Sanger sequencing might not give us the high-quality reads as exhibited in the chromatograms (Figures A.1–24).

To solve the problem of taxonomic classification using the data from gene-specific primers, the 18S rDNA data acquired from the higher-quality sequencing technique, Illumina, were utilized. In this method, the amplicons annotated as *Cladophora* sp. from all replicates were extracted, aligned, and used in phylogenetic estimation. The alignment of all the *Cladophora* sequences showed that they were identical, suggesting that, for the amplified regions, all the *Cladophora* hosts collected in this study were from the same algal population. Although the information obtained in this study was not sufficient for the identification of the *Cladophora* host to the species level, results from the maximum likelihood tree suggested that the alga was placed within the clade with other *Cladophora* species.

One interesting finding that arose from the taxonomic identification of the *Cladophora* host used in this study was that the *Cladophora* 18S rDNA sequences obtained from this study were not identical to that of other *Cladophora* samples previously reported from Thailand nor other countries in Southeast Asia (Boedeker et al., 2016; Thiamdao et al., 2012). This suggests that the diversity of *Cladophora* present in this region is higher than what we previously knew and has not yet been investigated.

5.2 Study of the diversity of epiphytic microbiota of Kai *Cladophora* sp.

The nature of having a high surface area of *Cladophora* made the alga an ecological engineer, which provides the microhabitats for other microorganisms. Investigation of *Cladophora* microbiota using light microscopy and amplicon analysis revealed congruent results as the epiphytic cyanobacteria, protozoa, and diatoms were observed both by microscopy and amplicon analysis.

For eukaryotic taxa, photosynthetic stramenopiles, namely diatoms, were abundantly present on the algal surface. The frequently observed taxa included *Cocconeis*, *Gomphonema*, *Synura*, *Synedra*, and *Navicula*. Among identified diatoms, *Cocconeis* and *Gomphonema* were CKD–PUA–TD1 shared taxa with a high abundance. The relative abundance of *Cocconeis* ranged from 4.9% at CKD to 27.4% at PUA, and *Gomphonema* ranged from 0.8% at PUA to 4.6% at TD1 (Table C.5).

The presence of dominant *Cocconeis* and *Gomphonema* were congruent with previous amplicon-based metagenomic studies of Lake Mendota *Cladophora* microbiome (Graham et al., 2015; Zulkifly et al., 2012), where they suggested that the cell shape of these diatoms allowed them to avoid grazing from other consumers and maintain a firm attachment to the algal filament.

For more details, according to results from amplicon analysis, there were various diatom genera present in the algal microbiota, such as *Achnanthidium*, *Amphora*, *Cocconeis*, *Craticula*, *Cymbella*, *Discostella*, *Epithemia*, *Fistulifera*, *Gomphonema*, *Melosira*, *Navicula*, *Nitzschia*, *Pinnularia*, *Planothidium*, *Pleurosigma*, *Pleurosira*, *Rhopalodia*, *Sellaphora*, *Thalassiosira* and *Ulnaria* (Table C.5). The presence of these diatoms might be explained by their capability to tolerate shading in the *Cladophora* tuft, which resulted from the densely algal filament growth that overshadows some regions of the algal filament. To tolerate shading, diatoms can maintain their abundance by upregulation of genes involved in transcription and photosynthesis to compensate for the lower light intensity (Buntha et al., 2020). The presence of these diatoms on the algal surface also enhances the diversity of the algal microbiota as these diatoms release extracellular polymeric substances, primarily as exopolysaccharides, which aided in biofilm formation, where groups of other minute eukaryotes and bacteria reside (Bahulikar & Kroth, 2007).

Amplicon analysis revealed a total of 575 eukaryotic genera and 698 bacterial genera, which were present variedly in the study sites. Among the identified bacteria, 227 genera were shared taxa, commonly present in all sites. Some were high abundant taxa those involved in various processes, i.e., cellulose degradation (*Bacteroides* and *Paludibacter*), vitamin B_{12} biosynthesis (*Flavobacterium*), fermentative hydrogen production (*Acetobacteroides* and *Clostridium sensu stricto* 12), ammonium oxidation (*Pirellula*), amino acid fermentation (*Acidaminobacter*), dissimilatory reduction of nitrate to ammonium (*Pelosinus*, *Aeromonas*, and *Lacunisphaera*), nitrite reduction (*Dechloromonas*), nitrate reduction (*Vogesella*), sulfur reduction (*Fusibacter*), polyphosphate accumulation (*Acinetobacter* and *Propionivibrio*), denitrifying phosphorus-accumulation (*Dechloromonas*), degradation of aromatic compounds (*Hydrogenophaga*), anaerobic chemoheterotrophy (*Fimbriiglobus*), and bacteria with no known specific ecological function, e.g., *Emticicia*, *Pseudarcobacter*, and WCHB1-32.

Among the bacterial genera, some fecal indicator bacteria in nuisance *Cladophora* were found with a low abundance, written here as the relative abundance present at CKD, PUA, and TD1, including *Escherichia-Shigella* (0.21%, 0.14%, and 0.03%), *Enterococcus* (0.02%, 0.02%, and 0.00%), *Streptococcus* (0.01%, 0.01%, and 0.00%), and *Salmonella* (0.14%, 0.02%, and 0.00%). The presence of these bacteria seemed to be habitat-specific as not all the *Cladophora* microbiota had these bacteria (Braus et al., 2017; Byappanahalli et al., 2003; Byappanahalli et al., 2007; Englebert et al., 2008a, 2008b; Graham et al., 2015; Olapade et al., 2006; Vanden Heuvel et al., 2010; Verhougstraete et al., 2010; Zulkifly et al., 2012). This suggested that only the *Cladophora* growing in clean habitats should be used for consumption, and it might be worthwhile to examine the presence of these bacteria before utilizing the alga.

The shared fungal taxa present in all sites were mainly involved in decomposition (e.g., *Avachytrium*, *Entophlyctis*, and *Glutinoglossum*), parasitism in algae, amoebae, and other fungi, plant-fungal interaction, predation, as well as mutualism as in the lichen-forming fungi. It is not yet clear at this stage whether these fungi were present temporarily (e.g., lichen-forming fungus, phylloplane fungi, and plant pathogens). However, it is hypothetically possible that the portion of these fungal taxa, mainly the decomposers, live in associations within the algal microbiome. In order to investigate whether the presence of these fungi was from the influx of the nearby floodplain, more temporal and spatial microbiome studies are needed.

The PCoA plots suggested that the microbiota were more similar between replicates than between sites, where the eukaryotic taxa appeared to be more diverse at CKD than at PUA and TDI, while bacterial taxa appeared to have the opposite fashion by being more diverse at PUA and TD1 than at CKD. It is not yet clear why the diversity of the microbiota differed between sites. Although it has been suggested that the trophic level does not affect the diversity of the organisms (Danilov & Ekelund, 1999; Lee & Liu, 2018; Spatharis et al., 2011), a more sufficient information of water quality index, dissolved oxygen, biological oxygen demand, and ammonia nitrogen content at the sampling sites, as well as the information of seasonal rainfall, the vegetation of the nearby floodplains and riverbanks are needed.

5.3 Comparative analysis of *Cladophora* microbiota

This study provided the first information of microbiota associated with *Cladophora* sp. living in a lotic, running water bodies, environment. Comparisons of the microbiota between this lotic habitat and other lentic, still water bodies, habitats (Braus et al., 2017; Chun et al., 2017; Graham et al., 2015; Zulkifly et al., 2012) revealed shared bacterial taxa among all studies. These shared taxa involved in vitamin B_{12} biosynthesis (*Flavobacterium* and *Pseudomonas*), extracellular polymeric substance secretion (*Terrimonas*), aerobic chemoorganotrophy (*Chryseobacterium* and *Novosphingobium*), anoxygenic phototrophy (*Rhodobacter*), polyphosphate accumulation (*Acinetobacter*), denitrification (*Acidovorax* and *Methylotenera*), degradation of alkane (*Aquabacterium* and *Hyphomicrobium*), and aromatic compounds (*Hydrogenophaga*). Innately, lakes are known for their significant methane emission to the atmosphere, resulting in global warming. Zulkifly et al. (2012) showed that the aerobic methanotrophs – *Methylotenera*, *Methylibium*, and *Methylobacter* – were present in the *Cladophora* microbiome from Lake Mendota in 2011. Later Braus et al. (2017) also showed that the methanotrophs – *Crenothrix*, *Methylobacter, Methylocaldum*, *Methylocella*, *Methylocyctis*, *Methylomonas*, and *Methylovulum* – were increasingly present in the *Cladophora* microbiome from Lake Mendota in 2014. These suggested the constant emission of methane from the lake sediments. Compared to this study, methanotrophs – *Gemmobacter,* OM43 clade, *Methylocystis,* and *Methylovulum* were low abundant genera. These indicated that the lotic Nan River might not be the methane hot spot like those lentic ecosystems. It is not clearly known why these taxa were commonly present in the *Cladophora* microbiome living in lentic and lotic environments and whether they play crucial roles in the dispersal and survival of this worldwide distributed algal genus. Afterall, it also could be from the wide distribution of the bacteria themselves. To answer the question, a further study of interactions between these bacteria and *Cladophora* is needed.

CHAPTER VI
CONCLUSION

6.1 Conclusions

In this study, the algal samples were collected from five collecting sites, four replicates per site, along Nan River, Nan Province, Thailand, in March 2020. The algal samples from Chiang Klang, Pua, and Tha Wang Pha districts were morphologically identified as *Cladophora*-dominant samples and performed phylogenetic analysis. Maximum likelihood trees of 18S rDNA, 23S rDNA, and ITS sequences suggested that *Cladophora* sequences obtained from Nan River were placed in clades consisting of *Cladophora* species. Then, *Cladophora*-dominant samples were further studied for the diversity of epiphytic microbiota using amplicon-based metagenomic analysis.

Results from 16S rDNA amplicon analysis showed that the identified bacterial taxa included 45 phyla, and the five most abundant phyla were Proteobacteria, Bacteroidota, Firmicutes, Verrucomicrobia, and Planctomycetota. These 45 bacterial phyla were classified into 689 distinct genera with varied relative abundance. Among the identified bacteria, 227 genera were shared taxa, commonly present in all sites. Some were high-abundant taxa those involved in various processes, i.e., cellulose degradation (*Bacteroides* and *Paludibacter*), vitamin B_{12} biosynthesis (*Flavobacterium*), and ammonium oxidation (*Pirellula*). Among these shared taxa, 17 genera were core taxa, present in *Cladophora* microbiota of this study and other previous studies from the USA. Some were bioinformatically inferred to play crucial roles in freshwater *Cladophora* microbiome, e.g., vitamin B_{12} biosynthesis (*Flavobacterium* and *Pseudomonas*), extracellular polymeric substance secretion (*Terrimonas*), and denitrification (*Acidovorax* and *Methylotenera*).

Results from 18S rDNA amplicon analysis showed that the identified eukaryotic taxa included six eukaryotic supergroups, and the five most abundant supergroups were Amorphea, Stramenopiles, Archaeplastida, Rhizaria, and Alveolata. These six eukaryotic supergroups were classified into 575 distinct genera with varied relative abundance. Among the identified genera, 51 genera were shared across all sites. Some were high-abundant taxa, which was congruent with results from microscopic observation. These taxa included photosynthetic stramenopiles (diatoms), *Cocconeis*, and *Gomphonema.*

Only one replicate of ITS amplicon analysis, which was the sample from TD1, was successful. The identified fungi included eight fungal phyla, of which the three most abundant phyla were Ascomycota, Chytridiomycota, and Basidiomycota. These eight fungal phyla were classified into 133 distinct genera with varied relative abundance. When comparing these genera with the results from 18S rDNA amplicon analysis, 22 genera were shared across all sites and were inferred to involve in various processes, i.e., decomposition, parasitism, plant-fungal interaction, and predation.

In conclusion, the information of the epiphytic microbiota of Kai *Cladophora* sp. obtained in this study allows us to have a better understanding of the taxonomic composition and the putative ecological functions of the epiphytic microbiota of *Cladophora* sp. in the Nan River, Nan Province, Thailand. Evidence observed in this study suggested that the *Cladophora* host and its microbiota thus play crucial roles in this freshwater ecosystem.

6.2 Perspectives

1. To predict the potential metabolites in associated epiphytic microbiota of Kai *Cladophora* sp. based on the amplicon-based metagenomic analysis.
2. To separately identify the core microbiota of the lentic and lotic ecosystem.

APPENDIX A

Phylogenetic estimation of *Cladophora* host

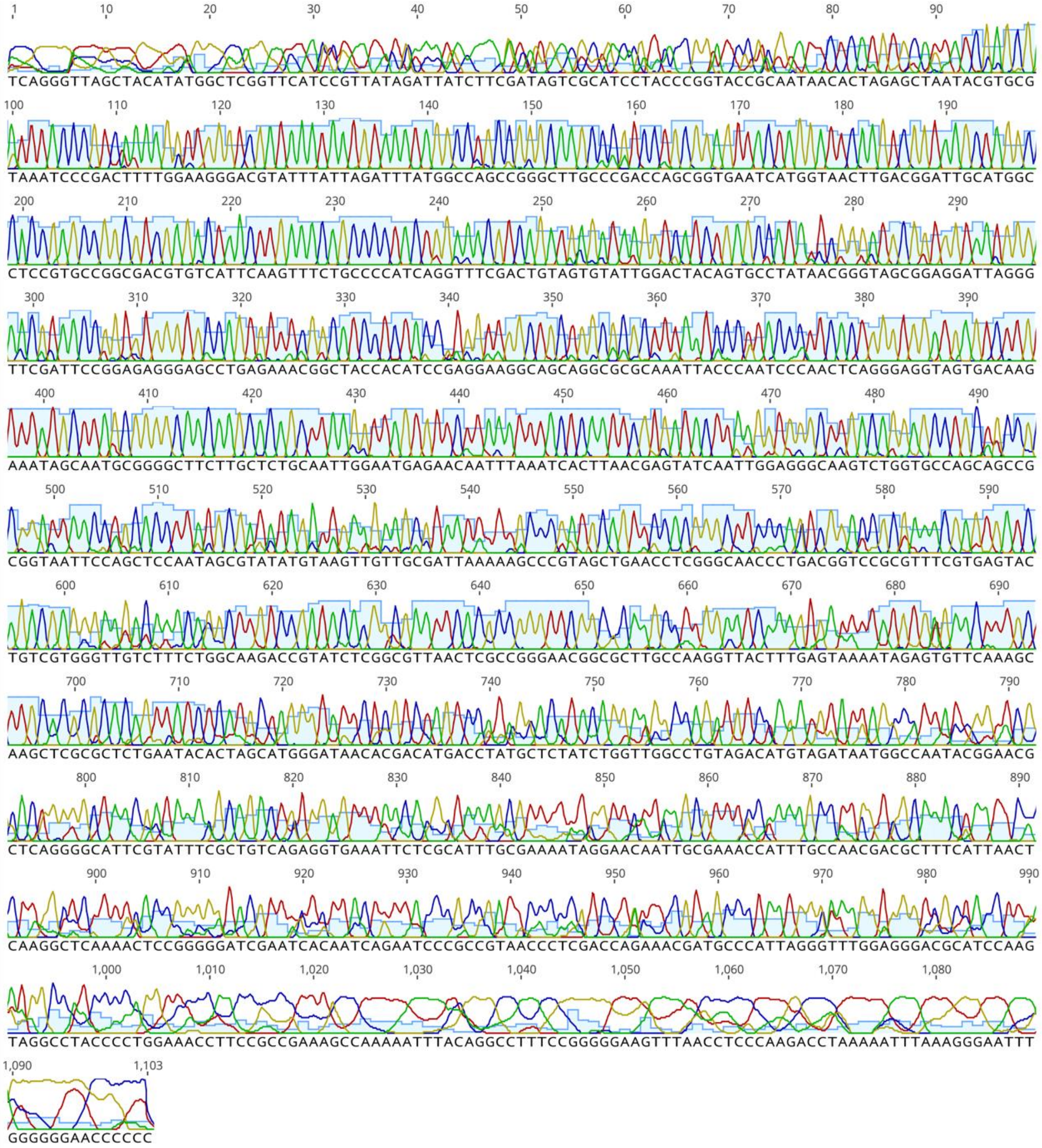


Figure A.1 Sanger chromatogram of 1,103 bp of 18S rDNA amplified from CKD sample sequenced in 5’–3’ direction (starting from the forward primer).

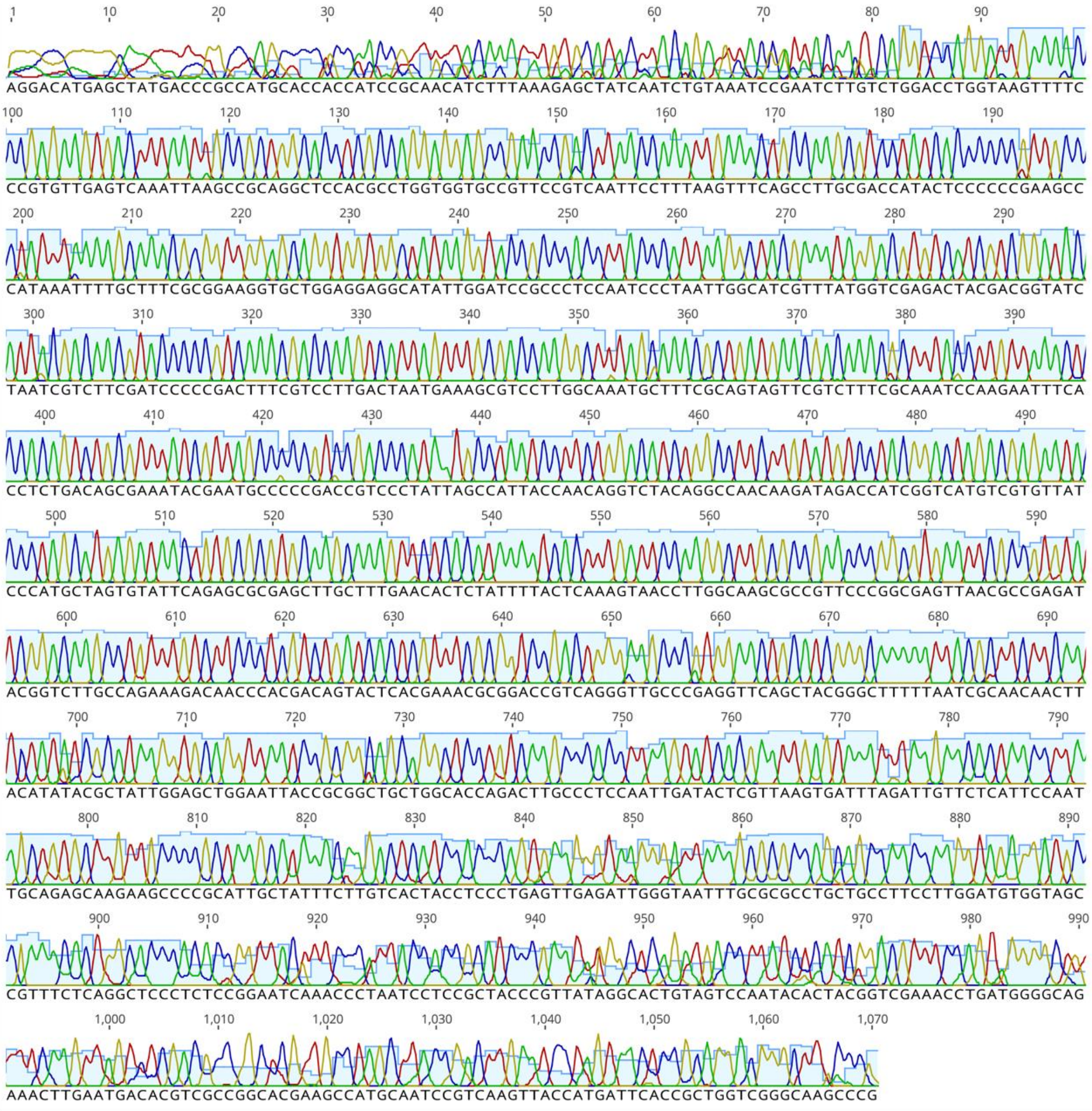


Figure A.2 Sanger chromatogram of 1,070 bp of 18S rDNA amplified from CKD sample sequenced in 3’–5’ direction (starting from the reverse primer).

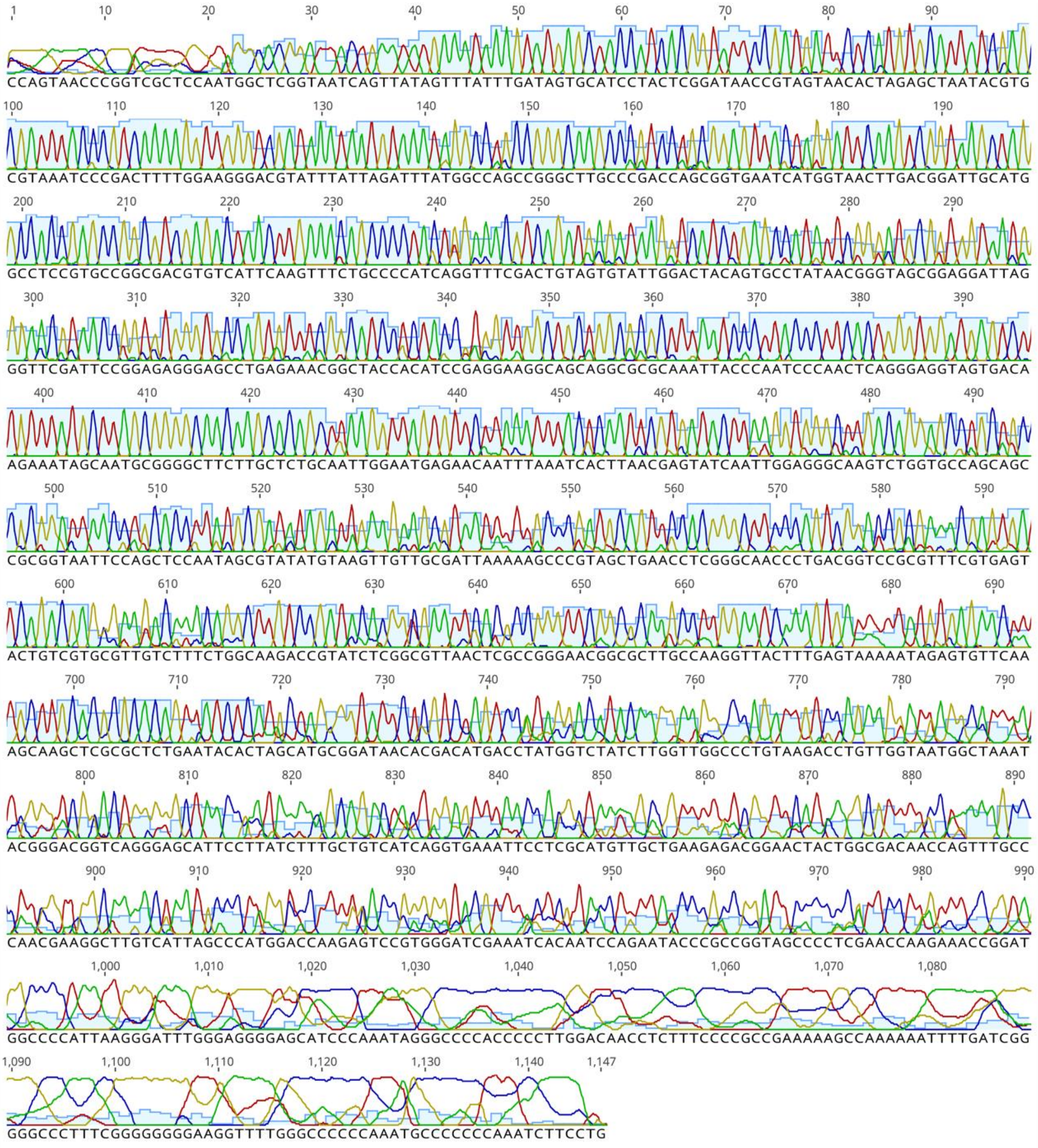


Figure A.3 Sanger chromatogram of 1,147 bp of 18S rDNA amplified from PUA sample sequenced in 5’–3’ direction (starting from the forward primer).

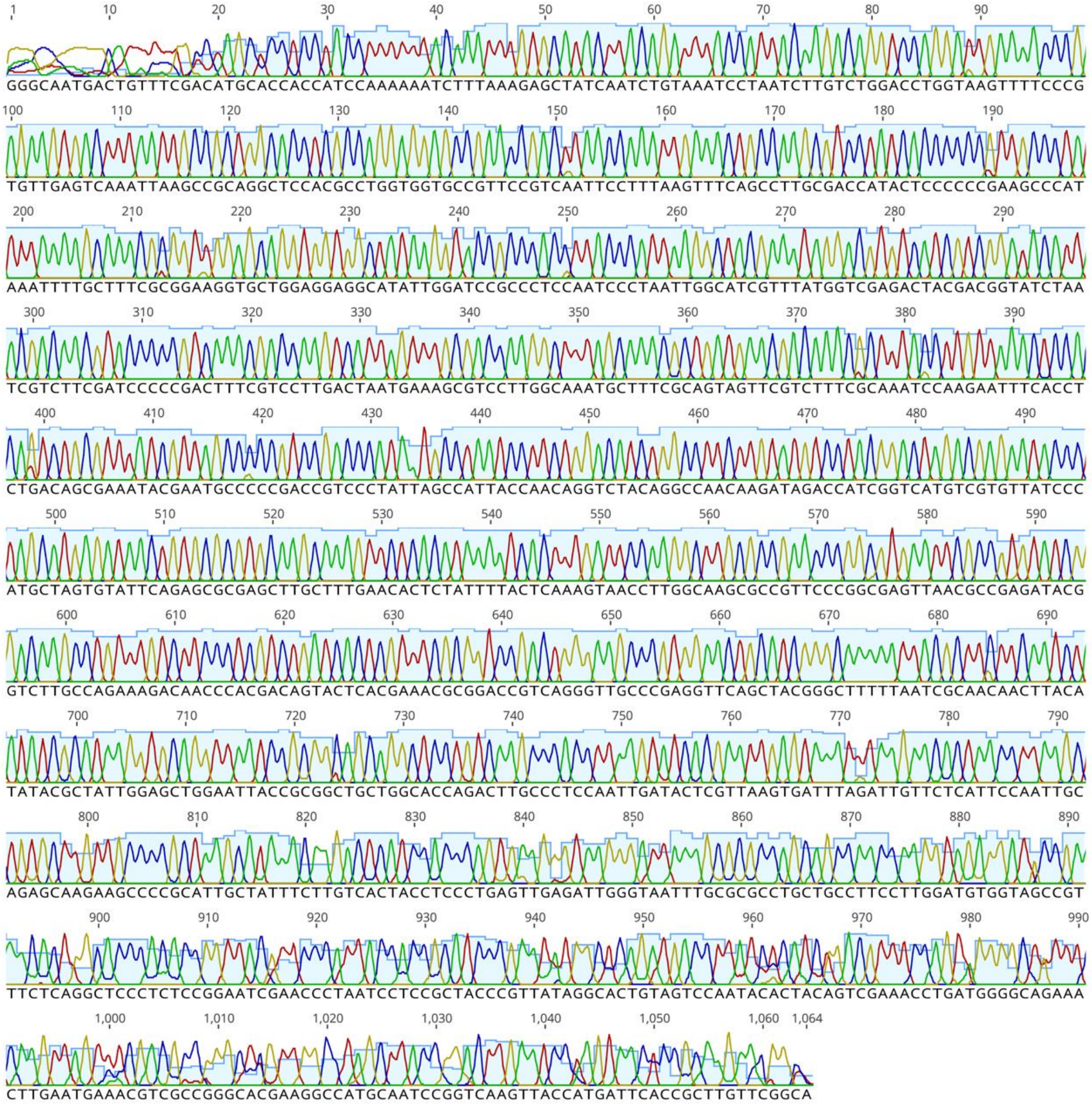


Figure A.4 Sanger chromatogram of 1,064 bp of 18S rDNA amplified from PUA sample sequenced in 3’–5’ direction (starting from the reverse primer).

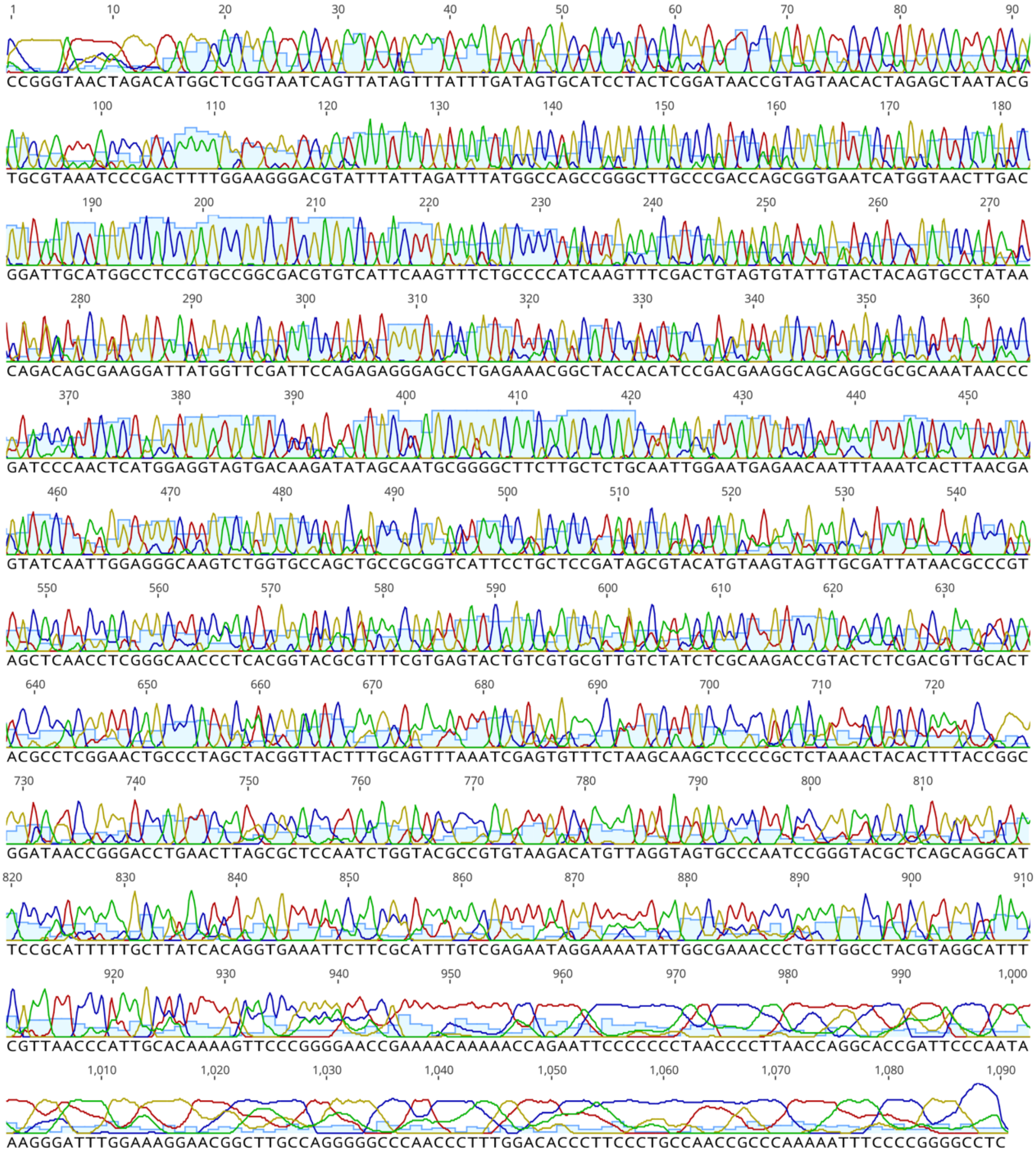


Figure A.5 Sanger chromatogram of 1,090 bp of 18S rDNA amplified from TD1 sample sequenced in 5’–3’ direction (starting from the forward primer).

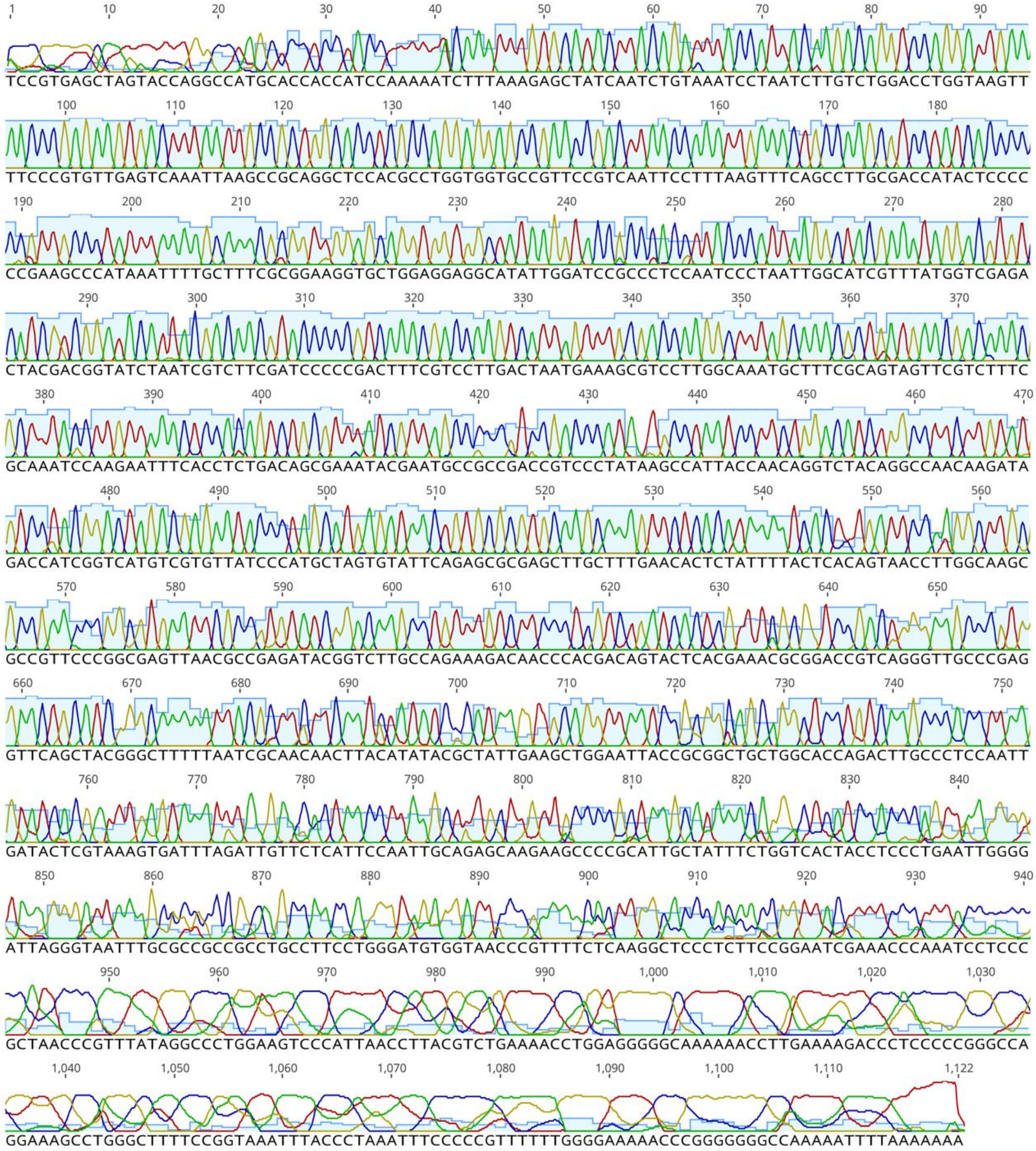


Figure A.6 Sanger chromatogram of 1,122 bp of 18S rDNA amplified from TD1 sample sequenced in 3’–5’ direction (starting from the reverse primer).

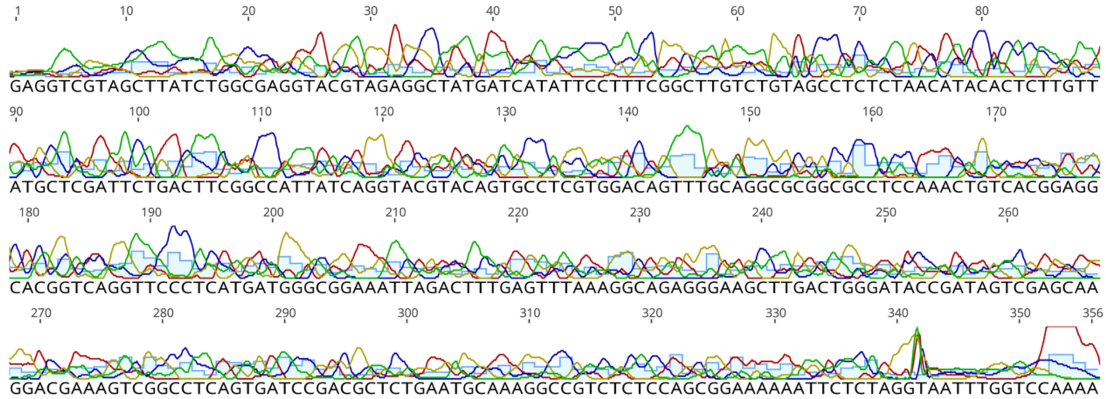


Figure A.7 Sanger chromatogram of 356 bp of 23S rDNA amplified from CKD sample sequenced in 5’–3’ direction (starting from the forward primer).

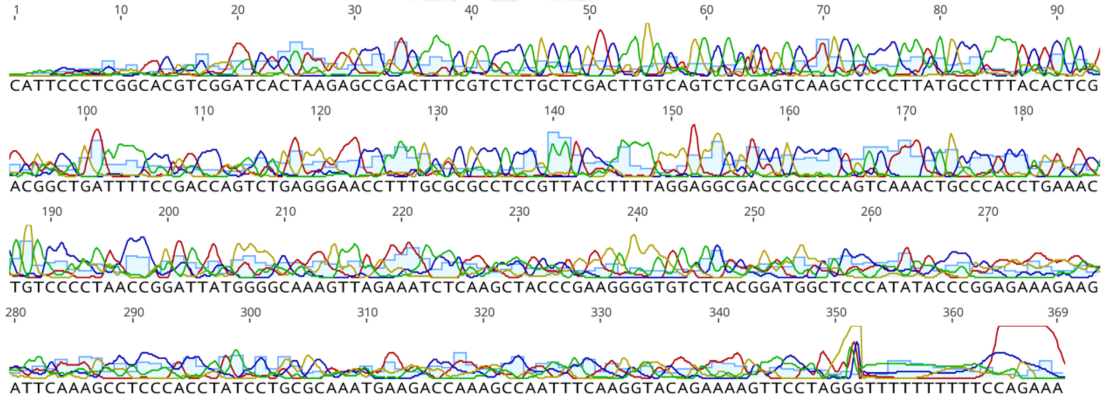


Figure A.8 Sanger chromatogram of 369 bp of 23S rDNA amplified from CKD sample sequenced in 3’–5’ direction (starting from the reverse primer).

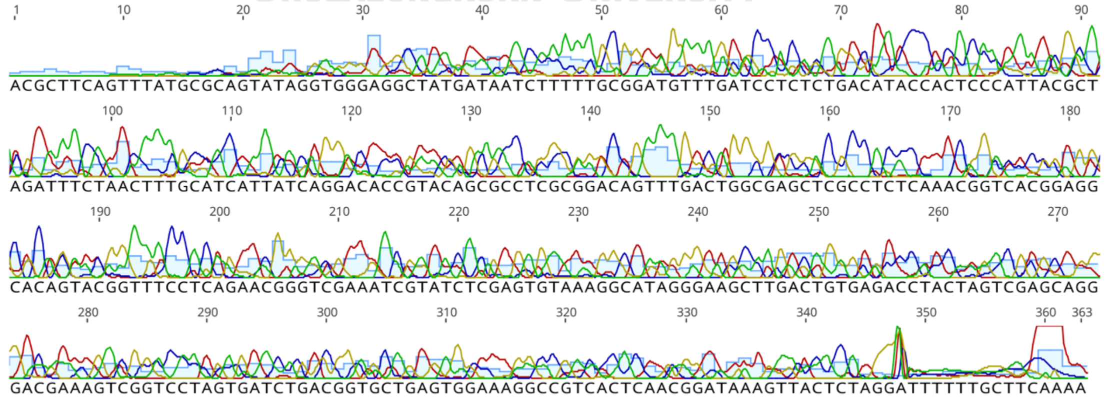


Figure A.9 Sanger chromatogram of 363 bp of 23S rDNA amplified from PUA sample sequenced in 5’–3’ direction (starting from the forward primer).

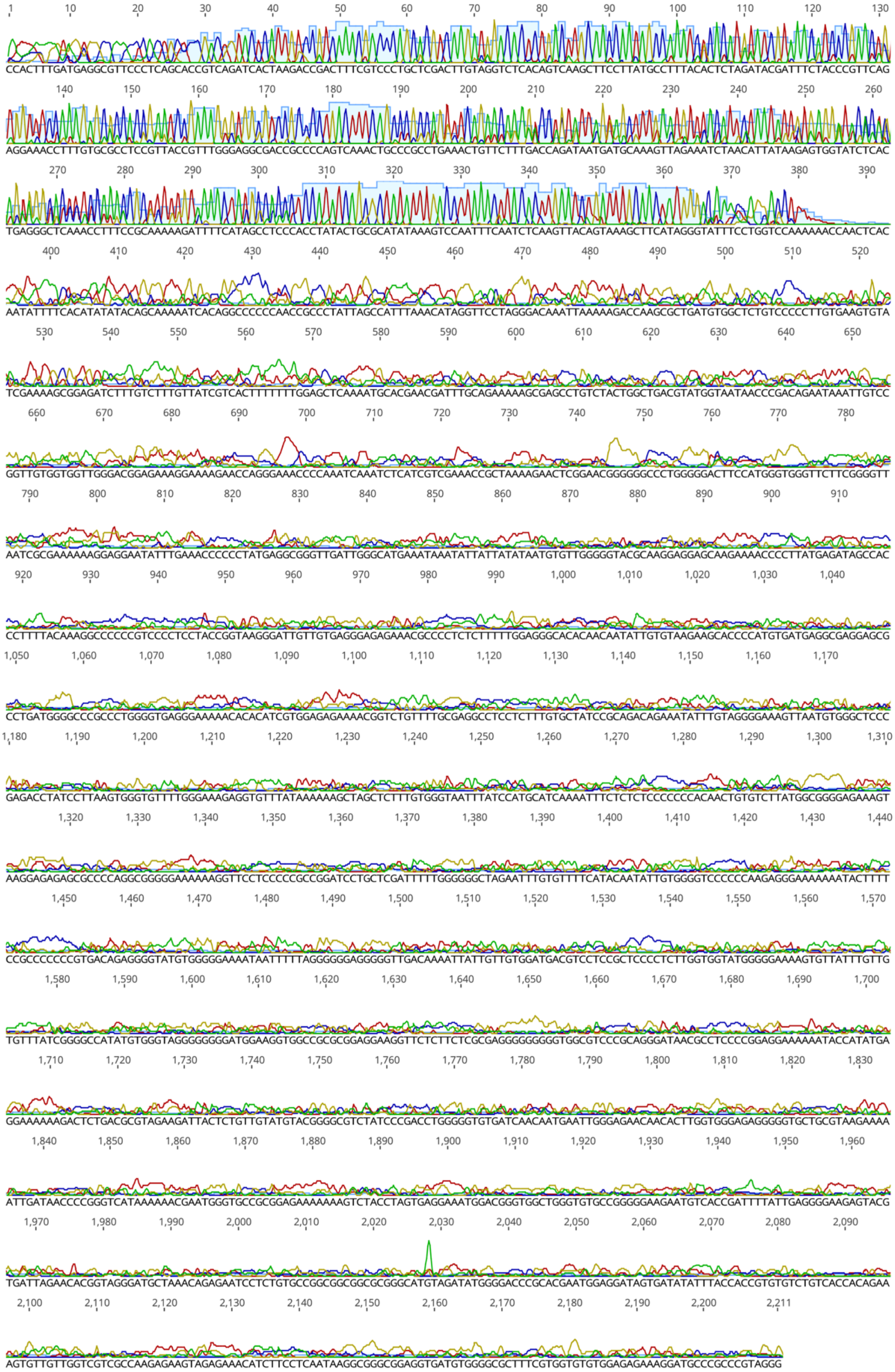


Figure A. 10 Sanger chromatogram of 2,211 bp of 23S rDNA amplified from PUA sample sequenced in 3’–5’ direction (starting from the reverse primer).

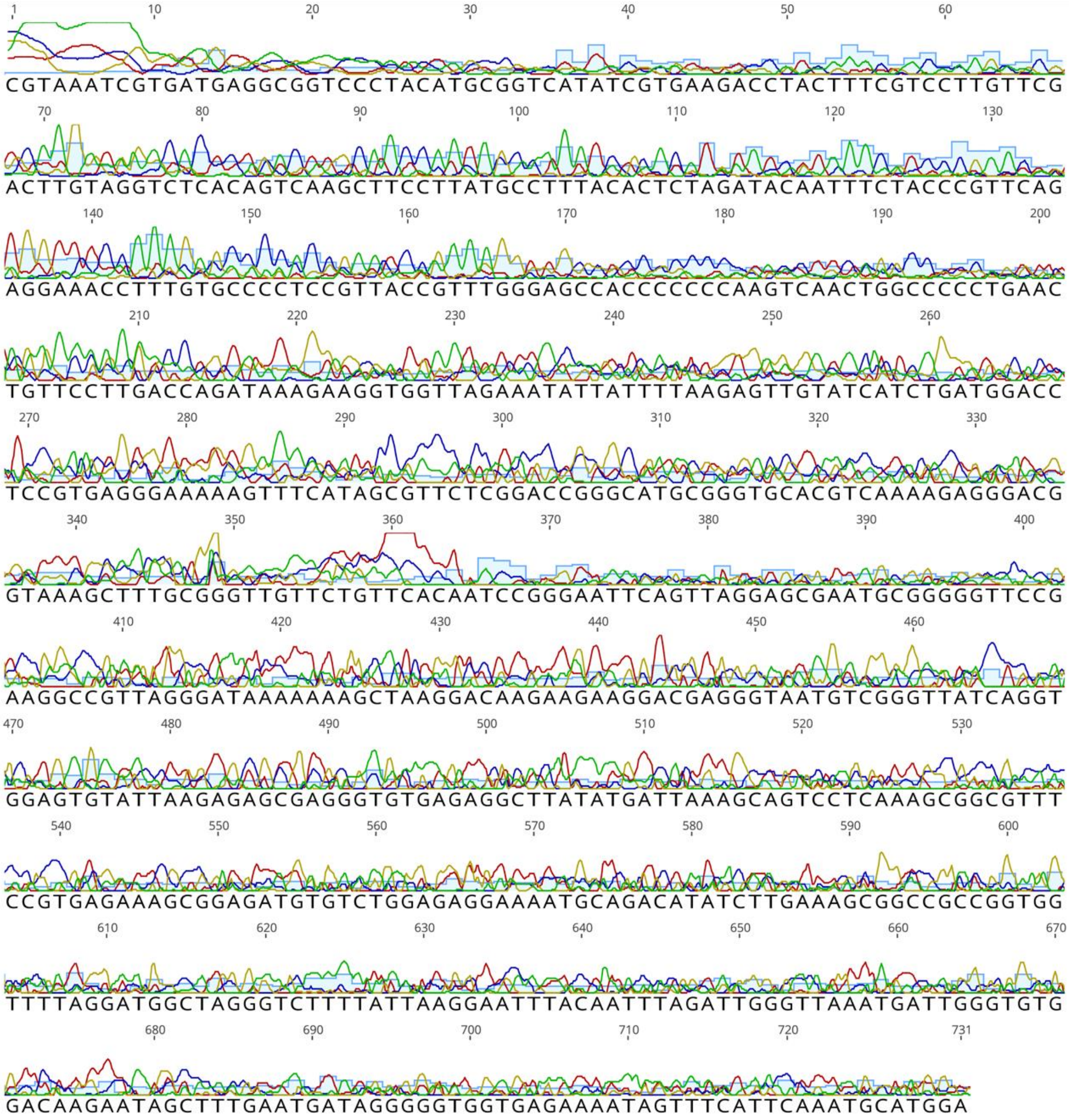


Figure A.11 Sanger chromatogram of 731 bp of 23S rDNA amplified from TD1 sample sequenced in 5’–3’ direction (starting from the forward primer).

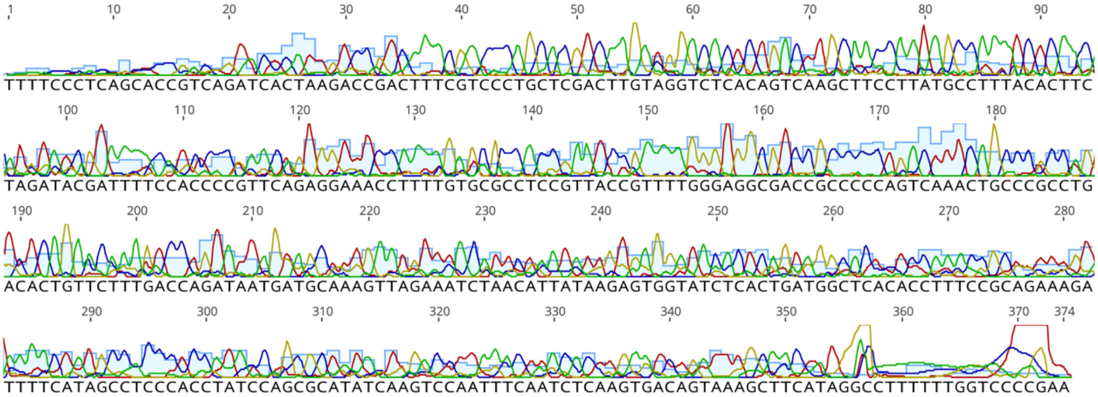


Figure A.12 Sanger chromatogram of 374 bp of 23S rDNA amplified from TD1 sample sequenced in 3’–5’ direction (starting from the reverse primer).

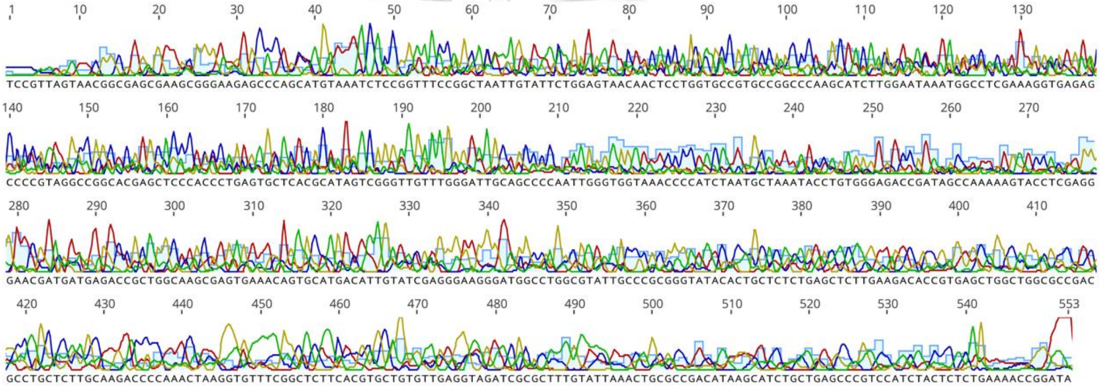


Figure A.13 Sanger chromatogram of 553 bp of 28S rDNA amplified from CKD sample sequenced in 5’–3’ direction (starting from the forward primer).

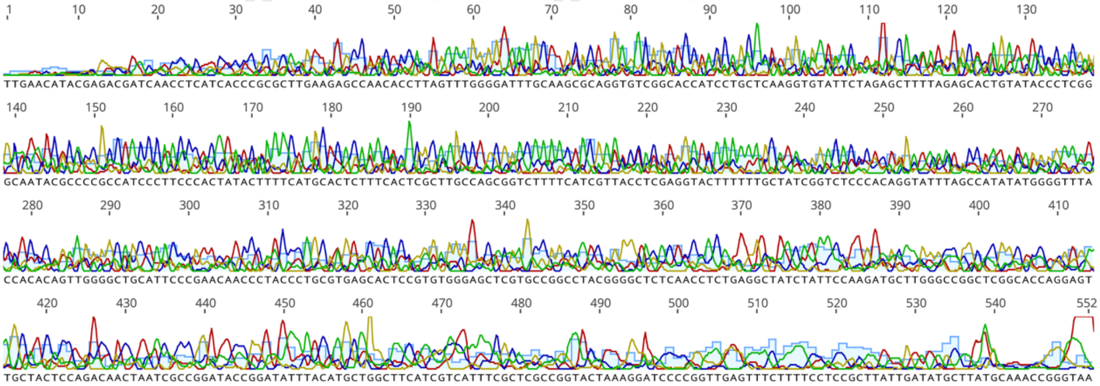


Figure A.14 Sanger chromatogram of 552 bp of 28S rDNA amplified from CKD sample sequenced in 3’–5’ direction (starting from the reverse primer).

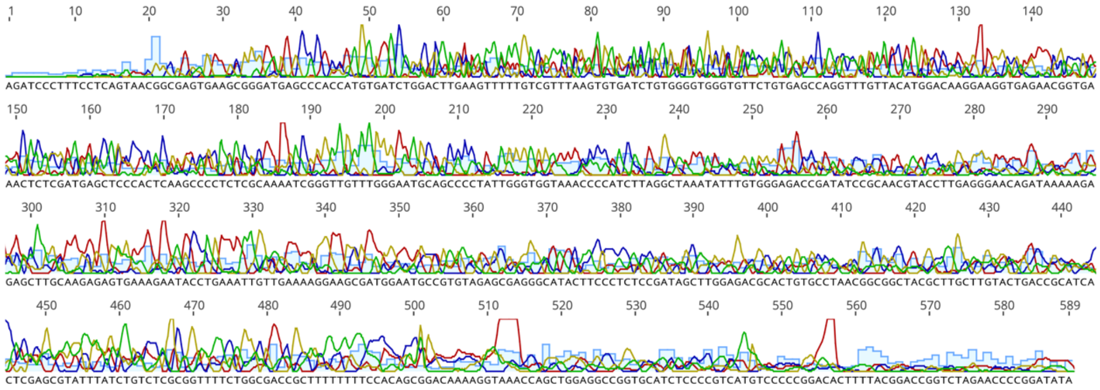


Figure A.15 Sanger chromatogram of 589 bp of 28S rDNA amplified from PUA sample sequenced in 5’–3’ direction (starting from the forward primer).

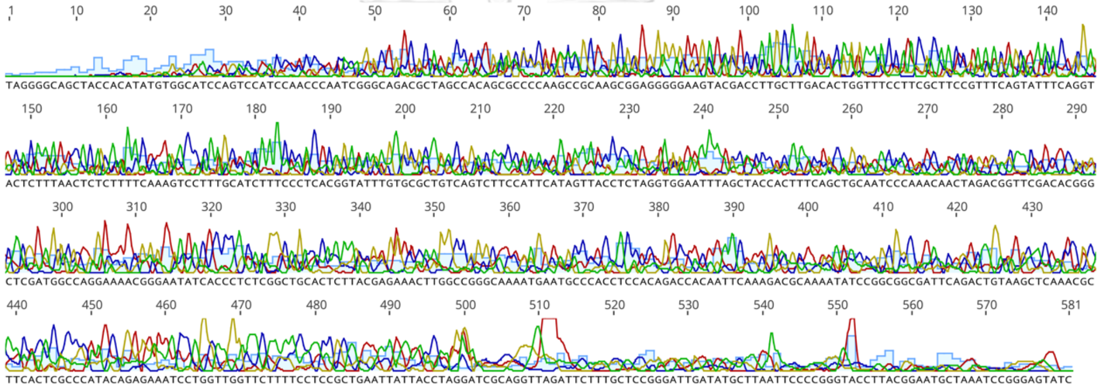


Figure A.16 Sanger chromatogram of 581 bp of 28S rDNA amplified from PUA sample sequenced in 3’–5’ direction (starting from the reverse primer).

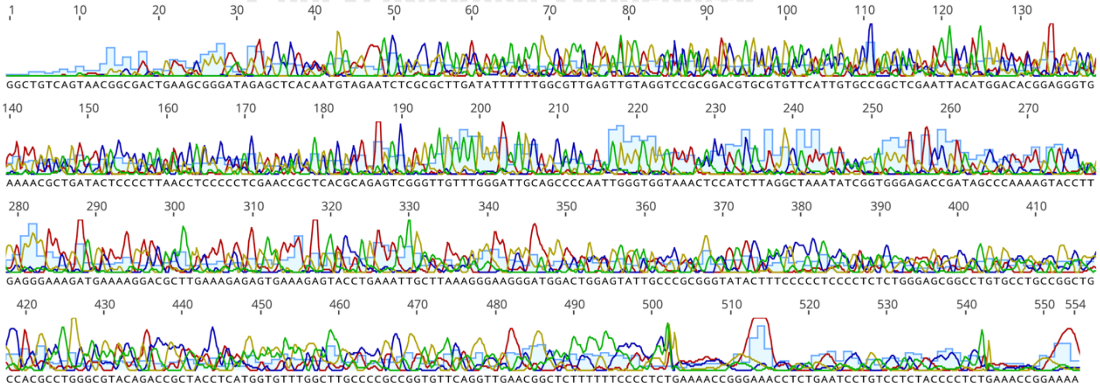


Figure A.17 Sanger chromatogram of 554 bp of 28S rDNA amplified from TD1 sample sequenced in 5’–3’ direction (starting from the forward primer).

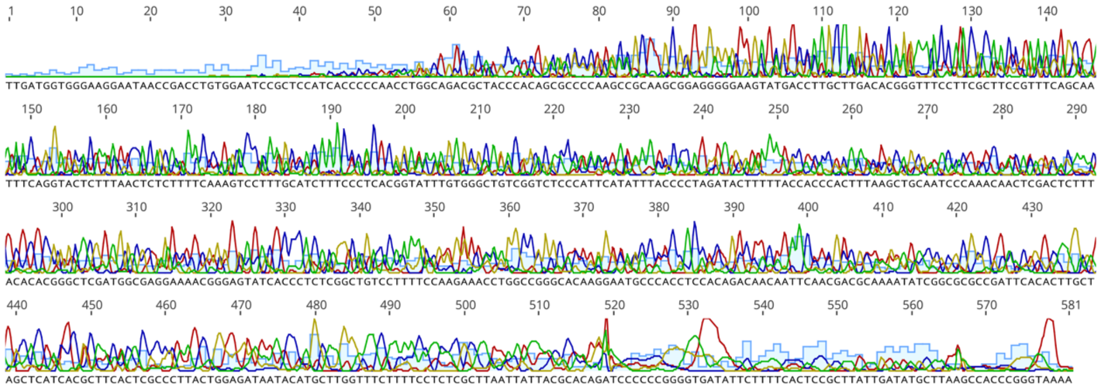


Figure A.18 Sanger chromatogram of 356 bp of 28S rDNA amplified from TD1 sample sequenced in 3’–5’ direction (starting from the reverse primer).

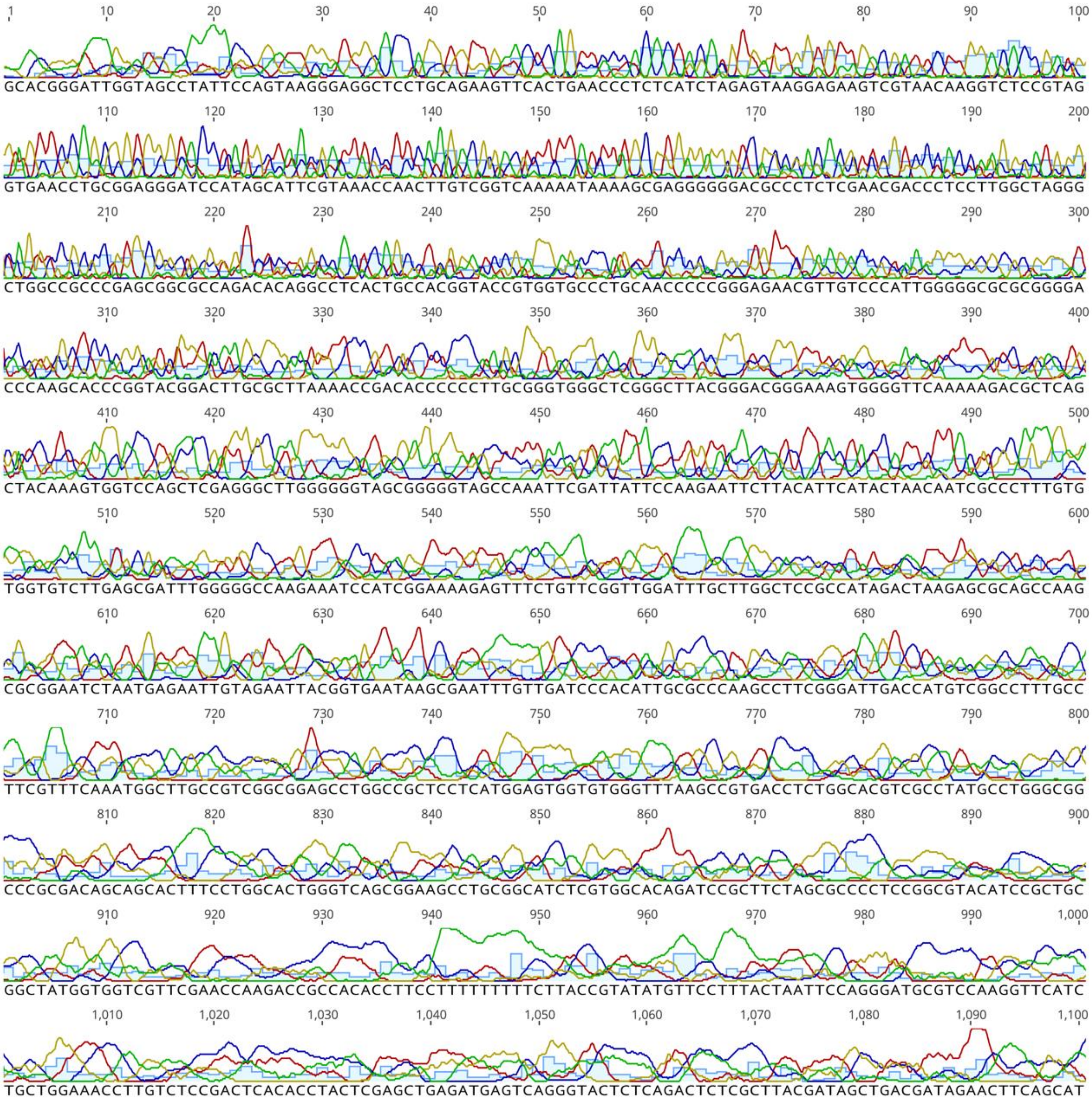


Figure A.19 Sanger chromatogram of 1,100 bp of ITS region amplified from CKD sample sequenced in 5’–3’ direction (starting from the forward primer).

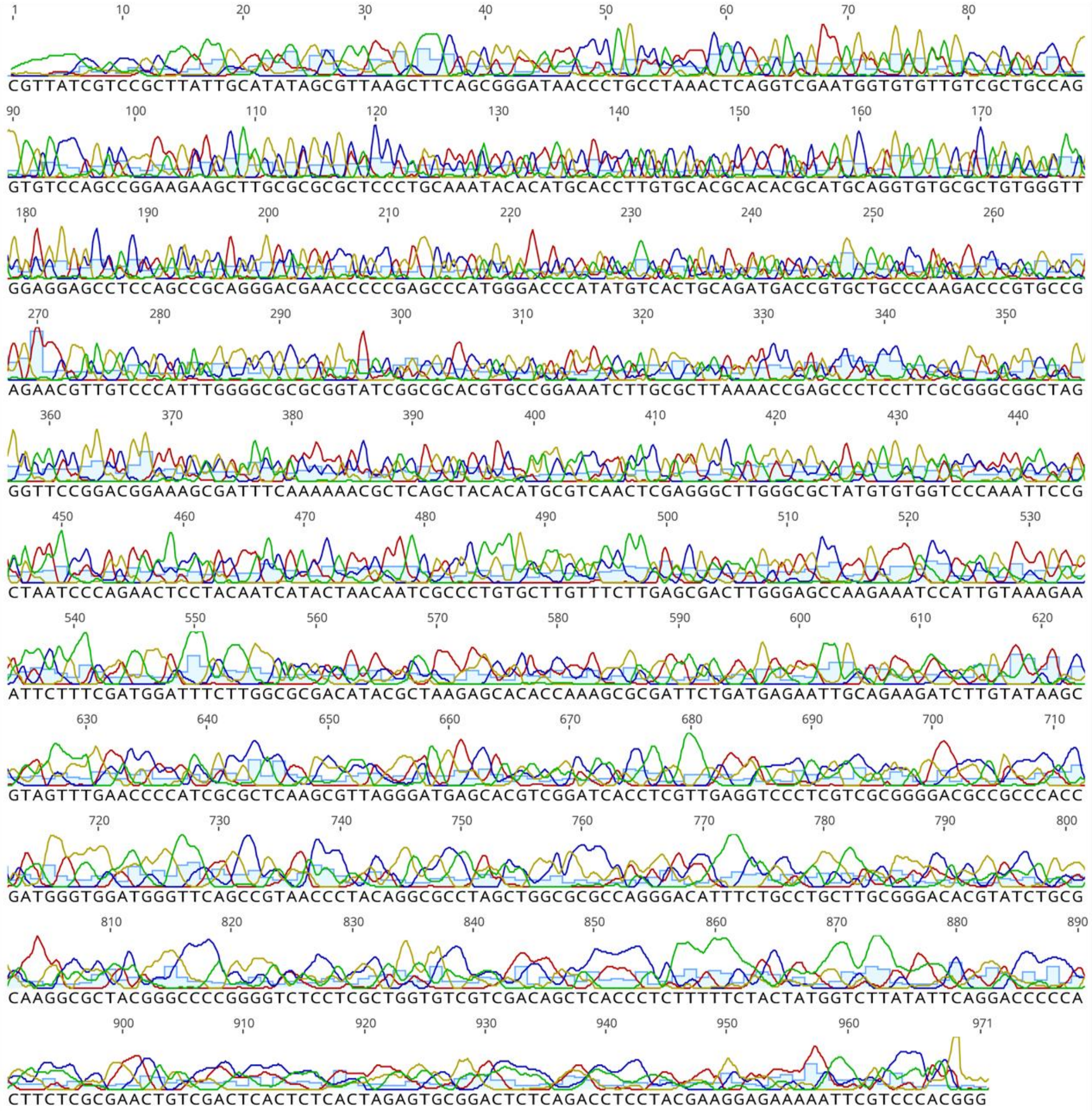


Figure A.20 Sanger chromatogram of 971 bp of ITS region amplified from CKD sample sequenced in 3’–5’ direction (starting from the reverse primer).

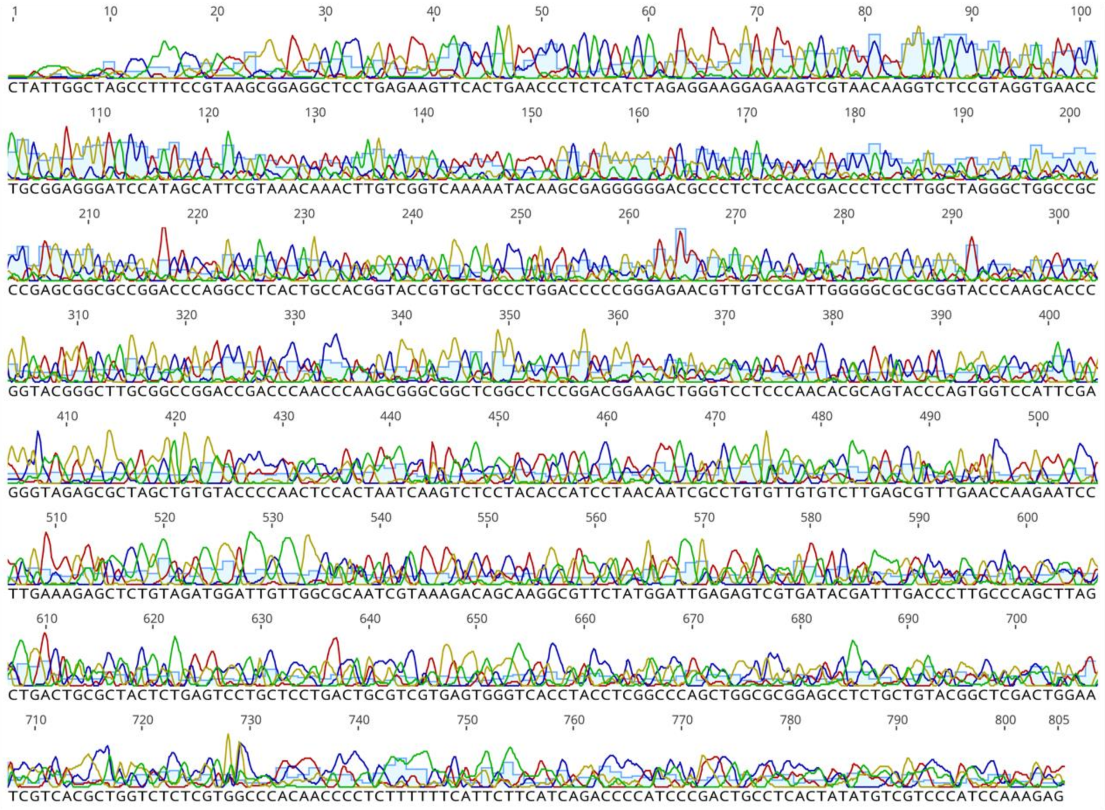


Figure A.21 Sanger chromatogram of 805 bp of ITS region amplified from PUA sample sequenced in 5’–3’ direction (starting from the forward primer).

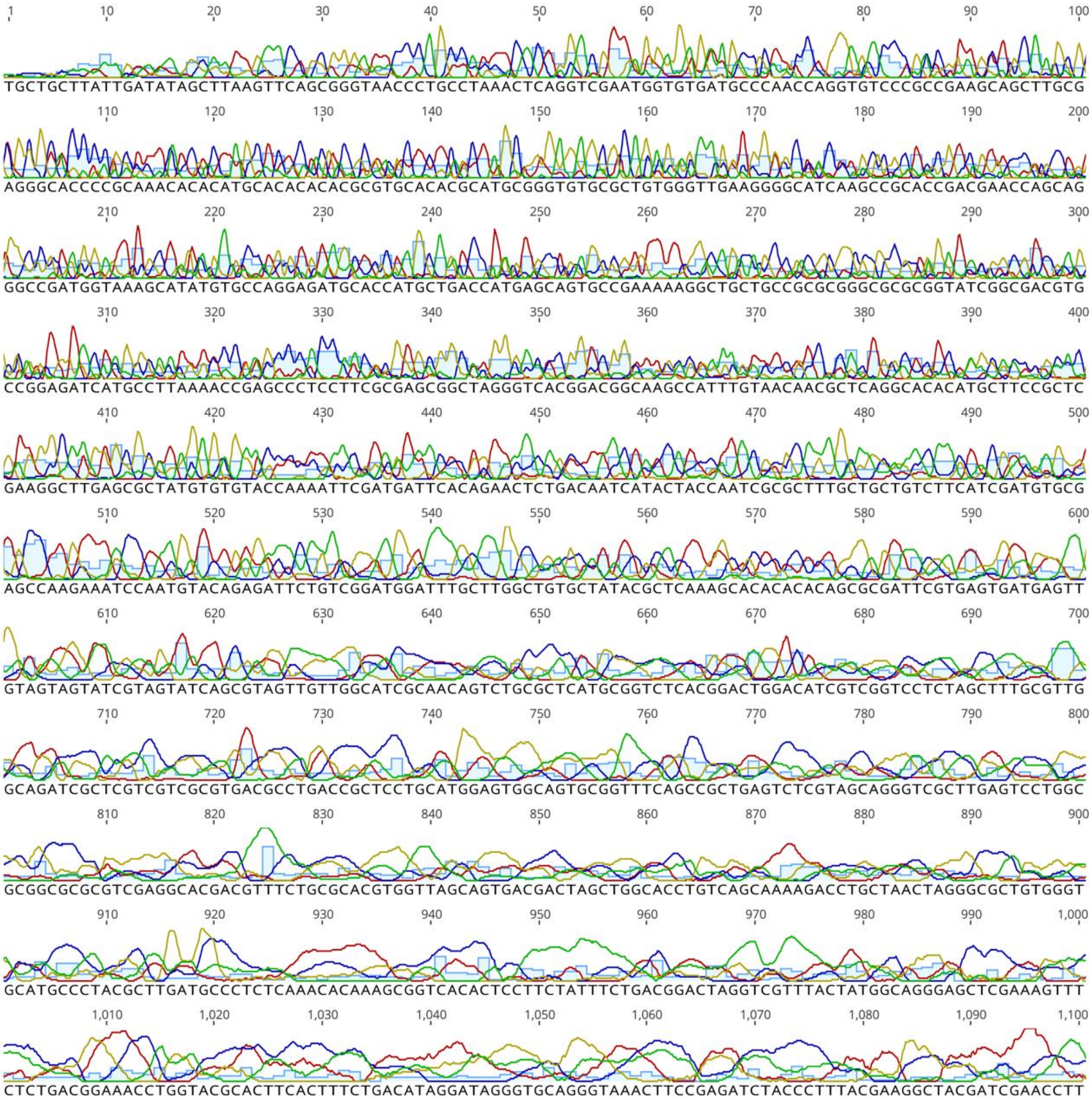


Figure A.22 Sanger chromatogram of 1,100 bp of ITS region amplified from PUA sample sequenced in 3’–5’ direction (starting from the reverse primer).

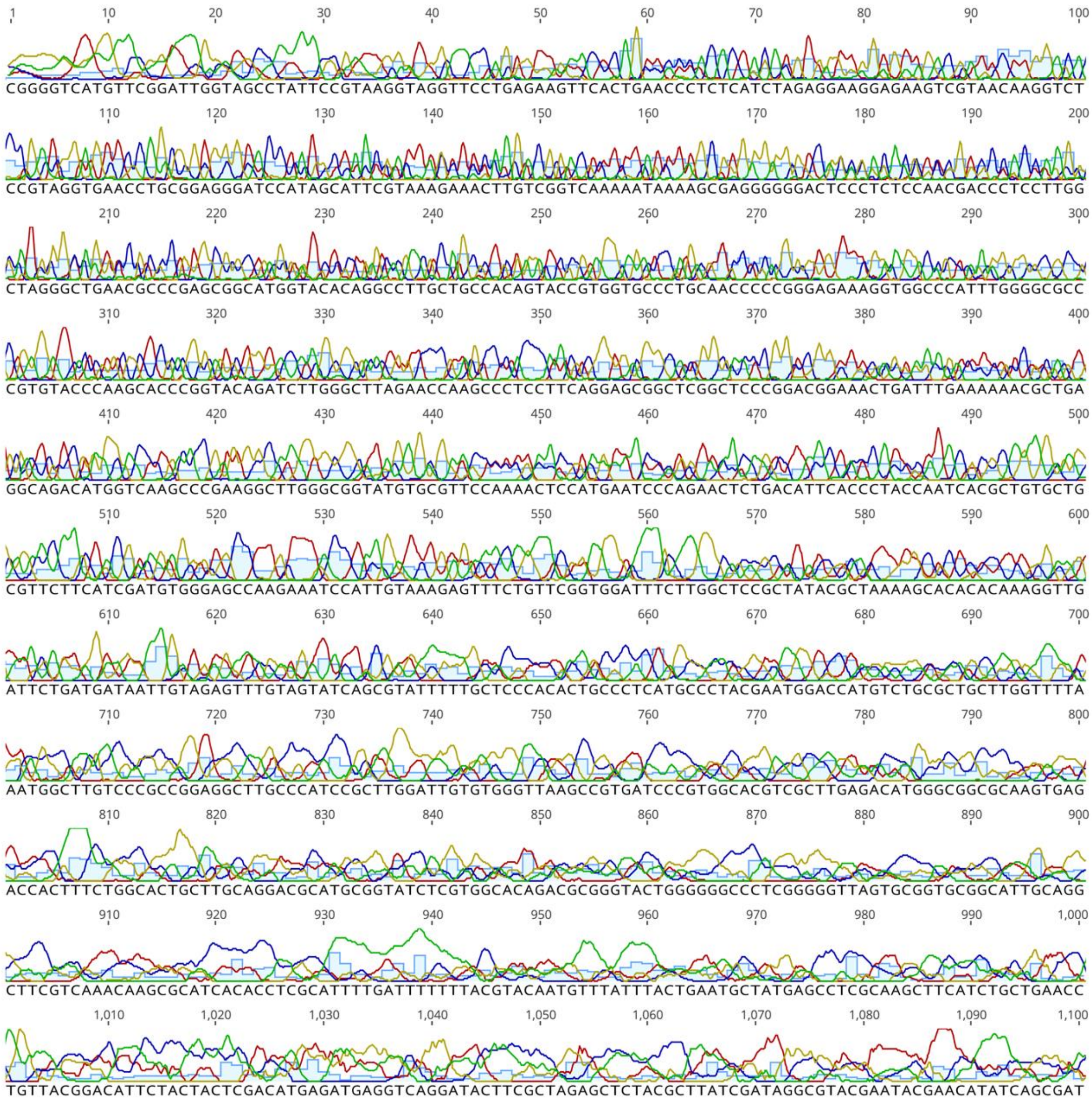


Figure A.23 Sanger chromatogram of 1,100 bp of ITS region amplified from TD1 sample sequenced in 5’–3’ direction (starting from the forward primer).

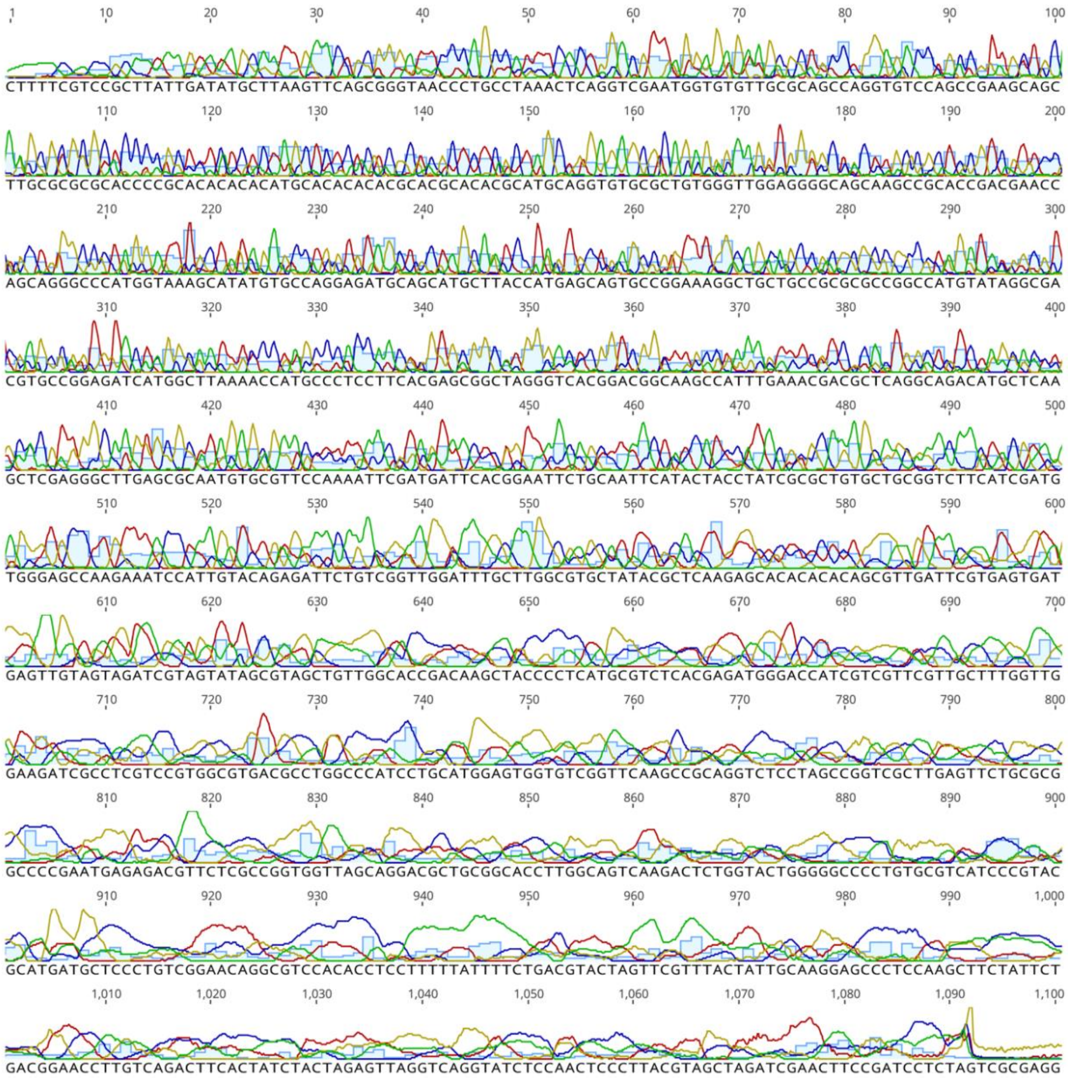


Figure A.24 Sanger chromatogram of 1,100 bp of ITS region amplified from TD1 sample sequenced in 3’–5’ direction (starting from the reverse primer).

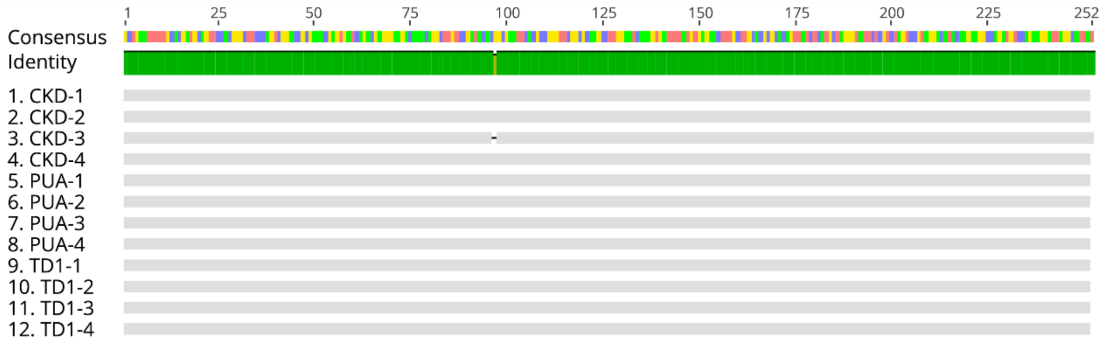


Figure A.25 Multiple sequence alignment of *Cladophora* 18S rDNA amplicons obtained from universal primers, sequenced by Illumina Miseq, and visualized using Geneious Prime 2021.1.1.

Table A.1 Top ten BLASTN search results of 18S rDNA amplicon sequenced by Sanger technology.

Sequence queries indicate collecting sites and sequencing directions. F indicates the direction starting from the 5’ terminal (forward primer). R indicates the direction starting from the 3’ terminal (reverse primer).

Query	No.	Accession number	Description	Total score	Query cover	E-value	Identity (%)
CKD-F	1	KU904705.1	*Cladophora* sp. HLJ1407 18S ribosomal RNA gene, partial sequence	1439	100%	0	95.57
	2	KU904721.1	*Cladophora* sp. TB1461 18S ribosomal RNA gene, partial sequence	1434	100%	0	95.46
	3	KU904720.1	*Cladophora* sp. TB1439 18S ribosomal RNA gene, partial sequence	1434	100%	0	95.46
	4	KU904718.1	*Cladophora* sp. TB1401 18S ribosomal RNA gene, partial sequence	1434	100%	0	95.46
	5	KU904717.1	*Cladophora* sp. YN1402 18S ribosomal RNA gene, partial sequence	1434	100%	0	95.46
	6	KU904716.1	*Cladophora* sp. YN1401 18S ribosomal RNA gene, partial sequence	1434	100%	0	95.46
	7	KU904715.1	*Cladophora* sp. YN1303 18S ribosomal RNA gene, partial sequence	1434	100%	0	95.46
	8	KU904713.1	*Cladophora* sp. FJ1301 18S ribosomal RNA gene, partial sequence	1434	100%	0	95.46
	9	KU904697.1	*Cladophora* sp. HEN1508 18S ribosomal RNA gene, partial sequence	1434	100%	0	95.46
	10	KU904705.1	*Cladophora* sp. HEN1504 18S ribosomal RNA gene, partial sequence	1434	100%	0	95.46
CKD-R	1	LS974894.1	*Cladophora* sp. QH1505 18S ribosomal RNA gene, partial sequence	1796	100%	0	99.39
	2	KU904733.1	*Cladophora* sp. QH1504 18S ribosomal RNA gene, partial sequence	1796	100%	0	99.39
	3	KU904732.1	*Cladophora* sp. QH1503 18S ribosomal RNA gene, partial sequence	1796	100%	0	99.39
	4	KU904731.1	*Cladophora* sp. QH1502 18S ribosomal RNA gene, partial sequence	1796	100%	0	99.39
	5	KU904730.1	*Cladophora* sp. QH1501 18S ribosomal RNA gene, partial sequence	1796	100%	0	99.39

Query	No.	Accession number	Description	Total score	Query cover	E-value	Identity (%)
CKD-R	6	KU904728.1	*Cladophora* sp. QH1403 18S ribosomal RNA gene, partial sequence	1796	100%	0	99.39
	7	KU904725.1	*Cladophora* sp. QH1304 18S ribosomal RNA gene, partial sequence	1796	100%	0	99.39
	8	KU904724.1	*Cladophora* sp. QH1303 18S ribosomal RNA gene, partial sequence	1796	100%	0	99.39
	9	KU904723.1	*Cladophora* sp. QH1302 18S ribosomal RNA gene, partial sequence	1796	100%	0	99.39
	10	KU904722.1	*Cladophora* sp. QH1301 18S ribosomal RNA gene, partial sequence	1796	100%	0	99.39
PUA-F	1	KU904719.1	*Cladophora* sp. TB1433 18S ribosomal RNA gene, partial sequence	1450	95%	0	95.55
	2	KU904650.1	*Cladophora* sp. HB1422 18S ribosomal RNA gene, partial sequence	1450	95%	0	95.55
	3	KU904689.1	*Cladophora* sp. HEN1416 18S ribosomal RNA gene, partial sequence	1447	95%	0	95.44
	4	KU904658.1	*Cladophora* sp. HUN1406 18S ribosomal RNA gene, partial sequence	1447	95%	0	95.44
	5	KU904721.1	*Cladophora* sp. TB1461 18S ribosomal RNA gene, partial sequence	1445	95%	0	95.44
	6	KU904720.1	*Cladophora* sp. TB1439 18S ribosomal RNA gene, partial sequence	1445	95%	0	95.44
	7	KU904718.1	*Cladophora* sp. TB1401 18S ribosomal RNA gene, partial sequence	1445	95%	0	95.44
	8	KU904717.1	*Cladophora* sp. YN1402 18S ribosomal RNA gene, partial sequence	1445	95%	0	95.44
	9	KU904716.1	*Cladophora* sp. YN1401 18S ribosomal RNA gene, partial sequence	1445	95%	0	95.44
	10	KU904715.1	*Cladophora* sp. YN1303 18S ribosomal RNA gene, partial sequence	1445	95%	0	95.44
PUA-R	1	KU904702.1	*Cladophora* sp. HLJ1404 18S ribosomal RNA gene, partial sequence	1882	99%	0	99.42
	2	KU904734.1	*Cladophora* sp. QH1505 18S ribosomal RNA gene, partial sequence	1881	99%	0	99.33
	3	KU904733.1	*Cladophora* sp. QH1504 18S ribosomal RNA gene, partial sequence	1881	99%	0	99.33

Query	No.	Accession number	Description	Total score	Query cover	E-value	Identity (%)
PUA-R	4	KU904732.1	*Cladophora* sp. QH1503 18S ribosomal RNA gene, partial sequence	1881	99%	0	99.33
	5	KU904731.1	*Cladophora* sp. QH1502 18S ribosomal RNA gene, partial sequence	1881	99%	0	99.33
	6	KU904730.1	*Cladophora* sp. QH1501 18S ribosomal RNA gene, partial sequence	1881	99%	0	99.33
	7	KU904728.1	*Cladophora* sp. QH1403 18S ribosomal RNA gene, partial sequence	1881	99%	0	99.33
	8	KU904725.1	*Cladophora* sp. QH1304 18S ribosomal RNA gene, partial sequence	1881	99%	0	99.33
	9	KU904724.1	*Cladophora* sp. QH1303 18S ribosomal RNA gene, partial sequence	1881	99%	0	99.33
	10	KU904723.1	*Cladophora* sp. QH1302 18S ribosomal RNA gene, partial sequence	1881	99%	0	99.33
TD1-F	1	KU904719.1	*Cladophora* sp. TB1433 18S ribosomal RNA gene, partial sequence	1016	94%	0	88.72
	2	KU904659.1	*Cladophora* sp. HUN1407 18S ribosomal RNA gene, partial sequence	1016	94%	0	88.72
	3	KU904650.1	*Cladophora* sp. HB1422 18S ribosomal RNA gene, partial sequence	1016	94%	0	88.72
	4	KU904721.1	*Cladophora* sp. TB1461 18S ribosomal RNA gene, partial sequence	1014	94%	0	88.72
	5	KU904720.1	*Cladophora* sp. TB1439 18S ribosomal RNA gene, partial sequence	1014	94%	0	88.72
	6	KU904718.1	*Cladophora* sp. TB1401 18S ribosomal RNA gene, partial sequence	1014	94%	0	88.72
	7	KU904717.1	Cladophora sp. YN1402 18S ribosomal RNA gene, partial sequence	1014	94%	0	88.72
	8	KU904716.1	*Cladophora* sp. YN1401 18S ribosomal RNA gene, partial sequence	1014	94%	0	88.72
	9	KU904715.1	*Cladophora* sp. YN1303 18S ribosomal RNA gene, partial sequence	1014	94%	0	88.72
	10	KU904713.1	*Cladophora* sp. FJ1301 18S ribosomal RNA gene, partial sequence	1014	94%	0	88.72

Query	No.	Accession number	Description	Total score	Query cover	E-value	Identity (%)
TD1-R	1	LS974894.1	*Cladophora* glomerata partial 18S rRNA gene, specimen voucher WELT: A033724, isolate Q41	1559	99%	0	97.7
	2	KU904720.1	*Cladophora* sp. TB1439 18S ribosomal RNA gene, partial sequence	1559	99%	0	97.7
	3	KU904718.1	*Cladophora* sp. TB1401 18S ribosomal RNA gene, partial sequence	1559	99%	0	97.7
	4	KU904717.1	*Cladophora* sp. YN1402 18S ribosomal RNA gene, partial sequence	1559	99%	0	97.7
	5	KU904716.1	*Cladophora* sp. YN1401 18S ribosomal RNA gene, partial sequence	1559	99%	0	97.7
	6	KU904715.1	*Cladophora* sp. YN1303 18S ribosomal RNA gene, partial sequence	1559	99%	0	97.7
	7	KU904713.1	*Cladophora* sp. FJ1301 18S ribosomal RNA gene, partial sequence	1559	99%	0	97.7
	8	KU904702.1	*Cladophora* sp. HLJ1404 18S ribosomal RNA gene, partial sequence	1559	99%	0	97.7
	9	KU904701.1	*Cladophora* sp. HLJ1403 18S ribosomal RNA gene, partial sequence	1559	99%	0	97.7
	10	KU904697.1	*Cladophora* sp. HEN1508 18S ribosomal RNA gene, partial sequence	1559	99%	0	97.7

Table A.2 Top ten BLASTN search results of 23S rDNA amplicon sequenced by Sanger technology.

Sequence queries indicate collecting sites and sequencing directions. F indicates the direction starting from the 5’ terminal (forward primer). R indicates the direction starting from the 3’ terminal (reverse primer).

Query	No.	Accession number	Description	Total score	Query cover	E-value	Identity (%)
CKD-R	1	MW675675.1	*Erythrotrichia longistipitata* chloroplast clone contig 32, complete genome	570	99%	3.00E-72	91.75
	2	HQ421333.1	*Erythrotrichia* sp. ARS-2011 voucher ARS04482 23S ribosomal RNA gene, partial sequence; plastid	285	99%	3.00E-72	91.75
	3	KF907445.1	*Cryptomonas* sp. Dumo2 100310C 23S large subunit ribosomal RNA gene, partial sequence; plastid	282	97%	1.00E-71	92.08
	4	KF907437.1	*Cryptomonas pyrenoidifera* strain CNUCRY 134 23S large subunit ribosomal RNA gene, partial sequence; plastid	282	97%	1.00E-71	92.08
	5	KF907429.1	*Cryptomonas ovata* strain CNUCRY 231 23S large subunit ribosomal RNA gene, partial sequence; plastid	282	97%	1.00E-71	92.08
	6	KF907426.1	*Cryptomonas obovata* strain Saenae080611D 23S large subunit ribosomal RNA gene, partial sequence; plastid	282	97%	1.00E-71	92.08
	7	KF907425.1	*Cryptomonas obovata* strain Hanjeong080610A 23S large subunit ribosomal RNA gene, partial sequence; plastid	282	97%	1.00E-71	92.08
	8	HQ421332.1	*Erythrotrichia* sp. ARS-2011 voucher ARS04481 23S ribosomal RNA gene, partial sequence; plastid	282	97%	1.00E-71	92.08
	9	CP021983.2	*Halomicronema hongdechloris* C2206 genome	561	99%	4.00E-71	91.26
	10	KY856939.1	*Cryptomonas curvata* strain CNUKR plastid, complete genome	556	97%	5.00E-70	91.58

Query	No.	Accession number	Description	Total score	Query cover	E-value	Identity (%)
PUA-F	1	MW648602.1	*Cocconeis euglypta* strain TCC720-sq1 large subunit ribosomal RNA gene, partial sequence; chloroplast	249	100%	3.00E-61	83.90
	2	MG021094.1	*Cladophora* sp. JIAC-WT-filamentous 2 large subunit ribosomal RNA gene, partial sequence; chloroplast	245	100%	3.00E-60	83.90
	3	KY916410.1	Uncultured organism clone UniqueSequence1835 23S ribosomal RNA gene, partial sequence	217	100%	4.00E-52	80.93
	4	KY916246.1	Uncultured organism clone UniqueSequence1671 23S ribosomal RNA gene, partial sequence	217	100%	4.00E-52	80.93
	5	KY915958.1	Uncultured organism clone UniqueSequence1383 23S ribosomal RNA gene, partial sequence	217	100%	4.00E-52	80.93
	6	KF803893.1	Uncultured organism clone QHS-288-1 23S ribosomal RNA gene, partial sequence	217	100%	4.00E-52	80.93
	7	KF803789.1	Uncultured organism clone QHS-22-4 23S ribosomal RNA gene, partial sequence	217	100%	4.00E-52	80.93
	8	MW648606.1	*Gomphonema parvulum* strain TCC888-sq1 large subunit ribosomal RNA gene, partial sequence; chloroplast	217	100%	4.00E-52	80.93
	9	KC284811.1	Uncultured organism clone DGCM54 23S ribosomal RNA gene, partial sequence	217	100%	4.00E-52	80.93
	10	KC284763.1	Uncultured organism clone C36 23S ribosomal RNA gene, partial sequence	217	100%	4.00E-52	80.93
PUA-R	1	MW648602.1	*Cocconeis euglypta* strain TCC720-sq1 large subunit ribosomal RNA gene, partial sequence; chloroplast	628	100%	1.00E-175	99.42
	2	MZ469850.1	*Aureoumbra* sp. strain DAD-ALGEUK-122 large subunit ribosomal RNA gene, partial sequence; chloroplast	568	100%	2.00E-157	96.24
	3	EU342146.1	Uncultured organism clone C6.71 23S ribosomal RNA gene, partial sequence	562	100%	1.00E-155	95.95
	4	NC_044464.1	*Halamphora calidilacuna* chloroplast, complete genome	1113	100%	5.00E-154	95.66
	5	NC_044463.1	*Halamphora americana* chloroplast, complete genome	1113	100%	5.00E-154	95.66
	6	MK045451.1	*Halamphora calidilacuna* chloroplast, complete genome	1113	100%	5.00E-154	95.66

Query	No.	Accession number	Description	Total score	Query cover	E-value	Identity (%)
PUA-R	7	MK045450.1	*Halamphora americana* chloroplast, complete genome	1113	100%	5.00E-154	95.66
	8	MZ469805.1	*Cerataulina* sp. strain DAD-ALGEUK-77 large subunit ribosomal RNA gene, partial sequence; chloroplast	556	100%	5.00E-154	95.66
	9	EU342166.1	Uncultured organism clone C6.11 23S ribosomal RNA gene, partial sequence	556	100%	5.00E-154	95.66
	10	EU342158.1	Uncultured organism clone C6.10 23S ribosomal RNA gene, partial sequence	556	100%	5.00E-154	95.66
TD1-F	1	KY915958.1	Uncultured organism clone UniqueSequence1383 23S ribosomal RNA gene, partial sequence	202	99%	7.00E-48	97.46
	2	KM458300.1	Uncultured Diadesmis isolate OTU 00008 23S ribosomal RNA gene, partial sequence; plastid	202	99%	7.00E-48	97.46
	3	KM458301.1	Uncultured Diadesmis isolate OTU 00009 23S ribosomal RNA gene, partial sequence; plastid	202	99%	7.00E-48	97.46
	4	KJ671835.1	*Chaetoceros didymus* strain 32 23S ribosomal RNA gene, partial sequence; chloroplast	202	99%	7.00E-48	97.46
	5	NC_044464.1	*Halamphora calidilacuna* chloroplast, complete genome	393	99%	3.00E-46	96.61
	6	NC_044463.1	*Halamphora americana* c chloroplast, complete genome	393	99%	3.00E-46	96.61
	7	MK045451.1	*Halamphora calidilacuna* chloroplast, complete genome	393	99%	3.00E-46	96.61
	8	MK045450.1	*Halamphora americana* chloroplast, complete genome	393	99%	3.00E-46	96.61
	9	NC_038001.1	*Entomoneis* sp. chloroplast, complete genome	393	99%	3.00E-46	96.61
	10	MG755800.1	*Entomoneis* sp. chloroplast, complete genome	393	99%	3.00E-46	96.61
TD1-R	1	MW648602.1	*Cocconeis euglypta* strain TCC720-sq1 large subunit ribosomal RNA gene, partial sequence; chloroplast	527	100%	4.00E-145	95.76
	2	MG021094.1	*Cladophora* sp. JIAC-WT-filamentous 2 large subunit ribosomal RNA gene, partial sequence; chloroplast	475	92%	1.00E-129	95.10
	3	NC_044464.1	*Halamphora calidilacuna* chloroplast, complete genome	932	100%	8.00E-127	92.42
	4	NC_044463.1	*Halamphora americana* chloroplast, complete genome	932	100%	8.00E-127	92.42
	5	MK045451.1	*Halamphora calidilacuna* chloroplast, complete genome	932	100%	8.00E-127	92.42

Query	No.	Accession number	Description	Total score	Query cover	E-value	Identity (%)
TD1-R	6	MK045450.1	*Halamphora americana* chloroplast, complete genome	932	100%	8.00E-127	92.42
	7	MZ469850.1	*Aureoumbra* sp. strain DAD-ALGEUK-122 large subunit ribosomal RNA gene, partial sequence; chloroplast	466	100%	8.00E-127	92.42
	8	KJ671831.1	*Amphiprora alata* strain J 23S ribosomal RNA gene, partial sequence; chloroplast	460	100%	4.00E-125	92.15
	9	EU342146.1	Uncultured organism clone C6.71 23S ribosomal RNA gene, partial sequence	460	100%	4.00E-125	92.15
	10	KY916472.1	Uncultured organism clone UniqueSequence1897 23S ribosomal RNA gene, partial sequence	457	99%	5.00E-124	92.07

Table A.3 Top ten BLASTN search results of 28S rDNA amplicon sequenced by Sanger technology.

Sequence queries indicate collecting sites and sequencing directions. F indicates the direction starting from the 5’ terminal (forward primer). R indicates the direction starting from the 3’ terminal (reverse primer).

Query	No.	Accession number	Description	Total score	Query cover	E-value	Identity (%)
CKD-F	1	LT607103.1	*Cladophora* cf. *rivularis* H28 partial 28S rRNA gene, specimen voucher WELT: A033264, isolate H28	684	100%	0	91.57
	2	LT607091.1	*Cladophora* cf. *glomerata* P02 partial 28S rRNA gene, specimen voucher WELT: A033290, isolate P02	671	100%	0	91.16
	3	KU904758.1	*Cladophora* sp. TB1461 28S ribosomal RNA gene, partial sequence	665	100%	0	90.96
	4	KU904745.1	*Cladophora* sp. HUN1440 28S ribosomal RNA gene, partial sequence	665	100%	0	90.96
	5	KU904743.1	*Cladophora* sp. HUN1436 28S ribosomal RNA gene, partial sequence	665	100%	0	90.96
	6	KU904738.1	*Cladophora* sp. HB1425 28S ribosomal RNA gene, partial sequence	665	100%	0	90.96
	7	LS974958.1	*Cladophora* sp. T52 partial 28S rRNA gene, specimen voucher WELT: A033729, isolate T52	665	100%	0	90.96

Query	No.	Accession number	Description	Total score	Query cover	E-value	Identity (%)
CKD-F	8	LS974948.1	*Cladophora glomerata* partial 28S rRNA gene, specimen voucher WELT: A033724, isolate Q41	665	100%	0	90.96
	9	LS974947.1	*Cladophora glomerata* partial 28S rRNA gene, specimen voucher WELT: A033722, isolate Q39	665	100%	0	90.96
	10	LS974946.1	*Cladophora glomerata* partial 28S rRNA gene, specimen voucher WELT: A033694, isolate P92	665	100%	0	90.96
CKD-R	1	LT607103.1	*Cladophora* cf. *rivularis* H28 partial 28S rRNA gene, specimen voucher WELT: A033264, isolate H28	654	98%	0	91.60
	2	KM676861.1	*Cladophora glomerata* voucher ARS07398_00001 28S ribosomal RNA gene, partial sequence	649	100%	0	90.91
	3	KU904758.1	*Cladophora* sp. TB1461 28S ribosomal RNA gene, partial sequence	647	100%	0	90.91
	4	KU904748.1	*Cladophora* sp. HEN1404 28S ribosomal RNA gene, partial sequence	647	100%	0	90.91
	5	KU904745.1	*Cladophora* sp. HUN1440 28S ribosomal RNA gene, partial sequence	647	100%	0	90.91
	6	KU904741.1	*Cladophora* sp. HUN1414 28S ribosomal RNA gene, partial sequence	647	100%	0	90.91
	7	KU904739.1	*Cladophora* sp. HB1503 28S ribosomal RNA gene, partial sequence	647	100%	0	90.91
	8	KU904738.1	*Cladophora* sp. HB1425 28S ribosomal RNA gene, partial sequence	647	100%	0	90.91
	9	KU866513.1	*Cladophora* sp. ZZ-2016 voucher TB1439 28S ribosomal RNA gene, partial sequence	647	100%	0	90.91
	10	KU866512.1	*Cladophora* sp. ZZ-2016 voucher TB1401 28S ribosomal RNA gene, partial sequence	647	100%	0	90.91
PUA-F	1	AM710566.1	*Cocconeis pediculus* partial 28S rRNA gene, strain AT-212.07	220	83%	3.00E-52	74.8
	2	AM710569.1	*Cocconeis placentula* partial 28S rRNA gene, strain AT-212Gel11	215	81%	3.00E-51	75.2

Query	No.	Accession number	Description	Total score	Query cover	E-value	Identity (%)
PUA-F	3	OL780369.1	*Cocconeis euglypta* strain CBac2019019 large subunit ribosomal RNA gene, partial sequence	212	82%	4.00E-50	74.4
	4	LT607077.1	*Chaetomorpha spiralis* partial 28S rRNA gene, specimen voucher WELT: A033220, isolate D52	198	44%	8.00E-46	82.41
	5	LT607060.1	*Chaetomorpha antennina* partial 28S rRNA gene, specimen voucher WELT: A033218, isolate D45	198	44%	8.00E-46	82.41
	6	LT607059.1	*Chaetomorpha antennina* partial 28S rRNA gene, specimen voucher LAF: BW02589, isolate Fx1141	198	44%	8.00E-46	82.41
	7	LT607218.1	*Chaetomorpha spiralis* partial 28S rRNA gene, specimen voucher GENT: F.0165 (D760), isolate Fx1353	198	44%	8.00E-46	82.41
	8	LT607217.1	*Chaetomorpha vieillardii* partial 28S rRNA gene, specimen voucher WELT: A033185, isolate B69, JW4416	198	44%	8.00E-46	82.41
	9	LT607216.1	*Chaetomorpha antennina* partial 28S rRNA gene, specimen voucher WELT: A033254, isolate G73	198	44%	8.00E-46	82.41
	10	LT607215.1	*Chaetomorpha antennina* partial 28S rRNA gene, specimen voucher GENT: GUAM118, isolate F701	198	44%	8.00E-46	82.41
PUA-R	1	AM710566.1	*Cocconeis pediculus* partial 28S rRNA gene, strain AT-212.07	567	100%	5.00E-157	87.29
	2	OL780369.1	*Cocconeis euglypta* strain CBac2019019 large subunit ribosomal RNA gene, partial sequence	516	100%	3.00E-141	84.96
	3	AM710569.1	*Cocconeis placentula* partial 28S rRNA gene, strain AT-212Gel11	437	100%	6.00E-118	81.36
	4	MK585217.1	*Phaeodactylum tricornutum* strain CCMA106 5.8S ribosomal RNA gene, partial sequence; internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence	343	93%	3.00E-89	78.78

Query	No.	Accession number	Description	Total score	Query cover	E-value	Identity (%)
PUA-R	5	KM407585.1	*Nitzschia* sp. LFS-2014 isolate Ant015-G03 28S ribosomal RNA gene, partial sequence	343	99%	3.00E-89	77.87
	6	OM859331.1	*Phaeodactylum tricornutum* strain OUCE6 large subunit ribosomal RNA gene, partial sequence	343	93%	3.00E-89	78.78
	7	OU594944.1	*Phaeodactylum tricornutum* isolate CCAP 1055/1 genome assembly, chromosome: 3	686	93%	3.00E-89	78.78
	8	EF553459.1	*Nitzschia closterium* f. *minutissima* strain MACC-B228 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	343	93%	3.00E-89	78.78
	9	EF553458.1	*Phaeodactylum tricornutum* strain CCAP 1055/1 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	343	93%	3.00E-89	78.78
	10	DQ085806.1	*Phaeodactylum tricornutum* strain CCMP631 5.8S ribosomal RNA gene, partial sequence; internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	343	93%	3.00E-89	78.78
TD1-F	1	LT969748.1	*Cladophora pellucida* partial 28S rRNA gene, specimen voucher GENT:SV0093	219	40%	4.00E-52	86.5
	2	FM205038.1	*Cladophora pellucidoidea* partial 28S rRNA gene, specimen voucher GENT:CpoT	219	40%	4.00E-52	86.5
	3	FM205037.1	*Cladophora pellucida* partial 28S rRNA gene, specimen voucher L:0793562	219	40%	4.00E-52	86.5
	4	FM205036.1	*Cladophora pellucida* partial 28S rRNA gene, specimen voucher GENT:HEC 15794	219	40%	4.00E-52	86.5
	5	AM503475.1	*Cladophora sibogae* partial 28S rRNA gene, specimen voucher FL910c (F430)	217	39%	1.00E-51	86.43

Query	No.	Accession number	Description	Total score	Query cover	E-value	Identity (%)
TD1-F	6	AJ544752.1	*Cladophora sibogae* partial large subunit ribosomal RNA gene, specimen voucher F61	217	39%	1.00E-51	86.43
	7	FM205029.1	*Cladophora echinus* partial 28S rRNA gene, specimen voucher UTEX:LB 1480 (Delepine no. 60/55)	213	40%	2.00E-50	86.14
	8	MH017624.1	*Tursiocola varicopulifera* voucher WGA voucher FLManM99TurWGA23 large subunit ribosomal RNA gene, partial sequence	195	33%	7.00E-45	87.95
	9	MH017623.1	*Tursiocola varicopulifera* voucher WGA voucher FLManM99TurWGA22 large subunit ribosomal RNA gene, partial sequence	195	33%	7.00E-45	87.95
	10	MH017616.1	*Tursiocola varicopulifera* voucher WGA voucher FLManM95TurWGA2 large subunit ribosomal RNA gene, partial sequence	195	33%	7.00E-45	87.95
TD1-R	1	AM710566.1	*Cocconeis pediculus* partial 28S rRNA gene, strain AT-212.07	570	88%	1.00E-157	89.11
	2	OL780369.1	*Cocconeis euglypta* strain CBac2019019 large subunit ribosomal RNA gene, partial sequence	535	88%	3.00E-147	87.53
	3	AM710569.1	*Cocconeis placentula* partial 28S rRNA gene, strain AT-212Gel11	471	88%	3.00E-128	84.44
	4	MK585217.1	*Phaeodactylum tricornutum* strain CCMA106 5.8S ribosomal RNA gene, partial sequence; internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence	376	88%	2.00E-99	80.53
	5	OM859331.1	*Phaeodactylum tricornutum* strain OUCE6 large subunit ribosomal RNA gene, partial sequence	376	88%	2.00E-99	80.53
	6	OU594944.1	*Phaeodactylum tricornutum* isolate CCAP 1055/1 genome assembly, chromosome: 3	752	88%	2.00E-99	80.53

Query	**No.**	**Accession number**	**Description**	**Total score**	**Query cover**	**E-value**	**Identity (%)**
TD1-R	7	EF553459.1	*Nitzschia closterium* f. *minutissima* strain MACC-B228 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	376	88%	2.00E-99	80.53
	8	EF553458.1	*Phaeodactylum tricornutum* strain CCAP 1055/1 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	376	88%	2.00E-99	80.53
	9	DQ085806.1	*Phaeodactylum tricornutum* strain CCMP631 5.8S ribosomal RNA gene, partial sequence; internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	376	88%	2.00E-99	80.53
	10	DQ085805.1	*Phaeodactylum tricornutum* strain CCMP630 5.8S ribosomal RNA gene, partial sequence; internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	376	88%	2.00E-99	80.53

Table A.4 Top ten BLASTN search results of ITS amplicon sequenced by Sanger technology.

Sequence queries indicate collecting sites and sequencing directions. F indicates the direction starting from the 5’ terminal (forward primer). R indicates the direction starting from the 3’ terminal (reverse primer).

Query	No.	Accession number	Description	Total score	Query cover	E-value	Identity (%)
CKD-F	1	LS975015.1	*Cladophora* sp. T52 genomic DNA sequence contains ITS1, 5.8S rRNA gene, ITS2, strain WELT: A033729, isolate T52	405	37%	5.00E-108	90.16
	2	KU186047.1	*Cladophora* sp. ZZ-2016 voucher HUN1435 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	403	37%	2.00E-107	90.16
	3	KU186020.1	*Cladophora* sp. ZZ-2016 voucher HEN1513 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	399	37%	2.00E-106	89.84
	4	KU186018.1	*Cladophora* sp. ZZ-2016 voucher HEN1509 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	399	37%	2.00E-106	89.84
	5	KU186015.1	*Cladophora* sp. ZZ-2016 voucher HEN1504 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	399	37%	2.00E-106	89.84

Query	No.	Accession number	Description	Total score	Query cover	E-value	Identity (%)
CKD-F	6	KU186008.1	*Cladophora* sp. ZZ-2016 voucher HEN1408 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	399	37%	2.00E-106	89.84
	7	KU186007.1	*Cladophora* sp. ZZ-2016 voucher HEN1406 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1 and 5.8S ribosomal RNA gene, complete sequence; and internal transcribed spacer 2, partial sequence	394	37%	1.00E-104	89.74
	8	KC914578.1	*Cladophora glomerat*a strain HB1211 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	394	32%	1.00E-104	92.45
	9	KU186017.1	*Cladophora* sp. ZZ-2016 voucher HEN1508 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	388	37%	5.00E-103	89.21
	10	LS975015.1	*Cladophora* sp. T52 genomic DNA sequence contains ITS1, 5.8S rRNA gene, ITS2, strain WELT: A033729, isolate T52	405	37%	5.00E-108	90.16
CKD-R	1	KU186015.1	*Cladophora* sp. ZZ-2016 voucher HEN1504 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	106	12%	4.00E-18	93.24

Query	**No.**	**Accession number**	**Description**	**Total score**	**Query cover**	**E-value**	**Identity (%)**
CKD-R	2	LC482135.1	*Cladophora glomerata* sp3-8 genes for 18S rRNA, ITS1, 5.8S rRNA, ITS2, 28S rRNA, partial and complete sequence	104	11%	1.00E-17	94.29
	3	LC482134.1	*Cladophora glomerata* sp3-7 genes for 18S rRNA, ITS1, 5.8S rRNA, ITS2, 28S rRNA, partial and complete sequence	104	11%	1.00E-17	94.29
	4	LC482131.1	*Cladophora glomerata* sp3-4 genes for 18S rRNA, ITS1, 5.8S rRNA, ITS2, 28S rRNA, partial and complete sequence	104	11%	1.00E-17	94.29
	5	LC482130.1	*Cladophora glomerata* sp3-3 genes for 18S rRNA, ITS1, 5.8S rRNA, ITS2, 28S rRNA, partial and complete sequence	104	11%	1.00E-17	94.29
	6	LC482129.1	*Cladophora glomerata* sp3-2 genes for 18S rRNA, ITS1, 5.8S rRNA, ITS2, 28S rRNA, partial and complete sequence	104	11%	1.00E-17	94.29
	7	LC482128.1	*Cladophora glomerata* sp3-1 genes for 18S rRNA, ITS1, 5.8S rRNA, ITS2, 28S rRNA, partial and complete sequence	104	11%	1.00E-17	94.29
	8	LS975015.1	*Cladophora* sp. T52 genomic DNA sequence contains ITS1, 5.8S rRNA gene, ITS2, strain WELT: A033729, isolate T52	104	11%	1.00E-17	94.29
	9	LC309740.1	Uncultured fungus gene for ITS1, 5.8S rRNA and ITS2, partial and complete sequence, OTU: Bi-F-460	104	11%	1.00E-17	94.29
	10	KU186020.1	*Cladophora* sp. ZZ-2016 voucher HEN1513 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	104	11%	1.00E-17	94.29

Query	No.	Accession number	Description	Total score	Query cover	E-value	Identity (%)
PUA-F	1	KU186047.1	*Cladophora* sp. ZZ-2016 voucher HUN1435 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	442	100%	1.00E-119	92.11
	2	LS975015.1	*Cladophora* sp. T52 genomic DNA sequence contains ITS1, 5.8S rRNA gene, ITS2, strain WELT: A033729, isolate T52	438	100%	2.00E-118	91.8
	3	KU186045.1	*Cladophora* sp. ZZ-2016 voucher HUN1432 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	433	100%	8.00E-117	91.25
	4	KU186020.1	*Cladophora* sp. ZZ-2016 voucher HEN1513 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	433	100%	8.00E-117	91.48
	5	KU186018.1	*Cladophora* sp. ZZ-2016 voucher HEN1509 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	433	100%	8.00E-117	91.48
	6	KU186015.1	*Cladophora* sp. ZZ-2016 voucher HEN1504 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	433	100%	8.00E-117	91.48

Query	No.	Accession number	Description	Total score	Query cover	E-value	Identity (%)
PUA-F	7	KU186008.1	*Cladophora* sp. ZZ-2016 voucher HEN1408 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	433	100%	8.00E-117	91.48
	8	KU186055.1	*Cladophora* sp. ZZ-2016 voucher YN1303 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	431	100%	3.00E-116	91.25
	9	KU186051.1	*Cladophora* sp. ZZ-2016 voucher HUN1441 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	431	100%	3.00E-116	91.25
	10	KU185995.1	*Cladophora* sp. ZZ-2016 voucher TS1307 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	431	100%	3.00E-116	91.25
PUA-R	1	KU186061.1	*Cladophora* sp. ZZ-2016 voucher TB1461 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	374	88%	1.00E-98	79.19

Query	No.	Accession number	Description	Total score	Query cover	E-value	Identity (%)
PUA-R	2	KU186058.1	*Cladophora* sp. ZZ-2016 voucher TB1419 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1 and 5.8S ribosomal RNA gene, complete sequence; and internal transcribed spacer 2, partial sequence	370	88%	1.00E-97	79.03
	3	LC482135.1	*Cladophora glomerata* sp3-8 genes for 18S rRNA, ITS1, 5.8S rRNA, ITS2, 28S rRNA, partial and complete sequence	80.5	8%	3.00E-10	91.38
	4	LC482134.1	*Cladophora glomerata* sp3-7 genes for 18S rRNA, ITS1, 5.8S rRNA, ITS2, 28S rRNA, partial and complete sequence	80.5	8%	3.00E-10	91.38
	5	LC482131.1	*Cladophora glomerata* sp3-4 genes for 18S rRNA, ITS1, 5.8S rRNA, ITS2, 28S rRNA, partial and complete sequence	80.5	8%	3.00E-10	91.38
	6	LC482130.1	*Cladophora glomerata* sp3-3 genes for 18S rRNA, ITS1, 5.8S rRNA, ITS2, 28S rRNA, partial and complete sequence	80.5	8%	3.00E-10	91.38
	7	LC482129.1	*Cladophora glomerata* sp3-2 genes for 18S rRNA, ITS1, 5.8S rRNA, ITS2, 28S rRNA, partial and complete sequence	80.5	8%	3.00E-10	91.38
	8	LC482128.1	*Cladophora glomerata* sp3-1 genes for 18S rRNA, ITS1, 5.8S rRNA, ITS2, 28S rRNA, partial and complete sequence	80.5	8%	3.00E-10	91.38
	9	LS975015.1	*Cladophora* sp. T52 genomic DNA sequence contains ITS1, 5.8S rRNA gene, ITS2, strain WELT: A033729, isolate T52	80.5	8%	3.00E-10	91.38
	10	LC309740.1	Uncultured fungus gene for ITS1, 5.8S rRNA and ITS2, partial and complete sequence, OTU: Bi-F-460	80.5	8%	3.00E-10	91.38

Query	No.	Accession number	Description	Total score	Query cover	E-value	Identity (%)
TD1-F	1	LS975015.1	*Cladophora* sp. T52 genomic DNA sequence contains ITS1, 5.8S rRNA gene, ITS2, strain WELT: A033729, isolate T52	517	98%	2.00E-84	88.56
	2	KU186020.1	*Cladophora* sp. ZZ-2016 voucher HEN1513 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	511	98%	1.00E-82	88.19
	3	KU186018.1	*Cladophora* sp. ZZ-2016 voucher HEN1509 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	511	98%	1.00E-82	88.19
	4	KU186015.1	*Cladophora* sp. ZZ-2016 voucher HEN1504 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	511	98%	1.00E-82	88.19
	5	KU186008.1	*Cladophora* sp. ZZ-2016 voucher HEN1408 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	511	98%	1.00E-82	88.19
	6	KU186055.1	*Cladophora* sp. ZZ-2016 voucher YN1303 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	502	93%	4.00E-82	87.96

Query	No.	Accession number	Description	Total score	Query cover	E-value	Identity (%)
TD1-F	7	KU186051.1	*Cladophora* sp. ZZ-2016 voucher HUN1441 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	497	93%	4.00E-82	87.96
	8	KU186047.1	*Cladophora* sp. ZZ-2016 voucher HUN1435 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	497	98%	4.00E-82	88.19
	9	KU186045.1	*Cladophora* sp. ZZ-2016 voucher HUN1432 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	497	93%	4.00E-82	87.96
	10	LS975015.1	*Cladophora* sp. ZZ-2016 voucher TS1307 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	497	93%	4.00E-82	87.96
TD1-R	1	KU186061.1	*Cladophora* sp. ZZ-2016 voucher TB1461 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	558	99%	2.00E-154	85.97
	2	KU186058.1	*Cladophora* sp. ZZ-2016 voucher TB1419 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1 and 5.8S ribosomal RNA gene, complete sequence; and internal transcribed spacer 2, partial sequence	547	98%	5.00E-151	85.82

Query	No.	Accession number	Description	Total score	Query cover	E-value	Identity (%)
TD1-R	3	KU904777.1	*Cladophora* sp. HB1503 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	585	82%	3.00E-128	89.42
	4	KU186036.1	*Cladophora* sp. ZZ-2016 voucher HUN1414 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	585	82%	3.00E-128	89.42
	5	KU186017.1	*Cladophora* sp. ZZ-2016 voucher HEN1508 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	595	82%	3.00E-128	89.42
	6	KU186015.1	*Cladophora* sp. ZZ-2016 voucher HEN1504 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	595	82%	3.00E-128	89.42
	7	KU186011.1	*Cladophora* sp. ZZ-2016 voucher HEN1417 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	580	82%	3.00E-128	89.42
	8	KU186010.1	*Cladophora* sp. ZZ-2016 voucher HEN1416 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	589	81%	3.00E-128	89.42

Query	**No.**	**Accession number**	**Description**	**Total score**	**Query cover**	**E-value**	**Identity (%)**
TD1-R	9	KU186009.1	*Cladophora* sp. ZZ-2016 voucher HEN1411 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	580	82%	3.00E-128	89.42
	10	KU185997.1	*Cladophora* sp. ZZ-2016 voucher HB1403 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	580	82%	3.00E-128	89.42

APPENDIX B

Reference sequences used in phylogenetic estimation

Table B.1 147 reference sequences used in maximum likelihood phylogenetic estimation using 18S rDNA gene.

Accession number	**Description**	**AlgaeBase nomenclature**
Z35317.1	*C.albida* (83.3) gene for 18S ribosomal RNA	*Cladophora albida* (Nees) Kutzing, 1843
Z35421.1	*C. albida* (A85.101) gene for 18S ribosomal RNA	*Cladophora albida* (Nees) Kutzing, 1843
LC650014.1	*Cladophora albida* 1A32G gene for 18S rRNA, partial sequence	*Cladophora albida* (Nees) Kutzing, 1843
AB665583.1	*Cladophora albida* gene for 18S rRNA, partial sequence, isolate: alb-6	*Cladophora albida* (Nees) Kutzing, 1843
LT607349.1	*Cladophora albida* partial 18S rRNA gene, specimen voucher WELT: A033275, isolate H87	*Cladophora albida* (Nees) Kutzing, 1843
JQ308264.1	*Cladophora albida* voucher AST2010006 18S ribosomal RNA gene, partial sequence	*Cladophora albida* (Nees) Kutzing, 1843
JQ308267.1	*Cladophora albida* voucher AST2010012 18S ribosomal RNA gene, partial sequence	*Cladophora albida* (Nees) Kutzing, 1843
JQ308274.1	*Cladophora albida* voucher AST2010031 18S ribosomal RNA gene, partial sequence	*Cladophora albida* (Nees) Kutzing, 1843
KX281853.1	*Cladophora albida* voucher NCweed-1509 18S small subunit ribosomal RNA gene, partial sequence	*Cladophora albida* (Nees) Kutzing, 1843
KX281856.1	*Cladophora albida* voucher NCweed-1520 18S small subunit ribosomal RNA gene, partial sequence	*Cladophora albida* (Nees) Kutzing, 1843
KX281850.1	*Cladophora albida* voucher NCweed-1548 18S small subunit ribosomal RNA gene, partial sequence	*Cladophora albida* (Nees) Kutzing, 1843
AM498747.1	*Cladophora aokii* partial 18S rRNA gene, strain C ryu 1 (F222)	*Cladophora aokii* Yamada, 1925
LT607351.1	*Cladophora capensis* partial 18S rRNA gene, specimen voucher WELT: A033161, isolate A69	*Cladophora capensis* (C.Agardh) De Toni, 1889
Z35418.1	*C. catenata* (CcatHJ) gene for 18S ribosomal RNA	*Cladophora catenata* Kützing, 1843
Z35315.1	*C. coelothrix* 87.1 gene for 18S ribosomal RNA	*Cladophora coelothrix* Kützing, 1843
FR865756.1	*Cladophora coelothrix* genomic DNA containing 18S rRNA gene, culture collection CCAP 505/10	*Cladophora coelothrix* Kützing, 1843
AM498748.1	*Cladophora coelothrix* partial 18S rRNA gene, strain C coel 2 (F275)	*Cladophora coelothrix* Kützing, 1843

Accession number	**Description**	**AlgaeBase nomenclature**
AM498749.1	*Cladophora coelothrix* partial 18S rRNA gene, strain PH 568 (F135)	*Cladophora coelothrix* Kützing, 1843
LT607353.1	*Cladophora columbiana* partial 18S rRNA gene, specimen voucher WELT: OR 1949, isolate H09	*Cladophora columbiana* F.S.Collins, 1903
LS974873.1	*Cladophora compacta* partial 18S rRNA gene, specimen voucher WELT: A033698, isolate Q05	*Cladophora compacta* (K.I.Meyer) K.I.Meyer, 1976
AB807628.1	*Cladophora flexuosa* gene for 18S ribosomal RNA, partial sequence	*Cladophora flexuosa* (O.F.Müller) Kützing, 1843
LS974879.1	*Cladophora floccosa* partial 18S rRNA gene, specimen voucher WELT: A033714, isolate Q23	*Cladophora floccosa* C.Meyer, 1927
LS974887.1	*Cladophora fracta* partial 18S rRNA gene, specimen voucher WELT: A033723, isolate Q40	*Cladophora fracta* (O.F.Müller ex Vahl) Kützing, 1843
LS974889.1	*Cladophora globula* partial 18S rRNA gene, specimen voucher WELT: A033718, isolate Q27	*Cladophora globula* (C.Meyer) C.Meyer, 1976
LS974888.1	*Cladophora globulina* partial 18S rRNA gene, specimen voucher WELT: A033721, isolate Q37	*Cladophora globulina* (Kützing) Kützing, 1845
LC536843.1	*Cladophora glomerata* 1-CL-2019 gene for 18S rRNA, partial sequence	*Cladophora glomerata* (Linnaeus) Kützing, 1843
GQ337527.1	*Cladophora glomerata* 18S ribosomal RNA gene, partial sequence	*Cladophora glomerata* (Linnaeus) Kützing, 1843
AB062706.1	*Cladophora glomerata* gene for 18S ribosomal RNA, partial sequence	*Cladophora glomerata* (Linnaeus) Kützing, 1843
AB665578.1	*Cladophora glomerata* gene for 18S rRNA, partial sequence, isolate: glo-3	*Cladophora glomerata* (Linnaeus) Kützing, 1843
LT607357.1	*Cladophora glomerata* partial 18S rRNA gene, isolate D06	*Cladophora glomerata* (Linnaeus) Kützing, 1843
LS974892.1	*Cladophora glomerata* partial 18S rRNA gene, specimen voucher WELT: A033694, isolate P92	*Cladophora glomerata* (Linnaeus) Kützing, 1843
JQ071989.1	*Cladophora glomerata* strain SOR11 small subunit ribosomal RNA gene, partial sequence	*Cladophora glomerata* (Linnaeus) Kützing, 1843
JQ072003.1	*Cladophora glomerata* strain SOR13 small subunit ribosomal RNA gene, partial sequence	*Cladophora glomerata* (Linnaeus) Kützing, 1843
JQ071990.1	*Cladophora glomerata* strain SOR16 small subunit ribosomal RNA gene, partial sequence	*Cladophora glomerata* (Linnaeus) Kützing, 1843
JQ071991.1	*Cladophora glomerata* strain SOR18 small subunit ribosomal RNA gene, partial sequence	*Cladophora glomerata* (Linnaeus) Kützing, 1843
JQ071992.1	*Cladophora glomerata* strain SOR20 small subunit ribosomal RNA gene, partial sequence	*Cladophora glomerata* (Linnaeus) Kützing, 1843
JQ071993.1	*Cladophora glomerata* strain SOR39 small subunit ribosomal RNA gene, partial sequence	*Cladophora glomerata* (Linnaeus) Kützing, 1843
JQ071994.1	*Cladophora glomerata* strain SOR41 small subunit ribosomal RNA gene, partial sequence	*Cladophora glomerata* (Linnaeus) Kützing, 1843

Accession number	Description	AlgaeBase nomenclature
JQ071995.1	*Cladophora glomerata* strain SOR45 small subunit ribosomal RNA gene, partial sequence	*Cladophora glomerata* (Linnaeus) Kützing, 1843
JQ071996.1	*Cladophora glomerata* strain SOR48 small subunit ribosomal RNA gene, partial sequence	*Cladophora glomerata* (Linnaeus) Kützing, 1843
JQ071987.1	*Cladophora glomerata* strain SOR5 small subunit ribosomal RNA gene, partial sequence	*Cladophora glomerata* (Linnaeus) Kützing, 1843
JQ072004.1	*Cladophora glomerata* strain SOR50 small subunit ribosomal RNA gene, partial sequence	*Cladophora glomerata* (Linnaeus) Kützing, 1843
JQ071997.1	*Cladophora glomerata* strain SOR53 small subunit ribosomal RNA gene, partial sequence	*Cladophora glomerata* (Linnaeus) Kützing, 1843
JQ071998.1	*Cladophora glomerata* strain SOR54 small subunit ribosomal RNA gene, partial sequence	*Cladophora glomerata* (Linnaeus) Kützing, 1843
JQ071999.1	*Cladophora glomerata* strain SOR56 small subunit ribosomal RNA gene, partial sequence	*Cladophora glomerata* (Linnaeus) Kützing, 1843
JQ072000.1	*Cladophora glomerata* strain SOR57 small subunit ribosomal RNA gene, partial sequence	*Cladophora glomerata* (Linnaeus) Kützing, 1843
JQ072001.1	*Cladophora glomerata* strain SOR58 small subunit ribosomal RNA gene, partial sequence	*Cladophora glomerata* (Linnaeus) Kützing, 1843
JQ071988.1	*Cladophora glomerata* strain SOR6 small subunit ribosomal RNA gene, partial sequence	*Cladophora glomerata* (Linnaeus) Kützing, 1843
JQ072002.1	*Cladophora glomerata* strain SOR66 small subunit ribosomal RNA gene, partial sequence	*Cladophora glomerata* (Linnaeus) Kützing, 1843
KM676884.1	*Cladophora glomerata* voucher ARS04069_00001 18S ribosomal RNA gene, partial sequence	*Cladophora glomerata* (Linnaeus) Kützing, 1843
KM676886.1	*Cladophora glomerata* voucher ARS04319_00001 18S ribosomal RNA gene, partial sequence	*Cladophora glomerata* (Linnaeus) Kützing, 1843
KM676887.1	*Cladophora glomerata* voucher ARS04369_00001 18S ribosomal RNA gene, partial sequence	*Cladophora glomerata* (Linnaeus) Kützing, 1843
KM676885.1	*Cladophora glomerata* voucher ARS04411_00001 18S ribosomal RNA gene, partial sequence	*Cladophora glomerata* (Linnaeus) Kützing, 1843
KM676991.1	*Cladophora glomerata* voucher ARS04678_00001 18S ribosomal RNA gene, partial sequence	*Cladophora glomerata* (Linnaeus) Kützing, 1843
KM676910.1	*Cladophora glomerata* voucher ARS04755_00001 18S ribosomal RNA gene, partial sequence	*Cladophora glomerata* (Linnaeus) Kützing, 1843
KM676889.1	*Cladophora glomerata* voucher ARS05449_00001 18S ribosomal RNA gene, partial sequence	*Cladophora glomerata* (Linnaeus) Kützing, 1843
KM676891.1	*Cladophora glomerata* voucher ARS05452_00001 18S ribosomal RNA gene, partial sequence	*Cladophora glomerata* (Linnaeus) Kützing, 1843
KM676911.1	*Cladophora glomerata* voucher ARS05456_00001 18S ribosomal RNA gene, partial sequence	*Cladophora glomerata* (Linnaeus) Kützing, 1843
KM676917.1	*Cladophora glomerata* voucher ARS07020_00001 18S ribosomal RNA gene, partial sequence	*Cladophora glomerata* (Linnaeus) Kützing, 1843
JN574840.1	*Cladophora gracilis* voucher AST2009001 18S ribosomal RNA gene, partial sequence	*Cladophora gracilis* Kützing, 1845

Accession number	Description	AlgaeBase nomenclature
AB807627.1	*Cladophora hutchinsiae* gene for 18S ribosomal RNA, partial sequence	*Cladophora hutchinsiae* (Dillwyn) Kützing, 1845
LT607362.1	*Cladophora hutchinsiae* partial 18S rRNA gene, specimen voucher WELT: A033249, isolate E85	*Cladophora hutchinsiae* (Dillwyn) Kützing, 1845
KX281848.1	*Cladophora hutchinsiae* voucher NCweed-1492 18S small subunit ribosomal RNA gene, partial sequence	*Cladophora hutchinsiae* (Dillwyn) Kützing, 1845
AB807629.1	*Cladophora hutchinsioides* gene for 18S ribosomal RNA, partial sequence	*Cladophora hutchinsioides* C.Hoek & Womersley, 1984
JQ308268.1	*Cladophora hutchinsioides* voucher AST2010013 18S ribosomal RNA gene, partial sequence	*Cladophora hutchinsioides* C.Hoek & Womersley, 1984
LS974895.1	*Cladophora kursanovii* partial 18S rRNA gene, specimen voucher WELT: A033674, isolate 475	*Cladophora kursanovii* Skabichevskij, 1976
AB665577.1	*Cladophora laetevirens* gene for 18S rRNA, partial sequence, isolate: lae-2	*Cladophora laetevirens* (Dillwyn) Kützing, 1843
LT607363.1	*Cladophora laetevirens* partial 18S rRNA gene, specimen voucher WELT: A033284, isolate J95	*Cladophora laetevirens* (Dillwyn) Kützing, 1843
LT607364.1	*Cladophora lehmanniana* partial 18S rRNA gene, specimen voucher WELT: A033279, isolate J33	*Cladophora lehmanniana* (Lindenberg) Kützing, 1843
Z35318.1	*C. liebetruthii* (VGA) gene for 18S ribosomal RNA	*Cladophora liebetruthii* Grunow, 1884
LS974898.1	*Cladophora meyeri* partial 18S rRNA gene, specimen voucher WELT: A033705, isolate Q12	*Cladophora meyeri* var. *gracilioir* (Meyer) Hollerbach
AB665581.1	*Cladophora oligocladoidea* gene for 18S rRNA, partial sequence, isolate: oli-3	*Cladophora oligocladoidea* C.Hoek & M.Chihara, 2000
AB665580.1	*Cladophora opaca* gene for 18S rRNA, partial sequence, isolate: opa-2	*Cladophora opaca* Sakai, 1964
JQ308265.1	*Cladophora opaca* voucher AST2010008 18S ribosomal RNA gene, partial sequence	*Cladophora opaca* Sakai, 1964
Z35422.1	*C. prolifera* (84.28) gene for 18S ribosomal RNA	*Cladophora prolifera* (Roth) Kützing, 1843
AM498750.1	*Cladophora prolifera* partial 18S rRNA gene, strain C prol 2 (F278)	*Cladophora prolifera* (Roth) Kützing, 1843
AF510145.1	*Cladophora prolifera* specimen-voucher BG-IT05-3 18S ribosomal RNA gene, partial sequence	*Cladophora prolifera* (Roth) Kützing, 1843
KX281852.1	*Cladophora prolifera* voucher NCweed-762 18S small subunit ribosomal RNA gene, partial sequence	*Cladophora prolifera* (Roth) Kützing, 1843
LS974902.1	*Cladophora pulvinata* partial 18S rRNA gene, specimen voucher WELT: A033676, isolate 627	*Cladophora pulvinata* (Meyer) Skabichevsky, 1976
LT607371.1	*Cladophora rivularis* partial 18S rRNA gene, specimen voucher WELT: A033234, isolate E20	*Cladophora rivularis* (Linnaeus) Kuntze, 1891
Z35319.1	*C. rupestris* (83.5) gene for 18S ribosomal RNA	*Cladophora rupestris* (Linnaeus) Kützing, 1843
LT607376.1	*Cladophora rupestris* partial 18S rRNA gene, specimen voucher WELT: A033257, isolate G92	*Cladophora rupestris* (Linnaeus) Kützing, 1843

Accession number	**Description**	**AlgaeBase nomenclature**
Z35320.1	*C. sericea* (85.35) gene for 18S ribosomal RNA	*Cladophora sericea* (Hudson) Kützing, 1843
LT607379.1	*Cladophora sericea* partial 18S rRNA gene, specimen voucher WELT: A033243, isolate E55	*Cladophora sericea* (Hudson) Kützing, 1843
Z35423.1	*C. socialis* (CPC2) gene for 18S ribosomal RNA	*Cladophora socialis* Kützing, 1849
AB971263.1	*Cladophora socialis* gene for 18S rRNA, partial sequence, strain: SCORL000358	*Cladophora socialis* Kützing, 1849
AM498753.1	*Cladophora socialis* partial 18S rRNA gene, strain C soc 1 (F224)	*Cladophora socialis* Kützing, 1849
AM498751.1	*Cladophora socialis* partial 18S rRNA gene, strain C soc 2 (F225)	*Cladophora socialis* Kützing, 1849
AM498752.1	*Cladophora socialis* partial 18S rRNA gene, strain Wysor 233 (F200)	*Cladophora socialis* Kützing, 1849
JQ308270.1	*Cladophora stimpsonii* voucher AST2010019 18S ribosomal RNA gene, partial sequence	*Cladophora stimpsonii* Harvey, 1860
KX281854.1	*Cladophora subtilissima* voucher NCweed-80 18S small subunit ribosomal RNA gene, partial sequence	*Cladophora subtilissima* R.L.Taylor & Freshwater, 2017
KX281855.1	*Cladophora subtilissima* voucher NCweed-82 18S small subunit ribosomal RNA gene, partial sequence	*Cladophora subtilissima* R.L.Taylor & Freshwater, 2017
KX281851.1	*Cladophora vadorum* voucher NCweed-1495 18S small subunit ribosomal RNA gene, partial sequence	*Cladophora vadorum* (Areschoug) Kützing, 1849
Z35316.1	*C. vagabunda* (83.17) gene for 18S ribosomal RNA	*Cladophora vagabunda* (Linnaeus) Hoek, 1963
JQ308269.1	*Cladophora fascicularis* voucher AST2010014 18S ribosomal RNA gene, partial sequence	*Cladophora vagabunda* (Linnaeus) Hoek, 1963
AB062710.1	*Cladophora vagabunda* gene for 18S ribosomal RNA, partial sequence	*Cladophora vagabunda* (Linnaeus) Hoek, 1963
AB665575.1	*Cladophora vagabunda* gene for 18S rRNA, partial sequence, isolate: vag-3	*Cladophora vagabunda* (Linnaeus) Hoek, 1963
LT607381.1	*Cladophora vagabunda* partial 18S rRNA gene, specimen voucher WELT: A033246, isolate E75	*Cladophora vagabunda* (Linnaeus) Hoek, 1963
KX281845.1	*Cladophora vagabunda* voucher NCweed-1240 18S small subunit ribosomal RNA gene, partial sequence	*Cladophora vagabunda* (Linnaeus) Hoek, 1963
KX281847.1	*Cladophora vagabunda* voucher NCweed-1508 18S small subunit ribosomal RNA gene, partial sequence	*Cladophora vagabunda* (Linnaeus) Hoek, 1963
KX281849.1	*Cladophora vagabunda* voucher NCweed-1639 18S small subunit ribosomal RNA gene, partial sequence	*Cladophora vagabunda* (Linnaeus) Hoek, 1963

Accession number	Description	AlgaeBase nomenclature
KF595076.1	*Cladophora wrightiana* strain KMP201308 18S ribosomal RNA gene, partial sequence	*Cladophora wrightiana* Harvey, 1860
FM205047.1	*Cladophora battersii* partial 18S rRNA gene, specimen voucher L:0793567	*Lychaete battersii* (C.Hoek) M.J.Wynne, 2017
FM205048.1	*Cladophora dotyana* partial 18S rRNA gene, specimen voucher US:DML40094	*Lychaete dotyana* (W.J.Gilbert) M.J.Wynne, 1965
FM205049.1	*Cladophora feredayi* partial 18S rRNA gene, specimen voucher L:0793564	*Lychaete feredayi* (Harvey) M.J.Wynne, 2017
Z35419.1	*C. zollingerii* (CloZ) gene for 18S ribosomal RNA	*Lychaete herpestica* (Montagne) M.J.Wynne, 2017
LT607360.1	*Cladophora herpestica* partial 18S rRNA gene, specimen voucher WELT: A033291, isolate P06	*Lychaete herpestica* (Montagne) M.J.Wynne, 2017
AB062707.1	*Cladophora japonica* gene for 18S ribosomal RNA, partial sequence	*Lychaete japonica* (Yamada) M.J.Wynne, 2017
FM205050.1	*Cladophora mirabilis* partial 18S rRNA gene, specimen voucher L:0793561	*Lychaete mirabilis* (C.Agardh) J.Agardh, 1846
AB062708.1	*Cladophora ohkuboana* gene for 18S ribosomal RNA, partial sequence	*Lychaete ohkuboana* (Holmes) M.J.Wynne, 2017
LT607367.1	*Cladophora ohkuboana* partial 18S rRNA gene, specimen voucher GENT: DHO151, isolate F172	*Lychaete ohkuboana* (Holmes) M.J.Wynne, 2017
Z35314.1	*C. pellucida* (84.23) gene for 18S ribosomal RNA	*Lychaete pellucida* (Hudson) M.J.Wynne
LT607370.1	*Cladophora pellucida* partial 18S rRNA gene, specimen voucher L: 0793562, isolate A25	*Lychaete pellucida* (Hudson) M.J.Wynne
Z35424.1	*C. pellucidoidea* (85.100) gene for 18S ribosomal RNA	*Lychaete pellucidoidea* (C.Hoek) M.J.Wynne, 2017
FM205051.1	*Cladophora pygmaea* partial 18S rRNA gene, specimen voucher L:0793569	*Lychaete pygmaea* (Reinke) M.J.Wynne, 2017
FM205052.1	*Cladophora radiosa* partial 18S rRNA gene, specimen voucher L:0793566	*Lychaete radiosa* (Suhr) M.J.Wynne, 2017
AB062709.1	*Cladophora sakaii* gene for 18S ribosomal RNA, partial sequence	*Lychaete sakaii* (I.A.Abbott) M.J.Wynne, 2017
LT607365.1	*Cladophora montagneana* partial 18S rRNA gene, specimen voucher WELT: A033289, isolate N80	*Willeella brachyclados* (Montagne) M.J.Wynne, 2016
Z35420.1	*C.* sp. (WC) gene for 18S ribosomal RNA	-
KU904710.1	*Cladophora* sp. CQ1410 18S ribosomal RNA gene, partial sequence	-
LT607385.1	*Cladophora* sp. D23 partial 18S rRNA gene, isolate D23	-

Accession number	Description	AlgaeBase nomenclature
LT607387.1	*Cladophora* sp. D64 partial 18S rRNA gene, specimen voucher WELT: A033221, isolate D64	-
LT607388.1	*Cladophora* sp. D73 partial 18S rRNA gene, specimen voucher WELT: A033223, isolate D73	-
LT607386.1	*Cladophora* sp. F653 partial 18S rRNA gene, specimen voucher US: DML 61670, isolate F653	-
KU904713.1	*Cladophora* sp. FJ1301 18S ribosomal RNA gene, partial sequence	-
KC898947.1	*Cladophora* sp. HB1206 18S ribosomal RNA gene, partial sequence	-
KU904647.1	*Cladophora* sp. HB1413 18S ribosomal RNA gene, partial sequence	-
KU904653.1	*Cladophora* sp. HB1501 18S ribosomal RNA gene, partial sequence	-
KU904681.1	*Cladophora* sp. HEN1401 18S ribosomal RNA gene, partial sequence	-
KU904692.1	*Cladophora* sp. HEN1501 18S ribosomal RNA gene, partial sequence	-
KU904698.1	*Cladophora* sp. HEN1513 18S ribosomal RNA gene, partial sequence	-
KU904699.1	*Cladophora* sp. HLJ1401 18S ribosomal RNA gene, partial sequence	-
KU904656.1	*Cladophora* sp. HUN1401 18S ribosomal RNA gene, partial sequence	-
KU904722.1	*Cladophora* sp. QH1301 18S ribosomal RNA gene, partial sequence	-
KU904726.1	*Cladophora* sp. QH1401 18S ribosomal RNA gene, partial sequence	-
KU904730.1	*Cladophora* sp. QH1501 18S ribosomal RNA gene, partial sequence	-
KU904711.1	*Cladophora* sp. SD1201 18S ribosomal RNA gene, partial sequence	-
KU904712.1	*Cladophora* sp. SD1401 18S ribosomal RNA gene, partial sequence	-
KU904714.1	*Cladophora* sp. SX1501 18S ribosomal RNA gene, partial sequence	-
KU904718.1	*Cladophora* sp. TB1401 18S ribosomal RNA gene, partial sequence	-
KU904719.1	*Cladophora* sp. TB1433 18S ribosomal RNA gene, partial sequence	-
KU904720.1	*Cladophora* sp. TB1439 18S ribosomal RNA gene, partial sequence	-
KU904721.1	*Cladophora* sp. TB1461 18S ribosomal RNA gene, partial sequence	-

Accession number	Description	AlgaeBase nomenclature
KU904644.1	*Cladophora* sp. TS1306 18S ribosomal RNA gene, partial sequence	-
KU904715.1	*Cladophora* sp. YN1303 18S ribosomal RNA gene, partial sequence	-
KU904716.1	*Cladophora* sp. YN1401 18S ribosomal RNA gene, partial sequence	-
KM892872.1*	*Rhizoclonium pachydermum* strain HUN1405 18S ribosomal RNA gene, partial sequence	*Rhizoclonium pachydermum* Kjellman, 1877

* represents the outgroup.

Table B.2 56 reference sequences used in maximum likelihood phylogenetic estimation using 23S rDNA gene.

Accession number	Description	AlgaeBase nomenclature
AJ544763.1	*Cladophora capensis* partial large subunit ribosomal RNA gene, specimen voucher F80	*Cladophora capensis* (C.Agardh) De Toni, 1889
AJ544754.1	*Cladophora coelothrix* partial large subunit ribosomal RNA gene, specimen voucher F58	*Cladophora coelothrix* Kützing, 1843
AJ544753.1	*Cladophora coelothrix* partial large subunit ribosomal RNA gene, specimen voucher F62	*Cladophora coelothrix* Kützing, 1843
MT558949.1	*Cladophora compacta* voucher C.B. 170 small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence	*Cladophora compacta* (K.I.Meyer) K.I.Meyer, 1976
MT558952.1	*Cladophora compacta* voucher C.B. 247 small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence	*Cladophora compacta* (K.I.Meyer) K.I.Meyer, 1976
MT558954.1	*Cladophora compacta* voucher C.B. 248 small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence	*Cladophora compacta* (K.I.Meyer) K.I.Meyer, 1976
MT558958.1	*Cladophora compacta* voucher C.B. 323 small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence	*Cladophora compacta* (K.I.Meyer) K.I.Meyer, 1976

Accession number	**Description**	**AlgaeBase nomenclature**
MT558948.1	*Cladophora compacta* voucher C.B. 344 small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence	*Cladophora compacta* (K.I.Meyer) K.I.Meyer, 1976
MT558950.1	*Cladophora compacta* voucher C.B. 345 small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence	*Cladophora compacta* (K.I.Meyer) K.I.Meyer, 1976
MT558956.1	*Cladophora compacta* voucher C.B. 364 small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence	*Cladophora compacta* (K.I.Meyer) K.I.Meyer, 1976
MT558955.1	*Cladophora compacta* voucher C.B. 369 small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence	*Cladophora compacta* (K.I.Meyer) K.I.Meyer, 1976
MT558957.1	*Cladophora compacta* voucher C.B. 397 small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence	*Cladophora compacta* (K.I.Meyer) K.I.Meyer, 1976
MT558947.1	*Cladophora compacta* voucher C.B. 682 small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence	*Cladophora compacta* (K.I.Meyer) K.I.Meyer, 1976
MT558951.1	*Cladophora compacta* voucher C.B. 683 small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence	*Cladophora compacta* (K.I.Meyer) K.I.Meyer, 1976
MT558953.1	*Cladophora compacta* voucher C.B. 848 small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence	*Cladophora compacta* (K.I.Meyer) K.I.Meyer, 1976

Accession number	**Description**	**AlgaeBase nomenclature**
MT558962.1	*Cladophora floccosa* subsp. *floccosa* voucher C.B. 28 small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence	*Cladophora floccosa* C.Meyer, 1927
MT558961.1	*Cladophora floccosa* subsp. *floccosa* voucher C.B. 304 small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence	*Cladophora floccosa* C.Meyer, 1927
MT558960.1	*Cladophora floccosa* subsp. *floccosa* voucher C.B. 676 small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence3	*Cladophora floccosa* C.Meyer, 1927
MT558959.1	*Cladophora floccosa* voucher C.B. 301 small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence	*Cladophora floccosa* C.Meyer, 1927
MT558964.1	*Cladophora floccosa* var. *irregularis* voucher C.B. 275 small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence	*Cladophora floccosa* var. *irregularis* Skabichevskij, 1934
MT558963.1	*Cladophora floccosa* var. *irregularis* voucher C.B. 477 small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence	*Cladophora floccosa* var. *irregularis* Skabichevskij, 1934
MT558978.1	*Cladophora kursanovii* voucher C.B. 148 small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence	*Cladophora kursanovii* Skabichevskij, 1976
MT558984.1	*Cladophora kursanovii* voucher C.B. 222 small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence	*Cladophora kursanovii* Skabichevskij, 1976

Accession number	**Description**	**AlgaeBase nomenclature**
MT558979.1	*Cladophora kursanovii* voucher C.B. 297 small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence	*Cladophora kursanovii* Skabichevskij, 1976
MT558980.1	*Cladophora kursanovii* voucher C.B. 384 small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence	*Cladophora kursanovii* Skabichevskij, 1976
MT558977.1	*Cladophora kursanovii* voucher C.B. 771 small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence	*Cladophora kursanovii* Skabichevskij, 1976
MT558982.1	*Cladophora kursanovii* voucher C.B. 804 small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence	*Cladophora kursanovii* Skabichevskij, 1976
MT558981.1	*Cladophora kursanovii* voucher C.B. 826 small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence	*Cladophora kursanovii* Skabichevskij, 1976
MT558983.1	*Cladophora kursanovii* voucher C.B. 865 small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence	*Cladophora kursanovii* Skabichevskij, 1976
AJ544761.1	*Cladophora laetevirens* partial large subunit ribosomal RNA gene, specimen voucher F29	*Cladophora laetevirens* (Dillwyn) Kützing, 1843
MT558966.1	*Cladophora meyeri* var. *gracilior* voucher C.B. 286 small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence	*Cladophora meyeri* var. *gracilioir* (Meyer) Hollerbach

Accession number	**Description**	**AlgaeBase nomenclature**
MT558965.1	*Cladophora meyeri* var. *gracilior* voucher C.B. 334 small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence	*Cladophora meyeri* var. *gracilioir* (Meyer) Hollerbach
MT558967.1	*Cladophora meyeri* subsp. meyeri voucher C.B. 264 small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence	*Cladophora meyerii* Skabitschevsky, 1976
MT558985.1	*Cladophora pulvinata* voucher C.B. 212 small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence	*Cladophora pulvinata* (Meyer) Skabichevsky, 1976
AJ544764.1	*Cladophora rupestris* partial large subunit ribosomal RNA gene, specimen voucher F44	*Cladophora rupestris* (Linnaeus) Kützing, 1843
AJ544752.1	*Cladophora sibogae* partial large subunit ribosomal RNA gene, specimen voucher F61	*Cladophora sibogae* Reinbold, 1905
AJ544760.1	*Cladophora vagabunda* partial large subunit ribosomal RNA gene, specimen voucher F5	*Cladophora vagabunda* (Linnaeus) Hoek, 1963
AJ544756.1	*Cladophora dotyana* partial large subunit ribosomal RNA gene, specimen voucher F31	*Lychaete dotyana* (W.J.Gilbert) M.J.Wynne, 1965
AJ544755.1	*Cladophora dotyana* partial large subunit ribosomal RNA gene, specimen voucher F57	*Lychaete dotyana* (W.J.Gilbert) M.J.Wynne, 1965
AJ544762.1	*Cladophora montagneana* partial large subunit ribosomal RNA gene, specimen voucher F30	*Willeella brachyclados* (Montagne) M.J.Wynne, 2016
AJ544757.1	*Cladophora ordinata* partial large subunit ribosomal RNA gene, specimen voucher F8	*Willeella ordinata* Børgesen, 1930
KX421223.1	*Cladophora* sp. JC1 large subunit ribosomal RNA gene, partial sequence	-
KX421224.1	*Cladophora* sp. JC2 large subunit ribosomal RNA gene, partial sequence	-
KX421225.1	*Cladophora* sp. JC3 large subunit ribosomal RNA gene, partial sequence	-
MG021092.1	*Cladophora* sp. JIAC-WT-filamentous 2 isolate JIAC-WT-Filamentous 2 large subunit ribosomal RNA gene, partial sequence	-

Accession number	**Description**	**AlgaeBase nomenclature**
MG021094.1	*Cladophora* sp. JIAC-WT-filamentous 2 large subunit ribosomal RNA gene, partial sequence; chloroplast	-
KX421226.1	*Cladophora* sp. LC1 large subunit ribosomal RNA gene, partial sequence	-
KX421227.1	*Cladophora* sp. LC2 large subunit ribosomal RNA gene, partial sequence	-
KX421228.1	*Cladophora* sp. SQW1 large subunit ribosomal RNA gene, partial sequence	-
KX421229.1	*Cladophora* sp. SQW2 large subunit ribosomal RNA gene, partial sequence	-
KX421230.1	*Cladophora* sp. SQW3 large subunit ribosomal RNA gene, partial sequence	-
KX421231.1	*Cladophora* sp. ST1 large subunit ribosomal RNA gene, partial sequence	-
KX421232.1	*Cladophora* sp. ST2 large subunit ribosomal RNA gene, partial sequence	-
KX421233.1	*Cladophora* sp. XA1 large subunit ribosomal RNA gene, partial sequence	-
KX421234.1	*Cladophora* sp. XA2 large subunit ribosomal RNA gene, partial sequence	-
AJ544763.1	*Cladophora capensis* partial large subunit ribosomal RNA gene, specimen voucher F80	*Cladophora capensis* (C.Agardh) De Toni, 1889
AJ544754.1	*Cladophora coelothrix* partial large subunit ribosomal RNA gene, specimen voucher F58	*Cladophora coelothrix* Kützing, 1843
AJ544753.1	*Cladophora coelothrix* partial large subunit ribosomal RNA gene, specimen voucher F62	*Cladophora coelothrix* Kützing, 1843
MT558949.1	*Cladophora compacta* voucher C.B. 170 small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence	*Cladophora compacta* (K.I.Meyer) K.I.Meyer, 1976
MT558952.1	*Cladophora compacta* voucher C.B. 247 small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence	*Cladophora compacta* (K.I.Meyer) K.I.Meyer, 1976
MT558954.1	*Cladophora compacta* voucher C.B. 248 small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence	*Cladophora compacta* (K.I.Meyer) K.I.Meyer, 1976

Accession number	Description	AlgaeBase nomenclature
MT558958.1	*Cladophora compacta* voucher C.B. 323 small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence	*Cladophora compacta* (K.I.Meyer) K.I.Meyer, 1976
MT558948.1	*Cladophora compacta* voucher C.B. 344 small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence	*Cladophora compacta* (K.I.Meyer) K.I.Meyer, 1976
MG021093.1*	*Rhizoclonium* sp. JIAC-WT-filamentous 3 isolate JIAC-WT-Filamentous 3 large subunit ribosomal RNA gene, partial sequence	-

* represents the outgroup.

Table B.3 145 reference sequences used in maximum likelihood phylogenetic estimation using 28S rDNA gene.

Accession number	Description	AlgaeBase nomenclature
AM503433.1	*Cladophora albida* partial 28S rRNA gene, specimen voucher A85.101/Calb3 (F516)	*Cladophora albida* (Nees) Kutzing, 1843
LT969747.1	*Cladophora albida* partial 28S rRNA gene, specimen voucher GENT:SV0092	*Cladophora albida* (Nees) Kutzing, 1843
LT969753.1	*Cladophora albida* partial 28S rRNA gene, specimen voucher GENT:SV0148	*Cladophora albida* (Nees) Kutzing, 1843
LT607282.1	*Cladophora albida* partial 28S rRNA gene, specimen voucher WELT: A033205, isolate C84	*Cladophora albida* (Nees) Kutzing, 1843
LT607081.1	*Cladophora albida* partial 28S rRNA gene, specimen voucher WELT: A033275, isolate H87	*Cladophora albida* (Nees) Kutzing, 1843
KX281869.1	*Cladophora albida* voucher NCweed-1509 28S ribosomal RNA gene, partial sequence	*Cladophora albida* (Nees) Kutzing, 1843
KX281872.1	*Cladophora albida* voucher NCweed-1520 28S ribosomal RNA gene, partial sequence	*Cladophora albida* (Nees) Kutzing, 1843
KX281865.1	*Cladophora albida* voucher NCweed-1548 28S ribosomal RNA gene, partial sequence	*Cladophora albida* (Nees) Kutzing, 1843
AM503434.1	*Cladophora aokii* partial 28S rRNA gene, specimen voucher CryHJ/Cryu1 (F222)	*Cladophora aokii* Yamada, 1925
LT607083.1	*Cladophora capensis* partial 28S rRNA gene, specimen voucher WELT: A033161, isolate A69	*Cladophora capensis* (C.Agardh) De Toni, 1889
AM503435.1	*Cladophora catenata* partial 28S rRNA gene, specimen voucher KZN454 (F114)	*Cladophora catenata* Kützing, 1843

Accession number	**Description**	**AlgaeBase nomenclature**
AM503441.1	*Cladophora coelothrix* partial 28S rRNA gene, specimen voucher JW4448 (B89)	*Cladophora coelothrix* Kützing, 1843
LT607288.1	*Cladophora columbiana* partial 28S rRNA gene, isolate H19	*Cladophora columbiana* F.S.Collins, 1903
LT607085.1	*Cladophora columbiana* partial 28S rRNA gene, specimen voucher WELT: OR 1949, isolate H09	*Cladophora columbiana* F.S.Collins, 1903
LS974930.1	*Cladophora compacta* partial 28S rRNA gene, specimen voucher Q35, isolate Q35	*Cladophora compacta* (K.I.Meyer) K.I.Meyer, 1976
LS974926.1	*Cladophora compacta* partial 28S rRNA gene, specimen voucher WELT: A033698, isolate Q05	*Cladophora compacta* K.I.Meyer) K.I.Meyer, 1976
LS974927.1	*Cladophora compacta* partial 28S rRNA gene, specimen voucher WELT: A033699, isolate Q06	*Cladophora compacta* K.I.Meyer) K.I.Meyer, 1976
LS974928.1	*Cladophora compacta* partial 28S rRNA gene, specimen voucher WELT: A033712, isolate Q21	*Cladophora compacta* K.I.Meyer) K.I.Meyer, 1976
LS974929.1	*Cladophora compacta* partial 28S rRNA gene, specimen voucher WELT: A033719, isolate Q34	*Cladophora compacta* K.I.Meyer) K.I.Meyer, 1976
KX890318.1	*Cladophora dalmatica* voucher L4132667 28S ribosomal RNA gene, partial sequence	*Cladophora dalmatica* Kützing, 1843
AB807616.1	*Cladophora flexuosa* gene for 28S ribosomal RNA, partial sequence	*Cladophora flexuosa* (O.F.Müller) Kützing, 1843
LS974931.1	*Cladophora floccosa* partial 28S rRNA gene, specimen voucher WELT: A033717, isolate Q26	*Cladophora floccosa* C.Meyer, 1927
LS974941.1	*Cladophora fracta* partial 28S rRNA gene, specimen voucher WELT: A033723, isolate Q40	*Cladophora fracta* (O.F.Müller ex Vahl) Kützing, 1843
LT607320.1	*Cladophora fracta* var. *intricata* partial 28S rRNA gene, isolate J53	*Cladophora fracta* var. *intricata* (Lyngbye) C.Hoek, 1963
LS974943.1	*Cladophora globula* partial 28S rRNA gene, specimen voucher WELT: A033718, isolate Q27	*Cladophora globula* (C.Meyer) C.Meyer, 1976
LS974942.1	*Cladophora globulina* partial 28S rRNA gene, specimen voucher WELT: A033721, isolate Q37	*Cladophora globulina* (Kützing) Kützing, 1845
AB807613.1	*Cladophora glomerata* gene for 28S ribosomal RNA, partial sequence	*Cladophora glomerata* (Linnaeus) Kützing, 1843
LT607089.1	*Cladophora glomerata* partial 28S rRNA gene, isolate D06	*Cladophora glomerata* (Linnaeus) Kützing, 1843
LN679067.1	*Cladophora glomerata* partial 28S rRNA gene, isolate K89	*Cladophora glomerata* (Linnaeus) Kützing, 1843
LT607311.1	*Cladophora glomerata* partial 28S rRNA gene, specimen voucher WELT: A033133, isolate A15	*Cladophora glomerata* (Linnaeus) Kützing, 1843
LT607316.1	*Cladophora glomerata* partial 28S rRNA gene, specimen voucher WELT: A033237, isolate E32	*Cladophora glomerata* (Linnaeus) Kützing, 1843
LS974946.1	*Cladophora glomerata* partial 28S rRNA gene, specimen voucher WELT: A033694, isolate P92	*Cladophora glomerata* (Linnaeus) Kützing, 1843

Accession number	**Description**	**AlgaeBase nomenclature**
KM676829.1	*Cladophora glomerata* voucher ARS04151_00001 28S ribosomal RNA gene, partial sequence	*Cladophora glomerata* (Linnaeus) Kützing, 1843
AB807612.1	*Cladophora hutchinsiae* gene for 28S ribosomal RNA, partial sequence	*Cladophora hutchinsiae* (Dillwyn) Kützing, 1845
LT607094.1	*Cladophora hutchinsiae* partial 28S rRNA gene, specimen voucher WELT: A033249, isolate E85	*Cladophora hutchinsiae* (Dillwyn) Kützing, 1845
KX281863.1	*Cladophora hutchinsiae* voucher NCweed-1492 28S ribosomal RNA gene, partial sequence	*Cladophora hutchinsiae* (Dillwyn) Kützing, 1845
AB807614.1	*Cladophora hutchinsioides* gene for 28S ribosomal RNA, partial sequence	*Cladophora hutchinsioides* C.Hoek & Womersley, 1984
LS974949.1	*Cladophora kursanovii* partial 28S rRNA gene, specimen voucher WELT: A033674, isolate 475	*Cladophora kursanovii* Skabichevskij, 1976
LT607247.1	*Cladophora laetevirens* partial 28S rRNA gene, isolate A71, Lt84.37	*Cladophora laetevirens* (Dillwyn) Kützing, 1843
LT607253.1	*Cladophora laetevirens* partial 28S rRNA gene, specimen voucher GENT: HV1269, isolate F711	*Cladophora laetevirens* (Dillwyn) Kützing, 1843
FM205033.1	*Cladophora laetevirens* partial 28S rRNA gene, specimen voucher GENT:Bunker4	*Cladophora laetevirens* (Dillwyn) Kützing, 1843
FM205032.1	*Cladophora laetevirens* partial 28S rRNA gene, specimen voucher L:0793552	*Cladophora laetevirens* (Dillwyn) Kützing, 1843
LT607245.1	*Cladophora laetevirens* partial 28S rRNA gene, specimen voucher WELT: A033136, isolate A21	*Cladophora laetevirens* (Dillwyn) Kützing, 1843
LT607095.1	*Cladophora laetevirens* partial 28S rRNA gene, specimen voucher WELT: A033284, isolate J95	*Cladophora laetevirens* (Dillwyn) Kützing, 1843
KX281859.1	*Cladophora laetevirens* voucher NCweed-1507 28S ribosomal RNA gene, partial sequence	*Cladophora laetevirens* (Dillwyn) Kützing, 1843
LT607261.1	*Cladophora lehmanniana* partial 28S rRNA gene, specimen voucher BM: 000840678, isolate J14	*Cladophora lehmanniana* (Lindenberg) Kützing, 1843
LT607096.1	*Cladophora lehmanniana* partial 28S rRNA gene, specimen voucher WELT: A033279, isolate J33	*Cladophora lehmanniana* (Lindenberg) Kützing, 1843
AM503461.1	*Cladophora liebetruthii* partial 28S rRNA gene, specimen voucher KZN802 (F127)	*Cladophora liebetruthii* Grunow, 1884
KX890319.1	*Cladophora liniformis* voucher L4123464 28S ribosomal RNA gene, partial sequence	*Cladophora liniformis* Kützing, 1849
LS974952.1	*Cladophora meyeri* partial 28S rRNA gene, specimen voucher WELT: A033705, isolate Q12	*Cladophora meyeri* var. *gracilioir* (Meyer) Hollerbach
AB807615.1	*Cladophora opaca* gene for 28S ribosomal RNA, partial sequence	*Cladophora opaca* Sakai, 1964
OU375458.1	*Cladophora prolifera* partial 28S rRNA gene, specimen voucher GENT: Barbara 19545	*Cladophora prolifera* (Roth) Kützing, 1843
LT969755.1	*Cladophora prolifera* partial 28S rRNA gene, specimen voucher GENT:SV0151	*Cladophora prolifera* (Roth) Kützing, 1843
AM503466.1	*Cladophora prolifera* partial 28S rRNA gene, specimen voucher ODC519 (F106)	*Cladophora prolifera* (Roth) Kützing, 1843

Accession number	**Description**	**AlgaeBase nomenclature**
KX281867.1	*Cladophora prolifera* voucher NCweed-762 28S ribosomal RNA gene, partial sequence	*Cladophora prolifera* (Roth) Kützing, 1843
LS974955.1	*Cladophora pulvinata* partial 28S rRNA gene, specimen voucher WELT: A033716, isolate Q25	*Cladophora pulvinata* (Meyer) Skabichevsky, 1976
LT607101.1	*Cladophora rivularis* partial 28S rRNA gene, specimen voucher WELT: A033234, isolate E20	*Cladophora rivularis* (Linnaeus) Kuntze, 1891
LN679069.1	*Cladophora ruchingeri* partial 28S rRNA gene, isolate C62	*Cladophora ruchingeri* (C.Agardh) Kützing, 1845
LN679071.1	*Cladophora ruchingeri* partial 28S rRNA gene, isolate CAW_01	*Cladophora ruchingeri* (C.Agardh) Kützing, 1845
LT607276.1	*Cladophora ruchingeri* partial 28S rRNA gene, specimen voucher GENT: Cruch3 = Ru84.60, isolate F220	*Cladophora ruchingeri* (C.Agardh) Kützing, 1845
LT607105.1	*Cladophora rupestris* partial 28S rRNA gene, isolate D04	*Cladophora rupestris* (Linnaeus) Kützing, 1843
LT607224.1	*Cladophora rupestris* partial 28S rRNA gene, specimen voucher WELT: A033173, isolate B24	*Cladophora rupestris* (Linnaeus) Kützing, 1843
AM503474.1	*Cladophora sericea* partial 28S rRNA gene, specimen voucher S84.35/Cser1 (F518)	*Cladophora sericea* (Hudson) Kützing, 1843
LT607107.1	*Cladophora sericea* partial 28S rRNA gene, specimen voucher WELT: A033180, isolate B47	*Cladophora sericea* (Hudson) Kützing, 1843
LT607265.1	*Cladophora sericea* partial 28S rRNA gene, specimen voucher WELT: A033208, isolate C91	*Cladophora sericea* (Hudson) Kützing, 1843
LT607270.1	*Cladophora sericea* partial 28S rRNA gene, specimen voucher WELT: A033259, isolate G95	*Cladophora sericea* (Hudson) Kützing, 1843
KX281894.1	*Cladophora sericea* voucher FHL14-085 28S ribosomal RNA gene, partial sequence	*Cladophora sericea* (Hudson) Kützing, 1843
AM503475.1	*Cladophora sibogae* partial 28S rRNA gene, specimen voucher FL910c (F430)	*Cladophora sibogae* Reinbold, 1905
AB971264.1	*Cladophora socialis* gene for 28S rRNA, partial sequence, strain: SCORL000358	*Cladophora socialis* Kützing, 1849
AM503440.1	*Cladophora socialis* partial 28S rRNA gene, specimen voucher B. Wysor233 (F200)	*Cladophora socialis* Kützing, 1849
AM503476.1	*Cladophora socialis* partial 28S rRNA gene, specimen voucher HV523 (F173)	*Cladophora socialis* Kützing, 1849
KX281870.1	*Cladophora subtilissima* voucher NCweed-80 28S ribosomal RNA gene, partial sequence	*Cladophora subtilissima* R.L.Taylor & Freshwater, 2017
KX281866.1	*Cladophora vadorum* voucher NCweed-1495 28S ribosomal RNA gene, partial sequence	*Cladophora vadorum* (Areschoug) Kützing, 1849
LN679068.1	*Cladophora vagabunda* partial 28S rRNA gene, isolate E62	*Cladophora vagabunda* (Linnaeus) Hoek, 1963

Accession number	**Description**	**AlgaeBase nomenclature**
LT607299.1	*Cladophora vagabunda* partial 28S rRNA gene, specimen voucher GENT: LT0060, isolate Fx1218	*Cladophora vagabunda* (Linnaeus) Hoek, 1963
AM503481.1	*Cladophora vagabunda* partial 28S rRNA gene, specimen voucher V83.17/Cvaga3 (F519)	*Cladophora vagabunda* (Linnaeus) Hoek, 1963
LT607293.1	*Cladophora vagabunda* partial 28S rRNA gene, specimen voucher WELT: A033209, isolate C93	*Cladophora vagabunda* (Linnaeus) Hoek, 1963
LT607110.1	*Cladophora vagabunda* partial 28S rRNA gene, specimen voucher WELT: A033246, isolate E75	*Cladophora vagabunda* (Linnaeus) Hoek, 1963
KX281896.1	*Cladophora vagabunda* voucher FHL14-065 28S ribosomal RNA gene, partial sequence	*Cladophora vagabunda* (Linnaeus) Hoek, 1963
KX281860.1	*Cladophora vagabunda* voucher NCweed-1240 28S ribosomal RNA gene, partial sequence	*Cladophora vagabunda* (Linnaeus) Hoek, 1963
KX281864.1	*Cladophora vagabunda* voucher NCweed-1639 28S ribosomal RNA gene, partial sequence	*Cladophora vagabunda* (Linnaeus) Hoek, 1963
OU375374.1	*Cladophora wrightiana* partial 28S rRNA gene, specimen voucher GENT:FL1242	*Cladophora wrightiana* Harvey, 1860
LT904845.1	*Cladophora dotyana* partial 28S rRNA gene, specimen voucher REU:ARV473	*Lychaete dotyana* (W.J.Gilbert) M.J.Wynne, 1965
FM205027.1	*Cladophora dotyana* partial 28S rRNA gene, specimen voucher US:DML40094	*Lychaete dotyana* (W.J.Gilbert) M.J.Wynne, 1965
FM205029.1	*Cladophora echinus* partial 28S rRNA gene, specimen voucher UTEX:LB 1480 (Delepine no. 60/55)	*Lychaete echinus* (Biasoletto) M.J.Wynne, 2017
FM205030.1	*Cladophora feredayi* partial 28S rRNA gene, specimen voucher L:0793564	*Lychaete feredayi* (Harvey) M.J.Wynne, 2017
LT607136.1	*Cladophora feredayi* partial 28S rRNA gene, specimen voucher WELT: A033158, isolate A59	*Lychaete feredayi* (Harvey) M.J.Wynne, 2017
AM503460.1	*Cladophora herpestica* partial 28S rRNA gene, specimen voucher Cloz20b3SJ/Cherp3 (F517)	*Lychaete herpestica* (Montagne) M.J.Wynne, 2017
FM205031.1	*Cladophora herpestica* partial 28S rRNA gene, specimen voucher GENT:ClozC007	*Lychaete herpestica* (Montagne) M.J.Wynne, 2017
LT969750.1	*Cladophora herpestica* partial 28S rRNA gene, specimen voucher GENT:SV0137	*Lychaete herpestica* (Montagne) M.J.Wynne, 2017
LT607092.1	*Cladophora herpestica* partial 28S rRNA gene, specimen voucher WELT: A033291, isolate P06	*Lychaete herpestica* (Montagne) M.J.Wynne, 2017
FM205034.1	*Cladophora mirabilis* partial 28S rRNA gene, specimen voucher L:0793561	*Lychaete mirabilis* (C.Agardh) J.Agardh, 1846

Accession number	**Description**	**AlgaeBase nomenclature**
FM205035.1	*Cladophora ohkuboana* partial 28S rRNA gene, specimen voucher GENT:DHO151	*Lychaete ohkuboana* (Holmes) M.J.Wynne, 2017
FM205036.1	*Cladophora pellucida* partial 28S rRNA gene, specimen voucher GENT:HEC 15794	*Lychaete pellucida* (Hudson) M.J.Wynne, 2017
LT969748.1	*Cladophora pellucida* partial 28S rRNA gene, specimen voucher GENT:SV0093	*Lychaete pellucida* (Hudson) M.J.Wynne, 2017
FM205037.1	*Cladophora pellucida* partial 28S rRNA gene, specimen voucher L:0793562	*Lychaete pellucida* (Hudson) M.J.Wynne, 2017
FM205038.1	*Cladophora pellucidoidea* partial 28S rRNA gene, specimen voucher GENT:CpoT	*Lychaete pellucidoidea* (C.Hoek) M.J.Wynne, 2017
FM205039.1	*Cladophora pygmaea* partial 28S rRNA gene, specimen voucher GENT:Bunker3	*Lychaete pygmaea* (Reinke) M.J.Wynne, 2017
FM205042.1	*Cladophora radiosa* partial 28S rRNA gene, specimen voucher L:0793566	*Lychaete radiosa* (Suhr) M.J.Wynne, 2017
FM205043.1	*Cladophora rhodolithicola* partial 28S rRNA gene, specimen voucher GENT:FL1036	*Lychaete rhodolithicola* (Leliaert) M.J.Wynne, 2017
FM205046.1	*Cladophora sakaii* partial 28S rRNA gene, specimen voucher GENT:CsakCJ	*Lychaete sakaii* (I.A.Abbott) M.J.Wynne, 2017
LT607097.1	*Cladophora montagneana* partial 28S rRNA gene, specimen voucher WELT: A033289, isolate N80	*Willeella brachyclados* (Montagne) M.J.Wynne, 2016
LT607099.1	*Cladophora ordinata* partial 28S rRNA gene, specimen voucher US: DML 64271, isolate F834	*Willeella ordinata* Børgesen, 1930
AM503480.1	*Cladophora* sp. 1-F224 partial 28S rRNA gene, specimen voucher CPSCr/Csoc1 (F224)	-
LT607246.1	*Cladophora* sp. A32_B partial 28S rRNA gene, specimen voucher WELT: A033141, isolate A32_B	-
LT607280.1	*Cladophora* sp. A42 partial 28S rRNA gene, specimen voucher WELT: A033148, isolate A42	-
LT607262.1	*Cladophora* sp. A54 partial 28S rRNA gene, isolate A54	-
LT607292.1	*Cladophora* sp. B25 partial 28S rRNA gene, specimen voucher WELT: A033174, isolate B25	-
LT607321.1	*Cladophora* sp. B28 partial 28S rRNA gene, specimen voucher WELT: A033176, isolate B28	-
LT607263.1	*Cladophora* sp. B48 partial 28S rRNA gene, specimen voucher WELT: A033181, isolate B48	-
LT607275.1	*Cladophora* sp. C08 partial 28S rRNA gene, specimen voucher WELT: A033193, isolate C08	-
LT607312.1	*Cladophora* sp. C77 partial 28S rRNA gene, specimen voucher WELT: A033203, isolate C77	-
KU904754.1	*Cladophora* sp. CQ1405 28S ribosomal RNA gene, partial sequence	-

Accession number	**Description**	**AlgaeBase nomenclature**
LT607114.1	*Cladophora* sp. D23 partial 28S rRNA gene, isolate D23	-
LT607249.1	*Cladophora* sp. D43 partial 28S rRNA gene, specimen voucher WELT: A033217, isolate D43	-
LT607267.1	*Cladophora* sp. E78 partial 28S rRNA gene, specimen voucher WELT: A033247, isolate E78	-
LT607254.1	*Cladophora* sp. F719 partial 28S rRNA gene, specimen voucher GENT: Bunker4, isolate F719	-
LT607290.1	*Cladophora* sp. Fx1234 partial 28S rRNA gene, specimen voucher GENT: HV1898, isolate Fx1234	-
LT607269.1	*Cladophora* sp. G83 partial 28S rRNA gene, specimen voucher WELT: A033256, isolate G83	-
LT607256.1	*Cladophora* sp. H84,ASF537 partial 28S rRNA gene, specimen voucher WELT: A033273, isolate H84	-
KU904738.1	*Cladophora* sp. HB1425 28S ribosomal RNA gene, partial sequence	-
KU904739.1	*Cladophora* sp. HB1503 28S ribosomal RNA gene, partial sequence	-
KU904748.1	*Cladophora* sp. HEN1404 28S ribosomal RNA gene, partial sequence	-
KU904740.1	*Cladophora* sp. HUN1413 28S ribosomal RNA gene, partial sequence	-
LT607272.1	*Cladophora* sp. J36 partial 28S rRNA gene, specimen voucher GENT: FL1028, isolate J36,FL1028	-
LT607295.1	*Cladophora* sp. J84 partial 28S rRNA gene, specimen voucher WELT: A033282, isolate J84	-
LT607287.1	*Cladophora* sp. J96 partial 28S rRNA gene, specimen voucher WELT: A033285, isolate J96	-
KU904759.1	*Cladophora* sp. QH1301 28S ribosomal RNA gene, partial sequence	-
LS974958.1	*Cladophora* sp. T52 partial 28S rRNA gene, specimen voucher WELT: A033729, isolate T52	-
KU904757.1	*Cladophora* sp. TB1433 28S ribosomal RNA gene, partial sequence	-
KU904755.1	*Cladophora* sp. YN1401 28S ribosomal RNA gene, partial sequence	-
KU866507.1	*Cladophora* sp. ZZ-2016 voucher CQ1409 28S ribosomal RNA gene, partial sequence	-
KU866510.1	*Cladophora* sp. ZZ-2016 voucher FJ1301 28S ribosomal RNA gene, partial sequence	-
KU866463.1	*Cladophora* sp. ZZ-2016 voucher HB1413 28S ribosomal RNA gene, partial sequence	-

Accession number	Description	AlgaeBase nomenclature
KU866466.1	*Cladophora* sp. ZZ-2016 voucher HB1501 28S ribosomal RNA gene, partial sequence	-
KU866486.1	*Cladophora* sp. ZZ-2016 voucher HEN1401 28S ribosomal RNA gene, partial sequence	-
KU866493.1	*Cladophora* sp. ZZ-2016 voucher HEN1501 28S ribosomal RNA gene, partial sequence	-
KU866499.1	*Cladophora* sp. ZZ-2016 voucher HLJ1401 28S ribosomal RNA gene, partial sequence	-
KU866468.1	*Cladophora* sp. ZZ-2016 voucher HUN1401 28S ribosomal RNA gene, partial sequence	-
KU866514.1	*Cladophora* sp. ZZ-2016 voucher QH1401 28S ribosomal RNA gene, partial sequence	-
KU866509.1	*Cladophora* sp. ZZ-2016 voucher SD1401 28S ribosomal RNA gene, partial sequence	-
KU866511.1	*Cladophora* sp. ZZ-2016 voucher SX1501 28S ribosomal RNA gene, partial sequence	-
KU866512.1	*Cladophora* sp. ZZ-2016 voucher TB1401 28S ribosomal RNA gene, partial sequence	-
KU866462.1	*Cladophora* sp. ZZ-2016 voucher TS1306 28S ribosomal RNA gene, partial sequence	-
FM205026.1*	*Chaetomorpha aerea* partial 28S rRNA gene, specimen voucher GENT:Bunker5	*Chaetomorpha aerea* (Dillwyn) Kützing, 1849

* represents the outgroup.

Table B.4 80 reference sequences used in maximum likelihood phylogenetic estimation using ITS region.

Accession number	Description	AlgaeBase nomenclature
AB665570.1	*Cladophora albida* genes for 18S rRNA, ITS1, 5.8S rRNA, ITS2, 28S rRNA, partial and complete sequence, isolate: alb-1	*Cladophora albida* (Nees) Kutzing, 1843
JQ308250.1	*Cladophora albida* voucher AST2010006 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, complete sequence; and internal transcribed spacer 2, partial sequence	*Cladophora albida* (Nees) Kutzing, 1843
JQ308253.1	*Cladophora albida* voucher AST2010012 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, complete sequence; and internal transcribed spacer 2, partial sequence	*Cladophora albida* (Nees) Kutzing, 1843
JQ308260.1	*Cladophora albida* voucher AST2010031 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, complete sequence; and internal transcribed spacer 2, partial sequence	*Cladophora albida* (Nees) Kutzing, 1843

Accession number	Description	AlgaeBase nomenclature
KX281887.1	*Cladophora albida* voucher NCweed-1509 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1 and 5.8S ribosomal RNA gene, complete sequence; and internal transcribed spacer 2, partial sequence	*Cladophora albida* (Nees) Kutzing, 1843
KX281880.1	*Cladophora albida* voucher NCweed-1581 internal transcribed spacer 1 and 5.8S ribosomal RNA gene, partial sequence	*Cladophora albida* (Nees) Kutzing, 1843
KX281891.1	*Cladophora albida* voucher NCweed-1624 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1 and 5.8S ribosomal RNA gene, complete sequence; and internal transcribed spacer 2, partial sequence	*Cladophora albida* (Nees) Kutzing, 1843
KX281889.1	*Cladophora albida* voucher NCweed-1625 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1 and 5.8S ribosomal RNA gene, complete sequence; and internal transcribed spacer 2, partial sequence	*Cladophora albida* (Nees) Kutzing, 1843
KX281885.1	*Cladophora albida* voucher NCweed-1627 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1 and 5.8S ribosomal RNA gene, complete sequence; and internal transcribed spacer 2, partial sequence	*Cladophora albida* (Nees) Kutzing, 1843
MT558947.1	*Cladophora compacta* voucher C.B. 682 small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence	*Cladophora compacta* (K.I.Meyer) K.I.Meyer, 1976
MT558959.1	*Cladophora floccosa* voucher C.B. 301 small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence	*Cladophora floccosa* C.Meyer, 1927
LC536858.1	*Cladophora glomerata* 1-CL-2019 genes for 18S rRNA, ITS1, 5.8S rRNA, ITS2, 28S rRNA, partial and complete sequence	*Cladophora glomerata* (Linnaeus) Kützing, 1843
LC536864.1	*Cladophora glomerata* 1-UL-2019 genes for 18S rRNA, ITS1, 5.8S rRNA, ITS2, 28S rRNA, partial and complete sequence	*Cladophora glomerata* (Linnaeus) Kützing, 1843
AB665565.1	*Cladophora glomerata* genes for 18S rRNA, ITS1, 5.8S rRNA, ITS2, 28S rRNA, partial and complete sequence, isolate: glo-1	*Cladophora glomerata* (Linnaeus) Kützing, 1843

Accession number	Description	AlgaeBase nomenclature
OM478590.1	*Cladophora glomerata* isolate Iso 1 internal transcribed spacer 1 and 5.8S ribosomal RNA gene, partial sequence	*Cladophora glomerata* (Linnaeus) Kützing, 1843
LC482128.1	*Cladophora glomerata* sp3-1 genes for 18S rRNA, ITS1, 5.8S rRNA, ITS2, 28S rRNA, partial and complete sequence	*Cladophora glomerata* (Linnaeus) Kützing, 1843
KC914578.1	*Cladophora glomerata* strain HB1211 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	*Cladophora glomerata* (Linnaeus) Kützing, 1843
JN574849.1	*Cladophora gracilis* voucher AST2009001 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, complete sequence; and internal transcribed spacer 2, partial sequence	*Cladophora gracilis* Kützing, 1845
KX281879.1	*Cladophora hutchinsiae* voucher NCweed-1492 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	*Cladophora hutchinsiae* (Dillwyn) Kützing, 1845
JQ308254.1	*Cladophora hutchinsioides* voucher AST2010013 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, complete sequence; and internal transcribed spacer 2, partial sequence	*Cladophora hutchinsioides* C.Hoek & Womersley, 1984
MT558977.1	*Cladophora kursanovii* voucher C.B. 771 small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence	*Cladophora kursanovii* Skabichevskij, 1976
AB665564.1	*Cladophora laetevirens* genes for 18S rRNA, ITS1, 5.8S rRNA, ITS2, 28S rRNA, partial and complete sequence, isolate: lae-1	*Cladophora laetevirens* (Dillwyn) Kützing, 1843
MT991608.1	*Cladophora laetevirens* isolate QM19122905 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence	*Cladophora laetevirens* (Dillwyn) Kützing, 1843

Accession number	**Description**	**AlgaeBase nomenclature**
KX281875.1	*Cladophora laetevirens* voucher NCweed-1507 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	*Cladophora laetevirens* (Dillwyn) Kützing, 1843
MT558965.1	*Cladophora meyeri* var. *gracilior* voucher C.B. 334 small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence	*Cladophora meyeri* var. *gracilioir* (Meyer) Hollerbach
AB665568.1	*Cladophora oligocladoidea* genes for 18S rRNA, ITS1, 5.8S rRNA, ITS2, 28S rRNA, partial and complete sequence, isolate: oli-1	*Cladophora oligocladoidea* C.Hoek & M.Chihara, 2000
AB665567.1	*Cladophora opaca* genes for 18S rRNA, ITS1, 5.8S rRNA, ITS2, 28S rRNA, partial and complete sequence, isolate: opa-1	*Cladophora opaca* Sakai, 1964
JQ308251.1	*Cladophora opaca* voucher AST2010008 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, complete sequence; and internal transcribed spacer 2, partial sequence	*Cladophora opaca* Sakai, 1964
KX281886.1	*Cladophora prolifera* voucher NCweed-762 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1 and 5.8S ribosomal RNA gene, complete sequence; and internal transcribed spacer 2, partial sequence	*Cladophora prolifera* (Roth) Kützing, 1843
MT558985.1	*Cladophora pulvinata* voucher C.B. 212 small subunit ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and large subunit ribosomal RNA gene, partial sequence	*Cladophora pulvinata* (Meyer) Skabichevsky, 1976
MK127549.1	*Cladophora socialis* voucher G-1240 small subunit ribosomal RNA gene and internal transcribed spacer 1, partial sequence	*Cladophora socialis* Kützing, 1849
JQ308256.1	*Cladophora stimpsonii* voucher AST2010019 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, complete sequence; and internal transcribed spacer 2, partial sequence	*Cladophora stimpsonii* Harvey, 1860

Accession number	Description	AlgaeBase nomenclature
KX281888.1	*Cladophora subtilissima* voucher NCweed-80 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	*Cladophora subtilissima* R.L.Taylor & Freshwater, 2017
KX281884.1	*Cladophora vadorum* voucher NCweed-1495 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1 and 5.8S ribosomal RNA gene, complete sequence; and internal transcribed spacer 2, partial sequence	*Cladophora vadorum* (Areschoug) Kützing, 1849
JQ308258.1	*Cladophora expansa* voucher AST2010023 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, complete sequence; and internal transcribed spacer 2, partial sequence	*Cladophora vagabunda* (Linnaeus) Hoek, 1963
JQ308255.1	*Cladophora fascicularis* voucher AST2010014 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, complete sequence; and internal transcribed spacer 2, partial sequence	*Cladophora vagabunda* (Linnaeus) Hoek, 1963
AB665562.1	*Cladophora vagabunda* genes for 18S rRNA, ITS1, 5.8S rRNA, ITS2, 28S rRNA, partial and complete sequence, isolate: vag-1	*Cladophora vagabunda* (Linnaeus) Hoek, 1963
LC482124.1	*Cladophora vagabunda* sp1-1 genes for 18S rRNA, ITS1, 5.8S rRNA, ITS2, 28S rRNA, partial and complete sequence	*Cladophora vagabunda* (Linnaeus) Hoek, 1963
KX281890.1	*Cladophora vagabunda* voucher NCweed-1518 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1 and 5.8S ribosomal RNA gene, complete sequence; and internal transcribed spacer 2, partial sequence	*Cladophora vagabunda* (Linnaeus) Hoek, 1963
KX281882.1	*Cladophora vagabunda* voucher NCweed-1618 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1 and 5.8S ribosomal RNA gene, complete sequence; and internal transcribed spacer 2, partial sequence	*Cladophora vagabunda* (Linnaeus) Hoek, 1963
KF595077.1	*Cladophora wrightiana* strain KMP201308 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	*Cladophora wrightiana* Harvey, 1860
FM205054.1	*Cladophora pygmaea* ITS1, 5.8S rRNA gene, ITS2 and 28S rRNA gene (partial), specimen voucher GENT:Bunker3	*Lychaete pygmaea* (Reinke) M.J.Wynne, 2017

Accession number	**Description**	**AlgaeBase nomenclature**
FM205055.1	*Cladophora rhodolithicola* ITS1, 5.8S rRNA gene, ITS2 and 28S rRNA gene (partial), specimen voucher GENT:Bunker1	*Lychaete rhodolithicola* (Leliaert) M.J.Wynne, 2017
GU384875.1	*Cladophora amphibia* 5.8S ribosomal RNA gene, partial sequence; internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	*Wittrockiella amphibia* (Collins) C.Boedeker & G.I.Hansen, 2010
OK642357.1	*Cladophora* sp. CUH/AL/FW 124 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, complete sequence; and internal transcribed spacer 2, partial sequence	-
OK642358.1	*Cladophora* sp. CUH/AL/FW 203 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, complete sequence; and internal transcribed spacer 2, partial sequence	-
OK642146.1	*Cladophora* sp. CUH/AL/FW 340 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, complete sequence; and internal transcribed spacer 2, partial sequence	-
MT752939.1	*Cladophora* sp. CUH/AL/FW193 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, complete sequence; and internal transcribed spacer 2, partial sequence	-
MT649492.1	*Cladophora* sp. CUH/AL/FW330870 voucher CUH/AL/FW330 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, complete sequence; and internal transcribed spacer 2, partial sequence	-
OK642145.1	*Cladophora* sp. CUH/AL/MW 216 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, complete sequence; and internal transcribed spacer 2, partial sequence	-
OK642356.1	*Cladophora* sp. CUH/AL/MW 260 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, complete sequence; and internal transcribed spacer 2, partial sequence	-
KF318887.1	*Cladophora* sp. FB-2013 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	-

Accession number	Description	AlgaeBase nomenclature
KC914574.1	*Cladophora* sp. HB1206 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	-
KU904777.1	*Cladophora* sp. HB1503 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	-
KU904789.1	*Cladophora* sp. HUN1443 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1 and 5.8S ribosomal RNA gene, complete sequence; and internal transcribed spacer 2, partial sequence	-
KU865580.1	*Cladophora* sp. JGDN5 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, complete sequence; and internal transcribed spacer 2, partial sequence	-
MG018619.1	*Cladophora* sp. JIAC-WT-filamentous 2 internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence	-
KU904803.1	*Cladophora* sp. QH1301 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	-
KU904807.1	*Cladophora* sp. QH1503 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	-
KU185989.1	*Cladophora* sp. ZZ-2016 voucher CQ1405 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	-
KU185993.1	*Cladophora* sp. ZZ-2016 voucher FJ1301 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	-
KU185997.1	*Cladophora* sp. ZZ-2016 voucher HB1403 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	-

Accession number	Description	AlgaeBase nomenclature
KU186001.1	*Cladophora* sp. ZZ-2016 voucher HB1501 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	-
KU186003.1	*Cladophora* sp. ZZ-2016 voucher HEN1401 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	-
KU186013.1	*Cladophora* sp. ZZ-2016 voucher HEN1501 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	-
KU186021.1	*Cladophora* sp. ZZ-2016 voucher HLJ1401 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1 and 5.8S ribosomal RNA gene, complete sequence; and internal transcribed spacer 2, partial sequence	-
KU186029.1	*Cladophora* sp. ZZ-2016 voucher HUN1401 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	-
KU186062.1	*Cladophora* sp. ZZ-2016 voucher QH1401 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	-
KU186053.1	*Cladophora* sp. ZZ-2016 voucher SD1202 internal transcribed spacer 1, partial sequence; 5.8S ribosomal RNA gene, complete sequence; and internal transcribed spacer 2, partial sequence	-
KU186054.1	*Cladophora* sp. ZZ-2016 voucher SD1401 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1 and 5.8S ribosomal RNA gene, complete sequence; and internal transcribed spacer 2, partial sequence	-
KU185992.1	*Cladophora* sp. ZZ-2016 voucher SX1501 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	-
KU186057.1	*Cladophora* sp. ZZ-2016 voucher TB1401 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1 and 5.8S ribosomal RNA gene, complete sequence; and internal transcribed spacer 2, partial sequence	-

Accession number	**Description**	**AlgaeBase nomenclature**
KU186058.1	*Cladophora* sp. ZZ-2016 voucher TB1419 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1 and 5.8S ribosomal RNA gene, complete sequence; and internal transcribed spacer 2, partial sequence	-
KU186059.1	*Cladophora* sp. ZZ-2016 voucher TB1433 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	-
KU186060.1	*Cladophora* sp. ZZ-2016 voucher TB1439 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	-
KU186061.1	*Cladophora* sp. ZZ-2016 voucher TB1461 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	-
KU185994.1	*Cladophora* sp. ZZ-2016 voucher TS1306 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	-
KU186055.1	*Cladophora* sp. ZZ-2016 voucher YN1303 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	-
KU186056.1	*Cladophora* sp. ZZ-2016 voucher YN1402 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2, complete sequence; and 28S ribosomal RNA gene, partial sequence	-
KF160859.1*	*Rhizophydium haynaldii* voucher DAOM_BR100 18S ribosomal RNA gene, partial sequence; internal transcribed spacer 1 and 5.8S ribosomal RNA gene, complete sequence; and internal transcribed spacer 2, partial sequence	*Rhizophydium littoreum* Amon, 1984

* represents the outgroup.

APPENDIX C

Amplicon-based metagenomic analysis

Table C.1 Statistics of raw reads obtained from Illumina Miseq sequencing.

Sample name	16S amplicon		18S amplicon		ITS amplicon	
	Number of reads	**Average read length**	**Number of reads**	**Average read length**	**Number of reads**	**Average read length**
CKD1-F	75,185	250.4 bp	23,218	240.7 bp	-	-
CKD1-R	75,185	250.7 bp	23,218	240.6 bp	-	-
CKD2-F	77,429	250.7 bp	28,759	240.8 bp	-	-
CKD2-R	77,429	250.8 bp	28,759	241.0 bp	-	-
CKD3-F	78,242	250.7 bp	59,219	239.4 bp	-	-
CKD3-R	78,242	250.8 bp	59,219	239.3 bp	-	-
CKD4-F	84,794	250.8 bp	68,406	248.8 bp	-	-
CKD-R	84,794	250.9 bp	68,406	248.6 bp	-	-
PUA1-F	79,314	250.8 bp	69,795	248.2 bp	-	-
PUA1-R	79,314	250.9 bp	69,795	248.1 bp	-	-
PUA2-F	73,818	250.8 bp	74,897	248.9 bp	-	-
PUA2-R	73,818	250.9 bp	74,897	248.8 bp	-	-
PUA3-F	79,595	250.7 bp	68,000	246.9 bp	-	-
PUA3-R	79,595	250.9 bp	68,000	246.9 bp	-	-
PUA4-F	100,136	250.8 bp	67,089	246.2 bp	-	-
PUA4-R	100,136	250.9 bp	67,089	246.1 bp	-	-
TD11-F	68,853	250.8 bp	72,608	249.0 bp	55,020	233.7 bp
TD11-R	68,853	250.8 bp	72,608	249.1 bp	55,020	234.6 bp
TD12-F	66,930	250.7 bp	75,656	248.0 bp	-	-
TD12-R	66,930	250.8 bp	75,656	248.1 bp	-	-
TD13-F	78,261	250.8 bp	67,373	247.8 bp	-	-
TD13-R	78,261	250.8 bp	67,373	247.9 bp	-	-
TD14-F	73,577	250.8 bp	73,919	250.4 bp	-	-
TD14-R	73,577	250.8 bp	73,919	250.4 bp	-	-

Figure C.1 Per base sequence quality plots generated from FastQC of one replicate of 16S amplicon from site CKD.

A. The raw reads originally obtained from Illumina MiSeq at Omics Sciences and Bioinformatics Center. B. The trimmed reads obtained by using Trimmomatic v. 0.39, with parameter SLIDINGWINDOW:4:30.

Table C.2 Identified bacterial phyla and their relative abundance obtained from 16S rDNA amplicon analysis.

No.	Bacterial phyla	Relative abundance		
		CKD	PUA	TD1
1	Abditibacteriota	0.02%	0.02%	0.01%
2	Acidobacteriota	2.14%	2.01%	2.45%
3	Actinobacteriota	2.64%	3.26%	2.41%
4	Armatimonadota	0.34%	0.58%	0.40%
5	Bacteroidota	21.07%	22.50%	24.37%
6	Bdellovibrionota	0.49%	1.16%	1.51%
7	Calditrichota	0.00%	0.01%	0.00%
8	Campylobacterota	2.44%	0.49%	0.76%
9	Chloroflexi	1.40%	1.75%	1.97%
10	Cloacimonadota	0.01%	0.00%	0.02%
11	Cyanobacteria	1.65%	6.20%	4.98%
12	Deferrisomatota	0.00%	0.02%	0.00%
13	Deinococcota	0.30%	1.38%	0.94%
14	Dependentiae	0.08%	0.12%	0.06%
15	Desulfobacterota	1.25%	0.90%	1.62%
16	Elusimicrobiota	0.01%	0.01%	0.04%
17	Euryarchaeota	0.01%	0.00%	0.00%
18	FCPU426	0.00%	0.00%	0.02%
19	Fibrobacterota	1.11%	0.52%	1.64%
20	Firmicutes	10.59%	10.86%	6.67%
21	Fusobacteriota	1.08%	1.30%	0.83%
22	Gemmatimonadota	0.25%	0.26%	0.32%
23	Halobacterota	0.00%	0.01%	0.02%
24	Hydrogenedentes	0.00%	0.02%	0.02%
25	Latescibacterota	0.12%	0.07%	0.09%
26	LCP-89	0.00%	0.03%	0.04%
27	Margulisbacteria	0.00%	0.02%	0.01%
28	MBNT15	0.02%	0.02%	0.02%
29	Methylomirabilota	0.03%	0.02%	0.03%
30	Myxococcota	0.88%	1.74%	2.42%
31	NB1-j	0.04%	0.07%	0.09%
32	Nitrospinota	0.00%	0.01%	0.02%
33	Nitrospirota	0.03%	0.03%	0.01%
34	Patescibacteria	1.36%	1.57%	3.12%
35	Planctomycetota	4.80%	6.88%	4.08%

No.	Bacterial phyla	Relative abundance		
		CKD	PUA	TD1
36	Proteobacteria	40.04%	29.29%	31.77%
37	RCP2-54	0.01%	0.02%	0.00%
38	SAR324 clade (Marine group B)	0.13%	0.28%	0.20%
39	Spirochaetota	0.21%	0.41%	0.57%
40	Sumerlaeota	0.02%	0.03%	0.04%
41	Sva0485	0.02%	0.00%	0.01%
42	Synergistota	0.03%	0.00%	0.03%
43	Verrucomicrobiota	5.30%	5.93%	6.22%
44	WPS-2	0.01%	0.10%	0.08%
45	Zixibacteria	0.00%	0.01%	0.02%
	unidentified taxa	0.07%	0.05%	0.08%

Table C.3 Identified bacterial genera and their relative abundance obtained from 16S rDNA amplicon analysis.

No.	Bacterial phyla	Bacterial genera	Relative abundance		
			CKD	PUA	TD1
1	Abditibacteriota	*Abditibacterium*	0.02%	0.02%	0.04%
2	Acidobacteriota	*Aridibacter*	0.02%	0.00%	0.02%
3	Acidobacteriota	*Blastocatella*	0.03%	0.09%	0.06%
4	Acidobacteriota	*Bryobacter*	0.18%	0.24%	0.24%
5	Acidobacteriota	*Candidatus* Solibacter	0.03%	0.03%	0.05%
6	Acidobacteriota	*Geothrix*	0.12%	0.01%	0.04%
7	Acidobacteriota	*Holophaga*	0.10%	0.02%	0.20%
8	Acidobacteriota	JGI 0001001-H03	0.04%	0.02%	0.01%
9	Acidobacteriota	*Luteitalea*	0.06%	0.04%	0.04%
10	Acidobacteriota	marine group	0.01%	0.00%	0.03%
11	Acidobacteriota	*Paludibaculum*	0.19%	0.19%	0.13%
12	Acidobacteriota	RB41	0.00%	0.01%	0.03%
13	Acidobacteriota	*Stenotrophobacter*	0.03%	0.03%	0.04%
14	Acidobacteriota	Subgroup 10	0.04%	0.09%	0.02%
15	Actinobacteriota	*Actinomycetospora*	0.01%	0.00%	0.00%
16	Actinobacteriota	*Actinotalea*	0.02%	0.01%	0.00%
17	Actinobacteriota	*Agromyces*	0.02%	0.00%	0.00%
18	Actinobacteriota	*Angustibacter*	0.00%	0.00%	0.01%
19	Actinobacteriota	*Arthrobacter*	0.01%	0.00%	0.00%
20	Actinobacteriota	*Cellulomonas*	0.02%	0.02%	0.00%
21	Actinobacteriota	*Chryseoglobus*	0.01%	0.01%	0.00%

No.	Bacterial phyla	Bacterial genera	Relative abundance		
			CKD	PUA	TD1
22	Actinobacteriota	CL500-29 marine group	0.28%	0.49%	0.33%
23	Actinobacteriota	*Collinsella*	0.00%	0.00%	0.00%
24	Actinobacteriota	*Conexibacter*	0.07%	0.07%	0.03%
25	Actinobacteriota	*Demequina*	0.05%	0.04%	0.01%
26	Actinobacteriota	*Gaiella*	0.06%	0.07%	0.04%
27	Actinobacteriota	*Glutamicibacter*	0.01%	0.00%	0.00%
28	Actinobacteriota	hgcI clade	0.00%	0.02%	0.01%
29	Actinobacteriota	*Iamia*	0.05%	0.07%	0.07%
30	Actinobacteriota	*Ilumatobacter*	0.07%	0.11%	0.06%
31	Actinobacteriota	IMCC26207	0.06%	0.09%	0.03%
32	Actinobacteriota	*Kineosporia*	0.00%	0.03%	0.02%
33	Actinobacteriota	*Longivirga*	0.06%	0.06%	0.04%
34	Actinobacteriota	*Marmoricola*	0.03%	0.00%	0.01%
35	Actinobacteriota	*Micromonospora*	0.02%	0.00%	0.00%
36	Actinobacteriota	MWH-Ta3	0.00%	0.00%	0.02%
37	Actinobacteriota	*Mycobacterium*	0.07%	0.07%	0.03%
38	Actinobacteriota	*Nakamurella*	0.01%	0.00%	0.00%
39	Actinobacteriota	*Nocardioides*	0.23%	0.09%	0.06%
40	Actinobacteriota	*Paenarthrobacter*	0.02%	0.00%	0.00%
41	Actinobacteriota	*Pedococcus-Phycicoccus*	0.03%	0.00%	0.00%
42	Actinobacteriota	*Pseudarthrobacter*	0.06%	0.00%	0.00%
43	Actinobacteriota	*Pseudonocardia*	0.01%	0.00%	0.00%
44	Actinobacteriota	*Rhodococcus*	0.02%	0.00%	0.00%
45	Actinobacteriota	*Rubrobacter*	0.00%	0.00%	0.03%
46	Actinobacteriota	*Sinomonas*	0.02%	0.00%	0.00%
47	Actinobacteriota	*Solirubrobacter*	0.02%	0.00%	0.00%
48	Actinobacteriota	*Sporichthya*	0.00%	0.03%	0.02%
49	Actinobacteriota	*Streptomyces*	0.05%	0.02%	0.02%
50	Actinobacteriota	Sva0996 marine group	0.00%	0.02%	0.00%
51	Actinobacteriota	*Tetrasphaera*	0.01%	0.00%	0.00%
52	Actinobacteriota	*Yonghaparkia*	0.02%	0.02%	0.00%
53	Armatimonadota	*Armatimonas*	0.18%	0.42%	0.17%
54	Bacteroidota	[*Cytophaga*] xylanolytica group	0.41%	0.32%	0.56%
55	Bacteroidota	*Acetobacteroides*	1.18%	1.15%	0.81%
56	Bacteroidota	*Adhaeribacter*	0.02%	0.00%	0.00%
57	Bacteroidota	*Algoriphagus*	0.00%	0.00%	0.03%
58	Bacteroidota	*Alistipes*	0.07%	0.06%	0.05%

No.	Bacterial phyla	Bacterial genera	Relative abundance		
			CKD	PUA	TD1
59	Bacteroidota	*Alloprevotella*	0.04%	0.02%	0.02%
60	Bacteroidota	*Arcicella*	0.05%	0.15%	0.06%
61	Bacteroidota	*Arsenicibacter*	0.00%	0.00%	0.00%
62	Bacteroidota	*Aurantisolimonas*	0.09%	0.11%	0.14%
63	Bacteroidota	*Aureispira*	0.00%	0.02%	0.04%
64	Bacteroidota	*Bacteroides*	1.66%	0.25%	0.05%
65	Bacteroidota	Blvii28 wastewater-sludge group	0.08%	0.02%	0.05%
66	Bacteroidota	BSV13	0.07%	0.03%	0.07%
67	Bacteroidota	*Candidatus* Amoebophilus	0.02%	0.07%	0.03%
68	Bacteroidota	*Carboxylicivirga*	0.01%	0.00%	0.02%
69	Bacteroidota	*Cesiribacter*	0.00%	0.00%	0.01%
70	Bacteroidota	*Chryseobacterium*	0.41%	0.16%	0.12%
71	Bacteroidota	*Chryseolinea*	0.01%	0.00%	0.00%
72	Bacteroidota	*Cloacibacterium*	0.69%	0.44%	0.11%
73	Bacteroidota	*Crocinitomix*	0.02%	0.00%	0.01%
74	Bacteroidota	*Cytophaga*	0.15%	0.20%	0.22%
75	Bacteroidota	*Dinghuibacter*	0.16%	0.19%	0.18%
76	Bacteroidota	*Dyadobacter*	0.01%	0.00%	0.09%
77	Bacteroidota	*Dysgonomonas*	0.02%	0.04%	0.02%
78	Bacteroidota	*Edaphobaculum*	0.04%	0.06%	0.09%
79	Bacteroidota	*Emticicia*	0.49%	0.89%	0.76%
80	Bacteroidota	*Ferruginibacter*	0.14%	0.21%	0.28%
81	Bacteroidota	*Filimonas*	0.00%	0.02%	0.02%
82	Bacteroidota	*Flaviaesturariibacter*	0.02%	0.01%	0.01%
83	Bacteroidota	*Flavisolibacter*	0.03%	0.04%	0.02%
84	Bacteroidota	*Flavobacterium*	2.67%	2.56%	1.86%
85	Bacteroidota	*Flectobacillus*	0.17%	0.43%	0.15%
86	Bacteroidota	*Flexibacter*	0.02%	0.11%	0.05%
87	Bacteroidota	*Flexithrix*	0.00%	0.04%	0.13%
88	Bacteroidota	*Fluviicola*	0.16%	0.30%	0.24%
89	Bacteroidota	*Fluviimonas*	0.00%	0.00%	0.01%
90	Bacteroidota	*Haliscomenobacter*	0.19%	0.30%	0.14%
91	Bacteroidota	*Hassallia*	0.04%	0.04%	0.02%
92	Bacteroidota	*Hymenobacter*	0.01%	0.00%	0.01%
93	Bacteroidota	*Lacibacter*	0.06%	0.03%	0.03%
94	Bacteroidota	*Lacihabitans*	0.35%	0.55%	0.44%
95	Bacteroidota	*Larkinella*	0.02%	0.02%	0.02%

No.	**Bacterial phyla**	**Bacterial genera**	**Relative abundance**		
			CKD	**PUA**	**TD1**
96	Bacteroidota	*Lentimicrobium*	0.02%	0.00%	0.06%
97	Bacteroidota	*Lewinella*	0.24%	0.05%	0.13%
98	Bacteroidota	*Macellibacteroides*	0.06%	0.04%	0.00%
99	Bacteroidota	*Mangroviflexus*	0.00%	0.01%	0.04%
100	Bacteroidota	*Marinifilum*	0.01%	0.00%	0.01%
101	Bacteroidota	*Mariniradius*	0.00%	0.00%	0.02%
102	Bacteroidota	*Ohtaekwangia*	0.05%	0.08%	0.05%
103	Bacteroidota	OLB12	0.11%	0.12%	0.08%
104	Bacteroidota	OLB8	0.00%	0.00%	0.00%
105	Bacteroidota	*Paludibacter*	4.40%	5.62%	6.57%
106	Bacteroidota	*Parabacteroides*	0.02%	0.02%	0.03%
107	Bacteroidota	*Parapedobacter*	0.02%	0.01%	0.03%
108	Bacteroidota	*Parasediminibacterium*	0.01%	0.02%	0.04%
109	Bacteroidota	*Parasegetibacter*	0.00%	0.00%	0.01%
110	Bacteroidota	*Pedobacter*	0.00%	0.00%	0.09%
111	Bacteroidota	*Phaeodactylibacter*	0.06%	0.19%	0.15%
112	Bacteroidota	*Pontibacter*	0.00%	0.00%	0.02%
113	Bacteroidota	*Porphyromonas*	0.03%	0.02%	0.04%
114	Bacteroidota	*Portibacter*	0.00%	0.02%	0.02%
115	Bacteroidota	*Prevotella*	0.08%	0.03%	0.00%
116	Bacteroidota	*Prevotella* 9	0.18%	0.10%	0.04%
117	Bacteroidota	Prevotellaceae NK3B31 group	0.03%	0.00%	0.00%
118	Bacteroidota	Prevotellaceae UCG-001	0.02%	0.00%	0.00%
119	Bacteroidota	*Pseudarcicella*	0.04%	0.04%	0.04%
120	Bacteroidota	*Puia*	0.00%	0.02%	0.02%
121	Bacteroidota	*Raineya*	0.00%	0.03%	0.03%
122	Bacteroidota	Rikenellaceae RC9 gut group	0.00%	0.02%	0.00%
123	Bacteroidota	*Roseimarinus*	0.02%	0.04%	0.06%
124	Bacteroidota	*Rubidimonas*	0.01%	0.02%	0.03%
125	Bacteroidota	*Runella*	0.09%	0.10%	0.14%
126	Bacteroidota	*Rurimicrobium*	0.03%	0.00%	0.02%
127	Bacteroidota	*Sediminibacterium*	0.04%	0.07%	0.11%
128	Bacteroidota	*Segetibacter*	0.00%	0.02%	0.03%
129	Bacteroidota	*Siphonobacter*	0.00%	0.00%	0.01%
130	Bacteroidota	*Solitalea*	0.03%	0.03%	0.08%
131	Bacteroidota	*Soortia*	0.00%	0.00%	0.00%
132	Bacteroidota	*Sphingobacterium*	0.01%	0.00%	0.01%
133	Bacteroidota	*Spirosoma*	0.00%	0.00%	0.01%

No.	Bacterial phyla	Bacterial genera	Relative abundance		
			CKD	PUA	TD1
134	Bacteroidota	*Sporocytophaga*	0.00%	0.03%	0.02%
135	Bacteroidota	*Sunxiuqinia*	0.00%	0.00%	0.00%
136	Bacteroidota	*Taibaiella*	0.01%	0.00%	0.00%
137	Bacteroidota	*Terrimonas*	0.22%	0.27%	0.23%
138	Bacteroidota	*Thermoflexibacter*	0.01%	0.04%	0.03%
139	Bacteroidota	WCHB1-32	1.01%	0.42%	0.88%
140	Bacteroidota	*Williamwhitmania*	0.36%	0.17%	0.47%
141	Bdellovibrionota	*Bacteriovorax*	0.04%	0.02%	0.12%
142	Bdellovibrionota	*Bdellovibrio*	0.06%	0.28%	0.48%
143	Bdellovibrionota	*Oligoflexus*	0.00%	0.01%	0.02%
144	Bdellovibrionota	OM27 clade	0.24%	0.39%	0.47%
145	Bdellovibrionota	*Peredibacter*	0.02%	0.09%	0.07%
146	Bdellovibrionota	*Silvanigrella*	0.00%	0.03%	0.01%
147	Campylobacterota	*Arcobacter*	0.02%	0.00%	0.00%
148	Campylobacterota	*Pseudarcobacter*	1.87%	0.16%	0.08%
149	Campylobacterota	*Sulfuricurvum*	0.04%	0.02%	0.03%
150	Campylobacterota	*Sulfurimonas*	0.02%	0.00%	0.00%
151	Campylobacterota	*Sulfurospirillum*	0.32%	0.24%	0.48%
152	Campylobacterota	*Sulfurovum*	0.02%	0.00%	0.00%
153	Chloroflexi	*Anaerolinea*	0.04%	0.00%	0.00%
154	Chloroflexi	Anaerolineaceae UCG-001	0.03%	0.00%	0.00%
155	Chloroflexi	*Candidatus* Chloroploca	0.00%	0.01%	0.03%
156	Chloroflexi	*Candidatus* Chlorothrix	0.00%	0.01%	0.00%
157	Chloroflexi	*Chloroflexus*	0.01%	0.03%	0.02%
158	Chloroflexi	*Chloronema*	0.00%	0.00%	0.02%
159	Chloroflexi	*Herpetosiphon*	0.14%	0.16%	0.19%
160	Chloroflexi	*Leptolinea*	0.00%	0.00%	0.00%
161	Chloroflexi	*Litorilinea*	0.00%	0.01%	0.00%
162	Chloroflexi	*Chloroflexi* bacterium OLB13	0.03%	0.02%	0.02%
163	Chloroflexi	*Oscillochloris*	0.00%	0.02%	0.02%
164	Cyanobacteria	*Aerosakkonema* Lao26	0.00%	0.00%	0.02%
165	Cyanobacteria	*Anabaena* HBU1	0.00%	0.01%	0.01%
166	Cyanobacteria	*Anabaena* XPORK15F	0.00%	0.00%	0.00%
167	Cyanobacteria	*Aphanizomenon* NIES81	0.00%	0.03%	0.00%
168	Cyanobacteria	*Calothrix* KVSF5	0.02%	0.49%	0.15%
169	Cyanobacteria	*Calothrix* PCC-6303	0.00%	0.11%	0.02%
170	Cyanobacteria	*Calothrix* UAM 374	0.00%	0.08%	0.01%
171	Cyanobacteria	CENA518	0.01%	0.00%	0.00%

No.	**Bacterial phyla**	**Bacterial genera**	**Relative abundance**		
			CKD	**PUA**	**TD1**
172	Cyanobacteria	*Cephalothrix* SAG 75.79	0.00%	0.00%	0.02%
173	Cyanobacteria	*Chamaesiphon* PCC-7430	0.04%	0.12%	0.05%
174	Cyanobacteria	*Chroococcidiopsis* PCC 7203	0.01%	0.00%	0.00%
175	Cyanobacteria	*Chroococcidiopsis* PCC-6712	0.00%	0.01%	0.03%
176	Cyanobacteria	*Chroococcidiopsis* SAG 2023	0.00%	0.00%	0.01%
177	Cyanobacteria	*Chroococcopsis*	0.00%	0.01%	0.00%
178	Cyanobacteria	*Cyanobium* PCC-6307	0.08%	0.06%	0.06%
179	Cyanobacteria	*Cyanothece* PCC 7425	0.00%	0.07%	0.04%
180	Cyanobacteria	*Cylindrospermopsis* CRJ1	0.00%	0.03%	0.00%
181	Cyanobacteria	*Cylindrospermum* PCC-7417	0.01%	0.02%	0.00%
182	Cyanobacteria	*Fischerella* PCC-9339	0.00%	0.04%	0.01%
183	Cyanobacteria	*Geminocystis* PCC-6308	0.00%	0.00%	0.00%
184	Cyanobacteria	*Gloeotrichia* PYH6	0.00%	0.04%	0.00%
185	Cyanobacteria	*Gloeotrichia* SAG 32.84	0.00%	0.00%	0.03%
186	Cyanobacteria	LB3-76	0.00%	0.09%	0.06%
187	Cyanobacteria	*Leptolyngbya* BN43	0.01%	0.00%	0.00%
188	Cyanobacteria	*Leptolyngbya* EcFYyyy-00	0.00%	0.01%	0.00%
189	Cyanobacteria	*Leptolyngbya* PCC-6306	0.00%	0.02%	0.00%
190	Cyanobacteria	*Limnothrix*	0.02%	0.08%	0.06%
191	Cyanobacteria	*Lyngbya* PCC-7419	0.00%	0.00%	0.01%
192	Cyanobacteria	*Mastigocladopsis* PCC-10914	0.00%	0.01%	0.00%
193	Cyanobacteria	*Nostoc* PCC-7107	0.00%	0.01%	0.00%
194	Cyanobacteria	*Nostoc* PCC-73102	0.00%	0.00%	0.00%
195	Cyanobacteria	*Oscillatoria* PCC-10802	0.00%	0.00%	0.02%
196	Cyanobacteria	*Oscillatoria* SAG 1459-8	0.00%	0.01%	0.00%
197	Cyanobacteria	*Phormidium* CYN64	0.00%	0.01%	0.00%
198	Cyanobacteria	*Phyllonema* CENA325	0.00%	0.01%	0.00%
199	Cyanobacteria	*Pleurocapsa* PCC-7319	0.01%	0.04%	0.00%
200	Cyanobacteria	*Potamolinea* 1PC	0.04%	0.09%	0.03%
201	Cyanobacteria	*Pseudanabaena* NgrPSln22	0.00%	0.03%	0.01%
202	Cyanobacteria	*Pseudanabaena* PCC-7429	0.00%	0.07%	0.08%
203	Cyanobacteria	*Roseofilum* AO1-A	0.00%	0.00%	0.03%
204	Cyanobacteria	*Schizothrix* LEGE 07164	0.02%	0.08%	0.04%
205	Cyanobacteria	*Sphaerospermopsis* BCCUSP55	0.02%	0.13%	0.00%
206	Cyanobacteria	SU2 symbiont group	0.01%	0.03%	0.00%
207	Cyanobacteria	*Synechocystis* PCC-6803	0.00%	0.00%	0.02%
208	Cyanobacteria	*Trichodesmium* IMS101	0.04%	0.00%	0.23%

No.	Bacterial phyla	Bacterial genera	Relative abundance		
			CKD	PUA	TD1
209	Cyanobacteria	*Tychonema* CCAP 1459-11B	0.01%	0.09%	0.00%
210	Deferrisomatota	*Deferrisoma*	0.00%	0.02%	0.00%
211	Deinococcota	*Deinococcus*	0.24%	0.66%	0.38%
212	Deinococcota	*Meiothermus*	0.02%	0.14%	0.04%
213	Deinococcota	*Truepera*	0.02%	0.30%	0.06%
214	Desulfobacterota	[*Desulfobacterium*] catecholicum group	0.03%	0.02%	0.03%
215	Desulfobacterota	*Bilophila*	0.02%	0.00%	0.00%
216	Desulfobacterota	*Citrifermentans*	0.17%	0.05%	0.22%
217	Desulfobacterota	*Desulfatirhabdium*	0.00%	0.00%	0.00%
218	Desulfobacterota	*Desulfobulbus*	0.13%	0.06%	0.06%
219	Desulfobacterota	*Desulfomicrobium*	0.13%	0.11%	0.14%
220	Desulfobacterota	*Desulfovibrio*	0.13%	0.13%	0.20%
221	Desulfobacterota	*Desulfuromonas*	0.03%	0.04%	0.09%
222	Desulfobacterota	*Geobacter*	0.20%	0.17%	0.80%
223	Desulfobacterota	*Geothermobacter*	0.04%	0.02%	0.06%
224	Desulfobacterota	Sva0081 sediment group	0.01%	0.01%	0.00%
225	Desulfobacterota	*Syntrophobacter*	0.01%	0.00%	0.00%
226	Desulfobacterota	*Syntrophorhabdus*	0.01%	0.00%	0.00%
227	Desulfobacterota	*Syntrophus*	0.00%	0.00%	0.01%
228	Euryarchaeota	*Methanobacterium*	0.01%	0.00%	0.00%
229	Fibrobacterota	*Fibrobacter*	0.20%	0.02%	0.03%
230	Fibrobacterota	possible genus 06	0.12%	0.01%	0.07%
231	Firmicutes	[*Anaerorhabdus*] furcosa group	0.01%	0.00%	0.02%
232	Firmicutes	[*Eubacterium*] brachy group	0.06%	0.04%	0.06%
233	Firmicutes	[*Eubacterium*] hallii group	0.01%	0.00%	0.00%
234	Firmicutes	[*Eubacterium*] ventriosum group	0.01%	0.00%	0.00%
235	Firmicutes	[*Ruminococcus*] gnavus group	0.01%	0.00%	0.00%
236	Firmicutes	[*Ruminococcus*] torques group	0.03%	0.01%	0.00%
237	Firmicutes	*Acetoanaerobium*	0.01%	0.00%	0.00%
238	Firmicutes	*Acetobacterium*	0.02%	0.03%	0.06%
239	Firmicutes	*Acidaminobacter*	0.54%	0.53%	1.49%
240	Firmicutes	*Acidaminococcus*	0.03%	0.02%	0.00%
241	Firmicutes	*Agathobacter*	0.02%	0.00%	0.01%
242	Firmicutes	*Amnipila*	0.03%	0.00%	0.00%

No.	Bacterial phyla	Bacterial genera	Relative abundance		
			CKD	**PUA**	**TD1**
243	Firmicutes	*Anaerobacillus*	0.00%	0.04%	0.00%
244	Firmicutes	*Anaerobacterium*	0.00%	0.00%	0.03%
245	Firmicutes	*Anaerobium*	0.02%	0.02%	0.00%
246	Firmicutes	*Anaerocolumna*	0.01%	0.00%	0.02%
247	Firmicutes	*Anaeromusa-Anaeroarcus*	0.14%	0.13%	0.10%
248	Firmicutes	*Anaerosinus*	0.02%	0.02%	0.00%
249	Firmicutes	*Anaerospora*	0.03%	0.05%	0.00%
250	Firmicutes	*Anaerosporobacter*	0.18%	0.05%	0.02%
251	Firmicutes	*Anaerosporomusa*	0.02%	0.04%	0.06%
252	Firmicutes	*Anaerostipes*	0.01%	0.00%	0.00%
253	Firmicutes	*Anaerovorax*	0.13%	0.13%	0.35%
254	Firmicutes	*Bacillus*	0.12%	0.47%	0.09%
255	Firmicutes	*Blautia*	0.03%	0.02%	0.00%
256	Firmicutes	*Butyricicoccus*	0.00%	0.02%	0.00%
257	Firmicutes	*Butyrivibrio*	0.02%	0.00%	0.00%
258	Firmicutes	*Candidatus* Soleaferrea	0.00%	0.02%	0.02%
259	Firmicutes	Christensenellaceae R-7 group	0.08%	0.05%	0.08%
260	Firmicutes	*Chryseomicrobium*	0.00%	0.00%	0.02%
261	Firmicutes	*Clostridium sensu stricto* 1	0.34%	0.31%	0.04%
262	Firmicutes	*Clostridium sensu stricto* 10	0.10%	0.26%	0.04%
263	Firmicutes	*Clostridium sensu stricto* 11	0.05%	0.00%	0.00%
264	Firmicutes	*Clostridium sensu stricto* 12	1.77%	0.77%	0.25%
265	Firmicutes	*Clostridium sensu stricto* 13	0.19%	0.16%	0.04%
266	Firmicutes	*Clostridium sensu stricto* 14	0.06%	0.02%	0.00%
267	Firmicutes	*Clostridium sensu stricto* 16	0.08%	0.09%	0.12%
268	Firmicutes	*Clostridium sensu stricto* 2	0.02%	0.01%	0.00%
269	Firmicutes	*Clostridium sensu stricto* 3	0.07%	0.09%	0.01%
270	Firmicutes	*Clostridium sensu stricto* 4	0.00%	0.02%	0.00%
271	Firmicutes	*Clostridium sensu stricto* 5	0.04%	0.12%	0.00%
272	Firmicutes	*Clostridium sensu stricto* 8	0.09%	0.15%	0.06%
273	Firmicutes	*Clostridium sensu stricto* 9	0.07%	0.10%	0.04%
274	Firmicutes	*Colidextribacter*	0.02%	0.03%	0.02%
275	Firmicutes	*Crassaminicella*	0.00%	0.00%	0.04%
276	Firmicutes	*Defluviitaleaceae* UCG-011	0.10%	0.18%	0.32%
277	Firmicutes	*Enterococcus*	0.02%	0.02%	0.00%
278	Firmicutes	*Epulopiscium*	0.05%	0.05%	0.00%
279	Firmicutes	*Erysipelothrix*	0.29%	0.27%	0.40%

No.	Bacterial phyla	Bacterial genera	Relative abundance		
			CKD	PUA	TD1
280	Firmicutes	*Exiguobacterium*	0.20%	0.49%	0.05%
281	Firmicutes	*Faecalibacterium*	0.06%	0.02%	0.04%
282	Firmicutes	*Fictibacillus*	0.00%	0.01%	0.00%
283	Firmicutes	*Fonticella*	0.02%	0.03%	0.04%
284	Firmicutes	*Fusibacter*	0.34%	0.63%	1.42%
285	Firmicutes	*Gracilibacillus*	0.00%	0.02%	0.00%
286	Firmicutes	*Herbinix*	0.04%	0.03%	0.04%
287	Firmicutes	HN-HF0106	0.01%	0.03%	0.07%
288	Firmicutes	*Holdemania*	0.00%	0.00%	0.00%
289	Firmicutes	*Jeotgalibacillus*	0.00%	0.06%	0.00%
290	Firmicutes	*Lachnoclostridium*	0.10%	0.14%	0.09%
291	Firmicutes	*Lachnospira*	0.00%	0.02%	0.00%
292	Firmicutes	*Lachnospiraceae* AC2044 group	0.01%	0.02%	0.07%
293	Firmicutes	*Lachnospiraceae* NK4A136 group	0.05%	0.04%	0.04%
294	Firmicutes	*Lachnospiraceae* UCG-008	0.02%	0.00%	0.00%
295	Firmicutes	*Lachnospiraceae* XPB1014 group	0.01%	0.01%	0.00%
296	Firmicutes	*Lactococcus*	0.03%	0.03%	0.00%
297	Firmicutes	*Lutispora*	0.00%	0.03%	0.06%
298	Firmicutes	*Megamonas*	0.02%	0.03%	0.00%
299	Firmicutes	*Megasphaera*	0.05%	0.03%	0.03%
300	Firmicutes	*Natranaerovirga*	0.00%	0.03%	0.09%
301	Firmicutes	NK4A214 group	0.04%	0.03%	0.03%
302	Firmicutes	*Oxobacter*	0.03%	0.02%	0.00%
303	Firmicutes	*Paenibacillus*	0.00%	0.05%	0.00%
304	Firmicutes	*Paludicola*	0.00%	0.01%	0.00%
305	Firmicutes	*Papillibacter*	0.00%	0.00%	0.02%
306	Firmicutes	*Paraclostridium*	0.02%	0.02%	0.00%
307	Firmicutes	*Pelosinus*	0.66%	0.97%	0.37%
308	Firmicutes	*Phascolarctobacterium*	0.09%	0.04%	0.01%
309	Firmicutes	*Propionispira*	0.12%	0.04%	0.00%
310	Firmicutes	*Proteiniclasticum*	0.05%	0.25%	0.02%
311	Firmicutes	*Proteocatella*	0.08%	0.03%	0.00%
312	Firmicutes	*Romboutsia*	0.03%	0.04%	0.02%
313	Firmicutes	*Roseburia*	0.03%	0.02%	0.00%
314	Firmicutes	*Ruminiclostridium*	0.02%	0.09%	0.09%

No.	Bacterial phyla	Bacterial genera	Relative abundance		
			CKD	PUA	TD1
315	Firmicutes	*Ruminococcus*	0.05%	0.14%	0.06%
316	Firmicutes	*Saccharofermentans*	0.06%	0.02%	0.04%
317	Firmicutes	*Sedimentibacter*	0.01%	0.01%	0.00%
318	Firmicutes	*Sediminibacillus*	0.00%	0.05%	0.00%
319	Firmicutes	*Sporacetigenium*	0.00%	0.03%	0.00%
320	Firmicutes	*Sporomusa*	0.07%	0.20%	0.11%
321	Firmicutes	*Streptococcus*	0.01%	0.01%	0.00%
322	Firmicutes	*Succiniclasticum*	0.01%	0.00%	0.00%
323	Firmicutes	TC1	0.00%	0.01%	0.00%
324	Firmicutes	*Terrisporobacter*	0.01%	0.02%	0.00%
325	Firmicutes	*Trichococcus*	0.08%	0.09%	0.00%
326	Firmicutes	*Tumebacillus*	0.00%	0.00%	0.01%
327	Firmicutes	*Turicibacter*	0.00%	0.02%	0.00%
328	Firmicutes	*Tyzzerella*	0.00%	0.01%	0.00%
329	Firmicutes	UBA1819	0.01%	0.00%	0.00%
330	Firmicutes	UCG-002	0.04%	0.03%	0.01%
331	Firmicutes	UCG-005	0.01%	0.00%	0.00%
332	Firmicutes	*Vallitalea*	0.02%	0.07%	0.09%
333	Firmicutes	*Veillonella*	0.08%	0.03%	0.00%
334	Firmicutes	XBB1006	0.02%	0.00%	0.00%
335	Firmicutes	*Youngiibacter*	0.02%	0.03%	0.00%
336	Firmicutes	ZOR0006	0.12%	0.21%	0.00%
337	Fusobacteriota	*Cetobacterium*	0.08%	0.04%	0.02%
338	Fusobacteriota	*Fusobacterium*	0.09%	0.04%	0.00%
339	Fusobacteriota	*Hypnocyclicus*	0.59%	0.78%	0.46%
340	Gemmatimonadota	*Gemmatimonas*	0.13%	0.13%	0.12%
341	Halobacterota	*Methanosaeta*	0.00%	0.00%	0.04%
342	Halobacterota	*Methanosarcina*	0.00%	0.01%	0.00%
343	Methylomirabilota	Sh765B-TzT-35	0.02%	0.00%	0.02%
344	Myxococcota	*Anaeromyxobacter*	0.10%	0.11%	0.16%
345	Myxococcota	*Byssovorax*	0.00%	0.02%	0.02%
346	Myxococcota	*Corallococcus*	0.00%	0.00%	0.00%
347	Myxococcota	*Haliangium*	0.15%	0.30%	0.28%
348	Myxococcota	KD3-10	0.00%	0.03%	0.06%
349	Myxococcota	*Labilithrix*	0.00%	0.02%	0.02%
350	Myxococcota	*Nannocystis*	0.00%	0.04%	0.04%
351	Myxococcota	P3OB-42	0.06%	0.08%	0.12%
352	Myxococcota	*Pajaroellobacter*	0.10%	0.22%	0.29%

No.	**Bacterial phyla**	**Bacterial genera**	**Relative abundance**		
			CKD	**PUA**	**TD1**
353	Myxococcota	*Phaselicystis*	0.10%	0.20%	0.25%
354	Myxococcota	*Polyangium*	0.02%	0.07%	0.10%
355	Myxococcota	*Sandaracinus*	0.02%	0.05%	0.11%
356	Myxococcota	*Sorangium*	0.01%	0.02%	0.05%
357	Nitrospirota	*Nitrospira*	0.03%	0.02%	0.00%
358	Patescibacteria	*Candidatus* Saccharimonas	0.00%	0.01%	0.01%
359	Patescibacteria	TM7a	0.01%	0.00%	0.00%
360	Planctomycetota	*Aquisphaera*	0.02%	0.03%	0.01%
361	Planctomycetota	*Blastopirellula*	0.14%	0.20%	0.13%
362	Planctomycetota	*Bythopirellula*	0.00%	0.02%	0.00%
363	Planctomycetota	*Candidatus* Anammoximicrobium	0.03%	0.01%	0.02%
364	Planctomycetota	CL500-3	0.00%	0.01%	0.00%
365	Planctomycetota	*Fimbriiglobus*	0.57%	1.17%	0.47%
366	Planctomycetota	*Gemmata*	0.52%	0.70%	0.43%
367	Planctomycetota	Pir4 lineage	0.09%	0.09%	0.08%
368	Planctomycetota	*Pirellula*	0.76%	0.95%	0.70%
369	Planctomycetota	*Planctomicrobium*	0.04%	0.05%	0.00%
370	Planctomycetota	*Planctopirus*	0.02%	0.06%	0.04%
371	Planctomycetota	*Rhodopirellula*	0.03%	0.06%	0.05%
372	Planctomycetota	*Schlesneria*	0.07%	0.05%	0.05%
373	Planctomycetota	*Planctomyces* sp. SH-PL14	0.21%	0.34%	0.19%
374	Planctomycetota	*Singulisphaera*	0.00%	0.02%	0.00%
375	Planctomycetota	Planctomycetes SM1A02	0.06%	0.08%	0.03%
376	Planctomycetota	*Telmatocola*	0.07%	0.13%	0.03%
377	Planctomycetota	*Tundrisphaera*	0.02%	0.04%	0.01%
378	Planctomycetota	*Zavarzinella*	0.07%	0.15%	0.08%
379	Proteobacteria	[*Agitococcus*] lubricus group	0.00%	0.07%	0.10%
380	Proteobacteria	AAP99	0.12%	0.35%	0.28%
381	Proteobacteria	*Acidibacter*	0.06%	0.15%	0.15%
382	Proteobacteria	*Acidiphilium*	0.02%	0.02%	0.03%
383	Proteobacteria	*Acidovorax*	0.32%	0.08%	0.21%
384	Proteobacteria	*Acinetobacter*	2.65%	0.32%	0.16%
385	Proteobacteria	*Aeromonas*	5.58%	2.95%	0.57%
386	Proteobacteria	*Aestuariibacter*	0.02%	0.01%	0.00%
387	Proteobacteria	*Ahniella*	0.10%	0.09%	0.13%
388	Proteobacteria	*Albidovulum*	0.02%	0.05%	0.04%
389	Proteobacteria	*Alkanindiges*	0.02%	0.08%	0.04%

No.	Bacterial phyla	Bacterial genera	Relative abundance		
			CKD	PUA	TD1
390	Proteobacteria	*Allorhizobium-Neorhizobium-Pararhizobium-Rhizobium*	0.39%	0.12%	0.31%
391	Proteobacteria	alphaI cluster	0.16%	0.13%	0.17%
392	Proteobacteria	*Altererythrobacter*	0.09%	0.11%	0.07%
393	Proteobacteria	*Alteromonas*	0.01%	0.01%	0.00%
394	Proteobacteria	*Amphiplicatus*	0.00%	0.02%	0.00%
395	Proteobacteria	*Ancalomicrobium*	0.01%	0.00%	0.00%
396	Proteobacteria	*Anderseniella*	0.00%	0.00%	0.02%
397	Proteobacteria	*Annwodia*	0.00%	0.00%	0.01%
398	Proteobacteria	*Aquabacterium*	0.15%	0.09%	0.11%
399	Proteobacteria	*Aquaspirillum*	0.00%	0.04%	0.22%
400	Proteobacteria	*Aquicella*	0.00%	0.02%	0.02%
401	Proteobacteria	*Aquimonas*	0.00%	0.00%	0.03%
402	Proteobacteria	*Aquitalea*	0.16%	0.03%	0.06%
403	Proteobacteria	*Arenimonas*	0.11%	0.10%	0.13%
404	Proteobacteria	*Asticcacaulis*	0.01%	0.01%	0.07%
405	Proteobacteria	*Aureimonas*	0.02%	0.00%	0.00%
406	Proteobacteria	*Azoarcus*	0.18%	0.05%	0.10%
407	Proteobacteria	*Azohydromonas*	0.02%	0.00%	0.10%
408	Proteobacteria	*Azonexus*	0.04%	0.01%	0.04%
409	Proteobacteria	*Azospira*	0.31%	0.13%	0.22%
410	Proteobacteria	*Azospirillum*	0.00%	0.01%	0.01%
411	Proteobacteria	*Azovibrio*	0.05%	0.02%	0.15%
412	Proteobacteria	*Bauldia*	0.00%	0.03%	0.00%
413	Proteobacteria	BD1-7 clade	0.09%	0.07%	0.07%
414	Proteobacteria	*Beggiatoa*	0.00%	0.02%	0.08%
415	Proteobacteria	BIyi10	0.03%	0.01%	0.00%
416	Proteobacteria	*Bordetella*	0.01%	0.00%	0.00%
417	Proteobacteria	*Bosea*	0.02%	0.02%	0.01%
418	Proteobacteria	*Brachymonas*	0.00%	0.00%	0.02%
419	Proteobacteria	*Bradyrhizobium*	0.00%	0.01%	0.04%
420	Proteobacteria	*Brevundimonas*	0.03%	0.01%	0.09%
421	Proteobacteria	*Burkholderia-Caballeronia-Paraburkholderia*	0.02%	0.02%	0.03%
422	Proteobacteria	*Buttiauxella*	0.03%	0.00%	0.00%
423	Proteobacteria	C39	0.02%	0.01%	0.00%
424	Proteobacteria	*Caenimonas*	0.01%	0.02%	0.05%

No.	**Bacterial phyla**	**Bacterial genera**	**Relative abundance**		
			CKD	**PUA**	**TD1**
425	Proteobacteria	*Candidatus* Accumulibacter	0.55%	0.33%	0.74%
426	Proteobacteria	*Candidatus* Alysiosphaera	0.03%	0.05%	0.06%
427	Proteobacteria	*Candidatus* Bealeia	0.00%	0.00%	0.00%
428	Proteobacteria	*Candidatus* Berkiella	0.02%	0.02%	0.03%
429	Proteobacteria	*Candidatus* Captivus	0.00%	0.00%	0.00%
430	Proteobacteria	*Candidatus* Jidaibacter	0.00%	0.00%	0.01%
431	Proteobacteria	*Candidatus* Megaira	0.00%	0.02%	0.01%
432	Proteobacteria	*Candidatus* Nitrotoga	0.05%	0.05%	0.08%
433	Proteobacteria	*Candidatus* Ovatusbacter	0.00%	0.01%	0.00%
434	Proteobacteria	*Candidatus* Paracaedibacter	0.01%	0.01%	0.02%
435	Proteobacteria	*Candidatus* Symbiobacter	0.06%	0.04%	0.14%
436	Proteobacteria	*Candidatus* Tenderia	0.03%	0.02%	0.01%
437	Proteobacteria	*Caulobacter*	0.06%	0.05%	0.11%
438	Proteobacteria	*Cellvibrio*	0.14%	0.23%	0.33%
439	Proteobacteria	*Cereibacter*	0.00%	0.00%	0.04%
440	Proteobacteria	*Chitinibacter*	0.07%	0.15%	0.07%
441	Proteobacteria	*Chitinilyticum*	0.03%	0.04%	0.00%
442	Proteobacteria	*Chitinimonas*	0.10%	0.07%	0.13%
443	Proteobacteria	*Chitinivorax*	0.03%	0.00%	0.03%
444	Proteobacteria	*Chromobacterium*	0.03%	0.02%	0.01%
445	Proteobacteria	*Chthonobacter*	0.04%	0.04%	0.07%
446	Proteobacteria	*Citrobacter*	0.15%	0.01%	0.00%
447	Proteobacteria	CM1G08	0.02%	0.03%	0.03%
448	Proteobacteria	*Collimonas*	0.01%	0.00%	0.00%
449	Proteobacteria	*Comamonas*	0.61%	0.32%	0.37%
450	Proteobacteria	*Coxiella*	0.00%	0.06%	0.01%
451	Proteobacteria	*Craurococcus-Caldovatus*	0.02%	0.00%	0.00%
452	Proteobacteria	*Crenobacter*	0.09%	0.03%	0.01%
453	Proteobacteria	*Crenothrix*	0.03%	0.01%	0.02%
454	Proteobacteria	*Cronobacter*	0.06%	0.01%	0.00%
455	Proteobacteria	*Cupriavidus*	0.02%	0.00%	0.00%
456	Proteobacteria	*Curvibacter*	0.33%	0.13%	0.25%
457	Proteobacteria	*Dechlorobacter*	0.05%	0.03%	0.09%
458	Proteobacteria	*Dechloromonas*	1.53%	0.47%	0.57%
459	Proteobacteria	*Dechlorosoma*	0.00%	0.00%	0.03%
460	Proteobacteria	*Deefgea*	0.04%	0.03%	0.00%
461	Proteobacteria	*Defluviimonas*	0.02%	0.01%	0.00%
462	Proteobacteria	*Delftia*	0.30%	0.13%	0.10%

No.	Bacterial phyla	Bacterial genera	Relative abundance		
			CKD	PUA	TD1
463	Proteobacteria	*Denitratisoma*	0.01%	0.01%	0.02%
464	Proteobacteria	*Devosia*	0.13%	0.06%	0.11%
465	Proteobacteria	*Diaphorobacter*	0.05%	0.02%	0.08%
466	Proteobacteria	*Dongia*	0.02%	0.00%	0.03%
467	Proteobacteria	DSSD61	0.02%	0.01%	0.03%
468	Proteobacteria	*Duganella*	0.13%	0.04%	0.07%
469	Proteobacteria	Ellin6055	0.01%	0.00%	0.00%
470	Proteobacteria	Ellin6067	0.19%	0.23%	0.24%
471	Proteobacteria	*Elstera*	0.02%	0.04%	0.04%
472	Proteobacteria	*Ensifer*	0.02%	0.00%	0.02%
473	Proteobacteria	*Enterobacillus*	0.04%	0.00%	0.00%
474	Proteobacteria	*Enterobacter*	0.32%	0.03%	0.00%
475	Proteobacteria	*Erwinia*	0.02%	0.00%	0.00%
476	Proteobacteria	*Erythrobacter*	0.00%	0.01%	0.03%
477	Proteobacteria	*Escherichia-Shigella*	0.21%	0.14%	0.03%
478	Proteobacteria	*Falsirhodobacter*	0.00%	0.00%	0.01%
479	Proteobacteria	*Ferribacterium*	0.03%	0.02%	0.09%
480	Proteobacteria	*Filomicrobium*	0.02%	0.04%	0.03%
481	Proteobacteria	*Flavimaricola*	0.00%	0.00%	0.01%
482	Proteobacteria	*Fluviicoccus*	0.00%	0.00%	0.03%
483	Proteobacteria	*Formivibrio*	0.12%	0.18%	0.12%
484	Proteobacteria	FukuN57	0.00%	0.00%	0.00%
485	Proteobacteria	*Gallionella*	0.01%	0.00%	0.00%
486	Proteobacteria	*Gemmobacter*	0.14%	0.14%	0.11%
487	Proteobacteria	*Giesbergeria*	0.05%	0.04%	0.04%
488	Proteobacteria	GKS98 freshwater group	0.00%	0.00%	0.02%
489	Proteobacteria	GOUTA6	0.00%	0.00%	0.01%
490	Proteobacteria	*Gulbenkiania*	0.02%	0.02%	0.01%
491	Proteobacteria	*Haemophilus*	0.01%	0.00%	0.00%
492	Proteobacteria	*Hafnia-Obesumbacterium*	0.10%	0.00%	0.00%
493	Proteobacteria	*Halomonas*	0.02%	0.02%	0.00%
494	Proteobacteria	*Herbaspirillum*	0.08%	0.04%	0.04%
495	Proteobacteria	*Herminiimonas*	0.01%	0.00%	0.01%
496	Proteobacteria	*Hirschia*	0.07%	0.04%	0.06%
497	Proteobacteria	*Hydrogenophaga*	0.45%	0.74%	1.36%
498	Proteobacteria	*Hyphomicrobium*	0.21%	0.58%	0.34%
499	Proteobacteria	*Hyphomonas*	0.04%	0.06%	0.04%
500	Proteobacteria	*Ideonella*	0.27%	0.27%	0.49%

No.	Bacterial phyla	Bacterial genera	Relative abundance		
			CKD	PUA	TD1
501	Proteobacteria	*Inhella*	0.24%	0.20%	0.54%
502	Proteobacteria	*Iodobacter*	0.04%	0.09%	0.00%
503	Proteobacteria	IS-44	0.00%	0.01%	0.01%
504	Proteobacteria	*Jannaschia*	0.00%	0.00%	0.01%
505	Proteobacteria	*Kingella*	0.02%	0.00%	0.00%
506	Proteobacteria	*Klebsiella*	0.50%	0.09%	0.05%
507	Proteobacteria	*Kluyvera*	0.03%	0.00%	0.00%
508	Proteobacteria	*Kosakonia*	0.02%	0.00%	0.00%
509	Proteobacteria	*Labrys*	0.00%	0.03%	0.00%
510	Proteobacteria	*Lautropia*	0.08%	0.17%	0.15%
511	Proteobacteria	*Leeia*	0.05%	0.03%	0.06%
512	Proteobacteria	*Legionella*	0.06%	0.16%	0.03%
513	Proteobacteria	*Lelliottia*	0.05%	0.00%	0.00%
514	Proteobacteria	*Leptothrix*	0.24%	0.37%	0.86%
515	Proteobacteria	*Limibaculum*	0.00%	0.00%	0.03%
516	Proteobacteria	*Limnobacter*	0.04%	0.10%	0.04%
517	Proteobacteria	*Limnohabitans*	0.23%	0.24%	0.33%
518	Proteobacteria	*Luteimonas*	0.03%	0.05%	0.03%
519	Proteobacteria	*Lysobacter*	0.23%	0.20%	0.19%
520	Proteobacteria	*Macromonas*	0.02%	0.04%	0.09%
521	Proteobacteria	*Magnetospirillum*	0.00%	0.00%	0.04%
522	Proteobacteria	*Malikia*	0.13%	0.02%	0.12%
523	Proteobacteria	*Massilia*	0.48%	0.17%	0.31%
524	Proteobacteria	MD3-55	0.00%	0.00%	0.01%
525	Proteobacteria	*Mesorhizobium*	0.04%	0.04%	0.01%
526	Proteobacteria	*Methylibium*	0.02%	0.02%	0.04%
527	Proteobacteria	*Methylobacterium-Methylorubrum*	0.01%	0.00%	0.02%
528	Proteobacteria	*Methylocaldum*	0.03%	0.01%	0.00%
529	Proteobacteria	*Methylocystis*	0.05%	0.03%	0.03%
530	Proteobacteria	*Methyloglobulus*	0.05%	0.11%	0.07%
531	Proteobacteria	*Methylomagnum*	0.00%	0.00%	0.01%
532	Proteobacteria	*Methylomicrobium*	0.01%	0.00%	0.00%
533	Proteobacteria	*Methylomonas*	0.00%	0.00%	0.05%
534	Proteobacteria	*Methyloparacoccus*	0.01%	0.00%	0.00%
535	Proteobacteria	*Methylophilus*	0.03%	0.00%	0.02%
536	Proteobacteria	*Methylotenera*	0.17%	0.08%	0.08%
537	Proteobacteria	*Methyloversatilis*	0.01%	0.01%	0.02%

No.	**Bacterial phyla**	**Bacterial genera**	**Relative abundance**		
			CKD	**PUA**	**TD1**
538	Proteobacteria	*Methylovirgula*	0.00%	0.01%	0.00%
539	Proteobacteria	*Methylovulum*	0.03%	0.04%	0.04%
540	Proteobacteria	*Microvirga*	0.04%	0.01%	0.04%
541	Proteobacteria	*Microvirgula*	0.03%	0.00%	0.00%
542	Proteobacteria	*Mitsuaria*	0.05%	0.00%	0.00%
543	Proteobacteria	mle1-7	0.06%	0.10%	0.10%
544	Proteobacteria	MM1	0.02%	0.00%	0.00%
545	Proteobacteria	MND1	0.02%	0.02%	0.03%
546	Proteobacteria	*Nitratireductor*	0.03%	0.03%	0.00%
547	Proteobacteria	*Niveibacterium*	0.32%	0.24%	0.35%
548	Proteobacteria	*Niveispirillum*	0.00%	0.00%	0.01%
549	Proteobacteria	*Nordella*	0.05%	0.10%	0.05%
550	Proteobacteria	*Noviherbaspirillum*	0.06%	0.18%	0.11%
551	Proteobacteria	*Novosphingobium*	0.31%	0.19%	0.11%
552	Proteobacteria	oc32	0.13%	0.06%	0.07%
553	Proteobacteria	*Ochrobactrum*	0.02%	0.00%	0.00%
554	Proteobacteria	*Oleiphilus*	0.00%	0.00%	0.04%
555	Proteobacteria	OM43 clade	0.02%	0.01%	0.01%
556	Proteobacteria	OM60(NOR5) clade	0.08%	0.09%	0.11%
557	Proteobacteria	*Orientia*	0.01%	0.00%	0.01%
558	Proteobacteria	*Ottowia*	0.02%	0.04%	0.07%
559	Proteobacteria	*Oxalobacter*	0.00%	0.02%	0.00%
560	Proteobacteria	*Paenochrobactrum*	0.00%	0.04%	0.00%
561	Proteobacteria	*Paludibacterium*	0.02%	0.02%	0.00%
562	Proteobacteria	*Pantoea*	0.06%	0.01%	0.02%
563	Proteobacteria	*Paracoccus*	0.04%	0.03%	0.04%
564	Proteobacteria	*Parasutterella*	0.01%	0.00%	0.00%
565	Proteobacteria	*Parvibium*	0.00%	0.03%	0.00%
566	Proteobacteria	*Paucibacter*	0.35%	0.10%	0.32%
567	Proteobacteria	*Pectobacterium*	0.02%	0.00%	0.00%
568	Proteobacteria	*Pedomicrobium*	0.11%	0.20%	0.07%
569	Proteobacteria	*Pelagibacterium*	0.00%	0.00%	0.00%
570	Proteobacteria	*Pelomonas*	0.47%	0.25%	0.59%
571	Proteobacteria	*Permianibacter*	0.02%	0.00%	0.01%
572	Proteobacteria	*Phenylobacterium*	0.03%	0.02%	0.04%
573	Proteobacteria	*Phreatobacter*	0.24%	0.61%	0.67%
574	Proteobacteria	*Piscinibacter*	0.05%	0.05%	0.06%
575	Proteobacteria	*Pleomorphomonas*	0.05%	0.00%	0.04%

No.	Bacterial phyla	Bacterial genera	Relative abundance		
			CKD	PUA	TD1
576	Proteobacteria	*Plesiomonas*	0.08%	0.09%	0.04%
577	Proteobacteria	*Polaromonas*	0.03%	0.03%	0.03%
578	Proteobacteria	*Polymorphobacter*	0.02%	0.01%	0.02%
579	Proteobacteria	*Polynucleobacter*	0.00%	0.05%	0.05%
580	Proteobacteria	*Porphyrobacter*	0.04%	0.06%	0.06%
581	Proteobacteria	*Porticoccus*	0.00%	0.00%	0.02%
582	Proteobacteria	*Pragia*	0.01%	0.00%	0.00%
583	Proteobacteria	*Propionivibrio*	1.15%	0.73%	1.75%
584	Proteobacteria	*Prosthecomicrobium*	0.02%	0.00%	0.04%
585	Proteobacteria	*Pseudoduganella*	0.02%	0.00%	0.00%
586	Proteobacteria	*Pseudomonas*	1.13%	0.25%	0.33%
587	Proteobacteria	*Pseudorhodobacter*	0.12%	0.16%	0.11%
588	Proteobacteria	*Pseudoxanthomonas*	0.02%	0.00%	0.03%
589	Proteobacteria	*Psychroglaciecola*	0.01%	0.03%	0.00%
590	Proteobacteria	*Psychromonas*	0.01%	0.00%	0.00%
591	Proteobacteria	*Quatrionicoccus*	0.01%	0.00%	0.00%
592	Proteobacteria	*Ralstonia*	0.00%	0.00%	0.01%
593	Proteobacteria	*Ramlibacter*	0.03%	0.05%	0.05%
594	Proteobacteria	*Raoultella*	0.05%	0.01%	0.00%
595	Proteobacteria	*Reyranella*	0.08%	0.07%	0.05%
596	Proteobacteria	*Rheinheimera*	0.41%	0.39%	0.55%
597	Proteobacteria	*Rhizobacter*	0.23%	0.16%	0.26%
598	Proteobacteria	*Rhizorhapis*	0.00%	0.04%	0.03%
599	Proteobacteria	*Rhodobacter*	0.48%	0.58%	0.51%
600	Proteobacteria	*Rhodoferax*	0.23%	0.22%	0.36%
601	Proteobacteria	*Rhodopila*	0.01%	0.01%	0.00%
602	Proteobacteria	*Rhodoplanes*	0.03%	0.03%	0.01%
603	Proteobacteria	*Rickettsia*	0.01%	0.01%	0.00%
604	Proteobacteria	*Rickettsiella*	0.00%	0.01%	0.01%
605	Proteobacteria	*Rivibacter*	0.01%	0.00%	0.02%
606	Proteobacteria	*Rivicola*	0.02%	0.03%	0.10%
607	Proteobacteria	*Roseateles*	0.02%	0.02%	0.02%
608	Proteobacteria	*Roseitalea*	0.00%	0.00%	0.01%
609	Proteobacteria	*Roseococcus*	0.02%	0.01%	0.02%
610	Proteobacteria	*Roseomonas*	0.17%	0.15%	0.19%
611	Proteobacteria	RS62 marine group	0.00%	0.00%	0.02%
612	Proteobacteria	*Rubellimicrobium*	0.05%	0.06%	0.10%
613	Proteobacteria	*Rubritepida*	0.00%	0.00%	0.02%

No.	Bacterial phyla	Bacterial genera	Relative abundance		
			CKD	**PUA**	**TD1**
614	Proteobacteria	*Rubrivivax*	0.17%	0.18%	0.19%
615	Proteobacteria	*Salmonella*	0.14%	0.02%	0.00%
616	Proteobacteria	*Sandaracinobacter*	0.03%	0.01%	0.01%
617	Proteobacteria	*Sandarakinorhabdus*	0.02%	0.02%	0.03%
618	Proteobacteria	*Schlegelella*	0.01%	0.01%	0.01%
619	Proteobacteria	*Serratia*	0.06%	0.02%	0.00%
620	Proteobacteria	*Shewanella*	0.38%	0.14%	0.07%
621	Proteobacteria	*Shinella*	0.03%	0.04%	0.04%
622	Proteobacteria	*Sideroxydans*	0.10%	0.10%	0.19%
623	Proteobacteria	*Silanimonas*	0.06%	0.09%	0.17%
624	Proteobacteria	*Simiduia*	0.00%	0.01%	0.00%
625	Proteobacteria	*Simplicispira*	0.05%	0.01%	0.02%
626	Proteobacteria	*Sphaerotilus*	0.04%	0.04%	0.11%
627	Proteobacteria	*Sphingobium*	0.02%	0.03%	0.01%
628	Proteobacteria	*Sphingomonas*	0.18%	0.13%	0.22%
629	Proteobacteria	*Sphingopyxis*	0.12%	0.13%	0.27%
630	Proteobacteria	*Sphingorhabdus*	0.02%	0.05%	0.08%
631	Proteobacteria	*Stenotrophomonas*	0.05%	0.00%	0.00%
632	Proteobacteria	*Steroidobacter*	0.02%	0.02%	0.00%
633	Proteobacteria	*Succinivibrio*	0.01%	0.01%	0.02%
634	Proteobacteria	*Sulfuritalea*	0.06%	0.08%	0.16%
635	Proteobacteria	*Sutterella*	0.02%	0.03%	0.00%
636	Proteobacteria	SWB02	0.06%	0.10%	0.10%
637	Proteobacteria	*Tabrizicola*	0.11%	0.15%	0.13%
638	Proteobacteria	*Tahibacter*	0.02%	0.00%	0.01%
639	Proteobacteria	*Thauera*	0.17%	0.12%	0.09%
640	Proteobacteria	*Thermomonas*	0.02%	0.00%	0.03%
641	Proteobacteria	*Thioalkalispira-Sulfurivermis*	0.02%	0.00%	0.00%
642	Proteobacteria	*Thiobacillus*	0.02%	0.00%	0.03%
643	Proteobacteria	*Thioclava*	0.00%	0.02%	0.03%
644	Proteobacteria	*Thiohalobacter*	0.00%	0.01%	0.00%
645	Proteobacteria	*Thiohalophilus*	0.02%	0.01%	0.00%
646	Proteobacteria	*Thiomonas*	0.00%	0.02%	0.03%
647	Proteobacteria	*Thiothrix*	0.00%	0.00%	0.00%
648	Proteobacteria	*Tolumonas*	0.81%	0.43%	0.04%
649	Proteobacteria	*Tropicimonas*	0.02%	0.04%	0.02%
650	Proteobacteria	UKL13-1	0.00%	0.01%	0.02%

No.	Bacterial phyla	Bacterial genera	Relative abundance		
			CKD	PUA	TD1
651	Proteobacteria	*Uliginosibacterium*	0.16%	0.07%	0.11%
652	Proteobacteria	*Undibacterium*	0.36%	0.13%	0.32%
653	Proteobacteria	*Variovorax*	0.01%	0.00%	0.00%
654	Proteobacteria	*Vogesella*	1.23%	0.87%	0.25%
655	Proteobacteria	*Vulcaniibacterium*	0.00%	0.00%	0.00%
656	Proteobacteria	*Woeseia*	0.01%	0.02%	0.00%
657	Proteobacteria	*Wolbachia*	0.00%	0.00%	0.01%
658	Proteobacteria	*Xylophilus*	0.01%	0.00%	0.00%
659	Proteobacteria	Z-35	0.02%	0.04%	0.07%
660	Proteobacteria	*Zoogloea*	0.18%	0.01%	0.26%
661	Spirochaetota	*Salinispira*	0.02%	0.11%	0.22%
662	Spirochaetota	*Spirochaeta* 2	0.06%	0.17%	0.21%
663	Spirochaetota	*Treponema*	0.04%	0.03%	0.17%
664	Spirochaetota	*Turneriella*	0.01%	0.02%	0.00%
665	Sumerlaeota	*Sumerlaea*	0.02%	0.03%	0.04%
666	Verrucomicrobiota	ADurb.Bin063-1	0.11%	0.06%	0.05%
667	Verrucomicrobiota	*Akkermansia*	0.02%	0.00%	0.00%
668	Verrucomicrobiota	*Alterococcus*	0.02%	0.01%	0.06%
669	Verrucomicrobiota	*Brevifollis*	0.03%	0.06%	0.09%
670	Verrucomicrobiota	*Candidatus* Omnitrophus	0.01%	0.03%	0.00%
671	Verrucomicrobiota	*Candidatus* Protochlamydia	0.00%	0.02%	0.01%
672	Verrucomicrobiota	*Candidatus* Udaeobacter	0.02%	0.06%	0.06%
673	Verrucomicrobiota	*Candidatus* Xiphinematobacter	0.05%	0.05%	0.05%
674	Verrucomicrobiota	*Cephaloticoccus*	0.05%	0.01%	0.11%
675	Verrucomicrobiota	*Cerasicoccus*	0.01%	0.02%	0.02%
676	Verrucomicrobiota	*Chthoniobacter*	0.44%	0.80%	0.42%
677	Verrucomicrobiota	DEV008	0.23%	0.10%	0.13%
678	Verrucomicrobiota	DEV114	0.08%	0.08%	0.10%
679	Verrucomicrobiota	Ellin516	0.06%	0.05%	0.02%
680	Verrucomicrobiota	*Haloferula*	0.04%	0.03%	0.02%
681	Verrucomicrobiota	IMCC26134	0.05%	0.08%	0.11%
682	Verrucomicrobiota	*Lacunisphaera*	0.46%	0.31%	0.88%
683	Verrucomicrobiota	*LD29*	0.02%	0.05%	0.03%
684	Verrucomicrobiota	*Lentimonas*	0.01%	0.02%	0.01%
685	Verrucomicrobiota	*Luteolibacter*	1.29%	1.33%	1.20%
686	Verrucomicrobiota	*Neochlamydia*	0.02%	0.03%	0.04%
687	Verrucomicrobiota	*Oikopleura*	0.06%	0.03%	0.03%

No.	Bacterial phyla	Bacterial genera	Relative abundance		
			CKD	PUA	TD1
688	Verrucomicrobiota	*Opitutus*	0.08%	0.10%	0.25%
689	Verrucomicrobiota	*Pedosphaera*	0.00%	0.00%	0.00%
690	Verrucomicrobiota	*Prosthecobacter*	0.10%	0.16%	0.07%
691	Verrucomicrobiota	*Roseibacillus*	0.01%	0.03%	0.00%
692	Verrucomicrobiota	*Roseimicrobium*	0.02%	0.04%	0.02%
693	Verrucomicrobiota	*Rubritalea*	0.00%	0.02%	0.00%
694	Verrucomicrobiota	SCGC AAA164-E04	0.02%	0.02%	0.02%
695	Verrucomicrobiota	SH3-11	0.17%	0.18%	0.26%
696	Verrucomicrobiota	*Terrimicrobium*	0.05%	0.06%	0.11%
697	Verrucomicrobiota	Verruc-01	0.00%	0.00%	0.02%
698	Verrucomicrobiota	*Verrucomicrobium*	0.02%	0.05%	0.02%
	unidentified taxa		26.95%	34.63%	35.38%

Table C.4 Identified eukaryotic supergroups and their relative abundance obtained from 18S rDNA amplicon analysis.

No.	Eukaryotic supergroups	Relative abundance		
		CKD	PUA	TD1
1	Alveolata	3.66%	4.01%	6.37%
2	Amorphea	46.19%	28.95%	27.83%
3	Archaeplastida	17.33%	15.71%	12.55%
4	Cryptophyceae	0.12%	0.12%	0.17%
5	Rhizaria	5.55%	5.33%	7.02%
6	Stramenopiles	27.10%	45.84%	46.04%
	unidentified taxa	0.05%	0.04%	0.02%

Table C.5 Identified eukaryotic genera and their relative abundance obtained from 18S rDNA amplicon analysis.

no.	Eukaryotic supergroups	Eukryotic genera	Relative abundance		
			CKD	**PUA**	**TD1**
1	Amorphea	*Schizoplasmodiopsis*	0.02%	0.00%	0.00%
2	Amorphea	*Choanocystis*	0.00%	0.00%	0.08%
3	Amorphea	*Pterocystis*	0.03%	0.14%	0.37%
4	Amorphea	*Chlamydaster*	0.07%	0.11%	0.17%
5	Amorphea	*Heterophrys*	0.03%	0.05%	0.06%
6	Amorphea	*Sphaerastrum*	0.00%	0.02%	0.04%
7	Amorphea	*Dictyostelium*	0.02%	0.00%	0.00%
8	Amorphea	*Angulamoeba*	0.06%	0.14%	0.05%
9	Amorphea	*Korotnevella*	0.06%	0.10%	0.25%
10	Amorphea	*Paramoeba*	0.00%	0.03%	0.03%
11	Amorphea	*Vermistella*	0.00%	0.04%	0.06%
12	Amorphea	*Platyamoeba*	0.00%	0.00%	0.02%
13	Amorphea	*Unda*	0.00%	0.00%	0.02%
14	Amorphea	*Vannella*	0.07%	0.07%	0.22%
15	Amorphea	*Acanthamoeba*	0.06%	0.00%	0.00%
16	Amorphea	*Filamoeba*	0.02%	0.00%	0.10%
17	Amorphea	*Flamella*	0.02%	0.00%	0.02%
18	Amorphea	*Telaepolella*	0.02%	0.02%	0.04%
19	Amorphea	*Amastigomonas*	0.00%	0.04%	0.07%
20	Amorphea	*Subulatomonas*	0.00%	0.00%	0.02%
21	Amorphea	*Dictyamoeba*	0.03%	0.05%	0.03%
22	Amorphea	*Ischnamoeba*	0.04%	0.00%	0.02%
23	Amorphea	*Micronuclearia*	0.04%	0.12%	0.12%
24	Amorphea	*Rigifila*	0.06%	0.05%	0.14%
25	Amorphea	*Mycamoeba*	0.03%	0.11%	0.10%
26	Amorphea	*Protosporangium*	0.02%	0.03%	0.00%
27	Amorphea	*Protostelium*	0.03%	0.02%	0.00%
28	Amorphea	*Schoutedamoeba*	0.06%	0.11%	0.29%
29	Amorphea	*Sorodiplophrys*	3.07%	2.70%	4.09%
30	Amorphea	*Phalansterium*	0.04%	0.22%	0.19%
31	Amorphea	*Schizoplasmodium*	0.03%	0.00%	0.00%
32	Amorphea	BOLA868	0.06%	0.05%	0.10%
33	Amorphea	*Pyxidicula*	0.00%	0.00%	0.06%
34	Amorphea	*Diaphanoeca*	0.02%	0.05%	0.09%
35	Amorphea	Marine Choanoflagellates 1	0.00%	0.00%	0.04%

no.	Eukaryotic supergroups	Eukryotic genera	Relative abundance		
			CKD	**PUA**	**TD1**
36	Amorphea	*Stephanoeca*	0.00%	0.04%	0.02%
37	Amorphea	*Codosiga*	0.00%	0.00%	0.09%
38	Amorphea	*Monosiga*	0.09%	0.07%	0.28%
39	Amorphea	*Sphaeroeca*	0.00%	0.00%	0.06%
40	Amorphea	Freshwater Choanoflagellates 1	0.45%	0.45%	0.42%
41	Amorphea	*Lagenoeca*	0.03%	0.07%	0.02%
42	Amorphea	Freshwater Choanoflagellates 2	0.17%	0.17%	0.21%
43	Amorphea	*Salpingoeca*	0.13%	0.22%	0.48%
44	Amorphea	*Stagondoeca*	0.03%	0.00%	0.00%
45	Amorphea	*Eccrinidus*	0.16%	0.03%	0.08%
46	Amorphea	AT4-11	0.00%	0.02%	0.03%
47	Amorphea	*Dermocystidium*	0.02%	0.02%	0.00%
48	Amorphea	*Rhinosporidium*	0.02%	0.00%	0.00%
49	Amorphea	Freshwater Ichthyosporeans 1	0.00%	0.05%	0.00%
50	Amorphea	*Paramoebidium*	0.08%	0.04%	0.02%
51	Amorphea	*Anurofeca*	0.06%	0.05%	0.07%
52	Amorphea	LKM51	0.12%	0.07%	0.04%
53	Amorphea	*Limnognathia*	0.00%	0.03%	0.00%
54	Amorphea	*Rotaria*	0.09%	0.00%	0.08%
55	Amorphea	*Aphelidium*	0.04%	0.18%	0.09%
56	Amorphea	*Paraphelidium*	0.22%	0.06%	0.03%
57	Amorphea	*Dendrochytridium*	0.00%	0.00%	0.09%
58	Amorphea	*Chytriomyces*	0.02%	0.05%	0.02%
59	Amorphea	*Rhizoclosmatium*	0.00%	0.02%	0.21%
60	Amorphea	*Nowakowskiella*	0.03%	0.06%	0.11%
61	Amorphea	*Catenomyces*	0.02%	0.00%	0.00%
62	Amorphea	*Clydaea*	0.03%	0.00%	0.00%
63	Amorphea	*Neokarlingia*	0.00%	0.00%	0.03%
64	Amorphea	*Polytrychium*	0.00%	0.00%	0.02%
65	Amorphea	*Rhizophlyctis*	0.04%	0.00%	0.09%
66	Amorphea	*Rhizophydium*	0.15%	0.18%	0.40%
67	Amorphea	*Olpidium*	0.02%	0.00%	0.00%
68	Amorphea	*Geranomyces*	0.03%	0.00%	0.02%
69	Amorphea	*Phlyctochytrium*	0.02%	0.02%	0.06%
70	Amorphea	*Spizellomyces*	0.03%	0.00%	0.00%
71	Amorphea	*Gonapodya*	0.03%	0.00%	0.00%
72	Amorphea	*Paramicrosporidium*	2.45%	1.05%	0.46%
73	Amorphea	*Rozella*	0.15%	0.09%	0.40%

no.	**Eukaryotic supergroups**	**Eukryotic genera**	**Relative abundance**		
			CKD	**PUA**	**TD1**
74	Amorphea	*Conidiocarpus*	0.02%	0.00%	0.00%
75	Amorphea	*Cladosporium*	0.22%	0.08%	0.10%
76	Amorphea	*Toxicocladosporium*	0.02%	0.00%	0.00%
77	Amorphea	*Vermiconia*	0.03%	0.00%	0.00%
78	Amorphea	*Cercospora*	0.02%	0.00%	0.00%
79	Amorphea	*Lecanosticta*	0.02%	0.00%	0.00%
80	Amorphea	*Neophloeospora*	0.02%	0.00%	0.00%
81	Amorphea	*Suberoteratosphaeria*	0.03%	0.00%	0.00%
82	Amorphea	*Phaeotheca*	0.00%	0.00%	0.02%
83	Amorphea	*Elsinoe*	0.03%	0.00%	0.00%
84	Amorphea	*Arthopyrenia*	0.05%	0.00%	0.00%
85	Amorphea	*Biatriospora*	0.04%	0.00%	0.00%
86	Amorphea	*Corynespora*	0.09%	0.00%	0.02%
87	Amorphea	*Pyrenochaeta*	0.07%	0.02%	0.02%
88	Amorphea	*Didymella*	0.10%	0.00%	0.03%
89	Amorphea	*Lentithecium*	0.03%	0.02%	0.02%
90	Amorphea	*Leptosphaeria*	0.03%	0.00%	0.00%
91	Amorphea	*Neophaeosphaeria*	0.03%	0.00%	0.00%
92	Amorphea	*Helicascus*	0.05%	0.02%	0.02%
93	Amorphea	*Periconia*	0.02%	0.00%	0.02%
94	Amorphea	*Ophiosphaerella*	0.07%	0.00%	0.00%
95	Amorphea	*Parastagonospora*	0.02%	0.02%	0.00%
96	Amorphea	*Alternaria*	0.03%	0.02%	0.02%
97	Amorphea	*Bipolaris*	0.13%	0.00%	0.02%
98	Amorphea	*Pleospora*	0.03%	0.00%	0.02%
99	Amorphea	*Preussia*	0.06%	0.00%	0.03%
100	Amorphea	*Westerdykella*	0.03%	0.00%	0.02%
101	Amorphea	*Neotestudina*	0.02%	0.02%	0.00%
102	Amorphea	*Torula*	0.03%	0.00%	0.00%
103	Amorphea	*Helicoma*	0.02%	0.00%	0.00%
104	Amorphea	*Wiesneriomyces*	0.03%	0.02%	0.02%
105	Amorphea	*Mycosisymbrium*	0.05%	0.02%	0.00%
106	Amorphea	*Ochroconis*	0.13%	0.02%	0.02%
107	Amorphea	*Cyphellophora*	0.05%	0.00%	0.02%
108	Amorphea	*Bradymyces*	0.03%	0.00%	0.00%
109	Amorphea	*Knufia*	0.04%	0.00%	0.02%
110	Amorphea	*Aspergillus*	0.09%	0.05%	0.02%
111	Amorphea	*Talaromyces*	0.03%	0.00%	0.00%

no.	Eukaryotic supergroups	Eukryotic genera	Relative abundance		
			CKD	PUA	TD1
112	Amorphea	*Lecophagus*	0.00%	0.00%	0.02%
113	Amorphea	*Arthrobotrys*	0.06%	0.00%	0.13%
114	Amorphea	*Neournula*	0.02%	0.00%	0.00%
115	Amorphea	*Peziza*	0.02%	0.00%	0.00%
116	Amorphea	*Phymatotrichopsis*	0.02%	0.00%	0.00%
117	Amorphea	*Diaporthe*	0.04%	0.00%	0.02%
118	Amorphea	*Colletotrichum*	0.03%	0.00%	0.00%
119	Amorphea	*Acrostalagmus*	0.02%	0.00%	0.00%
120	Amorphea	*Gibellulopsis*	0.09%	0.02%	0.03%
121	Amorphea	*Plectosphaerella*	0.05%	0.00%	0.00%
122	Amorphea	*Verticillium*	0.02%	0.00%	0.00%
123	Amorphea	*Ochronectria*	0.00%	0.00%	0.02%
124	Amorphea	*Conoideocrella*	0.03%	0.00%	0.00%
125	Amorphea	*Akanthomyces*	0.02%	0.00%	0.00%
126	Amorphea	*Parengyodontium*	0.02%	0.00%	0.00%
127	Amorphea	*Drechmeria*	0.04%	0.00%	0.00%
128	Amorphea	*Acremonium*	0.20%	0.02%	0.04%
129	Amorphea	*Emericellopsis*	0.02%	0.05%	0.02%
130	Amorphea	*Sarocladium*	0.13%	0.00%	0.02%
131	Amorphea	*Fusarium*	0.21%	0.08%	0.13%
132	Amorphea	*Mariannaea*	0.00%	0.00%	0.02%
133	Amorphea	*Hirsutella*	0.03%	0.00%	0.00%
134	Amorphea	*Septofusidium*	0.03%	0.00%	0.00%
135	Amorphea	*Myrothecium*	0.03%	0.00%	0.00%
136	Amorphea	*Lentomitella*	0.00%	0.02%	0.00%
137	Amorphea	*Lunulospora*	0.02%	0.00%	0.00%
138	Amorphea	*Kohlmeyeriopsis*	0.00%	0.00%	0.02%
139	Amorphea	*Lignincola*	0.03%	0.00%	0.02%
140	Amorphea	*Myrmecridium*	0.02%	0.00%	0.00%
141	Amorphea	*Chaetomium*	0.03%	0.00%	0.00%
142	Amorphea	*Pestalotia*	0.04%	0.00%	0.00%
143	Amorphea	*Arthrinium*	0.07%	0.02%	0.03%
144	Amorphea	*Circinotrichum*	0.03%	0.00%	0.02%
145	Amorphea	*Hyponectria*	0.02%	0.00%	0.02%
146	Amorphea	*Phialemoniopsis*	0.02%	0.00%	0.00%
147	Amorphea	*Hypoxylon*	0.03%	0.00%	0.00%
148	Amorphea	*Candida-Lodderomyces* clade	0.04%	0.00%	0.00%
149	Amorphea	*Kurtzmaniella-Candida* clade	0.02%	0.00%	0.00%

no.	Eukaryotic supergroups	Eukryotic genera	Relative abundance		
			CKD	**PUA**	**TD1**
150	Amorphea	*Galactomyces*	0.07%	0.03%	0.02%
151	Amorphea	*Geotrichum*	0.09%	0.03%	0.03%
152	Amorphea	*Yarrowia*	0.00%	0.00%	0.02%
153	Amorphea	*Ambrosiozyma*	0.00%	0.00%	0.02%
154	Amorphea	*Candida*	0.00%	0.00%	0.02%
155	Amorphea	*Middelhovenomyces*	0.03%	0.00%	0.00%
156	Amorphea	*Starmerella-Candida* clade	0.04%	0.00%	0.00%
157	Amorphea	*Clavispora-Candida* clade	0.02%	0.00%	0.00%
158	Amorphea	*Kodamaea*	0.00%	0.02%	0.00%
159	Amorphea	*Metschnikowia*	0.02%	0.00%	0.00%
160	Amorphea	*Barnettozyma-Candida* clade	0.02%	0.00%	0.00%
161	Amorphea	*Cyberlindnera-Candida* clade	0.00%	0.02%	0.00%
162	Amorphea	*Phaffomyces*	0.00%	0.02%	0.00%
163	Amorphea	*Martiniozyma*	0.02%	0.00%	0.00%
164	Amorphea	*Pichia*	0.20%	0.03%	0.04%
165	Amorphea	*Lachancea*	0.03%	0.00%	0.00%
166	Amorphea	*Nakaseomyces-Candida* clade	0.02%	0.00%	0.00%
167	Amorphea	*Hanseniaspora*	0.04%	0.00%	0.00%
168	Amorphea	*Saccharomycopsis*	0.03%	0.00%	0.00%
169	Amorphea	*Trichomonascus*	0.07%	0.00%	0.02%
170	Amorphea	*Neolecta*	0.02%	0.00%	0.00%
171	Amorphea	*Schizosaccharomyces*	0.00%	0.00%	0.02%
172	Amorphea	*Agaricus*	0.03%	0.00%	0.00%
173	Amorphea	*Leucoagaricus*	0.02%	0.00%	0.00%
174	Amorphea	*Macrolepiota*	0.02%	0.00%	0.00%
175	Amorphea	*Conocybe*	0.03%	0.00%	0.00%
176	Amorphea	*Coprinopsis*	0.03%	0.00%	0.00%
177	Amorphea	*Gymnopus*	0.02%	0.00%	0.00%
178	Amorphea	*Physalacria*	0.00%	0.00%	0.02%
179	Amorphea	*Pleurotus*	0.03%	0.00%	0.00%
180	Amorphea	*Psathyrella*	0.02%	0.00%	0.02%
181	Amorphea	*Boletellus*	0.02%	0.00%	0.00%
182	Amorphea	*Coniophora*	0.03%	0.00%	0.00%
183	Amorphea	*Tapinella*	0.03%	0.00%	0.00%
184	Amorphea	*Craterellus*	0.00%	0.02%	0.00%
185	Amorphea	*Rhizoctonia*	0.03%	0.00%	0.05%
186	Amorphea	*Thanatephorus*	0.02%	0.00%	0.00%
187	Amorphea	*Hyphodontia*	0.02%	0.00%	0.00%

no.	Eukaryotic supergroups	Eukryotic genera	Relative abundance		
			CKD	**PUA**	**TD1**
188	Amorphea	*Sistotrema*	0.02%	0.00%	0.00%
189	Amorphea	*Geastrum*	0.03%	0.00%	0.00%
190	Amorphea	*Phellinus*	0.02%	0.00%	0.00%
191	Amorphea	*Tropicoporus*	0.00%	0.02%	0.00%
192	Amorphea	*Phallus*	0.04%	0.00%	0.00%
193	Amorphea	*Donkia*	0.00%	0.00%	0.02%
194	Amorphea	*Ganoderma*	0.03%	0.00%	0.00%
195	Amorphea	*Hyphodermella*	0.00%	0.00%	0.02%
196	Amorphea	*Trametes*	0.00%	0.02%	0.00%
197	Amorphea	*Ceriporia*	0.03%	0.00%	0.00%
198	Amorphea	*Chaetospermum*	0.09%	0.03%	0.02%
199	Amorphea	*Sebacina*	0.02%	0.00%	0.00%
200	Amorphea	*Trechispora*	0.00%	0.00%	0.02%
201	Amorphea	*Dacrymyces*	0.02%	0.00%	0.00%
202	Amorphea	*Filobasidium*	0.00%	0.00%	0.02%
203	Amorphea	*Bullera*	0.03%	0.00%	0.00%
204	Amorphea	*Bulleribasidium*	0.00%	0.00%	0.02%
205	Amorphea	*Derxomyces*	0.02%	0.00%	0.00%
206	Amorphea	*Hannaella*	0.09%	0.02%	0.06%
207	Amorphea	*Vishniacozyma*	0.07%	0.04%	0.02%
208	Amorphea	*Papiliotrema*	0.06%	0.02%	0.00%
209	Amorphea	*Trichosporon*	0.02%	0.00%	0.00%
210	Amorphea	[*Sporobolomyces*] group	0.03%	0.00%	0.00%
211	Amorphea	*Occultifur*	0.02%	0.02%	0.02%
212	Amorphea	*Erythrobasidium*	0.04%	0.00%	0.05%
213	Amorphea	*Hasegawazyma*	0.00%	0.00%	0.02%
214	Amorphea	*Symmetrospora*	0.06%	0.02%	0.00%
215	Amorphea	*Spencerozyma*	0.00%	0.00%	0.03%
216	Amorphea	*Colacogloea*	0.02%	0.00%	0.00%
217	Amorphea	*Microbotryum*	0.00%	0.00%	0.02%
218	Amorphea	*Rhodosporidiobolus*	0.02%	0.00%	0.02%
219	Amorphea	*Rhodotorula*	0.07%	0.03%	0.02%
220	Amorphea	*Sporobolomyces*	0.12%	0.07%	0.10%
221	Amorphea	*Heterodoassansia*	0.00%	0.00%	0.02%
222	Amorphea	*Tilletiopsis*	0.00%	0.02%	0.00%
223	Amorphea	*Meira*	0.03%	0.00%	0.02%
224	Amorphea	*Sympodiomycopsis*	0.00%	0.00%	0.02%
225	Amorphea	*Malassezia*	0.05%	0.02%	0.00%

no.	Eukaryotic supergroups	Eukryotic genera	Relative abundance		
			CKD	PUA	TD1
226	Amorphea	*Fereydounia*	0.04%	0.00%	0.03%
227	Amorphea	*Anthracoidea*	0.00%	0.00%	0.02%
228	Amorphea	*Sporisorium*	0.03%	0.04%	0.02%
229	Amorphea	*Ustilago*	0.03%	0.00%	0.02%
230	Amorphea	*Acaulospora*	0.00%	0.00%	0.02%
231	Amorphea	*Glomus*	0.02%	0.00%	0.00%
232	Amorphea	*Rhizophagus*	0.00%	0.00%	0.02%
233	Amorphea	*Mortierella*	0.10%	0.06%	0.04%
234	Amorphea	*Endogone*	0.02%	0.02%	0.00%
235	Amorphea	*Piromyces*	0.05%	0.05%	0.08%
236	Amorphea	*Ramicandelaber*	0.03%	0.00%	0.00%
237	Amorphea	*Acaulopage*	0.38%	0.20%	0.07%
238	Amorphea	*Cochlonema*	0.07%	0.06%	0.02%
239	Amorphea	*Stylopage*	0.39%	0.12%	0.08%
240	Amorphea	*Nuclearia*	0.54%	0.47%	0.98%
241	Archaeplastida	*Tolypella*	0.00%	0.00%	0.02%
242	Archaeplastida	*Lejeunea*	0.00%	0.04%	0.00%
243	Archaeplastida	*Equisetum*	0.02%	0.00%	0.00%
244	Archaeplastida	*Azolla*	0.37%	0.02%	0.11%
245	Archaeplastida	*Gnetum*	0.13%	0.00%	0.00%
246	Archaeplastida	*Helianthus*	0.05%	0.00%	0.00%
247	Archaeplastida	*Batis*	0.02%	0.00%	0.00%
248	Archaeplastida	*Brassica*	0.12%	0.00%	0.00%
249	Archaeplastida	*Floerkea*	0.03%	0.00%	0.00%
250	Archaeplastida	*Nasturtium*	0.19%	0.05%	0.09%
251	Archaeplastida	*Chenopodium*	0.02%	0.00%	0.00%
252	Archaeplastida	*Persicaria*	0.00%	0.00%	0.02%
253	Archaeplastida	*Arachis*	0.00%	0.00%	0.02%
254	Archaeplastida	*Mezoneuron*	0.02%	0.00%	0.00%
255	Archaeplastida	*Oxygyne*	0.03%	0.00%	0.00%
256	Archaeplastida	*Potamogeton*	0.00%	0.00%	0.14%
257	Archaeplastida	*Eleusine*	0.02%	0.00%	0.00%
258	Archaeplastida	*Hordeum*	0.13%	0.00%	0.02%
259	Archaeplastida	*Sorghum*	0.02%	0.00%	0.00%
260	Archaeplastida	*Hevea*	0.03%	0.00%	0.00%
261	Archaeplastida	*Heywoodia*	0.02%	0.00%	0.00%
262	Archaeplastida	*Gossypium*	0.03%	0.02%	0.02%
263	Archaeplastida	*Broussonetia*	0.00%	0.02%	0.02%

no.	Eukaryotic supergroups	Eukryotic genera	Relative abundance		
			CKD	**PUA**	**TD1**
264	Archaeplastida	*Ficus*	0.04%	0.00%	0.02%
265	Archaeplastida	*Humulus*	0.02%	0.00%	0.00%
266	Archaeplastida	*Capsicum*	0.00%	0.02%	0.00%
267	Archaeplastida	*Ipomoea*	0.18%	0.04%	0.00%
268	Archaeplastida	*Pinus*	0.07%	0.02%	0.00%
269	Archaeplastida	*Closterium*	0.04%	0.05%	0.02%
270	Archaeplastida	*Cosmarium*	0.11%	0.02%	0.10%
271	Archaeplastida	*Gonatozygon*	0.02%	0.00%	0.00%
272	Archaeplastida	*Micrasterias*	0.00%	0.00%	0.02%
273	Archaeplastida	*Cylindrocystis*	0.02%	0.10%	0.00%
274	Archaeplastida	*Mesotaenium*	0.03%	0.02%	0.00%
275	Archaeplastida	*Sirogonium*	0.00%	0.02%	0.02%
276	Archaeplastida	*Spirogyra*	1.44%	4.27%	3.40%
277	Archaeplastida	*Tetraselmis*	0.06%	0.00%	0.04%
278	Archaeplastida	*Chaetopeltis*	0.00%	0.00%	0.04%
279	Archaeplastida	*Floydiella*	0.00%	0.02%	0.02%
280	Archaeplastida	*Aphanochaete*	0.11%	0.26%	0.18%
281	Archaeplastida	*Barranca*	0.02%	0.05%	0.00%
282	Archaeplastida	*Chaetophora*	0.04%	0.09%	0.06%
283	Archaeplastida	*Stigeoclonium*	0.05%	0.04%	0.02%
284	Archaeplastida	*Cephalomonas*	0.00%	0.00%	0.03%
285	Archaeplastida	*Characium*	0.09%	0.03%	0.09%
286	Archaeplastida	*Chlamydomonas*	0.27%	0.15%	0.09%
287	Archaeplastida	*Chlorococcum*	0.25%	0.23%	0.17%
288	Archaeplastida	*Chloromonas*	0.00%	0.02%	0.00%
289	Archaeplastida	*Deasonia*	0.70%	0.44%	0.19%
290	Archaeplastida	*Dysmorphococcus*	0.05%	0.00%	0.00%
291	Archaeplastida	*Golenkinia*	0.06%	0.02%	0.00%
292	Archaeplastida	*Gonium*	0.02%	0.00%	0.00%
293	Archaeplastida	*Hafniomonas*	0.00%	0.02%	0.00%
294	Archaeplastida	*Lobochlamys*	0.00%	0.02%	0.00%
295	Archaeplastida	*Lobomonas*	0.00%	0.02%	0.02%
296	Archaeplastida	*Neospongiococcum*	0.04%	0.00%	0.00%
297	Archaeplastida	*Oogamochlamys*	0.02%	0.00%	0.02%
298	Archaeplastida	*Pachycladella*	0.03%	0.00%	0.02%
299	Archaeplastida	*Phacotus*	0.05%	0.00%	0.00%
300	Archaeplastida	*Polytoma*	0.02%	0.00%	0.00%
301	Archaeplastida	*Protosiphon*	0.09%	0.19%	0.12%

no.	**Eukaryotic supergroups**	**Eukryotic genera**	**Relative abundance**		
			CKD	**PUA**	**TD1**
302	Archaeplastida	*Tetracystis*	0.06%	0.00%	0.00%
303	Archaeplastida	*Vitreochlamys*	0.02%	0.00%	0.00%
304	Archaeplastida	*Chlorosarcinopsis*	0.00%	0.00%	0.02%
305	Archaeplastida	*Neochlorosarcina*	0.03%	0.00%	0.02%
306	Archaeplastida	*Carteria*	0.00%	0.02%	0.00%
307	Archaeplastida	*Chlamydopodium*	0.03%	0.04%	0.06%
308	Archaeplastida	*Chlorochytrium*	0.00%	0.00%	0.03%
309	Archaeplastida	*Pleurastrum*	0.03%	0.02%	0.00%
310	Archaeplastida	*Oedocladium*	0.00%	0.02%	0.02%
311	Archaeplastida	*Oedogonium*	0.14%	0.36%	0.75%
312	Archaeplastida	*Acutodesmus*	0.03%	0.07%	0.07%
313	Archaeplastida	*Ankistrodesmus*	0.04%	0.02%	0.00%
314	Archaeplastida	*Auctodesmus*	0.15%	0.18%	0.12%
315	Archaeplastida	*Bracteacoccus*	0.09%	0.05%	0.06%
316	Archaeplastida	*Characiopodium*	0.28%	0.21%	0.20%
317	Archaeplastida	*Chlorolobion*	0.02%	0.00%	0.00%
318	Archaeplastida	*Chlorotetraedron*	0.14%	0.13%	0.07%
319	Archaeplastida	*Chromochloris*	0.18%	0.06%	0.05%
320	Archaeplastida	*Coelastrella*	0.10%	0.03%	0.03%
321	Archaeplastida	*Coelastrum*	0.11%	0.07%	0.06%
322	Archaeplastida	*Desmodesmus*	0.57%	0.36%	0.53%
323	Archaeplastida	*Hylodesmus*	0.00%	0.02%	0.00%
324	Archaeplastida	*Messastrum*	0.00%	0.02%	0.00%
325	Archaeplastida	*Monoraphidium*	0.23%	0.15%	0.17%
326	Archaeplastida	*Mychonastes*	0.07%	0.07%	0.10%
327	Archaeplastida	*Neochloris*	0.49%	0.30%	0.17%
328	Archaeplastida	*Neodesmus*	0.17%	0.13%	0.11%
329	Archaeplastida	*Pectodictyon*	0.02%	0.00%	0.08%
330	Archaeplastida	*Planktosphaeria*	0.97%	0.60%	0.21%
331	Archaeplastida	*Polyedriopsis*	0.02%	0.00%	0.00%
332	Archaeplastida	*Protodesmus*	0.00%	0.02%	0.00%
333	Archaeplastida	*Pseudomuriella*	0.12%	0.09%	0.11%
334	Archaeplastida	*Pseudopediastrum*	0.21%	0.11%	0.12%
335	Archaeplastida	*Pseudoschroederia*	0.14%	0.11%	0.06%
336	Archaeplastida	*Radiococcus*	0.10%	0.05%	0.00%
337	Archaeplastida	*Rotundella*	0.02%	0.00%	0.00%
338	Archaeplastida	*Scenedesmus*	0.15%	0.10%	0.11%
339	Archaeplastida	*Stauridium*	0.09%	0.05%	0.02%

no.	Eukaryotic supergroups	Eukryotic genera	Relative abundance		
			CKD	PUA	TD1
340	Archaeplastida	*Tetraedron*	0.11%	0.09%	0.10%
341	Archaeplastida	*Tetranephris*	0.08%	0.05%	0.05%
342	Archaeplastida	*Verrucodesmus*	0.05%	0.07%	0.09%
343	Archaeplastida	*Westella*	0.03%	0.02%	0.00%
344	Archaeplastida	*Asterococcus*	0.02%	0.00%	0.00%
345	Archaeplastida	*Fritschiella*	0.06%	0.13%	0.04%
346	Archaeplastida	*Bathycoccus*	0.00%	0.02%	0.00%
347	Archaeplastida	*Pedinomonas*	0.06%	0.00%	0.00%
348	Archaeplastida	*Chlorella*	0.14%	0.07%	0.09%
349	Archaeplastida	*Heveochlorella*	0.21%	0.02%	0.00%
350	Archaeplastida	*Makinoella*	0.08%	0.05%	0.11%
351	Archaeplastida	*Micractinium*	0.16%	0.09%	0.12%
352	Archaeplastida	*Nannochloris*	0.05%	0.04%	0.02%
353	Archaeplastida	*Oocystella*	0.02%	0.02%	0.06%
354	Archaeplastida	*Oocystis*	0.09%	0.03%	0.07%
355	Archaeplastida	*Prototheca*	0.02%	0.00%	0.00%
356	Archaeplastida	*Tetrastrum*	0.02%	0.00%	0.00%
357	Archaeplastida	*Choricystis*	0.13%	0.07%	0.06%
358	Archaeplastida	*Dictyosphaerium*	0.05%	0.05%	0.00%
359	Archaeplastida	*Heterochlorella*	0.32%	0.07%	0.00%
360	Archaeplastida	*Picochlorum*	0.02%	0.02%	0.00%
361	Archaeplastida	*Prasiola*	0.02%	0.00%	0.00%
362	Archaeplastida	*Stichococcus*	0.04%	0.00%	0.02%
363	Archaeplastida	*Trebouxia*	0.02%	0.00%	0.00%
364	Archaeplastida	*Bryopsis*	0.02%	0.00%	0.00%
365	Archaeplastida	*Desmochloris*	0.02%	0.00%	0.00%
366	Archaeplastida	*Cladophora*	0.16%	0.19%	0.11%
367	Archaeplastida	*Rhizoclonium*	0.56%	0.80%	0.24%
368	Archaeplastida	*Gloeotilopsis*	0.03%	0.02%	0.02%
369	Archaeplastida	*Pseudoneochloris*	0.02%	0.00%	0.00%
370	Archaeplastida	*Cloniophora*	0.02%	0.00%	0.00%
371	Archaeplastida	*Jaoa*	0.22%	0.92%	0.13%
372	Archaeplastida	*Pseudendoclonium*	0.00%	0.04%	0.00%
373	Archaeplastida	*Ulvella*	0.03%	0.08%	0.00%
374	Archaeplastida	*Nemalionopsis*	0.07%	0.05%	0.02%
375	Archaeplastida	*Sheathia*	0.02%	0.09%	0.00%
376	Archaeplastida	*Thorea*	0.08%	0.14%	0.00%
377	Archaeplastida	*Porphyridium*	0.05%	0.00%	0.05%

no.	**Eukaryotic supergroups**	**Eukryotic genera**	**Relative abundance**		
			CKD	**PUA**	**TD1**
378	Cryptophyceae	*Cryptomonas*	0.02%	0.03%	0.03%
379	Cryptophyceae	*Guillardia*	0.02%	0.00%	0.00%
380	Cryptophyceae	*Hanusia*	0.02%	0.00%	0.00%
381	Cryptophyceae	*Teleaulax*	0.03%	0.00%	0.00%
382	Cryptophyceae	*Goniomonas*	0.00%	0.04%	0.09%
383	Cryptophyceae	*Roombia*	0.03%	0.05%	0.03%
384	Excavata	*Notosolenus*	0.00%	0.00%	0.02%
385	Excavata	*Carpediemonas*	0.03%	0.00%	0.00%
386	Excavata	*Paratrimastix*	0.02%	0.00%	0.00%
387	Incertae Sedis	*Ancyromonas*	0.00%	0.02%	0.10%
388	Incertae Sedis	*Nutomonas*	0.00%	0.03%	0.08%
389	Incertae Sedis	*Palpitomonas*	0.02%	0.00%	0.02%
390	Alveolata	*Babesia*	0.00%	0.00%	0.02%
391	Alveolata	*Theileria*	0.03%	0.00%	0.00%
392	Alveolata	*Hepatozoon*	0.02%	0.05%	0.25%
393	Alveolata	*Aggregata*	0.02%	0.00%	0.00%
394	Alveolata	*Goussia*	0.09%	0.02%	0.07%
395	Alveolata	*Rhytidocystis*	0.03%	0.00%	0.02%
396	Alveolata	*Cryptosporidium*	0.26%	0.13%	0.10%
397	Alveolata	Elev-18S-1089	0.15%	0.02%	0.04%
398	Alveolata	*Hoplorhynchus*	0.00%	0.00%	0.02%
399	Alveolata	*Lecudina*	0.02%	0.00%	0.00%
400	Alveolata	*Paraschneideria*	0.03%	0.02%	0.00%
401	Alveolata	*Stylocephalus*	0.03%	0.00%	0.00%
402	Alveolata	*Syncystis*	0.14%	0.06%	0.03%
403	Alveolata	*Apicystis*	0.02%	0.00%	0.00%
404	Alveolata	*Bryometopus*	0.00%	0.02%	0.02%
405	Alveolata	*Bromeliothrix*	0.03%	0.00%	0.00%
406	Alveolata	*Colpoda*	0.02%	0.00%	0.00%
407	Alveolata	*Cyrtolophosis*	0.02%	0.06%	0.06%
408	Alveolata	*Microdiaphanosoma*	0.00%	0.00%	0.03%
409	Alveolata	*Zosterodasys*	0.02%	0.02%	0.00%
410	Alveolata	*Ichthyophthirius*	0.00%	0.00%	0.02%
411	Alveolata	*Tetrahymena*	0.06%	0.22%	0.20%
412	Alveolata	*Carchesium*	0.00%	0.00%	0.02%
413	Alveolata	*Cothurnia*	0.00%	0.06%	0.28%
414	Alveolata	*Epicarchesium*	0.00%	0.00%	0.02%
415	Alveolata	*Pseudepistylis*	0.05%	0.07%	0.00%

no.	Eukaryotic supergroups	Eukryotic genera	Relative abundance		
			CKD	**PUA**	**TD1**
416	Alveolata	*Pseudovorticella*	0.00%	0.00%	0.03%
417	Alveolata	*Telotrochidium*	0.14%	0.08%	0.13%
418	Alveolata	*Vaginicola*	0.04%	0.04%	0.05%
419	Alveolata	*Vorticella*	0.02%	0.61%	0.52%
420	Alveolata	*Cyclidium*	0.00%	0.05%	0.00%
421	Alveolata	*Hippocomos*	0.02%	0.00%	0.00%
422	Alveolata	*Chilodonella*	0.00%	0.07%	0.23%
423	Alveolata	*Phascolodon*	0.00%	0.00%	0.09%
424	Alveolata	*Pseudochilodonopsis*	0.00%	0.00%	0.02%
425	Alveolata	*Trithigmostoma*	0.00%	0.00%	0.07%
426	Alveolata	*Trochilia*	0.00%	0.46%	2.00%
427	Alveolata	*Heliophrya*	0.02%	0.00%	0.00%
428	Alveolata	*Trimyema*	0.02%	0.00%	0.00%
429	Alveolata	*Cryptocaryon*	0.00%	0.06%	0.05%
430	Alveolata	*Prorodon*	0.00%	0.00%	0.02%
431	Alveolata	*Acineria*	0.00%	0.00%	0.02%
432	Alveolata	*Amphileptus*	0.00%	0.07%	0.14%
433	Alveolata	*Chaenea*	0.00%	0.02%	0.00%
434	Alveolata	*Hemiophrys*	0.03%	0.00%	0.00%
435	Alveolata	*Litonotus*	0.00%	0.07%	0.06%
436	Alveolata	*Loxophyllum*	0.00%	0.02%	0.02%
437	Alveolata	*Bundleia*	0.03%	0.00%	0.00%
438	Alveolata	*Aspidisca*	0.00%	0.00%	0.02%
439	Alveolata	*Hemiurosomoida*	0.00%	0.00%	0.02%
440	Alveolata	*Holosticha*	0.00%	0.04%	0.06%
441	Alveolata	*Stylonychia*	0.00%	0.03%	0.02%
442	Alveolata	*Sinophysis*	0.00%	0.02%	0.00%
443	Alveolata	*Lepidodinium*	0.00%	0.00%	0.02%
444	Alveolata	*Cochlodinium*	0.02%	0.00%	0.00%
445	Alveolata	*Biecheleria*	0.03%	0.06%	0.00%
446	Alveolata	*Symbiodinium*	0.10%	0.06%	0.03%
447	Alveolata	*Azadinium*	0.00%	0.00%	0.03%
448	Alveolata	*Peridiniopsis*	0.07%	0.00%	0.02%
449	Alveolata	*Peridinium*	0.02%	0.00%	0.00%
450	Alveolata	*Roscoffia*	0.00%	0.00%	0.03%
451	Alveolata	*Cryptoperidiniopsis*	0.02%	0.05%	0.00%
452	Alveolata	*Pfiesteria*	0.00%	0.00%	0.02%
453	Alveolata	*Colponema*	0.03%	0.00%	0.00%

no.	Eukaryotic supergroups	Eukryotic genera	Relative abundance		
			CKD	**PUA**	**TD1**
454	Alveolata	*Chromera*	0.00%	0.02%	0.00%
455	Alveolata	*Colpodella*	0.00%	0.00%	0.03%
456	Alveolata	*Parvilucifera*	0.02%	0.00%	0.00%
457	Rhizaria	*Cavernomonas*	0.00%	0.02%	0.00%
458	Rhizaria	*Cercomonas*	0.14%	0.21%	0.56%
459	Rhizaria	*Eocercomonas*	0.00%	0.03%	0.02%
460	Rhizaria	*Paracercomonas*	0.09%	0.36%	0.42%
461	Rhizaria	*Allantion*	0.00%	0.00%	0.12%
462	Rhizaria	*Bodomorpha*	0.15%	0.17%	0.19%
463	Rhizaria	*Heteromita*	1.29%	0.43%	0.46%
464	Rhizaria	*Orciraptor*	0.05%	0.04%	0.00%
465	Rhizaria	*Viridiraptor*	0.03%	0.10%	0.11%
466	Rhizaria	*Massisteria*	0.05%	0.10%	0.19%
467	Rhizaria	*Pseudopirsonia*	0.00%	0.00%	0.02%
468	Rhizaria	*Nudifila*	0.00%	0.00%	0.02%
469	Rhizaria	*Euglypha*	0.03%	0.02%	0.03%
470	Rhizaria	*Tracheleuglypha*	0.07%	0.07%	0.05%
471	Rhizaria	*Paulinella*	0.11%	0.10%	0.02%
472	Rhizaria	*Trinema*	0.00%	0.02%	0.02%
473	Rhizaria	CCe	0.00%	0.02%	0.03%
474	Rhizaria	*Thaumatomonas*	0.00%	0.00%	0.09%
475	Rhizaria	*Spongomonas*	0.03%	0.07%	0.15%
476	Rhizaria	*Gymnophrys*	0.16%	0.36%	0.52%
477	Rhizaria	*Kraken*	0.03%	0.03%	0.09%
478	Rhizaria	*Aurigamonas*	0.00%	0.00%	0.04%
479	Rhizaria	*Rhogostoma*	0.38%	0.07%	0.32%
480	Rhizaria	*Lecythium*	0.02%	0.00%	0.04%
481	Rhizaria	*Pseudodifflugia*	0.05%	0.07%	0.00%
482	Rhizaria	*Collophidium*	0.02%	0.00%	0.00%
483	Stramenopiles	*Bicosoeca*	0.05%	0.05%	0.35%
484	Stramenopiles	*Siluania*	0.02%	0.00%	0.00%
485	Stramenopiles	*Blastocystis*	0.06%	0.02%	0.03%
486	Stramenopiles	*Pirsonia*	0.00%	0.00%	0.02%
487	Stramenopiles	*Labyrinthula*	0.04%	0.00%	0.05%
488	Stramenopiles	*Aplanochytrium*	0.03%	0.02%	0.00%
489	Stramenopiles	*Thraustochytrium*	0.00%	0.00%	0.02%
490	Stramenopiles	*Bolidomonas*	0.07%	0.00%	0.00%
491	Stramenopiles	*Chrysamoeba*	0.00%	0.07%	0.00%

no.	Eukaryotic supergroups	Eukryotic genera	Relative abundance		
			CKD	**PUA**	**TD1**
492	Stramenopiles	*Cyclonexis*	0.00%	0.00%	0.02%
493	Stramenopiles	JBNA46	0.04%	0.14%	0.13%
494	Stramenopiles	*Oikomonas*	0.09%	0.22%	0.13%
495	Stramenopiles	*Poterioochromonas*	0.00%	0.00%	0.10%
496	Stramenopiles	*Poteriospumella*	1.42%	1.26%	0.44%
497	Stramenopiles	*Spumella*	0.02%	0.06%	0.17%
498	Stramenopiles	*Chromophyton*	0.02%	0.00%	0.00%
499	Stramenopiles	*Lagynion*	0.00%	0.00%	0.02%
500	Stramenopiles	*Epipyxis*	0.00%	0.00%	0.02%
501	Stramenopiles	*Ochromonas*	3.64%	4.19%	3.06%
502	Stramenopiles	*Paraphysomonas*	0.43%	1.16%	1.12%
503	Stramenopiles	*Achnanthidium*	0.07%	0.12%	0.17%
504	Stramenopiles	*Amphora*	0.13%	0.09%	0.12%
505	Stramenopiles	*Bacillaria*	0.02%	0.02%	0.00%
506	Stramenopiles	*Cocconeis*	4.86%	27.43%	21.10%
507	Stramenopiles	*Craticula*	0.13%	0.07%	0.08%
508	Stramenopiles	*Cymbella*	0.34%	0.66%	0.42%
509	Stramenopiles	*Diadesmis*	0.05%	0.07%	0.00%
510	Stramenopiles	*Diploneis*	0.06%	0.00%	0.06%
511	Stramenopiles	*Encyonema*	0.00%	0.00%	0.06%
512	Stramenopiles	*Entomoneis*	0.03%	0.00%	0.00%
513	Stramenopiles	*Epithemia*	0.04%	0.07%	0.04%
514	Stramenopiles	*Fistulifera*	0.07%	0.04%	0.07%
515	Stramenopiles	*Geissleria*	0.00%	0.02%	0.00%
516	Stramenopiles	*Gomphonema*	2.28%	0.76%	4.59%
517	Stramenopiles	*Gyrosigma*	0.00%	0.00%	0.02%
518	Stramenopiles	*Lemnicula*	0.03%	0.00%	0.03%
519	Stramenopiles	*Navicula*	0.17%	0.25%	0.46%
520	Stramenopiles	*Neidium*	0.02%	0.00%	0.00%
521	Stramenopiles	*Nitzschia*	0.52%	0.50%	0.70%
522	Stramenopiles	*Pinnularia*	0.32%	0.14%	0.05%
523	Stramenopiles	*Placoneis*	0.03%	0.07%	0.02%
524	Stramenopiles	*Planothidium*	0.14%	0.24%	0.08%
525	Stramenopiles	*Pleurosigma*	0.10%	0.11%	0.00%
526	Stramenopiles	*Pseudo-nitzschia*	0.03%	0.03%	0.02%
527	Stramenopiles	*Rhaphoneis*	0.00%	0.00%	0.02%
528	Stramenopiles	*Rhopalodia*	0.49%	0.59%	0.22%
529	Stramenopiles	*Eolimna*	0.00%	0.00%	0.04%

no.	Eukaryotic supergroups	Eukryotic genera	Relative abundance		
			CKD	PUA	TD1
530	Stramenopiles	*Fallacia*	0.03%	0.02%	0.00%
531	Stramenopiles	*Sellaphora*	0.26%	0.24%	0.35%
532	Stramenopiles	*Staurotropis*	0.00%	0.02%	0.00%
533	Stramenopiles	*Surirella*	0.03%	0.04%	0.00%
534	Stramenopiles	*Chaetoceros*	0.03%	0.00%	0.00%
535	Stramenopiles	*Cyclotella*	0.05%	0.00%	0.07%
536	Stramenopiles	*Discostella*	0.14%	0.05%	0.05%
537	Stramenopiles	*Lauderia*	0.00%	0.00%	0.02%
538	Stramenopiles	*Pleurosira*	0.13%	0.05%	0.00%
539	Stramenopiles	*Skeletonema*	0.00%	0.03%	0.00%
540	Stramenopiles	*Stephanodiscus*	0.03%	0.00%	0.00%
541	Stramenopiles	*Thalassiosira*	0.14%	0.02%	0.05%
542	Stramenopiles	*Fragilaria*	0.02%	0.09%	0.00%
543	Stramenopiles	*Hyalosira*	0.03%	0.00%	0.02%
544	Stramenopiles	*Hyalosynedra*	0.00%	0.00%	0.02%
545	Stramenopiles	*Licmophora*	0.03%	0.04%	0.00%
546	Stramenopiles	*Stauroforma*	0.02%	0.00%	0.00%
547	Stramenopiles	*Staurosira*	0.02%	0.00%	0.04%
548	Stramenopiles	*Ulnaria*	0.30%	0.28%	0.40%
549	Stramenopiles	*Aulacoseira*	0.03%	0.00%	0.00%
550	Stramenopiles	*Melosira*	0.15%	0.04%	0.08%
551	Stramenopiles	*Tenuicylindrus*	0.00%	0.00%	0.02%
552	Stramenopiles	*Pteridomonas*	0.00%	0.00%	0.02%
553	Stramenopiles	*Goniochloris*	0.61%	0.31%	0.12%
554	Stramenopiles	*Monodus*	0.05%	0.02%	0.00%
555	Stramenopiles	*Pseudocharaciopsis*	0.58%	0.31%	0.36%
556	Stramenopiles	*Trachydiscus*	0.14%	0.08%	0.06%
557	Stramenopiles	*Vacuoliviride*	0.15%	0.03%	0.02%
558	Stramenopiles	*Vischeria*	0.02%	0.00%	0.00%
559	Stramenopiles	*Tetrasporopsis*	0.02%	0.00%	0.00%
560	Stramenopiles	*Bumilleriopsis*	0.14%	0.03%	0.00%
561	Stramenopiles	*Ophiocytium*	0.04%	0.02%	0.00%
562	Stramenopiles	*Tribonema*	0.03%	0.00%	0.00%
563	Stramenopiles	*Achlya*	0.03%	0.20%	0.10%
564	Stramenopiles	*Aphanomyces*	0.27%	0.88%	0.59%
565	Stramenopiles	*Apodachlya*	0.02%	0.00%	0.00%
566	Stramenopiles	*Halodaphnea*	0.00%	0.03%	0.02%
567	Stramenopiles	*Haptoglossa*	0.09%	0.08%	0.03%

no.	Eukaryotic supergroups	Eukryotic genera	Relative abundance		
			CKD	PUA	TD1
568	Stramenopiles	*Lagenidium*	0.03%	0.02%	0.08%
569	Stramenopiles	*Leptolegnia*	0.22%	0.18%	1.50%
570	Stramenopiles	*Olpidiopsis*	0.04%	0.10%	0.15%
571	Stramenopiles	*Phytophthora*	0.16%	0.00%	0.00%
572	Stramenopiles	*Pythium*	3.26%	0.56%	1.11%
573	Stramenopiles	*Salispina*	0.00%	0.00%	0.05%
574	Stramenopiles	*Saprolegnia*	0.06%	0.05%	0.35%
575	Stramenopiles	*Sapromyces*	0.02%	0.02%	0.00%
	unidentified taxa		43.45%	30.58%	27.82%

Table C.6 Identified fungal genera and their relative abundance obtained from ITS amplicon analysis from site TD1.

no.	Fungal phyla	Fungal genera	Relative abundance
1	Ascomycota	*Acremonium*	0.38%
2	Ascomycota	*Alternaria*	0.05%
3	Ascomycota	*Aquanectria*	0.01%
4	Ascomycota	*Aspergillus*	0.02%
5	Ascomycota	*Atractium*	0.01%
6	Ascomycota	*Candida*	0.21%
7	Ascomycota	*Capnobotryella*	2.54%
8	Ascomycota	*Cladosporium*	3.61%
9	Ascomycota	*Claussenomyces*	0.29%
10	Ascomycota	*Colletotrichum*	0.13%
11	Ascomycota	*Coniosporium*	0.03%
12	Ascomycota	*Cosmospora*	0.04%
13	Ascomycota	*Curvularia*	0.04%
14	Ascomycota	*Cyberlindnera*	0.01%
15	Ascomycota	*Cylindrocarpon*	0.01%
16	Ascomycota	*Cyphellophora*	0.05%
17	Ascomycota	*Debaryomyces*	0.02%
18	Ascomycota	*Devriesia*	0.06%
19	Ascomycota	*Didymella*	0.07%
20	Ascomycota	*Diutina*	0.06%
21	Ascomycota	*Erysiphe*	0.01%
22	Ascomycota	*Exophiala*	0.04%
23	Ascomycota	*Fusarium*	0.31%

no.	Fungal phyla	Fungal genera	Relative abundance
24	Ascomycota	*Fusicolla*	0.17%
25	Ascomycota	*Geosmithia*	0.02%
26	Ascomycota	*Gibberella*	0.07%
27	Ascomycota	*Gibellulopsis*	0.02%
28	Ascomycota	*Glutinoglossum*	2.33%
29	Ascomycota	*Hansfordia*	0.05%
30	Ascomycota	*Hirsutella*	0.01%
31	Ascomycota	*Knufia*	0.02%
32	Ascomycota	*Kodamaea*	0.02%
33	Ascomycota	*Microdochium*	0.01%
34	Ascomycota	*Murispora*	0.01%
35	Ascomycota	*Musicillium*	0.02%
36	Ascomycota	*Mycosphaerella*	0.08%
37	Ascomycota	*Nectria*	0.04%
38	Ascomycota	*Neodevriesia*	0.01%
39	Ascomycota	*Nigrospora*	0.05%
40	Ascomycota	*Ochroconis*	0.02%
41	Ascomycota	*Orbilia*	0.13%
42	Ascomycota	*Penicillium*	0.12%
43	Ascomycota	*Periconia*	0.06%
44	Ascomycota	*Phaeoacremonium*	0.01%
45	Ascomycota	*Phaeosphaeria*	0.13%
46	Ascomycota	*Phialemoniopsis*	0.04%
47	Ascomycota	*Phialophora*	0.02%
48	Ascomycota	*Phyllosticta*	0.03%
49	Ascomycota	*Plectosphaerella*	0.16%
50	Ascomycota	*Pseudocercospora*	0.04%
51	Ascomycota	*Pseudomassariosphaeria*	0.14%
52	Ascomycota	*Purpureocillium*	0.02%
53	Ascomycota	*Pyrenochaeta*	0.06%
54	Ascomycota	*Pyrenochaetopsis*	0.18%
55	Ascomycota	*Rhinocladiella*	0.02%
56	Ascomycota	*Roussoella*	0.03%
57	Ascomycota	*Saitoella*	0.17%
58	Ascomycota	*Sarocladium*	0.27%
59	Ascomycota	*Scedosporium*	0.02%
60	Ascomycota	*Setomelanomma*	0.03%
61	Ascomycota	*Setophaeosphaeria*	0.01%

no.	Fungal phyla	Fungal genera	Relative abundance
62	Ascomycota	*Setophoma*	0.05%
63	Ascomycota	*Sphaerulina*	0.02%
64	Ascomycota	*Stagonospora*	0.03%
65	Ascomycota	*Stephanonectria*	0.04%
66	Ascomycota	*Stilbella*	0.02%
67	Ascomycota	*Talaromyces*	0.05%
68	Ascomycota	*Thyronectria*	0.02%
69	Ascomycota	*Tingoldiago*	0.03%
70	Ascomycota	*Trichomerium*	0.06%
71	Ascomycota	*Uwebraunia*	0.02%
72	Ascomycota	*Valsonectria*	0.01%
73	Ascomycota	*Vermispora*	0.05%
74	Ascomycota	*Volutella*	0.02%
75	Basidiomycota	*Abortiporus*	0.03%
76	Basidiomycota	*Agaricus*	0.02%
77	Basidiomycota	*Amanita*	0.13%
78	Basidiomycota	*Boletus*	0.03%
79	Basidiomycota	*Bullera*	0.01%
80	Basidiomycota	*Bulleribasidium*	0.02%
81	Basidiomycota	*Calocera*	0.02%
82	Basidiomycota	*Clavulina*	0.07%
83	Basidiomycota	*Cryptococcus*	0.61%
84	Basidiomycota	*Derxomyces*	0.02%
85	Basidiomycota	*Dioszegia*	0.02%
86	Basidiomycota	*Erythrobasidium*	0.09%
87	Basidiomycota	*Exidia*	0.01%
88	Basidiomycota	*Gliophorus*	0.34%
89	Basidiomycota	*Goffeauzyma*	0.02%
90	Basidiomycota	*Grammothele*	0.01%
91	Basidiomycota	*Gymnopilus*	0.04%
92	Basidiomycota	*Hannaella*	2.40%
93	Basidiomycota	*Heterobasidion*	0.01%
94	Basidiomycota	*Hygrocybe*	0.04%
95	Basidiomycota	*Hypochnicium*	0.01%
96	Basidiomycota	*Inocybe*	0.96%
97	Basidiomycota	*Malassezia*	0.02%
98	Basidiomycota	*Microbotryozyma*	0.02%
99	Basidiomycota	*Microbotryum*	0.02%

no.	**Fungal phyla**	**Fungal genera**	**Relative abundance**
100	Basidiomycota	*Moesziomyces*	0.01%
101	Basidiomycota	*Occultifur*	0.10%
102	Basidiomycota	*Papiliotrema*	0.07%
103	Basidiomycota	*Phyllozyma*	0.02%
104	Basidiomycota	*Psathyrella*	0.02%
105	Basidiomycota	*Pycnopulvinus*	0.02%
106	Basidiomycota	*Rhodosporidiobolus*	0.09%
107	Basidiomycota	*Rhodotorula*	0.07%
108	Basidiomycota	*Saitozyma*	0.11%
109	Basidiomycota	*Sakaguchia*	0.01%
110	Basidiomycota	*Sampaiozyma*	0.06%
111	Basidiomycota	*Sirobasidium*	0.07%
112	Basidiomycota	*Sistotrema*	0.01%
113	Basidiomycota	*Sporisorium*	0.02%
114	Basidiomycota	*Sporobolomyces*	2.29%
115	Basidiomycota	*Symmetrospora*	0.11%
116	Basidiomycota	*Trechispora*	0.01%
117	Basidiomycota	*Tremella*	0.03%
118	Basidiomycota	*Vishniacozyma*	0.02%
119	Basidiomycota	*Wallemia*	0.05%
120	Chytridiomycota	*Alphamyces*	0.09%
121	Chytridiomycota	*Avachytrium*	18.57%
122	Chytridiomycota	*Cladochytrium*	0.02%
123	Chytridiomycota	*Delfinachytrium*	0.18%
124	Chytridiomycota	*Entophlyctis*	10.95%
125	Chytridiomycota	*Pateramyces*	0.03%
126	Chytridiomycota	*Powellomyces*	0.03%
127	Entomophthoromycota	*Basidiobolus*	0.01%
128	Entomophthoromycota	*Schizangiella*	0.10%
129	Glomeromycota	*Acaulospora*	0.03%
130	Glomeromycota	*Dentiscutata*	0.16%
131	Glomeromycota	*Diversispora*	0.04%
132	Mortierellomycota	*Mortierella*	0.06%
133	Mucoromycota	*Endogone*	0.04%
	unidentified taxa		48.43%

Table C.7 Identified fungal genera of *Cladophora* microbiota obtained from 18S rDNA and ITS amplicon analyses.

	18S amplicons			ITS amplicons
	CKD	PUA	TD1	TD1
22 fungal genera: *Acremonium*, *Aspergillus*, *Cladosporium*, *Hannaella*, *Helicascus*, *Mortierella*, *Nowakowskiella*, *Occultifur*, *Ochroconis*, *Paramicrosporidium*, *Paraphelidium*, *Phlyctochytrium*, *Pichia*, *Piromyces*, *Pyrenochaeta*, *Rhizophydium*, *Rhodotorula*, *Rozella*, *Sporisorium*, *Sporobolomyces*, *Vishniacozyma*, and *Wiesneriomyces*	✓	✓	✓	✓
14 fungal genera: *Acaulopage*, *Alternaria*, *Aphelidium*, *Arthrinium*, *Chaetospermum*, *Chytriomyces*, *Cochlonema*, *Emericellopsis*, *Fusarium*, *Galactomyces*, *Geotrichum*, *Gibellulopsis*, *Lentithecium*, and *Stylopage*	✓	✓	✓	X
4 fungal genera: *Neotestudina*, *Mycosisymbrium*, *Parastagonospora*, and *Symmetrospora*	✓	✓	X	✓
3 fungal genera: *Endogone*, *Malassezia*, and *Papiliotrema*	✓	✓	X	X
8 fungal genera: *Periconia*, *Pleospora*, *Preussia*, *Psathyrella*, *Rhizoctonia*, *Rhizophlyctis*, *Sarocladium*, and *Trichomonascus*	✓	X	✓	✓
17 fungal genera: *Arthrobotrys*, *Bipolaris*, *Circinotrichum*, *Corynespora*, *Cyphellophora*, *Diaporthe*, *Didymella*, *Erythrobasidium*, *Fereydounia*, *Geranomyces*, *Hyponectria*, *Knufia*, *Lignincola*, *Meira*, *Rhodosporidiobolus*, *Ustilago*, and *Westerdykella*	✓	X	✓	X
31 fungal genera: *Acrostalagmus*, *Agaricus*, *Ganoderma*, *Gymnopus*, *Hirsutella*, *Middelhovenomyces*, *Myrothecium*, *Nakaseomyces*/*Candida* clade, *Neolecta*, *Neophloeospora*, *Neournula*, *Ophiosphaerella*, *Parengyodontium*, *Pestalotia*, *Peziza*, *Phallus*, *Phellinus*, *Phialemoniopsis*, *Phymatotrichopsis*, *Plectosphaerella*, *Pleurotus*, *Ramicandelaber*, *Saccharomycopsis*, *Sebacina*, *Starmerella*/*Candida* clade, *Suberoteratosphaeria*, *Tapinella*, *Thanatephorus*, *Torula*, *Trichosporon*, and *Verticillium*	✓	X	X	✓
51 fungal genera: *Akanthomyces*, *Arthopyrenia*, *Barnettozyma*/*Candida* clade, *Biatriospora*, *Boletellus*, *Bradymyces*, *Bullera*, *Candida*/*Lodderomyces* clade, *Catenomyces*, *Cercospora*, *Ceriporia*, *Chaetomium*, *Clavispora*/*Candida* clade, *Clydaea*, *Colacogloea*, *Colletotrichum*, *Conidiocarpus*, *Coniophora*, *Conocybe*, *Conoideocrella*, *Coprinopsis*, *Dacrymyces*, *Derxomyces*, *Drechmeria*, *Elsinoe*, *Geastrum*, *Glomus*, *Gonapodya*, *Hanseniaspora*, *Helicoma*, *Hyphodontia*, *Hypoxylon*, *Kurtzmaniella/Candida* clade, *Lachancea*, *Lecanosticta*, *Leptosphaeria*, *Leucoagaricus*, *Lunulospora*, *Macrolepiota*, *Martiniozyma*, *Metschnikowia*, *Myrmecridium*, *Neophaeosphaeria*, *Olpidium*, *Septofusidium*, *Sistotrema*, *Spizellomyces*, *Talaromyces*, *Toxicocladosporium*, *Vermiconia*, and [*Sporobolomyces*] group	✓	X	X	X

	18S amplicons			ITS

				amplicons
	CKD	**PUA**	**TD1**	**TD1**
1 fungal genus: *Rhizoclosmatium*	X	✓	✓	✓
4 fungal genera: *Phaffomyces*, *Tilletiopsis*, *Trametes*, and *Tropicoporu*	X	✓	X	✓
4 fungal genera: *Craterellus*, *Cyberlindnera*/*Candida* clade, *Kodamaea*, and *Lentomitell*	X	✓	X	X
12 fungal genera: *Acaulospora*, *Hasegawazyma*, *Neokarlingia*, *Ochronectria*, *Phaeotheca*, *Physalacria*, *Polytrychium*, *Rhizophagus*, *Schizosaccharomyces*, *Spencerozyma*, *Sympodiomycopsis*, and *Yarrowia*	X	X	✓	✓
14 fungal genera: *Ambrosiozyma*, *Anthracoidea*, *Bulleribasidium*, *Candida*, *Dendrochytridium* , *Donkia*, *Filobasidium*, *Heterodoassansia*, *Hyphodermella*, *Kohlmeyeriopsis*, *Lecophagus*, *Mariannaea*, *Microbotryum*, and *Trechispora*	X	X	✓	X
51 fungal genera: *Goffeauzyma*, *Grammothele*, *Gymnopilus*, *Hansfordia*, *Microdochium*, *Moesziomyces*, *Murispora*, *Musicillium*, *Mycosphaerella*, *Nectria*, *Neodevriesia*, *Nigrospora*, *Orbilia*, *Pateramyces*, *Penicillium*, *Phaeoacremonium*, *Phaeosphaeria*, *Phialophora*, *Phyllosticta*, *Phyllozyma*, *Powellomyces*, *Pseudocercospora*, *Pseudomassariosphaeria*, *Purpureocillium*, *Pycnopulvinus*, *Pyrenochaetopsis*, *Rhinocladiella*, *Roussoella*, *Saitoella*, *Saitozyma*, *Sakaguchia*, *Sampaiozyma*, *Scedosporium*, *Schizangiella*, *Setomelanomma*, *Setophaeosphaeria*, *Setophoma*, *Sirobasidium*, *Sphaerulina*, *Stagonospora*, *Stephanonectria*, *Stilbella*, *Thyronectria*, *Tingoldiago*, *Tremella*, *Trichomerium*, *Uwebraunia*, *Valsonectria*, *Vermispora*, *Volutella*, and *Wallemia*	X	X	X	✓

Table C.8 Shared microbiota of freshwater *Cladophora.*

	Nan River (This study)	**Lake Mendota (Braus et al. 2017)**	**Lake Michigan (Chu et al. 2017)**	**Lake Mendota (Graham et al. 2015)**
17 bacterial genera: *Flavobacterium, Pseudorhodobacter, Pseudomonas, Terrimonas, Chryseobacterium, Bdellovibrio, Deinococcus, Hyphomicrobium, Rhodobacter, Altererythrobacter, Novosphingobium, Sphingopyxis, Acidovorax, Aquabacterium, Hydrogenophaga, Methylotenera,* and *Acinetobacter*	✓	✓	✓	✓
15 bacterial genera: *Arenimonas, Paludibacter, Ferruginibacter, Pseudarcicella, Porphyrobacter, Comamonas, Limnohabitans, Pelomonas, Rhodoferax,* AAP99, *Aeromonas, Cellvibrio,* NOR5/OM60 clade, *Luteimonas,* and *Luteolibacter*	✓	✓	✓	X
17 bacterial genera: *Paludibaculum, Lewinella, Haliscomenobacter, Phaeodactylibacter, Fluviicola, Meiothermus, Gemmatimonas, Haliangium, Planctomyces* sp. SH-PL14, *Hyphomonas, Sphingomonas ,Lautropia, Leptothrix, Rhizobacter, Rubrivivax,* mle1-7, and *Dechloromonas*	✓	✓	X	✓
57 bacterial genera: *Armatimonas, Bryobacter,* CL500-29 marine group, *Ilumatobacter, Aurantisolimonas, Dinghuibacter, Flavisolibacter, Lacibacter, Cytophaga,* OLB12, *Arcicella, Emticicia, Lacihabitans, Runella,* OM27 clade, *Chamaesiphon, Cyanobium ,Truepera,* P3OB-42, *Phaselicystis, Pajaroellobacter,* Planctomycetes SM1A02, *Fimbriiglobus, Blastopirellula, Pirellula, Roseomonas, Hirschia, alphaI* cluster, *Pedomicrobium, Phreatobacter,Gemmobacter, Tabrizicola, Sphingorhabdus, Limnobacter, Chitinibacter, Ideonella, Inhella, Polaromonas, Ramlibacter,* Ellin6067, CM1G08, *Massilia, Noviherbaspirillum, Candidatus* Accumulibacter, *Niveibacterium, Propionivibrio, Sulfuritalea, Rheinheimera, Acidibacter, Methylocystis, Methylovulum,* BD1-7 clade, *Ahniella, Lysobacter, Chthoniobacter,* DEV114, and SH3-11	✓	✓	X	X
9 bacterial taxa: *Bacillus, Vogesella,* Christensenellaceae R-7 group, *Bacteroides, Mesorhizobium, Sphaerotilus, Klebsiella, Plesiomonas,* and *Shewanella*	✓	X	✓	✓
16 bacterial taxa: *Acidaminobacter, Fusibacter, Nocardioides, Prevotella* 9, *Exiguobacterium, Clostridium sensu stricto* 1, *Clostridium sensu stricto* 12, *Anaeromusa and Anaeroarcus, Devosia, Rhizobium, Shinella, Vogesella, Curvibacter, Delftia, Ottowia,* and *Paucibacter*	✓	X	✓	X
20 bacterial taxa: *Mycobacterium, Chloroflexus, Porphyromonas, Desulfovibrio, Haloferula,* IMCC26207, *Alistipes, Hassallia, Cloacibacterium, Herpetosiphon, Erysipelothrix, Lachnoclostridium, Anaeromyxobacter, Chromobacterium, Leeia, Azoarcus, Thauera, Treponema,* IMCC26134, and *Lacunisphaera* **4 eukaryotic taxa:** *Spirogyra, Pseudopediastrum, Cercomonas,* and *Aphanomyces*	✓	X	X	✓

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