Final Project Evaluation Report

Your Details	
Full Name	Tran Thi Lan Anh
Project Title	Investigating diversity and ecology of indigenous green seaweed *Ulva* (Chlorophyta, *Ulva*ceae) in Southwest regions, Vietnam for future bioremediation purposes
Application ID	23562
Grant Amount	4875£
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1. Indicate the level of achievement of the project's original objectives and include any relevant comments on factors affecting this.

Objective	Not achieved	Partially achieved	Fully achieved	Comments
Field sampling			■	Three sampling campaigns were conducted in the course of this project including one pilot sampling and two subsequently seasonal samplings, covering both natural and aquaculture settings. The availability of *Ulva* spp. does not depend only on seasonality but also by current farming activities. For several shrimp farms with intensive aquaculture activities, we could not gain access easily. Nonetheless, we managed to access or to choose alternative sites with support from our local assistants, so our sampling was followed almost exactly as what we planned. Over 80 *Ulva* samples were collected.
Morphological observation			■	Since morphology of *Ulva* species is flexible, changing according to environmental conditions and the developmental stages, we recorded in-situ environmental characteristics to assist further identification. Subsequently, morphological observation was carried out at Institute of Tropical Biology using Olympus BX41 microscope. However, other characteristics that we want to observe seems be changed according to preservation condition, particularly a long preservation in dark and cold condition. Therefore, we noted these conditions and preservation period as reference for morphological comparison afterward. Herbarium of these samples are deposited at aforementioned institute.
DNA-based identification		■		A piece of each sample is preserved in silica gel for genetic analysis at Phycology Research Group, Gent university. DNA was successful amplified for 95% of our samples. However, the identify of most of the sequences could not be revealed since one sequence can match with more than one names in GenBank (https://www.ncbi.nlm.nih.gov/genbank/). It reflects the remaining problem of *Ulva*

				taxonomy due to their simple morphology and the shortage of genetic information of *Ulva* in tropical region. Therefore, we could only be able to identify three *Ulva* species which are *U. tepida, U. meridionalis* and *U. limnetica* in our Vietnamese *Ulva* samples. There are four remaining distinct genetic clusters of *Ulva* in Ca Mau and Kien Giang. We will continue spending effort in assigning names on these clusters and preparation for publication to widen the circulation of information.
Dissemination of the result				We will send our final report to inform different stakeholders involved in our sampling. We aimed to publish our results on a local journal and integrate it to a larger dataset for an international journal. However, due to the difficulty arose in DNA-based identification, we could not achieve this result within the course of our project. We are confident that we will be able to publish our result next year.

2. Please explain any unforeseen difficulties that arose during the project and how these were tackled.

During our project, we encountered two main obstacles preventing us from achieving our project goals which occurred during our sampling and DNA-based identification work.

For our sampling campaigns, we had identified our sampling sites where the occurrence of *Ulva* species was recorded. However, when we were collecting *Ulva* samples, the owners of several shrimp farms did not allow us to enter. Since there are on-going farming activities at these sites and the owner thought that our sampling will affect their farming. We had anticipated this obstacle because there is limited awareness of people in some remote regions of Vietnam, so we were prepared to convince the farm owners together with our local assistance. However, this process was more difficult and consumed more time than we expected. In addition, three provisional sampling sites which were observed the occurrence of *Ulva* spp. had no *Ulva* at the time we sampled because the owner either had cleaned their ponds or had treated water with chemicals to prevent the growth of macrophytes in their shrimp ponds to control the productivity. For these difficulties, we switched to ponds nearby provided that there were *Ulva* species growing there.

The most difficult challenge that we encountered was, to be honest, an unexpected obstacle. We expect that DNA-based identification will help us identify well *Ulva* species since this method has been successfully employed in many studies of *Ulva* species. We did not face any big problem until, nonetheless, the last step which is identification by matching our sequences to public sequences on GenBank. Each sequence can match with sequences having different names. It is owing to the fact

that there is a taxonomic problem remained in some groups of *Ulva* species and the lack of quality control for sequences uploaded on GenBank database. Among seven clusters, 1 cluster did not have a close match with any sequences on GenBank. It might be because of the lack of reference database for tropical *Ulva*.

We finally decided to manually curate the sequences we retrieved from GenBank. There are three sequences matched to our sequences which have high level of confidence. Therefore, we decided to apply these names, which are *U. tepida, U. meridionalis* and *U. limnetica*, to our samples. The remaining samples were just temporarily divided to distinct genetic clusters.

3. Briefly describe the three most important outcomes of your project.

a) Improve understanding:

Current aquaculture practice in these two provinces releases a huge amount of wastewater to the environment, possibly posing harmful effects to regional biodiversity. Aquaculture practice in the area are mainly at family scale and are operated based on farmers' experience or based on techniques introduced in local workshops. There is a lack of monitoring the input of chemicals, pesticides, growth hormones and antibiotics into aquaculture systems. Moreover, aquaculture facilities there are poor and often lack of an extra pond for water treatment before releasing wastewater to the environment. However, almost all farmers we met did neither monitor the water quality in their pond regularly nor aware of the harmful effects that waste water discharged from their aquaculture practice would pose towards the environment. Yet, aquaculture is the main source of income to many households there and it has been practiced for many years. The measurements of the water quality at 53 sites during two sampling seasons shows that water parameters including salinity, pH, nitrate (NO_3^-) and ammonium (NH_4^+) are suitable for aquaculture practice (Boyd 1998). However, the nitrate level in three sites at sea in Kien Giang and 16 sites in Ca Mau (11 aquaculture ponds and five channels) are higher than the nitrate criteria for conservation of aquatic resources (0.2 mg/L - QCVN 10-MT:2015/BTNMT) in dried season. Similarly, for ammonium, its concentration was detected at higher level than Vietnamese standard at eight aquaculture ponds, in which most of them are intensive aquaculture ponds, and four channels in dried season (0.3 mg/L - QCVN 10-MT:2015/BTNMT). For phosphate (PO_4^{3-}), 15 ponds in dried season and the other 4 ponds in rainy season were observed to have higher phosphate than the standard recommended for aquaculture (Boyd 1998). Particularly, three sites locate in Kien Giang and 16 others situate in Ca Mau. In natural environments, 17 sites, including eight sites in Kien Giang and nine sites in Ca Mau, have higher phosphate concentration than the phosphate criteria for conservation of aquatic resources in dried season. No site was observed with elevated phosphate level compared to the standard in rainy season. We showed local farmers the negative effects of overusing chemicals and additive food in aquaculture and encouraged them to practice monitoring water quality regularly. We had informed part of these results (in rainy season – collected during our first campaign) to their group leaders regarding the current status of pollution and possible negative effects pollutions can cause to the environment.

b) **Raising awareness**:

We introduced to every farmer that we met about Integrated multi-trophic aquaculture (IMTA) as a simple and low-cost technique but sustainable practice that has potential to replace their current practice. Many young farmers were interested in our idea because it is a solution to harmony their needs and remediate pollution to further sustain regional biodiversity. On the one hand, *Ulva* species can absorb excess nutrient from wastewater of shrimp production activities, thus reducing the nutrient load discharged into the environment. On the other hand, IMTA is easy to install and might help them to improve their life by increasing shrimps' quality or providing to them a by-product which is *Ulva* biomass collected after the purification of extra nutrient from water column.

c) **Establish the first DNA-based diversity of *Ulva* spp. in the region**:

To facilitate the development of IMTA, the first step is to understand the diversity of *Ulva* spp. in Ca Mau and Kien Giang. Our project is the first and comprehensive study of *Ulva* spp. diversity in Ca Mau and Kien Giang employing genetic tool. It reshapes out understanding about *Ulva* diversity in the region. Our analysis based on three genes which includes elongation factor Tu (tufA), ribulose bisphosphate carboxylase large subunit (rbcL), and internal transcribed spacer (ITS) suggests that there are seven *Ulva* species occurring in Ca Mau and Kien Giang which are *U. limnetica*, *U. meridionalis, and U. tepida* and four other unnamed *Ulva* which show close relationship to *U.* simplex, two sub species in the *U.* flexuosa group and one species which potentially an undescribed species. All most all the species occurring there was not mentioned in our textbook of Algae diversity of the South of Vietnam. Details of these species are described as follow:

1. *U.* tepida: green, branching from the basal region and then widening in the upper part. Thalli are up to 4 cm height and 8 mm width. Mainly found attached on rock or sand beach.
2. *U.* mediorinalis: light green to yellowish, branching from the basal region and then gradually widening in the upper part. Thalli are up to 6 cm height and 5 mm width. Mainly found attached on rock or sand beach.
3. *Ulva* sp1: green, filamentous, branching from the basal region; basal and thallus are at the same size. Species are up to 2 cm height and 1 mm width. Mainly found attached on rock.
4. *U. limnetica*: tubular, having many small branches along the main thallus, fragile and wrinkled, often found floating in brackish channels having salinity from 2 to 24 ppt.
5. *U. flexuosa* sub sp1: green, filamentous, branching at the basal part. Thalli are slightly bigger than rhizoids, having width ranging from 2-4 mm and can have up to 20 cm long. Found both in aquaculture pond and stagnant channels.
6. *U. flexuosa* sub sp2: having similar morphology to *U. flexuosa* sub sp1. Found in aquaculture ponds.
7. *Ulva* sp2: green, filamentous, branching at the basal part. Thalli and rhizoids have similar size, having width ranging from 1-3 mm and can have up to 14 cm long. Only found 1 record in an abandon pond.

My project acted as a baseline and initial steps assisting further studies on potential of *Ulva* in these area for sustainable aquaculture practices. For ITMA, the first criterion is to choose the algal species from native flora to minimize the impacts that can be resulted from introduced species. Among the species recorded in our investigation, *U. tepida* and *U. meridionalis* are the most abundant species occurring in both rainy and dried seasons. Thus, they have a potential to be used in bioremediation and will be used in our experiment to test for their nutrient extraction efficiency.

4. Briefly describe the involvement of local communities and how they have benefitted from the project.

Our project was carried out in many different areas of Kien Giang and Ca Mau provinces. During our work, we, together with the local guide, had talked to many farmers of the shrimp ponds that we visited to gain knowledge about their current practices, their understanding about regional biodiversity and the effect of unsustainable aquaculture activity to regional biodiversity, and their willingness to improve their current aquaculture practice. We had shared our knowledge about the effect of unsustainable aquaculture practice to biodiversity, had showed and had encourage them to apply some feasible practices of sustainable aquaculture.

We had shared to local governors and farmers we met about our idea of ITMA. They, mainly local governors and young farmers were highly interested in our idea and were willing to participate in our further activities. Therefore, we are planning to engage them in our future project, which composes of trial implementation of ITMA in the region and bring ITMA into practice.

5. Are there any plans to continue this work?

Our next step will be investigation on which *Ulva* species/strain is most fit for bioremediation purposes in the southwest of Vietnam. Initially, we will use *U. tepida* and *U. meridionalis* to perform our lab experiment. Criteria to select the most suitable species includes their efficiency in extracting excess nutrient (nitrate and phosphate) from water column and their tolerance to environmental changes. Species which perform better will be induced for gametes/spores which subsequently will be seeded on ropes. After 2-4 weeks, these ropes seeded with young thalli of *Ulva* will be implemented in trial phases in situ at selected aquaculture ponds in Ca Mau and Kien Giang areas to test for its effectiveness in removing excess nutrients in water column.

6. How do you plan to share the results of your work with others?

This final report and all the progress reports from the project will be shared to the community and various stakeholders working in the area.

We will send a summary reporting regional water quality and *Ulva* diversity to local governors, the head of the farmers and the farmers that we met during our project. The diversity of *Ulva* recorded in this Rufford project is integrated to a bigger dataset of Vietnamese *Ulva* to public in a peer-review journal. We are preparing the manuscript and it will be submitted next year. If possible, these data will be included

in a guidebook about seaweed in Southwest sea of Vietnam. The result of the publications will be informed to RSG when they are published.

7. **Timescale: Over what period was the grant used? How does this compare to the anticipated or actual length of the project?**

The implementation of this project was proposed to be used during the period from December 2017 to December 2018. Timely delivery and implementation of all the activities except for the DNA-based identification were accordingly to the provisional plan thanks to the contribution of all the project team members. We had 10 months extension of our project due to the difficulty arose in the DNA-based identification.

8. Budget: Provide a breakdown of budgeted versus actual expenditure and the reasons for any differences. All figures should be in £ sterling, indicating the local exchange rate used. It is important that you retain the management accounts and all paid invoices relating to the project for at least 2 years as these may be required for inspection at our discretion.

Item	Budgeted Amount	Actual Amount	Difference	Comments
Consumables (Materials and other tools for fieldwork, herbarium collection, DNA collection, DNA extraction, PCR)	300	102	-198	We made use of available facilities at Phycology Research Group, Gent university for DNA extraction and PCR so we did not purchase materials for these purposes.
Lab expenses	900	642	-258	We had sequenced more samples (4 plates) due to the indistinguishable of *Ulva* morphology. However, we got the cheaper price for sequencing, so the final cost is less than what we expected.
Field equipment DR890 Multiparameter Portable Colorimeter = 800£ Phosphate test (1 pack - 100 tests x 1) = 20£ Nitrate test (1 pack - 50 tests x 2) = 130£ Nitrite test (1 pack - 50 tests x 3) = 85£ Ammonia test (1 pack - 50 tests x 3) = 140	1175	1138	-37	We decided not to measure nitrite concentration in the water, so we did not purchase nitrite test. The price of phosphate test was higher than our estimation.

Digital camera		328	+328	Our camera was accidently felt and was broken during our first field trip, so we had to buy one to replace it.
Transportation (from our institute to the field): 2 times travel to the field in the work team of 2 persons Price is estimated for 1 field work (10 days): Transportation (Bus, motorbike, boat) 500£ Accommodation 2 persons = 260£ Allowance (2 persons + 1 field assistance) = 250£	2020	2020		
Field work in advance: For selecting sampling plots Transportation (Bus, motorbike, boat) 250 £ Accommodation 2 persons x 5 days = 130 Allowance (2 persons x 5 days) = 100 £	480	477	-3	
Total	**4875**	**4707**	**-168**	**1 GBP = 30.533 VND**

9. **Looking ahead, what do you feel are the important next steps?**

Moving forward, focusing on raising awareness of stake holders involving in management and shrimp farming should be one of our priorities considering the low perception of farmers on environment and biodiversity conservation. Although many farmers show their interests in our work, it is difficult to change their mindset as well as the practice that they do for many years. Thus, to facilitate our implementation of ITMA in Ca Mau and Kien Giang, we defined raising awareness of local farmers is also one of our priorities together with our previous plan.

10. Did you use The Rufford Foundation logo in any materials produced in relation to this project? Did the Foundation receive any publicity during the course of your work?

We introduced to people we met during our sampling campaigns that our work is sponsored by Rufford foundation which is a foundation dedicated to nature conservation. As aforementioned, we will also mention Rufford Foundation in our prepared manuscript about Ulva diversity of Vietnam as one of our sponsors.

11. Please provide a full list of all the members of your team and briefly what was their role in the project.

Tran Thi Lan Anh: coordinator of all the activities related to the project including fieldwork, lab work, reporting and financial management.

Nguyen Van Tu: assisted our paper works, reviewing our sampling plan and facilitating our work at Institute of Tropical Biology.

Tran Van Tien: main coordinator the field surveys. He also helped in data collection and data analyses including the analysis of water quality and morphological observation of *Ulva* samples.

Le Thi Trang: assisting us on our second sampling campaign.

Lu Anh Pha: local guide who advised us on our sampling design and assisted us during our sampling campaigns in Kien Giang province.

Le Si Hieu: local guide who advised us on our sampling design and assisted us during our sampling campaigns in Ca Mau province.
Working together, we were able to achieve most of the project objectives. Additionally, we would like to thank Sofie D'hont and Professor Olivier De Clerck, Gent university for their support for molecular analyses.

12. Any other comments?

We wish to express our wholehearted thank The Rufford Foundation for their financial support which is crucial for the success of our project. We look forward to more engagement with Rufford Foundation in the future to promote the conservation activities in Vietnam.