

## Sequence-based Phylogeography of Seaweeds: How Current Distribution is Shaped by Accumulation of Past?

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### Abstract

State-of-the-art techniques of phylogeography are now routinely used to assess changes at DNA level accumulated over time, and thereby to study forces that might have influenced distribution patterns of organisms. Presented in this mini-review is the works on phylogeography of edible green seaweed *Monostroma* in Japan and how the current distribution pattern of this algae might have been influenced with the history of medieval Japan. Also presented herein is the striking revelations in ancient Japanese *waka* – a form of short poetry – that documents historical distribution patterns of this algae, and how these are congruent with findings in phylogeography reconstruction.

Key words: Kuroshio Current; *hitoegusa*; *Monostroma kuroshiensis*; phylogeography; waka; haiku; India

### Introduction

One of my favorite saying is “Sometimes Taking Time is a Shortcut” by Haruki Murakami in his recent work “What I talk about when I talk about running”. It took almost 8 years since I got my master’s degree in 2002 for completing my Ph.D. in 2010. However, as I now contemplate, it was a Ph.D. well worth pursuing for, and in a topic I passionately wanted to work in.

I had been to Japan as Japanese Govt. *Monbukagakusho* Scholar in 2005, nominated by Ministry of Human Resources and Development, Govt. of India and registered for Ph.D. at the graduate school of Kuroshio Sciences, Kochi University. As part of the compulsory coursework, I remember having a week-long seminar series on Kuroshio Sciences at Kochi University Agricultural Sciences campus where I came to know how vast the field of Kuroshio Science really is; encompassing knowledge disciplines including human biology, economics, history, oceanography and marine biology; spanning across the entire university system. I was altogether fascinated with this interdisciplinary nature of my graduate school; unification of knowledge from all spheres. This experience would soon set as an inspiration for my later works on linguistic phylogeny-application of computational phylogenetics in inferring evolutionary heritage of languages, which indeed is a

marriage between computational biology and linguistics. Before then I was having an impression that the sole purpose of this graduate school is to study Kuroshio Current – the warm water Western Pacific oceanic current – which comes under the scope of oceanography. In a way, the purpose and the current direction of this graduate school emphasizes how intricately forces of nature like Kuroshio Current influences humanity as a whole. During the course of my Ph.D. programme I got opportunities to visit some of the renowned institutions of higher education and research in Marine Biology, including Hopkins Marine Station of Stanford University, Monterey Bay Aquarium Research Institute in California, Friday Harbor Laboratory of University of Washington and Natural History Museum in London. I must affirm that the facilities and the kind of professional atmosphere that I experienced at Kuroshio Sciences Graduate School was indeed world-class, and no doubt the best research laboratory I had ever been to.

### Phylogeography of *Monostroma* in Japan

No civilizations like that of Japan exist anywhere in which seaweeds are so much integrated into gastronomy, culture and literature. Needless to say Japan is the epicenter for phycological research, with most of the well-known phycologists in the world hailing from Japan. I

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started my works on various aspects of *Monostroma latissimum* (green seaweed referred as *hitoegusa* in Japanese) distributed in Tosa Bay, Kochi Prefecture. This edible seaweed ranks second, next to “nori” (*Porphyra*), in terms of net commercial cultivation and market value in the world, however, remain absolutely obscured in scientific literature. My pioneer studies on life histories, seasonality (Bast *et al.* 2009c), ecophysiology, sex ratios (Bast *et al.* 2009a), ontogeny (Bast *et al.* 2009a; Bast and Okuda 2010) and DNA barcoding lead to a general hypothesis on how marine green algae respond to a changing saline environment. I serendipitously discovered a green alga near Inoshiri marina at Uranouchi Inlet, Tosa Bay which is the only asexually reproducing monostromatic algae reported in the world till date (Bast *et al.* 2009b). This alga, to my surprise, belong to the same species of sexually reproducing *Monostroma*, as evidenced by DNA barcoding at ITS (Internal Transcribed Spacer) and *rbcL* (RUBISCO Large subunit) genomic loci. This discovery had a profound impact on understanding life history evolution in eukaryotes, as this is the first report of any eukaryotes having sexual and asexual life histories with no substitutions in highly variable ITS sequences. In other words, this study reported “existence of two different lifecycles in the algae belong to the same species” (as an analogy to the famous Darwin’s similarly entitled work on flowering plants (Darwin 1877)). My later investigations sought to understand factors responsible for phylogeographical patterns and revealed an emerging sympatric speciation in the Japanese algal isolates (Bast 2010). Phylogeographical studies under robust statistical framework of Bayesian Inference lead to a conclusion that *Hitoegusa* originated in Ise Bay, Mie prefecture and spread elsewhere in Japan, as evidenced by greater genetic heterogeneity of the isolates collected from this area (Bast 2010). This finding could also be related to the history of Japan with areas covering Ise Bay having played a major role in the cultural upheaval of Japanese Empire, with largest port in Japan, Nagoya and holiest imperial Shinto shrine in the world, Ise Grand Shrine, situated on the banks of this bay.

The topic on the phylogeography of *M. latissimum* indeed helped to satiate my inner desire to travel throughout Japan; I had travelled almost every prefectures right from Hokkaido in North-East to Kagoshima in South-West in *futsu densha* (=ordinary trains; being a railway enthusiast, I absolutely loved them). No one other than passionate *ferroequinophiles* in Japan might had really been to a little-known train station, Maibara – set aside as a brief stopover in Bullet Trains – where I spent eight long nights on various occasions for transfer

between JR (Japan Railway)-Central and JR-West in Tōkaidō Main Line! Sampling expeditions using JR’s *Seishun Jyuhachi Kippu* (Youth-18-Ticket) gifted me fond memories of places and people that I came across, that would etch in my mind rest of my life. This included overnight stay at Arao bus stop (Kumamoto Prefecture), amid chilling December Kyushu, but with distant beating of the surf-a lullaby to be soon interrupted by cops who wanted to see my “Alien” card! (In Japan, foreigners are termed “Aliens” with the official “Alien registration cards” issued by the government to all of its foreign nationals). I also remember near-death experience while on search of seaweed, when I realized I’m going deeper into the mud at Kiire Cho river mouth, Kagoshima Prefecture, and efforts to retract from this “hell”-which is reminiscent of *Jigoku Meguri* that I have taken at Beppu-is in fact taking me more and more deeper to the other world! When a couple of old fishermen came to rescue my ill-fate with sticks and ropes, I felt I am born once again.

During my days in Japan I had been an avid *Haiku* lover (one of my creation appeared in *Mainichi Shinbun* (Bast 2009)), probably only form of poetry that enthralls me even today. Needless to say that my choice of phylogeography sampling sites was also influenced by Haiku. For example, I came across Suma, Kobe Prefecture first time from *Genji Monogatari*:

“The world of fisher folk:  
Might I hear it from afar?  
On the beach at Suma,  
Seaweed-salt droplets fell,  
For who, if not you... “

*Monostroma latissimum*, which I collected from Suma when I went to this place, neither did not grow in the culture attempt nor gave any PCR-amplifiable genomic DNA, unfortunately indeed. On the other hand, I first came across Ise from Gosenshu Waka:

In the sea at Ise  
A frolicking fisherman is what  
I would become, for then  
I’d plunge between the waves  
To harvest (*hitoegusa*) algae-and a glimpse of you.  
- Ariwara no Narihira (825-880)

And soon DNA sequence of the isolates that I collected from Ise Bay (Toba and Matsusaka) would reveal how this holy place had been the center of *Hitoegusa* cultivation in the days of yore and how it got introduced elsewhere in Japan! A number of references on the “Jeweled Seaweed” (*Tamamo*, 玉藻 can be seen in medieval haiku texts which most probably refers *M. latissimum*

*simum*, because of the distinct iridescent green sheen of this seaweed resembling emerald (*Ryoku gyoku*, 緑玉) upon drying. This epithet have also influenced me to title my first book that I published in 2011 as “*Monostroma*: The Jeweled Seaweed for Future” that deal with its cultivation methods, ecophysiology and phylogeography (Bast 2011). A critical analysis of medieval *Waka* with respect to importance of seaweeds in Japanese literature, culture and even romance has recently been accepted for publication (Bast 2013b).

### Renaming the alga as a homage to Kuroshio Science

A quick glance at Fig. 1 will reveal an interesting trend in the locations of sampling collections that I have included in phylogeographic assessment of *Monostroma latissimum*; that all these sites are located on Kuroshio coast. Reason is clear; this alga have never been documented either in Oyashio or in Tsushima current regions and therefore distribution of which is presumed to have something to do with warm-water Kuroshio Current. However, my phylogeographic studies could not relate routes of dispersal of this alga with the direction of Kuroshio Current and therefore it is highly likely that this edible species got deliberately introduced elsewhere

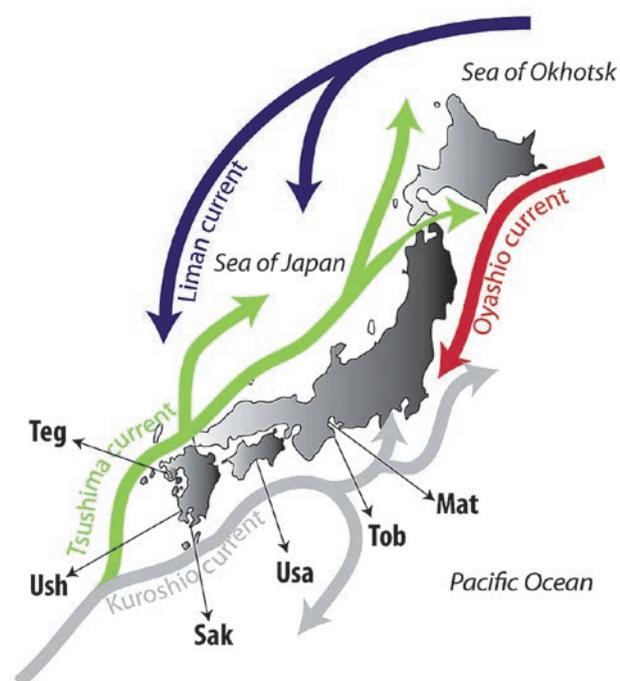


Fig. 1. Map indicating sampling locations of isolates included in my Ph.D. study and major oceanic currents in the region. Teg=Teguma, Ush=Ushinohama, Sak=Sakurajima, Tob=Toba and Mat=Matsusaka. Directions of the currents as per JAMSTEC (2009). Note that *Monostroma kuroshiensis* is distributed only along Kuroshio Current coast.

from Ise Bay for the cultivation purposes. Careful review of taxonomy of this species by following step-by-step revisions – mostly in either Swedish or French – lead to a conclusion that *Monostroma latissimum* is an illegitimate taxonomic synonym of *Monostroma nitidum* (as per Bliding’s synonymy in place (Bliding 1963)). Japanese alga commonly referred to as *hitoegusa* is indeed a new species, which is different from *Monostroma nitidum* Wittrock (Swedish species) in a number of ways, including culture characteristics (thallus ontological patterns) and multi-local molecular assessment (DNA sequence homology at ITS1, 5.8S, ITS2 and *rbcL* loci). Having convinced that this species is new and yet to be named, I was absolutely excited to search for a new name for it, like a father looking for a name for his newborn child! I then befittingly realized that the credit for discovering this species-albeit wrongly as *M. latissimum*-should be given to Yendo who in 1917 working at Mie Prefectural Marine Laboratory described this species for first time from Ise Bay, Japan (Yendo 1917). Given its unique biogeographic distribution pattern that is confined only to Kuroshio Coast, I renamed this species as *Monostroma kuroshiensis* (Yendo) Bast *et al.* Sp. Nov., as a homage to Kuroshio Sciences, and involuntarily as a tribute to my own and very special eponymous graduate school!

### Concluding Remarks

I am now mid-way through Murakami’s IQ84, lost in the dream-like historical fiction (or history and fiction?), and wondering what would happen to *Aomame* and *Tengo*! Influence of my life and time in Japan had been enormous for my later years. I would soon marry my dream (means “*Swapna*” in Malayalam!) Railway-Environmental Engineer, name my daughter as Haritha (or *Midori*, Green!) as a tribute to nature in general (Bast 2013a) and green seaweeds in particular. My research is now targeted to systematically catalogue seaweed diversity and phylogeography in India, with multi-faceted approach that I learned as a student for first time in Kuroshio Sciences Graduate School, encompassing culture studies, ultrastructural microscopy, morphometry and DNA barcoding. India is a big nation- seventh largest in the world. I remember most of my Japanese friends being skeptic about my claim that whole of the Western Europe will go inside India area-wise! Unfortunately seaweeds in India remains one of the poorly documented plant groups and enormous potential exists in cataloguing its cryptic biodiversity. Some of the algae that I have collected – barcoding and phylogenetics of which is



Fig.2. Seaweed diversity in India. Collected, identified and photographed by Felix Bast © 2013.

presently progressing – are presented in Fig. 2.

I am looking at forces of nature that might have shaped the current distribution patterns of these aquatic plants, by quantifying and analyzing molecular changes accumulated over time. As Murakami said it right:

“What we call the present is given shape by an accumulation of the past.”

— Haruki Murakami, 1Q84

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