

Explore genetic relationship of plants along Suzhou creek through DNA barcoding

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Abstract

Suzhou Creek is a river that flows into Huangpu River. The origin of it is the Lake Tai, which locates in Jiangsu province. As the Suzhou Creek flows through this wide range, the genetic relationship of plants along Suzhou creek could be a feasible topic to be explored by utilizing DNA barcoding technique. Here, we collected several plant specimens from two specific locations on the river bank. Comparing PHYLIP ML to construct systematic evolutionary relationship between allopathy plant specimens. The eliminated aim was to demonstrate the influence of water flow on plant migration by comparing morphological and genetic similarities in the herbarium specimens.

Introduction

The total length of Suzhou Creek is 53.1km, it seemed impossible to propagate offsprings in this wide range for these rooted plants. It is difficult to identify these plants belong to which species or genus by observing them. However, DNA barcoding technique now has been invented for species identification that even non-professionals could utilize it and contribute to modern taxonomy. The integral protocols sound easy to understand, collecting plants samples, then do PCR amplification and DNA sequencing. The Barcode of Life Datasystem(BOLD) is a recommended platform to compare DNA sequencing result and existing data of herbarium in database.

For us, we choose to use DNA subway to generate precise DNA sequences, the website will analyze DNA sequences in a more intelligent way. For instance, using 'sequence trimmer' is a way eliminate the need for manual inspection, automatically cut out inappropriate DNA segments. Two plastidial genes *rbcl* (ribulose-1,5- bisphosphate carboxylase/oxygenase) and *matK* (maturase K) were adopted as core primers.

In a word, our purpose is to identify species of plants and explore genetic relationship by analyzing phylogenetic tree. Meanwhile, we suppose that geographical discrepancy might affect the evolutionary course of plants, but objectively this can be interfered with by various external factors.

Materials & Methods

Purposed in collecting plant specimens of upstream and downstream of the Suzhou Creek. Sampling spots were parks along the Suzhou Creek in Suzhou and Shanghai. More specifically, at grove along the river. Based on quadrat method, on Jul 24, we collected 28 specimens in 7 quadrats separately. Collected as whole plants and several leaves. They represent the biological feature of each place. Mostly, the features of upstream and downstream. Every specimen was photographed for record and plant AI identification. Then specimen's information was recorded as a sheet. All samples were stored with temperature of 277K for 3 days. Later, DNA extraction was produced at DNALC.

Extract DNA of YRT-001 to YRT-028 using rapid method. Prepare PCR primer mix of *rbcl* primer. Add 2μL of extracted DNA TE solution into primer mix, then produce PCR. PCR stages including: exponential amplification, leveling off stage, plateau^[4]. Through gel electrophoresis, we knew Fig.2.

Then Select potential samples (YRT-001, YRT-002, YRT-003, YRT-004, YRT-006, YRT-007, YRT-008, YRT-011, YRT-012, YRT-013, YRT-014, YRT-015, YRT-016, YRT-019, YRT-023, YRT-024, YRT-025, YRT-028) to Genewiz for two-way sequencing.

Re-extract failed samples (YRT-005, YRT-009, YRT-010, YRT017, YRT-018, YRT-020, YRT-021, YRT-022, YRT-026, YRT-027) with silica method. Retry these steps as mentioned. Then send the successful samples (YRT-005, YRT-018, YRT-022) to Genewiz for secondary sequencing. Result turned out that YRT-018 is successful.

Then build Phylip ML tree for next analysis.

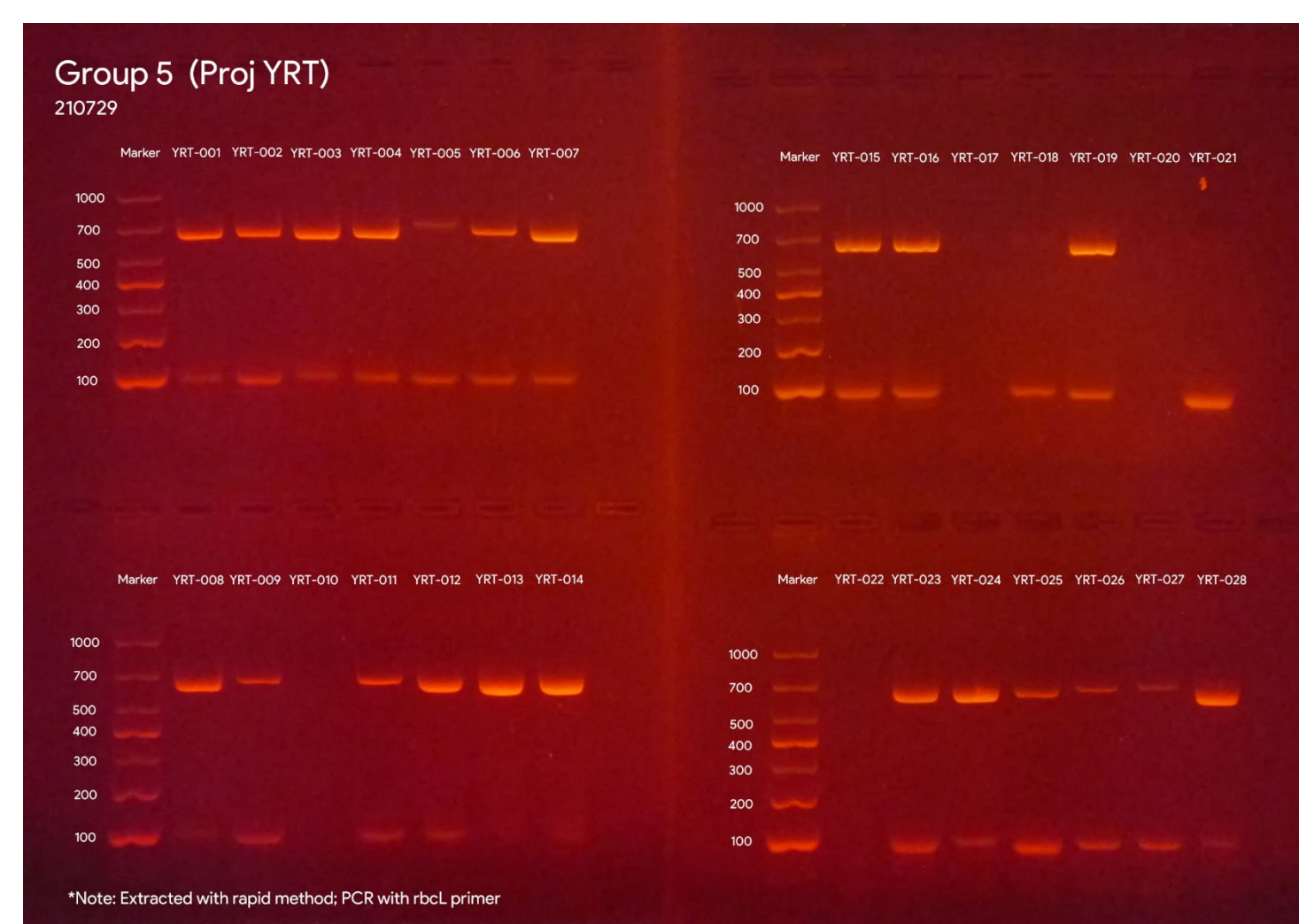


Fig.2 gel electrophoresis result

- Product in low concentration (YRT-005, YRT-009, YRT-026, YRT-027)
- Primer issues (YRT-010, YRT017, YRT-020, YRT-022)
- Product not amplified (YRT-018, YRT-021)

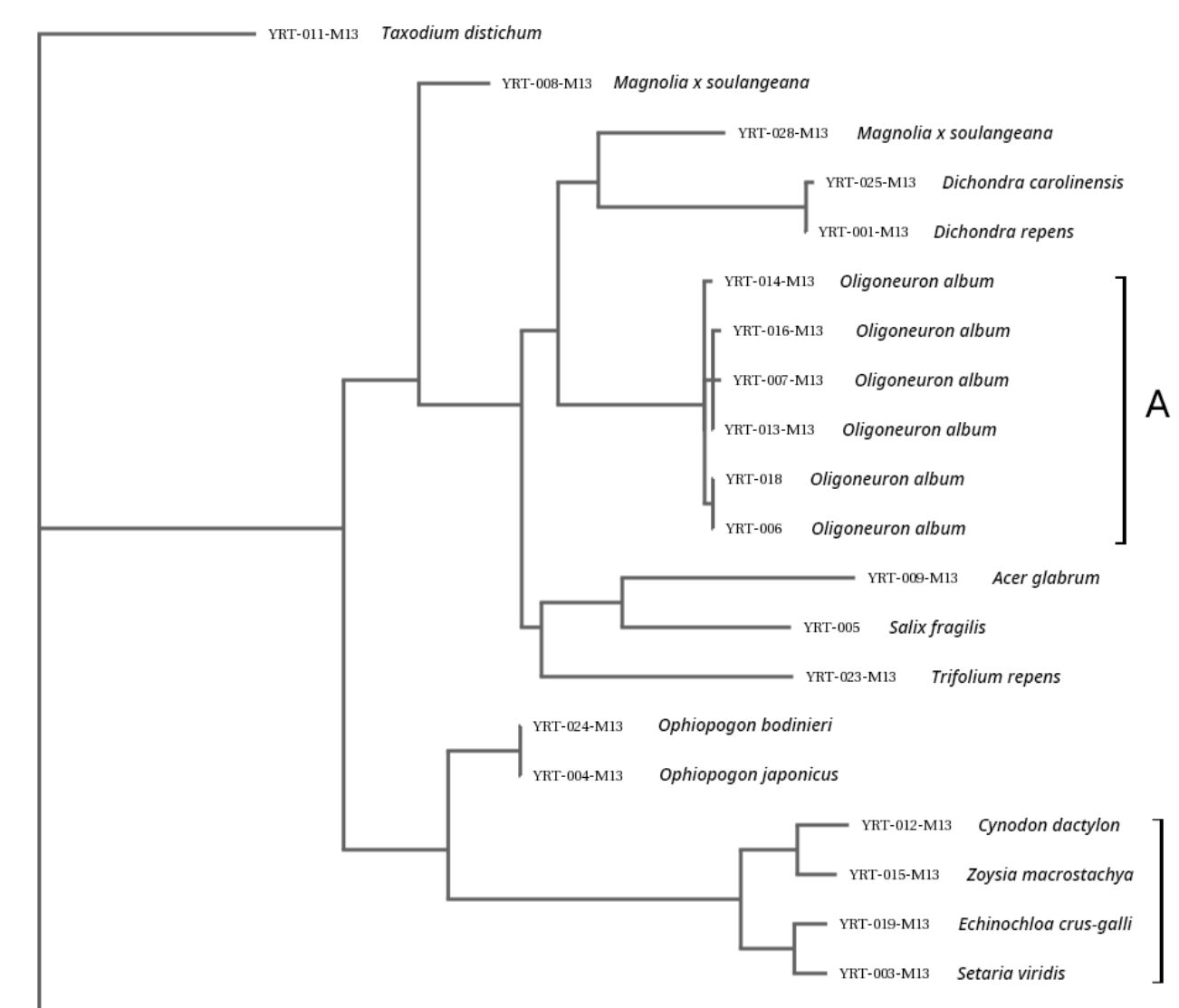


Fig.4 Phylip ML tree

Cluster A represents difference in morphology and similarity in gene relationship
Cluster B represents both similarity in morphology and gene relationship

Results

Analyzing the species distribution of the sequenced samples, the results showed that among the 21 samples, 2 belonged to the *Convolvulaceae*, 1 belonged to the *Bryophyllaceae*, 4 belonged to the *Gramineae*, 2 belonged to the *Asparagaceae* family, and 6 belonged to the *Compositae* family. , 1 belongs to *Willow* family, 1 belongs to *Soappinaceae*, 1 belongs to *Cupressaceae*, 1 belongs to *Leguminous* family, 1 belongs to *Apocynaceae*.As to Fig.2.

Referring to the drawn Philip ML image, it can be seen that there are two clusters of plant samples in the Fig.3, cluster A and cluster B. Cluster A includes samples YRT006, YRT007, YRT013, YRT014, YRT016, YRT018 which collected along the coast of the Jiangsu section of Suzhou Creek, which express quite different in morphology. Nonetheless, after testing they are identical at the genetic level and belong to the family *Compositae*. Simultaneously the three groups of samples YRT004 and YRT024, YRT001 and YRT025, YRT003 and YRT019 and YRT012 and YRT015 of cluster B collected along the coast of Jiangsu and Shanghai sections of Wusong present similar morphological characteristics and genetic sequence, they belong to the *Asparagaceae*. Flower family, *Gramineae*.

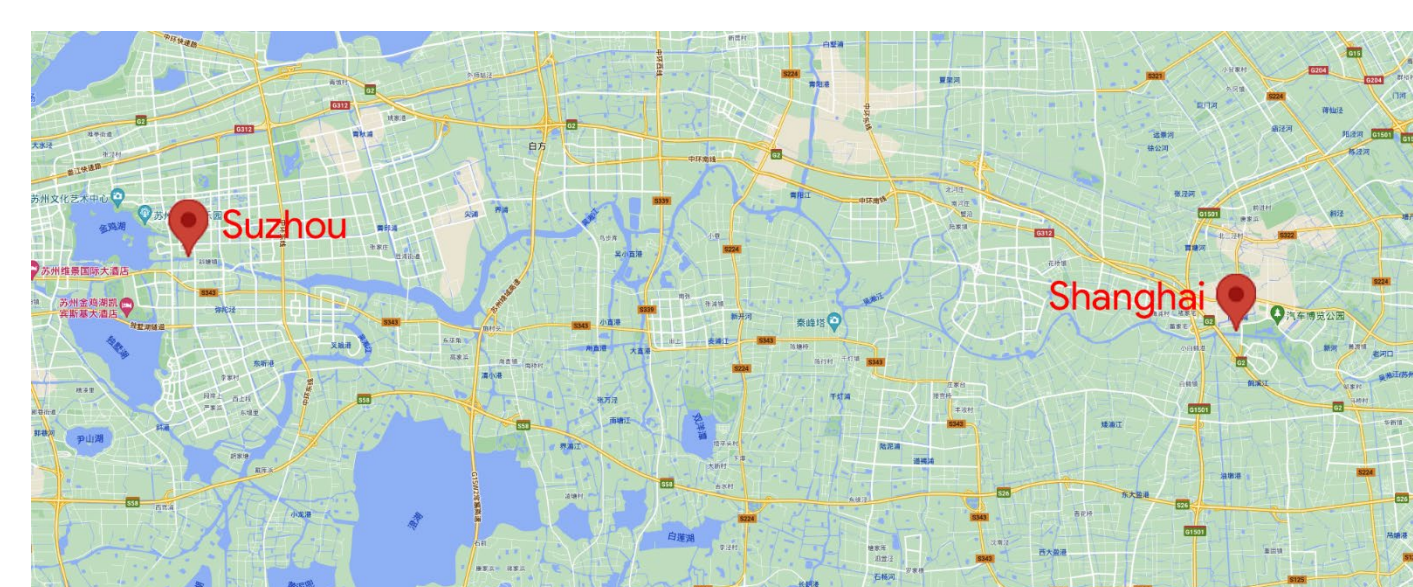


Fig.1 Sampling Spots in Suzhou and Shanghai

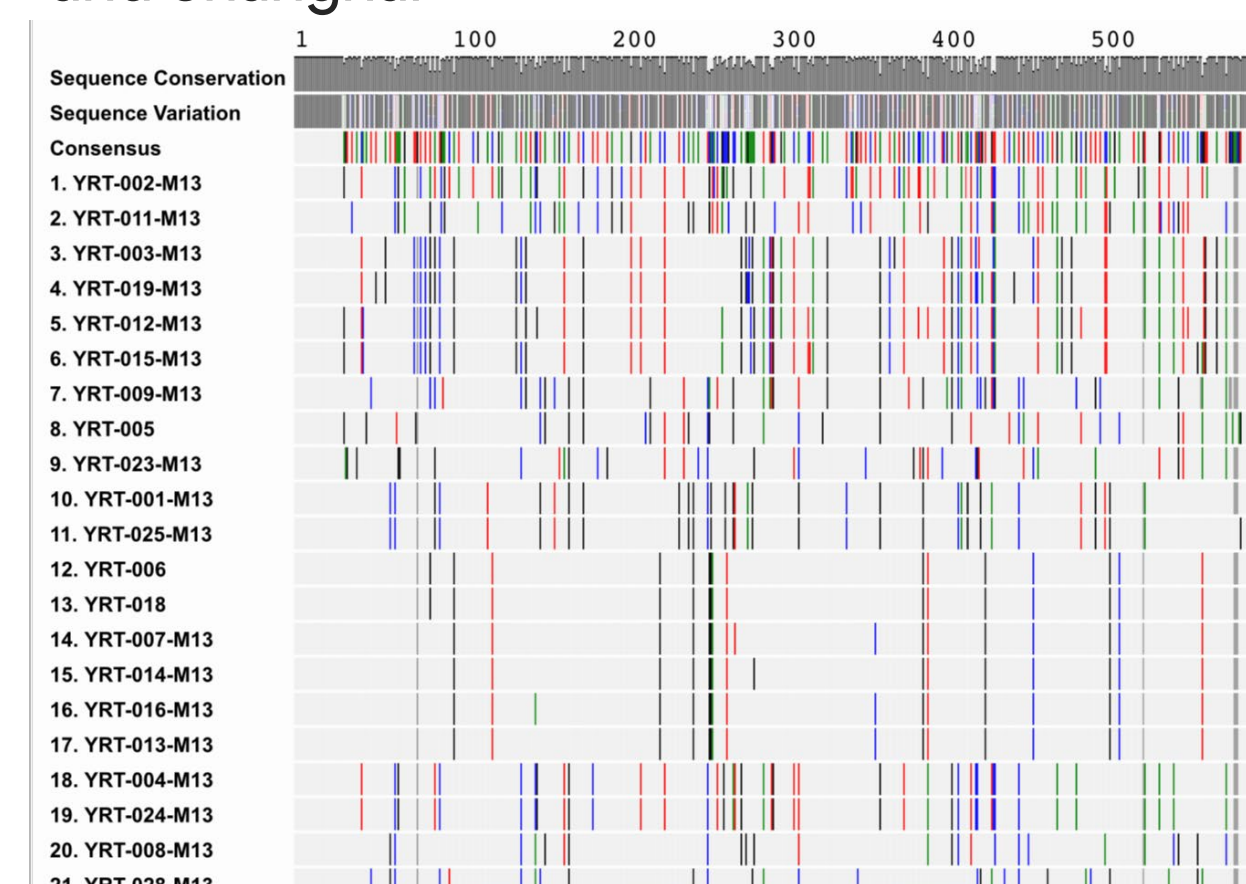


Fig.3 Alignment View

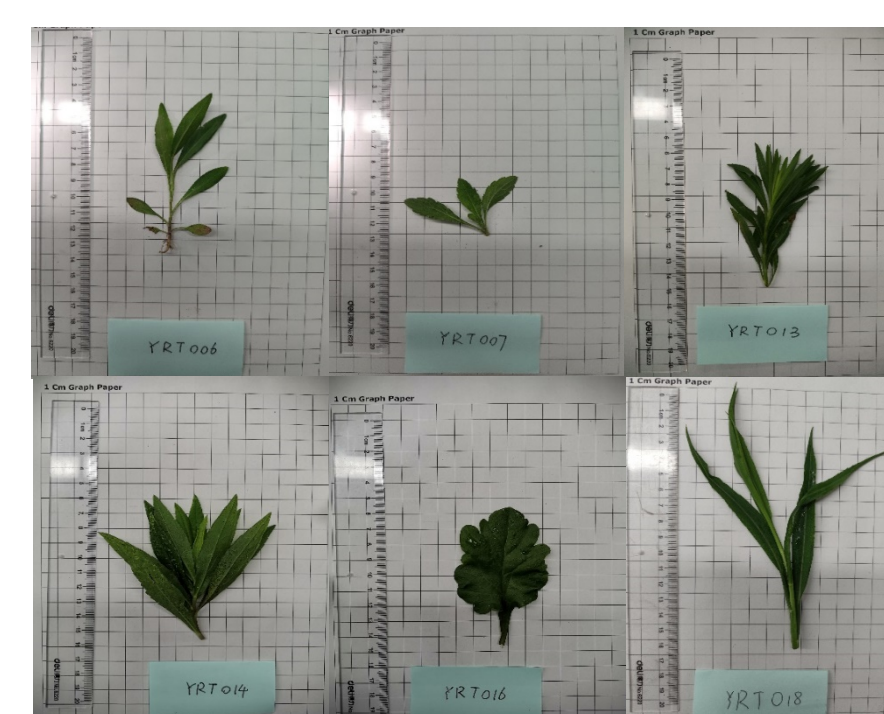


Fig.5 Samples in Cluster A Morphologically different



Fig.6 Samples in Cluster B Morphologically similar

Discussion

We guessed that our samples have a common ancestor in the past. Through water media of Suzhou Creek, they evolved separately and finally developed reproductive isolation because of geographic isolation. In another aspect, Suzhou and Shanghai's climate is highly similar, geographic distribution and terrain is the same. As a result, the living environment is almost the same. Plants' morphological features are also similar. However, by the influence of human activity, difference in living environment is unavoidable. In the cause of natural selection with tiny difference. Population-based evolutionary behavior appears in plants as time flows. Under geographic isolation, the gene from two city cannot communicate in time. Finally caused the morphological similarity and the genetic difference. In the same time, this situation also also proves that plants can migrate in a relatively short period of time under the action of water flow media.

Along with the development of high-throughput sequencing technologies, cost of DNA sequencing has reduced recently, thus it lead to a large increase in numbers of gene sequence in public databases, this enhance people's identification of animals and plants' gene sequences. Analysis of the gene sequence of the plants contribute to reveal the plant functional characteristics, evolutionary relationships, variation of geological history and human activity. Modern taxonomy has also been boosted by rapidly filling databases.

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