Study and Comparative Analysis of Biodiversity in Different Areas in East of Jinji Lake during the Rainstorm Caused by Severe Typhoon In-Fa

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Abstract

During the rainstorm caused by Typhoon "In-Fa" in 2021, our group used DNA Barcoding technology to analyze the genetic relationship between samples, established the phylogenetic tree and compared the similarities and differences of DNA barcode.
The final results showed that human activities, heavy rainfall, and changes in environmental condition will lead to the decline of biodiversity and the invasion of alien species.

1. Sample Collection
The 30 samples were collected from the waters and land of Jinji Lake (31°18 '31 "N °43' 3" E ° 120°43 '3 "E) in the Suzhou Industrial Park, Jiangsu Province, before and after the rainstorm caused by Severe Typhoon In-fa. Our team searched for three representative sites within Jinji Lake, including fungi-parasitic rotten wood, shallow water and egg carrying vegetation, and collected samples including plants, fungi, invertebrates and fish (Fig.1). After the collected samples are classified, photographed and recorded, the samples are used for DNA extraction.
2. DNA Extraction
After dissection and cutting, our group used Silica method to extract DNA from some samples, and Tissue Kit and Plant Kit methods were used to extract DNA from other samples that were not suitable for Silica method (Fig.1).
3. DNA Amplification
In order to carry out DNA Barcoding, our group used PCR technology. rbcL (plants and fungi) and COI (invertebrates and fish) primers (Fig.1) to amplify the rbcL and COI genes for recording.
4. Gel Electrophoresis
To determine the results, the amplified DNA sequences were separated by 2% agarose gel electrophoresis.
5. DNA Sequencing
The resulting DNA bands were sent to a company for sequencing, and results were imported website (https://dnasubway.cyverse.org/) (https://www.ncbi.nlm.nih.gov/guide/sitemap/?ivk_sa=1024320u) for further analysis, and our group mapped the phylogenetic trees.

2021/7/28-2021/7/29

Number	Scientific Name	名 称	Extraction Method	Primer
CRT-001	*Anisolabis maritima*	蠼螋	Silica	COI
CRT-002	*Pseudozizeeriz maha*	蝴蝶	Tissue Kit	COI
CRT-003	*Scutigera Coleotrata*	蚰蜒	Tissue Kit	COI
CRT-004	*Oophana heudei*	蜗牛	Tissue Kit	COI
CRT-005	*Caridina gracilipes(big)*	河虾（大）	Tissue Kit	COI
CRT-006	*Caridina gracilipes(small)*	河虾（小）	Silica	COI
CRT-007	*Meghinatium bilineatum*	蛞蝓	Tissue Kit	COI
CRT-008	*Meghinatium bilineatum*	蛞蝓	Silica	COI
CRT-009	*Cheiracanthium trivile(Insect Egg)*	虫卵	Tissue Kit	COI
CRT-010	*Cheiracanthium trivile*	蜘蛛	Silica	COI
CRT-011	*Lasioderma serricorne*	烟草甲虫	Silica	COI
CRT-012	*Iwogumoa interuna*	水蜘蛛	Silica	COI
CRT-013	*Euphaedusa planostriata*	钉螺	Silica	COI
CRT-014	*Porcellio sp.*	鼠妇	Tissue Kit	COI
CRT-015	*Ozyptila praticola*	蜘蛛	Silica	COI
CRT-016	*Iwogumoa interuna*	蜘蛛	Silica	COI
CRT-017	*Sinotaia quadrata*	螺蛳	Tissue Kit	COI
CRT-018	*Sinotaia quadrata*	螺蛳	Tissue Kit	COI
CRT-019	*Pardosa laura*	蜘蛛	Silica	COI
CRT-020	*Armadillidium vulgare*	鼠妇	Silica	COI
CRT-021	*Rhinogobius similis*	鱼	Tissue Kit	COI
CRT-022	*Venturiella sinensis(red)*	树蘚（红）	Silica	rbcl
CRT-023	*Venturiella sinensis(green)*	树蘚（绿）	Silica	rbcl
CRT-024	*Najas gracillima*	水草	Silica	rbcl
CRT-025	*Derbesia solier*	水藻	Silica	rbcl
CRT-026	*Nymphoides peltata*	浮萍	Silica	rbcl
CRT-027	*Ganoderma applanatum*	树舌灵芝	Plant Kit+Silica	rbcl
CRT-028	*Ganoderma applanatum(red)*	树舌（红）	Plant Kit+Silica	rbcl
CRT-029	*Ganoderma applanatum(white)*	树舌（白）	Plant Kit+Silica	rbcl
CRT-030	*Cercis glabra*	树叶	Silica	rbcl

Tab.1: A statistical table of samples, including sample names, methods of DNA extraction and primers used

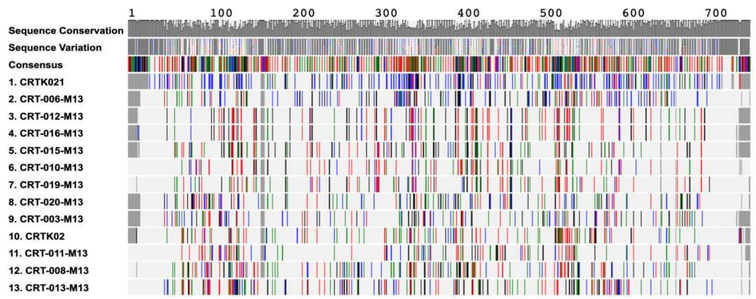

Fig.2: MUSCLE result shown in Alignment Viewer for invertebrates and fish

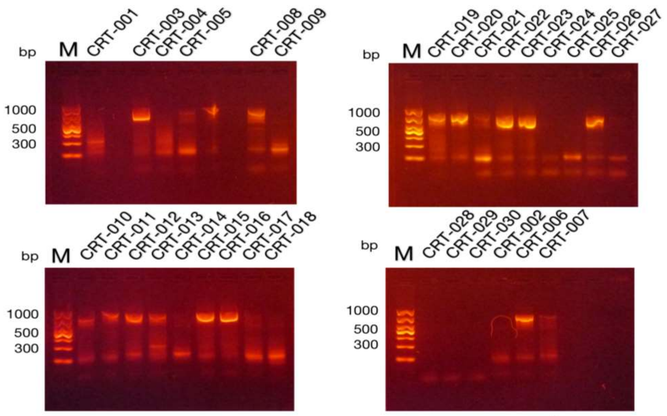

Fig.1: Restriction patterns of genomeic DNAs isolated from 30 'CRT' samples. The restriction digests were separated on 0.8% agarose gels. M = bp marker

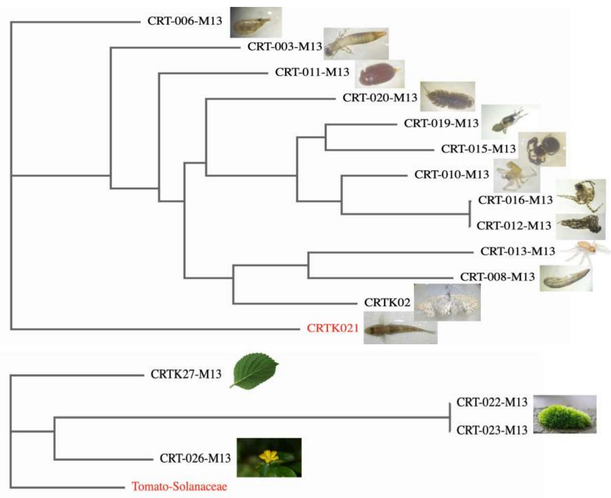

Fig.2: Two ML tree. The two marked red are used as out groups to construct two trees respectively

Materials & Methods

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Results

For some samples that failed to extract DNA during the experiment, our group made a summary, mainly divided into technical problems and operational problems:
1. Lack of specificity and functionality of primers
The primers used for the whole set of experimental procedures are rbcL and COI gene fragments, which may have a lack of specificity and functionality. For example, the primers required for DNA amplification of fungi are not rbcL gene fragments but ITS fragments, resulting in unsatisfactory sequencing of fungal DNA by my group.

2. Deficiencies of the sample itself
In the second experiment, our group use the best DNA extraction position of the samples, for example, used the body and foot part instead of the wings of butterfly. We found that more bands were obtained by gel electrophoresis, indicating that different parts have significant effect on the results of the experiment. In future experiments, we should pay attention to understanding the characteristics of each sample before conducting experiments.

3. Improper operation
When our group first performed grinding, we took the same grinding method as other samples for the difficult-to-grind samples such as Ganoderma applanatum, which resulted in insufficient cell lysis and could not extract DNA for amplification even after using a mortar and pestle; and a large number of fibers were found when grinding algae, which also caused an impact on DNA extraction and amplification.
4. DNA and primer properties
Primers can bind to other primers at room temperature and form dimers, so stray bands will be seen in gel electrophoresis; DNA may also be partially denatured during transport leading to sequencing failure, and accurate sequencing cannot be performed when the sample DNA concentration is too low.

Discussion

The main location of insect activity we observed before the rainstorm was on the trees and vegetations on land. After heavy rains, we found it difficult to find complete insect populations on land vegetation. Because of the heavy rainfall, many land animals had to leave the most suitable habitats and go to higher altitudes. As a result, they lost their life and the population of life was decimated. According to our observations, after the rain, a lot of the creatures flocked to man-made habitats such as telephone poles. Clearly, forcing dozens of species of animals that didn't live together to gather together in a habitat was not suitable for them and might have a huge impact on biodiversity. There was even a symbiosis between predators (spiders) and prey (ants). Many of the spiders we observed were unable to build their webs to feed, and eventually starved to death during weeks or even months of heavy rain. We also observed that the heavy rain washed a large amount of nitrogen, phosphorus and other nutrients needed by organisms in fertilizers into Jinji Lake , causing the rapid reproduction of algae and other plankton. However, due to the short observation time, the eutrophication has not developed to the extent of endangering aquatic animals. But in just four days, the algae in Jinji Lake is in stark contrast to what it was before the rain.

Reference

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