Barcoding diversity of crab apple trees found on Governors Island, NY

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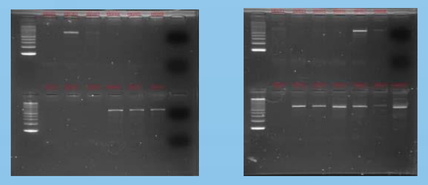
Abstract: Crabapple trees (*Malus* spp.) are well known ornamental trees. Nowadays, there are nearly 25 to 47 different species of crabapples worldwide. Until now, there has been no research ever done on diversities of crabapple on Governors Island, NY except for one research done by MCNDHS students, "Genetic Variations of Crabapples (*Malus* spp.) found on Governors Island and NYC area by Jianri Chen, Zinan Ma, Iulius Sergiu Moldovan and Xuanzhi Zhao. Their research showed that there were four different species of Malus on the Island. However, their result was not conclusive and we hypothesized that there may be more than that number of species on the island. Therefore, our research objective was to determine if there were more than 4 species of crabapple on Governors Island that the previous research affirmed. We collected crabapples' fruits and leaves in all different locations on the Governors Island, NY. Our DNA barcode result showed that two additional *Malus* species apart from the 4 *Malus* species found by Chen *et al*.

Introduction: Crab apple trees are fruit-bearing and flowering trees that belong to Genus *Malus*. They are planted as ornamental trees. They are most favorite trees for gardeners as they help gardeners in pollination of their trees because their blossoms are quite attractive to all types of pollinators. Crabapples (*Malus* species) are native to North America, Europe and Asia and there are about 25 species." Nearly 1000 different types are estimated to exist and among them only 100 different kinds are popularly grown nationwide. Our research focus was to determine diversity of crabapples on Governors Island based on previous research. In 2015, the summary report of their research **"Genetic Variation of Crabapples (*Malus* spp.) found on Governors Island and NYC Area by Jianri Chen, Zinan Ma, Iulius Sergiu Moldovan and Xuanzhi Zhao"** states that samples of the crabapple trees that they collected from Governors Island belong to ***Malus Baccata, Malus Micromalus, Malus Domestica, Malus Pumila***. Their result was based on 25 leave samples and 6 from fruit tissue samples. We hypothesized that there may be more than four different species of crabapples on the Governors Island as mentioned above. We hypothesized that those crabapple trees around this area must have been planted recently and they may have come from different locations and different species. Therefore, we sought to find out if the crabapple trees from the Governors Island belong to more than four *Malus* species or other types of *Malus* through DNA barcoding. We also think that understanding diversity of different species in one particular area, we can learn how to preserve those diversity. Biodiversity brings balance of ecosystem, promotes productivity of our society, and can help prevent natural disaster.

Method: Altogether, samples from 24 crabapple trees were collected from different locations on the Governors Island, NY in October 2017, and properly documented and stored in a refrigerator at MCNDHS. Then we took the samples to DNA Learning Center to extract DNA from our samples. For barcoding purpose, we isolated chloroplast DNA from the leaf and fruit samples using the DNA Barcoding 101 protocol. We used *rbcL* primer to amplify the samples' DNA, and we ran Gel Electrophoresis to analyze DNA from the PCR products to see if we obtained good amount of DNA to barcode. Then, we requested the DNA Learning Center to send our DNA for sequencing. Later we analyzed the DNA samples using DNA Subway's "Blue Line".

Samples collection of Crabapple trees from Governors Island, NY.



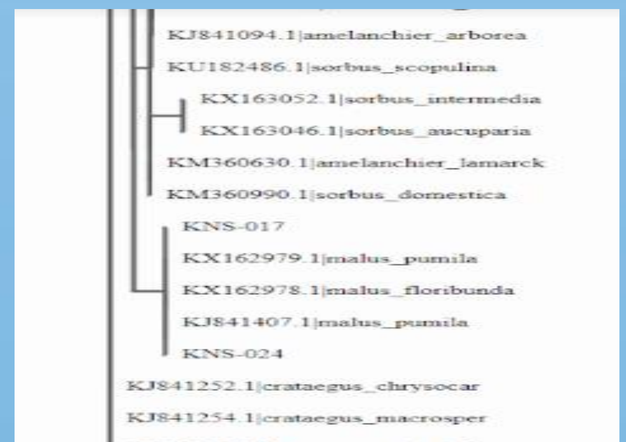
Agarose gels showing positive PCR amplification.

Database #	Common Name	Genus/species	new species?	longitude	latitude
KNS-001	Crabapple	Malus sp	No	-74.0134661799	40.6888583984
KNS-002	Crabapple	Malus cf. x robusta OMH0009	New	-74.0134661799	40.6888583984
KNS-003	Crabapple	Malus sp	No	-74.01591	40.6874617
KNS-004	Crabapple	Malus sp	No	-74.0136429372	40.68866966
KNS-005	Crabapple	Malus sp	No	-74.0136429372	40.68866966
KNS-006	Crabapple	Malus sp	No	-74.02	40.68
KNS-007	Crabapple	Malus sp	No	-74.022	40.686
KNS-008	Crabapple	Malus sp	No	-74.026	40.685
KNS-009	Crabapple	Malus sp	No	-74.0222	40.6871
KNS-010	Crabapple	Malus sp	No	-74.0222	40.687
KNS-011	Crabapple	Malus floribunda	New	-74.0222	40.687
KNS-012	Crabapple	Malus floribunda	New	-74.0222	40.687
KNS-013	Crabapple	Malus sp	No	-74.0222	40.6871
KNS-014	Crabapple	Malus sp	No	-74.0244169327	40.6868314041
KNS-015	Crabapple	Malus sp	No	-74.0244169327	40.6868314041
KNS-016	Crabapple	Malus sp	No	-74.0244169327	40.6868314041
KNS-017	Crabapple	Malus cf. x robusta OMH0009	New	-74.0244169327	40.6868314041
KNS-018	Crabapple	Malus sp	No	-74.0244169327	40.6868314041
KNS-019	Crabapple	Malus sp	No	-74.0244169327	40.6868314041
KNS-020	Crabapple	Malus sp	No	-74.0244169327	40.6868314041
KNS-021	Crabapple	Malus sp	New	-74.0244169327	40.6868314041
KNS-022	Crabapple	Malus sp	No	-74.0244169327	40.6868314041
KNS-023	Crabapple	Malus floribunda	New	-74.0244169327	40.6868314041

Results: Our results showed that the samples, which we collected from Governors Island, New York City, belong to ***Malus baccata***, ***Malus micromalus***, ***Malus domestica***, ***Malus pumila***, ***Malus floribunda, Malus cf. x robusta*** OMH0009. However, due to some inconveniences and lack of proper laboratory equipment, our results were not very accurate.

Sources	Total # Sample of Crabapple Collected from Governor Island, NY	DNA Barcode Result (Type of Crabapple Species)	Common Name
2014 MCNDHS Crabapple DNA Barcode Research Result	13	• Malus baccata • Malus micromalus • Malus domestica • Malus pumila	• Siberian crab apple • Midget crabapple or Kaido crabapple • Apple • Paradise apple
2018 MCNDHS Crabapple Team Research Result	24	• Malus baccata • Malus micromalus • Malus domestica • Malus pumila • Malus floribunda • Malus cf. x robusta OMH0009	• Siberian crab apple • Midget crabapple or Kaido crabapple • Apple • Paradise apple • Japanese flowering crabapple • No

#	Accession #	Details	Aln. Length	Bit Score	e	Mis-matches
4(4).	KX162979.1	**Malus pumila** - Malus pumila voucher OMH0003 ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (rbcL) gene, partial cds	599	1081	0.0	0
5(5).	KX162978.1	**Malus floribunda** - Malus floribunda voucher OMH0016 ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (rbcL) gene, partial cds	599	1081	0.0	0
6(6).	KX162977.1	**Malus floribunda** - Malus floribunda voucher OMH0011 ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (rbcL) gene, partial cds	599	1081	0.0	0
7(7).	KX162976.1	**Malus cf. x robusta OMH0009** - Malus cf. x robusta OMH0009 ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (rbcL)gene, partial cds	599	1081	0.0	0
3(3).	KX162979.1	**Malus pumila** - Malus pumila voucher OMH0003 ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (rbcL) gene, partial cds	599	1081	0.0	0
4(4).	KX162978.1	**Malus floribunda** - Malus floribunda voucher OMH0016 ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (rbcL) gene, partial cds	599	1081	0.0	0
5(5).	KX162977.1	**Malus floribunda** - Malus floribunda voucher OMH0011 ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (rbcL) gene, partial cds	599	1081	0.0	0
6(6).	KX162976.1	**Malus cf. x robusta OMH0009** - Malus cf. x robusta OMH0009 ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (rbcL)gene, partial cds	599	1081	0.0	0



Discussion: Before we started planning to collect samples, we hypothesized that there are maybe more than four species of crabapple in Governors Island in New York City. Our results indicated there are two more new species of crabapple that the previous team did not find, which are Malus floribunda and ***Malus cf. x robusta*** OMH0009. This result supported our hypothesis. Possible reason that previous DNA Barcode team did not find them might be because when they went to Governors Island for sample collections, they did not have access to the areas where the park and the Outlook Hill were under construction. We believed that the landscapers may have chosen ***Malus floribunda***, commonly known as Japanese crab apple to plant around the hill because of its' bright and beautiful flowers and fruits. Out of 24 samples we collected, 12 samples had good DNA sequence result.

Conclusion: In conclusion, our research was not definitive because the 12 samples that we did not have DNA may belong to different other species of *Malus*. If the time were permitted, we would spend more time on Governors Island, NY and thoroughly look for more crabapple trees and collect larger samples to barcode. We are convinced that our research may contribute to some extant to the DNA Database of NYC Biodiversity.

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