

Management of an Invasive Species

Seed Distribution of *Corydalis incisa* Along the Bronx River

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Abstract

Corydalis incisa is an invasive plant species that is currently spreading southward along the Bronx River at an alarming rate. The Bronx River is a unique habitat that exists within New York City and invasive species have recently become a larger part of management planning. *C. incisa* is known to have two mechanisms of reproduction, dehiscent fruit and tubers. *C. incisa* is thought to use river floodwater as part of its seed dispersal. Soil samples were collected for seed germination and identification by both morphology and DNA barcode. Seed identification included 15 different genera in which 22 species were represented. The seed collection did not include any *C. incisa*.

Introduction

Corydalis incisa is an invasive plant species that is rapidly spreading southward along the Bronx River. *C. incisa* was first discovered in the New York City area in 2005 by Michael Sundue near the Burke Avenue Bridge, Bronx County (Atha, D. 2014). Later, specimens from this area were collected and vouchered by Steven Glenn (Lamont, E. 2011). Since then *C. incisa* has been spotted thriving further south along the river. In 2010, Daniel Atha (2014) examined the Bronx River and found the second population of the same species on the Northern grounds of the New York Botanical Garden. That same year, Atha (2014) revisited the original 2005 site and found the initial population to have colonized both sides of the river.

C. incisa is a biennial plant commonly found in Korea, Taiwan, Japan, and Eastern China (Fig.1). It takes two years to grow into a fruiting plant. The fruit becomes dehiscent when ripe, dropping its seeds, and water can disperse the seeds to a greater distance. After dispersing its seeds, the adult plant dies back. Alternatively it spreads by tubers, which can grow from first year plants.



Fig 1. Photograph of *C. incisa*, December 13, 2015, Burke Avenue Bridge, Bronx River Parkway.

C. incisa usually has purple flowers with white being a rare variant. The height is between 10-50cm. When the *C. incisa* is less than a year old, it can be confused with other seedlings including *Cryptotaenia canadensis*, *Corydalis sempervirens*, *Corydalis flavula*, and *Corydalis aurea* (Atha, D. 2014) For this reason, amateur botanists might find it difficult to distinguish the *C. incisa*. Also interpreting a dichotomous key is difficult because of the advanced descriptive language used. These complications can be eliminated through the use of Barcode (Hollingsworth, P. 2009).

This project examined the seed distribution of *C. incisa* in unmanaged area along the Bronx River using DNA barcode. In 2014-15 a study of *C. incisa* was conducted on managed land in the New York Botanical Garden using DNA barcode and a seed collection. The results of this survey found 23 genera, in which 29 different species were represented. In the 2014-15 survey no *C. incisa* was identified.

Materials and Methods



Fig 2. Area Surveyed. (A) Walking survey conducted to locate *C. incisa*. (B) Map showing the survey site (red dot) selected for soil sampling. (C) Photo of students surveying.

Area Surveyed The study area was located along the Bronx River Parkway, north of New York Botanical Garden, Bronx, NY, on property managed by New York City Department of Parks and Recreation. An exhaustive walking survey was first conducted to locate any *C. incisa* along the river's edge and north of the Burke Avenue Bridge. Next, a single site within this first survey was selected for the soil sampling (Fig.2). Three transects (A, B, and C) were laid with one origin point. The control transect C was parallel to the river and placed on multiple identified *C. incisa*. Using a metal cylinder (3" diameter), 24 soil samples were collected every 1.5m along each transect and latitude and longitude recorded (Fig.3). Each soil sample was a volume of 0.12L. Samples were not taken at 9m and 7.5m, Transect A, or on Transect B at 10.5m and 12m due to a footpath.

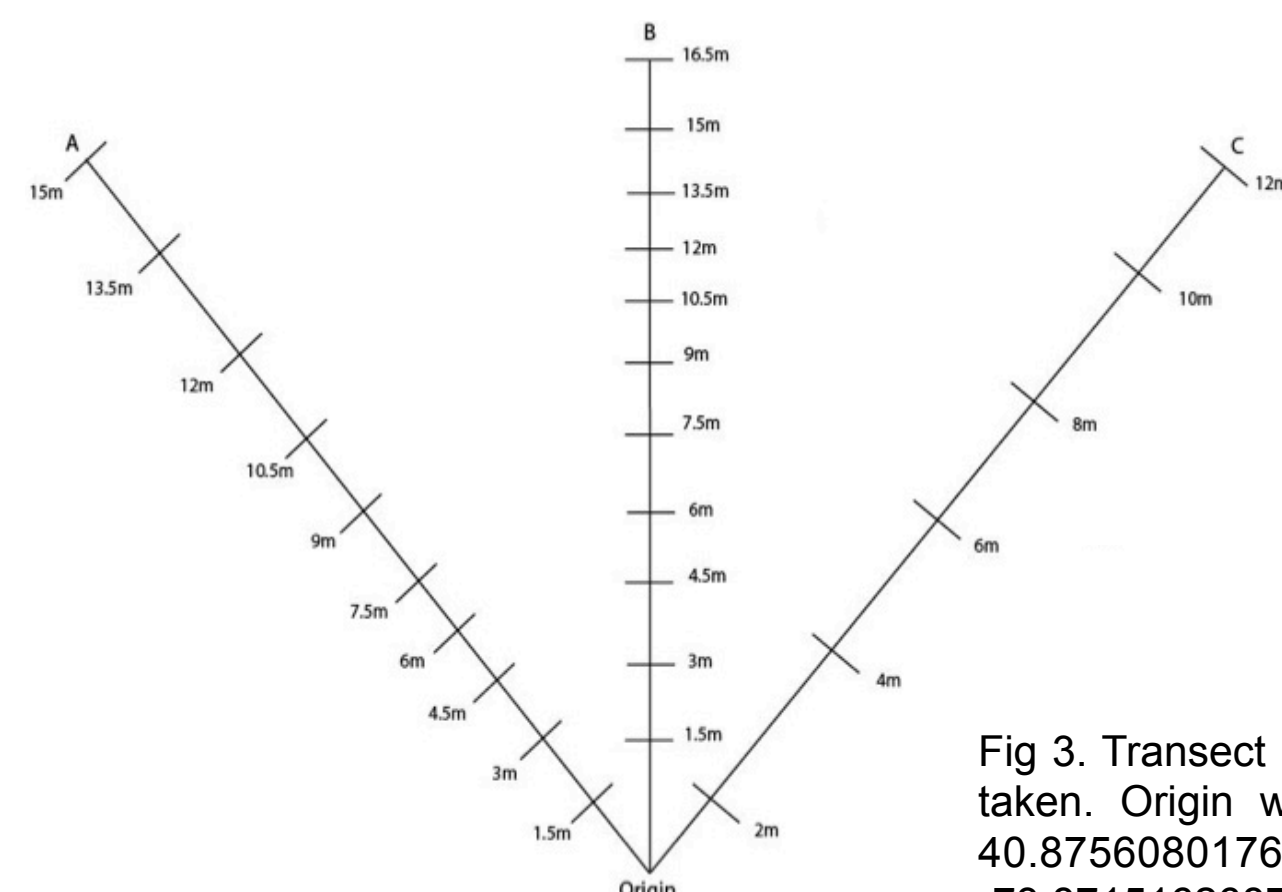


Fig 3. Transect map of all soil samples taken. Origin was located at latitude 40.875608017668128 and longitude -73.871516268700361.

Growing of Seedlings The soil samples were dried at 47°C for 48 hours and stored at 4°C until planted. Potting soil was mixed with vermiculite and baked at 85°C for 30min. 1 liter of sterile potting soil was mixed with each dried soil sample and poured into a growing tray (11.25"L x 7.75"W x 2"H). The dilution factor for each tray was 9.3 (1.12L/0.12L). Each soil sample was grown for ≈6 weeks. Seedlings were harvested when the second set of true leaves appeared, and stored in 91% isopropyl alcohol at 0°C. All seedlings collected were morphotyped (Fig.4).

Fig. 4. Morphological Features Used to Identify Seedlings

Shape of Leaf		Tip of Leaf		Vein Origin	Leaf Arrang't
Jagged	Toothed	Cordate	Blunt	Basal	Alternate
Smooth	Lobed	Rounded	Pointed	Midvein/Rib	Opposite

DNA Extraction, PCR, and Sequencing A silica protocol was used to extract DNA. A short segment of the ribulose-bisphosphate carboxylase gene (*rbcl*) was amplified (Hollingsworth, P., et.al. 2009). Sequence chromatograms were edited with Sequencher®, DNA analysis software. Final results were matched to sequences found in NCBI's GenBank database using BRONX (Little, D. 2001). The final Raw Score in this analysis indicates the number of base positions that match *rbcl* gene segment. The highest possible match is 595.

Results

DNA Barcode

17 of the 24 soil samples had seedlings that were removed, morphotyped, and DNA extracted. The morphotyping resulted in 39 distinct groups. 30 of the 39 morphological groups had DNA extracted. 15 Morphological groups differed in *rbcl* sequence. 70% of sample DNA was sequenced. All sequenced DNA had BRONX Raw Scores that ranged between 480 and 549 with the highest level of frequency occurring between 530 and 539 (Fig. 5).

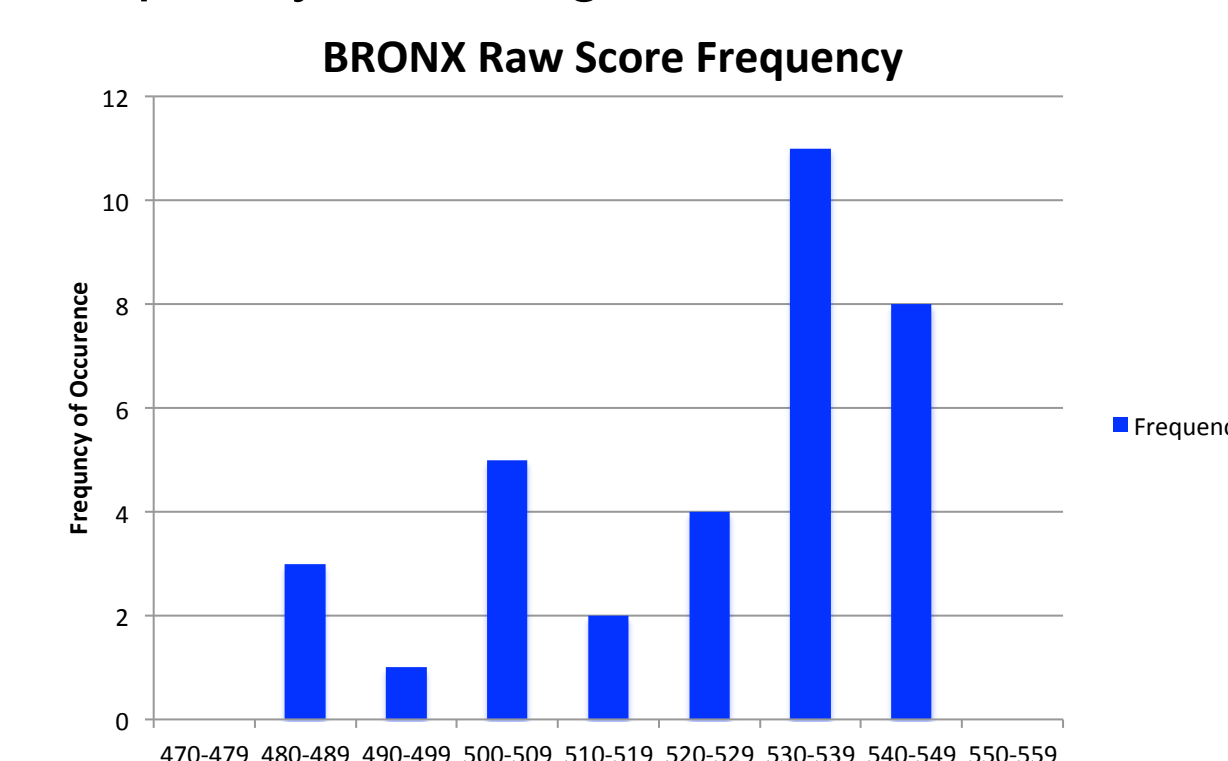


Fig. 5. Histogram of BRONX Raw Score.

15 different genera were determined by barcode analysis: 36% identified a single species; the other 64% had identified multiple species (Table 2). No *C. incisa* was found.

Database #	Group	Raw Score	Genus Species	Native/ Non Native	Perennial/Annual	Stem Type
KXW-003	2	488	<i>Chenopodium cristatum</i>	Non Native	Annual	Herbaceous
KXW-004	3	524	<i>Cardamine flexuosa</i>	Non Native	Both	Herbaceous
KXW-004	3	524	<i>Cardamine impatiens</i>	Non Native	Annual	Herbaceous
KXW-004	3	524	<i>Cardamine pensylvanica</i>	Native	Both	Herbaceous
KXW-005	4	543	<i>Portulaca oleracea</i>	Undetermined	Annual	Herbaceous
KXW-006	5	542	<i>Mollugo enneandra</i>	No Data	No Data	Herbaceous
KXW-006	5	542	<i>Mollugo verticillata</i>	Native	Annual	Herbaceous
KXW-007	6	543	<i>Trifolium repens</i>	Non Native	Perennial	Herbaceous
KXW-008	7	539	<i>Amaranthus hybridus</i>	Native	Annual	Herbaceous
KXW-009	8	488	<i>Chenopodium cristatum</i>	Unreported	Annual	Herbaceous
KXW-010	9	539	<i>Amaranthus hybridus</i>	Native	Annual	Herbaceous
KXW-011	10	507	<i>Polygonum cuspidatum</i>	Non Native	Perennial	Herbaceous
KXW-012	11	512	<i>Persicaria longisteta</i>	Non Native	Annual	Herbaceous
KXW-012	11	512	<i>Persicaria lindtiora</i>	No Data	No Data	No Data
KXW-013	12	537	<i>Mollugo enneandra</i>	No Data	No Data	No Data
KXW-013	12	537	<i>Mollugo verticillata</i>	Native	Annual	Herbaceous
KXW-014	13	502	<i>Fallopia japonica</i>	Non Native	Perennial	Herbaceous
KXW-014	13	502	<i>Polygonum cuspidatum</i>	Non Native	Perennial	Herbaceous
KXW-015	17	537	<i>Mollugo enneandra</i>	No Data	No Data	No Data
KXW-015	17	537	<i>Mollugo verticillata</i>	Native	Annual	Herbaceous
KXW-016	14	543	<i>Ageratum luciae-brunae</i>	Unreported	Perennial	Herbaceous
KXW-017	15	536	<i>Eragrostis pectinacea</i>	Native	Both	Herbaceous
KXW-023	21	488	<i>Chenopodium cristatum</i>	Unreported	Annual	Herbaceous
KXW-024	22	508	<i>Sagina apetala</i>	Non Native	Annual	Herbaceous
KXW-024	22	508	<i>Sagina procumbens</i>	Non Native	Perennial	Herbaceous
KXW-025	23	543	<i>Morus alba</i>	Non Native	Perennial	Woody
KXW-025	23	543	<i>Morus indica</i>	No Data	No Data	No Data
KXW-026	27	493	<i>Polygonum cuspidatum</i>	Non Native	Perennial	Herbaceous
KXW-027	4	543	<i>Portulaca oleracea</i>	Undetermined	Annual	Herbaceous
KXW-028	4	538	<i>Portulaca oleracea</i>	Undetermined	Annual	Herbaceous
KXW-029	32	537	<i>Mollugo enneandra</i>	No Data	No Data	No Data
KXW-029	32	537	<i>Mollugo verticillata</i>	Native	Annual	Herbaceous
KXW-030	34	524	<i>Cardamine hirsuta</i>	Non Native	Annual	Herbaceous
KXW-031	33	538	<i>Erigeron annuus</i>	Native	Annual	Herbaceous

Table 2. BRONX Raw Score, Identification and Plant Type.

Morphotype

While 39 morphological groups were recognized, 15 differed by barcode sequence. (Table 3).



Table 3. Photographs of Seedlings from 39 Identified Morphological Groups.

Groups 8 and 21 are now categorized as group 2, group 9 became part of group 7, and group 32 collapsed into group 5. 9 morphological groups are still in need of DNA extraction and analysis.

Discussion

DNA sequencing reduced the total morphological groups from 30 to 15. Misidentification based on morphology might be due to attempts at identifying a damaged seedling or misinterpreting the morphological features of the seedlings. For example, one feature that was difficult to interpret was distinguishing between toothed or smooth for the leaf shape. Another possible explanation may be that the morphological groups are true but *rbcl* is not variable enough to detect that distinction.

Among the 15 morphological groups identified through sequencing, *C. incisa* was not identified. This contradicts the methodical survey conducted, which found *C. incisa* in abundance. There were 78 points marked on the survey map indicating *C. incisa*. 50% of the soil samples analyzed were within 20cm of *C. incisa*. One possible explanation is that *C. incisa* is not reproducing through seeds but only through tubers. Another is that seeds are present but need a longer cold period for germination than used.

In the 2014-15 survey there were 24% non-native species while in 2015-16 67% were non-native (USDA plant databases). With a distance of only ≈3500ft between surveyed areas similarities in the data set are expected. One possible reason for the difference is the area usage. This year's surveyed area contains heavily used pathways while 2014-2015's area did not and is more isolated.

Additionally, 93% of the identified species in 2015-16 survey were non-native herbaceous, representing a highly disturbed area, while 2014-15's were 76% native. The native species of 2014-15's survey contained 16% woody and 45% herbaceous. This reflects a slightly different ecosystem that is more protected in the New York Botanical Garden.

Future Direction

One future study would be aimed at better understanding the *C. incisa*'s reproductive cycle. Seeds would be collected from mature plants and grown to serve as a control for the current protocol. This also would provide evidence that *C. incisa* is producing viable seeds.

References

Atha, D., Schuler, J., & Tobing, S. (2014). *CORYDALIS INCISA (FUMARIACEAE) IN BRONX AND WESTCHESTER COUNTIES, NEW YORK*

Bronx River Alliance, (n.p)(n.d.). Bronx River Forest, Burke Avenue Bridge. Retrieved November 10, 2015, from http://bronxriver.org/puma/images/usersubmitted/file/030_BxRForest.pdf

Hollingsworth, et al (2009, May 27). A DNA barcode for land plants.

Lamont, E., Glenn, S., & Young, S. (2011). Noteworthy plants reported from the Torrey Range—2009 and 2010. *The Journal of the Torrey Botanical Society*.

Little, D.P. 2010. DNA barcode sequences identification incorporating taxonomic hierarchy and within taxon variability. *PLoS ONE* 6 (8): e20552.

USDA plant databases, (n.p)(n.d.). Plant database. Retrieved May, 22, 2016, from <http://plants.usda.gov/java/>

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