



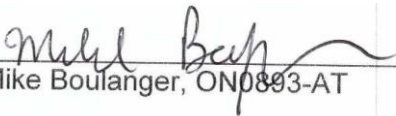
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WILDWOOD
TREE SERVICES LTD

— 905-337-TREE —

320 Pine Cove Road Burlington, Ontario Arborist Report & Tree Protection Plan

Report to:	City of Burlington Forestry Department	Submitted by:	Wildwood Tree Services Michael Boulanger ISA Certified Arborist info@wildwoodtree.ca 905-337-8733
Requested by:	Stuart Riley stuart@stuartriley.ca	Report Author:	Jennifer Kreller info@wildwoodtree.ca
Re:	Building Permit Application		
Date:	April 12, 2022		
# of Pages:	27		


Mike Boulanger, ON0893-AT

THIS REPORT IS STILL UNDER REVIEW BY STAFF AND IS NOT CONSIDERED THE FINAL APPROVED DOCUMENT.

THIS DOCUMENT IS PROVIDED FOR INFORMATIONAL PURPOSES TO SUPPORT THE PUBLIC TREE REMOVAL REPORT TO COUNCIL RPF 27-22 FOR TREE #6 THE PUBLIC CEDAR TREE.

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Site & Report Background:

To obtain a permit to construct a 2-storey dwelling at 320 Pine Cove Road in Burlington.

I have visited the above site and collected all relevant data pertaining to the tree protection on site and any relevant trees within 4 meters of the property line. Data was collected on March 11, 2022 by Mike Boulanger ISA Certified Arborist.

Observations & Recommendations:

There are 7 trees identified on this report; any municipal trees located within 4 meters of the property line and all privately owned trees over 20cm in DBH within 4 meters of the property line.

All remaining trees to be preserved and protected throughout construction in accordance with the City of Burlington's private tree bylaw.

This report has been written in accordance the City of Burlington's Tree Protection and Preservation during Construction Procedures. Additional information can be found at https://www.burlington.ca/en/services-for-you/resources/Forestry%20Operations/Tree_Protection_and_Preservation/Tree_protection_and_preservtion.pdf

Inspection of the trees on site was limited to a visual assessment from the ground only, unless stated otherwise. No inspection via climbing, exploration below grade, probing, or coring were conducted. Any observations and data collected from site are based on conditions at the time of inspection. Diameters of trees located on neighbouring properties were estimated to avoid trespassing.

Tree Inventory

Tree No.	Species	DBH (cm)	Crown Reserve (m)	Health	Structural Condition	Preservation Rating	TPZ (m)	Ownership	Observations/ Comments/ Recommendations
1	Japanese Walnut, <i>Juglans ailantifolia</i>	71	18.3	P	P	L REMOVE	N/R	Private	PD; requires removal. Permit required. Large DW. CD. LCR >20%. IB. Sample ID #1: CCDBFR0692.
2	Japanese Walnut, <i>Juglans ailantifolia</i>	91	21.6	P	P	L REMOVE	N/R	Private	PD; requires removal. Permit required. CD. Large DW. TD.LCR >30%. Sample ID #2: CCDBFR0693.
3	Blue Spruce, <i>Picea pungens</i>	25	5	G	G	L REMOVE	N/R	Private	PP. Poor pruning cuts. PD; requires removal. Permit required.
4	Japanese Walnut, <i>Juglans ailantifolia</i>	45	15.7	F	F	H PRESERVE	3.0	Neighbour	CD. Large DW. PP. Sample ID #3: CCDBFR0694.
5	Eastern White Cedar, <i>Thuja occidentalis</i>	55	8.9	G	F	H PRESERVE	3.6	Municipal	Previously topped. Pruned for hydro clearance. Slightly LN. Air spading & root pruning recommended for new driveway.
6	Eastern White Cedar, <i>Thuja occidentalis</i>	46	10.9	G	F	L REMOVE	N/R 3.0	Municipal	PP; Pruned for hydro clearance. Slightly LN. PD; requires removal to accommodate proposed driveway. Permit and city's permission required.
7	Eastern White Cedar, <i>Thuja occidentalis</i>	49	12	G	F	H PRESERVE	3.0	Municipal	PP; Pruned for hydro clearance. Slightly LN.

*DBH Estimated due to access restraints
PWF = Protected with fencing

N/R= Tree Protection Fencing Not Required
Requires attention

TREE INVENTORY METHEDOLOGY

DBH (cm): Diameter at breast height, standard of 1.4m above ground.

Height (m): Height of tree from ground to top of crown.

Crown Reserve (m): Diameter of crown, the trees canopy width.

Health: General overall health of the tree. Rated as (E) excellent, (G) good, (F) fair, (P) poor, (D) dead.

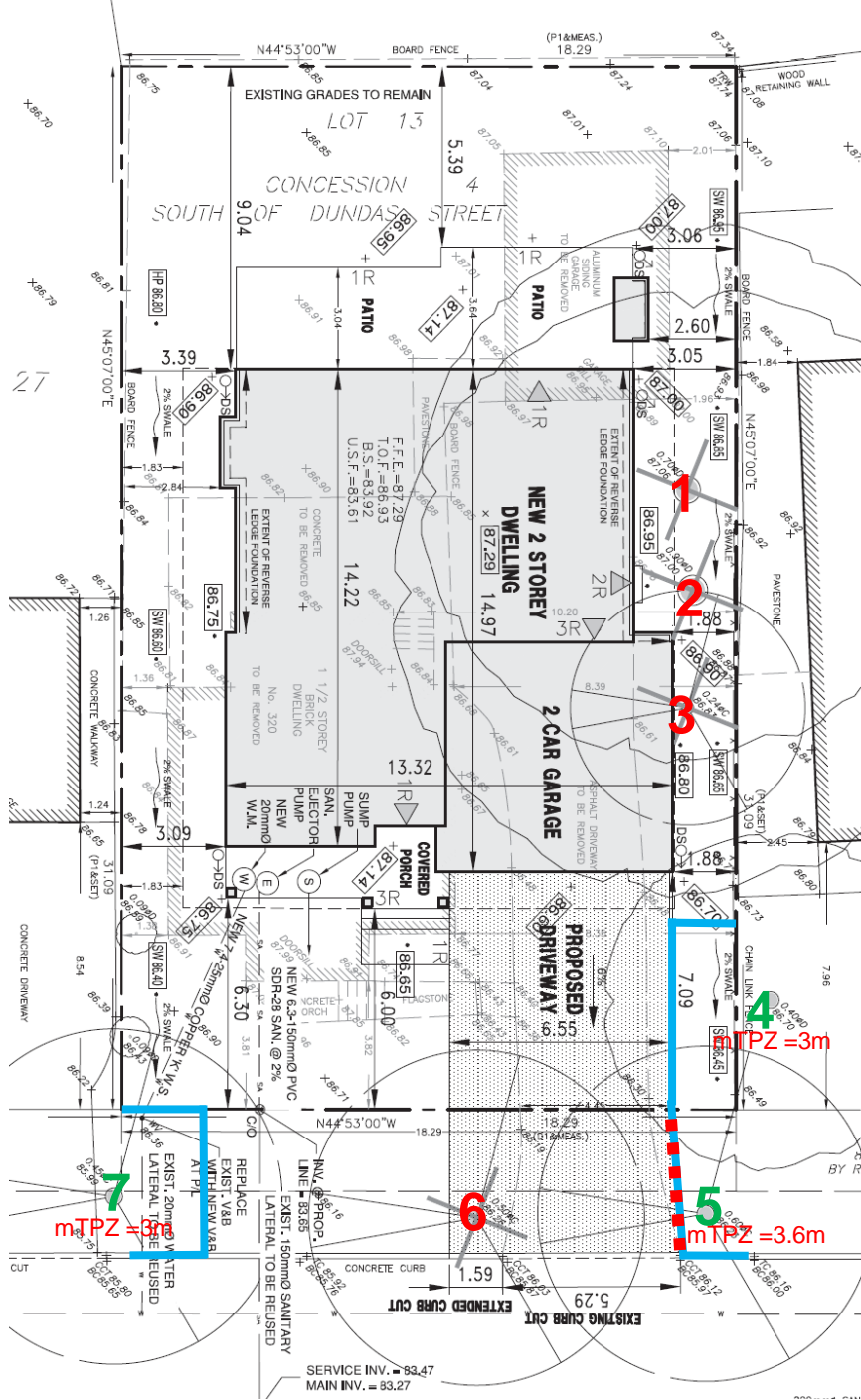
Structural Condition: Based on structural faults or defects Rated as (E) excellent, (G) good, (F) fair, (P) poor.

Preservation Rating: A trees projected survival based on the existing conditions. Rated as (H) high, (M) moderate, (L) low.

Observations, Comments, and Recommendations:

BC= broken crown	LN= leaning
BN= bark necrosis (dead/dying bark)	LS= light suppressed
BR= branch	MS= multiple stems
BS= bark split	PC= pollarded crown
BT= bent trunk	PL= poor leader development
CD= crown die back	PP= passed pruning
CN= crown	PTH= planted high
DC= tree in decline	PD= proposed development near tree
DD= dead	PS= prune away from structure
DF= defoliated	PT= preserve tree
DS= diseased	PTL= planted low
DW= deadwood	RB= remove basket
EC= elevate canopy	RC= requires cabling
ER= exposed roots	RM= remove plant
EX= existing cable	RP= requires pruning
FC= frost cracks	RS= remove stakes or wire
FS= plant in footprint of proposed structure	TD= trunk decay
GC= impact from grade changes	TP= transplanting potential
GR= girdling roots	TRS= transplant stress
HT= hazardous tree	TS= trunk split
IB= included bark	UT= understory tree

Site Plan & Tree Protection Measures: (tree canopies and locations are approximate and not to scale)



■■■■■ Air Spade & Root Pruning by certified arborist prior to excavation

— Tree Protection Fencing / Vertical Hoarding is indicated in BLUE

■ Root Protection Layer of woodchips and plywood or Steel Plating

TREE NUMBERS IN RED = REMOVAL GREEN = PRESERVE

Please see the Inventory Page to determine the recommended TPZ (Tree Protection Zone) requirements for each tree.

Site Photos



Wildwood Tree Services Ltd. Favored Tree Species for Urban Settings.

Large Shade Trees.

- Sugar Maple, *Acer saccharum*
- Red Oak, *Quercus rubra*
- American Beech, *Fagus grandifolia*
- European Beech, *Fagus sylvatica*
- Tulip Tree, *Liriodendron tulipifera*
- Kentucky Coffeetree, *Gymnocladus dioicus*

Evergreens (Coniferous)

- Silver Fir, *Abies concolor*
- Canadian Hemlock, *Tsuga canadensis*
- Eastern White Pine, *Pinus strobus*
- Colorado Blue Spruce, *Picea pungens*
- Hicks Yew, *Taxus media hicksii*
- Black Cedar, *Thuja Occidentalis Nigra*

Small and Ornamental Trees.

- Hackberry, *Celtis occidentalis*
- River Birch, *Betula nigra*
- Chanticleer Pear, *Pyrus calleryana chanticleer*
- Bradford Pear, *Pyrus calleryana bradford*
- Paperbark Maple, *Acer griseum*
- Bloodgood Japanese Maple, *Acer palmatum bloodgood*
- Siberian Crab Tree, *Malus baccata*
- Pyramidal English Oak, *Quercus robur fastigiata*
- Dawyck Purple Beech, *Fagus Sylvatica dawyck purple*
- Tricoloured Beech, *Fagus sylvatica 'Tricolor'*
- Florida Dogwood, *Cornus florida Std.*

It is best to consult an arborist to ensure you are selecting a species that is suitable for its location. A well maintained and carefully selected tree will ensure a tree that is both beautiful and sustainable.

**"The true meaning of life is to plant trees,
under whose shade you do not expect to sit."
~Nelson Henderson**

CORPORATION OF THE CITY OF BURLINGTON
SPECIFICATIONS
INDEX FOR
Tree Protection and Preservation
(SPEC NO. SS12A)

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Tree Protection and Preservation Specification No.: SS12A

February 2013

1. Scope

This specification describes the measures required to protect trees not designated for removal for all works within the City of Burlington's road right-of-way.

2. Definitions

For the purpose of this specification, the following definitions apply:

- a) **Certified Arborist:**
An arborist certified by the International Society of Arboriculture (ISA) who has a diploma (minimum) in arboriculture or urban forestry. They may be required to provide their ISA Certification number. The arborist must have minimum job experience of at least 6 years in Tree Protection and Mitigation;
- b) **Barrier:**
A fence placed around a single tree or group of trees to protect them from removal and injury;
- c) **Dripline:**
The location on the ground surface directly beneath the theoretical vertical line from the tips of the outermost branches of the trees.

3. Application of Policy

- a) Tree protection measures are required for City trees and other trees protected by City Tree by-law 19-1975 as amended and are recommended for all trees which may be impacted by construction activities;
- b) All trees situated on City property are protected under provisions of the City Tree by-law. Some trees situated on private property are also protected by the City Tree bylaw or as conditions of approvals granted by the City under site plan approval or subdivision agreements;
- c) Trees protected by the City Tree by-law may not be removed, injured or destroyed in any way without authorization from the City Arborist. Note that the term "tree" refers to all parts of the tree, including all roots. In certain cases, City Council will be required pursuant to the Tree By-law;
- d) The specifications set out in this policy shall be the standard specifications for tree protection measures during construction whenever tree protection measures are required by the City and in every instance where construction activities may result in damage to trees;
- e) Higher standards of tree protection may be imposed where warranted in the opinion of the City Arborist for municipal trees and Project Arborist for private trees with regard to the size, variety, location, and health of the tree and any circumstances surrounding the construction which may pose a particular hazard to the tree;
- f) Lesser standards of tree protection shall only be permitted on the recommendation of a Certified Arborist (as defined in this policy) and with the written consent of the City Arborist;
- g) Anyone failing to adhere to the tree protection policies and specifications outlined below will be financially responsible for any resulting damage to trees.

4. General Policy

Construction activities near trees may result in injury to the trunk, limbs or roots of trees causing damage or death of the tree. In order to prevent such damage:

- a) Trees within or adjacent to a construction area must be protected during construction by means of a barrier surrounding the Minimum Tree Protection Zone (MTPZ) as outlined in Sections 5 & 6 of this standard specification;
- b) Activities which are likely to injure or destroy the tree are not permitted within the MTPZ. Equipment or vehicles shall not be operated, parked, repaired or refueled within the dripline of trees.
- c) Tree pruning or root cutting of City owned trees is to be done in accordance with Section 8 of this specification.

**Tree Protection and Preservation
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- d) No objects may be attached to trees protected by the City Tree by-law without written authorization by the City. Construction material, supplies, or equipment and earth shall not be stockpiled within the dripline of any tree.
- e) No City tree or tree protected by the City Tree by-law may be removed without the written permission of the City Arborist.
- f) Exposed roots from excavation must be covered with soil, mulch or burlap and watered within 24 hours of exposure to prevent drying out.

5. The Minimum Tree Protection Zone (MTPZ)

The following is a chart showing minimum required distances for determining a Minimum Tree Protection Zone. Some trees and some site conditions may require a larger Minimum Tree Protection Zone at the discretion of the City.

Table 1 – Minimum Tree Protection Zones

Trunk Diameter (DBH) ²	Minimum Tree Protection Zone (MTPZ) Distances Required ³	Critical Root Zone (CRZ) Distances Required ^{3&4}
< 10 cm	1.8 m	1.8 m
11 - 40 cm	2.4 m	4.0 m
41 - 50 cm	3.0 m	5.0 m
51 - 60 cm	3.6 m	6.0 m
61 - 70 cm	4.2 m	7.0 m
71 - 80 cm	4.8 m	8.0 m
81 - 90 cm	5.4 m	9.0 m
91 - 100+ cm	6.0 m	10.0 m

NOTES:

- ¹ The roots of a tree can extend from the trunk to approximately 2-3 times the distance of the drip line.
- ² Diameter at breast height (DBH) measurement of tree trunk taken at 1.4 metres above ground.
- ³ Minimum Tree Protection Zone and Critical Root Zone distances are to be measured from the outside edge of the tree base towards the drip line and may be limited by an existing paved surface, provided the existing paved surface remains intact throughout the construction work and is subject to Section 6 of this specification.
- ⁴ Where work is being performed beyond the Minimum Tree Protection Zone but within the Critical Root Zone the works are subject to Section 8 of this specification.

6. Tree Protection Barriers

Trees within Minimum Tree Protection Zones shall be protected by means of a “tree protection barrier” meeting the following specifications:

- a) The required barrier is a 1.2 metre (4 ft) high orange plastic web snow fencing on 2” x 4” frame. Where orange plastic web snow fencing creates a restriction to sightlines, page wire fencing with reflective tape can be used.
- b) Tree protection barriers are to be erected prior to the commencement of any construction or grading activities on the site and are to remain in place throughout the entire duration of the project. The barriers shall be maintained erect and in good repair throughout the duration of construction operations with breaks and unsupported sections repaired immediately. Tree protection may be not be removed prior to the completion of construction without written authorization from the City Arborist.
- c) All supports and bracing used to safely secure the barrier should be located outside the MTPZ. All supports and bracing should minimize damage to roots.
- d) Where some fill or excavated material must be temporarily located near a MTPZ, a wooden barrier with silt fencing must be used to ensure no material enters the MTPZ.

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- e) No materials or fill may be stored within the MTPZ.
- f) Equipment or vehicles shall not be operated, parked, repaired, or refueled within the MTPZ.
- g) No construction activity, grade changes, surface treatment or excavations of any kind is permitted within the MTPZ without written authorization from the City Arborist.
- h) A laminated Minimum Tree Protection Zone sign (see attached) must be attached to the most visible side of the Tree Protection, where it will be visible by those persons entering the site. Minimum size must be 10"x14".

7. Tree Removal and Relocation

Any requests for removal, cutting, pruning or relocating a tree must be made in writing to the City. If approval is granted for removal of a tree, the contractor will assume all costs involved. In most instances tree replacement or compensation will be required.

8. Site Services or Excavations within the Minimum Tree Protection Zone

It is recognized that there are cases where trees are growing overtop existing underground utilities that may require inspection and/or new installation. While the guidelines in this standard specification still apply, in these cases some modification to the Minimum Tree Protection Zone, in addition to pre-construction pruning, may be required where work within or near the MTPZ is approved by the City Arborist. The objective is to avoid severance of anchor roots and or major branches and minimize damage to the tree(s) and to maintain the natural form of the crown.

- a) Above ground clearance for overhanging branches in the work zone must be anticipated. The contractor is required to have a City approved tree service raise the crown of all branches to provide adequate clearance for construction equipment. The cost of above ground pre-construction pruning is to be borne by the party that requires the work to be completed.
- b) When excavation is required for inspection (only) of private water and sanitary lateral connections at the property line, are within the limits of the MTPZ and the CRZ and have the potential to damage tree roots, excavation must be completed by a qualified arborist or under an arborist's supervision using a hydro vacuum unit or air powered soil excavation tools in order to preserve the integrity of the tree's roots. When new private water and sanitary lines must be installed, and there is not a suitable location outside of the MTPZ and the CRZ, directional micro tunneling and boring is the preferred option for installation within the MTPZ and CRZ.
- c) When any site works, are within the limits of the MTPZ and the CRZ and have the potential to damage tree roots, root pruning using a hydro vacuum unit or air powered soil excavation tools is acceptable, provided it is operated by a qualified arborist or under an arborist's supervision, and completed to a depth of 300mm. Roots are to be cut a maximum of 150 mm from the edge of excavation (grading or removals). The limit of excavation, grading or removals is to be minimized to the greatest extent possible and is to include the use of excavation shoring, smaller excavation equipment or rubber tired machines. See Detail TP-2 (attached as an appendix) for further information.

9. Tree Repair

Trees damaged by construction operations shall be repaired as follows, within five days of the damage:

- a) Branches 25 mm or greater in diameter that are broken shall be cut back cleanly on the tree side of the break or to within 10 mm of their base, if a substantial portion of the branch is damaged.
- b) Bark that is damaged shall be neatly trimmed back to the uninjured bark without causing further injury to the tree.
- c) Root pruning within the Minimum Tree Protection Zone of any tree requires root exploration via supersonic air tool or hydro vacuum unit to first remove the soil and expose the roots.

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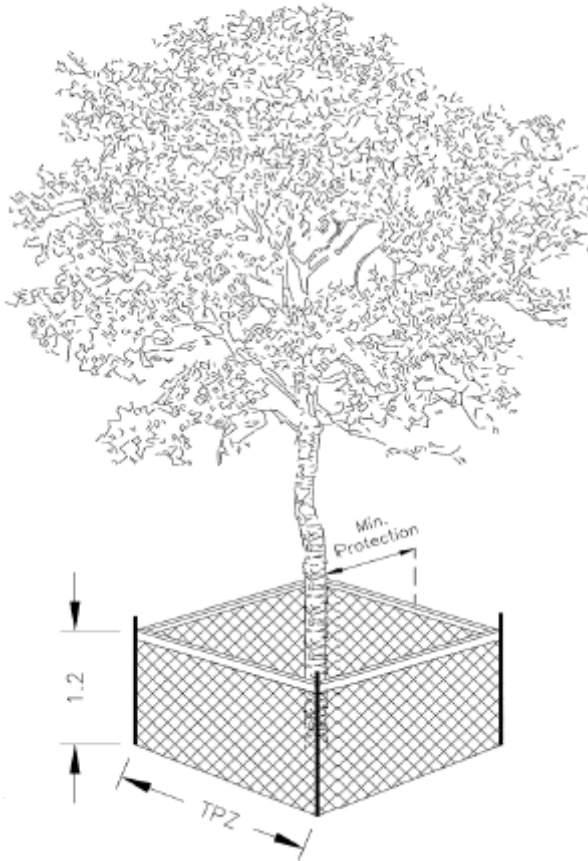
- d) Roots under 2 cm in diameter can be pruned using a sharpened tool such as hand pruners or a sharpened spade under the supervision of the Construction Inspector.
- e) Roots between 2 and 8 cm in diameter can be pruned by the arborist using a sharp tool, such as a handsaw, hand pruner or loppers and under the supervision of the Construction Inspector and the advisement of the Project Arborist.
- f) All roots over 8 cm in diameter must be assessed by the Project Arborist prior to pruning unless the arborist on-site can confidently assess the effect of the removal of the root as not detrimental to the tree.
- g) Root pruning within the Critical Root Zone and outside of the MTPZ, typically requires the use of a sharpened garden spade, cutting a line to a depth of about 30 cm by the on-site arborist under the advisement of the Project Arborist if needed. However, the same pruning protocol for the size of roots encountered (in the MTPZ) applies to the roots found within this area.
- h) The trenches are typically backfilled with the same excavated soil or new topsoil or compost and hoarding should be installed along this trench to project the remaining roots.

10. Securities for Non-Compliance

Where a tree has been damaged through non-compliance with the Tree Protection and Preservation Specification, any security provided for tree preservation or replacement may be held by the City for up to three (3) years from the date of the final inspection of the construction project. If the tree dies or needs to be replaced within those three years, the Owner shall pay for replacement planting with nursery stock according to the City of Burlington's Aggregate Caliper Formula. Replacement trees must have a minimum height of 180cm for coniferous trees and 80mm caliper for deciduous replacements. Replacements must be made to the satisfaction of the City Arborist. If the Owner meets their financial obligations and there is no further need to care for the tree, after three (3) years, the City shall return the deposit(s) or the balance of the deposit(s) if the City had to use some of the money for monitoring, preservation or replacement of the tree. If the tree does not recover to the satisfaction of the City Arborist, then the City shall retain the deposit(s) for the continued maintenance, preservation or eventual replacement of the tree. The Owner may be required to post additional securities if, in the opinion of the City Arborist, the saved tree is very damaged and will need on-going monitoring, preservation or replacement that will exceed the cost of the deposit(s) mentioned above.

Securities may be released prior to the expiry of the three year period provided the City is satisfied that the tree has not been damaged. Applicants for the early release or reduction of securities shall submit a report from a Certified Arborist certifying that the tree is in a state of vigorous health and has not been injured or destroyed as a result of the construction activities.

Detail TP-1 – Tree Protection Detail.



Trunk Diameter (DBH) ²	Minimum Tree Protection Zone (MTPZ) Distances Required ³	Critical Root Zone (CRZ) Distances Required ^{3&4}
< 10 cm	1.8 m	1.8 m
11 - 40 cm	2.4 m	4.0 m
41 - 50 cm	3.0 m	5.0 m
51 - 60 cm	3.6 m	6.0 m
61 - 70 cm	4.2 m	7.0 m
71 - 80 cm	4.8 m	8.0 m
81 - 90 cm	5.4 m	9.0 m
91 - 100+ cm	6.0 m	10.0 m

NOTES:

¹ The roots of a tree can extend from the trunk to approximately 2-3 times the distance of the drip line.

² Diameter at breast height (DBH) is the measurement of tree trunk taken at 1.4 metres above ground.

³ Minimum Tree Protection Zone and Critical Root Zone distances are to be measured from the outside edge of the tree base towards the drip line and may be limited by an existing paved surface, provided the existing paved surface remains intact throughout the construction work and is subject to Section 6 of this specification.

⁴ Where work is being performed beyond the Minimum Tree Protection Zone but within the Critical Root Zone the works are subject to Section 8 of this specification.

TREE PROTECTION BARRIER

1. The required barrier is a 1.2 metre (4 ft) high orange plastic web snow fencing on 2" x 4" frame. Where orange plastic web snow fencing creates a restriction to sightlines, page wire fencing with reflective tape can be used.
2. Tree protection barriers are to be erected prior to the commencement of any construction or grading activities on the site and are to remain in place throughout the entire duration of the project. The barriers shall be maintained erect and in good repair throughout the duration of construction operations with breaks and unsupported sections repaired immediately. Tree protection may be not be removed prior to the completion of construction without written authorization from the City Arborist.
3. All supports and bracing used to safely secure the barrier should be located outside the MTPZ. All supports and bracing should minimize damage to roots.
4. Where some fill or excavated material must be temporarily located near a MTPZ, a wooden barrier with silt fencing must be used to ensure no material enters the MTPZ.
5. No materials or fill may be stored within the MTPZ.
6. Equipment or vehicles shall not be operated, parked, repaired, or refueled within the MTPZ.
7. No construction activity, grade changes, surface treatment or excavations of any kind is permitted within the MTPZ without written authorization from the City Arborist.
8. A laminated Minimum Tree Protection Zone sign (See Detail TP-3 – Minimum Tree Protection Zone Sign) must be attached to the side of the Tree Protection where it will be visible by persons entering the site. Minimum size must be 10"x14".

Date of issue: April 05, 2022

CLIENT INFORMATION

Client Name: Mike Boulanger
Client Address: Wildwood Tree Services Ltd.
3448 Ninth Line, Oakville, Ontario L6H 7A9
Contact Name: Jenn/Mike Boulanger (info@wildwoodtree.ca)

ITEMS

Description: Three samples (twigs) from putative butternut submitted for hybrid detection.

Sample ID	Sample ID provided	Process ID
CCDBFR0692	1	ABCBF711-22
CCDBFR0693	2	ABCBF712-22
CCDBFR0694	3	ABCBF713-22

Dates Received: March 16, 2022

Sample Received by: Maria Kuzmina – Research Associate, Plant Lead from Mike Boulanger by Xpresspost

Dates of Analysis: March 17-30, 2021

Analyses by: Nguyen NguyenTX. / Canadian Centre for DNA Barcoding, Biodiversity of Ontario, University of Guelph, 50 Stone Road East, Guelph

METHODS

To ascertain the identity of the species from the submitted samples, an approximate 1mm by 1mm area of bud from samples CCDBFR0692, CCDBFR0693, and CCDBFR0694 was subsampled using sterile techniques. The samples were ground to a fine powder and then lysed. Total genomic DNA was extracted using validated spin column DNA extraction protocol. Two target genetic markers: the second internal transcribed spacer from the nuclear ribosomal DNA (ITS2), and an intergenic spacer between the chloroplast genes *trnL* and *trnF* (*trnL-trnF*) were amplified by using the Polymerase Chain Reaction (PCR) with the primer cocktails ITS_S2F/ITS4 and *trnFGAA-f/trnLUAA-c*, respectively; followed by cycle sequencing with standardized commercially available BigDye Terminator v3.1 kit. Sequencing reactions were analyzed by high-voltage capillary electrophoresis using the automated ABI 3730xL DNA Analyzer. Bidirectional forward and reverse sequences were generated from the samples. Resulting trace files were assembled into contigs, manually edited, and aligned in CodonCode Aligner (version 4.1.1.) software. The DNA sequences recovered from the samples were compared against the ITS2 and *trnL-trnF* reference libraries in the Barcode of Life Data System (BOLD) accessible at <http://www.boldsystems.org/>.

IMAGING

The items were photographed in the Photography Lab Area by Nguyen NguyenTX., using a Canon ELPH 300 HS, 12.1 megapixels. Pictures were uploaded to the BOLD website into a secure project called "CCDB forensic sampling [ABCBF]". See Appendix 1 for item images.

INTERPRETATION

Based on the number of nucleotide substitutions between sequence from the test sample and reference DNA barcode, the closest match was used to infer species identity for the corresponding test samples provided by the contributor. The sequence trace base calls for diagnostic sites in ITS2 were used to resolve hybridization.

The ITS2 marker demonstrated five nucleotide substitutions between *Juglans cinerea* (white walnut) and *Juglans ailantifolia* (Japanese walnut) reference sequences across the amplified ~344 base pair length. Unlike the plastid genome, ribosomal nuclear DNA is inherited by both maternal and paternal organisms. Thus, hybridization events are supported by presence of mixed signals at the characteristic nucleotide positions in the trace file chromatograms. Therefore, the ITS2 DNA barcode can be used to identify hybridization events between species.

The *trnL-trnF* marker demonstrated five nucleotide substitutions between *Juglans cinerea* (white walnut) and *Juglans ailantifolia* (Japanese walnut) reference sequences across the target ~950 base pair length. This marker is a part of the chloroplast genome and is inherited maternally. It is therefore not suitable for hybridization testing but as it can only verify the maternal lineage. Here it was used as a supplementary marker.

RESULTS

The full length ITS2 and *trnL-trnF* genetic markers (~344 base pairs and ~950 base pairs, respectively) for the samples CCDBFR0692, CCDBFR0693, and CCDBFR0694 were aligned with the known reference sequences for *Juglans cinerea* (white walnut, butternut), *Juglans ailantifolia* (Japanese walnut), and their hybrid *Juglans* CCDB, BIO, UofG, 50 Stone Rd E, Building 138, Guelph, ON, Canada N1G 2W1 Tel: 1-519-824-4120 Fax: 1-519-824-5703

cinerea x *Juglans ailantifolia*. The alignments were analyzed by visual comparison, and by building the Neighbor Joining (NJ) phylogenetic trees using BOLD (Figures 1-8).

ITS2

The chromatograms for the samples CCDBFR0692, CCDBFR0693, and CCDBFR0694 match with the reference of hybrid *Juglans cinerea* x *Juglans ailantifolia* (Figures 1, 2, and 3). All three analyzed samples revealed mixed basecalls at the nucleotide positions which are diagnostic for each of the two species in questions. This basecall pattern is indicative of hybridization between two parental organisms of *Juglans cinerea* and *Juglans ailantifolia*. Therefore, our analysis confirms that samples CCDBFR0692, CCDBFR0693, and CCDBFR0694 are of hybrid origin.

trnL-trnF

The analysis of *trnL-trnF* sequences showed that the maternal organism for the provided samples belong to *Juglans ailantifolia* with five nucleotide substitutions (across the amplified length of the marker) differentiating it from *Juglans cinerea*. As shown in Figures 5, 6, and 7, samples CCDBFR0692, CCDBFR0693, and CCDBFR0694 match those of reference sequences for *Juglans ailantifolia*.

CONCLUSIONS

The present testing indicated that the provided samples CCDBFR0692, CCDBFR0693, and CCDBFR0694 are hybrids between *Juglans cinerea* x *Juglans ailantifolia*. The maternal organism for these samples was *Juglans ailantifolia* (Japanese walnut).

FIGURES



Figure 1. ITS2 sequence comparison of samples CCDBFR0692, CCDBFR0693, and CCDBFR0694, and *Juglans cinerea*, *Juglans ailantifolia*, and hybrid *Juglans cinerea x Juglans ailantifolia* reference sequences from the BOLD reference library at the base ~98 and ~103.



Figure 2. ITS2 sequence comparison of samples CCDBFR0692, CCDBFR0693, and CCDBFR0694, and *Juglans cinerea*, *Juglans ailantifolia*, and hybrid *Juglans cinerea x Juglans ailantifolia* reference sequences from the BOLD reference library at the base ~182.



Figure 3. ITS2 sequence comparison of samples CCDBFR0692, CCDBFR0693, and CCDBFR0694, and *Juglans cinerea*, *Juglans ailantifolia*, and hybrid *Juglans cinerea x Juglans ailantifolia* reference sequences from the BOLD reference library at the base ~242 and ~264.

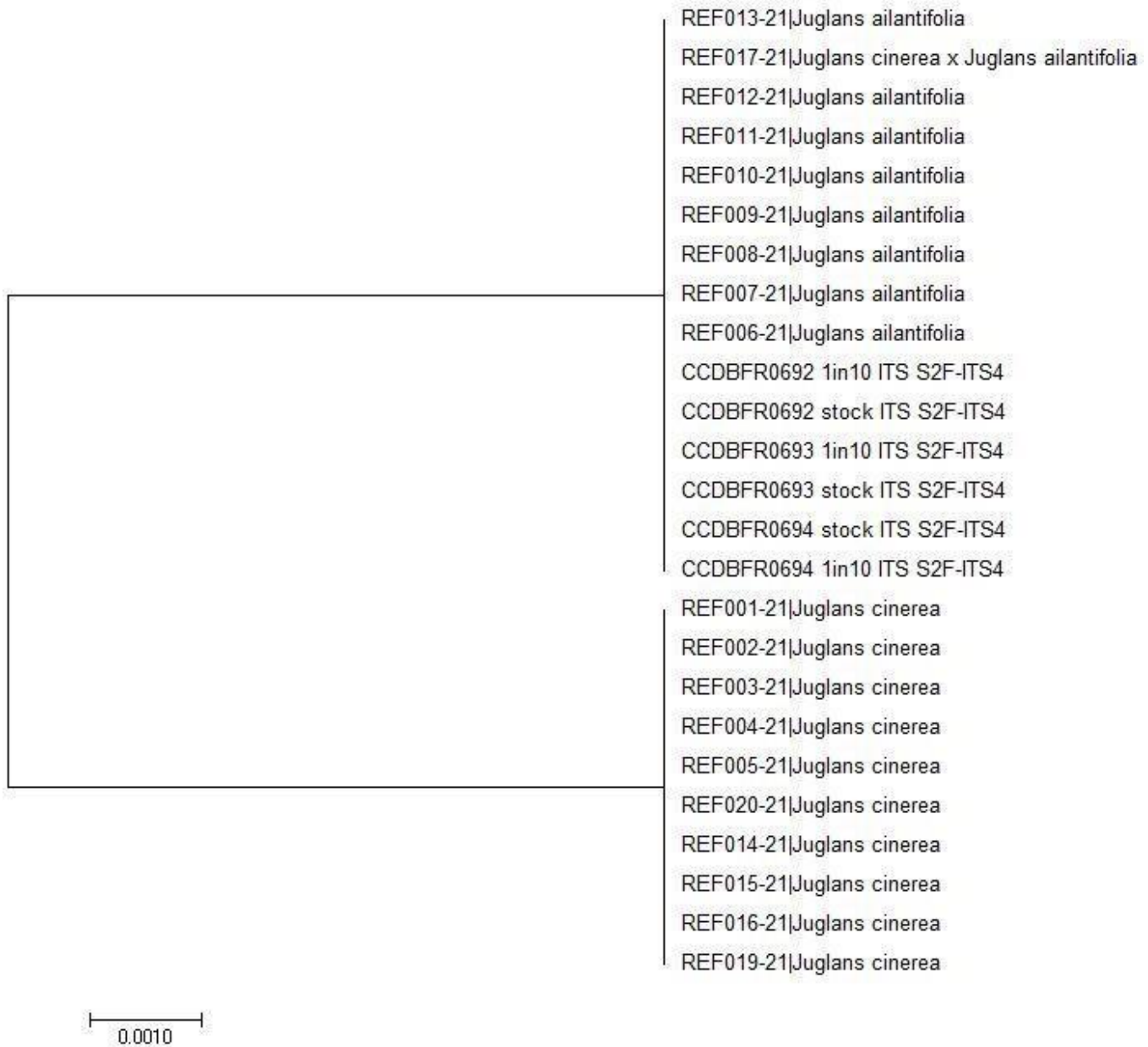


Figure 4. NJ phylogenetic tree of ITS2 sample sequences CCDBFR0692, CCDBFR0693, and CCDBFR0694, and *Juglans cinerea*, *Juglans ailantifolia*, and hybrid *Juglans cinerea* x *Juglans ailantifolia* reference sequences from the BOLD reference library.



Figure 5. *trnL-trnF* sequence comparison of samples CCDBFR0692, CCDBFR0693, and CCDBFR0694, and *Juglans cinerea*, *Juglans ailantifolia*, and hybrid *Juglans cinerea* x *Juglans ailantifolia* reference sequences from the BOLD reference library at the base ~200 and ~276.

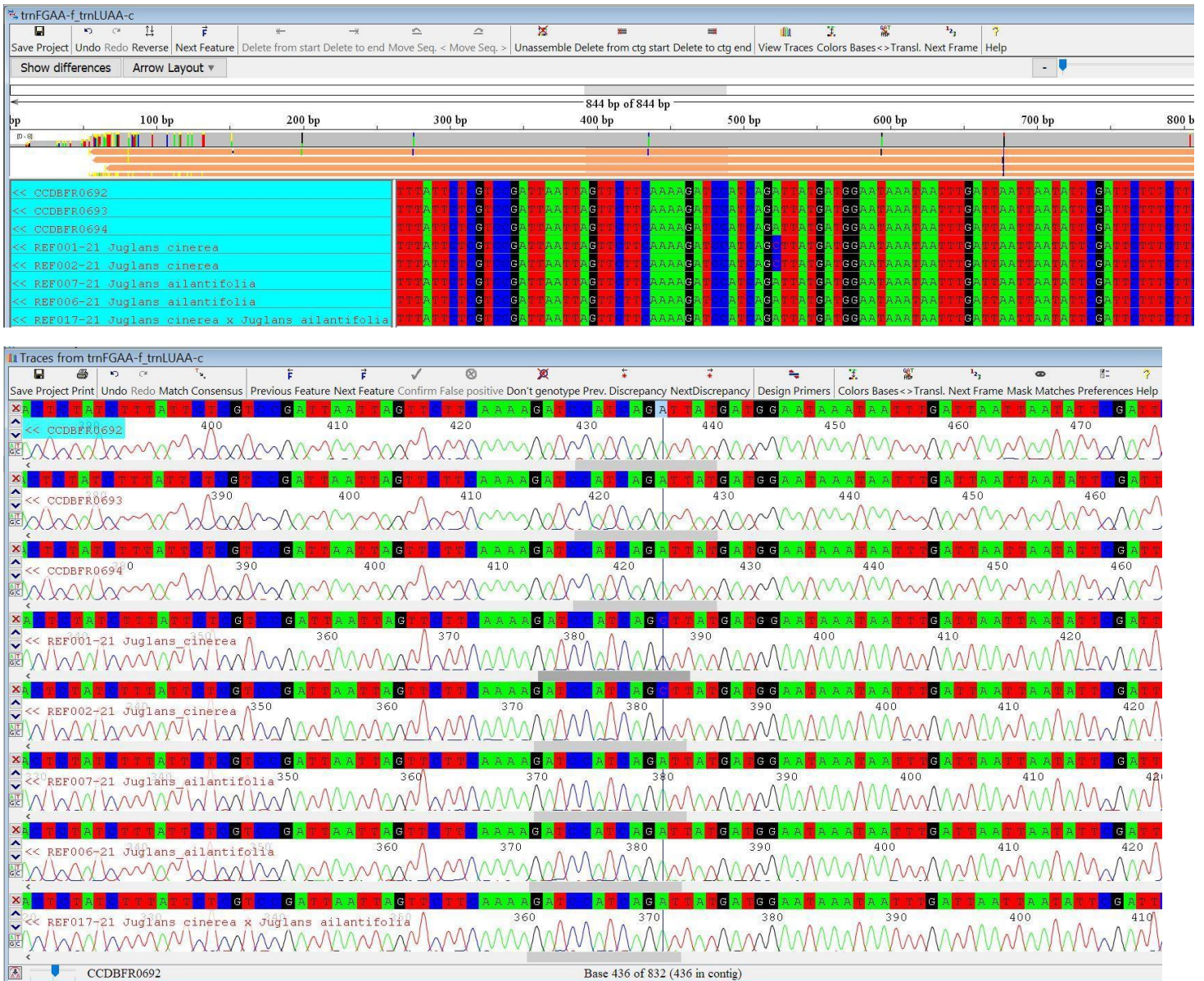


Figure 6. *trnL-trnF* sequence comparison of samples CCDBFR0692, CCDBFR0693, and CCDBFR0694, and *Juglans cinerea*, *Juglans ailantifolia*, and hybrid *Juglans cinerea x Juglans ailantifolia* reference sequences from the BOLD reference library at the base ~436.



Figure 7. *trnL-trnF* sequence comparison of samples CCDBFR0692, CCDBFR0693, and CCDBFR0694, and *Juglans cinerea*, *Juglans ailantifolia*, and hybrid *Juglans cinerea* x *Juglans ailantifolia* reference sequences from the BOLD reference library at the base ~595 and ~678.

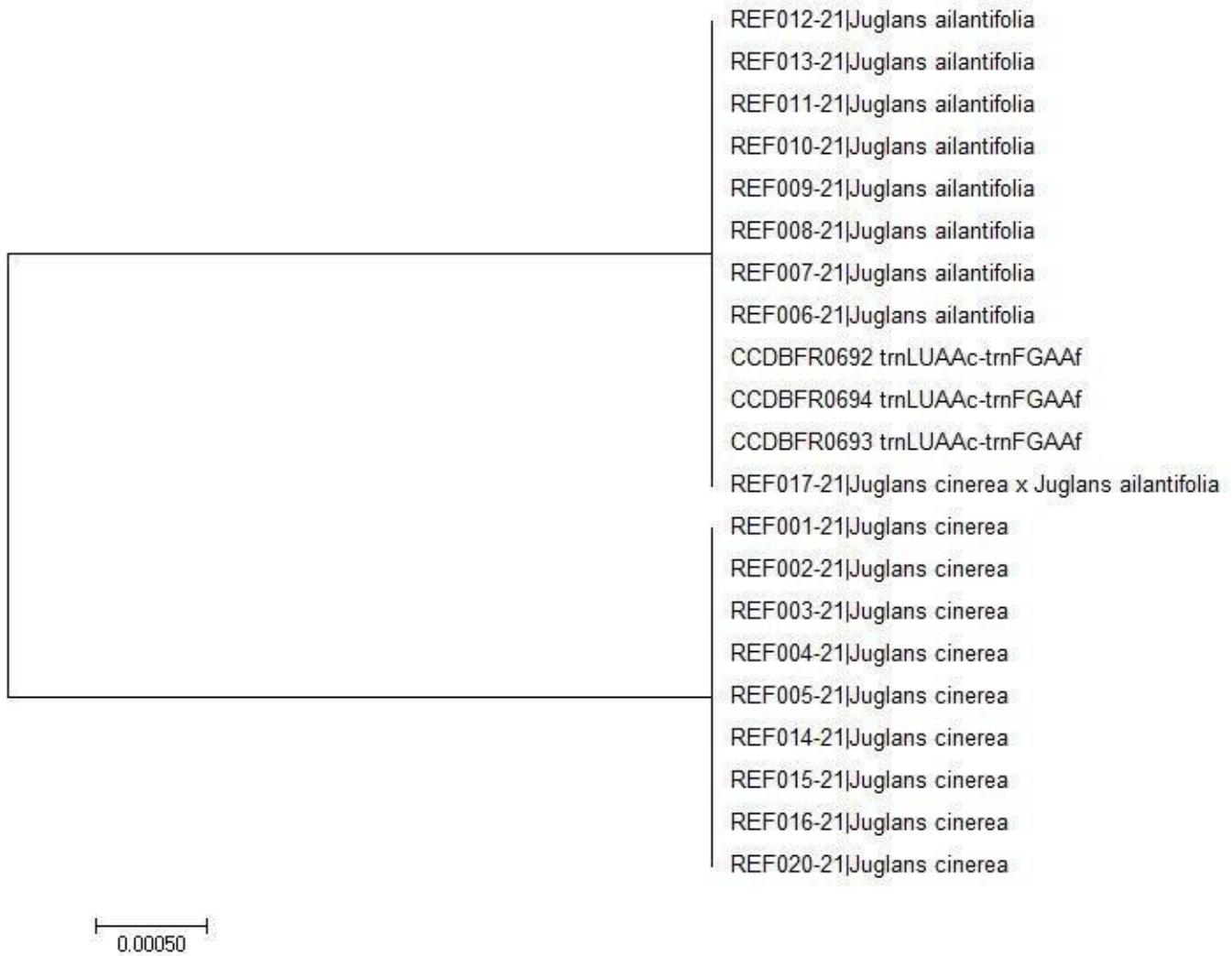


Figure 8. NJ phylogenetic tree of *trnL-trnF* sample sequences CCDBFR0692, CCDBFR0693, and CCDBFR0694, and *Juglans cinerea*, *Juglans ailantifolia*, and hybrid *Juglans cinerea x Juglans ailantifolia* reference sequences from the BOLD reference library.

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Appendix 1. Image Inventory



Image 1 – Samples CCDBFR0692, CCDBFR0693, and CCDBFR0694.