

ARBORIST REPORT
HIGHWAY 124 & POPLAR SIDE ROAD
TOWN OF COLLINGWOOD

PREPARED FOR:
CHARIS DEVELOPMENTS LTD.

PREPARED BY:
C.F. CROZIER & ASSOCIATES INC.

70 HURON STREET, SUITE 100
COLLINGWOOD, ON L9Y 4L4

SEPTEMBER 2024

CFCA FILE NO. 2199-6198-2

The material in this report reflects best judgment in light of the information available at the time of preparation. Any use which a third party makes of this report, or any reliance on or decisions made based on it, are the responsibilities of such third parties. C.F. Crozier & Associates Inc. accepts no responsibility for damages, if any, suffered by any third party as a result of decisions made or actions based on this report.



Revision Number	Date	Comments
Rev. 0	September 17, 2024	Issued for OPA/ZBA

TABLE OF CONTENTS

1.0 INTRODUCTION.....1

2.0 METHODOLOGY.....1

3.0 SUMMARY OF TREES INVENTORIED2

 3.1 Development Impacts/Tree Removal2

 3.2 Tree Retention and Protection2

4.0 TREE REPLACEMENT3

5.0 SUMMARY AND RECOMMENDATIONS.....3

LIST OF APPENDICES

- Appendix 1:** Tree Inventory Chart
- Appendix 2:** Aerial Photo
- Appendix 3:** Tree Protection Plan
- Appendix 4:** DNA Test Results for *Juglans* spp.

1.0 Introduction

C.F. Crozier & Associates was retained to provide a tree inventory and assessment of existing trees within the boundaries of the property located at the South-East Corner of Highway 124 and Poplar Side Road as they pertain to the Town of Collingwood Tree By-law and Development Standards. The site is located at the south end of the Town of Collingwood adjacent to an existing residential neighbourhood to the East and a commercial neighborhood to the West. Commercial, retail is proposed for phase 1 and 2 and residential and office space is proposed for phase 3 on the site.

Field work was completed on August 20, 2024, and this report relates to the condition of the trees as observed on that date. The report is meant to describe the health and composition of the existing trees on site, the evaluation of which will inform the Tree Protection Plan.

2.0 Methodology

The following Town of Collingwood policies and by-laws were referred to for the purposes of completing the Arborist report and Tree Protection Plan:

- By-Law No. 2012-084 Being a By-Law to Prohibit and Regulate the Destruction or Injury of trees in the Town of Collingwood, June 25, 2012
- Town of Collingwood Development Standards, July 2007
- Town of Collingwood Urban Design Manual, December 2010

Existing trees of all sizes were inventoried and evaluated using Trimble GPS and a current survey depicting edge of row of trees only, dated September 22, 2021. The inventory includes all trees within the site boundary, all trees with a DBH of 15cm or more, within 10.0 meters of the site boundary and all Town owned trees along the adjacent boulevards. Species at risk/endangered species were searched for and have been documented.

This inventory is summarized graphically in the Tree Protection Plan, which is to be read together with this report and shall form part of this report. For the purposes of this report, trees and groupings of trees are identified in terms of:

- i. Tree ID number – number assigned to the tree.
- ii. Tree species – botanical and common names provided.
- iii. Diameter breast height (DBH) – measured in cm 1.4m above ground.
- iv. Canopy radius – in metres.
- v. Arboricultural condition – condition of tree considering trunk integrity, crown structure and crown vigor.
- vi. Directive – Tree to be retained or removed.
- vii. Minimum tree protection zones (TPZ) for retained trees – Minimum Tree Protection Zone in meters, using similar methods to many other municipalities.
- viii. Comments – Additional information regarding the tree or grouping.

The following rating system was used in describing the arboricultural condition of the trees inventoried:

Good: Indicates a condition of vigor and no major concerns.

Fair: Indicates an adequate tree, which may have some minor issues.

Poor: Indicates declining health, poor form, or other more serious issues.

Dead: Indicates a dead tree that should be removed.

3.0 Summary of Trees Inventoried

The following section discusses the reason for removal and the retention of trees within and adjacent to the subject site. Please refer to Appendix 1 for the tree inventory chart that provides further information regarding each tree, Appendix 2 for an aerial photo of the property, Appendix 3 for the Tree Protection Plan identifying the locations of each tree discussed, and Appendix 4 for the DNA test results for tree #9.

3.1 Development Impacts/Tree Removal

A total of ninety (90) trees and five (5) tree groupings were inventoried and assessed, of which seventy-three (73) trees and five (5) groupings were determined to be in conflict with the proposed development. Trees and vegetation within the property will be removed to facilitate placement of fill and re grading of the property.

Trees #2 to 9, 13, 30, 31, and 32 are along the eastern property boundary and will be retained. Preliminary grading indicates that there is potential they may be injured/harmed due to the placement of fill and grading within the drip line to match grade at the eastern property line. Measures to mitigate these potential grading impacts will be considered and implemented where possible during detailed design.

3.2 Tree Retention and Protection

Tree #1 is in the ROW along Poplar Sideroad and will be retained although it is in poor condition. Trees 61 and 62 are in the ROW on Hurontario Street and will be retained and are in fair condition.

Trees #2 to 9, 13, 30, 31, and 32 are along the eastern property boundary and will be retained to provide a buffer between the commercial development and adjacent residential land use. Trees 4, and 31 will be retained and are in poor condition. Trees #2, 3, 5, 6, 7, 8, 9, 32, 35 will be retained and are in fair condition. Trees 13 and 30 will be retained and are in good condition.

Tree #9 has been identified as a *Juglans* species. As such a DNA test was undertaken and has confirmed that the tree is a hybrid between white and Japanese walnut species, *Juglans cinerea* x *Juglans ailantifolia*. See Appendix 4 for the DNA test results. The tree will be retained and is in fair condition.

As the above-mentioned trees are being retained the establishment of a Tree Protection Zone around them is required. The purpose of the tree protection zone is to prevent root damage, soil compaction and soil contamination during construction activities and as such workers and machinery shall not disturb or move the tree protection zone in any way. To prevent damage and access to the roots tree protection hoarding shall be installed as per Town of Collingwood Tree Protection Fence STD No. 1110. The tree protection hoarding shall be maintained in good condition for the duration of construction and shall not be removed until all construction activities have been completed. No fill, excavation, equipment, vehicles, supplies, or waste are permitted within the tree protection zone. Tree protection signage shall be attached to all sides of the tree protection barrier and shall be a minimum of 40cm x 60cm and made of white corrugated plastic board or equivalent material.

The Town of Collingwood shall be contacted to obtain approval prior to any changes to the location of the approved TPZ or sediment control, or where temporary access to the TPZ is proposed. Before, during and post construction a Landscape Architect or certified consulting

Arborist (I.S.A.) should make periodic visits to ensure tree protection barriers are being properly utilized. Any hazard trees or limbs should be identified for removal by the Developer if required.

4.0 Tree Replacement

As per the Town of Collingwood Urban Design Manual, 30% tree canopy cover at maturity is required for site plan approval. Refer to the Conceptual Landscape Plan prepared by C.F. Crozier & Associates Inc., September 17, 2024, for the location of proposed replacement trees. Canopy coverage meets and exceeds the 30% tree canopy coverage requirement.

5.0 Summary and Recommendations

In total, ninety (90) individual trees and five (5) tree groupings were inventoried and assessed on and within 10.0m of the subject property. We have based our recommendations for retention and removal of trees on the current site plan drawings and the health and condition of the trees while assuming best practices during construction.

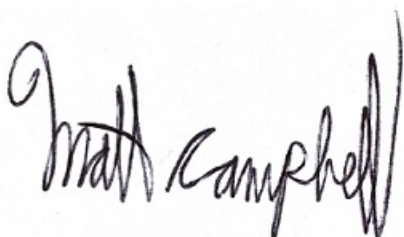
A total of seventy-three (73) individual trees and five (5) tree groupings were determined to be in conflict with the proposed development or in poor/dead condition. The remaining seventeen (17) trees will be retained and protected by tree protection hoarding the locations of which are shown on the Tree Protection Plan (Appendix 3).

The following recommendations are made to minimize impacts on those trees to be retained:

- Tree protection hoarding shall be installed and maintained in good condition for the duration of construction and shall not be removed until all construction activities have been completed.
- No fill, excavation, equipment, vehicles, supplies, or waste are permitted within the tree protection zone.
- Before, during and post construction a Landscape Architect or certified Arborist (I.S.A.) should make periodic visits to ensure tree protection barriers are being properly utilized.

Respectfully submitted,

C.F. CROZIER & ASSOCIATES INC.



Matthew Campbell, OALA, CSLA, ISA Certified Arborist ON-3008A, TRAQ
Senior Contract Administrator

\\CROZIER-FILES\Projects\2100\2199- Charis Developments\6198- The Gateway Centre\Reports\Arboriculture\2199-6198
Arborist Report_2024.08.30.docx

APPENDIX 1

TREE INVENTORY CHARTS

APPENDIX 1: TREE INVENTORY CHART

ID No.	Botanical Name	Common Name	DBH (cm) (Diameter at Breast Height)	Canopy Radius (m)	Condition	Directive	Comment
1	<i>Pinus sylvestris</i>	Scots pine	21	1.5	Poor	Retain	Under hydro utility line, topped
2	<i>Acer platanoides</i>	Norway maple	15	2.5	Fair	Retain	Boundary tree, grape ivy
3	<i>Ulmus pumila</i>	Siberian elm	25	3.5	Fair	Retain	Boundary tree, fence inclusion, codominant at 1.4m
4	<i>Ulmus pumila</i>	Siberian elm	57	6	Poor	Retain	Boundary tree, dead wood in canopy, evidence of rot
5	<i>Ulmus pumila</i>	Siberian elm	31	4	Fair	Retain	Boundary tree, fill on root zone, poor canopy
6	<i>Ulmus pumila</i>	Siberian elm	42	6	Fair	Retain	Boundary tree, dead wood in canopy
7	<i>Ulmus pumila</i>	Siberian elm	34	6	Fair	Retain	Boundary tree, multi-stem: 34, 32, & 30cm DBH
8	<i>Ulmus pumila</i>	Siberian elm	50	7	Fair	Retain	Boundary tree, irregular crown, long horizontal branches within site
9	<i>Juglans spp.</i>	Hybrid between white and Japanese Walnut	32	5	Fair	Retain	Boundary tree, leaves are blistering, one sided canopy. DNA testing confirmed species is a hybrid. Refer to DNA report.
10	<i>Populus alba</i>	White poplar	21	4	Good	Remove	
11	<i>Populus alba</i>	White poplar	25	3	Good	Remove	Growing on bottom of fill pile.
12	<i>Populus alba</i>	White poplar	16	3.5	Good	Remove	Growing on bottom of fill pile.
13	<i>Acer platanoides</i>	Norway maple	34	5	Good	Retain	Multi-stem: 34, 34, 18, 25, & 20cm DBH
14	<i>Ulmus pumila</i>	Siberian elm	20	4	Good	Remove	Growing on fill pile
15	<i>Ulmus pumila</i>	Siberian elm	21	4	Good	Remove	Growing on fill pile
16	<i>Populus alba</i>	White poplar	15	4.5	Good	Remove	
17	<i>Ulmus pumila</i>	Siberian elm	15	4.5	Fair	Remove	Multi-stem: 15 & 15cm DBH, growing on fill pile
18	<i>Ulmus pumila</i>	Siberian elm	15	3.5	Poor	Remove	2 stems, growing on fill pile, diseased
19	<i>Populus nigra</i>	Black poplar	30	1	Poor	Remove	Columnar form
20	<i>Populus nigra</i>	Black poplar	22	1	Poor	Remove	Multi-stem: 22 & 22cm DBH
21	<i>Populus nigra</i>	Black poplar	36	1	Poor	Remove	Multi-stem: 36 & 22cm DBH
22	<i>Ulmus pumila</i>	Siberian elm	15	2	Fair	Remove	
23	<i>Fraxinus spp.</i>	Ash species	28	0	Dead	Remove	Multi-stem: 28 & 22cm DBH
24	<i>Fraxinus spp.</i>	Ash species	22	0	Dead	Remove	
25	<i>Salix spp.</i>	Willow spp.	17	2.5	Good	Remove	
26	<i>Fraxinus spp.</i>	Ash species	18	0	Dead	Remove	
27	<i>Fraxinus spp.</i>	Ash species	22	0	Dead	Remove	
28	<i>Fraxinus spp.</i>	Ash species	22	0	Dead	Remove	
29	<i>Salix spp.</i>	Willow spp.	28	5.5	Fair	Remove	Multi-stem: 28cm DBH x 5
30	<i>Acer platanoides</i>	Norway maple	31	4	Good	Retain	Boundary tree
31	<i>Malus spp.</i>	Common apple	16	3	Poor	Retain	Boundary tree
32	<i>Malus spp.</i>	Common apple	23	4	Fair	Retain	Boundary tree
33	<i>Pyrus spp.</i>	Pear spp.	22	2	Fair	Remove	
34	<i>Pyrus spp.</i>	Pear spp.	24	3.5	Good	Remove	
35	<i>Malus spp.</i>	Apple spp.	18	3	Fair	Retain 2/ Remove 3	5 Malus spp. All roughly same size.
36	<i>Fraxinus spp.</i>	Ash species	16	0	Dead	Remove	4 dead Ash spp with over 15cm DBH. Located at the edge of wet area
37	<i>Salix nigra</i>	Black willow	70	12	Fair	Remove	Multi-stem: 70, 60, 50, & 60cm DBH with dead wood in canopy
38	<i>Salix nigra</i>	Black willow	65	10	Fair	Remove	
39	<i>Acer negundo</i>	Manitoba maple	30	4	Fair	Remove	
40	<i>Fraxinus spp.</i>	Ash species	20	0	Dead	Remove	
41	<i>Malus spp.</i>	Common apple	21	3.5	Fair	Remove	Multi-stem: 21 & 15cm DBH, with one sided crowed
42	<i>Pinus strobus</i>	Eastern white pine	38	4.5	Fair	Remove	Split leader and thin canopy
43	<i>Fraxinus spp.</i>	Ash species	19	0	Dead	Remove	
44	<i>Fraxinus spp.</i>	Ash species	18	0	Dead	Remove	
45	<i>Acer platanoides</i>	Norway maple	19	4.5	Good	Remove	In hedgerow
46	<i>Fraxinus spp.</i>	Ash species	15	0	Dead	Remove	
47	<i>Fraxinus spp.</i>	Ash species	19	0	Dead	Remove	
48	<i>Acer platanoides</i>	Norway maple	17	4	Fair	Remove	Poor form
49	<i>Fraxinus spp.</i>	Ash species	17	0	Dead	Remove	
50	<i>Fraxinus spp.</i>	Ash species	21	0	Dead	Remove	
51	<i>Fraxinus spp.</i>	Ash species	22	0	Dead	Remove	
52	<i>Fraxinus spp.</i>	Ash species	20	0	Dead	Remove	
53	<i>Acer platanoides</i>	Norway maple	20	3.5	Good	Remove	
54	<i>Fraxinus spp.</i>	Ash species	18	0	Dead	Remove	Group of three
55	<i>Fraxinus spp.</i>	Ash species	20	0	Dead	Remove	

APPENDIX 1: TREE INVENTORY CHART

ID No.	Botanical Name	Common Name	DBH (cm) (Diameter at Breast Height)	Canopy Radius (m)	Condition	Directive	Comment
56	<i>Acer platanoides</i>	Norway maple	26	5	Good	Remove	
57	<i>Fraxinus spp.</i>	Ash species	22	0	Dead	Remove	
58	<i>Fraxinus spp.</i>	Ash species	22	0	Dead	Remove	
59	<i>Robinia pseudoacacia</i>	Black locust	33	4	Good	Remove	Co-dominant at 1.4
60	<i>Robinia pseudoacacia</i>	Black locust	50	6	Fair	Remove	Dead wood in canopy
61	<i>Robinia pseudoacacia</i>	Black locust	26	6	Fair	Retain	Located at ROW. Multi-stem: 2 x 26, 20, & 12cm DBH
62	<i>Robinia pseudoacacia</i>	Black locust	24	5	Fair	Retain	Located at ROW. Multi-stem: 24, 20, & 25cm DBH
63	<i>Fraxinus spp.</i>	Ash species	51	0	Dead	Remove	
64	<i>Picea pungens</i>	Blue spruce	35	3.5	Fair	Remove	Growing in shade
65	<i>Pinus sylvestris</i>	Scots pine	44	4.5	Fair	Remove	
66	<i>Fraxinus spp.</i>	Ash species	39		Dead	Remove	
67	<i>Acer negundo</i>	Manitoba maple	26	6	Poor	Remove	Leaning heavily to the north, irregular canopy
68	<i>Thuja occidentalis</i>	Eastern white cedar	15	3	Good	Remove	Cedar grouping, 15 cm DBH and under, 22 stems
69	<i>Pyrus spp.</i>	Pear spp.	18	4	Fair	Remove	Co-dominant at 1 metre. Multi-stem: 15 & 18cm DBH
70	<i>Pyrus spp.</i>	Pear spp.	21	5	Good	Remove	
71	<i>Prunus spp.</i>	Cherry spp.	23	3.5	Poor	Remove	Clump form
72	<i>Prunus spp.</i>	Plum spp.	23	2.5	Poor	Remove	Dead wood in canopy with poor form
73	<i>Prunus spp.</i>	Plum spp.	19	1.5	Poor	Remove	Dying and dead wood in canopy
74	<i>Elaeagnus angustifolia</i>	Russian olive		6	Poor	Remove	Multi-stem: 16, 21, & 14 with irregular canopy
75	<i>Thuja occidentalis</i>	Eastern white cedar	15	3	Good	Remove	Clump of four with 15cm DBH
76	<i>Pinus sylvestris</i>	Scots pine	35	2.5	Fair	Remove	Sparse crown
77	<i>Pinus sylvestris</i>	Scots pine	26	3.5	Fair	Remove	6 trees in corner: 16, 26, 20, 17, 16, & 26cm DBH
78	<i>Pinus sylvestris</i>	Scots pine	36	6	Fair	Remove	Hedgerow
79	<i>Pinus sylvestris</i>	Scots pine	34	6	Fair	Remove	
80	<i>Fraxinus spp.</i>	Ash species	23	0	Dead	Remove	
81	<i>Fraxinus spp.</i>	Ash species	15	0	Dead	Remove	
82	<i>Fraxinus spp.</i>	Ash species	25	0	Dead	Remove	
83	<i>Pinus strobus</i>	Eastern white pine	28	4	Fair	Remove	
84	<i>Fraxinus spp.</i>	Ash species	18	0	Dead	Remove	
85	<i>Salix babylonica</i>	Weeping willow	50	7	Fair	Remove	
86	<i>Salix spp.</i>	Willow spp.	16	2	Good	Remove	

ID No.	Name	Condition	Directive	Comment
87	Tree Grouping #1	Dead	Remove	Ash spp.
88	Tree Grouping #2	Fair/ Dead	Remove	Willow spp. & Malus spp. with dead Ash spp.
89	Tree Grouping #3	Fair/ Dead	Retain	Outside of property. Willow spp. & Malus spp. with dead Ash spp.
90	Tree Grouping #4	Dead	Remove	Ash spp.
91	Tree Grouping #5	Dead	Remove	Ash spp.

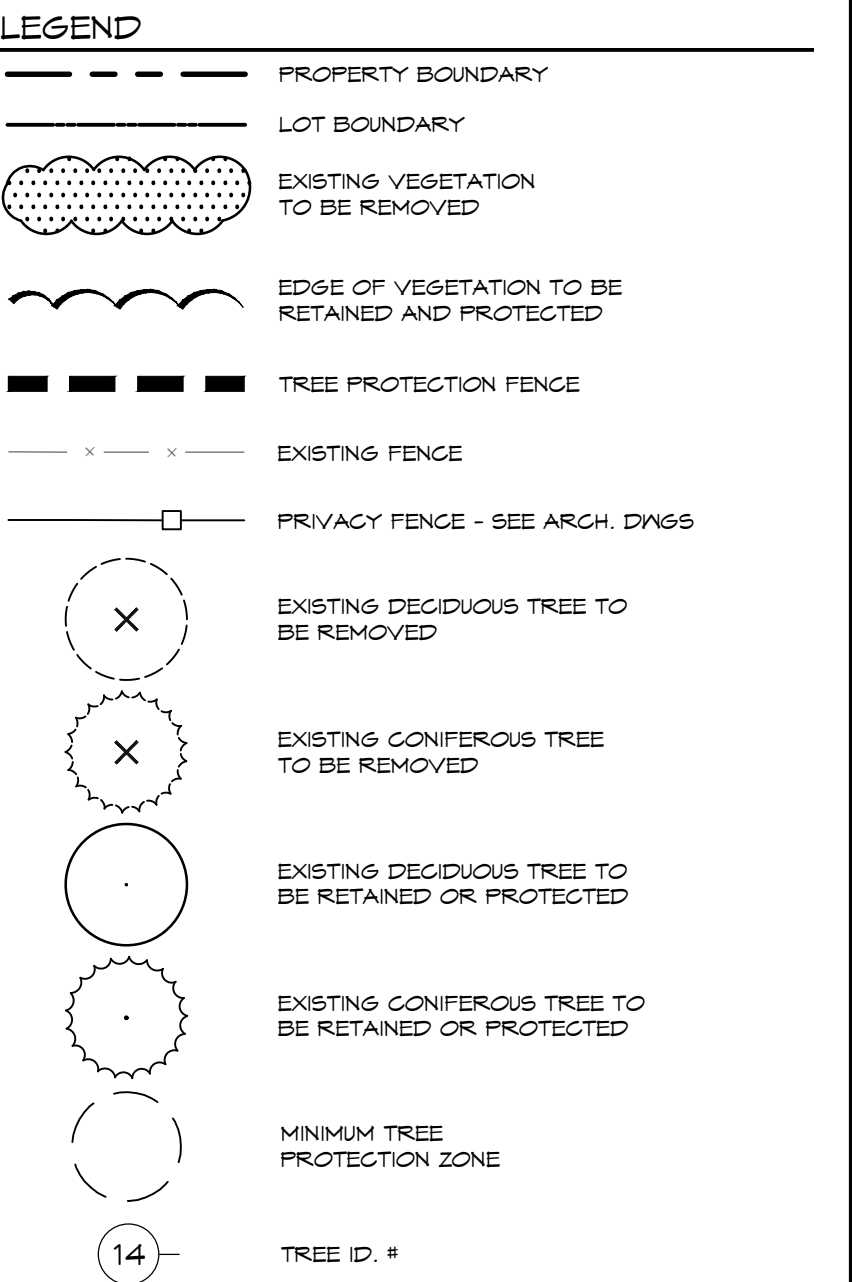
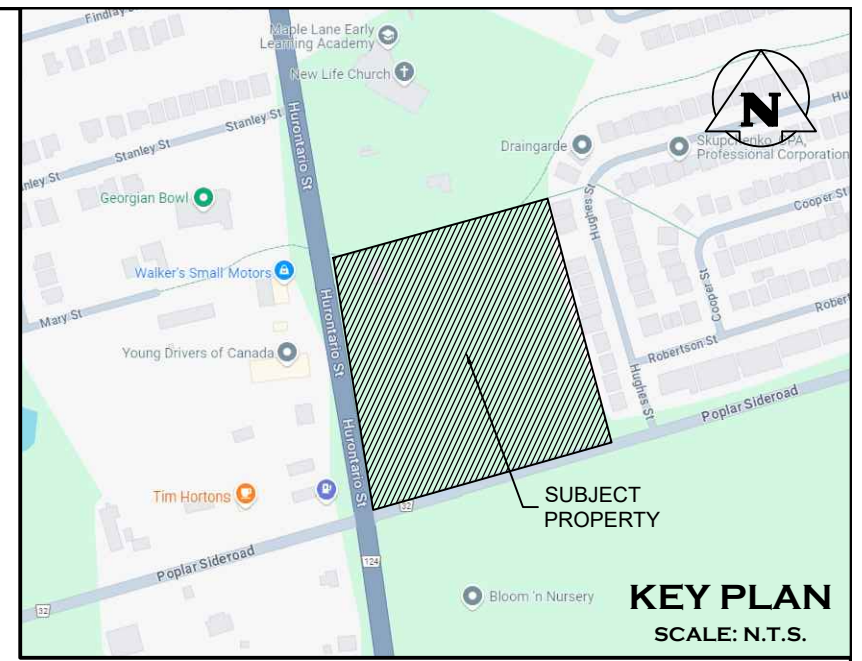
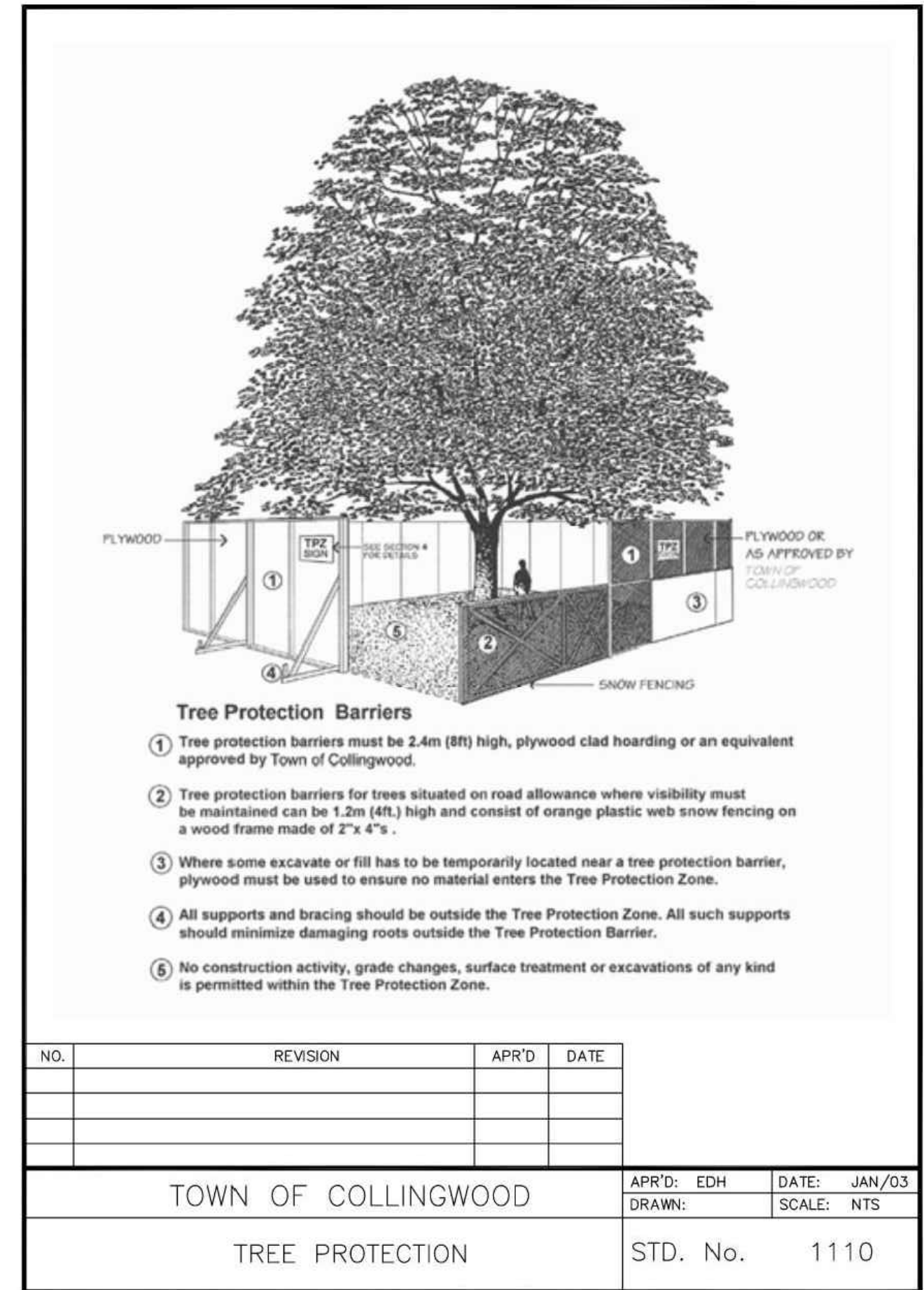
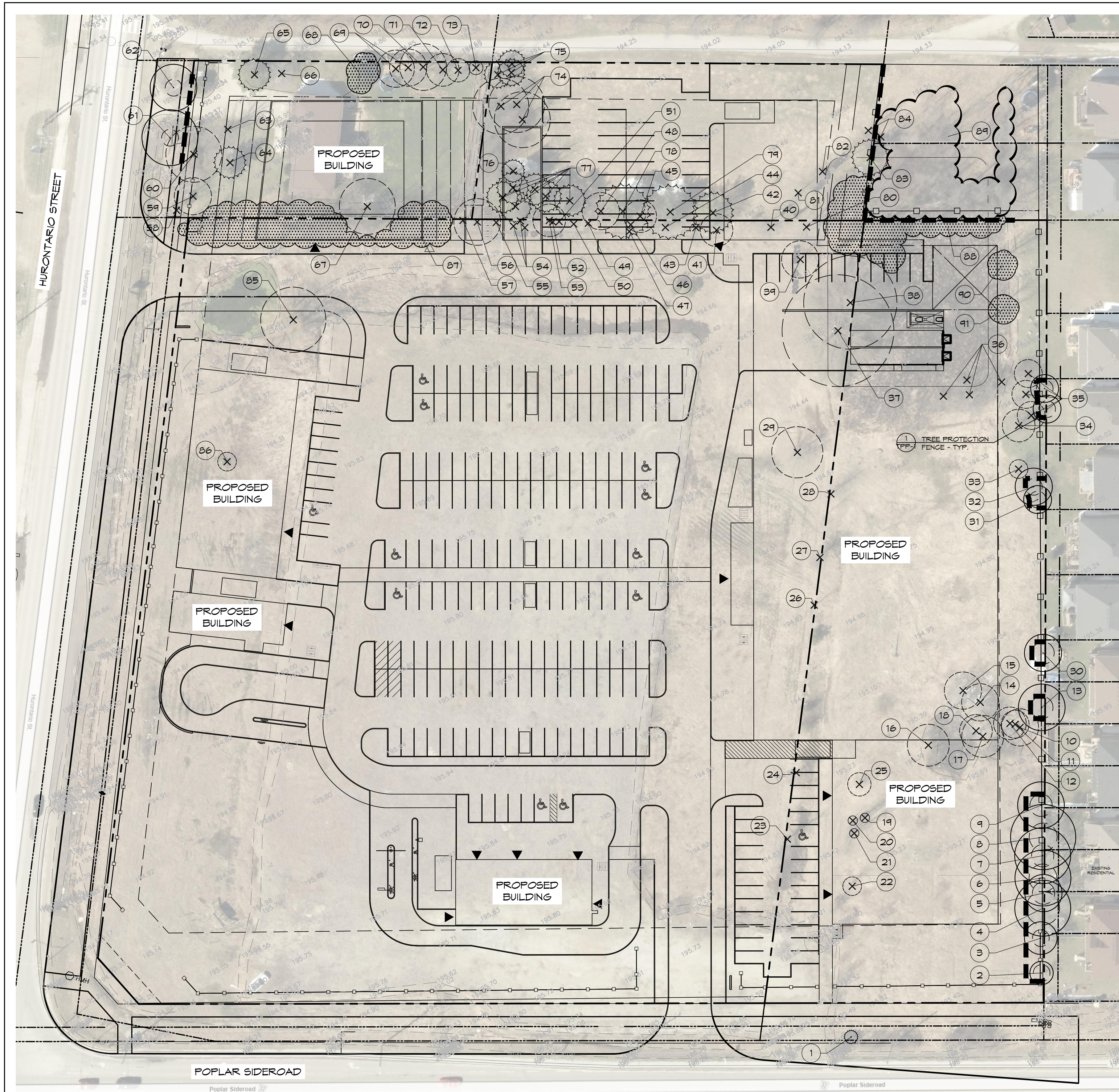
APPENDIX 2

AERIAL PHOTO



APPENDIX 3

TREE PROTECTION PLAN



1 TREE PROTECTION FENCE N.T.S.

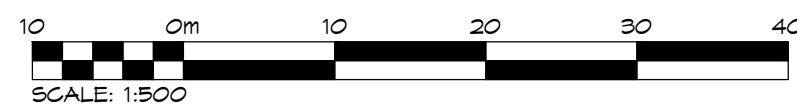
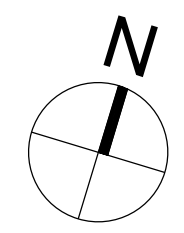
NOTES:

1.0 TREE REMOVAL RATIONAL

- ALL EXISTING TREES SHOWN TO BE REMOVED AND LOCATED WITHIN THE VEGETATION TO BE REMOVED AREA ARE TO BE REMOVED DUE TO CONFLICTS WITH THE PROPOSED GRADING AND/OR SERVING OF THE SITE DEVELOPMENT. THE CONFLICTS ARE UNAVOIDABLE.
- FOR SERVING, GRADING AND DRAINAGE INFORMATION SEE ENGINEERING DRAWINGS.
- THE CONTRACTOR SHALL CHECK AND VERIFY ALL EXISTING AND PROPOSED GRADES AND CONDITIONS OF THE PROJECT AND IMMEDIATELY REPORT ANY DISCREPANCIES TO THE CONSULTANT BEFORE PROCEEDING WITH ANY REMOVALS.
- TREE REMOVALS SHALL CONFORM WITH THE MIGRATORY BIRDS CONVENTION ACT AND MUNICIPAL BY-LAWS.

2.0 GENERAL

- FOR TREE INVENTORY CHART AND TREE GROUPING INVENTORY CHART, REFER TO CHARTS ON DRAWING TPP-2.



1. THIS DRAWING IS THE EXCLUSIVE PROPERTY OF C.F. CROZIER & ASSOCIATES INC. AND THE MODIFICATION AND/OR REPRODUCTION OF ANY PART OF THIS DRAWING IS STRICTLY PROHIBITED WITHOUT WRITTEN AUTHORIZATION FROM THIS OFFICE.
2. THE DIGITAL FILES CONTAIN INTELLECTUAL AND DIGITAL DATA PROPERTY THAT IS THE EXCLUSIVE PROPERTY OF C.F. CROZIER & ASSOCIATES INC.
3. THE CONTRACTOR SHALL VERIFY ALL DIMENSIONS, LEVELS, AND DATUMS ON SITE AND REPORT ANY DISCREPANCIES OR OMISSIONS TO C.F. CROZIER & ASSOCIATES INC. PRIOR TO CONSTRUCTION.
4. THIS DRAWING IS TO BE READ AND UNDERSTOOD IN CONJUNCTION WITH ALL OTHER PLANS AND DOCUMENTS APPLICABLE TO THIS PROJECT.
5. ALL EXISTING UNDERGROUND UTILITIES TO BE VERIFIED IN THE FIELD BY THE CONTRACTOR PRIOR TO CONSTRUCTION.
6. DO NOT SCALE DRAWINGS.

NOTES:

- TOPOGRAPHIC SURVEY PROVIDED BY J.D. BARNES LIMITED (RECEIVED ON 2024-07-24).
- BASE INFORMATION AND SITE PLAN PROVIDED BY CHARIS DEVELOPMENTS LTD. (RECEIVED ON 2024-08-23).
- ALL DIMENSIONS ARE IN METERS UNLESS OTHERWISE NOTED.

Town

No.	ISSUE	DATE: YYYY/MM/DD
0	ISSUED FOR SUBMISSION - OPA/ZBA	2024/04/17

ARBORIST



MATTHEW CAMPBELL,
OALA, C.S.A.
CERTIFIED ARBORIST
(ISA ON-3008-A), TRAG

LANDSCAPE ARCHITECT



Project

THE GATEWAY CENTRE
TOWN OF COLLINGWOOD

Drawing

TREE PROTECTION PLAN



Drawn By	K.C.	Design By	Project	2199-6198
Check By	A.P.	Check By	M.C.	Drawing
				TPP-1

APPENDIX 4

DNA REPORT FOR TREE #9

CANADIAN CENTRE FOR DNA BARCODING
DNA Testing Laboratory Report

Page 1 of 9

Date of issue: September 6, 2024

CLIENT INFORMATION

Client Name: Alison Phillips
Client Address: Crozier Consulting Engineers
1 First Street, Suite 200
Collingwood, ON
L9Y 1A1
Contact Name: Alison Phillips (aphillips@cfcrozier.ca)

ITEMS

Description: One plant sample (fresh leaves) from putative butternut species submitted for hybrid detection Sample ID: CCDBFR1478; Process ID: ABCBF1088-24
Dates Received: August 23, 2024
Dates of Analysis: August 26 – 30, 2024
Sample Received and Analyzed 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 sample, an approximate 2mm by 2mm area of leave from sample was subsampled using sterile techniques. Sample was 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 primers *ITS_S2F/ITS4* and *trnLUAA-c/trnFGAA-f*, 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 for each amplicon. Resulting trace files were assembled into contigs and consensus sequences, and then manually edited in CodonCode Aligner (version 9.1.1.) software. The sequences of ITS2 and *trnL-trnF* were compared against the BOLD reference libraries. Based on the percentage of nucleotide sequence divergence (a number of nucleotide substitutions) between sequences from the test samples and reference DNA barcodes, the closest match was used to infer species identity of the corresponding test sample provided by the contributor. The quality of the sequence traces for ITS2 was done by visual inspection to resolve hybridization. Images, sequences, and their associated trace files with quality scores were uploaded to the secure BOLD project called "CCDB forensic sampling [ABCBF]".

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

The ITS2 marker demonstrates five nucleotide substitutions between *Juglans cinerea* (white walnut, commonly known as butternut) 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 reflected in the trace file chromatograms as mixed signals at the characteristic nucleotide positions.

The *trnL-trnF* marker demonstrates five nucleotide substitutions between *Juglans cinerea* (white walnut) and *Juglans ailantifolia* (Japanese walnut) reference sequences across the amplified ~950 base pair length. The marker is used as supplementary evidence to confirm species identity. The *trnL-trnF* marker is a part of the chloroplast genome and is inherited maternally. Therefore, this marker confirms the maternal lineage in a hybrid but on its own does not detect a hybridization event.

RESULTS

Sample ID	Process ID	Hybridity detected	Maternal organism
CCDBFR1478	ABCBF1088-24	Yes	<i>Juglans ailantifolia</i> (Japanese walnut)

ITS2 sequences of 344 base pairs were obtained for sample CCDBFR1478. Sample sequences were aligned against the known reference sequences for *Juglans cinerea* (white walnut), *Juglans ailantifolia* (Japanese walnut), and their hybrid *Juglans cinerea* x *Juglans ailantifolia*.

Across the amplified length of the ITS2 marker, five diagnostic nucleotide substitutions differentiate *Juglans cinerea* (white walnut) and *Juglans ailantifolia* (Japanese walnut). At these five nucleotide positions, sample CCDBFR1478 were identical to references for hybrid *Juglans cinerea* x *Juglans ailantifolia*. The chromatogram traces for these sequences showed evidence of mixed base calls at the diagnostic nucleotide positions, which confirms that sample CCDBFR1478 is of hybrid origin (Figures 1). The phylogenetic tree of the ITS2 marker for sample is shown in Figure 2.

trnL-trnF

Across the amplified length of the *trnL-trnF* marker, five diagnostic nucleotide substitutions differentiate *Juglans cinerea* (white walnut) and *Juglans ailantifolia* (Japanese walnut). At these five nucleotide positions, sample CCDBFR1478 was identical to references for *Juglans ailantifolia* (Japanese walnut). As the chloroplast genome is inherited maternally, our results indicate that *Juglans ailantifolia* (Japanese walnut) was the maternal organism for sample CCDBFR1478 (Figures 4). The phylogenetic tree of the *trnL-trnF* marker for this sample is shown in Figure 5.

CONCLUSIONS

The present testing has indicated that sample CCDBFR1478 is a hybrid between white and Japanese walnut species: *Juglans cinerea* x *Juglans ailantifolia*. The maternal organism belongs to *Juglans ailantifolia* (Japanese walnut).

Based on appropriate statistical BOLD match calculations and a reasonable degree of scientific certainty of the BOLD reference library, the taxonomic identity of the detected DNA source in these samples is considered practically proven.

RESULTS REPORTED BY:



Nguyen NguyenT.X., MSc; Wildlife Forensic Technician

RESULTS REVIEWED BY:



Maria Kuzmina, PhD; Plant Lead



Evgeny V. Zakharov, PhD; Director, CCDB

All inquiries pertaining to this report should be directed to Nguyen NguyenT.X. (n.nguyen@uoguelph.ca) and Evgeny V. Zakharov (zakharov@uoguelph.ca). This report should not be reproduced, except in full, without written approval of the CCDB.

Disclaimer: "THIS REPORT AND ALL ATTACHMENTS HERETO ARE CONFIDENTIAL AND SUBJECT TO SOLICITOR-CLIENT PRIVILEGE. DO NOT FORWARD, CIRCULATE, DISTRIBUTE, COPY OR DUPLICATE THIS REPORT OR ANY ATTACHMENT HERETO WITHOUT WRITTEN AUTHORIZATION."

FIGURES

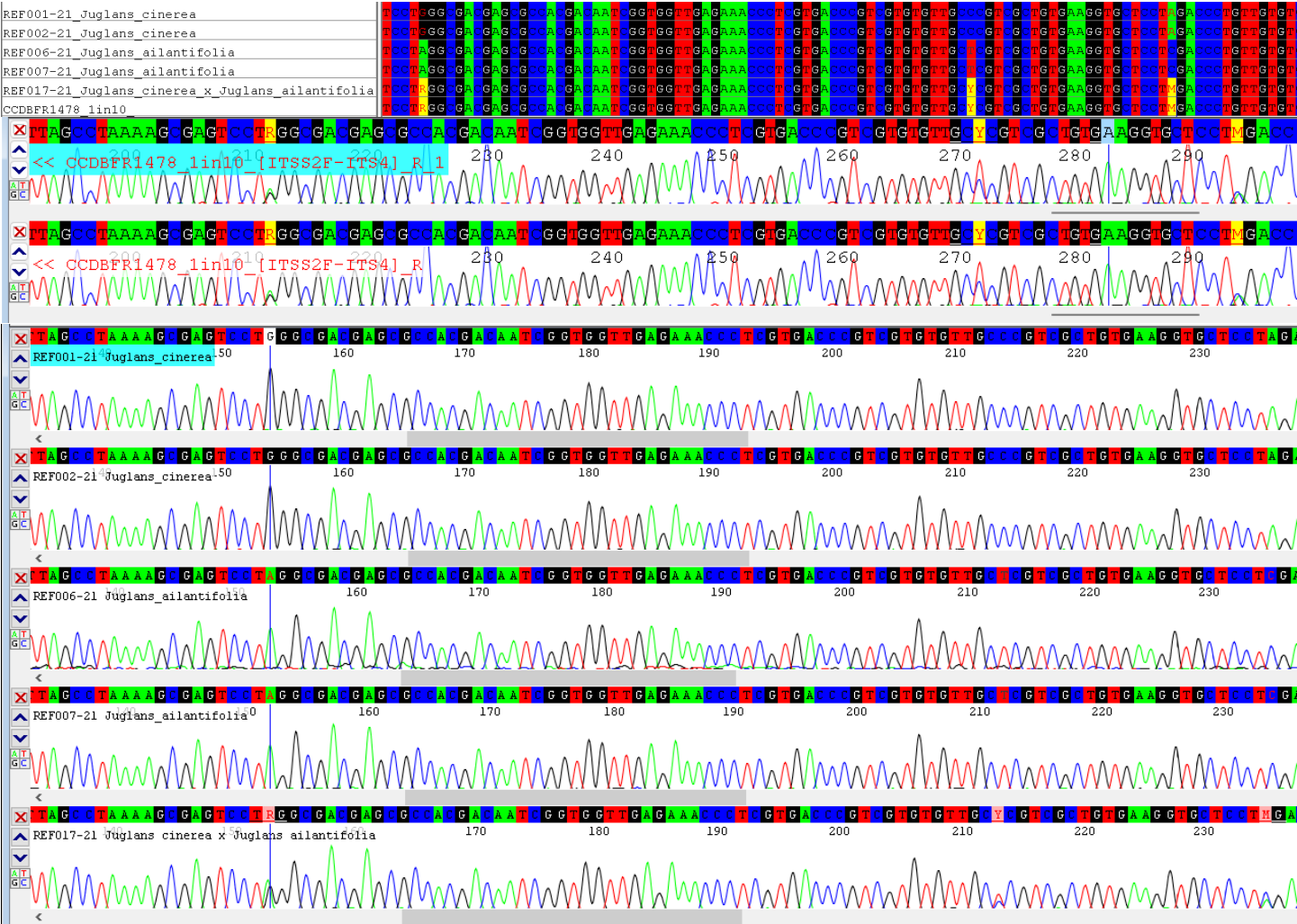


Figure 1 – ITS2 sequence comparison of sample CCDBFR1478 with references for *Juglans cinerea*, *Juglans ailantifolia*, and their hybrid *Juglans cinerea* x *Juglans ailantifolia*. Diagnostic nucleotide positions 3, 4 and 5 are shown.

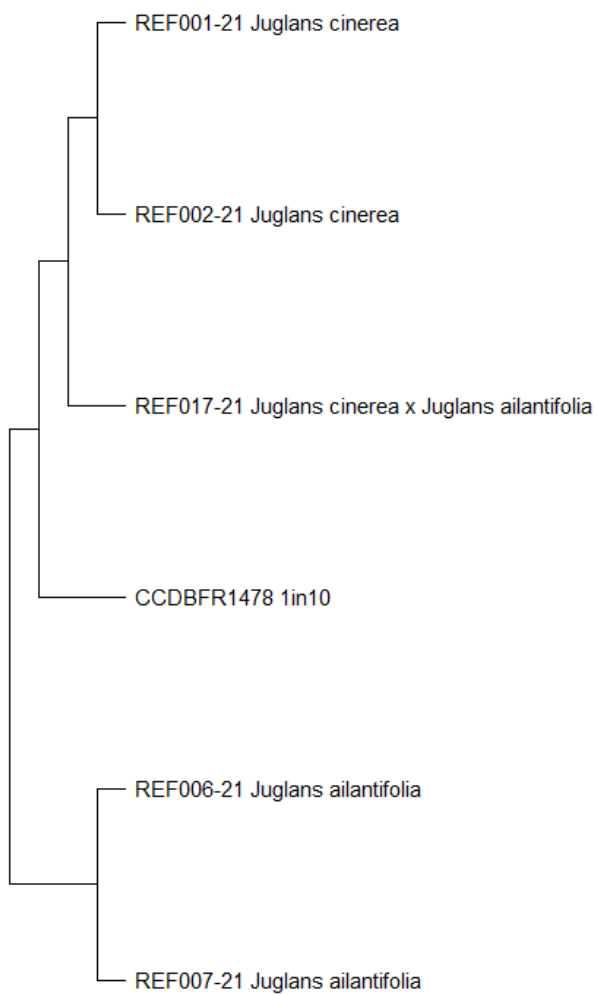


Figure 2 – Neighbour joining phylogenetic tree of ITS2 sequences of the query sample CCDBFR1478, and their references: *Juglans cinerea*, *Juglans ailantifolia*, and hybrid *Juglans cinerea* x *Juglans ailantifolia*.

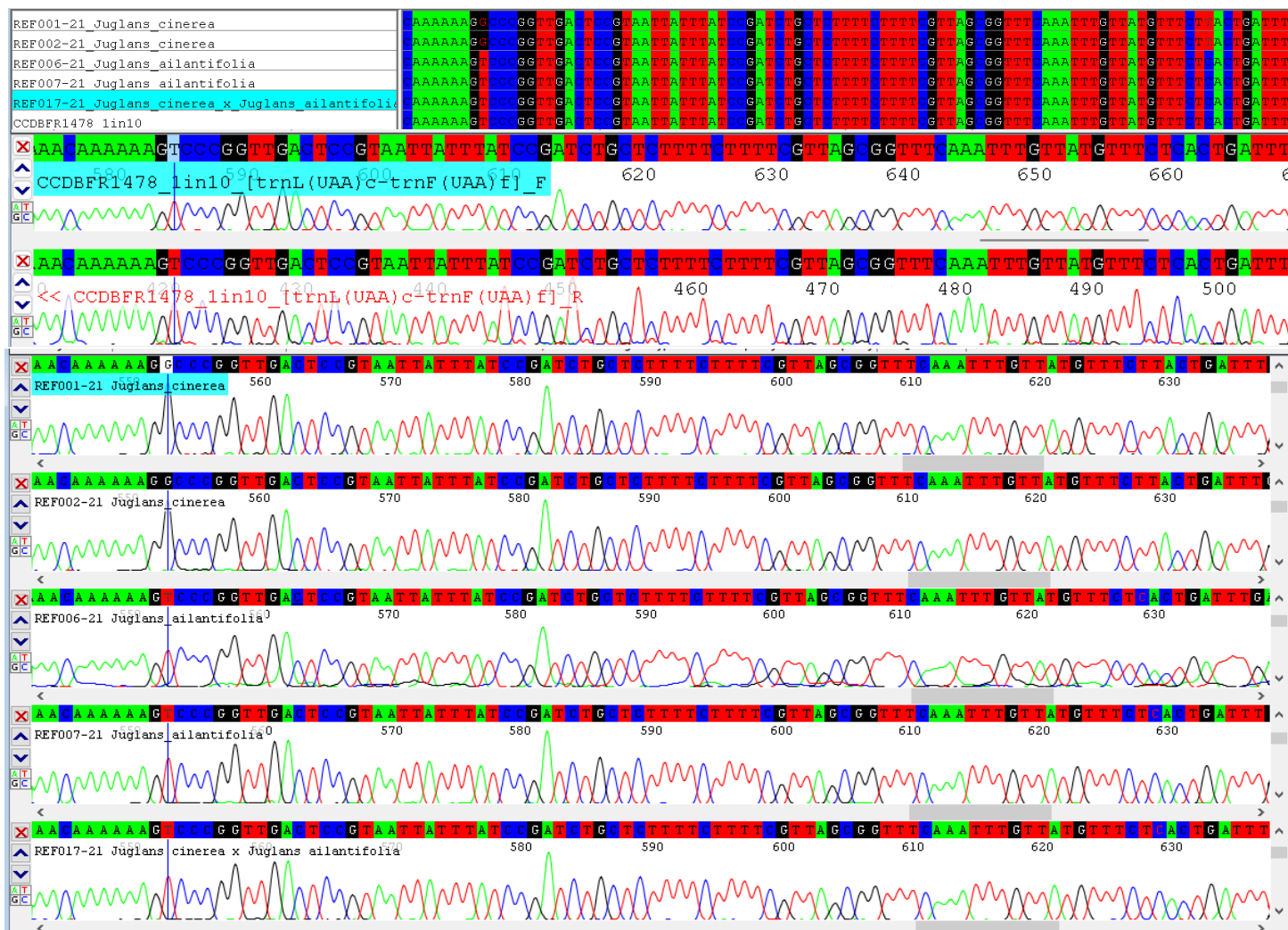


Figure 3 – trnL-trnF sequence comparison of sample CCDBFR1478 with references for *Juglans cinerea*, *Juglans ailantifolia*, and hybrid *Juglans cinerea* x *Juglans ailantifolia*. Diagnostic nucleotide positions 4 and 5 are shown.

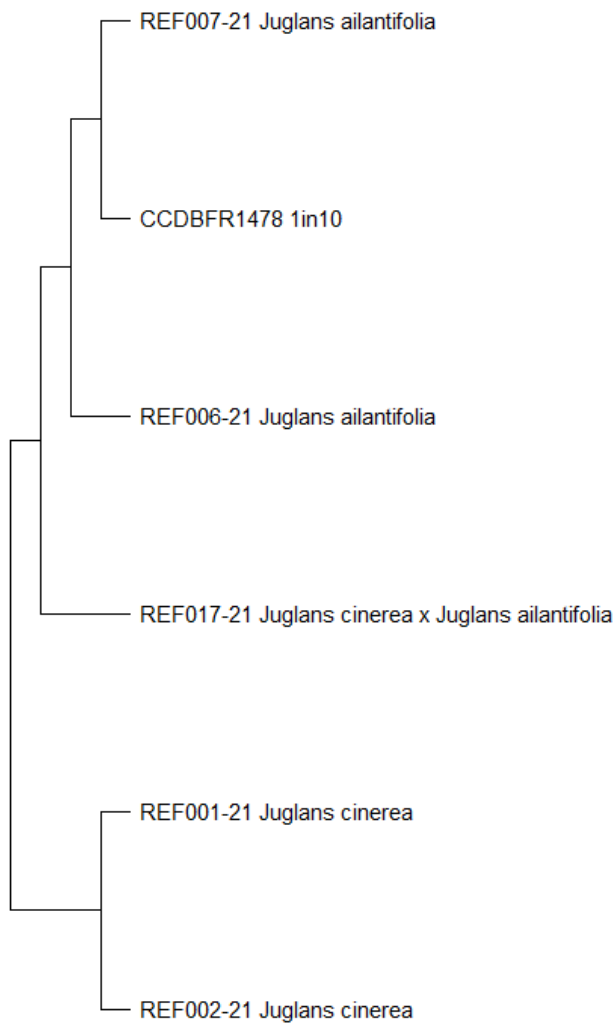


Figure 4 – Neighbour joining phylogenetic tree of *trnL-trnF* sequences of the query sample CCDBFR1478, and their references: *Juglans cinerea*, *Juglans ailantifolia*, and hybrid *Juglans cinerea* x *Juglans ailantifolia*.

Appendix 1. Image Inventory



Image1: Sample CCDBFR1478.