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Assessing grey squirrel dispersal patterns within the landscape using DNA sequence variation.

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3 1 Assessing grey squirrel dispersal patterns within the landscape using sequence variation.
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8 3 ABSTRACT
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10 4 The grey squirrel *Sciurus carolinensis* is thought to have contributed to the decline of
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12 5 red squirrel *S. vulgaris* populations in the UK through resource competition and disease
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14 6 spread. This study used mtDNA sequencing to assess patterns of grey squirrel dispersal in the
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16 7 UK. Patterns of genetic variation within the dloop sequence were characterised for seven grey
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18 8 squirrel populations. Infiltration directions and potential barriers to dispersal are identified
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20 9 and discussed, with a focus on Cumbria, a county at the forefront of grey squirrel expansion.
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22 10 Understanding the dynamics of grey squirrel dispersal will aid their management at a
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24 11 landscape scale and enhance the conservation of red squirrels.
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34 13 INTRODUCTION
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36 14 The Eastern grey squirrel *Sciurus carolinensis* was first introduced to Britain between
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38 15 1876 and 1929 (Middleton 1931; Shorten 1954; Lloyd 1983). Subsequent successful
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40 16 introductions and translocations occurred within the UK and by the 1930's populations were
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42 17 established in southeast England and rapidly spreading through the country (Shorten 1954).
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44 18 This expansion occurred simultaneously with the decline in native red squirrel *Sciurus*
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46 19 *vulgaris* populations (Lloyd 1962; Lloyd 1983). Studies suggest that interspecific resource
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48 20 competition occurs (Wauters et al. 2000; Gurnell et al. 2004) and that this, along with the
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50 21 effects of a particularly virulent squirrelpox virus (SQPV), carried by the grey squirrel, has
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52 22 caused the decline and extirpation of many red squirrel populations (Tompkins et al. 2003;
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54 23 Gurnell et al. 2004; Carroll et al. 2009).

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57 24 Cumbria in North West England has been found to hold genetically unique
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59 25 populations of red squirrel which contain high levels of genetic diversity (Hale et al. 2004).
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3 26 Concerns have been raised over the impact of expanding grey squirrel populations are having
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5 27 on red squirrel populations. Interspecific competition and SQPV transmission are thought to
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8 28 be highest at times of grey squirrel dispersal (Sainsbury et al. 2008). An understanding of the
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10 29 dispersal ecology and directional movements of the grey squirrel will aid grey squirrel
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12 30 management decisions and red squirrel conservation.

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15 31 Recent work (Stevenson et al., in review) has indicated that the Cumbrian Mountain
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17 32 range is acting as a barrier to dispersal. Grey squirrel populations within Cumbria have been
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19 33 derived from the infiltration of individuals from two directions; to south Cumbria from
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21 34 Lancashire and to north Cumbria from Northumberland/ Scottish Borders. Hale et al. (2001)
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23 35 and Trizio et al. (2005) both suggest genetic analysis has enabled the identification of land
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25 36 cover types which either facilitate dispersal or provide barriers to red squirrels over large
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27 37 geographic scales. In this paper we report on variation within Cytochrome b (Cytb) and Dloop
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29 38 DNA sequences found within grey squirrel mtDNA, the first such report to date.
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34 39 35 36 40 METHODOLOGY 37

38 41 Two grey squirrel accessions were collected from each of four known introduction
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40 42 points (see Middleton, 1931); Balloch near Loch Long; Dalkeith in Edinburgh; Henbury in
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42 43 Cheshire, and from Alice Holt Forest in Surrey. In addition, samples were collected from
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44 44 three established/ emerging populations within the UK; Doune, in Stirlingshire; Millom, in
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46 45 south Cumbria and Brampton, in north Cumbria (Figure 1).
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50 46 DNA was extracted from the leg muscle tissue of 14 individuals using the QIAGEN
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52 47 DNeasy blood and tissue extraction kit and following the manufactures instructions
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54 48 (QIAGEN Ltd). Fragments of the two mtDNA sequence encoding Cytb and Dloop were
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56 49 amplified by polymerase chain reaction (PCR). Primers for *Sciurus carolinensis* Cytb were
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58 50 available from Meece et al. (2005), BM1 (5'-CCCCTCAGAATGATATTTGTCCTCA) and
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3 51 BM2 (5'-CCATCCAACATCTCAGCATGATGAAA). Each PCR reaction had a reaction
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5 52 volume of 25 µl and containing 1 µl 1:10 mtDNA, 12.5 µl AmpliTaq Gold PCR master mix
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8 53 (Roche, USA), 1 µl 1:10 BM1 primer to distilled water, 1 µl 1:10 BM2, 9.5 µl distilled water.
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10 54 PCR amplification followed the protocol of Meece et al. (2005): denaturation of 3.5 min at
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12 55 95°C followed by 36 cycles of 30s at 95°C, annealing 50s at 60 °C, extension 40s at 72°C,
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14 56 final extension of 5min at 72 °C.

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17 57 Primers for *Sciurus carolinensis* Dloop were taken from the published sequence of the
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19 58 Dloop (GenBank Accession no. AF111027; Barrett et al. 1999), Dloop forward primer 5'-
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21 59 GCCACCCCAAGTTAAATGG-3' and Dloop reverse primer 5'-
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23 60 ATTCGTGCATTAATGCACTATCC-3'. Each PCR reaction for Dloop contained the same
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25 61 relative quantities of components as outlined above for cytb amplification apart from 5 µl of
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27 62 each primer was used. PCR amplification for Dloop sequence followed the protocol of Trizio
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29 63 et al. (2005), denaturation of 1 min at 94°C followed by 40 cycles of 30s at 94°C, annealing
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31 64 30s at 52 °C, extension 1min at 72°C, final extension of 5min at 72 °C.

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34 65 Electrophoresis on 1% agarose gels were used to check the success of the PCR
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36 66 reactions for each sample. All PCR products were then cleaned following the methodology of
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38 67 the QIAquick PCR purification kit (QIAGEN Ltd). Sequencing of PCR products was carried
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40 68 out (DBS Genomics, Durham, UK).

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43 69 Sequences for cytb and Dloop from each individual were aligned manually using
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45 70 BioEdit_R v7.0.4.1 Freeware (Ibis Therapeutics, California,USA) and Sequence Analysis
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47 71 5.2.0 (Applied Biosystems) using the default settings within the programmes. A sequence
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49 72 from *S. vulgaris* (GenBank Accession no. AJ238588) was also included as an outgroup
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51 73 accession in the analysis. The sequences were entered into Paup 4.0 Beta 10 (Swofford,
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53 74 Illinois Natural History Survey, Illinois, USA) for phylogeny reconstruction and a 50%
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3 75 majority rule consensus tree was created with 1000 bootstrap and jackknife replicates (Harrison
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5 76 and Langdale 2006).
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9 10 78 RESULTS

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12 79 MtDNA was successfully extracted from 14 grey squirrel individuals from selected
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14 80 locations around the UK. Cytb sequence data was analysed for all 14 accessions with no
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16 81 sequence variation observed. The 325bp sequence generated, demonstrated a 99% similarity
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18 82 match for grey squirrel cytochrome b gene in the Genbank BLAST search tool (Accession no.
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20 83 AY509680). Similarly, the 329bp Dloop sequence generated showed a 98% similarity match
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22 84 to that of grey squirrel (Accession no. AF111027). Dloop sequences were aligned in BioEdit
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24 85 and discrete points of variation were detected at 16 unique sites, representing 4.9 % of the
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26 86 overall sequence.
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31 87 Parsimony analysis generated a 50% majority rule consensus tree from the grey
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33 88 squirrel dloop sequence data which was rooted against red squirrel (GenBank Accession no.
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35 89 AJ238588). The consensus tree (Figure 2) indicated that the grey squirrel samples are
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37 90 separated into four distinct clades; Clade I; Henbury, Balloch and accession S8 from Alice
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39 91 Holt; Clade II; Doune and accession S7 from Alice Holt; Clade III; Dalkeith and Brampton
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41 92 (north Cumbria); Clade IV; Millom (south Cumbria). The branching of samples from
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43 93 Dalkeith and Brampton is strongly supported with bootstrap values of 73% and jackknife
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45 94 values of 56%.
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52 53 96 DISCUSSION

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55 97 This study examines DNA sequence variation and its utility in assessing patterns of
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57 98 grey squirrel dispersal in the UK. Cytb sequences generated in this study demonstrated no
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59 99 detectable differences across the range of populations sampled. This may be due to the low
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3 100 mutation rate within the cytb coding sequence and the relatively short time frame of
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5 101 introduction and dispersal within the UK. Conversely, sequence variation was detected
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8 102 within the dloop sequence for these same accessions. Dloop sequence analysis showed
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10 103 significant statistical support for the distinct separation of north and south Cumbria grey
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12 104 squirrel populations.

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15 105 Accessions from north Cumbria grouped with accessions from Dalkeith in Edinburgh.
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17 106 Individual accessions within north Cumbria may not necessarily have been derived from
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19 107 individuals dispersing south from Edinburgh. However, as they are both within the same
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21 108 clade they may have been derived from the same progenitor individuals from another location
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23 109 such as northeast England (Stevenson et al., in review). Similar patterns of dispersal have
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25 110 been seen in red squirrels, mediated by the afforestation of woodland between Cumbria and
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27 111 the Northeast (Hale & Lurz 2003).

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31 112 The two samples taken from each population were generally grouped together within
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33 113 the phylogenetic tree, however, samples from Alice Holt Forest did not follow this pattern and
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35 114 were grouped separately with samples from elsewhere. David-Gray et al. (1998) study found
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37 115 high levels of genetic diversity within grey squirrel populations from Alice Holt Forest and
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39 116 attributed this to numerous introduction sites and translocations from different source
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41 117 populations, which could explain the results demonstrated in Figure 2 in this study.

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45 118 The initial findings presented here demonstrate support for the hypotheses that the
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47 119 Cumbrian Mountain range is acting as a barrier to dispersal and that invasion into the area is
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49 120 coming from multiple directions. If grey squirrels colonised Cumbria with a northerly
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51 121 advance as suggested (Lowe 1993; Skelcher 1997) accessions from north and south Cumbria
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53 122 should be grouped together within a phylogenetic reconstruction. However, this is not the
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55 123 case, and the two populations separate out in the tree. This points towards separate
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57 124 populations and the effectiveness of the Cumbrian Mountain range as a barrier. Whilst it is

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3 125 acknowledged that a small number of individuals may try to disperse over this mountain
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5 126 barrier, the majority of individuals will choose a lower cost dispersal route. Further validation
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7 127 of prospective incursion directions will require an increase in the sample size for each of the
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9 128 seven locations and additional accessions from within Cumbria and adjoining areas.

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12 129 This study has provided the first evidence of Dloop sequence variation within UK
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14 130 grey squirrel populations. Despite the small sample size, it supports the suggestion that the
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16 131 Cumbrian Mountains provide a barrier to grey squirrel dispersal and infers both northerly and
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18 132 southerly grey squirrel incursion routes into the county. Ultimately, further knowledge of
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20 133 dispersal and colonisation of grey squirrels will inform conservation policy and can be used to
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22 134 target resources for grey squirrel control and enable better protection for vulnerable red
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24 135 squirrel populations. We have shown here, that landscape genetics can provide evidence of
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26 136 population origin and genetic differences. Although this study has focused on grey squirrels in
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28 137 Cumbria, the techniques are equally applicable to other landscapes, validating and
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30 138 highlighting dispersal routes of invasive species and species of conservation concern.

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Figure 1. Geographical location of grey squirrel sample sites within the UK.

186x273mm (300 x 300 DPI)

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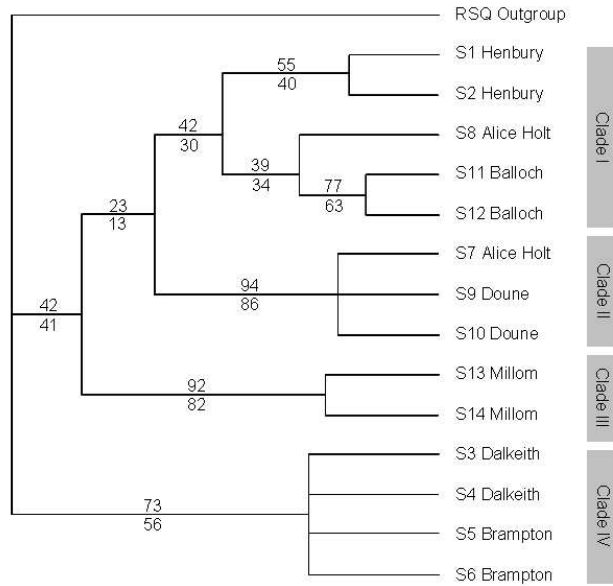


Figure 2. Phylogenetic tree showing Bootstrap and Jackknife analysis results above and below each group separation.

254x190mm (96 x 96 DPI)