

MICROSATELLITE LOCI IN TWO EPIPHYTIC LICHENS WITH CONTRASTING DISPERSAL MODES: *NEPHROMA LAEVIGATUM* AND *N. PARILE* (NEPHROMATACEAE)¹

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- *Premise of the study:* Microsatellite markers were characterized for two epiphytic cyanolichens, *Nephroma laevigatum* and *N. parile* (Nephromataceae), and will be used to investigate population structure and estimate gene flow among populations of these two closely related species with contrasting dispersal modes.
- *Methods and Results:* Twelve and 14 microsatellite loci were characterized for *N. laevigatum* and *N. parile*, respectively. Allele number in *N. laevigatum* ranged from three to 13 per locus, while in *N. parile* there were from two to six alleles per locus. As expected, the sexually reproducing *N. laevigatum* had higher genetic diversity than the predominantly asexual *N. parile*.
- *Conclusions:* This new set of markers is suitable for studying population structure and providing insights into gene flow among populations and for understanding processes of diversification. Compared between the species, they will facilitate an understanding of the influence of contrasting reproductive strategies on population and community structure.

Key words: Ascomycetes; lichen-forming fungi; microsatellites; *Nephroma laevigatum*; *Nephroma parile*; Nephromataceae.

Dispersal among populations can explain gene flow patterns, can help in understanding the processes driving genetic structure and diversity, and can explain population persistence under different ecological and environmental scenarios (Lowe et al., 2004). Dispersal traits (i.e., reproductive strategy and propagule size) reflect a trade-off between dispersal ability and establishment success, and they have important consequences for population genetic structure (Hartl and Clark, 1997). In lichens, the contrast between sexual vs. asexual reproductive modes has provided a convenient system for evaluating consequent patterns of genetic structure and diversity (Werth, 2010); this is especially the case for lichen epiphytes that occur on trees mimicking discrete habitats (islands) of measurable size and age, and that are linked through dispersal dynamics to a network of surrounding trees, and at a larger scale, to adjacent woodland stands. Asexually reproducing lichen species disperse both their symbionts together (fungus and photosynthetic alga or cyanobacteria), but their propagules are larger compared to sexually reproducing species, which have a higher likelihood of long-distance dispersal (Seaward, 2008). However, this broad generalization in dispersal ability and its consequences for gene flow

remain contentious owing to contradictory results (Werth et al., 2014). In particular, the majority of epiphyte studies have focused on a single model species—*Lobaria pulmonaria* (L.) Hoffm. (e.g., Scheidegger and Werth, 2009)—that can reproduce both sexually and asexually, making it therefore problematic to unequivocally partition the ecological consequences of contrasting dispersal modes.

The focus of this paper is to describe microsatellite markers developed for two codistributed epiphytic lichens with different reproductive strategies and dispersal modes: i.e., *Nephroma laevigatum* Ach. and *N. parile* (Ach.) Ach. Both species are morphologically very similar and have a *Nostoc* photobiont, but they have developed contrasting reproductive traits. *Nephroma laevigatum* typically reproduces via sexual ascospores (spores = 18.5 × 6 μm), while the predominantly asexually reproducing *N. parile* normally produces marginal soredia (soredia = 150 μm) and only very infrequent apothecia (Smith et al., 2009). It has been shown using multigene phylogenies that these ecologically similar foliose lichens are each monophyletic and closely related to each other, although they are not sister species (Lohtander et al., 2002; Sérusiaux et al., 2011). Both species have oceanic to boreal-montane distributions, are common in ancient woodlands in Europe, and are sensitive to SO₂ pollution (Smith et al., 2009).

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METHODS AND RESULTS

For primer design, fungal genomic DNA was extracted from eight individuals per lichen species collected from four different populations across a climatic gradient in Scotland (Appendix 1). DNA was isolated from 20 mg of dried thallus material using the DNeasy Plant Mini Kit (QIAGEN, Hilden, Germany), following the manufacturer's protocol modified by increasing initial lysis incubation to 1 h at 70°C. Microsatellite sequences were isolated and primers were designed by Ecogenics GmbH (Zurich, Switzerland) using magnetic streptavidin beads and

TABLE 1. Characteristics of 26 microsatellite loci developed in *Nephroma laevigatum* and *N. parile*.

| Locus | Primer sequences (5'–3') | Repeat motif | Multiplex ^a | Dye label | Allele size range (bp) | A | GenBank accession no. |
|--------|--|----------------------|------------------------|-----------|------------------------|---|-----------------------|
| NLae01 | F: TCCGATACGTAATGCAAACGG R: TCGCTGTTGTGCGCAAGAAAC | (TCA) ₁₂ | M-NL1 | PET | 192–231 | 8 | KM361439 |
| NLae02 | F: ACAACGGTAGGGCAGATCG R: GTCTTGTACTCTGTAACCATGTC | (ACAT) ₈ | M-NL2 | NED | 198–237 | 7 | KM361440 |
| NLae03 | F: CATGGATGAAACGGTCTCGG R: TCTTTGTGTATGTCCACTCC | (AC) ₁₂ | M-NL2 | 6-FAM | 230–234 | 3 | KM361441 |
| NLae04 | F: AGGTCCCACAAACAAAAGC R: TCCCATAGGGGAAGTCAAGCC | (CAGA) ₉ | M-NL2 | VIC | 142–165 | 5 | KM361442 |
| NLae05 | F: ATACCTTACCGACCCACGTC R: GAGCCGGAGTGAATCCAAC | (GA) ₁₁ | M-NL1 | NED | 254–258 | 4 | KM361443 |
| NLae06 | F: ATAACCAGGACGAGGAAGT R: TCCGTCTACACCTGCAAAG | (GGA) ₉ | M-NL1 | PET | 89–129 | 8 | KM361444 |
| NLae07 | F: ACATGAAAAGCACCTGCCAC R: CTTTGGCTCTCGGTAAGTC | (TG) ₁₁ | M-NL2 | VIC | 240–284 | 7 | KM361445 |
| NLae08 | F: GGGCTTAGGAAAAGTGCCG R: AGTGCTGTATATATCACCTTTGG | (CTTG) ₁₅ | M-NL1 | 6-FAM | 207–240 | 3 | KM361446 |
| NLae09 | F: AGATAACGTCGAGGTGGTGG R: AGCAGTTTGGTCACTTCTC | (CAT) ₁₂ | M-NL2 | 6-FAM | 126–168 | 7 | KM361447 |
| NLae10 | F: TCAGTCTTCCATTACCGGGG R: GCCTGAGTAGAAGGCTGGAC | (TC) ₁₁ | M-NL1 | 6-FAM | 137–155 | 6 | KM361448 |
| NLae11 | F: TCGTCCAAGACACGAAAGGG R: GACCTGCAATATGCGACCTG | (GGAT) ₇ | M-NL2 | PET | 199–229 | 4 | KM361449 |
| NLae12 | F: AGCTGGTCATGTTATCCCC R: GGAATCAGTGCAGTTGCTGG | (CTT) ₉ | M-NL1 | VIC | 205–212 | 3 | KM361450 |
| NPar01 | F: CCGAGAGTACCCACTGAAGG R: TGAGATTGTGGAGGTGGCTC | (CA) ₁₁ | M-NP2 | VIC | 207–221 | 3 | KM361451 |
| NPar02 | F: CTAGACTCCGAGGAGACGC R: GCATGGAGCTGAACCACATC | (TGG) ₈ | M-NP1 | PET | 234–241 | 3 | KM361452 |
| NPar03 | F: GGCGCAACGAGGAGAATATG R: GTC AACAGGTCACTGCATCG | (AC) ₁₃ | M-NP2 | 6-FAM | 230–242 | 4 | KM361453 |
| NPar04 | F: GGTACTTAGGGCCTTTAAATGACTG R: CGTCACTGAAGAAGAATCAGACG | (TGA) ₉ | M-NP1 | NED | 170–176 | 3 | KM361454 |
| NPar05 | F: TTCCCTAACGCAAAATGCTCG R: CGCGGAAGTCACCATTCATC | (GA) ₁₃ | M-NP1 | VIC | 244–246 | 2 | KM361455 |
| NPar06 | F: AGCCATGCGATTTACACAG R: CGATACCTGATCGCAGGAGG | (TGG) ₈ | M-NP2 | NED | 194–224 | 4 | KM361456 |
| NPar07 | F: TGTTTGCTTCTCGACGTGTG R: TCGATAGGGCTCCTGTTC | (GT) ₁₁ | M-NP1 | NED | 233–244 | 3 | KM361457 |
| NPar08 | F: GCCTTTCAGGGGTTGGATTG R: GGTACCATGGCTTGCATACG | (CAT) ₉ | M-NP1 | 6-FAM | 192–215 | 4 | KM361458 |
| NPar09 | F: ACCGGGGGTTTTTAATTGGTG R: CAAAGCATTTGCTGCTACCC | (GT) ₁₁ | M-NP2 | NED | 109–116 | 3 | KM361459 |
| NPar10 | F: CTTCCGGTGGACTTTCGTTTCG R: TCCTTTCATCTGCATACCCC | (CGT) ₇ | M-NP2 | PET | 113–116 | 2 | KM361460 |
| NPar11 | F: TTATGTGTGCCCCGACTGGAG R: GTTTTCTTCCGAGTTCCCGC | (GA) ₁₁ | M-NP1 | 6-FAM | 115–125 | 2 | KM361461 |
| NPar12 | F: AGCGATTTTGTGGGTCGTTG R: GTTCTCCGAAAGCGCTCC | (GGT) ₈ | M-NP2 | VIC | 145–151 | 3 | KM361462 |
| NPar13 | F: TCCTACCCGACACAATTCCT R: GTATGCGGGCTGGATAAAC | (CA) ₁₃ | M-NP1 | NED | 102–108 | 4 | KM361463 |
| NPar14 | F: GTAAACGCAATCCCTGGACG R: TACGTCAAACGCACAGTACG | (GAG) ₇ | M-NP2 | PET | 147–172 | 3 | KM361464 |

Note: A = number of alleles.

^aMultiplex indicates loci that were mixed in the same capillary electrophoresis run. Annealing temperatures were 56°C.

biotin-labeled CT and GT repeat oligonucleotides. The genomic library was enriched for simple sequence repeats (SSRs) prior to 454 sequencing. The SSR content is species dependent, but usually less than 2% of the sequence read pool contains a microsatellite insert without enrichment (Ecogenics GmbH). The SSR-enriched library was analyzed on a Roche 454 platform using GS FLX Titanium reagents (454 Life Sciences, a Roche Company, Branford, Connecticut, USA). Based on fragment length, repeat motif, and no poly N present in each amplicon, the most promising markers were chosen for screening in *N. laevigatum* and *N. parile* using a Primer3 core code (available from <http://primer3.sourceforge.net/releases.php>; Rozen and Skaletsky, 2000). The remaining sequences were not suitable for primer design because flanking regions were absent or too short. In the end, the

most promising markers were proved (1) to amplify, and (2) to be repeatable and polymorphic (i.e., bearing at least two alleles per population); the remaining were monomorphic, nonamplifying in some individuals, or too unspecific. For *N. laevigatum*, a total of 19,431 reads had an average length of 404 base pairs. Of these, 630 contained a microsatellite insert with a tetra- or a trinucleotide of at least six repeat units or a dinucleotide of at least 10 repeat units. Suitable primer design was possible in 265 reads (i.e., read sequence is not starting or ending with the repeat motif). In the case of *N. parile*, a total of 21,136 reads had an average length of 396 base pairs. Of these, 1386 contained a microsatellite insert with a tetra- or a trinucleotide of at least six repeat units or a dinucleotide of at least 10 repeat units. Suitable primer design was possible in 488 reads. Primers

TABLE 2. Results of microsatellite screening in four populations of *Nephroma laevigatum*.^{a,b}

| Locus | Total | | | Dulsie (n = 19) | | | Torboll (n = 14) | | | Glen Nant (n = 17) | | | Ardery (n = 16) | | |
|-----------------|-------|------|----------------|-----------------|------|----------------|------------------|------|----------------|--------------------|------|----------------|-----------------|------|----------------|
| | n | A | H _e | n | A | H _e | n | A | H _e | n | A | H _e | n | A | H _e |
| NLae02 | 66 | 10 | 0.75 | 19 | 4 | 0.71 | 14 | 4 | 0.57 | 17 | 6 | 0.85 | 16 | 7 | 0.87 |
| NLae03 | 65 | 3 | 0.39 | 19 | 2 | 0.35 | 13 | 1 | 0.00 | 17 | 3 | 0.69 | 16 | 3 | 0.51 |
| NLae04 | 64 | 7 | 0.50 | 18 | 2 | 0.20 | 13 | 3 | 0.50 | 17 | 4 | 0.57 | 16 | 6 | 0.75 |
| NLae05 | 66 | 4 | 0.26 | 19 | 2 | 0.11 | 14 | 1 | 0.00 | 17 | 3 | 0.49 | 16 | 4 | 0.44 |
| NLae07 | 66 | 13 | 0.77 | 19 | 6 | 0.80 | 14 | 4 | 0.74 | 17 | 7 | 0.82 | 16 | 6 | 0.73 |
| NLae08 | 66 | 6 | 0.61 | 19 | 3 | 0.51 | 14 | 3 | 0.27 | 17 | 6 | 0.83 | 16 | 6 | 0.84 |
| NLae09 | 66 | 10 | 0.83 | 19 | 5 | 0.77 | 14 | 6 | 0.86 | 17 | 9 | 0.89 | 16 | 6 | 0.83 |
| NLae10 | 66 | 6 | 0.70 | 19 | 4 | 0.70 | 14 | 3 | 0.60 | 17 | 5 | 0.78 | 16 | 5 | 0.73 |
| NLae11 | 66 | 5 | 0.53 | 19 | 3 | 0.62 | 14 | 2 | 0.26 | 17 | 4 | 0.49 | 16 | 4 | 0.73 |
| NLae12 | 66 | 4 | 0.24 | 19 | 1 | 0.00 | 14 | 1 | 0.00 | 17 | 2 | 0.31 | 16 | 4 | 0.64 |
| Mean | | 6.80 | 0.56 | | 3.20 | 0.47 | | 2.80 | 0.38 | | 4.90 | 0.67 | | 5.10 | 0.71 |
| Private alleles | | | | | 5 | | | 1 | | | 4 | | | 9 | |

Note: A = number of alleles; H_e = Nei's unbiased gene diversity; n = total number of samples analyzed.

^a See Appendix 1 for populations used in this study.

^b For analyses, markers with a high percentage of nonamplifications from the total number of samples tested were removed: NLae01 (9%), NLae06 (8%).

were designed for 12 microsatellite inserts for *N. laevigatum* and 14 microsatellite inserts for *N. parile* and tested for polymorphism. Although few simple sequence repeats are expected in bacterial genomes, an additional BLAST search was included for all primer candidates to exclude any that matched the available *Nostoc* reference genome before testing for polymorphism.

Polymorphisms within the 26 microsatellite loci were determined by EcoGenics GmbH using a test set of 15 samples for each species collected from four Scottish populations (Appendix 1), and using M13-tailed forward primers and universal M13 fluorescent-labeled primer following Schuelke (2000). For these samples, genomic DNA was extracted from dried thallus material with the QIAGEN DNeasy Plant Mini Kit, and loci were PCR amplified in the Veriti 96-Well Thermal Cycler (Applied Biosystems, Carlsbad, California, USA). Primers that showed clear amplification profiles and reliable amplification and that were polymorphic across samples are described in Table 1.

Of the set of 26 polymorphic primers obtained from the polymorphism test, a larger screen of marker variation was completed using four populations for each species (Tables 2, 3), totaling more than 60 samples per species. All PCR products obtained were multiplexed (Table 1). PCR reactions were performed in a total volume of 10 µL containing 1 µL of genomic DNA, 2 µL of water, 5 µL of HotStarTaq (QIAGEN), and 2 µL of primer mix (1 µM each of forward and reverse primer concentration). Cycling conditions were: 15 min at 95°C, 35× (30 s at 94°C, 90 s at 56°C, 60 s at 72°C), and a final extension of 30 min at 72°C. For the fragment analyses, 1 µL of 1:20 diluted multiplex products was used. PCR products were sized on an ABI3730 Genetic Analyzer (Applied Biosystems), and

genotypes were assigned with a GeneScan 500 LIZ Size Standard (Life Technologies, Carlsbad, California, USA). Of the initial 26 microsatellite primer pairs, four were not included in the results of this screen due to their low amplification success on the evaluated samples (>8% nonamplifications; NLae01, NLae06, NPar04, NPar14). The number of alleles and unbiased genetic diversity were generated using GenAlEx 6.501 (Peakall and Smouse, 2006; Tables 2, 3). For the sample of 66 specimens for *N. laevigatum*, all 10 loci evaluated were polymorphic with between three and 13 alleles per locus, a total of 68 alleles across loci, and altogether comprising 46 multilocus genotypes detected over all populations. From the sample of 61 specimens of *N. parile*, all 12 loci evaluated were polymorphic with two to six alleles per locus, a total number of 48 alleles across loci, and 16 multilocus genotypes detected over all populations. Average gene diversities varied from 0.38 to 0.71 and 0.01 to 0.50 for *N. laevigatum* and *N. parile*, respectively (Tables 2, 3). A small sample of specimens (four per species) was repeated to check for reproducibility, and all yielded identical allele lengths.

CONCLUSIONS

Twelve and 14 microsatellite primers were characterized to investigate population structure and gene flow in *N. laevigatum* and *N. parile*, respectively. The markers demonstrate high-resolution variability at even a relatively small geographic

TABLE 3. Results of microsatellite screening in four populations of *Nephroma parile*.^{a,b}

| Locus | Total | | | Dundonnell (n = 16) | | | Glen Nant (n = 16) | | | Dreggie (n = 14) | | | Dulsie (n = 15) | | |
|-----------------|-------|------|----------------|---------------------|------|----------------|--------------------|------|----------------|------------------|------|----------------|-----------------|------|----------------|
| | n | A | H _e | n | A | H _e | n | A | H _e | n | A | H _e | n | A | H _e |
| NPar01 | 59 | 3 | 0.42 | 16 | 1 | 0.00 | 14 | 3 | 0.62 | 14 | 3 | 0.70 | 15 | 2 | 0.34 |
| NPar02 | 61 | 3 | 0.28 | 16 | 1 | 0.00 | 16 | 2 | 0.33 | 14 | 2 | 0.44 | 15 | 2 | 0.34 |
| NPar03 | 61 | 5 | 0.32 | 16 | 1 | 0.00 | 16 | 2 | 0.33 | 14 | 3 | 0.62 | 15 | 2 | 0.34 |
| NPar05 | 61 | 2 | 0.23 | 16 | 1 | 0.00 | 16 | 2 | 0.13 | 14 | 2 | 0.44 | 15 | 2 | 0.34 |
| NPar06 | 61 | 6 | 0.44 | 16 | 1 | 0.00 | 16 | 5 | 0.73 | 14 | 5 | 0.80 | 15 | 2 | 0.25 |
| NPar07 | 61 | 5 | 0.43 | 16 | 1 | 0.00 | 16 | 4 | 0.68 | 14 | 3 | 0.70 | 15 | 2 | 0.34 |
| NPar08 | 61 | 6 | 0.43 | 16 | 2 | 0.13 | 16 | 3 | 0.43 | 14 | 5 | 0.84 | 15 | 2 | 0.34 |
| NPar09 | 61 | 2 | 0.06 | 16 | 1 | 0.00 | 16 | 2 | 0.23 | 14 | 1 | 0.00 | 15 | 1 | 0.00 |
| NPar10 | 61 | 2 | 0.06 | 16 | 1 | 0.00 | 16 | 2 | 0.23 | 14 | 1 | 0.00 | 15 | 1 | 0.00 |
| NPar11 | 61 | 2 | 0.09 | 16 | 1 | 0.00 | 16 | 1 | 0.00 | 14 | 1 | 0.00 | 15 | 2 | 0.34 |
| Npar12 | 61 | 3 | 0.40 | 16 | 1 | 0.21 | 16 | 3 | 0.63 | 14 | 3 | 0.62 | 15 | 2 | 0.34 |
| NPar13 | 60 | 6 | 0.43 | 16 | 1 | 0.00 | 16 | 3 | 0.58 | 13 | 4 | 0.79 | 15 | 2 | 0.34 |
| Mean | | 3.75 | 0.30 | | 1.08 | 0.01 | | 2.67 | 0.41 | | 2.75 | 0.50 | | 1.83 | 0.28 |
| Private alleles | | | | | 1 | | | 6 | | | 6 | | | 4 | |

Note: A = number of alleles; H_e = Nei's unbiased gene diversity; n = total number of samples analyzed.

^a See Appendix 1 for populations used in this study.

^b For analyses, markers with a high percentage of nonamplifications from the total number of samples tested were removed: NPar04 (17%), NPar14 (34%).

sampling scale, with no shared genotypes among populations for the sexual species, in contrast to the predominantly asexual *N. parile*. We conclude that the markers can potentially provide insights contributing to an improved understanding of population genetic processes, and they are currently being used to analyze population genetic structure in *N. laevigatum* and *N. parile* for variable habitats across a steep climatic gradient.

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APPENDIX 1. Location information, number of individuals sampled, and voucher specimens of *Nephroma* species used in this study. All specimens were sampled as epiphytes from broadleaved woodlands.^a

| Species | Voucher ^b | Locality ^c | Latitude | Longitude | <i>n</i> | Collection date |
|----------------------------|----------------------|--|-----------------|-------------------|----------------------------------|-----------------|
| <i>Nephroma laevigatum</i> | RB1 | Ard Trilleachan SSSI, 31 m a.s.l. | 56°33'09.8100"N | −005°05'08.1874"W | 2 ^d | 15 Oct. 2012 |
| <i>Nephroma laevigatum</i> | RB2 | Glen Nant SSSI, 82 m a.s.l. | 56°23'46.8924"N | −005°12'38.0419"W | 2 ^d , 17 ^f | 20 Oct. 2012 |
| <i>Nephroma laevigatum</i> | RB3 | Birks of Abberfeldy SSSI, 241 m a.s.l. | 56°36'25.4736"N | −003°52'18.4706"W | 2 ^d | 10 Jan. 2013 |
| <i>Nephroma laevigatum</i> | RB4 | Glen Tilt SSSI, 221 m a.s.l. | 56°48'22.2876"N | −003°50'09.0647"W | 2 ^d | 15 Jan. 2013 |
| <i>Nephroma laevigatum</i> | RB5 | Ardura SSSI, 48 m a.s.l. | 56°23'56.9184"N | −005°45'26.3063"W | 4 ^e | 25 Oct. 2012 |
| <i>Nephroma laevigatum</i> | RB6 | Kyles Wood, 45 m a.s.l. | 56°48'08.9964"N | −005°48'51.8998"W | 4 ^e | 1 Nov. 2012 |
| <i>Nephroma laevigatum</i> | RB7 | Dulsie Bridge, 182 m a.s.l. | 57°26'44.6460"N | −003°47'10.0759"W | 4 ^e , 19 ^f | 18 Jan. 2013 |
| <i>Nephroma laevigatum</i> | RB8 | Dreggie Aspen Wood, 262 m a.s.l. | 57°20'01.7592"N | −003°37'28.3181"W | 3 ^e | 23 Jan. 2013 |
| <i>Nephroma laevigatum</i> | RB9 | Torboll Woods SSSI, 72 m a.s.l. | 57°57'18.0036"N | −004°07'07.2178"W | 14 ^f | 15 Apr. 2013 |
| <i>Nephroma laevigatum</i> | RB10 | Ardery, 36 m a.s.l. | 56°41'38.2884"N | −005°40'09.1535"W | 16 ^f | 5 Oct. 2012 |
| <i>Nephroma parile</i> | RB11 | Ard Trilleachan SSSI, 31 m a.s.l. | 56°33'09.8100"N | −005°05'08.1874"W | 2 ^d | 15 Oct. 2012 |
| <i>Nephroma parile</i> | RB12 | Glen Nant SSSI, 82 m a.s.l. | 56°23'46.8924"N | −005°12'38.0419"W | 2 ^d , 16 ^f | 20 Oct. 2012 |
| <i>Nephroma parile</i> | RB13 | Kindrogan Field Centre, 280 m a.s.l. | 56°44'50.1936"N | −003°32'41.6249"W | 2 ^d | 15 Nov. 2013 |
| <i>Nephroma parile</i> | RB14 | Glen Tilt SSSI, 221 m a.s.l. | 56°48'22.2876"N | −003°50'09.0647"W | 2 ^d | 15 Jan. 2013 |
| <i>Nephroma parile</i> | RB15 | Ardura SSSI, 48 m a.s.l. | 56°23'56.9184"N | −005°45'26.3063"W | 4 ^e | 25 Oct. 2012 |
| <i>Nephroma parile</i> | RB16 | Kyles Wood, 45 m a.s.l. | 56°48'08.9964"N | −005°48'51.8998"W | 3 ^e | 1 Nov. 2012 |
| <i>Nephroma parile</i> | RB17 | Dulsie Bridge, 182 m a.s.l. | 57°26'44.6460"N | −003°47'10.0759"W | 4 ^e , 15 ^f | 18 Jan. 2013 |
| <i>Nephroma parile</i> | RB18 | Dreggie Aspen Wood, 262 m a.s.l. | 57°20'01.7592"N | −003°37'28.3181"W | 4 ^e , 14 ^f | 23 Jan. 2013 |
| <i>Nephroma parile</i> | RB19 | Dundonnell Woods SSSI, 26 m a.s.l. | 57°50'18.0378"N | −005°11'16.818"W | 16 ^f | 1 May 2013 |

Note: *n* = number of samples analyzed; SSSI = Site of Special Scientific Interest.

^aAll specimens were collected by R.B.

^bVouchers deposited at the Herbarium of the Royal Botanic Garden Edinburgh (E).

^cLocality in Scotland.

^dSpecimens used for shotgun sequencing.

^eSpecimens used for polymorphism testing.

^fSpecimens used for microsatellite screening.