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Authors: McDaniel, Stuart F., Baren, Marijke J. van, Jones, Kelly S., Payton, Adam C., and Quatrano, Ralph S.

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PRIMER NOTE

ESTIMATING THE NUCLEOTIDE DIVERSITY IN *CERATODON PURPUREUS* (DITRICHACEAE) FROM 218 CONSERVED EXON-PRIMED, INTRON-SPANNING NUCLEAR LOCI[1]

STUART F. MCDANIEL[2,3,5], MARIJKE J. VAN BAREN[4,6], KELLY S. JONES[2], ADAM C. PAYTON[2], AND RALPH S. QUATRANO[3]

[2]Biology Department, University of Florida, Gainesville, Florida 32611 USA; [3]Biology Department, Washington University in St. Louis, St. Louis, Missouri 63130 USA; and [4]Center for Genome Sciences, Washington University in St. Louis, St. Louis, Missouri 63130 USA

- *Premise of the study:* We developed and tested primers for 218 nuclear loci for studying population genetics, phylogeography, and genome evolution in bryophytes.
- *Methods and Results:* We aligned expressed sequence tags (ESTs) from *Ceratodon purpureus* to the *Physcomitrella patens* genome sequence, and designed primers that are homologous to conserved exons but span introns in the *P. patens* genome. We tested these primers on four isolates from New York, USA; Otavalo, Ecuador; and two laboratory isolates from Austria (WT4 and GG1). The median genome-wide nucleotide diversity was 0.008 substitutions/site, but the range was large (0–0.14), illustrating the among-locus heterogeneity in the species.
- *Conclusions:* These loci provide a valuable resource for finely resolved, genome-wide population genetic and species-level phylogenetic analyses of *C. purpureus* and its relatives.

Key words: chromosomal inversion; expressed sequence tag (EST); phylogeography; *Physcomitrella patens*; sex-linked loci.

Over the past 15 yr, our understanding of the microevolutionary processes that shape variation within bryophyte populations has been revolutionized by the use of DNA sequence variation. Most of these inferences have been drawn from variation in a small number of loci, principally from the chloroplast and nuclear ribosomal regions (Stech and Quandt, 2010). However, these loci may be difficult to align, they may lack sufficient variation to answer many questions, and they may not reflect the full complexity of the organismal history (McDaniel et al., 2010; Vanderpoorten and Shaw, 2010).

To develop new loci for phylogeographic and population genetic inference in *Ceratodon purpureus* (Hedw.) Brid., we have generated primers for exon-primed intron-spanning loci, based on an alignment of expressed sequence tags (ESTs) from *C. purpureus* to the *Physcomitrella patens* (Hedw.) Bruch & Schimp. genome. The common ancestor of *P. patens* and *C. purpureus* represents the common ancestor of nearly all of the arthrodontous mosses, comprising ~95% of moss species (Cox et al., 2010). Thus, although we designed these primers specifically for use in *C. purpureus* and its relatives, by choosing conserved priming sites we have maximized the chance that these loci will amplify homologous regions in other bryophyte species.

METHODS AND RESULTS

To develop primers for nuclear loci in *C. purpureus*, we screened the 1677 ESTs available on GenBank at the time. We first clustered the ESTs into 850 unigenes, and aligned them to the *P. patens* genome using the software BLAT (Kent, 2002; http://genome.ucsc.edu/goldenPath/help/blatSpec.html). This resulted in 450 aligned unigenes, or 1050 aligned ESTs. Using the software Primer3 (Rozen and Skaletsky, 2000), we designed pairs of primers that were homologous to the *C. purpureus* sequence and that spanned a single intron in the *P. patens* genome. We designed a set of primers with their 3′ end at least 25 bp from the beginning of the intron. This resulted in primers for 212 nuclear loci. On the intron-spanning unigenes that failed the primer design process, we also designed a set of primers with their 3′ ends at least 5 bp from the beginning of the intron. This resulted in primers for an additional 33 nuclear loci (all primer details are in Table 1). In some cases, the unigene spanned multiple introns, and we designed separate pairs of primers for each intron. Where possible, we also designed alternate primers for each intron in the complete unigene set.

To evaluate the full set of 245 loci, we sequenced each of these gene regions in the female laboratory strain GG1 (collected from Gross Gerunds, Austria, by D. J. Cove), the male laboratory strains WT4 (collected in Wispertal, Austria, by E. Hartmann) and R40 (collected by S.F.M. in Rensselaer County, New York, USA), and an isolate from Otavalo, Ecuador (collected by S.F.M.). Live cultures of all of these individuals are available from the authors. DNA was extracted from 7-d-old protonemal grown under standard conditions (Cove et al., 2009) using the Nucleon PhytoPure Genomic DNA Extraction Kit (Amersham Biosciences, Piscataway, New Jersey, USA) following the manufacturer's

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[5]Author for correspondence: stuartmcdaniel@ufl.edu

[6]Current address: Monterey Bay Aquarium Research Institute, Moss Landing, California 95039 USA

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TABLE 1. *Ceratodon purpureus* EST intron primer sequences.

EST–intron start position	5′ Primer	3′ Primer	Forward T_m[a]	Reverse T_m[b]	Prod. size[c]
Primer >25 nucleotides from intron					
AW098097–137	CCAATAGCAAAAGAATACAATCAAAGC	CGCGTTGTTCCTGGAATGG	62	64	318
AF233229–229	CCGAACTTGAACAGGACAGTTATGG	TGATCACGATTCCGACTGAGG	65	63	458
AF233229–433	GCTCCCAACTCAGGAACACCC	GGAGGCCTGGAGATGGTAACG	65	65	510
AW098318–384	AATCGTCACCGAATGAGAGGG	CAATGTTGGCATGCTCCC	63	61	467
AW098631–273	ATGTGTTGTGCTTTCCCAGG	TGGACAGTCACAACTCCTCTCCC	60	65	396
AW086794–139	TATCGGCGTGTGCAAGGG	GCTGGCTTCTCGAATGTGGG	64	65	342
AW086770–115	TCCGCGAGCTCTGAGTGG	AACAACTTCACCACATCTGCACG	63	64	308
AW086590–267	AACTGTGCACAAACAGCGGC	TGTACGACCATCCAGACTAAGAAGG	64	62	593
AW098359–150	GCGAAGTCCAAGAACCATACCG	AGCCTCGACCTCATCGGC	65	63	407
AW087018–146	CGACATTGCAGTACGAACTTTGC	CAAGTGGAGCCTGTCATCTTTGC	64	65	375
AW087018–260	CTGGTCATGGATCTGGTCGC	CAGTCCTCTCAGCATCCAGG	64	60	298
AW086837–165	CTTGGACGTGCATGGAGGC	GTGTTGAACCGCATGTTGTCG	65	64	316
AW086837–243	ATCAGCTTCGGACCCTTCACC	CTATGTGTGGGGCCAGCG	64	63	307
AW086675–86	CCGCATGTAGAAGAGCTACGCC	TCTCGTCGCAATGCAGGC	65	64	277
AW086645–117	ATGCTTGCAACGCTTGATGG	GTCAGGTTTCGGTGGTCAGTCC	64	65	466
AW098393–199	AAGGCTGAGGCTGAGGGC	AGTGCGGGAAGCCACACG	63	65	284
AW098679–454	CCTTGATGTGGTAGTAATCTGCG	CGAAATTGGTGGTGTGAGGG	60	63	438
AW098679–532	CATCGGGATTGAAAGCAAGAGC	ATTGGTGGTGGTCAAGAGGC	65	62	426
AW098515–203	CTGAAGAATTGGAACCAGTCCG	TTAAGCTTCGCGCAGTCCG	63	64	452
AW086579–194	ATCGGACGACTTCCTGCC	TTCAGGAATACGGTCGGCG	61	64	223
AW086579–332	TGCAACTCCCAAAGCTGCC	TGTTTTGGCACGAGACATGG	64	63	412
AW086736–262	GAGGTCCGAAGAGAGCCACC	TCTTGCATGTGGCAGACACG	63	64	404
AW097953–298	GCCCCTTCAAGAGACTTTGGC	GGCAAGAACATCTTGTCTTTGATGC	64	65	354
AW098418–107	GAAGAAGGAGACGCCCGC	AGACGGTCGGACAACACGG	63	64	320
AW098974–394	TTGGAAGCCAATTTCTCCTTGG	CAGGCACAGGAAGACTGTGAACC	64	65	510
AW086877–265	TTGATCCTCGGCTAGTTGCTCC	TCCAGTGCTTCCAGCAGCC	64	65	511
AW086877–378	ATGGAGATGCGGCTGCTGG	ACCAAGTGAATGCGGTCATATCC	67	64	339
AW086549–119	ACAAGGAAAGACGCAATTATTTATAGG	TGTGTCCTCCGGCTCACG	61	64	394
AW086549–238	AGAGCACAGGCATGACGGC	GCAGAATTGTATGCGCGACG	64	64	323
AW086868–74	CGATGTGGTTTACAACGCGTCC	ACCAGGTACGGAAGGGGGC	66	65	377
AW086868–247	TCGATGCTGCCCCCTTCC	CAGAACGGCCGCATTGG	66	64	392
AW086594–170	TGCTCCAACAAGTCACCAATGC	TTCCCATTGACTTGCGCC	65	63	337
AW086594–327	GCTTGGTCAACCTCCTGCG	TGAGAGTTGCCAAGGTCACTGG	64	64	479
AW098340–132	GCCCAGCAACAGCAAGGG	CACCACCCTGGCCAACTCC	65	66	441
AW098700–545	CCACTTCTGCCATGGTGGG	GCTTCGGTGTGGTTCTGTGG	64	64	350
AW086759–94	CCGTAGCTTCTGCAATATGTTTGAGG	TGGCAAGGCGACCAAAGC	65	65	486
AW098624–132	GACAAGGCCAGTTTGTACGCC	TGGTCTTAGCCTTATTGTGAAAGACG	64	63	269
AW098020–216	TCCGTTCAATTCAGCTCGG	TGACGCAGCTTAGCGGGC	62	65	262
AW098462–157	TGACGATAGAAGGTGTGCGCC	AACAGGAAGGCCAGCCTATGG	65	64	576
AW086686–370	CACTCCCTTTTTGAGATCTTCAGGG	ATGGGGTGCAGATTGGGG	65	64	396
AW098568–216	ACCACATCCATTTCGGACGC	TTCCCAGCTCGACAACATGG	65	64	327
AW086975–261	ACTTATTTCGCCAGAGGATCTGC	TCACTTTGACTCACAGACTGAATGACC	63	65	391
AW086975–331	TTGCGCAAGTTTGACAGTGG	GCAGGAGCCATTCCCAAGG	63	65	362
AW098234–164	TGGAGCTTGCTCACATCGC	CAGCAATACCCACTGCACGG	63	64	396
AW098407–97	GGCGAAGGCTGTGATGGG	TTTTTGCAAATGCAACCTGGG	65	65	392
AW086555–375	GCCATAGTCGCATCAAAATTGG	GGTTGTGGTCAGCAGCCG	63	63	438
AW097994–187	ACGAAGCTGGAGGCTGTGG	CAATGTCTATGGAACCAATCTTTGC	63	62	396
AW086517–380	TTGGTCTTCCTCCGCAGC	TCCGTGGATGATGATCTGGC	62	64	473
AW086517–457	CATGTCTTGCTTCAAGGATGC	TCTGCATTGGAGGCAGAATCCG	60	68	267
AW098225–85	AGGCCAGAGTTGCTGAACCG	ACTCACCCGCCAGCAAGG	65	64	531
AW098839–86	TGACTAGATGCTTTTATTGCTGAAAGG	ATCACATCGAACGGGCAGC	63	64	493
AW098839–177	CAACCTGGTCCAGATCACGC	TACGGTGGCCGCAAGAGC	64	65	428
AW086694–137	TCTTCATGACCTTAGCCACAGCC	AGATGATCTTGATGCGAGGG	64	60	304
AW086845–149	AGGGGAAAGGCACCAGGG	AGTTCACGGTTCCGGTGAGG	64	64	679
AW086858–91	CATCAAGGGAGTGGGGCG	TCAGCTTCATGTCCAAGGCG	64	64	451
AW086858–280	TGGTGATCGTTGCCAACCC	CACCGACGGTTCTTCCACG	65	64	406
BI894288–61	TGGAAGAAGCCTTTTGCTGG	TTCAAACAACCATCGTTGGGG	62	65	285
BI894288–188	GGCTAAGCAGCCCAACTCTGC	TCTCCCACGGCATGTCCC	65	65	353
AW087023–114	GGATCCACAAGGCCAAGTTGC	CATTTCGACGAACGGTGGC	66	64	433
AW087023–239	AGCCATAAACGCAATTCGGG	TGACTGAACCCGTCCGGC	64	65	399
AW097931–294	AGAAGTTCCAAGTCGGGTGGC	TCCGTTGGCGTTCTTCAGC	64	64	500
AW098613–99	CTTTGGCTTGAGAAACAAGGGG	GCCGGCGATACAAATGAAACC	64	65	251
AW086659–247	TTCCAGGTGGGTGGGAGC	AGGGTTTGACCCCGTCGC	64	65	494
AW086999–142	AGCTATTAGGGCGAGTGAAAGCG	GGCTGGAGTGAATCATTTTGGG	65	64	368
AW086548–172	AAATTCGGCAAATAAGAGGGG	CCGGGCAAATCGTTCAGG	61	64	428
AW098157–126	CCTGCGAGGAAGATGACGC	AAGTTTCCACATTCAGAAGAGCCG	64	64	514
AJ250735–923	TCAGCCAGTTTGATGGGC	GGTTTCCATCCGGGGAGC	60	65	723
AJ250735–1460	TCAGGCCCGAGTTGACCC	AAGTGCCCGAAGCCATGC	64	64	747

Table 1. Continued.

EST–intron start position	5′ Primer	3′ Primer	Forward T_m[a]	Reverse T_m[b]	Prod. size[c]
AW097975–117	GAAGGGAAAGCCTGATTTGCG	GATGGGTCCGGTGATGGC	65	64	564
AW098252–97	CAAGGAGGTGGCGACTGC	CCGGCGAACTGCAAAACC	63	64	624
AW086519–461	CAGCGAAAATCAGACGCTGC	CCGCAAGGACCATCACCC	64	64	1025
AW098432–169	ATGAGTCGGCCGCATGG	TGCAGAGCCTTACACGACGC	64	64	759
AW087005–110	GCTATGTGGTGGCGTCCG	GAACAGCAAAATATTGCCTCTCGG	63	65	385
AW098158–174	TCCCTTGCCCTTCTTGTCTCC	TGTGCTGAGGTTGCAGTGCC	64	65	216
AW098158–249	TGCTGTGTCACGTGCTTGC	GGAAGCAGTCAGGATACGGAGG	63	64	443
AW098158–317	CGGGCTTGGTCTGTCCTCC	TCTTCTGCCCTGGGAAGGC	65	65	471
AW098372–71	TCCATTTGATCGCTGTTCCG	GAACTCAGGCAATCAAAGCAAGC	64	64	257
AW097984–288	GTGGGCTGAAGCGCAAGC	TGATCATGTTGCGTAGGTGGG	65	64	237
AW098512–413	TCGCAACTCAGCCCTCACG	TTGTTCCCGCAAGTGGGC	66	65	319
AW098442–86	AGCTCTGCACTGGGCTGG	TGGAACCGTGAAAGCCCG	62	65	279
AW098442–198	GGATAAGCAGACGCGCCG	CGAAAATGCAACCCCAGGC	65	65	271
AW098442–292	CCCAAAGTTCTTCTTGGGGC	GAAAGATGTGAAGAAGCTGTTGAAGC	63	63	252
AW098349–75	AGATGATGCTGAGATGGAAGCG	TCAAGAGAGGCACCCTCGG	63	63	400
AW098572–84	TGGGCTCTTGATGTTCTGGG	ACCGATCCTCAGCCTGCC	63	63	381
AW098572–303	CCACAAATGGATCGGCAGG	TTGCCTTCAAGCACAGCAGC	64	65	316
AW086786–104	ACGTGCTTGTTGCAAGGGC	AACAGCACGTGTCGGTCAGC	64	64	291
AW086989–141	AAGTGGCGCGGTTTGAGG	GTTTGACAGGAACGGGCAGG	64	65	759
AW098304–85	ATGGAAGCATGTGGTTTTGGG	CGACGCATGTCCTTCGGC	64	66	560
AW086668–76	CAACAGGCCTCTAAATCTTGGCG	TGGAGATTCTCACAGCCCCC	66	64	307
AW098058–71	CCAAGCGGGGCTTGTATGC	GTCCAGCTTGCGTTCGGG	66	65	414
AW098449–310	CTGCCAAGACCATTTTGAGTGG	AAAAAGTCACGACTAATGCTGTGC	64	61	369
AW097976–116	CATGTGCTGGTCTCACATTCCC	GCATTTTGTCTCAAGCCTTCG	65	62	485
AW098621–172	TCGTGGTTGAGTTGAACGGG	TCCCAAAACCTTGCCTCCG	64	65	407
AW097924–87	GCGTGCGTATGTTGTGATTAAGG	GCTCTCTGCTACAGCAGCCTCG	63	66	347
AW097924–222	CATGTTGCGCGAATCAGAGG	AACACAGAGTCTTGAATCTCCAGGG	65	64	323
AW086546–344	TCCTTCTCGGCAGCCTCG	CAACCGTCTTAACACCTACAGGGC	65	65	504
AW086789–334	GACCCCCAGAGCATCTTCG	CCTTGCGCTCCTTGATCTGC	63	65	358
AW098343–365	CCAACGACGCTTCTTCGC	GAGGACGGGATTTGTTCATGC	63	63	674
AW098256–171	TGATGACGAGCTGCTCAGAGG	GCCTTCTCAGCTTTGGCTGC	64	64	310
AW098256–290	AAGTGGCCGTGGAGATACAAGC	GCTGCTCTCTCCTTCTCACGC	64	64	393
AW097983–255	TGCAAGCCCAGCTCTCTGC	TCAAATTCCATTTGCTCGTCC	65	62	455
AW097983–358	GTGGCAATATTAAAGAGGCACCG	GGAATACATGCTGCTCCTTTCTCC	63	64	307
AW087043–267	CCCTTGCCCTTCTTCTTCTGG	TTCTCACTTGGAGGATCAGTTTGC	64	63	764
AW086539–362	GTTCCTGCAGCGCAGTCG	ACTTGCGCCAAGGAGAATGG	64	64	425
AW098322–137	CTTGGGAAGACGTGGCCC	AAGCGCTTCACCCAGGC	63	62	339
AW098322–275	CGTCATTGATGCTGAGGCG	CAAGCCGACGCCAGTTGC	63	66	398
AW097946–284	CGACGCCGACATTCATGC	AGGTCTGCATTCAGCAGATTACCC	64	64	370
AW098391–123	AGCCGAGGATTTAGGGCAGG	AGACCGGTGCCTCATCCG	64	64	287
AW098391–191	AGGTGCTTGGACTGCTCAGG	GGAATGCCAAAGGCGTCG	62	64	297
AW097948–171	GGCCCAAATCACCTACGCC	GTTCTTCAGATGACGACGAGCC	64	63	408
AW097948–251	TGGTCTTCTTTTGGCTCGTCG	CGTCCAACAGAGCCCTAAATGG	64	65	244
AW098786–108	GCACCGGTGGAAAGACCG	ACGTATCACGAGGGCCACC	65	63	349
AW098786–315	ATGGGAAGACTTGCGCGG	TTTGTGACCCATTCGCCC	64	62	324
AW098048–269	TCCCAGGTATCATTGCCCG	AATGAAAGTGGCTGCTATCCAAGC	64	64	606
AW098048–348	ACAGATGTGGCTTGGATAGCAGC	CCGATGTAAGTGTCTCTGCTGATGG	64	66	333
AW098804–195	GGCGGAGACAGGCACATACC	GAGAATTCAGGCCGCTCCG	65	65	405
AW086917–156	GAATGGCCTCTTCCGGGC	TGCAATCTGCACCGACTTGG	65	65	333
AW086712–250	TTACTGCCTTGCTGGGGTCC	AACCAGCCCGCTAAGCTGC	64	64	378
AW087002–282	TTATCGCCAGAGGACAGCG	GCCCATATTTGACAAGGCCG	62	64	402
AW086924–329	GCAGCACCTTGACGTCGC	AGCTTCAAGGCCTGAGAAATGC	64	64	600
AW098761–71	CATGGCGCAGACCAATACG	AAGTATGGGATCAAAGAGTTGTAGAGC	63	61	294
AW086619–136	CACACCTGCCTGTGGATTTGG	CAAGCTCGACAACCCGGC	65	65	602
AW087029–297	CATGGGGTTCTGAGGCCG	GGTCGGGATGAAATGCAAGC	64	64	486
AW086641–183	GGTTGCCTCCCTCAATCGC	GAAGGCTGTCGGCATCTGG	65	64	248
AW086641–266	AAGGTGTAAGCACCTCCGGC	GAAAGCCGGAATCGTCGG	63	63	501
AW086618–269	TGCCTCCTTCGCCTGATCC	AGACATCGGAAAAGAAGTCGAACG	66	64	513
AW098004–106	GGATTGGGCGAAAGAAGCG	AGAGAAGCACAACAGGGCCG	65	65	363
AW098004–215	CCATGGCTCGGAAGGAGG	CCTCCGTAAGGCTGACTGTCC	64	63	373
AW098782–68	GCTTGCTCCTGCTTTGACTGC	CTGAAGGGCTCCCAAATAGCC	64	64	422
AW098479–502	CTGGACAGCCAGTCAAGGC	CCGATGCAATGAATGCCG	62	64	281
AW086833–142	AAGGATCCGCGACAAGTACCC	TTGATGCGCTTCCTTATGACG	64	63	298
AW098949–71	GATTTCAAGACGCATCAGTTGCC	CGAAGATGTCGCCCCTGG	65	65	466
AW098949–266	TGGCGCTATTACCAGGGGC	CTGTCCAGGCATGGTGGG	65	63	406
AW098098–311	CCCCTGATCTTCTTGGCACG	GACCCCCAGAGCATCTTCG	65	63	349
AW086738–110	CATGCAGAACGAGGAGGGC	CCTGAGCGCGAACGAAGC	64	65	528
AW086979–128	TACACATTGCAGCGCATGG	CCATAACAATGAAGGCGCACG	62	65	342
AW086878–120	TCTACCCCGCCGATGACG	GAGAAGGCCAGACTCCAACTGC	65	64	654

Table 1. Continued.

EST–intron start position	5′ Primer	3′ Primer	Forward T_m[a]	Reverse T_m[b]	Prod. size[c]
AW087022–123	CGCCTTCACAGGCACAGG	CTTGGAAGCCAATTTCTCCTTGG	64	65	502
AW086647–346	AGATGATGGGGGTGGTGTAAGC	GCAACCCCATTGAGAAAGCG	64	65	395
AW098140–138	CCTAGTCACATGCCATGACCACC	CGCTCAGGGAGATACTGACAGC	65	63	412
AW097944–86	AAGGCCGTGTCCAGCTCC	TCGGGGCTAACAATGCACC	63	64	236
AW097944–242	CGTAACGGTGAGCGCAGG	TTGCCTGTGGCAGACACG	63	63	538
AW087030–122	ACTGTTGGAGGATGGTCGTCG	CCTCCATCAACAGCCGAGG	64	64	536
AW086635–160	TTGACTGCGAACTTCCTCATGG	GGCTTTCAATCGCCCAGG	64	63	380
AW098279–292	TTTCGCGACATGGATGGG	AAGTCTCTACCTCTATTGCATCAAGCC	64	63	260
AW086909–334	ACGTCCTCTGCGTCCTCCC	TCCGCTCGTGTCACTGGG	65	64	412
AW086827–136	CTCGGATCCGCGTGTTGC	CGTACGCGGACTCTGGTGG	66	65	790
AW086674–69	CTGCTCTTGCCAGTCTTGAACC	GAACACTGTGACTGCTGAGAAGTTGG	63	65	352
AW098248–320	TTGGACGCCATCTTCGGC	CTGCCTCTCCTGTGACAAAAGC	65	63	571
AW098328–149	TCAAGATGGAGGTGGGAATCG	GCCCCGTCCATGATTTCG	64	64	335
AW097937–210	TCAAATGCGATGAATGTCGC	CTGGACAGCCAGTCAAGGC	63	62	291
AW086669–414	AACGACCAGCGTAGGTGCC	GCTGAGAAGGGTGAAGATGCG	63	64	290
AW098185–232	TATTGCGACTGCCCCACG	GGGGTTATGCTACGGCACG	64	63	166
AW087017–243	CGATCCTGAAACAGGCCACC	GAACTTTGCCTCAAACTTTCCAGC	65	64	445
AW087017–331	TTGGTGGTGGTCAAGAGGC	AAAGCAAGAGCATTGATAGGTCCG	62	64	382
AW086830–254	TTTGGGTGGCCTTCTTCAGC	GGGAGACACCCAACCACTTCG	64	66	533
AW086566–259	TGGGTAGCTCCAATACCGGG	TTCCAGCGTGGGTTGAAGC	64	64	431
AW086566–452	CGATGATCATGTTGCGGAGG	TCCGGGGAGTGGATCTCG	64	64	606
AW098560–101	ATCCGCAAGTGCAAGCCC	TCATCCGTGGTGATTTTCGG	64	64	420
AW098281–150	GTACTTCGAAGACAATGCGGG	CATTCGCAGCACTAGCAATCC	61	63	291
AW098587–180	AGGAAGTTCGTGGTCGTGGG	TTGTGGCGTCCACATCGG	64	65	436
AW086962–158	TGGACTATTCCTTGGGCTTCTCG	CCCCGATTGCGCAGTATCC	65	65	287
AW086962–273	TCGAGCAAGGCAAGTCATAAGG	TCCGATGTTTCCTTCAGCGG	63	65	310
AW086962–333	TTCTCTACATGCTTGCCGC	TTTGATAGCACAGCAGGGTAATGC	60	64	272
AW098337–225	GAACCTGCACCACGACAAAGC	AAGCGTCGGCATTGCTCC	65	64	304
AW098364–121	TGGATATGGGTTGGCGGG	AGCTGCTTGATGTCGGCTCC	64	65	518
AW098799–185	TCAGGCTTTGCCTTGGTGG	AAGGTTGCTGGAGAAAGTAGCCC	64	64	354
AW098651–169	GCAATCCTGCTCTACCTTGCG	CGCTCCAACGGGTAAGGG	64	63	307
AW098361–258	AAATGCAGGCTGTGCGAGC	GCCATGACGACCTCCACG	64	63	834
AW086544–267	TTTCTTCGCAGAGCCCACG	GTGTTGCATCCTGGTCGTGC	64	65	331
AW086990–246	CCCAGACGGCATTCGAGG	TCATCCATTCGCATCTTCGC	65	64	499
AW086531–354	TTCAGCTGTCATGGCCTTCG	ACGCAAGCTTTGGCACCC	64	64	373
AW086934–65	TTTTTCAACGAAAATAGCGAGCC	CGAAGCTTTTGCAGGAACTCCC	63	66	265
AW086934–136	TCGAAAGGGACAAACGGGG	AAGGCTCTCTAATATGGGGGTCG	65	63	314
AW087013–133	CTTCGGAGCCACCATCCC	TTGTTGCCGAATGGGTCG	63	64	465
AW098823–115	AGCGAGCTTGCCTGCACC	GCTTGGCAAAGAGACCAGGC	65	64	393
AW098823–199	GCAACTTTGGTGAAGGCCG	CGGTCGCGGCAGTAGTAGC	63	64	308
AW098056–70	AAGAATGCAGTGTTTGGTGACAGC	CCACATCCGCCTTGAGAGC	64	64	542
AW097956–276	CCGCGACCAATGAGACCC	TGATCTGGATGCACGGGG	65	64	710
AW098620–189	GATGAACAACGCTCGCGG	TGGTGTCGGAGATGTGGGG	63	65	326
AW097987–87	GTCTTAACCTTGGATGGCCGC	GAGTGCTTTGAGTCATCGCTTCC	65	64	268
AW097987–167	TGTGGAGCCAACAGAGATTGAGG	TGCATGCCTGCAAATCAGC	65	64	328
AW097987–313	CATGCAGCCGTTAAGGAAAGG	TCAGCAATCCATTTTCAACCG	64	63	279
AW087075–240	TTGGTGATGCATACTCAAAGGTGG	GGTCATCGAGCTCTCCTGGC	65	64	452
AW087075–332	TCAACTGGCGATCAGAAGCC	CTGGCTGCACTAACACTGCC	63	61	232
AW098367–359	CAATTATCTCCAACGGCGGC	AGTGGTAATCAGCCGTCATCTCG	64	64	421
AW086525–450	ACTCCTGGTTGGTGGCCG	CTTCTTGAGGAAGTTGCAGAAGG	64	61	674
AW086525–514	TGATGGCAAGAAGGTGTCCG	ACCGCATGAAGTTGTGGGC	64	64	318
AW098078–239	CCTCAACACCAGGCTCAATGG	TGATCAAGGGCGCCAAGG	65	65	482
AW086765–100	TGACCCATGTGTTGATGATGAGG	ATTTCAATGCCAGATTCCAGC	65	61	484
AF309562–421	TCCCCTTCGAGGAAAACCC	CTGGCGACCAAAGCTCCG	63	65	425
AF309562–538	TGCTGGCGTTACAGACGACC	GAGCAGCAGCCCTCTGAGC	64	63	323
AW087021–183	GGTTGAAGCTGTGAAGTTGTTCG	GAGGACCTCTCTGGATGGGG	63	63	310
AW087021–312	AGCTTCTGCCATCCTCACTGC	AGTTGCGACGACGAGACGG	64	64	315
AW098247–82	ACGCGTCCATCTGCCAGG	AAGGTCGCCTCCACCTCG	65	63	461
AW086944–155	CTGCCATCCTTCCCGTCC	AAGCAGTTGGTGTTGGCGG	63	64	353
AW098672–254	TTCCTTGGGGGCCTCAGC	ACTGGCCTGGTCTCTTTGCC	65	63	313
AW087053–85	CGTCGTGAGCGTGAGGAGG	AGGTATCCAAGCTTCTCATTGTAGTCC	65	63	474
AW098317–317	TGATATGGGGTCTTCCAGGTCC	CGTTTTAATAAGAGGTCGACAGTGGC	64	65	498
AW086622–280	GCGAAGAGTGGGTAGCTCCG	AAGCCCGAGCCTGTGAGG	64	63	346
AW086841–85	TTCGGAAGCACAAAACTGACG	TCTCATCGCCGTTTAGCCG	63	64	279
AW098024–169	GGAAAGCTGTGACTGCACTTACCC	AATCTGGGCCTGGCCTTACC	65	64	578
AW086649–182	TGCATGAATCACAATGAAGCCC	ACGCATGCGCCATCTGC	65	65	463
AW098284–284	GCAGGACTGAGGAGTCGTCG	TTTTCCTAGTCCCGCACGC	63	63	309
AW098191–439	GACCCGCCACATGAATGC	CTTCCAGCTTGTGGACGGC	63	64	572
AW098191–543	TTGGAAAGGTTGTCAGCTTCTGC	TTCGAAGCATTTGGACCAGG	64	63	478

Table 1. Continued.

EST–intron start position	5′ Primer	3′ Primer	Forward T_m[a]	Reverse T_m[b]	Prod. size[c]
AW086824–186	GAACGAGGGCAAAACAACACG	TTGTTCGTATCATGAGTCCTTATTTGC	64	63	282
AW086824–253	GTGTACGTCATGGTGTCGATTGG	TGCTGGGTGGAGATGGTCC	64	64	456
AW086779–195	TGGTGCCAGTTTGAGGAAGC	AGCACCTCCGGCTTTGACC	63	64	431
AW098026–111	TGGCAGTGGAAAGCTGCG	CTCATTGGGCATGTGGATGG	64	64	395
AW086929–81	ATGCACACTGCATCCCTTTGC	AAATATGAAAGGAGGGGTCGC	65	61	407
AW086753–293	GGCAGCCCAATTTCATGAGG	ATTGCTTGGAGCCTCTCAATGG	64	64	458
AW086737–66	AACCTACTGGTCGACAAGAACTGG	CGAAGCACTTCCGGTGCC	62	65	464
AW098283–167	TGATGGATGCGCTTGTGG	TCTGCAAGAGAGCCTACCTTGACC	62	65	426
AW098019–120	ATCGGGATTAGGACCAAGGC	GATTCTGCCAGCGCATCC	62	62	271
AW098019–199	TGGAAAGGATGCGCTGGC	ATTGCGCATCATCCATACCG	65	63	265
AW098019–297	CTTGCGTGAGGACTATTTGGC	TTCTGCCCCTCAGAACCAGC	62	65	325
AW098233–116	TTATGCGCAGGAGCTTGGC	CCTCCTCCCGCTACCAACG	64	65	453
AW098233–233	GCAAATCCTGATGGCCGC	CCCGCATATTTGCCAATCC	65	63	281
AW086758–136	AAGGCTTTTGCACTGCACTCG	TGCTGCTCGCACTGGAGG	64	65	299
AW087065–177	CGGGAGCACTTAACGACGC	TCACACCTTCTGCTGTCTGG	64	60	575
AW098770–113	CCGTGAAGGACTGGGACAGG	TTGCACGCCTTGTATCCTCG	65	64	282
AW098258–180	TTTGCACCCATTGCCAACC	GCCGGGTTGTAAGCGAAGC	65	65	390
AW098797–116	AGGATCGGACTCCCTGCC	TTCCCAACTTGTCAACTGCCC	62	64	490
AW098643–123	GGAGGCTTTTGAGGCGAGG	CAATCAGCTGGCAATGAGCG	64	65	508
AW086790–163	TGGCAGAGTTTGATCGAGGC	TGAAGGTGCTCTGGAGCGG	63	65	591
AW098780–75	AGGCGTCAGTCTACGGAATTGG	TGCTCGCAAATCTTGCCC	64	63	379
AW098780–211	CCCACATCCCGATCCACG	ACAATTTTCGCTTCAACAAGATCTCC	65	64	324
AW086556–413	AAGGCAGACTCTCGGGCG	GCGTTCAGAAGGCCAATGC	64	64	458
AW098831–338	TTCAGTGGACGCGCTACCC	TCCACAGTTGAGTTCCGGTGC	64	65	456
AW086710–390	TTTCTCCGGGCTTCCATCC	AGGGCATTGCTCTCAGGGG	64	64	531
AW086768–261	CCTCCTCCCCATACGCTCC	CACAACAACAGCACAAAGCTGC	64	64	427
AW087072–205	CCGCGACCAATGAGACCC	TTGCGAGAAGTTGACCGTGG	65	64	387
AW087072–269	GCAGAAGGTCAAGAAGGCCC	CCGTTGAGAGTGAGGTCACGG	63	65	373
AW086870–57	AACCCCGCCTGCTTCACC	GGTGCATTTGGCTGTGCC	65	63	318
AW098197–197	ACGTGGATTTCAGGCGGC	CGTGCTTCTTCTTCAGTAGCAGGG	64	65	489
AW098074–139	CAGGGGAAAGGCACCTGG	CGGTTCCAGTGAGGATGCG	63	65	520
AW098521–456	ACTTCTCAGACGAGTTGGGGC	CGTGCCGAAAAGGTGCG	63	64	288
AW098301–160	GCTGTGCAGGCGTTGTGG	AGAAAGATGACGCAGATGGCG	65	64	599
AW087027–88	TGGGACTTCTACAAGCAAAAGTTGG	CAGCGCTTGATGCTTCG	64	60	268
AW087027–159	AGGTGCCAGAGTACAAGGATGACC	CTTCTGTTCCTGCACCCTCG	64	63	374
AW087027–246	GCTAAGGCCTCAGAGCAAGAGG	CTTCACTTGTGGGTGCTTTGC	63	63	291
AW086848–172	CAACCCTTTCATGCACGCC	CGCAACTCAGCCCTCACG	64	64	513
AW098187–139	AGTACAATCAGGCTGCCACGC	TTGAGTTGAACTTCATTTACCTGCC	64	62	306
AW086969–51	TCGCATTTATGGCAGAGCAGG	AGATTCTTCAGACGAACAGCCG	65	63	280
AW086692–252	CCGAATCATCAGATGCCAGG	GTCTCGCAGCCGAGTTGG	63	63	420
BI894286–170	TGGACGAGCTGAGCGAGG	TTACGCCATGTCCTTCGCC	63	64	494
AW086973–252	GGATGATTCCGCCAAACAGG	TCTACGACGGCATCAGGGC	64	64	309
AW098153–431	CGAAGACGGCCTTGCACC	CAAATTGGAGCAGCTGTGGC	65	64	448
AW098812–178	ACTGGCCCAGCCTTTCCG	AGAAGTAGCCCCACTGCATCG	65	63	430
AW098812–326	TGAGATTGGGTTGTTCGATGG	CCGCCGTCCACAATCTCG	63	66	457
AW086545–249	TTCCTTGGGGGCCTCAGC	GCCGGTGTGCAAATTGAGG	65	64	330
AW086575–297	CATCTAGGTATTGTCGAGTCCCG	TGAAAGTGGCAAGATGACCAAGG	62	65	459
AW086575–420	AGCAACTGCATCAATAAATTCCTCG	TGGACCCGTGGTCTTAGCC	64	63	317
AW086781–77	CTCGCGTTGCTGGTGTCG	TTCTGCGCATCTTCTTTTTGC	65	62	421
AW087048–137	GAAGGCTGCAATTCAGGAAACG	TGAAAGAGGTTTCTTTGGTTTGTTGG	65	65	257
AW087048–215	GAGAAGAAGAAGGCCAGGGACC	GTGGGCATTCGTTTCCGC	64	64	515
AW098758–58	TATTTGCTCCAGGATGCTGATGG	GGCTTCATCGTCAGTCACGC	65	64	319
AW086711–314	TGAATCCGGCTGTCAAATCG	GCGAGCTGCTGGTTCTGAGG	64	65	300
AW086711–405	TAGATGAGGTCACTCAAACGCTGC	AAATCGGTATGGATGATGCG	64	60	438
AW086711–484	TTGCGCATCATCCATACCG	AGTTGCAAAACTCTTGCGTGAGG	63	64	305
AW098776–59	GTCAGGCTCTGGCTGACG	GCCAGCCTTTGCTTGATGTCC	60	66	314
AW098776–152	GCCAAGACGAACATGAATGTGG	GCAGGAACCTGATGCTGGC	64	64	359
AW098585–138	CCTCGCCTCATGTCCTGC	AGCACGTGCGTAGTTCCCG	63	64	335
AW098746–319	GGTTTCATCTCTGGCGCTTCG	CCCATGAGGTCAAAGATGAGGG	66	65	249
AW098746–414	CCACCATGGTCCACTTCATAGC	CTGAAGCGATCCCCCACC	63	63	421
AW098409–116	CAGAGATGGTCGTGGGTTGG	CCCAACATCATCGTCTGAGGG	63	64	447
Primer <25 nucleotides from intron					
AW086551–330	TTCTGTTTCCAACAGGCCG	GTGCGCAAATTCACAGAGCG	62	65	307
AW086551–425	TCTCGCTCTGTGAATTTGCG	CGATGTCAGAAGGCAGGTGG	62	64	231
AW086636–421	TCAGCTGACTTCGCGTTTGC	GTTGGCTCAAGGAAAGGAGC	65	61	314
AW086700–323	AGTCAAGCGGGCCCTTCC	ATGTGAAGTGCCAGGGCTGC	64	65	301
AW086752–361	AACCAGAGCCCCAACCCG	ACAATCAGCGTGACCTCAAACG	65	64	395
AW086783–208	CGTCTCATCATGCGCAACG	CTTCGCGCTCAGACTCAAGG	65	63	665
AW086798–387	GCATGGAACTCCCGGAACC	CATCAAACTCCCACAACTCATTTCC	65	64	393

Table 1. Continued.

EST–intron start position	5′ Primer	3′ Primer	Forward T_m[a]	Reverse T_m[b]	Prod. size[c]
AW086855–57	GTGATCATGGCGGGGAGG	TCCTCCGTCCTACAGTCGCC	64	64	263
AW086855–188	CCTGTTGTCGCAGCCAGC	CGTCGTCAACGATTGTAGGG	64	61	321
AW086856–213	TCACGGATCTTTGGCCCG	TTGGGGCCCCTCTTCTTACC	65	64	276
AW087036–57	AGAAGGAGTCTACTCTGCATCTGG	CCTCAAGCTGCTTTCCAGCG	60	65	425
AW087051–351	TTCACAACAGGCAGACCCG	GACAGTGGCTCTCTGGAAGGC	63	63	328
AW087074–125	CAGGCTGGTCCGCTTTGG	CCGTTAACCTGAATATCCTTCACACC	65	64	395
AW087074–171	TGTGAAGGATATTCAGGTTAACGGC	TGATGGTTGAGCTCCGACG	64	63	237
AW097915–117	GGGAGCAACCTGACCCTTCC	CGCTCGAGATCGTAGCCTTGC	65	66	252
AW097968–35	AGTGCGGTGCCAACTGCG	CCGCACTTGCCGTTCTCC	67	65	418
AW097979–42	GATGGCTCCAGCCCTTCG	AGCTCTCCCCTGATGTTTTCAGC	64	65	318
AW097979–92	AACTGGCTGAAAACATCAGGGG	AGTGACGCTGGCAACTCCC	64	63	361
AW098011–47	TGTTGCAGCTTGTGTACTTTCTGTACC	ATGGGTGCGCCTGAAATCG	64	66	303
AW098051–84	GATTCCGTCACTGTCGGTGG	GTGCCGAAACTGGTGCCC	63	65	519
AW098200–145	CAATGACACCAGGCTCCAACC	TGCACCTTGTGGGGCAGC	65	66	275
AW098203–61	CAAGTTCTTGCAGGAAACATTTGG	CCATGCTTGCTGACCGGG	64	66	464
AW098216–110	AGGGTGTCAATACGTCCAAGG	TGGCACCGAGAGACGAAGG	61	64	460
AW098262–128	ACTACGTTGCCCACGACGC	TTCGTAAACATCCTTGCCAGC	64	62	233
AW098262–171	GGATGCAACTGGTGCTGGC	GATCAGATCCGAAGTCGAAACTCC	65	64	224
AW098263–244	TAGCATGGAGCACTGATCGGG	CAATCCAACGTCCAAAGTAAACTGC	65	64	281
AW098272–223	CATTTGTGTGTTCCTTCTGCCG	TGAGTCTCTTGAAGTCTCTTCATTTCC	64	62	190
AW098291–131	TGTAGGCCGAATAGCACTTGG	TGGACCAGTAGTCATGTTGAATGC	62	63	245
AW098300–108	TGCAACGGTGTCGTTGTGC	ACCCGTGAATCTTGATGAGGTCC	65	65	453
AW098306–52	GTTGCGCAGGGTTGAGCC	TGGCCTCTCTGTTGCCAGC	65	65	450
AW098419–60	CGCCAGATTCAGGAGCAACC	CTGGGGTACATGAACACCCTTACG	65	65	223
AW098448–78	ATGTCAAGTGCCAAGGATGC	CACAGCACCGTGGAGCAGC	61	66	276
AW098461–262	CACACTCCTTTCCAATGGGGC	ACAATCGGGAGTCATTGTGACC	66	63	269
AW098461–303	GGGGTCACAATGACTCCCG	CAAGCCCTGAGGCGCAAAGC	63	69	330
AW098472–354	GTAGGATACGGTTTGAGGGCTGC	GCTCTTGAAGAAGAAGTTCGGG	65	61	493
AW098535–258	TCGAACAAGCTGAAGCCC	GAAGTTCGCGTCTGTGCCC	60	64	431
AW098580–204	CAAGACCCCACCATCTACAGGC	CCCTTCACCTTCTCCACAGAGC	65	64	315
AW098597–63	GGAGCTGGTGACAGTGTGAAGG	AGCAGCCATCAGACCCCC	64	63	273
AW098717–46	TCACGGCTCAAACTCTGATTAAATAGC	AAATCCAAGGCCAGAACCCC	64	64	334

[a] Melting temperature of the forward primer.

[b] Melting temperature of the reverse primer.

[c] Prod. size indicates the predicted PCR product size on the *Physcomitrella patens* genome sequence.

instructions. PCR was accomplished using GoTaq Green Master Mix (Promega Corporation, Madison, Wisconsin, USA) in 16-μL reactions. The cycling conditions were 94°C for 120 s, then 10 cycles of 94°C for 15 s, an annealing temperature of 65°C that decreased one degree each cycle, and 72°C for 60 s, followed by 20 cycles of 94°C for 15 s, 56°C for 30 s, and 72°C for 60 s. The PCR products were cleaned using the QIAquick PCR Purification Kit (QIAGEN Sciences, Germantown, Maryland, USA). Sequencing used BigDye Terminator version 3.1 chemistry and was accomplished on an ABI 3100 capillary sequencer (Applied Biosystems, Carlsbad, California, USA). Forward and reverse sequence fragments were edited and assembled using Sequencher 4.0 (Gene Codes Corporation, Ann Arbor, Michigan, USA), and all polymorphisms were checked from the chromatograms.

We generated high-quality sequence data for 218 of the 245 loci. We used the software DnaSP (Librado and Rozas, 2009) to estimate the distribution of the per-site genome-wide nucleotide variation (θ, an estimate of $N_e\mu$ [where N_e is the effective population size and μ is the per-site nucleotide mutation rate]) in *C. purpureus* (mean: 0.014, median: 0.008, range: 0.0–0.14; Fig. 1, Table 2). Although these data were generated from a modest sample, this stands as the most complete estimate of this fundamental parameter in any bryophyte, and forms a benchmark for further comparisons. It is possible that this estimate of θ is biased upward, by cryptic population structure in our sample, or downward by our small sample size. However, many loci showed no variation among intercontinentally disjunct samples, consistent with previous work (McDaniel and Shaw, 2005), suggesting that the loci that are more diverged reflect locus-specific rather than genome-wide evolutionary processes. For example, loci at the low end of the distribution may be linked to loci that have experienced a selective sweep (McDaniel and Shaw, 2005), while loci on the high end of the distribution may be linked to the sex chromosomes or loci linked to local adaptation (McDaniel et al., 2007, 2008). This degree of variation illustrates the among-locus heterogeneity in evolutionary history within this species. While sampling more individuals would quantitatively improve this estimate, the concordance between this and previous estimates suggests that the median value is unlikely to be qualitatively improved without a much larger sample.

We have identified more than 50 loci with $\theta = 0.02$, a value more than twice the species median. This value is also equivalent to the most variable nuclear loci used for phylogeographic inference in any bryophyte species to date. Using the PCR and sequencing strategy outlined above, we chose 12 loci

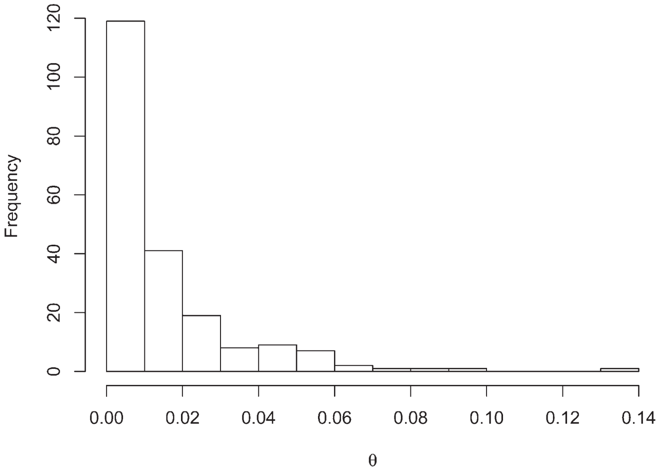

Fig. 1. A frequency histogram of θ (an estimate of $4N_e\mu$) from the 218 loci in *Ceratodon purpureus.*

TABLE 2. Nucleotide diversity at sequenced loci in four geographically widespread accessions of *Ceratodon purpureus*.[a]

EST accession	Gene name	Length[b]	Theta[c]	GenBank accession no.
AF233229	auxin binding protein 1-like protein (abp1)	1138	0.000843	JY262836, JY262996, JY263192, JY262676
AF309562	hemoglobin mRNA	820	0.007737	JY262947, JY263101, JY263271, JY262786
AJ250735	delta 6-fatty acid desaturase	1264	0.043478	JY262851, JY263008, JY263206, JY262691
AW086517	similar to SW:IM30_PEA Q03943 CHLOROPLAST MEMBRANE-ASSOCIATED 30 KD PROTEIN PRECURSOR	1164	0	JY262832, JY262991, JY263187, JY262671
AW086519	similar to gb:gb|U77939.1|PVU77939 Phaseolus vulgaris ubiquitin-like (PLANT)	357	0.019055	JY262883, JY263033, JY263228, JY262722
AW086525	similar to TR:O76968 O76968 RIBOSOMAL PROTEIN L18A	1171	0.042882	JY262920, JY263071, JY263259, JY262758
AW086531	similar to SW:SYY_BACST P00952 TYROSYL-TRNA SYNTHETASE	436	0.00489	JY262928, JY263079, JY262765
AW086539	**Moss EST library CPU Ceratodon purpureus cDNA clone PEP_SOURCE_ID:CPU010318 3-, mRNA sequence**	**364**	**0.057971**	JY263102, JY263272, JY262787
AW086546	similar to SW:RL13_ARATH P41127 60S RIBOSOMAL PROTEIN L13	628	0.015856	JY262894, JY263042, JY263239, JY262731
AW086548	similar to SW:RK24_TOBAC Q02764 50S RIBOSOMAL PROTEIN L24, CHLOROPLAST PRECURSOR	468	0.009183	JY262834, JY262993, JY263189, JY262673
AW086549	similar to SW:SYRP_LACBI P87068 SYMBIOSIS-RELATED PROTEIN	286*	0.008734	JY262855, JY262696
AW086551	similar to TR:O48891 O48891 ATP-DEPENDENT CLP PROTEASE PROTEOLYTIC SUBUNIT.	787	0.001285	JY263156, JY263316
AW086555	similar to TR:O04619 O04619 SIMILAR TO MITOCHONDRIAL CARRIER FAMILY	509	0.006367	JY262870, JY263022, JY263219, JY262710
AW086556	similar to TR:O65731 O65731 40S RIBOSOMAL PROTEIN S5	392	0.020997	JY262973, JY263135, JY262814
AW086566	similar to TR:O65059 O65059 PROBABLE 40S RIBOSOMAL PROTEIN S15	762	0.035361	JY262939, JY263090
AW086575	similar to TR:P93133 P93133 NADP-ISOCITRATE DEHYDROGENASE	830	0.003851	JY263138, JY263302
AW086579	similar to SW:LDLC_HUMAN Q14746 LDLC PROTEIN	595	0.001778	JY262854, JY263010, JY262695
AW086590	similar to SW:RL2B_FRIAG O22644 60S RIBOSOMAL PROTEIN L23A	483	0	JY262886, JY263231, JY262725
AW086594	similar to TR:O80626 O80626 PUTATIVE RIBOSOMAL PROTEIN L35	594	0	JY262830, JY262989, JY263185, JY262669
AW086594	similar to TR:O80626 O80626 PUTATIVE RIBOSOMAL PROTEIN L35	429	0	JY262950, JY263105, JY262790
AW086618	similar to SW:SYV_BACSU Q05873 VALYL-TRNA SYNTHETASE	604	0.002098	JY262937, JY263088, JY263268, JY262774
AW086619	similar to TR:O82413 O82413 HISTIDYL-TRNA SYNTHETASE	524	0.046074	JY262906, JY263056, JY263251, JY262745
AW086636	Moss EST library CPU Ceratodon purpureus cDNA clone PEP_SOURCE_ID:CPU010708 3-, mRNA sequence	283	0.021739	JY263160, JY263320
AW086641	2similar to TR:Q9ZRS8 Q9ZRS8 RIBOSOMAL PROTEIN L37A	981	0.012953	JY262923, JY263074, JY263262, JY262761
AW086647	similar to SW:SPEE_COFAR O82147 SPERMIDINE SYNTHASE	327	0.008439	JY262916, JY263067, JY262754
AW086659	similar to SW:RS13_ARATH P49203 40S RIBOSOMAL PROTEIN S13	502	0.011655	JY262882, JY263032, JY263227, JY262721
AW086668	similar to SW:RS3A_BRARA P49396 40S RIBOSOMAL PROTEIN S3A	277	0.013468	JY262995, JY263191, JY262675
AW086669	similar to SW:COXG_YEAST Q01519 CYTOCHROME C OXIDASE POLYPEPTIDE VIB	535	0	JY263106, JY262791
AW086674	similar to SW:RL27_PYRST Q02984 60S RIBOSOMAL PROTEIN L27	439	0.018667	JY262926, JY263077, JY263263
AW086675	similar to TR:O48691 O48691 F3I6.17 PROTEIN	286	0.003115	JY262867, JY263217, JY262707
AW086686	similar to TR:P93359 P93359 PUTATIVE PRE-PRO-CYSTEINE PROTEINASE	306	0.007755	JY262889, JY263037, JY263234, JY262727
AW086692	similar to TR:O16619 O16619 F36H9.3 PROTEIN	356	0.002924	JY263146, JY263308
AW086694	similar to TR:O65088 O65088 TAT-BINDING PROTEIN HOMOLOG	339	0.004295	JY262864, JY263017, JY263215, JY262703
AW086700	similar to TR:Q9ZNS3 Q9ZNS3 RIBOSOMAL PROTEIN S27	322	0.090909	JY263164, JY263324
AW086710	similar to SW:BAS1_HORVU Q96468 2-CYS PEROXIREDOXIN BAS1 PRECURSOR	388	0.011459	JY262982, JY263149, JY263311, JY262824
AW086736	similar to TR:O81925 O81925 40S RIBOSOMAL PROTEIN S6	608	0	JY262868, JY263020, JY262708
AW086737	similar to TR:O23719 O23719 MAP3K DELTA-1 PROTEIN KINASE	421	0.004364	JY262971, JY263133, JY263298, JY262812
AW086738	similar to SW:RS21_ORYSA P35687 40S RIBOSOMAL PROTEIN S21	806	0.055219	JY263096, JY263270, JY262781

TABLE 2. Continued.

EST accession	Gene name	Length[b]	Theta[c]	GenBank accession no.
AW086752	similar to TR:Q9ZU52 Q9ZU52 PUTATIVE ALDOLASE	285*	0	JY263168, JY263328
AW086753	similar to TR:O04202 O04202 26S PROTEASOME REGULATORY SUBUNIT S12 ISOLOG	319	0.009603	JY262967, JY263128, JY263295, JY262809
AW086758	similar to SW:SYK_CRILO P37879 LYSYL-TRNA SYNTHETASE	316	0.004399	JY262968, JY263129, JY263296, JY262810
AW086759	similar to SW:YGLA_SYNP2 P28606 HYPOTHETICAL 34.1 KD PROTEIN IN GLNA 3-REGION	729	0.017016	JY262856, JY263011, JY263210, JY262697
AW086765	similar to TR:O82229 O82229 PUTATIVE SERINE CARBOXYPEPTIDASE	420	0.025048	JY262941, JY263092, JY262777
AW086768	similar to TR:O04820 O04820 HYPOTHETICAL 9.1 KD PROTEIN		–	JY262985
AW086770	similar to TR:Q55649 Q55649 ABC TRANSPORTER	619	0.005089	JY262876, JY263028, JY262715
AW086781	similar to TR:Q9ZU75 Q9ZU75 UBIQUITIN-CONJUGATING ENZYME E2	751	0.015385	JY263151, JY263312
AW086783	similar to SW:RS28_MAIZE P46302 40S RIBOSOMAL PROTEIN S28	663	0.017572	JY263172, JY263331
AW086786	similar to TR:O65583 O65583 PUTATIVE URACIL PHOSPHORIBOSYL TRANSFERASE	271	0.005742	JY262874, JY263026, JY263222, JY262713
AW086789	similar to SW:RS24_HUMAN P16632 40S RIBOSOMAL PROTEIN S24	552	0.020856	JY262895, JY263043, JY263240, JY262732
AW086790	similar to SW:RS3A_CATRO P33444 40S RIBOSOMAL PROTEIN S3A	506	0.026438	JY262960, JY263119, JY263286, JY262802
AW086798	similar to TR:Q55953 Q55953 HYPOTHETICAL 18.6 KD PROTEIN	595	0.003401	JY263176, JY263335
AW086824	Moss EST library CPU Ceratodon purpureus cDNA clone PEP_SOURCE_ID:CPU020404 5-, mRNA sequence	230	0	JY262983, JY263152, JY263313, JY262825
AW086827	similar to SW:RL31_PICMA O65071 60S RIBOSOMAL PROTEIN L31	704	0.005418	JY262917, JY263068, JY263257, JY262755
AW086830	similar to SW:RL4_ARATH P49691 60S RIBOSOMAL PROTEIN L4	580	0.003736	JY262927, JY263078, JY263264, JY262764
AW086833	similar to gb:gb|AF016284.1|AF016284 Arabidopsis thaliana (PLANT)	323	0.002403	JY262915, JY263066, JY263256, JY262753
AW086837	similar to SW:SYL_BACSU P36430 LEUCYL-TRNA SYNTHETASE	540	0.001699	JY262853, JY263009, JY263209, JY262694
AW086841	similar to TR:O04002 O04002 CDSP32 PROTEIN	264	0.004587	JY262953, JY262795
AW086845	similar to SW:RS11_SOYBN P17093 40S RIBOSOMAL PROTEIN S11	700	0.062613	JY262871, JY263023, JY263220, JY262711
AW086855	similar to TR:Q9ZRT5 Q9ZRT5 GLUTATHIONE TRANSFERASE ATGST 11	564	0.002141	JY263157, JY263317
AW086858	**similar to gb:emb|Z23165.1|ATRBPS18A A.thaliana ribosomal protein gene (PLANT)**	**719**	**0.012759**	JY262881, JY263031, JY263226, JY262720
AW086868	similar to SW:RS8_ORYSA P49199 40S RIBOSOMAL PROTEIN S7	417	0.019656	JY262878, JY263029, JY263224, JY262717
AW086870	similar to TR:Q43548 Q43548 GOLDEN DELICIOUS APPLE FRUIT EXPRESSED	304	0.007576	JY262964, JY263125, JY263292, JY262806
AW086877	similar to SW:CG1C_ORYSA P93411 G1/S-SPECIFIC CYCLIN C-TYPE	397	0	JY262838, JY262998, JY263194, JY262678
AW086877	similar to SW:CG1C_ORYSA P93411 G1/S-SPECIFIC CYCLIN C-TYPE	336	0.003704	JY262847, JY263005, JY262687
AW086878	similar to SW:RL6_MESCR P34091 60S RIBOSOMAL PROTEIN L6	527	0.010703	JY263048, JY263245, JY262737
AW086917	similar to SW:ARF_ORYSA P51823 ADP-RIBOSYLATION FACTOR	189*	0.011364	JY262931, JY263081, JY263266, JY262767
AW086924	Moss EST library CPU Ceratodon purpureus cDNA clone PEP_SOURCE_ID:CPU030116 3-, mRNA sequence	425	0.031648	JY262948, JY263103, JY263273, JY262788
AW086929	similar to WP:F10G7.1 CE02624	416	0.045455	JY262962, JY263123, JY263290, JY262804
AW086934	similar to SW:YKQ0_YEAST P36053 HYPOTHETICAL 16.2 KD PROTEIN IN PIR3-APE2 INTERGENIC REGION	220	0.00813	JY263084, JY262770
AW086944	similar to TR:O48773 O48773 HYPOTHETICAL 47.8 KD PROTEIN	342	0.005782	JY262965, JY263126, JY263293, JY262807
AW086962	similar to TR:O81846 O81846 PHOSPHATIDYLINOSITOL SYNTHASE	699	0.011111	JY262908, JY263059, JY262747
AW086969	similar to TR:Q43275 Q43275 PLASMA MEMBRANE H+-ATPASE	185	0	JY263137, JY263301
AW086973	similar to TR:O22972 O22972 HSP90 ISOLOG	142*	0	JY263154, JY263315
AW086975	similar to WP:F17C11.8 CE05655	708	0	JY262840, JY263000, JY263196, JY262680
AW086979	similar to TR:Q9ZQN8 Q9ZQN8 PUTATIVE GLUCOSYLTRANSFERASE	316	0.012397	JY262949, JY263104, JY263274, JY262789
AW086989	**similar to TR:Q14692 Q14692 KIAA0187 PROTEIN**	**570**	**0.007792**	JY262885, JY263035, JY263230, JY262724

Table 2. Continued.

EST accession	Gene name	Length^b	Theta^c	GenBank accession no.
AW086990	Moss EST library CPU Ceratodon purpureus cDNA clone PEP_SOURCE_ID:CPU030623 5-, mRNA sequence	572	0.017001	JY262918, JY263069, JY262756
AW086999	similar to SW:SMD2_HUMAN P43330 SMALL NUCLEAR RIBONUCLEOPROTEIN SM D2	352	0.012636	JY262890, JY263038, JY263235, JY262728
AW087002	similar to gb:gb|AF068690.1|AF068690 Citrullus lanatus peroxisomal (PLANT)	458	0	JY263094, JY262779
AW087005	**similar to TR:O65606 O65606 HYPOTHETICAL 23.9 KD PROTEIN**	**253**	**0.035573**	JY262835, JY262994, JY263190, JY262674
AW087013	similar to gb:dbj|D00571.1|PYPLHABBP Pyrus pyrifolia mRNA for light harvesting (PLANT)	192	0.047619	JY262946, JY263100, JY262785
AW087017	similar to TR:O82341 O82341 PUTATIVE TGF-BETA RECEPTOR INTERACTING PROTEIN	306	–	JY263058
AW087018	similar to SW:PPCE_HUMAN P48147 PROLYL ENDOPEPTIDASE	606	0.052876	JY262837, JY262997, JY263193, JY262677
AW087021	Moss EST library CPU Ceratodon purpureus cDNA clone PEP_SOURCE_ID:CPU031003 5-, mRNA sequence	578	0.016376	JY262952, JY263109, JY263276, JY262794
AW087023	Moss EST library CPU Ceratodon purpureus cDNA clone PEP_SOURCE_ID:CPU031009 5-, mRNA sequence	760	0.004662	JY262850, JY263007, JY263205, JY262690
AW087027	Moss EST library CPU Ceratodon purpureus cDNA clone PEP_SOURCE_ID:CPU031019 5-, mRNA sequence	817	0.006417	JY262956, JY263112, JY263279, JY262798
AW087029	similar to SW:RS8_ORYSA P49199 40S RIBOSOMAL PROTEIN S8	482	0.005482	JY262914, JY263065, JY263255, JY262752
AW087030	similar to WP:C41C4.4 CE01519 PUTATIVE SERINE/THREONINE-PROTEIN KINASE C41C4.4 IN CHROMOSOME II PRECURSOR	498	0.010417	JY262943, JY263097, JY262782
AW087034	similar to SW:YML4_ARATH O22815 HYPOTHETICAL MLO-LIKE PROTEIN	226	–	JY263139
AW087043	similar to SW:RS8_ORYSA P49199 40S RIBOSOMAL PROTEIN S8	349	0.086154	JY263093, JY262778
AW087048	similar to TR:O80383 O80383 98B	511	0.007026	JY263114, JY263281
AW087051	similar to TR:O80644 O80644 F12L6.23 PROTEIN	74*	0	JY263165, JY263325
AW087053	similar to SW:IFE1_WHEAT P29557 EUKARYOTIC TRANSLATION INITIATION FACTOR 4E	227	0.017408	JY262975, JY263140, JY263303, JY262816
AW087065	similar to SW:SYS_HELAN O81983 SERYL-TRNA SYNTHETASE	524	0.004272	JY262972, JY263134, JY263299, JY262813
AW087074	similar to TR:O65686 O65686 PUTATIVE RIBOSOMAL PROTEIN S16	700	0	JY263169, JY263329
AW087075	Moss EST library CPU Ceratodon purpureus cDNA clone PEP_SOURCE_ID:CPU031418 5-, mRNA sequence	646	0.014107	JY262905, JY263054, JY263250, JY262743
AW097915	**Moss EST library CPU Ceratodon purpureus cDNA clone PEP_SOURCE_ID:CPU011109 5-**	**379**	**0.028902**	JY263177, JY263336
AW097924	**Moss EST library CPU Ceratodon purpureus cDNA clone PEP_SOURCE_ID:CPU011114 5-, mRNA sequence**	**631**	**0.009688**	JY262875, JY263027, JY263223, JY262714
AW097946	similar to TR:O81362 O81362 SULFITE REDUCTASE	176*	0.004545	JY262912, JY263063, JY263254, JY262751
AW097948	similar to SW:RL5_ORYSA P49625 60S RIBOSOMAL PROTEIN L5	641	0.009762	JY262936, JY263087, JY263267, JY262773
AW097953	similar to SW:R13A_PICMA O65055 60S RIBOSOMAL PROTEIN L13A	183*	0	JY262877, JY262716
AW097956	similar to SW:RL1X_ARATH P51418 60S RIBOSOMAL PROTEIN L18A	901	0.004234	JY262919, JY263070, JY263258, JY262757
AW097975	similar to TR:O65068 O65068 60S RIBOSOMAL PROTEIN L17	700	0.008681	JY262865, JY262704
AW097976	similar to TR:O24447 O24447 CARBAMOYL PHOSPHATE SYNTHETASE SMALL SUBUNIT	512	0.007593	JY262860, JY263015, JY263213
AW097979	similar to TR:O82808 O82808 F17H15.2 PROTEIN	609	0	JY263158, JY263318
AW097983	similar to SW:GYP7_YARLI P09379 PROBABLE GYP7 PROTEIN	733	0.004071	JY262930, JY263080, JY263265, JY262766
AW097984	similar to TR:O65059 O65059 PROBABLE 40S RIBOSOMAL PROTEIN S15	554	0.002037	JY262873, JY263025, JY263221, JY262712
AW097987	similar to TR:Q96337 Q96337 AMP-BINDING PROTEIN	1031	0.034648	JY262934, JY263085, JY262771
AW097994	similar to TR:O65462 O65462 RECEPTOR LIKE PROTEIN	359	0	JY262880, JY262719
AW098004	similar to TR:O66573 O66573 ALDEHYDE DEHYDROGENASE	673	0.008141	JY262942, JY263095, JY263269, JY262780
AW098011	similar to TR:Q14997 Q14997 KIAA0077 PROTEIN	491	0.010417	JY263161, JY263321
AW098019	similar to SW:VATA_MAIZE P49087 VACUOLAR ATP SYNTHASE CATALYTIC SUBUNIT A	1081	0.008511	JY262954, JY263110, JY263277, JY262796
AW098020	similar to TR:O82204 O82204 F6F22.24 PROTEIN	601	0.02139	JY262869, JY263021, JY263218, JY262709
AW098024	similar to TR:Q9ZWB8 Q9ZWB8 F21M11.8 PROTEIN	687	0.010737	JY262957, JY263116, JY263283, JY262799
AW098026	similar to SW:RS3_MOUSE P17073 40S RIBOSOMAL PROTEIN S3	397	0.036855	JY262958, JY263117, JY263284, JY262800

Table 2. Continued.

EST accession	Gene name	Length[b]	Theta[c]	GenBank accession no.
AW098048	similar to TR:O65023 O65023 HYPOTHETICAL 41.8 KD PROTEIN	534	0.003115	JY263055, JY262744
AW098048	similar to TR:O65023 O65023 HYPOTHETICAL 41.8 KD PROTEIN	57*	0	JY262913, JY263064
AW098051	Moss EST library CPU Ceratodon purpureus cDNA clone PEP_SOURCE_ID:CPU011520 5-	424	0.131034	JY263166, JY263326
AW098056	similar to TR:O23984 O23984 EXPRESSED SEQUENCE TAG	388	0.053872	JY262909, JY263060, JY262748
AW098058	similar to SW:RM24_YEAST P36525 60S RIBOSOMAL PROTEIN L24, MITOCHONDRIAL PRECURSOR	439	0.033613	JY262845, JY263004, JY263201, JY262685
AW098074	similar to gb:gb|L28831.1|SOYRIPR Glycine max ribosomal protein S11 gene, (PLANT)	706	0.037273	JY262974, JY263136, JY263300, JY262815
AW098078	similar to SW:RS20_ORYSA P35686 40S RIBOSOMAL PROTEIN S20	470	0.01982	JY262935, JY263086, JY262772
AW098097	similar to TR:O65583 O65583 PUTATIVE URACIL PHOSPHORIBOSYL TRANSFERASE	210*	0.005076	JY262827, JY262665
AW098140	similar to SW:RS21_ORYSA P35687 40S RIBOSOMAL PROTEIN S21	106*	0.014815	JY262925, JY263076, JY262763
AW098153	similar to TR:O82505 O82505 F2P3.12 PROTEIN	529	0	JY263113, JY263280
AW098157	similar to SW:RL37_ARATH Q43292 60S RIBOSOMAL PROTEIN L37	801	0.026701	JY262842, JY263002, JY263198, JY262682
AW098158	similar to SW:RL44_GOSHI Q96499 60S RIBOSOMAL PROTEIN L44	688	0.017544	JY262843, JY263003, JY263199, JY262683
AW098158	similar to SW:RL44_GOSHI Q96499 60S RIBOSOMAL PROTEIN L44	446	0.054795	JY262858, JY263013, JY262699
AW098185	Moss EST library CPU Ceratodon purpureus cDNA clone PEP_SOURCE_ID:CPU030823 3-, mRNA sequence	151*	0	JY262901, JY263050, JY262739
AW098187	similar to SW:GLYM_PEA P34899 SERINE HYDROXYMETHYLTRANSFERASE, MITOCHONDRIAL PRECURSOR	*	–	JY263131
AW098191	similar to TR:O49336 O49336 T11J7.10 PROTEIN	330	0	JY262970, JY263132
AW098191	similar to TR:O49336 O49336 T11J7.10 PROTEIN	392	0	JY262976, JY263141, JY262817
AW098197	similar to TR:O49337 O49337 T11J7.11 PROTEIN	747	0.002204	JY262969, JY263130, JY263297, JY262811
AW098200	Moss EST library CPU *Ceratodon purpureus* cDNA clone PEP_SOURCE_ID:CPU021119 5-		–	JY263170
AW098203	similar to TR:Q9ZTW0 Q9ZTW0 ABA-RESPONSIVE PROTEIN	479	0.004264	JY263173, JY263332
AW098216	similar to SW:R33B_YEAST P41056 60S RIBOSOMAL PROTEIN L33-B	194*	0	JY263178, JY263337
AW098225	similar to SW:ERD1_ARATH P42762 ERD1 PROTEIN PRECURSOR	454	0.008905	JY262841, JY263001, JY263197, JY262681
AW098233	similar to TR:O80526 O80526 F19J9.9 PROTEIN	708	0.006442	JY262959, JY263118, JY263285, JY262801
AW098234	similar to SW:GLYM_PEA P34899 SERINE HYDROXYMETHYLTRANSFERASE, MITOCHONDRIAL PRECURSOR	278	0.053292	JY262857, JY263012, JY263211, JY262698
AW098247	similar to SW:ALFD_PEA Q01517 FRUCTOSE-BISPHOSPHATE ALDOLASE 2, CHLOROPLAST	569	0.005981	JY262961, JY263122, JY263289, JY262803
AW098252	similar to SW:RS2_ARATH P49688 40S RIBOSOMAL PROTEIN S2	86*	0	JY262872, JY263024
AW098256	similar to TR:O80799 O80799 T8F5.5 PROTEIN	517	0.002727	JY262911, JY263062, JY263253, JY262750
AW098258	similar to TR:O22215 O22215 PUTATIVE ESTERASE D	619	0.043062	JY262981, JY263148, JY263310, JY262823
AW098262	similar to TR:Q9ZV56 Q9ZV56 PUTATIVE PHOSPHOCHOLINE CYTIDYLYLTRANSFERASE	540	0.001946	JY263159, JY263319
AW098272	similar to TR:O49379 O49379 HYPOTHETICAL 16.4 KD PROTEIN	211	0.023952	JY263162, JY263322
AW098279	Moss EST library CPU Ceratodon purpureus cDNA clone PEP_SOURCE_ID:CPU021506 5-, mRNA sequence	141*	0	JY262900, JY263049, JY263246, JY262738
AW098281	similar to TR:O65068 O65068 60S RIBOSOMAL PROTEIN L17	348	0.044199	JY263107, JY263275, JY262792
AW098283	**similar to TR:O64720 O64720 PUTATIVE TBP-BINDING PROTEIN**	**431**	**0.004885**	JY262977, JY263142, JY263304, JY262818
AW098284	similar to TR:Q40922 Q40922 PSEUDOTZAIN	154*	0	JY262966, JY263127, JY263294, JY262808
AW098291	similar to SW:YAUB_SCHPO Q10166 HYPOTHETICAL 35.7 KD PROTEIN C26A3.11 IN CHROMOSOME I	286	0.02952	JY263167, JY263327
AW098300	similar to SW:SUI1_MAIZE P56330 PROTEIN TRANSLATION FACTOR SUI1 HOMOLOG	537	0.072549	JY263171, JY263330
AW098304	similar to SW:PRL1_ARATH Q42384 PP1/PP2A PHOSPHATASES PLEIOTROPIC REGULATOR PRL1	385	0.019139	JY262893, JY263041, JY263238, JY262730
AW098306	similar to SW:RL7_ARATH Q42208 60S RIBOSOMAL PROTEIN L7	203*	0	JY263174, JY263333

TABLE 2. Continued.

EST accession	Gene name	Length[b]	Theta[c]	GenBank accession no.
AW098317	Moss EST library CPU Ceratodon purpureus cDNA clone PEP_SOURCE_ID:CPU030319 5-, mRNA sequence	508	0	JY262980, JY262822
AW098318	Moss EST library CPU Ceratodon purpureus cDNA clone PEP_SOURCE_ID:CPU030321 5-, mRNA sequence	128*	0	JY262852, JY263208, JY262693
AW098322	similar to TR:O23601 O23601 HYPOTHETICAL 40.2 KD PROTEIN	735	0.023511	JY262896, JY263044, JY263241, JY262733
AW098328	similar to TR:Q23920 Q23920 PEPA	429	0.024938	JY262938, JY263089, JY262775
AW098337	similar to SW:P2A_HELAN P48579 SERINE/THREONINE PROTEIN PHOSPHATASE PP2A CATALYTIC SUBUNIT	293	0.004065	JY262933, JY263083, JY262769
AW098340	Moss EST library CPU Ceratodon purpureus cDNA clone PEP_SOURCE_ID:CPU030420 5-, mRNA sequence	434	0.011223	JY262839, JY262999, JY263195, JY262679
AW098349	Moss EST library CPU Ceratodon purpureus cDNA clone PEP_SOURCE_ID:CPU030717 5-, mRNA sequence	329	0	JY263207, JY262692
AW098359	similar to SW:RL29_RAT P25886 60S RIBOSOMAL PROTEIN L29	334	0.023392	JY262986, JY263182, JY262666
AW098361	similar to TR:O80800 O80800 T8F5.6 PROTEIN	849	0.012285	JY262903, JY263052, JY263248, JY262741
AW098364	similar to TR:O65636 O65636 HYPOTHETICAL 15.1 KD PROTEIN	442	0.004902	JY262940, JY263091, JY262776
AW098367	similar to TR:Q42809 Q42809 GMCK1P	453	0.010195	JY262910, JY263061, JY263252, JY262749
AW098372	Moss EST library CPU Ceratodon purpureus cDNA clone PEP_SOURCE_ID:CPU030814 5-, mRNA sequence	147*	0.008658	JY262866, JY263018, JY262705
AW098391	similar to TR:O30618 O30618 ACYL-COA OXIDASE	530	0.010414	JY262921, JY263072, JY263260, JY262759
AW098393	similar to SW:RL7A_ORYSA P35685 60S RIBOSOMAL PROTEIN L7A	211	0.020408	JY262887, JY263232
AW098407	similar to TR:O81046 O81046 AXI 1-LIKE PROTEIN	727	0.022222	JY262863, JY263214, JY262702
AW098418	similar to SW:RS25_LYCES P46301 40S RIBOSOMAL PROTEIN S25	312	0.011988	JY262888, JY263036, JY263233, JY262726
AW098419	similar to SW:R35A_MOUSE O55142 60S RIBOSOMAL PROTEIN L35A	550	0.01165	JY263179, JY263338
AW098432	Moss EST library CPU Ceratodon purpureus cDNA clone PEP_SOURCE_ID:CPU031514 5-, mRNA sequence	598	0	JY262891, JY263039, JY263236, JY262729
AW098442	similar to TR:P93321 P93321 CDC2 KINASE HOMOLOGUE, CDC2MSD	290	0.011252	JY262844, JY263200, JY262684
AW098442	**similar to TR:P93321 P93321 CDC2 KINASE HOMOLOGUE, CDC2MSD**	**473**	**0.029591**	JY262892, JY263040, JY263237
AW098448	similar to TR:Q9ZQX9 Q9ZQX9 40S RIBOSOMAL PROTEIN S27 HOMOLOG	274	0	JY263181, JY263340
AW098462	**similar to SW:RS1A_ARATH P42798 40S RIBOSOMAL PROTEIN S15A**	**215**	**0.044709**	JY262879, JY263030, JY263225, JY262718
AW098472	Moss EST library CPU Ceratodon purpureus cDNA clone PEP_SOURCE_ID:CPU011207 3-	446	0	JY263163, JY263323
AW098479	similar to TR:O48649 O48649 ADP-RIBOSYLATION FACTOR	251	0.029268	JY262907, JY263057, JY262746
AW098512	similar to SW:ILV5_SPIOL Q01292 KETOL-ACID REDUCTOISOMERASE PRECURSOR	645	0.019108	JY262884, JY263034, JY263229, JY262723
AW098515	similar to TR:O04556 O04556 T7N9.9	442	0.00391	JY262846, JY263202, JY262686
AW098521	similar to TR:O82203 O82203 PUTATIVE RIBOSOMAL PROTEIN	286	0	JY263145, JY263307, JY262821
AW098560	similar to TR:O13870 O13870 PROBABLE TRANSCRIPTIONAL REGULATOR C1B3.05	458	0.008354	JY262944, JY263098, JY262783
AW098568	similar to SW:DHE3_RHISN Q53199 PROBABLE GLUTAMATE DEHYDROGENASE	330	0.007305	JY262831, JY262990, JY263186, JY262670
AW098572	similar to TR:Q9ZUL5 Q9ZUL5 PUTATIVE DNA-BINDING PROTEIN	619	0.002573	JY262859, JY263014, JY263212, JY262700
AW098585	similar to SW:FKB7_WHEAT Q43207 70 KD PEPTIDYLPROLYL ISOMERASE		–	JY263155
AW098587	similar to SW:OAT_EMENI Q92413 ORNITHINE AMINOTRANSFERASE	547	0.017842	JY262902, JY263051, JY263247, JY262740
AW098597	similar to TR:Q56987 Q56987 HYPOTHETICAL 23.2 KD PROTEIN	254	0	JY263175, JY263334
AW098620	similar to TR:Q9ZRI8 Q9ZRI8 FORMATE DEHYDROGENASE	606	0	JY262929, JY263341
AW098621	**similar to SW:RS1A_ARATH P42798 40S RIBOSOMAL PROTEIN S15A**	**732**	**0.044058**	JY263019, JY263216, JY262706
AW098624	similar to SW:RL44_GOSHI Q96499 60S RIBOSOMAL PROTEIN L44		–	JY262862
AW098631	similar to SW:SPEE_COFAR O82147 SPERMIDINE SYNTHASE	161*	0	JY262861, JY263016, JY262701
AW098643	similar to TR:Q9ZQP2 Q9ZQP2 PUTATIVE ACYL COENZYME A OXIDASE, PEROXISOMAL COMPONENT	456	0.019697	JY262955, JY263111, JY263278, JY262797

TABLE 2. Continued.

EST accession	Gene name	Length[b]	Theta[c]	GenBank accession no.
AW098651	**similar to TR:Q45073 Q45073 HYPOTHETICAL 23.3 KD PROTEIN**	**337**	**0.008584**	JY262951, JY263108, JY262793
AW098679	similar to TR:O82342 O82342 PUTATIVE TGF-BETA RECEPTOR INTERACTING PROTEIN	807	0.054728	JY262828, JY262987, JY263183, JY262667
AW098700	similar to TR:Q9ZQR3 Q9ZQR3 PUTATIVE RECEPTOR PROTEIN KINASE	272	0.023622	JY262848, JY263203, JY262688
AW098717	similar to TR:O82204 O82204 F6F22.24 PROTEIN	173*	0.006211	JY263180, JY263339
AW098746	similar to TR:O22662 O22662 PROTEIN PHOSPHATASE U	532	0	JY263115, JY263282
AW098758	similar to SW:TRP2_ARATH P25269 TRYPTOPHAN SYNTHASE BETA CHAIN 2 PRECURSOR	369	0	JY263121, JY263288
AW098761	similar to TR:Q9ZDU0 Q9ZDU0 30S RIBOSOMAL PROTEIN S9	134*	0.031169	JY262898, JY263046, JY263243, JY262735
AW098770	similar to WP:D1054.3 CE05522	603	0.003924	JY262978, JY263143, JY263305, JY262819
AW098776	similar to TR:Q41024 Q41024 SMALL GTP-BINDING PROTEIN	599	0.003643	JY263147, JY263309
AW098780	similar to SW:IMB3_HUMAN O00410 IMPORTIN BETA-3 SUBUNIT	656	0	JY262963, JY263124, JY263291, JY262805
AW098782	similar to TR:Q9ZVS6 Q9ZVS6 F15K9.15 PROTEIN	310	0.012212	JY262899, JY263047, JY263244, JY262736
AW098786	similar to SW:LE22_ARCFU O28084 PUTATIVE 3-ISOPROPYLMALATE DEHYDRATASE LARGE SUBUNIT	569	0.006545	JY262897, JY263045, JY263242, JY262734
AW098797	similar to SW:CAHC_HORVU P40880 CARBONIC ANHYDRASE, CHLOROPLAST PRECURSOR	410	0.001855	JY262984, JY263153, JY263314, JY262826
AW098799	similar to WP:F41C3.4 CE02732	347	0.004489	JY262945, JY263099, JY262784
AW098804	similar to TR:O81808 O81808 HYPOTHETICAL 62.6 KD PROTEIN	512	0.017094	JY262922, JY263073, JY263261, JY262760
AW098812	similar to TR:O81468 O81468 T15F16.12 PROTEIN	747	0.007407	JY263120, JY263287
AW098823	similar to SW:RLA1_MAIZE P52855 60S ACIDIC RIBOSOMAL PROTEIN P1	852	0.005929	JY262904, JY263053, JY263249, JY262742
AW098831	similar to TR:O75865 O75865 R32611_2	485	0.005742	JY262979, JY263144, JY263306, JY262820
AW098839	similar to SW:RS19_ORYSA P40978 40S RIBOSOMAL PROTEIN S18	857	0	JY262849, JY263006, JY263204, JY262689
AW098949	similar to TR:O04033 O04033 F7G19.16	389	0.002045	JY262924, JY263075, JY262762
AW098949	similar to TR:O04033 O04033 F7G19.15	411	0.003956	JY262932, JY263082, JY262768
AW098974	similar to SW:RL34_TOBAC P41098 60S RIBOSOMAL PROTEIN L34	514	0.063425	JY262829, JY262988, JY263184, JY262668
BI894286	similar to SW:ATP2_ACTCH P43395 ATP SYNTHASE BETA CHAIN, MITOCHONDRIAL	272	–	JY263150
BI894288	**similar to SW:RS23_FRAAN P46297 40S RIBOSOMAL PROTEIN S23**	**565**	**0**	JY262833, JY262992, JY263188, JY262672
CZ893596	CEPU109		–	
CZ894207	CEPU105	86	0.026316	
CZ894357	CEPU108	243	0.0125	

[a] Accessions studied were: *C. purpureus*: WT4 (Wispertal, Austria), GG1 (Grossgerunds, Austria), R40 (Petersburg Pass, Renssalaer, New York), and Otavalo, Ecuador (McDaniel E112, DUKE). Additional isolates were sequenced for the loci in bold in *C. purpureus* (Ros29.10.2009-1 [UF], Ros29.10.2009-2 [UF], Antarctica, Robinson&Wasley 99/00 [DUKE]); *T. cylindricus* (DUKE11365, DUKE65082, DUKE65084); and *C. chloropus* (Werner&Ros 14024 [DUKE], Guerra15.4.2001 [DUKE], Ros29.10.2009-1 [UF], Ros29.10.2009-2 [UF]).

[b] Length of the amplified PCR product in *C. purpureus*; an "*" indicates that *C. purpureus* lacks the *P. patens* intron.

[c] A "–" indicates that a single PCR product was generated; a "0" indicates an absence of variation among isolates.

to sequence in isolates of *C. purpureus* from the Sierra Nevadas, Spain; Casey Station, Antarctica; and Wollongong, Australia, and 1–2 isolates of the sister groups to *C. purpureus*, *Trichodon cylindricus* (Hedw.) Schimp., and *Cheilothela chloropus* (Brid.) Broth. (Table 2). The PCR products were nearly the same length in all three species, and produced sequences with unambiguous chromatograms. In all cases, the introns were alignable among the three species, but the species differed at ~10–20% of the intron sites, suggesting that these loci may be useful for phylogeographic and species-level phylogenetic studies. In the complete panel of loci, we also found 23 introns that were present in the *P. patens* genome that were absent in the *C. purpureus* genome (Table 2). Using a PCR length variation test, we determined that the intron absence was shared by many species in the Dicranidae (McDaniel and Neubig, unpublished data). These presence/absence polymorphisms may be useful phylogenetic markers (Goffinet et al., 2007). We expect that this panel of primers will be valuable for the bryophyte evolutionary genetics community as a whole.

CONCLUSIONS

In this study, we have generated primers for more than 200 loci, based on comparisons from ESTs from *C. purpureus* and the genome of *P. patens*. We have used these loci to estimate the genome-wide distribution of nucleotide diversity within *C. purpureus*. Because these primers were designed to be homologous to exonic regions that are conserved between species that diverged long ago, these primers may amplify the target region in a wide variety of mosses. We anticipate that these loci will form a valuable addition to the bryophyte molecular ecology toolkit, enabling more detailed phylogeographic and population genetic studies of a variety of focal species.

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