


Development and characterization of simple sequence repeat markers for the invasive tetraploid waterweed *Elodea nuttallii* (Hydrocharitaceae)

Isabell Weickardt^{1,3} , Andreas Zehnsdorf¹, and Walter Durka^{2,3} 

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¹ Centre for Environmental Biotechnology, Helmholtz Centre for Environmental Research–UFZ, Permoserstraße 15, D-04318 Leipzig, Germany

² Department of Community Ecology, Helmholtz Centre for Environmental Research–UFZ, Theodor-Lieser-Straße 4, D-06120 Halle, Germany

³ Authors for correspondence: isabell.weickardt@ufz.de, walter.durka@ufz.de

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PREMISE OF THE STUDY: To enhance the understanding of the recent invasion process of the clonal waterweed *Elodea nuttallii* (Hydrocharitaceae), analyses of population structure and genotypic diversity need to be undertaken, for which genetic markers are needed.

METHODS AND RESULTS: High-throughput sequencing of DNA enriched for microsatellites was used to develop 24 loci that were characterized in *E. nuttallii*, 21 of which were polymorphic, with the number of alleles ranging from two to 10. In two populations, expected heterozygosity ranged among loci between zero and 0.796. In the congener *E. canadensis*, all markers yielded PCR products, 19 of which were polymorphic, with two to nine alleles and expected heterozygosity ranging from zero to 0.690 in two populations.

CONCLUSIONS: The markers described should be useful for future studies of population structure and clonal diversity of *E. nuttallii* as well as *E. canadensis* in their native and invasive range.

KEY WORDS clonality; *Elodea canadensis*; *Elodea nuttallii*; Hydrocharitaceae; invasive species.

Western waterweed (*Elodea nuttallii* (Planch.) H. St. John, Hydrocharitaceae) is a tetraploid, dioecious, submerged freshwater macrophyte native to North America that is invasive in Europe and Japan. In its invasive range, almost exclusively female plants have been found and the reproduction is primarily vegetative (Cook and Urmi-König, 1985). In Europe, it was first found in 1939 in Belgium and has quickly spread to 19 other European countries (Cook and Urmi-König, 1985; Hussner, 2012). Because it can form dense dominant stands, *E. nuttallii* constrains water flow in rivers and the recreational use of lakes. Furthermore, the aggressive growth of this species may influence abiotic factors, outcompete native plants, change the makeup of the aquatic vegetation, and thus impact fish occurrence (Carey et al., 2016). Because no effective, species-specific management options are known (Zehnsdorf et al., 2015), more information about the species' biology and population structure is urgently needed. The analysis of genetic variation and population structure can give insights into reproduction mode, dispersal patterns, and the invasion process in general and may allow the identification of source regions in the native range (e.g., Durka et al., 2005; Voss et al., 2012).

Microsatellite markers are available for *E. canadensis* Michx. (Huotari et al., 2010); however, cross-species amplification in *E. nuttallii* had not been tested (T. Huotari and H. Korpelainen,

University of Helsinki, Helsinki, Finland, personal communication). Moreover, 60% of these markers only amplified in *E. canadensis* samples from invasive but not from native populations (Huotari et al., 2011), and preliminary tests revealed no or nonreproducible amplification products in our samples from both *E. canadensis* and *E. nuttallii*. Additionally, the available markers have limited power to discriminate clones because of low number of alleles (mean number of alleles = 2.8; Huotari et al., 2011) and their limited number ($N = 10$). Therefore, we developed new markers specifically for *E. nuttallii*. We demonstrate their utility for native and invasive samples as well as trans-species amplification in *E. canadensis*, thus allowing for comparative analyses of clonal variation between native and invasive range and among species.

METHODS AND RESULTS

Total genomic DNA from *E. nuttallii* collected in Großer Goitzschensee, Germany (51°37'12"N, 12°23'59"E), was isolated using the cetyltrimethylammonium bromide (CTAB) extraction procedure of Doyle (1991) and sent to GenoScreen (Lille, France). One microgram of DNA was used for the development of microsatellites through 454 GS-FLX Titanium pyrosequencing (Roche Applied

TABLE 1. Characteristics of 24 microsatellite markers developed in *Elodea nuttallii* and cross-species amplification in *E. canadensis*.

| Locus | Primer sequences (5'-3') ^a | Repeat motif | Fluorescent dye | PCR mix | Fragment analysis mix | <i>E. nuttallii</i> (N = 262) ^b | | | <i>E. canadensis</i> (N = 92) ^b | | | GenBank accession no. | | |
|-------|---|---------------------|-----------------|---------|-----------------------|--|----|------------------|--|------------------------|---|-----------------------|------------------|-----------------|
| | | | | | | Allele size range (bp) | A | A _{max} | P _{NA} | Allele size range (bp) | A | | A _{max} | P _{NA} |
| Enu01 | F: CAGTCGGGGTCAATAGGTTGGAAGATGACCCGTAAG R: GTTTGTCGAGTAGGCACGTCGAA | (GA) ₇ | NED | e | 4 | 119 | 1 | 1 | 0 | 119 | 1 | 1 | 0 | MG272338 |
| Enu04 | F: GGAAACAGCTATGACCAITAGGCTCTCATGCTTTCC R: GTTTCCGGTAGTCAGCAGTGTGGT | (TC) ₁₀ | PET | b | 1 | 121–129 | 4 | 3 | 0 | 123–129 | 3 | 1 | 1.1 | MG272339 |
| Enu05 | F: CAGTCGGGGTCAATCAGATCTGGACCCAAAGCGAA R: GTTTTCAGATAGAGTGGTCTTCGCCA | (TC) ₇ | FAM | — | 3 | 93–128 | 10 | 3 | 0 | 93–116 | 3 | 2 | 0 | MG272340 |
| Enu06 | F: GGAAACAGCTATGACCAITAGGCTCTCATGCTTTCC R: GTTTAGCCACTGCATCATGCTTTG | (TC) ₈ | VIC | — | 2 | 101–107 | 3 | 2 | 0 | 101–109 | 5 | 3 | 0 | MG272341 |
| Enu07 | F: CAGTCGGGGTCAATGAGTGGTGGACCAAGTATC R: GTTTTCATATGACACCGAGATTGCAC | (CT) ₈ | VIC | a | 1 | 106–114 | 4 | 3 | 0 | 110–114 | 3 | 3 | 0 | MG272342 |
| Enu09 | F: GGAAACAGCTATGACCAITAGGCTCTCATGCTTTCC R: GTTTCTCCAAAGAAAGCGCAATC | (TCT) ₉ | VIC | — | 4 | 108–126 | 7 | 4 | 0 | 108–129 | 6 | 4 | 0 | MG272343 |
| Enu10 | F: CAGTCGGGGTCAATGAGTGGTGGACCAAGTATC R: GTTTGGTCAAGCAGCATGCTCTTTT | (GAG) ₇ | NED | d | 3 | 106–118 | 5 | 3 | 0.8 | 109–118 | 3 | 2 | 2.2 | MG272344 |
| Enu12 | F: CAGTCGGGGTCAATGAGTGGTGGACCAAGTATC R: GTTTGGGAGAGCCATACCTTGT | (TTC) ₁₁ | NED | c | 2 | 104–128 | 9 | 4 | 0 | 104–119 | 4 | 3 | 0 | MG272345 |
| Enu13 | F: GGAAACAGCTATGACCAITAGGCTCTCATGCTTTCC R: GTTTGACAGTCCGATGGTGAAGT | (CTT) ₉ | VIC | — | 3 | 108–135 | 9 | 4 | 0 | 111–135 | 7 | 3 | 0 | MG272346 |
| Enu15 | F: CAGTCGGGGTCAATGAGTGGTGGACCAAGTATC R: GTTTCTTTGGGGTCTAGGGAGGG | (TC) ₆ | FAM | — | 4 | 150–160 | 3 | 3 | 0 | 138–160 | 3 | 2 | 0 | MG272347 |
| Enu19 | F: CAGTCGGGGTCAATGAGTGGTGGACCAAGTATC R: GTTTAAGCAATAAAGCGAAAGCCA | (GA) ₆ | NED | c | 2 | 155–162 | 3 | 2 | 0.4 | 155–162 | 3 | 2 | 91.3 | MG272348 |
| Enu20 | F: GGAAACAGCTATGACCAITAGGCTCTCATGCTTTCC R: GTTTAAGCAATAAAGCGAAAGCCA | (CT) ₆ | PET | d | 3 | 156–164 | 5 | 3 | 0 | 156–158 | 2 | 1 | 89.1 | MG272349 |
| Enu21 | F: CAGTCGGGGTCAATGAGTGGTGGACCAAGTATC R: GTTTGAGGCTCTGGACTCCAATCA | (TC) ₆ | NED | b | 1 | 132–170 | 7 | 3 | 1.5 | 150–170 | 4 | 3 | 4.4 | MG272350 |
| Enu22 | F: CAGTCGGGGTCAATGAGTGGTGGACCAAGTATC R: GTTTCAAGAAAGACCCCAAGGA | (TCC) ₇ | FAM | f | 1 | 141 | 1 | 1 | 0 | 141 | 1 | 1 | 5.4 | MG272351 |
| Enu24 | F: CAGTCGGGGTCAATGAGTGGTGGACCAAGTATC R: GTTTGAAGCTGCAGAAAGCAACAACA | (CTT) ₉ | VIC | a | 1 | 160 | 1 | 1 | 0 | 160 | 1 | 1 | 0 | MG272352 |
| Enu26 | F: CAGTCGGGGTCAATGAGTGGTGGACCAAGTATC R: GTTTGTCGAGTGGGGTCTAAGG | (TTC) ₁₁ | FAM | — | 2 | 121–149 | 10 | 4 | 0 | 121–146 | 3 | 2 | 0 | MG272353 |
| Enu28 | F: CAGTCGGGGTCAATGAGTGGTGGACCAAGTATC R: GTTTCTTATGCAAGAGGGAATCA | (TCT) ₆ | NED | e | 4 | 149–158 | 3 | 3 | 3.8 | 149–158 | 3 | 2 | 4.4 | MG272354 |
| Enu30 | F: GGAAACAGCTATGACCAITAGGCTCTCATGCTTTCC R: GTTTGCGCCATAGGCACGATGAT | (GA) ₈ | PET | c | 2 | 218–228 | 3 | 2 | 3.4 | 222 | 1 | 1 | 97.8 | MG272355 |
| Enu35 | F: CAGTCGGGGTCAATGAGTGGTGGACCAAGTATC R: GTTTCTGCGACCACTACTGCTT | (GGA) ₆ | FAM | f | 1 | 188–200 | 4 | 2 | 0.4 | 188–203 | 4 | 3 | 0 | MG272356 |
| Enu36 | F: GGAAACAGCTATGACCAITAGGCTCTCATGCTTTCC R: GTTTGTAGCTGTTCCATTTTC | (GAA) ₇ | PET | e | 4 | 184–188 | 2 | 2 | 1.1 | 185 | 1 | 1 | 1.1 | MG272357 |
| Enu37 | F: CAGTCGGGGTCAATGAGTGGTGGACCAAGTATC R: GTTTGACCTTCTCTCTTGGTG | (TTC) ₁₁ | VIC | a | 1 | 185–216 | 9 | 3 | 1.1 | 185–198 | 3 | 2 | 91.3 | MG272358 |
| Enu38 | F: GGAAACAGCTATGACCAITAGGCTCTCATGCTTTCC R: GTTTGTGATGCTCTCTTTTGTGG | (AGT) ₁₅ | PET | b | 1 | 180–219 | 9 | 3 | 0 | 183–216 | 9 | 4 | 0 | MG272359 |

(continues)

TABLE 1. (Continued)

| Locus | Primer sequences (5'-3') ^a | Repeat motif | Fluorescent dye | PCR mix | Fragment analysis mix | <i>E. nuttallii</i> (N = 262) ^b | | | <i>E. canadensis</i> (N = 92) ^b | | | GenBank accession no. | | |
|-------|--|---------------------|-----------------|---------|-----------------------|--|---|------------------|--|------------------------|---|-----------------------|------------------|-----------------|
| | | | | | | Allele size range (bp) | A | A _{max} | P _{NA} | Allele size range (bp) | A | | A _{max} | P _{NA} |
| Enu39 | F: CAGTCGGGGCGTCACGATCGTCAGAGACCTCACA R: GTTTATCACGTGAAATGCCAGTCA | (CTT) ₁₀ | NED | d | 3 | 214–232 | 7 | 4 | 3.0 | 220–232 | 5 | 3 | 0 | MG272360 |
| Enu41 | F: CAGTCGGGGCGTCACGATCGTCAGAGACCTCACA R: GTTTAATACATTGGCCGGGTATC | (CTT) ₁₄ | NED | b | 1 | 230–257 | 8 | 4 | 2.0 | 223–254 | 9 | 4 | 3.3 | MG272361 |

Note: — = singleplex reaction; A = number of alleles; A_{max} = maximal number of alleles detected per individual; N = number of individuals sampled; P_{NA} = percentage homozygous null alleles.
^aForward primer sequences include CAG and M13R tags and reverse primers include GTTT PIG-tails (in italics).
^bGeographic coordinates for the populations and voucher information are given in Appendix 1.

Science, Basel, Switzerland) of enriched DNA libraries following Malausa et al. (2011). Briefly, total DNA was mechanically fragmented and enriched for AG, AC, AAC, AAG, AGG, ACG, ACAT, and ATCT repeat motifs. Enriched fragments were subsequently amplified. PCR products were purified and quantified, and GsFLX libraries were then prepared following the manufacturer's protocols and sequenced on a GsFLX PTP (Roche Applied Science). Using QDD (Megléczy et al., 2010), adapters and vectors were removed, microsatellites were detected, their redundancy and association with mobile elements were tested, sequences with target microsatellites were selected, and primers were designed.

Among 2815 sequences comprising a microsatellite motif, 168 bioinformatically validated primer sets were designed on perfect motif microsatellites. We chose 41 primer pairs based on their motif, repeat number, and length of expected amplification product. We aimed to equally represent dinucleotide and trinucleotide repeats as well as short (90–140 bp), medium (140–190 bp), and long (190–250 bp) amplification products. Furthermore, microsatellites with high repeat numbers were favored because they often display a higher mutation rate.

These 41 primer pairs were ordered from Eurofins (Ebersberg, Germany) and were screened using the method of Schuelke (2000), i.e., adding CAG- or M13R-tags to forward primers, adding GTTT-tags to reverse primers (Brownstein et al., 1996), and using fluorescent-labeled CAG and M13R primers. The PCR reaction contained 5 µL QIAGEN Multiplex PCR Master Mix (QIAGEN, Hilden, Germany), 1 µL of 2.5 µM tagged forward primer and 0.5 µM reverse primer, 1 µL of 2.5 µM fluorescent-labeled CAG- or M13R-tag, 2 µL of ultrapure water (Carl Roth GmbH, Karlsruhe, Germany), and 1 µL of DNA (~400 ng/µL). A touchdown protocol was used with cycles as follows: 95°C for 15 min; 20 cycles of 94°C for 30 s, 60°C for 30 s (decreasing 0.5°C per annealing cycle), and 72°C for 90 s; 20 cycles of 94°C for 30 s, 50°C for 30 s, and 72°C for 90 s; and a final elongation step of 10 min at 72°C. PCR products (1 µL) were multiplexed into four mixes (Table 1) with 10 µL of Hi-Di Formamide (Thermo Fisher Scientific, Waltham, Massachusetts, USA) for fragment length analysis on an ABI 3130xl genetic analyzer (Thermo Fisher Scientific) with GeneScan 500 LIZ Size Standard (Thermo Fisher Scientific). Genotyping was performed using GeneMapper Software 5 (Thermo Fisher Scientific), allowing for a maximum of four alleles, assuming tetraploidy (Cook and Urmi-König, 1985; Di Nino, 2008).

The 41 primers were tested for polymorphism on 40 individuals from eastern Germany, and 24 loci yielded PCR products in the expected size range (Table 1). Sequences containing these primers were deposited in the National Center for Biotechnology Information's GenBank database. The 24 markers were amplified in six multiplex and six singleplex reactions (Table 1). In case no PCR product was detected, at least two replicate analyses were performed; if no PCR product was found in the replicate analyses, the presence of a fixed null allele was assumed. Because of clonal reproduction and invasive spread, single populations harbored only a small amount of total allelic variation. Therefore, to assess genetic variation at the population level, we analyzed two well-sampled invasive populations for both *E. nuttallii* and *E. canadensis*. For these, we report the number of genotypes (N_{gr}) and used GenoDive version 2.0b23 (Meirmans and Van Tienderen, 2004) to calculate values of observed heterozygosity (H_o) and expected heterozygosity (H_e) correcting for unknown allele dosage of polyploids. To assess allelic variation at the species level, we included additional samples

TABLE 2. Genotypic and genetic variation of 24 newly developed microsatellites at population and overall level in two invasive populations each of *Elodea nuttallii* and *E. canadensis*.^a

| Locus | <i>E. nuttallii</i> | | | | | | <i>E. canadensis</i> | | | | | |
|---------|-------------------------|-------|-------|-------------------------------|-------|-------|--------------------------------|-------|-------|-----------------------|-------|-------|
| | Cospudener See (N = 21) | | | Großer Goitzschensee (N = 43) | | | Gravel pit Kleinpösna (N = 27) | | | Parthe River (N = 11) | | |
| | N_{gt} | H_o | H_e | N_{gt} | H_o | H_e | N_{gt} | H_o | H_e | N_{gt} | H_o | H_e |
| Enu01 | 1 | 0 | 0.000 | 1 | 0 | 0.000 | 1 | 0 | 0.000 | 1 | 0 | 0.000 |
| Enu04 | 1 | 0 | 0.000 | 4 | 0.095 | 0.343 | 1 | 0 | 0.000 | 1 | 0 | 0.000 |
| Enu05 | 1 | 0 | 0.000 | 3 | 0.048 | 0.084 | 1 | 0 | 0.000 | 1 | 0 | 0.000 |
| Enu06 | 1 | 0 | 0.000 | 2 | 0.024 | 0.015 | 2 | 0 | 0.074 | 2 | 0 | 0.533 |
| Enu07 | 1 | 1 | 0.677 | 1 | 1 | 0.506 | 1 | 1 | 0.675 | 1 | 1 | 0.690 |
| Enu09 | 1 | 1 | 0.677 | 7 | 1 | 0.715 | 1 | 0 | 0.000 | 1 | 0 | 0.000 |
| Enu10 | 1 | 1 | 0.512 | 2 | 1 | 0.506 | 1 | 0 | 0.000 | 1 | 0 | 0.000 |
| Enu12 | 1 | 1 | 0.677 | 9 | 1 | 0.720 | 1 | 1 | 0.509 | 1 | 1 | 0.526 |
| Enu13 | 1 | 1 | 0.512 | 3 | 1 | 0.570 | 4 | 1 | 0.614 | 2 | 0.8 | 0.490 |
| Enu15 | 1 | 1 | 0.512 | 7 | 0.667 | 0.616 | 1 | 0 | 0.000 | 1 | 0 | 0.000 |
| Enu19 | 1 | 0 | 0.000 | 3 | 0.357 | 0.385 | — | — | — | — | — | — |
| Enu20 | 1 | 1 | 0.512 | 2 | 0.857 | 0.485 | — | — | — | — | — | — |
| Enu21 | 1 | 1 | 0.512 | 2 | 0.976 | 0.505 | 1 | 0 | 0.000 | 1 | 0 | 0.000 |
| Enu22 | 1 | 0 | 0.000 | 1 | 0 | 0.000 | 1 | 0 | 0.000 | 1 | 0 | 0.000 |
| Enu24 | 1 | 0 | 0.000 | 1 | 0 | 0.000 | 1 | 0 | 0.000 | 1 | 0 | 0.000 |
| Enu26 | 1 | 1 | 0.759 | 12 | 1 | 0.796 | 1 | 0 | 0.000 | 1 | 0 | 0.000 |
| Enu28 | 1 | 0 | 0.000 | 1 | 0 | 0.000 | 1 | 1 | 0.509 | 1 | 1 | 0.526 |
| Enu30 | 1 | 0 | 0.000 | 2 | 0.095 | 0.060 | — | — | — | — | — | — |
| Enu35 | 1 | 0 | 0.000 | 1 | 0 | 0.000 | 1 | 0 | 0.000 | 2 | 0.8 | 0.490 |
| Enu36 | 1 | 1 | 0.512 | 2 | 0.619 | 0.378 | 1 | 0 | 0.000 | 1 | 0 | 0.000 |
| Enu37 | 1 | 1 | 0.512 | 5 | 0.81 | 0.479 | — | — | — | — | — | — |
| Enu38 | 2 | 1 | 0.677 | 4 | 0.952 | 0.604 | 1 | 1 | 0.675 | 2 | 1 | 0.689 |
| Enu39 | 1 | 1 | 0.677 | 5 | 0.929 | 0.563 | 2 | 0.185 | 0.127 | 3 | 1 | 0.608 |
| Enu41 | 1 | 1 | 0.677 | 8 | 0.929 | 0.688 | 1 | 1 | 0.675 | 1 | 1 | 0.690 |
| Average | 1.04 | 0.583 | 0.350 | 3.67 | 0.557 | 0.376 | 1.25 | 0.309 | 0.193 | 1.30 | 0.38 | 0.262 |

Note: — = no amplification; H_e = expected heterozygosity corrected for allele dosage; H_o = observed heterozygosity; N = number of individuals sampled; N_{gt} = number of genotypes.
^aGeographic coordinates for the populations and voucher information are given in Appendix 1.

from other native and invasive sites, totaling 262 ramets from 53 sites in *E. nuttallii* and 92 ramets from 30 sites in *E. canadensis* (Appendix 1).

In *E. nuttallii*, 21 microsatellites were polymorphic, with the number of alleles ranging from two to 10 (average 5.3), totaling 127 alleles (Table 1). At population level, moderate levels of genetic diversity were found in two populations (mean H_e = 0.350 and 0.376), but genotypic diversity was either very low (mean N_{gt} = 1.04) or high (N_{gt} = 3.67; Table 2).

Cross-species amplification in *E. canadensis* was successful, revealing 19 polymorphic loci with two to nine alleles per locus (average 3.6) and 87 alleles in total (Table 1). However, four loci had high null allele frequencies. At population level, low levels of both genetic diversity (mean H_e = 0.193 and 0.262; Table 2) and genotypic diversity (mean N_{gt} = 1.25 and 1.30) were found in two populations. In both species, the number of alleles detected per individual and locus ranged between one and four, as expected for a tetraploid species (Table 1).

CONCLUSIONS

The microsatellite markers developed for *E. nuttallii* were also proved to be useful in *E. canadensis*. Allelic diversity was generally higher in invasive populations of *E. nuttallii* compared to invasive populations of *E. canadensis*. This suggests that different processes may drive the invasion of these two morphologically and ecologically highly similar species. The new markers will be useful

for further analysis of clonal diversity and genetic structure of *E. nuttallii* and *E. canadensis* and, in particular, to investigate their European invasion history.

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APPENDIX 1. Locality and voucher information for *Elodea* populations used in this study.

| Species | Country, municipality/state, waterbody | Geographic coordinates | No. of ramets | Voucher ^a |
|---|---|--------------------------|---------------|----------------------|
| <i>E. canadensis</i> Michx. | Germany, Bavaria, Chiemsee | 47°52'21"N, 12°27'36"E | 1 | EMT_41 |
| <i>E. canadensis</i> | Germany, Bavaria, Ferchensee | 47°26'19"N, 11°12'49"E | 1 | EMT_42 |
| <i>E. canadensis</i> | Germany, Bavaria, Froschhauser See | 47°41'13"N, 11°13'28"E | 1 | EMT_37 |
| <i>E. canadensis</i> | Germany, Bavaria, Moosach | 48°23'38"N, 11°43'27"E | 1 | EMT_38 |
| <i>E. canadensis</i> | Germany, Bavaria, Starnberge See | 47°54'45"N, 11°18'25"E | 1 | EMT_43 |
| <i>E. canadensis</i> | Germany, Lower Saxony, Hauptkanal | 52°53'20"N, 8°58'53"E | 1 | EMT_28 |
| <i>E. canadensis</i> | Germany, North Rhine-Westphalia, Diersfordter Waldsee | 51°41'46"N, 6°31'54"E | 1 | EMT_70 |
| <i>E. canadensis</i> | Germany, North Rhine-Westphalia, Tenderingsee | 51°35'43"N, 6°43'30"E | 1 | EMT_67 |
| <i>E. canadensis</i> | Germany, Saxony, Delinkateich | 51°24'25"N, 14°45'00"E | 1 | EMT_11 |
| <i>E. canadensis</i> | Germany, Saxony, gravel pit Kleinpösna | 51°18'43"N, 12°31'51"E | 27 | EMT_130 |
| <i>E. canadensis</i> | Germany, Saxony, Kleinliebenau | 51°22'01"N, 12°13'31"E | 1 | EMT_328 |
| <i>E. canadensis</i> | Germany, Saxony, Kulkwitzer See | 51°17'52"N, 12°15'14"E | 1 | EMT_51 |
| <i>E. canadensis</i> | Germany, Saxony, Cospudener See | 51°17'06"N, 12°21'38"E | 1 | EMT_323 |
| <i>E. canadensis</i> | Germany, Saxony, Leipzig | 51°19'41"N, 12°17'27"E | 2 | EMT_324 |
| <i>E. canadensis</i> | Germany, Saxony, Nangteich | 51°24'30"N, 14°45'01"E | 1 | EMT_329 |
| <i>E. canadensis</i> | Germany, Saxony, Parthe | 51°22'09"N, 12°24'47"E | 11 | EMT_266 |
| <i>E. canadensis</i> | Germany, Saxony-Anhalt, Hasselvorsperre | 51°42'33"N, 10°49'51"E | 1 | EMT_50 |
| <i>E. canadensis</i> | Germany, Saxony-Anhalt, Mulde | 51°40'51"N, 12°17'41"E | 2 | EMT_249 |
| <i>E. canadensis</i> | Germany, Saxony-Anhalt, Raßnitzer See | 51°22'0"N, 12°44'1"E | 5 | EMT_87 |
| <i>E. canadensis</i> | Norway, Buskerud, Tyrifjorden | 59°57'51"N, 9°59'47"E | 1 | EMT_317 |
| <i>E. canadensis</i> | Austria, Salzkammergut, Grundlsee | 47°37'58"N, 13°51'52"E | 1 | EMT_39 |
| <i>E. canadensis</i> | Peru, Cusco, Oropesa | −13°33'37"N, −71°51'40"E | 1 | EMT_338 |
| <i>E. canadensis</i> | Purchased online (Nymphaion) | | 6 | EMT_347 |
| <i>E. canadensis</i> | Slovenia, Podravska, Ptujsko jezero | 46°23'14"N, 15°54'25"E | 1 | EMT_307 |
| <i>E. canadensis</i> | USA, Alaska, Alexander Lake | 61°44'38"N, −150°53'31"E | 1 | EMT_61 |
| <i>E. canadensis</i> | USA, Maryland, Conococheague Creek | 39°41'38"N, −77°48'34"E | 2 | EMT_308 |
| <i>E. canadensis</i> | USA, New York, Collins Lake | 42°49'38"N, −73°57'15"E | 6 | EMT_284 |
| <i>E. canadensis</i> | USA, New York, Lake Ontario | 43°18'43"N, −77°43'13"E | 9 | EMT_285 |
| <i>E. canadensis</i> | USA, New York, Niagara River | 43°06'26"N, −78°58'41"E | 1 | EMT_300 |
| <i>E. canadensis</i> | USA, Pennsylvania, Erie See | 42°07'41"N, −80°08'33"E | 1 | EMT_303 |
| <i>E. nuttallii</i> (Planch.) H. St. John | Germany, Baden-Württemberg, Bodensee | 47°44'59"N, 9°07'54"E | 3 | EMT_359 |
| <i>E. nuttallii</i> | Germany, Baden-Württemberg, Brigach | 47°59'51"N, 8°27'48"E | 1 | EMT_32 |
| <i>E. nuttallii</i> | Germany, Baden-Württemberg, Donau | 47°54'57"N, 8°34'9"E | 2 | EMT_30 |
| <i>E. nuttallii</i> | Germany, Baden-Württemberg, Kapuzinergraben | 48°17'26"N, 7°47'46"E | 3 | EMT_354 |
| <i>E. nuttallii</i> | Germany, Baden-Württemberg, Rhein | 48°12'26"N, 7°39'31"E | 14 | EMT_356 |
| <i>E. nuttallii</i> | Germany, Bavaria, Chiemsee | 47°51'49"N, 12°24'58"E | 1 | EMT_40 |
| <i>E. nuttallii</i> | Germany, Bavaria, Ilm | 48°25'58"N, 11°23'55"E | 1 | EMT_35 |
| <i>E. nuttallii</i> | Germany, Bavaria, Kleine Vils | 48°29'8"N, 12°18'18"E | 1 | EMT_36 |
| <i>E. nuttallii</i> | Germany, Bavaria, Starnberge See | 47°54'53"N, 11°17'41"E | 3 | EMT_34 |
| <i>E. nuttallii</i> | Germany, Berlin, Tegeler See | 52°35'33"N, 13°15'44"E | 1 | EMT_274 |
| <i>E. nuttallii</i> | Germany, North Rhine-Westphalia, Baldeneysee | 51°23'56"N, 7°02'56"E | 1 | EMT_208 |
| <i>E. nuttallii</i> | Germany, North Rhine-Westphalia, Hürther Waldsee | 50°52'26"N, 6°50'36"E | 1 | EMT_64 |
| <i>E. nuttallii</i> | Germany, North Rhine-Westphalia, Kemnader See | 51°25'20"N, 7°16'00"E | 4 | EMT_213 |
| <i>E. nuttallii</i> | Germany, North Rhine-Westphalia, Lippe | 51°41'43"N, 7°50'28"E | 1 | EMT_254 |
| <i>E. nuttallii</i> | Germany, North Rhine-Westphalia, Rotbach | 51°34'24"N, 6°47'40"E | 1 | EMT_237 |
| <i>E. nuttallii</i> | Germany, Rhineland-Palatinate, Dreifelder Weiher | 50°35'27"N, 7°49'33"E | 1 | EMT_66 |
| <i>E. nuttallii</i> | Germany, Rhineland-Palatinate, Laacher See | 50°24'48"N, 7°16'20"E | 1 | EMT_69 |

(continues)

APPENDIX 1. (Continued)

| Species | Country, municipality/state, waterbody | Geographic coordinates | No. of ramets | Voucher ^a |
|---------------------|--|--------------------------|---------------|----------------------|
| <i>E. nuttallii</i> | Germany, Rhineland-Palatinate, Rhein | 49°17'9"N, 8°27'21"E | 2 | EMT_65 |
| <i>E. nuttallii</i> | Germany, Rhineland-Palatinate, Schäferweiher | 50°33'40"N, 7°44'01"E | 1 | EMT_68 |
| <i>E. nuttallii</i> | Germany, Saxony, Berzdorfer See | 51°06'13"N, 14°58'35"E | 1 | EMT_45 |
| <i>E. nuttallii</i> | Germany, Saxony, Chausseeteich | 51°24'38"N, 14°47'19"E | 2 | EMT_268 |
| <i>E. nuttallii</i> | Germany, Saxony, Cospudener See | 51°15'37"N, 12°20'19"E | 21 | EMT_252 |
| <i>E. nuttallii</i> | Germany, Saxony, gravel pit Kleinliebenau | 51°22'08"N, 12°11'59"E | 3 | EMT_337 |
| <i>E. nuttallii</i> | Germany, Saxony, gravel pit Kleinpösna | 51°18'35"N, 12°31'45"E | 23 | EMT_104 |
| <i>E. nuttallii</i> | Germany, Saxony, Heideteich | 51°24'36"N, 14°45'33"E | 2 | EMT_23 |
| <i>E. nuttallii</i> | Germany, Saxony, Leipzig | 51°15'45"N, 12°21'21"E | 2 | EMT_321 |
| <i>E. nuttallii</i> | Germany, Saxony, Mulde | 51°31'40"N, 12°36'28"E | 1 | EMT_275 |
| <i>E. nuttallii</i> | Germany, Saxony, Mylau | 50°37'12"N, 12°16'33"E | 1 | EMT_331 |
| <i>E. nuttallii</i> | Germany, Saxony, Parthe | 51°21'40"N, 12°24'28"E | 9 | EMT_266 |
| <i>E. nuttallii</i> | Germany, Saxony, Unterer Oberteich | 51°24'36"N, 14°47'17"E | 3 | EMT_21 |
| <i>E. nuttallii</i> | Germany, Saxony, Weiße Elster | 51°12'19"N, 12°18'16"E | 20 | EMT_234 |
| <i>E. nuttallii</i> | Germany, Saxony, Weiße Schöps | 51°22'57"N, 14°44'01"E | 1 | EMT_335 |
| <i>E. nuttallii</i> | Germany, Saxony, Winterteich | 51°23'16"N, 14°51'42"E | 1 | EMT_267 |
| <i>E. nuttallii</i> | Germany, Saxony, Zwenkauer See | 51°12'57"N, 12°21'15"E | 12 | EMT_261 |
| <i>E. nuttallii</i> | Germany, Saxony-Anhalt, Elbe | 52°02'01"N, 1°52'39"E | 4 | EMT_256 |
| <i>E. nuttallii</i> | Germany, Saxony-Anhalt, Großer Goitzschensee | 51°37'12"N, 12°23'59"E | 43 | EMT_185 |
| <i>E. nuttallii</i> | Germany, Saxony-Anhalt, Mulde | 51°35'44"N, 12°30'03"E | 7 | EMT_204 |
| <i>E. nuttallii</i> | Germany, Saxony-Anhalt, Muldestausee | 51°37'16"N, 12°25'01"E | 15 | EMT_201 |
| <i>E. nuttallii</i> | Germany, Saxony-Anhalt, Raßnitzer See | 51°21'59"N, 12°4'59"E | 19 | EMT_76 |
| <i>E. nuttallii</i> | Germany, Saxony-Anhalt, Saale | 51°34'52"N, 11°48'46"E | 1 | EMT_242 |
| <i>E. nuttallii</i> | Germany, Saxony-Anhalt, Seelhausener See | 51°35'19"N, 12°26'27"E | 9 | EMT_56 |
| <i>E. nuttallii</i> | Peru, Cusco, Oropesa | -13°33'37"N, -71°51'40"E | 1 | EMT_340 |
| <i>E. nuttallii</i> | Purchased online (Nymphaion) | | 4 | EMT_343 |
| <i>E. nuttallii</i> | Slovenia, Podravska, Drava | 46°24'17"N, 16°08'59"E | 1 | EMT_306 |
| <i>E. nuttallii</i> | Slovenia, Oresje | 45°53'11"N, 15°16'07"E | 1 | EMT_305 |
| <i>E. nuttallii</i> | USA, Alaska, Potter Marsh | 61°03'47"N, -149°47'55"E | 1 | EMT_60 |
| <i>E. nuttallii</i> | USA, Maryland, Potomac River | 39°36'27"N, -77°58'08"E | 1 | EMT_310 |
| <i>E. nuttallii</i> | USA, New York, Collins Lake | 42°49'38"N, -73°57'15"E | 1 | EMT_284.2 |
| <i>E. nuttallii</i> | USA, New York, Lake Ontario | 43°21'22"N, -77°56'16"E | 1 | EMT_298 |
| <i>E. nuttallii</i> | USA, New York, Niagara River | 43°03'36"N, -78°58'41"E | 2 | EMT_301 |
| <i>E. nuttallii</i> | USA, Pennsylvania, Lake Erie | 42°07'41"N, -80°08'25"E | 1 | EMT_302 |
| <i>E. nuttallii</i> | USA, Pennsylvania, Quaker Lake | 41°58'39"N, -75°55'08"E | 2 | EMT_314 |
| <i>E. nuttallii</i> | USA, Virginia, Gunston Cove | 38°40'30"N, -77°09'20"E | 3 | EMT_311 |

^aVouchers are deposited at the Helmholtz Centre for Environmental Research, Permoserstraße 15, 04318 Leipzig, Germany.