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Development of polymorphic microsatellite markers for a rare dragonfly, *Cordulegaster sarracenia* (Odonata: Cordulegastridae), with notes on population structure and genetic diversity

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We isolated and characterized a total of 13 microsatellite loci from *Cordulegaster sarracenia* (Odonata: Cordulegastridae). Loci were screened in 24 individuals from Louisiana and Texas. Within *C. sarracenia*, the number of alleles per locus ranged from 0 to 5, and observed and expected heterozygosities ranged from 0.000 to 0.556 and 0.000 to 0.613, respectively. Overall differentiation among study populations was very high ($F_{ST} = 0.423$), suggesting significant geographic population structure with low diversity within populations. Twelve of the 13 primers amplified in *C. sayi*, *C. diastatops*, *C. maculata*, and *C. obliqua* and polymorphism levels are reported. These new genetic markers will provide tools for addressing a number of population genetic and demographic questions relating to conservation of this rare dragonfly species.

Keywords: Illumina; PCR primers; SSR; Texas; Louisiana; F_{ST}; heterozygosity

Introduction

The family Cordulegastridae currently contains about 55 species in three genera and is Holarctic with two species, *Cordulegaster diadema* Selys and *C. virginiae* Novelo-Gutiérrez, extending into Central America (Novelo-Gutiérrez, 2018). All 10 North American species are currently placed in *Cordulegaster*, of which five closely related species make up the *diastatops*-group (*C. bilineata* (Carle), *C. diastatops* (Selys), *C. sarracenia* Abbott & Hibbitts, *C. sayi* Selys and *C. talaria* Tennessen). Habitat of the nymphs of the *diastatops*-group can be characterized as small woodland streams and seepages throughout the eastern USA (Needham, Westfall, & May, 2014). Adults fly in adjacent habitats to the stream. Three species, *C. sarracenia*, *C. sayi*, and *C. talaria* have restricted ranges (9400 km^2, 55,500 km^2, and 1400 km^2, respectively) as compared to the more widespread *C. bilineata* and *C. diastatops* that have ranges extending throughout the eastern USA. This is presumably a result of either narrow habitat requirements or habitat loss.

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Abbott and Hibbitts (2011) described *C. sarracenia* from southeast Texas and western Louisiana where it has similar habitat requirements as the pitcher plant, *Sarracenia alata* (Alph. Wood), for which it was named. *Cordulegaster sarracenia* was overlooked for years presumably because of its very early flight season (15 March to 29 April; Abbott, 2015a), its habitat requirements and its general rarity. *C. sarracenia* is found in small rivulets of water among large swampy bog systems that are generally very difficult to navigate. Given the limited range of *C. sarracenia*, there is federal and state concern that the species is potentially imperiled and is thus a good candidate for conservation measures (Abbott, 2015b). Population genetic data can be critical for informing the conservation of threatened species (Laikre et al., 2010), but such data are lacking for *C. sarracenia* and many of its relatives. To incorporate genetic data into the design of appropriate conservation measures for *C. sarracenia*, we collected tissue samples from across its range to develop novel microsatellite loci and perform preliminary characterization of geographic population structure and genetic diversity of this rare species.

Methods

Total DNA was extracted from whole thoracic muscle tissue from one adult *C. sarracenia* using a modified Qiagen DNeasy extraction (Lozier, 2014) for use in isolation of microsatellite loci. Forty-eight primer pairs were tested for amplification and polymorphism using methods described in O'Bryhim et al. (2013). Briefly, a paired-end dual-indexed Illumina sequencing library was prepared and sequenced on an Illumina HiSeq 3000 with 150 bp paired-end reads. Five million reads were analyzed to search for 2-6mer repeat motifs with PAL_FINDER v 0.02.04 (Castoe et al., 2012) primers were designed for 48 candidate loci using Primer3 2.0.0

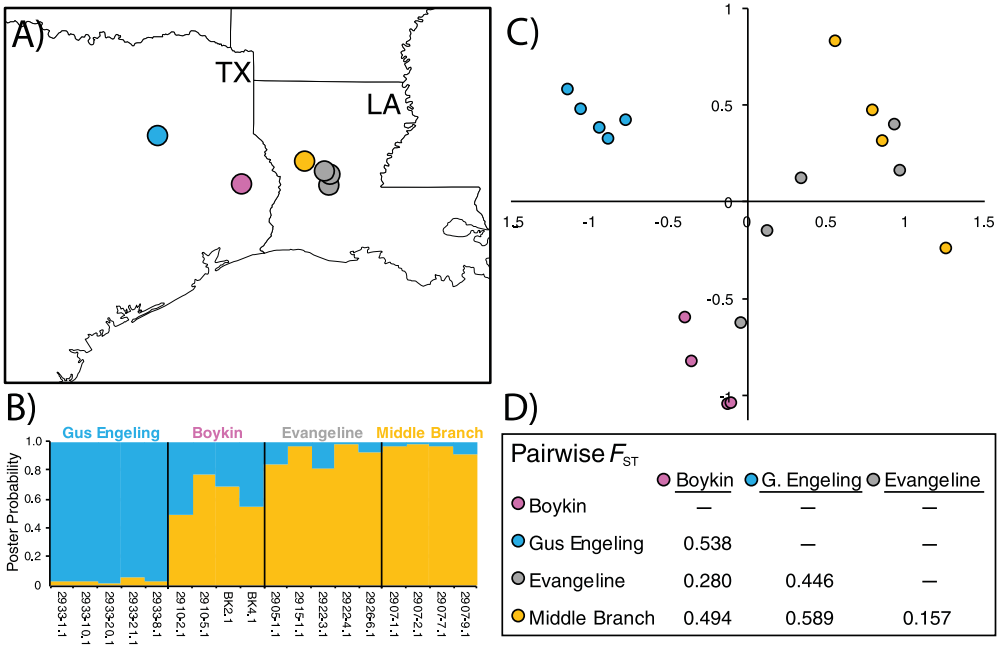

Figure 1. Evaluation of population genetic structure in *Cordulegaster sarracenia*. (A) Map of sampled localities; (B) STRUCTURE plot showing assignment probability of each genotype to $K = 2$ clusters; (C) a principal coordinates analysis on a standardized covariance matrix from individual codominant genotypic distances (conducted in GENALEX) from four populations; (D) a table of pairwise F_{ST} (all significant at $P < 0.005$).

Table 1. Details for 13 microsatellite loci developed for *Cordulegaster sarracenia*, with repeat motif and size of repeat region detected in the *C. sarracenia* sample used for locus development. Size range indicates the range of observed alleles in base pairs (including length of the primer tags); number of individuals genotyped is N; k is number of alleles observed; H_o and H_e are observed and expected heterozygosity, respectively; HWE P is the significance of deviations from HWE (without Bonferroni correction); P_{ID} is the probability of identity for each locus. For analyses presented here, all loci were amplified with the TD65 touchdown protocol (see text).

Locus	5′-3′ Primer sequence[a]	Repeat motif (repeat bp)	Size range (bp)	N	k	H_o	H_e	HWE P[b]	P_{ID}[c]
Cosa19	F: *CTTGTCGGAATTTCGTGTCG R: *GGGAAAAGTGAGTTGAATAACGC	AGGG(28)	157–165	17	2	0.000	0.291	0.001	0.545
Cosa23	F: *CCCCTTTCTCCCCATCTACC R: *TTTTGGCACGAGAAAAGACG	TTTC(28)	233–237	18	2	0.111	0.475	0.001	0.388
Cosa27	F: *GCGGTGAGATGTGAGAGGG R: *TTCCTTTGGGAAATCAACGC	TTTC(32)	221–225	18	2	0.111	0.475	0.001	0.388
Cosa28	F: *CTGCACGTGAATCATTTCCC R: *ACTTGCACCTCGTAAGTGGG	ATAG(28)	201–213	17	3	0.412	0.455	ns	0.377
Cosa29	F:*ATTTGTTGACACAGATGCGG R: *AATTTTCCTGAATTTTCTGAACG	AAAT(32)	371–395	18	5	0.556	0.613	ns	0.221
Cosa30	F: *CATACGTGCATGTGTGGGC R: *CACGCACACGAGTTCAAACC	ATGT(44)	457–469	18	3	0.278	0.245	ns	0.589
Cosa31	F: *AATTTTCCGTCTTCCTCCCC R: *GCTTATGTTAGGACCAGGAATGC	AAAT(28)	342	18	1	0.000	0.000	–	1.000
Cosa36	F: *TCCTCTCCCACCTCATTTCC R: *TGTGGTATTGGAGGGTGTCG	TCCC(28)	258	18	1	0.000	0.000	–	1.000
Cosa4	F: *CATTGTGCTGTTTTGGGACG R: *GTCACACAGTTTTGGCGACC	ATTT(28)	197	18	1	0.000	0.000	–	1.000
Cosa42	F: *ACACTGTGGCAGAAAGAGCG R: *AAGAGAGCGAAGGATATGGGC	ATGT(28)	200–212	18	2	0.389	0.313	ns	0.521
Cosa43	F:*CGTGATATTTGACAAGAATAAACTACCC R: *TGTAATCGCGGAAACATTCG	TCTG(36)	290–298	18	2	0.111	0.494	0.001	0.378
Cosa8	F:*CCATCGGGTATAACAGACCCC R: *CGAGTAATTTCGCTATCTAATATATCAAGG	ATGT(36)	338–342	13	2	0.538	0.500	ns	0.375
Cosa9	F: *TGTAAACGAAAGGAGCAACCG R: *AAGGGTACCATGGAAGTGGC	ATGT(28)	341	18	1	0.000	0.000	–	1.000

[a]The asterisks indicate CAG tag (5′-CAGTCGGGCGTCATCA-3′) label on F primer and 5′-GTTT-3′ pigtail on R primer.
[b] P-values for deviations from Hardy–Weinberg expectations; ns = not significant.
[c] Total probability of identity $= 3 \times 10^{-4}$ across variable loci.

(Untergasser et al., 2012), searching for primers with an optimal size of 20 bp (minimum 18 bp, maximum 30 bp), optimal melting temperature of 62°C (range 58–65°C) with a maximum 2°C difference between pairs, product size range of 60–500 bp, a GC content of 30–80%, GC clamp = 2, and maximum number of Gs or Cs allowed in the last five 3′ bases = 5, and maximum monopolymer length = 5. Microsatellite primers were modified with universal 5′-CAG tag (5′-CAGTCGGGCGTCATCA-3′) on "forward" primers for fluorescently labeled third primer amplification and a 5′-GTTT pigtail on "reverse" primers to improve allele-scoring consistency. Of these, we assessed the variability of 13 consistently amplifying tetranucleotide repeat loci in 24 *Cordulegaster* specimens: 18 *C. sarracenia* collected across all known populations (Figure 1A), and exemplars from four additional species (one *C. sayi*, one *C. diastatops*, one *C. obliqua*, and three *C. maculata*). Polymerase chain reactions used conditions and TD65 thermocycling as described in O'Bryhim et al. (2013). Reactions were performed individually for each primer pair, including a universal fluorescently labeled third primer (identical to the CAG tag on forward primers), in 12.5 μl volumes using the following concentrations: 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 0.5 U AmpliTaq Gold® Polymerase (Applied Biosystems, Foster City, CA, USA), 0.4 µM reverse primer, 0.04 µM forward primer, 0.36 µM universal dye-labeled primer (CAG-fam), 0.3 mM $MgCl_2$, 0.8 mM dNTPs, 25µg ml^{-1} BSA, and 20 ng of DNA template. The TD65 thermocycling conditions consisted of 95°C for 5 min; 20 0.5°C annealing temperature touchdown cycles of 95°C for 30 s, 65–55°C for 30 s, and 72°C for 30 s; 20 standard cycles of 95°C for 30 s, 55°C for 30 s, and 72°C for 30 s; and a final extension step of 72°C for 30 s). Products were visualized for fragment size analysis using an ABI-3130xl sequencer. Characteristics of the loci are provided in Table 1.

We tested for linkage disequilibrium and deviations from Hardy–Weinberg equilibrium using GENEPOP v 4.0 with default settings (Rousset, 2008). We performed most other statistical analyses in GENALEX 6.503 (Peakall & Smouse, 2012), including calculation of summary statistics, F_{ST} (significance tested with 999 permutations), probability of identity (P_{ID}) for each locus and overall, and principal coordinates analysis on a covariance matrix of pairwise individual genotypic distances. We also performed individual genotype clustering using STRUCTURE v2.3.4 (Falush, Stephens, & Pritchard, 2003) with 100,000 burn-in steps followed by 500,000 samples and other parameters as default (allowing for correlated allele frequencies and admixture). We investigated a range of values for the putative number of STRUCTURE clusters (K) from 2 to 5, however only report results for $K = 2$ as higher values were uninformative (e.g. for all larger K the Gus Engeling genotypes formed one strong cluster while remaining genotypes were each split evenly into the remaining clusters).

Results and discussion

We present a set of novel microsatellite loci developed for the dragonfly *C. sarracenia* and tested across several congeners, representing the first microsatellites developed for the genus *Cordulegaster*. Nine of the 13 most consistently amplifying loci were polymorphic within the *C. sarracenia* screening panel, with the remainder being polymorphic across species. Within *C. sarracenia*, expected (H_e) and observed (H_O) heterozygosities were generally low across loci, with ranges of 0.000–0.613 and 0.000–0.556, respectively, with 0–5 alleles per locus (k). More complete sampling of *C. sarracenia* populations is needed to fully characterize allelic variation at these loci; however, considering the low H_e detected even for polymorphic microsatellites, low genetic diversity may be a general feature of *C. sarracenia* and not a locus-specific concern. Such low diversity is not necessarily surprising for this species, which is rare across a small and fragmented geographic range, and other rare odonates have been shown to exhibit low

genetic diversity, especially in isolated populations (Monroe & Britten, 2014; Watts, Saccheri, Kemp, & Thompson, 2006). Four of the nine variable loci deviated significantly from Hardy–Weinberg equilibrium in *C. sarracenia* and linkage disequilibrium was detected in one locus pair following Bonferroni correction (Cosa23–Cosa27). Such deviations, together with incomplete amplification of some loci (e.g. Cosa8), could be explained in part by the presence of null alleles (e.g. Watts, 2009), but may also be explained by strong population structure in the samples used for initial screening.

To evaluate such structure and test the utility of the markers to address common population genetic questions, we determined genetic differentiation using F_{ST}. Global F_{ST} among study populations was indeed high (0.423; $P \leq 0.001$) as were all pairwise F_{ST} values (all significant at $P \leq 0.005$) (Figure 1). In part, these high values reflect the low diversity in *C. sarracenia* and small sample size, but overall support strong geographic isolation among fragmented habitat patches that make up the range of this species, as illustrated by clustering of individual genotypes in STRUCTURE and principal coordinates analyses (Figure 1B, C). The westernmost population (Gus Engeling Wildlife Management Area; Tennessee Colony, Texas) is especially distinct, which is consistent with its spatial separation and disjunct habitat relative to other known *C. sarracenia* populations.

Other studies have investigated the population genetics of dragonfly species that are more globally distributed and found little to no genetic population structure (Alvial, Veliz, Vargas, Esquivel, & Vila, 2017; Troast, Suhling, Jinguji, Sahlén, & Ware, 2016). However, a study on a damselfly found that large distances (> 24 km) presented a significant enough barrier to create population structure and isolation of some populations (Lorenzo-Carballa et al., 2015). Dragonflies are capable of flying longer distances than most damselflies; therefore, distance alone may not be as much of a geographic barrier. In *Somatochlora hineana* (endangered according to US Fish and Wildlife Service, 2001), Monroe and Britten (2014) found that no genetic isolation was observed at relatively small scales (< 69 km), but distinct population structuring was observed for samples separated by larger distances (214 km). The genetic distinctiveness of the Gus Engeling, TX population in our data reinforces that separation by distances greater than 200 km can likewise produce genetic isolation in *C. sarracenia* (Figure 1). Interestingly, the Boykin, TX population is separated from the Louisiana populations by 167 km, yet appears genetically mixed between western and eastern genotypes (Figure 1B, C). We suspect that there are undiscovered populations between Boykin Springs and the known breeding populations in Louisiana. Breeding populations connecting Boykin Spring with Gus Engeling are unlikely, but there is clearly still some genetic affinity between dragonflies in these two populations (Figure 1B). These results demonstrate that, even with a small sample of only 18 dragonflies, these novel markers can reveal significant population structure that matches the spatial distribution of the samples, and that more comprehensive sampling should have sufficient power to detect more complex population genetic patterns.

We also investigated the potential utility of the novel microsatellites in other *Cordulegaster* species. Our multispecies screen demonstrates that subsets of these microsatellite markers can be applied to different *Cordulegaster* species (Table 2). All loci in the five species had polymorphic alleles in the expected ranges (four base pair motifs). Interestingly, although microsatellites were isolated from *C. sarracenia*, the four monomorphic loci within *C. sarracenia* were polymorphic within at least one of the other species (*C. sayi*, *C. disatatops*, *C. maculata*, or *C. obliqua*) despite the far more limited screening sets. These new microsatellites may prove especially useful for comparing patterns of structure among the rare *Cordulegaster* species like *C. sarracenia*, *C. sayi*, and *C. talaria* against more widespread species in the group.

Finally, for species of conservation concern, non-lethal sampling is often desirable (e.g. DNA isolated from tarsal clips) (Fincke & Hadrys, 2001; Hadrys, Schroth, Schierwater, Streit, & Fincke, 2005; Watts, Thompson, Daguet, & Kemp, 2005). For data in this study, amplifications

Table 2. Details for cross-species amplification for 13 microsatellite loci developed for *Cordulegaster sarracenia*. The species and sample size are provided with the fraction of samples for which each locus amplified. Loci that did not amplify are indicated with – and PCR product size is in parentheses after each fraction.

	C. sayi (1)	*C. diastatops* (1)	*C. maculata* (3)	*C. obliqua* (1)
Cosa19	1/1 (141)	1/1 (141–149)	3/3 (137)	–
Cosa23	1/1 (221)	–	2/3 (213–237)	–
Cosa27	–	1/1 (205)	–	1/1 (213)
Cosa28	–	–	2/3 (185)	–
Cosa29	–	–	–	–
Cosa30	–	–	1/3 (465)	–
Cosa31	–	1/1 (318)	2/3 (318–342)	1/1 (306 –310)
Cosa36	1/1 (258)	1/1 (242–258)	3/3 (254–270)	1/1(238–242)
Cosa4	1/1 (193)	1/1 (193)	1/3 (169)	1/1 (193)
Cosa42	1/1 (200–204)	1/1 (196)	3/3 (192)	1/1 (180)
Cosa43	–	–	3/3 (278)	–
Cosa8	1/1 (318)	1/1 (318)	–	–
Cosa9	1/1 (341)	1/1 (325–333)	–	–

were initially optimized for large amounts of DNA ($\sim 100\,\text{ng}\,\mu\text{l}^{-1}$) isolated from thoracic muscle. However, our goal is ultimately to conduct our full study of *C. sarracenia* using non-lethal techniques, and we have subsequently tested primers on DNA isolated from tarsal clips (as low as $0.6\,\text{ng}\,\mu\text{l}^{-1}$) from nymphs and adults. Our initial tests suggest that PCR amplification for all primers performs well for these much lower DNA template conditions by modifying thermocycling protocols to slightly less stringent conditions (e.g. a TD58 touchdown protocol similar to the TD65 protocol in Methods but with a 58–48°C touchdown; modified from Cerqueira-Silva et al., 2014). Individual labs will likely have to re-optimize PCR conditions depending on the exact amount and nature of template material; however, our preliminary assessments suggest that these microsatellite markers will be useful for DNA templates of varying concentration and quality.

In conclusion, preliminary population characterization of the novel microsatellites presented here suggests these markers will aid larger-scale conservation genetic studies of *C. sarracenia.* Despite overall low variation, the low total P_{ID} across loci (3×10^{-4}) and detection of significant geographic population structure even with limited sampling, suggest that these novel loci will be powerful tools for addressing a number of population genetic and demographic questions relating to conservation of this rare dragonfly species. Future analysis will be necessary to look at sibling/cohort structure within the population, given that nymphs were often collected from the same area and could be siblings. The next steps are to analyze the entire range of *C. sarracenia*, sampling a larger number of individuals to get a more accurate characterization of geographic population structure and genetic diversity of this rare species.

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References

Abbott, J. C. (2015a). *Dragonflies of Texas: A field guide*. Austin, TX: University of Texas Press.

Abbott, J. C. (2015b). *Distribution and conservation status for two rare odonates in Southeast Texas*. Austin, TX: Texas Parks and Wildlife Report for Wildlife Conservation Grant.

Abbott, J. C., & Hibbitts, T. D. (2011). *Cordulegaster sarracenia*, n. sp. (Odonata: Cordulegastridae) from East Texas and Western Louisiana, with a key to adult Cordulegastridae of the New World. *Zootaxa,* 2899, 60–68. http://www.mapress.com/j/zt/article/view/11215

Alvial, I., Veliz, D., Vargas, H., Esquivel, C., & Vila, I. (2017). Lack of genetic structure in *Pantala flavescens* among Central and South American localities (Odonata:Libellulidae). *Odonatologica*, *46*(1/2), 67–82. doi:10.5281/zenodo.572357

Castoe, T. A., Poole, A.W., de Koning, A. P. J., Jones, K. L., Tomback, D. F., Oyler-McCance, S. J., Fike, J. A., Lance, S. L., Streicher, J. W., Smith, E. N., & Pollack, D. D. (2012). Rapid microsatellite identification from Illumina paired-end genomic sequencing in two birds and a snake. *PLoS ONE*, *7*(2), e30953. doi.org/10.1371/journal.pone.0136465

Cerqueira-Silva, C. B. M., Santos, E. S. L., Vieira, J. G. P., Mori, G. M., Jesus, O. N., Corrêa, R. X., & Souza, A. P. (2014), New microsatellite markers for wild and commercial species of *Passiflora* (Passifloraceae) and cross-amplification. *Applications in Plant Sciences*, *2*: 1300061. doi:10.3732/apps.1300061

Falush, D., Stephens, M., & Pritchard, J. K. (2003). Inference of population structure using multilocus genotype data: linked loci and correlated allele frequencies. *Genetics*, *164*:1567–1587. http://www.genetics.org/content/164/4/1567

Fincke, O. M. & Hadrys, H. (2001). Unpredictable offspring survivorship in the damselfly, *Megaloprepus coerulaus*, shapes parental behavior, constrains sexual selection, and challenges traditional fitness estimates. *Evolution*, *55*, 762–772. doi:10.1111/j.0014-3820.2001.tb00812.x

Hadrys, H., Schroth, W., Schierwater, B., Streit, B., & Fincke, O. M. (2005). Tree hole odonates as environmental monitors: Non-invasive isolation of polymorphic microsatellites from the neotropical damselfly *Megaloprepus caerulatus*. *Conservation Genetics*, *6*, 481–483. doi:10.1007/s10592-005-4971-5

Laikre, L., Allendorf, F. W., Aroner, L. C., Baker, C. S., Gregovich, D. P., Hansen, M. M., Jackson, J. A., Kendall, K. C., McKelvey, K., Neel, M. C., Olivieri, I., Ryman, N., Schwartz, M. K., Bull, R. S., Stetz, J. B., Tallmon, D. A., Taylor, B. L., Vojta, C. D., Waller, D. M., & Waples, R. S. (2010). Neglect of genetic diversity in implementation of the convention on biological diversity. *Conservation Biology*, *24*, 86–88. doi:10.1111/j.1523-1739.2009.01425.x

Lorenzo-Carballa, M. O., Ferreira, S., Sims, A. M., Thompson, D. J., Watts, P. C., Cher, Y., Damoy, V., Evrard, A., Gelez, W., & Vanappelghem, C. (2015). Impact of landscape on spatial genetic structure and diversity of *Coenagrion mercuriale* (Zygoptera:Coenagrionidae) in northern France. *Freshwater Science*, *34*, 1065–1078. doi.org/10.1086/682687

Lozier, J. D. (2014). Revisiting comparisons of genetic diversity in stable and declining species: assessing genome-wide polymorphism in North American bumble bees using RAD sequencing. *Molecular Ecology*, *23*, 788–801. doi:10.1111/mec.12636

Monroe, E. M., & Britten, H. B. (2014). Conservation in Hine's sight: the conservation genetics of the federally endangered Hine's emerald dragonfly, *Somatochlora hineana*. *Journal Insect Conservation*, *18*, 353–363. doi:10.1007/s10841-014-9643-7

Needham, J. G., Westfall, M. J., Jr., & May, M. L. (2014). *Dragonflies of North America* (3rd ed.). Gainesville, FL: Scientific Publishers.

Novelo-Gutiérrez, R. (2018). *Cordulegaster virginiae* sp. nov. from Mexico, including a comparison with *C. diadema* Selys, 1868, and a redescription of its larva (Odonata: Cordulegastridae). *Zootaxa*, *4394*, 371–382. https://doi.org/10.11646/zootaxa.4394.3.3

O'Bryhim, J., Somers, C., Lance, S. L., Yau, M., Boreham, D. R., Jones, K. L., & Taylor, E. B. (2013). Development and characterization of twenty-two novel microsatellite markers for the mountain whitefish, *Prosopium williamsoni* and cross-amplification in the round whitefish, *P. cylindraceum*, using paired-end Illumina shotgun sequencing. *Conservation Genetic Resources*, *5*, 89–91. http://doi:10.1007/s12686-012-9740-4

Peakall, R., & Smouse, P. E. (2012). GenALEx 6.5: Genetic analysis in Excel. Population genetic software for teaching and research-an update. *Bioinformatics*, *28*, 2537–2539. doi:10.1093/bioinformatics/bts460

Rousset, F. (2008). GENEPOP'007: a complete re-implementation of the GENEPOP software for Windows and Linux. *Molecular Ecology Research*, *8*, 103–106. doi:10.1111/j.1471-8286.2007.01931.x

Troast, D., Suhling, F., Jinguji, H., Sahlén, G., & Ware, J. (2016). A global population genetic study of *Pantala flavescens*. *PLoS ONE*, *11*(3), e0148949. doi:10.1371/journal.pone.0148949

Untergasser, A., Cutcutache, I., Koressaar, T., Ye, J., Faircloth, B. C., Remm, M., & Rozen, S. G. (2012). Primer3 – new capabilities and interfaces. *Nucleic Acids Research*, *40*(15):e115. doi:10.1093/nar/gks596

US Fish and Wildlife Service. (2001). *Hine's emerald dragonfly (*Somatochlora hineana*) recovery plan*. Fort Snelling, MN: US Fish and Wildlife Service.

Watts, P. C. (2009). Characteristics of microsatellite loci in Odonata. *International Journal of Odonatology*, *12*(2), 275–286. doi:10.1080/13887890.2009.9748345

Watts, P.C., Saccheri, I. J., Kemp, S. J., & Thompson, D. J. (2006). Population structure and the impact of regional and local habitat isolation upon levels of genetic diversity of the endangered damselfly *Coenagrion mercuriale* (Odonata:Zygoptera). *Freshwater Biology*, *51*, 193–205. doi:10.1111/j.1365-2427.2005.01478.x

Watts, P. C., Thompson, D. J., Daguet, D., & Kemp, S. J. (2005). Exuviae as a reliable source of DNA for population-genetic analysis of odonates. *Odonatologica*, *34*, 193–187.