



Developing a Standard Operating Procedure for Species Identifications of Dragonfly Larvae Using DNA Barcoding

A Contribution of the Dragonfly Mercury Project

Natural Resource Report NPS/NRSS/ARD/NRR—2022/2362





ON THIS PAGE

Prepared dragonfly tissue samples begin the extraction process prior to DNA amplification and sequencing.
Image credit Sarah Nelson

ON THE COVER

Co-authors M. Marion and M. Little prepare samples for DNA barcoding in the laboratory.
Image credit Sarah Nelson

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Executive Summary

Identification of larval dragonflies can be challenging even for experts, especially when larvae are in their earlier life stages (Tennessen 2019). DNA barcoding provides a promising new approach to species identification, where the DNA sequences of difficult-to-identify larval dragonflies are matched to a public, global DNA database based on adult dragonflies that are more often studied and collected, and more easily identified with formal taxonomy. The outcome is a species identification inferred solely from its DNA. Here we used DNA barcoding to identify species of individual dragonfly larvae that were collected as part of the Dragonfly Mercury Project (DMP), a citizen engagement initiative that enables long-term monitoring and research documenting mercury contamination in national parks and other protected lands nationwide. The DMP is currently managed by the National Park Service (NPS) and US Geological Survey (USGS), with collaborative engagement from the Appalachian Mountain Club (AMC) and Dartmouth College. Over the past 10 years, the project has assessed mercury concentrations in dragonfly larvae in more than 500 waterbodies across more than 100 U.S. national parks and other federal, state, and local protected areas. Citizen scientists collect dragonfly larvae from freshwaters in park units around the U.S., under supervision of park or partner staff, who may or may not have scientific backgrounds.

Our interpretation of mercury concentrations determined in the DMP is hindered by the potentially confounding influence of dragonfly species identity. Specifically, there are important differences among species in three key aspects that influence mercury bioaccumulation: 1) diet, which directly controls the magnification of mercury with increasing trophic level; 2) the length of aquatic life stage, which influences the lifetime accumulation of mercury; and 3) habitat preference.

Traditionally, identification to the genus or species level relies on a skilled, expert taxonomist who uses dichotomous keys and other relevant literature to visually identify specimens. In this project, we identified dragonfly larvae to the finest taxonomic level possible (genus or species) in 2013–2014, with visually-based identification by a taxonomist in 2013 and DNA barcoding in 2014. We developed DNA barcoding methodology and produced an SOP (Standard Operating Procedure) to allow the process to be replicated in other studies. Here we present results of these analyses and the detailed SOP for use in future work. In brief:

- We identified 61 unique dragonfly species, representing the six major dragonfly families in North America, from a sample set of 401 individual dragonfly larvae (Figure E-1), collected from 84 distinct freshwater sites.
- Visual identification resulted in 100% identification to genus, and further 59% identification to species. For DNA barcoding, of the 54% of samples that produced a DNA sequence, 87% were identified to species. Thus, both methods produced similar overall percentages of species identities; visual identification was more likely to produce identification to at least the genus level for all samples, but DNA barcoding had a greater success rate moving from genus- to species-level identification when DNA sequences were produced.

- The Standard Operating Procedure (Little et al. 2021) can be used and refined as the field of DNA barcoding progresses and associated DNA databases are expanded, to identify new specimens. This method was promising as 97–100% of the DNA-based species identities were corroborated by visual identifications.

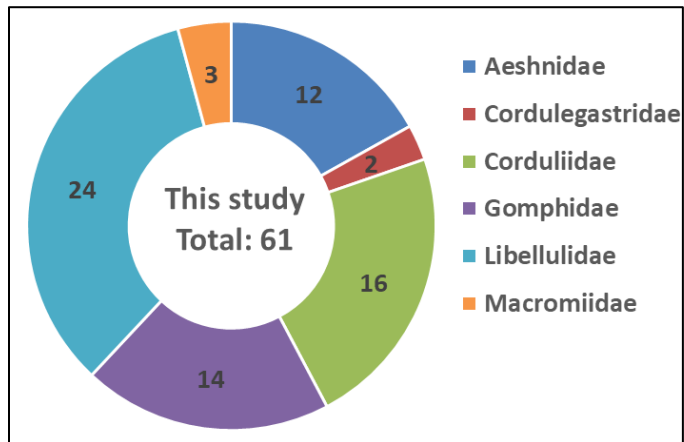


Figure E-1. The total number of dragonfly (Odonata: Anisoptera) species identified in this study, shown as proportion of species by family.

Acknowledgments

Funding for the Dragonfly Mercury Project through the National Park Service Natural Resource Fund Source (NRFS) supported taxonomic identification in 2013. University of Maine faculty research funds to Sarah Nelson and Hamish Greig supported the DNA barcoding effort. The Senator George J. Mitchell Center for Sustainability Solutions and The MDI Biological Laboratory provided laboratory space and support for this project. We thank Katherine Ko of the Appalachian Mountain Club and National Park Service for assistance with data coordination and report formatting. Two peer reviewers representing both the NPS and the University of Colorado – Boulder provided constructive feedback that improved this report. The United States Geological Survey and the National Park Service are core collaborators in the Dragonfly Mercury Project, which provided the dragonfly samples used in this research. Staff from the NPS, its partners, and over 824 citizen scientists collected samples across the US in 2014.

Bronco Quick provided invaluable assistance beyond the taxonomic identifications he contributed to the project dataset: Mr. Quick assisted with pilot efforts that were the seed of the Dragonfly Mercury Project; he provided background information, connections with Odonate scientists, consultations on dragonfly ecology and behavior, and a huge collection of preserved specimens and exuviae used as a teaching tool for the project PI and some participating parks. His depth of natural history knowledge and interest in fostering understanding and appreciation of dragonflies and damselflies were crucial to this project and the broader DMP.

Introduction

In biodiversity science, the difficulty of identifying organisms, compounded by a dearth of taxonomic expertise, the “taxonomic impediment,” limits the capacity of research (e.g. ecology, evolution and systematics) and its applications (e.g. conservation) (CBD 2008). The taxonomic impediment is compounded in the context of citizen science. Projects that engage citizen scientists in the identification of biological specimens, such as the DMP or ‘bioblitzes’ (Ruch et al. 2010), are limited by the amount of time and training required for participants to gain the skills they need to identify species. These projects usually rely heavily on taxonomic experts in the laboratory after collection, or in the field during bioblitzes, to both supervise participants while they identify collected specimens and also to validate and complete these identifications. These limitations impose a bottleneck, the “specimen identification bottleneck,” in the scientific process that can preclude the involvement of citizen scientists in subsequent steps of scientific investigations like analysis and interpretation.

The Dragonfly Mercury Project (DMP) is one such citizen science effort that provides data on where and how much mercury contamination is affecting national parks through the collection and analysis of dragonfly larvae for mercury (Eagles-Smith et al. 2020). This project also connects people to parks, creates biodiversity discovery opportunities, and advances the National Park Service (NPS) educational mission (Flanagan Pritz and Nelson 2017, Watkins et al. 2018). More than 100 park units and 5,000 citizen scientists have participated in the DMP since its inception (go.nps.gov/dragonflymercury). The DMP’s dragonfly larvae biosentinels have been used to assess relative risk among waterbodies within and across parks at the continental scale (Eagles-Smith et al. 2020). Further, the data are being used to test hypotheses about whether and how mercury concentrations vary with dragonfly larvae body size, taxonomic family, or a collection site’s landscape characteristics. Significant differences were observed among the six taxonomic families of dragonfly larvae collected in the project, but these family differences could be statistically modeled and addressed. Even within a family, dragonfly larvae can vary widely in ecological characteristics, including feeding strategy and diet, length of time in the aquatic phase, and habitat preferences. Thus, the project team sought to explore methodology by which we could determine finer taxonomic resolution to the species level.

For dragonfly larvae, the taxonomic impediment is immense. There are few experts in identification; only recently has a definitive text with identification of the larval (nymph) stage been released (Damm et al. 2010, Tennessen 2019). However, their charismatic and brightly-colored adult forms are well-studied by enthusiastic scientists and volunteers, and are more easily identified than the juvenile form. Because of this great interest in adult dragonflies, many voucher specimens exist in collections worldwide, including those in databases that catalog genetic sequences. Thus, we proposed that DNA-assisted species identification (“DNA barcoding”) could be employed in the DMP to help extend taxonomic expertise to empower researchers using citizen-collected specimens to identify organisms (Hebert et al. 2005, Ellis et al. 2010). DNA barcoding involves obtaining the sequence from (“sequencing”) a short stretch of a specimen’s DNA (part of the mitochondrial *COI* gene for animals, or parts of the chloroplast genes *rbcL* and *matK* for plants) from a biological

specimen. This query sequence is then compared to a reference library of *COI* or *rbcL* and *matK* sequences (“DNA barcodes”) linked to voucher specimens that have been identified by professional taxonomic specialists. The outcome is a DNA-assisted species identification, that is, a species-level name for the query specimen, inferred solely from its DNA.

DNA barcoding of insects has been used to address the taxonomic impediment, for example identifying species present in a geographic area (e.g., Koroiva et al. 2017, Galimberti et al. 2021), but also to investigate species interactions and trophic relationships, and in applied science such as agricultural and epidemiological investigations (Jinbo et al. 2011). An important benefit of this methodology is that the amount of tissue needed for analysis is small, so non-lethal sampling is feasible. For rare species, wing clips, a subset of legs (which later regenerate), or even exuviae can be used successfully (butterflies: Hamm et al. 2010, Marschalek et al. 2013; bees: Châline et al. 2004, Oi et al. 2013), although living tissues provided more reliable results than exuviae in one study (Ožana et al. 2020). Drawbacks of DNA barcoding include difficulties determining species identity for very close relatives or for species whose reference sequences are not yet present in the Barcode of Life Data Systems (BOLD) or other databases (Damm et al. 2010, Galimberti et al. 2021).

DNA-assisted species identification has the potential to combine the research-boosting opportunities of DNA barcoding and citizen science. However, when this project was envisioned, the specific methodological details around sample preparation, extraction, and amplification for DNA barcoding dragonfly larvae had not yet been determined. Further, identification using traditional visual (morphological) methods by a project taxonomist was already underway for one project year. Thus, this project had three primary goals: 1) identify representative dragonfly larvae samples from each national park using visual methods; 2) develop detailed methodology (SOP, or Standard Operating Procedure) that fine-tuned the DNA barcoding process for dragonfly larvae; 3) assess relative strengths and weaknesses of both identification methods: traditional visual methods and DNA barcoding. In this report, we describe and compare results of both visual identifications and DNA barcoding. Detailed methods we developed are available in the accompanying SOP (Little et al. 2021).

Methods

Identifying representative dragonfly larvae

We employed two techniques to identify dragonfly larvae to genus and/or species level: (1) a trained, specialist taxonomist using a visual identification method relying on morphology, taxonomic keys, and literature, and (2) DNA barcoding. The taxonomist was Mr. Bronco Quick, who had previously been employed in identification of specimens for other projects, including the Maine Damselfly and Dragonfly Survey (<https://www.maine.gov/ifw/fish-wildlife/wildlife/species-information/invertebrates/damselfly-dragonfly.html>), and funded research at the University of Maine. Mr. Quick identified specimens that had been frozen as part of the sample set for the Dragonfly Mercury Project (DMP), and after thawing, preserved specimens in isopropyl alcohol or ethanol. Taxonomic identifications were made by visual inspection, comparing to published identification materials, reference textbooks, and taxonomic keys. For this project, we piloted and developed a protocol for DNA-assisted species identification through DNA barcoding. The process involves tissue preparation, then extracting, amplifying, and sequencing a number of base pairs of the *COI* gene from an unknown specimen and comparing it to a reference library of *COI* sequences from previously identified specimens. Assembling this reference library from newly-identified specimens collected in the project of interest and/or from publicly-available sequences is the starting point of any DNA barcoding project.

DNA Barcoding Protocol Development

In the last decade, over three million reference DNA barcoding sequences from specimens of known species have been deposited in the Barcode of Life Data Systems (BOLD) database. We first identified Odonate species known in our 2013 visually-identified dataset to query the BOLD database to determine the extent of the existing reference library. When species sequences were not found in the existing records, we consulted additional references and online databases (Table 1) to determine whether species or genus nomenclature had been updated and verified the alternate nomenclature in BOLD (Table 1). Initial comparison of the 33 Odonata species that were the focus of this scoping study (Table 1) revealed that only five were not yet represented by at least one *COI* sequence in BOLD. We extracted DNA from one to three representative specimens of these five species (already collected and identified) using the Qiagen Dneasy Blood and Tissue kit, amplified 658 base pairs of the *COI* gene using primers that have previously been optimized for use in Odonata, OdoF1_t1 (5' – TGT AAA ACG ACG GCC AGT ATT CAA CHA ATC ATA ARG ATA TTG G) and OdoR1_t1 (5' – CAG GAA ACA GCT ATG ACT AAA CTT CTG GAT GYC CRA ARA AYC A) (Semotok, unpublished; used in e.g., Koroiva et al. 2017, Maggioni et al. 2021), determined the DNA sequence of the amplified *COI* fragments using an Applied Biosystems 3130xl genetic analyzer, and uploaded specimen and sequence data to BOLD, completing the reference library for our Odonata checklist.

Table 1. Reference library query results, indicating additional species nomenclature references consulted to validate synonymous or updated naming.

Family	Genus	Species	IN BOLD	BOLD Bar-code	ITIS Match	ITIS valid name in BOLD	BOLD Bar-code	Other GNR	Other GNR Database	BOLD Bar-code	BOLD search name	BOLD Barcode
Aeshnidae	<i>Aeshna</i>	<i>juncea</i>	YES	YES	N/A	–	–	–	–	–	–	–
Aeshnidae	<i>Aeshna</i>	<i>subarctica</i>	YES	YES	N/A	–	–	–	–	–	–	–
Aeshnidae	<i>Boyeria</i>	<i>vinosa</i>	YES	YES	N/A	–	–	–	–	–	–	–
Aeshnidae	<i>Aeshna</i>	<i>vinosa</i>	NO	N/A	NO	–	–	<i>Aeschna vinosa</i>	BioLib.cz	NO	–	–
Aeshnidae	<i>Basiaeshna</i>	<i>janata</i>	NO	N/A	NO	–	–	<i>Basiaeschna janata</i>	Catalogue of Life	YES	–	–
Aeshnidae	<i>Nasiaeschna</i>	<i>pentacantha</i>	NO	N/A	NO	–	–	<i>Nasiaeschna pentacantha</i>	Catalogue of Life	YES	–	–
Cordulegastridae	<i>Cordulegaster</i>	<i>diastatops</i>	YES	YES	N/A	–	–	–	–	–	–	–
Cordulegastridae	<i>Cordulegaster</i>	<i>maculata</i>	YES	YES	N/A	–	–	–	–	–	–	–
Cordulegastridae	<i>Cordulegaster</i>	<i>erronea</i>	NO	N/A	VALID	–	–	–	–	–	–	–
Corduliidae	<i>Dorocordulia</i>	<i>lepida</i>	YES	YES	N/A	–	–	–	–	–	–	–
Corduliidae	<i>Cordulia</i>	<i>shurtleffi</i>	NO	N/A	NO	–	–	<i>Cordulia shurtleffi</i>	spelling in BOLD search	YES	–	–
Corduliidae	<i>Epicordulia</i>	<i>princeps</i>	NO	N/A	INVALID	<i>Epithea princeps</i>	YES	–	–	–	–	–
Corduliidae	<i>Tetragoneuria</i>	<i>cynosura</i>	NO	N/A	INVALID	<i>Epithea cynosura</i>	YES	–	–	–	–	–
Corduliidae	<i>Tetragoneuria</i>	<i>epithea</i>	NO	N/A	NO	–	NO	<i>Tetragoneuria</i>	Catalogue of Life	NO	–	–
Gomphidae	<i>Gomphus</i>	<i>borealis</i>	YES	YES	N/A	–	–	–	–	–	–	–
Gomphidae	<i>Gomphus</i>	<i>descriptus</i>	YES	YES	N/A	–	–	–	–	–	–	–
Gomphidae	<i>Gomphus</i>	<i>exilis</i>	YES	YES	N/A	–	–	–	–	–	–	–
Gomphidae	<i>Gomphus</i>	<i>spicatus</i>	YES	YES	N/A	–	–	–	–	–	–	–
Gomphidae	<i>Lanthus</i>	<i>vernalis</i>	YES	NO	N/A	–	–	–	–	–	–	–
Gomphidae	<i>Lanthus</i>	<i>parvulus</i>	YES	YES	N/A	–	–	–	–	–	–	–

Table 1 (continued). Reference library query results, indicating additional species nomenclature references consulted to validate synonymous or updated naming.

Family	Genus	Species	IN BOLD	BOLD Bar-code	ITIS Match	ITIS valid name in BOLD	BOLD Bar-code	Other GNR	Other GNR Database	BOLD Bar-code	BOLD search name	BOLD Barcode
Gomphidae	<i>Arigomphus</i>	<i>furcifer</i>	NO	N/A	VALID	–	–	–	–	–	–	–
Gomphidae	<i>Gomphus</i>	<i>stylurus</i>	NO	N/A	NO	–	–	NO	–	–	–	–
Libellulidae	<i>Erythemis</i>	<i>simplicicollis</i>	YES	YES	N/A	–	–	–	–	–	–	–
Libellulidae	<i>Leucorrhinia</i>	<i>frigida</i>	YES	YES	N/A	–	–	–	–	–	–	–
Libellulidae	<i>Leucorrhinia</i>	<i>intacta</i>	YES	YES	N/A	–	–	–	–	–	–	–
Libellulidae	<i>Libellula</i>	<i>auripennis</i>	YES	YES	N/A	–	–	–	–	–	–	–
Libellulidae	<i>Libellula</i>	<i>incesta</i>	YES	YES	N/A	–	–	–	–	–	–	–
Libellulidae	<i>Libellula</i>	<i>pulchella</i>	YES	YES	N/A	–	–	–	–	–	–	–
Libellulidae	<i>Pachydiplax</i>	<i>longipennis</i>	YES	YES	N/A	–	–	–	–	–	–	–
Libellulidae	<i>Perithemis</i>	<i>tenera</i>	YES	YES	N/A	–	–	–	–	–	–	–
Libellulidae	<i>Sympetrum</i>	<i>illotum</i>	YES	YES	N/A	–	–	–	–	–	–	–
Libellulidae	<i>Leucorrhinia</i>	<i>glacialis</i>	NO	N/A	NO	–	–	Leucorrhinia glacialis	Index of Organism Names	NO	Leucorrhinia glacialis	YES
Macromiidae	<i>Macromia</i>	<i>illinoensis</i>	YES	YES	N/A	–	–	–	–	–	–	–

Table 2. Details of references consulted to validate additional synonymous or updated taxonomic names.

TSN	Scientific Name	Author	Rank Name	Credibility	Name Usage	Accepted TSN	Accepted Name
101773	<i>Arigomphus furcifer</i>	Hagen in Selys, 1878	Species	Reviewed	valid	–	–
102029	<i>Cordulegaster erronea</i>	Hagen in Selys, 1878	Species	Reviewed	valid	–	–
102031	<i>Cordulegaster maculata</i>	Selys, 1854	Species	Reviewed	valid	–	–
101863	<i>Epicordulia princeps</i>	Hagen, 1861	Species	Reviewed	invalid	206630	<i>Epitheca princeps princeps</i>
101768	<i>Lanthus parvulus</i>	Selys, 1854	Species	Reviewed	valid	–	–
101996	<i>Tetragoneuria cynosura</i>	Say, 1839	Species	Reviewed	invalid	185986	<i>Epitheca cynosura</i>

The progression of steps in DNA barcoding follows the same sequence, regardless of tissue type: tissue preparation, extraction, amplification, sequencing, comparison to reference database, such as BOLD. However, at each step, specific details can vary among different taxa and to address constraints for specific projects. For our project, a goal was to identify the specimen without contaminating or destroying most of the tissue, to allow for future mercury analysis or additional identification if desired. For dragonfly larvae, we first tested the amount of tissue needed for successful extraction and amplification; through trial and error, we determined that three legs were required from each larva to limit the potential for failed amplification. Legs could be dissected from frozen samples with minimal handling using pre-rinsed (with de-ionized (DI) water) implements and Nitrile gloves, minimizing potential for mercury contamination. Best practices dictate that laboratory implements are sterilized (e.g., with ethanol, UV) to minimize contamination from previously handled specimens. However, we were unable to test the implications of these sterilization techniques for potential future Hg analyses on these same individuals, so we used the accepted Hg method of DI rinsing. Sanger sequencing was unlikely to be influenced by this carryover but there could be issues with other high-throughput methods. We recommend removing all three legs from the same side of each specimen, which preserves the other full set of legs and allows for later verification of identity by morphology, if required. This also allows most of the tissue mass to remain uncontaminated and preserved frozen for future mercury analysis. Specific primers optimized for Odonata were used in the amplification step: OdoF1_t1 and OdoR1_t1 (Semotok, unpublished). We used the final protocol, Developing a Standard Operating Procedure for Species Identifications of Dragonfly Larvae Using DNA Barcoding: Standard Operating Procedure, Version 1.0 (Little et al. 2021), to identify the remaining samples targeted for identification by the DMP team. These dragonfly larvae represent eastern national parks that were part of the DMP in 2014.

Comparing visual identifications with DNA barcoding results

To evaluate the utility of the reference library for identifying unknown Odonata samples, we extracted, amplified, and sequenced a segment of the *COI* gene using the methods described above (see Little et al. 2021 for detailed methods) from an additional 16 Odonata specimens already collected and identified visually by the taxonomist. We challenged the library to identify these dummy query sequences. The success rate of the library in supporting the correct identification of these dummy sequences was used to establish a baseline expectation for how accurately we can expect the procedure to identify real query sequences (i.e., from unidentified specimens) in the future. The presence of sequences from many additional Odonata species in BOLD from the same genera as our 33 pilot test study species enabled us not only to rule in the identity of these specimens by strong sequence similarity to reference specimens (greater than 90%), but also rule out other species identifications. We compared results from both visual and DNA barcoding methods on an individual sample basis and evaluated overall agreement for the sample set (see Results section, Table 4).

Results

Identifying representative dragonfly larvae from national parks

We attempted identification of 501 individual dragonfly larvae in this project and achieved success in identifying to at least the genus level in 402 of these samples. For samples collected in 2013 and 2014, we definitively identified 61 different species of larval dragonfly specimens using the two methods (Table 3, Appendix A). In four additional instances, species identification was resolved to a choice of two potential identities, with four of the eight total possible species identities representing novel additions to the definitive list; however, two of these are unlikely identifications due to the range of candidate species, as indicated in Table 3.

Table 3. Listing of the 12 genus-level and 61 species-level identifications determined in this project for 2013–2014 samples, using both identification techniques.

Identification	Number of individuals	Tentative species identifications ¹
Aeshna ²	60	–
<i>Aeshna canadensis</i>	2	–
<i>Aeshna eremita</i>	2	–
<i>Aeshna subarctica</i> or <i>juncea</i>	1	1
<i>Aeshna umbrosa</i>	6	–
<i>Aeshna vinosa</i>	1	–
Anax ²	2	–
<i>Anax junius</i>	6	–
Arigomphus ²	2	–
<i>Arigomphus furcifer</i>	2	–
Basiaeschna ²	2	–
<i>Basiaeschna janata</i>	15	–
Boyeria ²	1	–
<i>Boyeria grafiana</i>	1	–
<i>Boyeria grafiana</i> or <i>vinosa</i>	3	3
<i>Boyeria vinosa</i>	2	–
<i>Celithemis elisa</i>	3	–
<i>Cordulegaster erronea</i>	3	2
<i>Cordulegaster maculata</i> or <i>diastatops</i>	2	2

¹ Tentative species identification indicates the number of individual specimens in each species that had tentative identifications, either marked in the dataset (Appendix A) with a “?” or ending with a couplet of potential species.

² Genus-level identification, also shown in a bold font style.

³ Tentative identification for which later investigation suggests identity is unlikely due to the range located far outside the region of the park where the species was collected.

Table 3 (continued). Listing of the 12 genus-level and 61 species-level identifications determined in this project for 2013–2014 samples, using both identification techniques.

Identification	Number of individuals	Tentative species identifications ¹
<i>Cordulia shurtleffi</i>	12	–
<i>Didymops transversa</i>	1	–
<i>Dorocordulia lepida</i>	2	–
<i>Dorocordulia libera</i>	1	–
<i>Dromogomphus spinosus</i>	3	2
<i>Epicordulia princeps</i>	1	–
<i>Epitheca/Tetragoneuria</i> ²	9	–
<i>Epitheca canis</i>	1	–
<i>Epitheca cynosura</i> or <i>spinigera</i>	5	5
<i>Tetragoneuria cynosura</i>	4	–
<i>Tetragoneuria spinigera</i>	1	–
<i>Tetragoneuria spinosa</i>	1	–
<i>Erythemis simplicicollis</i>	6	–
<i>Gomphus</i> ²	18	–
<i>Gomphus borealis</i>	1	–
<i>Gomphus descriptus</i>	1	1
<i>Gomphus exilis</i>	16	–
<i>Gomphus graslinellus</i>	1	–
<i>Gomphus spicatus</i>	3	–
<i>Gomphus stylurus</i>	7	–
<i>Hagenius brevistylus</i>	7	–
<i>Helocordulia uhleri</i>	1	–
<i>Ladona deplanata</i>	1	–
<i>Ladona julia</i>	3	–
<i>Lanthus parvulus</i>	1	–
<i>Lanthus parvulus</i> or <i>vernalis</i>	1	1
<i>Leucorrhinia</i> ²	2	–
<i>Leucorrhinia frigida</i>	15	–
<i>Leucorrhinia glacialis</i>	4	1
<i>Leucorrhinia intacta</i>	3	–

¹ Tentative species identification indicates the number of individual specimens in each species that had tentative identifications, either marked in the dataset (Appendix A) with a “?” or ending with a couplet of potential species.

² Genus-level identification, also shown in a bold font style.

³ Tentative identification for which later investigation suggests identity is unlikely due to the range located far outside the region of the park where the species was collected.

Table 3 (continued). Listing of the 12 genus-level and 61 species-level identifications determined in this project for 2013–2014 samples, using both identification techniques.

Identification	Number of individuals	Tentative species identifications ¹
<i>Leucorrhinia proxima</i>	1	–
<i>Libellula</i> ²	8	–
<i>Libellula auripennis</i>	9	–
<i>Libellula incesta</i>	9	–
<i>Libellula incesta</i> or <i>jesseana</i> ³	4	1
<i>Libellula needhami</i> ³	1	1
<i>Libellula pulchella</i>	4	–
<i>Libellula saturata</i>	4	–
<i>Macromia</i> ²	4	–
<i>Macromia illinoensis</i>	12	–
<i>Macromia taeniolata</i>	1	–
<i>Nasiaeschna pentacantha</i>	4	–
<i>Ophiogomphus</i> ²	20	–
<i>Ophiogomphus colubrinus</i>	1	–
<i>Orthemis ferruginea</i>	4	–
<i>Pachydiplax longipennis</i>	34	1
<i>Perithemis tenera</i>	3	–
<i>Plathemis lydia</i>	2	–
<i>Rhionaeschna multicolor</i>	2	–
<i>Somatochlora</i> ²	3	–
<i>Somatochlora elongata</i>	3	–
<i>Somatochlora franklinii</i>	1	–
<i>Somatochlora minor</i>	1	–
<i>Stylogomphus albistylus</i>	1	–
<i>Stylurus spiniceps</i>	1	–
<i>Sympetrum illotum</i>	8	–
<i>Sympetrum madidum</i>	2	–
<i>Sympetrum vicinum</i>	4	–
<i>Tamea carolina</i>	1	–
<i>Tamea lacerata</i>	2	–

¹ Tentative species identification indicates the number of individual specimens in each species that had tentative identifications, either marked in the dataset (Appendix A) with a “?” or ending with a couplet of potential species.

² Genus-level identification, also shown in a bold font style.

³ Tentative identification for which later investigation suggests identity is unlikely due to the range located far outside the region of the park where the species was collected.

Species identified in this project were proportionally representative of those present in North America. As of 2018, 331 dragonfly species were tallied in A Checklist of North American Odonata (Paulson and Dunkle, 2018). The 61 species positively identified here represent 18% of known North American species (Figure 1). Further, the number of species we identified within each taxonomic family was relatively proportional to the number of species in North America for the same families, ranging between 14–33% of North American species, excluding the rare Petaluridae (two known species), which were not identified in any of our sample sets. Of the samples identified to species, according to NatureServe (<https://www.natureserve.org/>), all but five species were classified as G5–Secure; the remaining five species were classified as G4–Apparently Secure in terms of their global conservation status. Appendix B contains links to the NatureServe entry for each identified species, which can be consulted for further state-specific information as well as general ecology for each species.

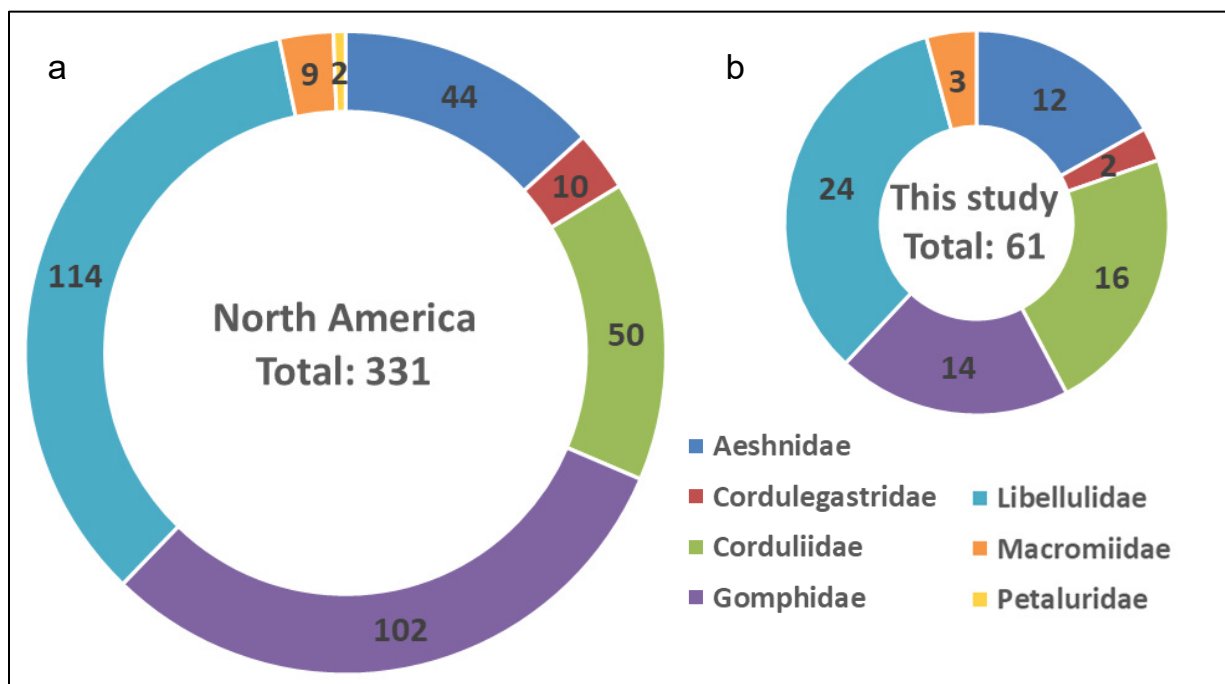


Figure 1. The total number of dragonfly (Odonata: Anisoptera) species in (a) North America, and (b) identified in this study, shown as proportion of species by family. Data for North America (a) from Paulson and Dunkle, 2018.

DNA Barcoding Protocol Development: Identifications

The initial generation of dummy query sequences that were tested against the BOLD reference library resulted in 10 (of 16) viable sequences. Technical failures in the initial tests failed at the PCR (polymerase chain reaction) step. Our early work to develop the protocol was refined to include more tissue, three legs of each dragonfly larva, but the issue of failed PCR amplification persisted throughout the project.

Comparing visual identifications with DNA barcoding results

For the overlap dataset of the 10 individuals that were first identified by traditional visual methods then DNA barcoding (Table 4), these identifications agreed in seven cases, and in an eighth instance, BOLD conflicts included one species that matched the visual ID, thus we consider it marginally successful. For the visually-identified Corduliidae *Cordulia shurtleffi* sample that was identified as Corduliidae *Somatochlora* sp. in BOLD (Table 4), we noted that the *C. shurtleffi* sequence was present in BOLD, based on other positive identifications later in the project. The percent match in BOLD for that sample was less than 90%. Overall, the percent accuracy between visual identification and DNA barcoding can be characterized as 70–80%, based on this small test set.

Table 4. Results of the initial dummy query test for 16 dragonfly larvae. BOLD ID is the DNA barcoding result. % Match-BOLD indicates similarity between sequences of the sample and in BOLD. Agreement indicates whether (Y) or not (N) the visual identification and DNA barcoding identification matched. Conflicts list other possible identifications provided in BOLD; “None” indicates that there were no potential alternative matches in BOLD; “na” indicates the conflict check was not applicable at this level of identification; “Synonym” notes when identification appears to be a mismatch but taxonomic names are synonymous, thus the identifications do indeed match. In one case, presence or absence of a conflict was not noted in the project dataset, thus it is labeled “-”.

Park	Visual ID	BOLD ID	% Match-BOLD	Agreement: Visual-DNA	BOLD Conflicts (% Match>98%)
SACN	Gomphidae <i>Dromogomphus spinosus</i>	Gomphidae <i>Dromogomphus spinosus</i>	99.31	Y	None
JELA	Libellulidae <i>Erythemis simplicicollis</i>	Libellulidae <i>Erythemis simplicicollis</i>	99.17	Y	None
UNK	Libellulidae <i>Pachydiplax longipennis</i>	Libellulidae <i>Pachydiplax longipennis</i>	91.67	Y	–
UNK	Libellulidae <i>Pachydiplax longipennis</i>	Libellulidae <i>Pachydiplax longipennis</i>	97.01	Y	None
GRSM	Gomphidae Lanthus parvulus or vernalis	Gomphidae Lanthus parvulus	99.49	Y, resolved couplet ¹	None
GRSM	Cordulegastridae <i>Cordulegaster erronea</i>	Cordulegastridae <i>Kalypogaster erronea</i>	96.32	Y	Synonym
GRSM	Cordulegastridae <i>Cordulegaster erronea</i>	Cordulegastridae <i>Kalypogaster erronea</i>	96.35	Y	Synonym
CACO or VOYA	Libellulidae <i>Leucorrhinia frigida</i>	Libellulidae <i>Leucorrhinia proxima</i>	100	Y, with conflict ²	<i>Leucorrhinia glacialis</i> , <i>hudsonica</i> , <i>frigida</i>

¹ The visual identification provided two possible species identities; BOLD confirmed one of these options. We consider this a matched identification.

² Visual and DNA barcoding identities matched when including potential alternative identities (conflicts) in BOLD.

Table 4 (continued). Results of the initial dummy query test for 16 dragonfly larvae. BOLD ID is the DNA barcoding result. % Match-BOLD indicates similarity between sequences of the sample and in BOLD. Agreement indicates whether (Y) or not (N) the visual identification and DNA barcoding identification matched. Conflicts list other possible identifications provided in BOLD; “None” indicates that there were no potential alternative matches in BOLD; “na” indicates the conflict check was not applicable at this level of identification; “Synonym” notes when identification appears to be a mismatch but taxonomic names are synonymous, thus the identifications do indeed match. In one case, presence or absence of a conflict was not noted in the project dataset, thus it is labeled “-”.

Park	Visual ID	BOLD ID	% Match-BOLD	Agreement: Visual-DNA	BOLD Conflicts (% Match>98%)
UNK	Corduliidae <i>Cordulia shurtleffi</i>	Corduliidae <i>Somatochlora sp.</i>	87.67	N	na
CUVA	Aeshnidae <i>Aeshna vinosa</i>	Sequence too short	na	na	na
CUVA	Gomphidae <i>Arigomphus furcifer</i>	Sequence too short	na	na	na
UNK	Libellulidae <i>Pachydiplax longipennis</i>	Sequence too short	na	na	na
UNK	Corduliidae <i>Cordulia shurtleffi</i>	Sequence too short	na	na	na
SACN	Aeshnidae <i>Boyeria grafiana</i>	Sequence too short	na	na	na
YELL	Libellulidae <i>Libellula saturata</i>	Sequence too short	na	na	na

¹ The visual identification provided two possible species identities; BOLD confirmed one of these options. We consider this a matched identification.

² Visual and DNA barcoding identities matched when including potential alternative identities (conflicts) in BOLD.

In 2013, all 286 specimens submitted were identified by the taxonomist using visual methods to at least the genus level. Of these, 170 were further identified to the species level; three of these had two possible species identities, and nine species identifications were tentative, as noted in Table 3. In 2014, 215 samples were prepared and analyzed according to the methods in the Standard Operating Procedure (Little et al. 2021). Of these, 116 were identified to genus and 101 to species using DNA barcoding; 13 of these had two possible species identities. Percent similarity, an indication of the accuracy of the BOLD match, ranged from 97–100% for all species identifications (Appendix A), above the 90% threshold proposed after inspection of the pilot query study (Table 4). For the other 99 samples, 8 had no match in BOLD, PCR failures accounted for 25 un-identified samples, and sequencing failures (one or two attempts) precluded identification in the remaining 66 cases. Thus, of the samples with attempted identifications, 100% of the visually-identified sample set was positively identified to genus; 54% of the DNA barcoding sample set produced a DNA sequence that was subsequently identified to genus. Of the full sample set for each method, 59% (visual) and 47% (DNA barcoding) were further identified to species. Notably, 54% of the individuals that the

Taxonomist identified to the genus level were further resolved to the species level using visual methods, whereas 87% of the individuals that produced a DNA sequence and were resolved to genus were further resolved to species by DNA barcoding. The full list of species identifications by park and site is in Appendix A.

Summary and Discussion

Dragonfly larvae species identifications

We identified 61 unique dragonfly species, representing the six major dragonfly families in North America, from a sample set of 401 individual dragonfly larvae from 24 national park units and one state park (Figure 1, Appendix A). Species identified in this project were proportionally representative of those present in North America, and the number of species we identified within each taxonomic family was relatively proportional to the number of species in North America for the same families. Of the samples identified to species, according to NatureServe (<https://www.natureserve.org/>), all were classified as “secure” or “relatively secure” with respect to their conservation status. It was not surprising that we identified common species, as the DMP’s primary goal is rapid collection of samples of any Anisoptera species for mercury determination, not quantitative assessment of biodiversity metrics for odonates. Our skew toward relatively common species likely led to few identification failures due to lack of reference sequences. When DNA barcoding failed, it was generally due to failed PCR amplification and can be addressed in future work with methodological refinements. For example, in order to submit samples with relatively larger body size for mercury determination, our specimens for DNA barcoding were generally very small individuals and it is possible that we lacked adequate tissue mass. Using larger individuals or more legs could improve results, although Ožana et al. (2020) noted that legs were typically among the most efficacious tissue for this purpose. When the PCR step did not fail, DNA barcoding produced an 87% success rate in identification to species (Table 5), similar to the 94% reported in Koroiva et al. (2017).

Table 5. Summary of challenges to dragonfly larvae identification, and relative comparison of strengths and weaknesses of DNA barcoding and visual identification methods in addressing these challenges.

Challenge	DNA Barcoding	Visual identification
<i>Specialized expertise needed: invertebrate/odonate taxonomist</i>	Not needed	Essential
<i>Access to laboratory support for DNA preparation and analysis</i>	Essential	n/a
<i>Successful identification to Genus</i>	~50% of attempts	100%
<i>Successful identification to Species once genus determined</i>	87%	54%
<i>Overall success rate: all attempted identities ultimately resolved to species, including failed sequences</i>	47%	59%—
<i>Dominant cause of lack of identification</i>	Sequencing failure, method improvements could address	Lack of resolution to species level; lack of morphometric distinction at larval stage; small individual size

DNA barcoding methodology development

To enable DNA barcoding for these dragonfly larvae, we developed a Standard Operating Protocol (Little et al. 2021), that provides specific details aimed at successful barcoding, but also takes into account the need to preserve most of the dragonfly tissue for potential mercury analysis. Prior to establishing the DNA barcoding approach, samples set aside for taxonomic identification were rendered useless for mercury determination, due to contamination and different preservation requirements (typically, ethanol) for taxonomic identification. Thus, DNA barcoding can be employed to determine mercury concentration at the species level. Length of the DNA sequence we used, 658 base pairs, is typical for insect DNA barcoding, but there is always the potential for a lack of overlap with a reference sequence. Maggioni et al. (2021) compared a different (3') DNA sequence that was 629 base pairs to the more common 658 base pair (5') segment in odonates; the 658 base pair sequence was marginally more successful in identifications but testing alternative or longer sequences could potentially improve success rates. Method refinement to reduce technical failures, including trials of different PCR primers, longer sequences, and fine-tuning other protocol steps, could lead to better efficiency in amplification and sequencing results.

Assessing the relative strengths of traditional visual methods vs. DNA barcoding for dragonfly larvae identification

The barcoding method was promising as all of the species it identified with a BOLD (DNA barcoding database) match greater than 90% corroborated visual identifications, in a small overlap set. In addition, DNA barcoding offers the ability to reveal species-level identity without taxonomist support for visual/morphometric analysis, which can be challenging to secure as professionals in this field are limited and declining in number. However, our methodology was not successful for nearly half of the samples, with failed amplification or sequences. When sequences were produced, however, DNA barcoding identification was more successful in resolving the step from genus to species than taxonomist visual identification (87% and 54%). Visual identification has the benefit of allowing family identity confirmation, and the taxonomist was able to resolve to the genus level visually for 100% of attempted specimens, whereas technical failures in the sample preparation and analysis steps in DNA barcoding limited the overall number of genus or genus-species identifications that were possible to produce. Damm et al. (2010) thus recommend combining identification methodologies, including newly-developed molecular methods and species ecology, to ensure more definitive identifications. In our overlap study (Table 4), each method documented a case where the other method resolved a couplet or potential conflict, indicating that the two methods in tandem could be used to provide confirmation of these difficult to identify taxa. In summary, both methods produced similar overall percentages of species identities; visual identification was more likely to produce identification to at least the genus level for all submitted samples, but DNA barcoding had a greater success rate moving from genus- to species-level identification when DNA sequences were produced.

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Appendix A. Dragonfly larvae taxonomic identifications

Table A-1. Metadata for dragonfly larvae species identifications. Data provided online:
<https://irma.nps.gov/DataStore/Reference/Profile/2282155>.

Park	Four-letter code for National Park Service unit abbreviation.
Site	Site name as originally reported in the Dragonfly Mercury Project.
SitePrimaryKey	Unique site identifier from DMP master database (see Eagles-Smith et al. 2020)
Collection Year	Year samples were collected.
Collection date	Date or dates samples were collected.
Sample ID	Incoming sample identification code.
Length (mm)	Length (tip of mentum to end of tail spine) of individual dragonfly larva in millimeters, from field sheets (not lab-validated).
ID Method	Indicates whether taxonomic identification was attempted using visual methods or the DNA barcoding approach.
Odonate Family	Taxonomic family, identified by Dragonfly Mercury Project staff and for visually-identified samples, rechecked by taxonomist.
Odonate Genus	Genus, as identified using one of the two methods.
Odonate Species	Species, as identified using one of the two methods. When inconclusive, couplets are separated by "or"; uncertain identifications followed by "?".
ID Failure Reason	For DNA barcoding, note indicating step in process that failed.
BOLD Match	Percent similarity statistic between sample query and BOLD sequences.
ID Notes	Notes relevant to species identity, provided by Taxonomist for visually-identified samples (notes about samples) or S. Nelson (notes about range of species).

Appendix B. Links to NatureServe information for species identified in this work

Table B-1. Information on global conservation status and links to more information for each species with a definitive identification in this study. Status G5: Secure; Status G4: Apparently Secure.

Identification	Status	NatureServe Link
<i>Aeshna canadensis</i>	G5	https://explorer.natureserve.org/Taxon/ELEMENT_GLOBAL.2.121119/Aeshna_canadensis
<i>Aeshna eremita</i>	G5	https://explorer.natureserve.org/Taxon/ELEMENT_GLOBAL.2.116134/Aeshna_eremita
<i>Aeshna umbrosa</i>	G5	https://explorer.natureserve.org/Taxon/ELEMENT_GLOBAL.2.118551/Aeshna_umbrosa
<i>Aeshna vinosa</i>	n/a	n/a
<i>Anax junius</i>	G5	https://explorer.natureserve.org/Taxon/ELEMENT_GLOBAL.2.116554/Anax_junius
<i>Arigomphus furcifer</i>	G5	https://explorer.natureserve.org/Taxon/ELEMENT_GLOBAL.2.116184/Arigomphus_furcifer
<i>Basiaeschna janata</i>	G5	https://explorer.natureserve.org/Taxon/ELEMENT_GLOBAL.2.119506/Basiaeschna_janata
<i>Boyeria grafiana</i>	G5	https://explorer.natureserve.org/Taxon/ELEMENT_GLOBAL.2.116183/Boyeria_grafiana
<i>Boyeria vinosa</i>	G5	https://explorer.natureserve.org/Taxon/ELEMENT_GLOBAL.2.114935/Boyeria_vinosa
<i>Celithemis elisa</i>	G5	https://explorer.natureserve.org/Taxon/ELEMENT_GLOBAL.2.109207/Celithemis_elisa
<i>Cordulegaster erronea</i>	G4	https://explorer.natureserve.org/Taxon/ELEMENT_GLOBAL.2.114245/Cordulegaster_erronea
<i>Cordulia shurtleffi</i>	G5	https://explorer.natureserve.org/Taxon/ELEMENT_GLOBAL.2.119786/Cordulia_shurtleffi
<i>Didymops transversa</i>	G5	https://explorer.natureserve.org/Taxon/ELEMENT_GLOBAL.2.119139/Didymops_transversa
<i>Dorocordulia lepida</i>	G5	https://explorer.natureserve.org/Taxon/ELEMENT_GLOBAL.2.109149/Dorocordulia_lepida
<i>Dorocordulia libera</i>	G5	https://explorer.natureserve.org/Taxon/ELEMENT_GLOBAL.2.117107/Dorocordulia_libera
<i>Dromogomphus spinosus</i>	G5	https://explorer.natureserve.org/Taxon/ELEMENT_GLOBAL.2.111864/Dromogomphus_spinosus
<i>Epicordulia princeps</i>	G5	https://explorer.natureserve.org/Taxon/ELEMENT_GLOBAL.2.114450/Epithea_princeps
<i>Epithea canis</i>	G5	https://explorer.natureserve.org/Taxon/ELEMENT_GLOBAL.2.111224/Epithea_canis
<i>Erythemis simplicicollis</i>	G5	https://explorer.natureserve.org/Taxon/ELEMENT_GLOBAL.2.108317/Erythemis_simplicicollis

Table B-1 (continued). Information on global conservation status and links to more information for each species with a definitive identification in this study. Status G5: Secure; Status G4: Apparently Secure.

Identification	Status	NatureServe Link
<i>Tetragoneuria cynosura</i>	G5	https://explorer.natureserve.org/Taxon/ELEMENT_GLOBAL.2.117238/Epitheca_cynosura
<i>Tetragoneuria spinigera</i>	G5	https://explorer.natureserve.org/Taxon/ELEMENT_GLOBAL.2.112978/Epitheca_spinigera
<i>Tetragoneuria spinosa</i>	G4	https://explorer.natureserve.org/Taxon/ELEMENT_GLOBAL.2.114314/Epitheca_spinosa
<i>Gomphus borealis</i>	G5	https://explorer.natureserve.org/Taxon/ELEMENT_GLOBAL.2.110913/Phanogomphus_borealis
<i>Gomphus descriptus</i>	G4	https://explorer.natureserve.org/Taxon/ELEMENT_GLOBAL.2.109772/Phanogomphus_descriptus
<i>Gomphus exilis</i>	G5	https://explorer.natureserve.org/Taxon/ELEMENT_GLOBAL.2.114260/Phanogomphus_exilis
<i>Gomphus graslinellus</i>	G5	https://explorer.natureserve.org/Taxon/ELEMENT_GLOBAL.2.111346/Phanogomphus_graslinellus
<i>Gomphus spicatus</i>	G5	https://explorer.natureserve.org/Taxon/ELEMENT_GLOBAL.2.116369/Phanogomphus_spicatus
<i>Hagenius brevistylus</i>	G5	https://explorer.natureserve.org/Taxon/ELEMENT_GLOBAL.2.112805/Hagenius_brevistylus
<i>Helocordulia uhleri</i>	G5	https://explorer.natureserve.org/Taxon/ELEMENT_GLOBAL.2.111879/Helocordulia_uhleri
<i>Ladona deplanata</i>	G5	https://explorer.natureserve.org/Taxon/ELEMENT_GLOBAL.2.107784/Ladona_deplanata
<i>Ladona julia</i>	G5	https://explorer.natureserve.org/Taxon/ELEMENT_GLOBAL.2.118032/Ladona_julia
<i>Lanthus parvulus</i>	G4	https://explorer.natureserve.org/Taxon/ELEMENT_GLOBAL.2.112808/Lanthus_parvulus
<i>Leucorrhinia frigida</i>	G5	https://explorer.natureserve.org/Taxon/ELEMENT_GLOBAL.2.113552/Leucorrhinia_frigida
<i>Leucorrhinia glacialis</i>	G5	https://explorer.natureserve.org/Taxon/ELEMENT_GLOBAL.2.120738/Leucorrhinia_glacialis
<i>Leucorrhinia intacta</i>	G5	https://explorer.natureserve.org/Taxon/ELEMENT_GLOBAL.2.107011/Leucorrhinia_intacta
<i>Leucorrhinia proxima</i>	G5	https://explorer.natureserve.org/Taxon/ELEMENT_GLOBAL.2.116693/Leucorrhinia_proxima
<i>Libellula auripennis</i>	G5	https://explorer.natureserve.org/Taxon/ELEMENT_GLOBAL.2.111159/Libellula_auripennis
<i>Libellula incesta</i>	G5	https://explorer.natureserve.org/Taxon/ELEMENT_GLOBAL.2.114789/Libellula_incesta
<i>Libellula pulchella</i>	G5	https://explorer.natureserve.org/Taxon/ELEMENT_GLOBAL.2.107466/Libellula_pulchella
<i>Libellula saturata</i>	G5	https://explorer.natureserve.org/Taxon/ELEMENT_GLOBAL.2.107393/Libellula_saturata

Table B-1 (continued). Information on global conservation status and links to more information for each species with a definitive identification in this study. Status G5: Secure; Status G4: Apparently Secure.

Identification	Status	NatureServe Link
<i>Macromia illinoensis</i>	G5	https://explorer.natureserve.org/Taxon/ELEMENT_GLOBAL.2.109358/Macromia_illinoensis
<i>Macromia taeniolata</i>	G5	https://explorer.natureserve.org/Taxon/ELEMENT_GLOBAL.2.116046/Macromia_taeniolata
<i>Nasiaeschna pentacantha</i>	G5	https://explorer.natureserve.org/Taxon/ELEMENT_GLOBAL.2.110359/Nasiaeschna_pentacantha
<i>Ophiogomphus colubrinus</i>	G5	https://explorer.natureserve.org/Taxon/ELEMENT_GLOBAL.2.115945/Ophiogomphus_colubrinus
<i>Orthemis ferruginea</i>	G5	https://explorer.natureserve.org/Taxon/ELEMENT_GLOBAL.2.111415/Orthemis_ferruginea
<i>Pachydiplax longipennis</i>	G5	https://explorer.natureserve.org/Taxon/ELEMENT_GLOBAL.2.110139/Pachydiplax_longipennis
<i>Perithemis tenera</i>	G5	https://explorer.natureserve.org/Taxon/ELEMENT_GLOBAL.2.117133/Perithemis_tenera
<i>Plathemis lydia</i>	G5	https://explorer.natureserve.org/Taxon/ELEMENT_GLOBAL.2.112429/Plathemis_lydia
<i>Rhionaeschna multicolor</i>	G5	https://explorer.natureserve.org/Taxon/ELEMENT_GLOBAL.2.114579/Rhionaeschna_multicolor
<i>Somatochlora elongata</i>	G5	https://explorer.natureserve.org/Taxon/ELEMENT_GLOBAL.2.118364/Somatochlora_elongata
<i>Somatochlora franklinii</i>	G5	https://explorer.natureserve.org/Taxon/ELEMENT_GLOBAL.2.106803/Somatochlora_franklinii
<i>Somatochlora minor</i>	G5	https://explorer.natureserve.org/Taxon/ELEMENT_GLOBAL.2.116005/Somatochlora_minor
<i>Stylogomphus albistylus</i>	G5	https://explorer.natureserve.org/Taxon/ELEMENT_GLOBAL.2.736644/Stylogomphus_albistylus
<i>Stylurus spiniceps</i>	G5	https://explorer.natureserve.org/Taxon/ELEMENT_GLOBAL.2.117792/Stylurus_spiniceps
<i>Sympetrum illotum</i>	G5	https://explorer.natureserve.org/Taxon/ELEMENT_GLOBAL.2.113095/Sympetrum_illotum
<i>Sympetrum madidum</i>	G5	https://explorer.natureserve.org/Taxon/ELEMENT_GLOBAL.2.115218/Sympetrum_madidum
<i>Sympetrum vicinum</i>	G5	https://explorer.natureserve.org/Taxon/ELEMENT_GLOBAL.2.106621/Sympetrum_vicinum
<i>Tamea carolina</i>	G5	https://explorer.natureserve.org/Taxon/ELEMENT_GLOBAL.2.116098/Tamea_carolina
<i>Tamea lacerata</i>	G5	https://explorer.natureserve.org/Taxon/ELEMENT_GLOBAL.2.121110/Tamea_lacerata

The Department of the Interior protects and manages the nation's natural resources and cultural heritage; provides scientific and other information about those resources; and honors its special responsibilities to American Indians, Alaska Natives, and affiliated Island Communities.

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National Park Service
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