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DOI:
https://doi.org/10.1111/syen.12672

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Received: 12 August 2024 | Accepted: 19 December 2024

DOI: 10.1111/syen.12672

ORIGINAL ARTICLE

Systematic Entomology

Royal Entomological Society

Systematics and biogeography of the Holarctic dragonfly genus *Somatochlora* (Anisoptera: Corduliidae)

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Funding information
National Science Foundation (NSF) Awards, Grant/Award Numbers: 2002489, 2002473, 2002432, 2002457

Abstract

The striped emeralds (*Somatochlora* Selys) are a Holarctic group of medium-sized metallic green dragonflies that mainly inhabit bogs and seepages, alpine streams, lakes, channels and lowland brooks. With 42 species they are the most diverse genus within Corduliidae (Odonata: Anisoptera). Systematic, taxonomic and biogeographic resolution within *Somatochlora* remains unclear, with numerous hypotheses of relatedness based on wing veins, male claspers (epiproct and paraprocts) and nymphs. Furthermore, *Somatochlora borisi* was recently described as a new genus (*Corduliochlora*) based on 17 morphological characters, but its position with respect to *Somatochlora* is unclear. We present a phylogenetic reconstruction of *Somatochlora* using Anchored Hybrid Enrichment (AHE) sequences of 40/42 *Somatochlora* species (including *Corduliochlora borisi*). Our data recover the monophyly of *Somatochlora*, with *C. borisi* recovered as sister to the remaining *Somatochlora*. We also recover three highly supported clades and one of mixed support; this lack of resolution is most likely due to incomplete lineage sorting, third-codon position saturation based on iterative analyses run on variations of our dataset and hybridization. Furthermore, we constructed a dataset for all species based on 20 morphological characters from the literature which were used to evaluate phylogenetic groups recovered with molecular data; the data support the validity of *Corduliochlora* as a genus distinct from *Somatochlora*. Finally, divergence time estimation and biogeographic analysis indicate *Somatochlora* originated in the Western North Hemisphere during the Miocene, with three dispersal events to the Eastern North Hemisphere (11, 7 and 5 Ma, respectively) across the Beringian Land Bridge.

KEYWORDS

Anchored Hybrid Enrichment, Cavilabiata, classification, *Corduliochlora*, emerald dragonflies, phylogenetics, taxonomy

INTRODUCTION

The striped emeralds (*Somatochlora* Selys) are a group of medium-sized dragonflies, most commonly recognized by their metallic green, brown or black bodies, emerald coloured eyes, a series of dull to yellow coloured marks along the thorax and abdomen, and a distinctive bulbous swelling on the first and second abdominal segments (Burmeister, 1839; Dunkle, 2000; Needham et al., 2000; Walker, 1925) (Figure 1).

Somatochlora is the most speciose genus within the family Corduliidae (Odonata: Anisoptera), with 42 currently recognized species (odonatacentral.org). Habitat preferences vary greatly, with larvae

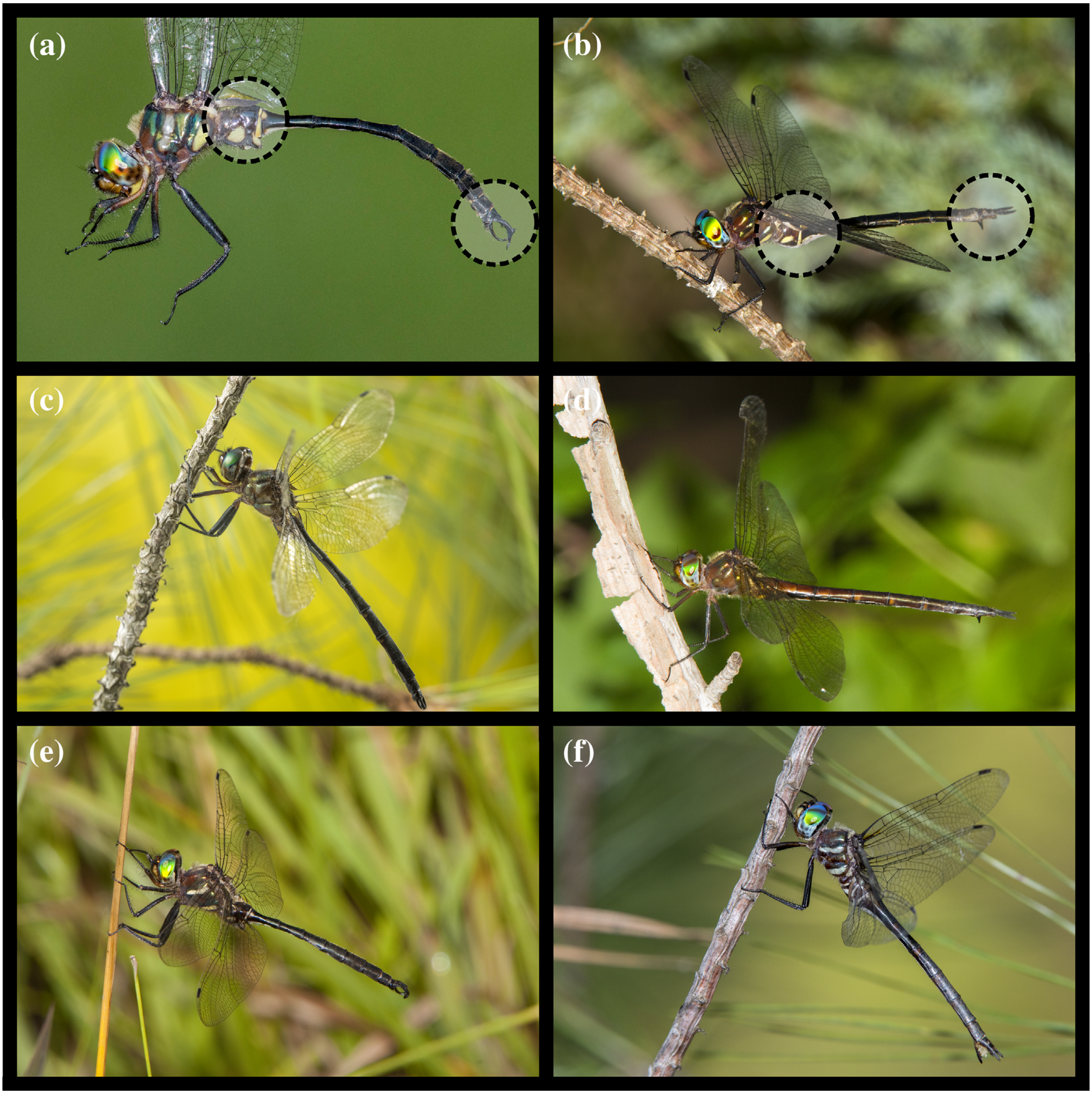

FIGURE 1 Diversity of North American *Somatochlora* species. Black circles indicate some key characters for distinguishing species within the genus including a distinctive bulbous swelling on the first and second abdominal segments, shape of the male cerci and elongation and shape of the female subgenital plate. Species presented are as follows: Clamp-tipped Emerald (*Somatochlora tenebrosa*) male (a) and female (b), Mocha Emerald (*Somatochlora linearis*), male (c) and female (d), United States federally endangered Hine's Emerald (*Somatochlora hineana*), male (e) and the rare Texas Emerald (*Somatochlora margarita*), female (f). Photos provided by J. Abbott.

(nymphs) inhabiting either alpine streams, bogs, fens or lakes (Needham et al., 2000; Tennessen, 2019). The genus resides throughout the Northern Hemisphere (North America, Europe, Northern Asia), with four residing within or extending to eastern and southeastern Asia (Vietnam, Taiwan, Japan) (Dijkstra & Schröter, 2020; Kalkman et al., 2007; Paulson, 2011). Despite their generally wide geographic range, some species are rare, examples being Hine's Emerald *Somatochlora hineana*, Williamson, 1931 which is federally endangered in the United States and the Texas Emerald *Somatochlora margarita*, Donnelly 1962 (Abbott, 2001, 2015; Abbott & Mynhardt, 2007; Foster & Soluk, 2006; Jackson et al., 2018; Kijowski, 2014; Monroe & Britten, 2014; Vogt & Cashatt, 1994) (Figure 1). Ranges of species can extend past the arctic tree line (i.e., *Somatochlora sahlbergi*, Trybom 1889) which possesses one of the northernmost breeding ranges among dragonflies (Cannings & Cannings, 1985; Kohli et al., 2018; Schröter, 2011).

Species-level relationships within *Somatochlora* remains unclear. Original taxonomic works on *Somatochlora* relied heavily on wing vein and nymphal characters (Garrison et al., 2006; Needham et al., 2000; Walker, 1925; Walker & Corbet, 1975). Additional external body characters commonly used for diagnosis of this group include the shape of the male cerci, as well as the degree of elongation and shape of the female subgenital plate; some species possess an elongate genital plate acting as an ovipositor to lay eggs into mud (Paulson, 2011) (Figure 1). European species have previously been grouped into a southern *metallica* group (*S. metallica*, *S. meridionalis* and *S. flavomaculata*), which share the traits of middorsal abdominal hooks in nymphs and glabrous metallic adult bodies, and a northern *arctica* group (*S. alpestris*, *S. arctica*) whose nymphs possess no dorsal hooks, with adults possessing dull black bodies (Fleck et al., 2007). However, all these characters are highly prone to convergence (Carle, 1982, 1995; Carle et al., 2008; Dijkstra & Vick, 2006; Hovmöller, 2006; Hovmöller & Johansson, 2004; Pilgrim & Von Dohlen, 2008), necessitating molecular analyses to determine the interspecific relationships and morphological homologies within *Somatochlora*.

Continuing advances in molecular sequencing have allowed for the capture and analysis of thousands of loci in short amounts of time and the ability to sequence samples collected over 100 years ago despite genetic degradation (Faircloth et al., 2012; Goodman et al., 2023; Lemmon et al., 2012). *Somatochlora* have been included in phylogenetic studies using multi-locus and high-throughput sequencing. However, these studies have focused on broader relationships within Anisoptera and did not address *Somatochlora* specifically (Bybee et al., 2008, 2021; Carle et al., 2015; Fleck et al., 2008; Kohli, Letsch, et al., 2021; Misof et al., 2001; Suvorov et al., 2022; Ware et al., 2007).

The first aim of this paper is to reconstruct the phylogeny of *Somatochlora* utilizing high-throughput DNA sequencing, with special attention to the tentative generic status of the Balkan species *C. borisi* (Marinov, 2001). Original descriptions of *C. borisi* differentiated it from other *Somatochlora* species citing earlier emergence times (between mid-May and late June), an absence of mesotibial keels and the presence of two cubital-anal crossveins in the hind wing (Marinov, 2001). Boudot et al. (2004) determined that the vulvar scale of female *C. borisi* is deeper and more narrowly divided than in other *Somatochlora* species, as well as its putative closest relative *Cordulia*; however, they discouraged the elevation of the species to a new genus without the incorporation of molecular data. Nymphal characters by Fleck et al. (2007) suggested that *C. borisi* is within the *metallica* group of European *Somatochlora*, but only compared *C. borisi* with *S. meridionalis* and *S. metallica*. Furthermore, karyotype analysis by Grozeva (2007) revealed that *C. borisi* possesses a new chromosomal formula for both *Somatochlora* and Corduliidae ($2n = 20 + XX/XY$). Finally, Marinov and Seidenbusch (2007) proposed *Corduliochlora gen. nov*. to accommodate *C. borisi* by identifying 17 adult morphological characters which differentiated it from *Somatochlora*, *Cordulia*, *Neurocordulia*, *Helocordulia*, *Dorocordulia* and *Epitheca* (based on wing venation, male and female external genitalia and metallic coloration on the body). Subsequent morphometric analysis of *C. borisi* exuviae by Seidenbusch (2010) suggested large differences that distinguished *C. borisi* from other *Somatochlora*, *Cordulia* and *Epitheca* species. Despite all of this, the absence of a comprehensive species-level molecular phylogeny of *Somatochlora* leaves the status of *Corduliochlora* unresolved.

The second aim of this paper is to infer the divergence time and biogeographic history of *Somatochlora*. Most *Somatochlora* are Nearctic, with only 11 species endemic within the Palearctic, and *S. sahlbergi* inhabiting both regions with a circumboreal distribution. Molecular studies by Kohli et al. (2018) suggest that *S. sahlbergi* constitutes a single species, possessing no variation among European and North American populations and exhibiting high amounts of interbreeding across its range despite the geographic barriers across the arctic. Furthermore, Kohli et al. (2018) suggest that connectivity among populations of *S. sahlbergi* persisted until relatively recently with the disappearance of the Beringian land bridge (~11.6 kya) (Brigham-Grette, 2001; Hopkins, 1973; Scholl et al., 1968). Transcriptomic data suggest that the family Corduliidae originated ~45 Ma (Kohli, Letsch, et al., 2021), corresponding with the peak of the Eocene warming period (Norris & Röhl, 1999; West et al., 2020). Unfortunately, no *Somatochlora* species were used within this analysis. If *Somatochlora* originated during the Eocene, or more recently, its current distribution may be the result of dispersal across the North American Land Bridges (NALB) including Beringia, which underwent episodic periods of opening and closing since the Cenozoic (Denk et al., 2011; Graham, 2018; Scharff, 1909). A wealth of research pertaining to dispersal across Beringia is present for plants, birds and mammals, including humans, while studies on arthropods are generally lacking with the exception of butterflies and moths (Boyd et al., 2022; Haas et al., 2020; Hoffecker et al., 2016; Jiang et al., 2019; Kurata et al., 2022; Maguilla et al., 2018; Mills et al., 2023; Rubinoff & Doorenweerd, 2020; Shao et al., 2019; Stubbs et al., 2020; Toussaint, Ellis, et al., 2021; Wooller et al., 2018). Furthermore, cyclic glaciation events during the Pleistocene (~2 Ma) could have contributed to subsequent pulses of diversification within *Somatochlora* within North America, Europe and Asia after dispersal across Beringia. Dispersal south or to local refugia during glaciation events, followed by pulses of diversification post-glaciation, is observed in many damselfly species both in North America and Europe (Bernard et al., 2011; Bernard & Daraż, 2010; Dumont et al., 2021; Turgeon et al., 2005).

METHODS

Taxon sampling

We acquired specimens of *Somatochlora* from natural history collections. Specimens sampled from collections in the American Museum of Natural History (AMNH), Florida State Collection of Arthropods (FSCA), Naturalis Biodiversity Center (RMNH), National Museum of Natural History Museum (NMNH) and Monte L. Bean Life Sciences

Museum at Brigham Young University (BYU). In total, we sampled 40 of the 42 current species of *Somatochlora*. We selected a large number of outgroups to establish a robust phylogenetic placement for the *Somatochlora*. We sampled outgroups from Corduliidae (*Hemicordulia*, *Procordulia*, *Guadalca*, *Paracordulia*, *Neurocordulia*, *Navicordulia*, *Helocordulia*, *Cordulia*, *Epitheca*, *Metaphya*, *Pentathemis*, *Aeschnosoma* and *Antipodochlora*), other established families within the superfamily Libelluloidea (Libellulidae: *Pantala*, *Libellula*, *Orthetrum*; Macromiidae: *Macromia*, *Epophthalmia*; Synthemistidae: *Eusynthemis*, *Choristhemis*, *Gomphomacromia*) and families within Cavilabiata (Chlorogomphidae: *Chlorogomphus*; Cordulegastridae: *Cordulegaster*, *Anotogaster*; Neopetalia: *Neopetalia punctata*). Specimen provenance data, including locality, date, author, collector and determiner, are listed in Table S1.

DNA extraction and sequencing

We removed the hind leg from individual specimens of each species using sterilized forceps and extracted DNA using ZYMOBIOMICS DNA miniprep kits (Irvine, CA). We quantified DNA yield using a Qubit 4 fluorometer and sent DNA extracts to RAPID Genomics (Gainesville, FL) for library preparation and sequencing. Loci were amplified using Anchored Hybrid Enrichment (AHE) probes modified from Bybee et al. (2021), consisting of 1306 loci (Goodman et al., 2023). Probes sets were originally created by scanning for 941 exons commonly shared across insects using published data from 24 odonate transcriptomes (Futahashi et al., 2015; Suvorov et al., 2017) as well as two assembled genomes from Bybee et al. (2021). An additional 211 functional loci were sequenced, focusing on vision, flight and immunity (Bybee et al., 2021; Goodman et al., 2023). We sequenced loci of representatives of each genus using the full 1306 probe set (500 kb), while a subset of 92 loci (20 kb) was sequenced for the remaining species. Raw AHE reads can be obtained from Dryad digital repository number: https://doi.org/10.5061/dryad.prr4xgxw5, while loci coverage for each species can be obtained from Table S1.

AHE assembly and analysis

We trimmed adaptors from raw reads using *fastp* (Tang & Wong 2001) and checked for quality using *multiQC* (Ewels et al., 2016). We followed methods outlined in Breinholt et al. (2018) to assemble and assign orthology to each target capture locus with a few modifications. In brief, we assembled each locus individually using iterative baited assembly with *SPAdes* (Prjibelski et al., 2020) and reference loci from the chromosome-length genome assembly of *Tanypteryx hageni* (Petaluridae) (Tolman, Beatty, Bush, Kohli, Moreno, et al., 2023). We then screened each locus for orthology by first ensuring that the locus did not have BLAST hits to multiple places in the genome and secondly by ensuring best reciprocal hits between the reference and the query sequence. We performed subsequent analyses using assemblies of the probe region of our loci, as preliminary analyses recovered increased noise and reduced phylogenetic support using probe + flanking regions if a low number of loci was recovered from any taxon, as well as flanking regions expressing high variability in alignment.

Phylogenetic analysis

We generated multiple sequence alignments for each locus using the ‘MAFFT-linsi’ algorithm in *MAFFT v.7.475* (Katoh & Standley, 2013) and trimmed alignments using a 0.75 threshold cutoff using *trimAl v1.2* (Capella-Gutiérrez et al., 2009). We concatenated the alignment using *FASconCAT v1.11* (Kück & Meusemann, 2010) and generated an initial partitioning scheme using relaxed clustering with the model fixed to GTR + G for each subset in *IQtree v2.1.3* (Minh, Schmidt, et al., 2020). We then selected the best nucleotide substitution model for each subset in the partitioning scheme using ModelFinder and estimated a maximum likelihood (ML) tree. We estimated branch support using SH-like approximate likelihood ratio tests (SH-aLRT) and 1000 ultrafast bootstrap replicates (UFboot) in *IQtree v2.1.3* (Guindon et al., 2010; Kalyaanamoorthy et al., 2017). To assess the degree of incomplete lineage sorting (ILS), we first reconstructed ML trees for each locus with 1000 ultrafast bootstrap replicates and performed a coalescent-based species-tree estimation in *ASTRAL2 v5.6.1* using local posterior probabilities (LPPs) (Mirarab & Warnow, 2015). As an additional metric to assess the degree of ILS as well as introgression (hybridization), we calculated gene concordance factors (gCFs) and site concordance factors (sCFs), using our concatenated ML tree and our loci trees (Minh, Hahn, et al., 2020); gCF and sCF calculates the proportion of genes and informative sites, respectively, which support the bipartition (split) defined by the branches within our ML tree (Minh, Hahn, et al., 2020). We identify nodes of high support possessing bootstrap (BS) and SH-aLRT values >90, and LPP values >0.90. We identify regions of high gene and site concordance (gCF and sCF, respectively) possessing values >0.7. We rooted the tree using Neopetaliidae.

Additional analyses of phylogeny

Preliminary phylogenetic analyses recovered *Somatochlora georgiana* (coppery emerald) as sister to Libellulidae with mixed support (SH-alrt: 100 UFBoot: 100, LPP: 0.39). We checked for contaminants among aligned loci sequenced for *S. georgiana* by blasting them to the National Center for Biotechnology Information (NCBI) Basic Local Alignment Search Tool (BLAST) Database using the *blastn* command (Y. Chen et al., 2015). Top predicted identity blast hits (percent identity) for our loci belonged to annotated assemblies of Zygoptera including *Ischnura elegans* (Price et al., 2022), *Pantala flavescens* (Liu et al., 2022), as well as other arthropod genomes including *Megalopta genalis* (Hymenoptera: Halictidae), *Zootermopsis nevadensis* (Blattodea: Archotermopsidae), *Cryptotermes secundus* (Blattodea: Kalotermitidae) and *Neodiprion fabricii* (Hymenoptera: Tenthredinoidea) (Herrig et al., 2023; Jones et al., 2015; Kapheim et al., 2020; Lin et al., 2021; Terrapon et al., 2014). Furthermore, 24 of our loci did not recover any blast hits, most likely due to locus fragment motifs being unique among Anisoptera (Bybee et al., 2021; Goodman et al., 2023) and lacking arthropod homologues within GenBank. However, *S. hineana* was recovered as the top predicted blast hit for two legacy genes (Cytochrome c oxidase subunit 1: Locus 2001 and Cytochrome b: Locus 2000), suggesting genes are still usable to place *S. georgiana* within the genus. To utilize *S. georgiana* within our analysis, we first reconstructed ML trees for each locus with 1000 ultrafast bootstrap replicates. Using a custom-built python script, we (1) rooted each gene tree with Neopetaliidae, (2) determined the most closely related taxa to *S. georgiana* using branch length as a proxy, (3) calculated the bootstrap support values of *S. georgiana* + sister taxon, (4) retained the sequences of *S. georgiana* from loci where it is sister to a *Somatochlora* species with high bootstrap support, (5) realigned, trimmed and concatenated our revised locus list as outlined in our methods and (6) reran our ML phylogenetic tree in *IQtree*. We verified the results of our code through visual inspection of our gene trees. In total, we retained 37/92 loci for *S. georgiana*, recovering it within *Somatochlora* with high support across metrics (SH-alrt: 100 UFBoot: 100, LPP: 1.0). As this method of loci pruning is new and heavily dependent on correct taxonomy of species, we verified our specimen of *S. georgiana* using dichotomous keys from Garrison et al. (2006) and Walker (1925). All subsequent analyses utilize this new pruned-loci dataset, which herein will be referred to as our '*no-coded*' dataset.

Preliminary analyses also recovered discrepancies in topology of *Somatochlora* between our *no-coded* ML and ASTRAL trees suggesting ILS and hybridization, as also made evident by our low gCF and sCF scores across *Somatochlora* (Figure 2). However, we also hypothesize that these differences are the result of 3rd codon site saturation, where the signal to noise ratio is skewed, reducing resolution (Parvathy et al., 2022; Simmons et al., 2006; Yang, 1996). Codon usage bias has been more commonly observed in high-throughput molecular datasets as well as insect genomes (Behura & Severson, 2012, 2013; Breinholt & Kawahara, 2013; Galtier et al., 2018; Sharma & Uddin, 2014). To test for 3rd codon site saturation, and using another custom-built python script, we conducted two analyses. We set the reading frame of our *no-coded* alignments of all our loci to reduce the amount of stop codons. Next, we (1) replaced the third-codon position to 'R' for purine nucleotides (A and G), and 'Y' for pyrimidines (C and T) across all our loci (herein referred to as '*RY-third*' analysis), (2) recoded all nucleotides as R's or Y's for all of our loci (herein referred to as '*RY-all*' analysis), (3) for each of these two datasets, we realigned, trimmed and concatenated our loci as outlined in our methods and (4) reconstructed new ML and ASTRAL trees for both of our RY-coded loci datasets. RY-coding of nucleotides is common practice to reduce noise in data as it reflects signal coming from transitions and transversions (J.-N. Chen et al., 2014; Harshman et al., 2008; Kück & Meusemann, 2010; Phillips et al., 2004; Simmons, 2017; Timmermans et al., 2016; White et al., 2011; Woese et al., 1991). Custom-built python and bash scripts were created with the assistance of ChatGPT (OpenAI, 2023) and provided in the Supplemental information.

Fossil selection and time divergence analysis

Taxonomy of odonate fossils relies predominantly on wing characters due to their high preservation potential, and plethora of venational traits (Fraser & Tillyard, 1957). However, most researchers acknowledge that wing venation is highly prone to convergence and should be used in conjunction with other traits if available (Fleck et al., 2008; Fraser & Tillyard, 1957; Gloyd, 1959; Hennig, 1981). Amber fossils of adult and nymphal Odonata are rare in the fossil record (Bechly, 1996; Boudet et al., 2023; Karr & Clapham, 2015; Schädel & Bechly, 2016; Wighton & Wilson, 1986; Zheng & Jarzembowski, 2020) (see tab. 1 in Schäedel et al., 2020) (paleodb.com), limiting analyses pertaining to accessory genitalic, thoracic, penile or nymphal traits. Kohli et al. (2016) published a list of vetted fossil calibrations for Odonata, as part of the Fossil Calibration Database (fossilcalibrations.org), providing recommendations for fossil selection covering the breadth of taxa in our phylogeny. We chose fossil calibrations for the crown nodes for Cavilabiata, Chlorogomphidae + Cordulegastridae, Macromiidae, Corduliidae, Libellulidae and Corduliidae + Libellulidae. Phylogenetic and age justifications for divergence time estimation of our fossils are outlined in Kohli et al. (2016) and Kohli, Letsch, et al. (2021) (Table 1).

Fossil validation

We surveyed two additional tentative *Somatochlora* fossils as calibration points, extending our sampling beyond Kohli, Letsch, et al. (2021). We used the five principles outlined by Ksepka et al. (2015) and Parham et al. (2012) as best calibration practices. In brief, the five criteria are as follows: (1) Fossil accession number for fossil and referrals, (2) apomorphy-based or phylogenetic analysis, (3) reconciliation of morphological and molecular data, (4) locality and stratigraphic data for fossil taxa and (5) radioisotopic age or numeric age references for fossils.

Two putative fossils of *Somatochlora* exist, the older being *Somatochlora oregonica* Cockerell, 1927 from Central Oregon, which is estimated to be Oligocene in origin (33.9–28.4 Ma). However, we are sceptical of this identification since the fossil is only of the upper half of either the forewing or hindwing, from the nodus to the pterostigma, possessing the first radial vein (R_1), and the first and second medial veins (M_1 and M_2) (Needham et al., 2000). Within his diagnosis, Cockerell (1927) draws similarities of the fossil to other species of *Somatochlora* by the first two postnodal crossveins distal to the pterostigma being obliquely angled, while the subnodal veins are perpendicular. Cockerell (1927) also states that doubling of cells occurs at the 7th crossvein between R_1 and M_2. However, these traits are quite variable among North American *Somatochlora* (Garrison et al., 2006; Needham, 1930; Needham et al., 2000; Walker, 1925; Walker & Corbet, 1975). Cockerell (1927) also states that the first subnodal cell is very long, roughly equal to three postnodal cells. Although this is the case with some *Somatochlora* species, other North American corduliid genera also possess this trait including *Dorocordulia*, *Epitheca*, *Helocordulia* and *Neurocordulia* (Garrison et al., 2006; Needham

TABLE 1 Fossil calibration points used in our *no-coded* time-calibrated phylogeny, as well as calibration points for different scenarios for the placement of *Somatochlora brisaci*.

		Fossil age			
	Fossil species	Prior lower bound (Ma)	Prior upper bound (Ma)	Reference	Catalogue number
Tree node (full analysis)					
Cavilabiata (Root)	*Juralibellula ningchengensis*	158.1	158.1	Huang and Nel (2007)	NIGP 143439
Chlorogomphidae + Cordulegasteridae	*Araripechlorogomphus muratai*	112.6	158.1	Bechly and Ueda (2002)	KMNHIP000004
Macromiidae	*Epophthalmia biordinata*	15.5	158.1	Lewis (1969)	UC Boulder MC 1a
Libellulidae	*Tauriphila cerestensis*	29.2	158.1	Nel and Paicheler (1993)	MNHM PE 2014/4
Libellulidae + Corduliidae	*Palaeolibellula zherikhini*	89.5	158.1	Fleck et al. (1999)	PIN 2041/1
Corduliidae	*Croatocordulia platyptera*	12.7	158.1	Charpentier (1843)	No collection number
Tree node (Scenarios 2–4)					
Antipodochlora (Corduliidae)	*S. brisaci*	8.7	158.1	Nel et al. (1996)	MNHN-LP-R.10420
Guadalca insularis (Corduliidae)	*S. brisaci*	8.7	158.1	Nel et al. (1996)	MNHN-LP-R.10420
Somatochlora (Corduliidae)	*S. brisaci*	8.7	158.1	Nel et al. (1996)	MNHN-LP-R.10420

Note: Table includes name of the fossil taxa, the family/genus node placement of the fossil (Kohli et al., 2016; Kohli, Letsch, et al., 2021; Nel et al., 1996), the lower and upper bound age of the fossils (Kohli, Letsch, et al., 2021), publication which describes the fossil and museum/university catalogue number. Phylogenetic justifications for fossils included in full analysis are outlined in Kohli et al. (2016), Kohli, Djernæs, et al. (2021) and Kohli, Letsch, et al. (2021), while phylogenetic justification for *S. brisaci* is included in Appendix.

et al., 2000). Finally, Cockerell (1927) states that at the second cell below the pterostigma possesses an oblique crossvein which can be found in *S. arctica*. However, no *Somatochlora* species, including *S. arctica* possess a second crossvein below the pterostigma. Overall, we exclude this fossil for calibration, as the traits mentioned do not provide strong enough synapomorphies to place the fossil into *Somatochlora*.

The second fossil, *Somatochlora brisaci* (Nel et al., 1996), is significantly younger, with a Miocene origin (8.7–5.3 Ma), discovered in a deposit from Southeastern France. The fossil is a near-complete hindwing except for a few posterior regions of the wing margin missing near the third and fourth medial veins, the cubital (C) and anal (A) veins. Within the description, Nel et al. (1996) performed a parsimony analysis on the fossil, comparing it with other corduliid, synthemistid and macromiid genera. The authors conclude that the *S. brisaci* does seem to be related to *Somatochlora* but forms an unresolved trichotomy with the genera *Antipodochlora* and *Guadalca*. *Somatochlora brisaci* was temporarily attributed to *Somatochlora* because the arculus is midway between the first two antenodal crossveins, but the authors acknowledge several key traits which separate it from recent species; the most noticeable being the triangle possessing four cells (see Table S2 and Appendix).

Using our *no-coded* ML phylogeny, we performed several additional morphological analyses to verify the fossil placement of *S. brisaci*. Using two independent lists of wing trait characters from Nel et al. (1996) and Ware (2008), we scored the wings of all extant species within our phylogeny. We traced each trait onto our ML phylogeny using *Mesquite v3.81* (W. Maddison & Maddison, 2007) using a parsimony-based reconstruction of character history, retaining characters which possessed high phylogenetic signal within families and genera and excluding the ones which exhibited homoplasy (Retention Index > 0.70) (Farris, 1989; Källersjö et al., 1999). After removing homoplasious characters, we then combined both revised Ware et al. and Nel et al. datasets; if the two datasets had similar characters, such redundancies were removed. We then scored the wing traits of *S. brisaci* using this morphological trait set and performed phylogenetic analyses using parsimony in *TNT v1.6* (Goloboff et al., 2008), Bayesian inference (BI) using *MrBayes v3.2* (Huelsenbeck & Ronquist, 2001) and ML using *IQtree v2.1.3* (see Supplemental information). We applied an MK model of discrete character evolution for our BI and ML analyses (Lewis, 2001).

Since fossil choice and placement can drastically affect the outcome of divergence time analysis (Kohli, Letsch, et al., 2021), we ran divergence time estimation under four different scenarios. The first scenario was designated as '*No Fossil*'; we exclude *S. brisaci* from our analysis for a total of six remaining fossil calibrations. The second scenario was designated as '*Somatochlora node*'; we place *S. brisaci* on the node of the *Somatochlora*. The third scenario was designated as '*Guadalca node*'; we place *S. brisaci* on the node of *Guadalca*. The fourth scenario was designated as '*Antipodochlora node*'; we place *S. brisaci* on the node of *Antipodochlora* (Table 1).

All divergence time analyses were conducted on the nucleotide dataset in MCMCtree as implemented in the software package *PAML v.4.7a* (Yang, 2007) using an ultrametric (equal branch lengths) version of our *no-coded* ML tree. We used our full unpartitioned dataset due to computational limits since our dataset consists of over 1000 loci. Fossil calibrations were set using uniform prior distributions with hard upper and lower bounds (Table 1). Our root maximum age was set at 158.1 million years, based on the earliest fossil within Cavilabiata (*Juralibellula ningchengensis*) (Huang & Nel, 2007). We set default parameters for defining prior distribution and used the general time reversible (GTR) nucleotide substitution model for calculating the hessian matrix for our dataset. For each scenario, we performed two independent MCMC runs with 500,000 iterations, sampling every 100 trees with a 2000 tree burn-in, and checked for convergence using *Tracer v. 1.6* (Drummond & Rambaut, 2007). Finally, we examined prior distributions of each run to ensure reasonable fossil choices and placement on the tree (Warnock et al., 2012). Divergence time estimates and outputs from all the four scenarios are provided in our Supplemental information.

Biogeographic analysis

We estimated the ancestral range of *Somatochlora* excluding outgroups with the R package *BioGeoBears v1.1.2* (Matzke, 2013) using our *no-coded* Bayesian-estimated time-calibrated phylogeny from MCMCtree. We chose BioGeoBears due to its customization of dispersal rates, time stratification events and comparison of different likelihood and Bayesian dispersal models. Furthermore, BioGeoBears incorporates a new parameter called the founder-event speciation (J), which allows for the possibility that a new population could colonize a new area via a 'jumping dispersal event' (Matzke, 2013). We conducted the analysis three ways: (1) using the dispersal extinction cladogenesis (DEC, DEC + j) model (Ree & Smith, 2008), (2) using a likelihood implementation of the dispersal-vicariance analysis (DIVA, DIVA + j) (Ronquist, 1997) and (3) using a Bayesian-like implementation of area estimation (BayArea-like, BayArea + j) (Matzke, 2013). Although Ree and Sanmartín (2018) highlight conceptual and statistical issues with the DEC + j model, recent work has validated +j models as valid in AIC$_c$ comparisons (Matzke, 2022). Wallace's biogeographic regions are commonly used when estimating ancestral ranges for insects (Kawahara et al., 2023; Lohman et al., 2011; Toussaint, Chiba, et al., 2021; Toussaint, Dias, et al., 2019; Toussaint, Ellis, et al., 2021; Toussaint, Vila, et al., 2019; Tseng et al., 2022), but delineations among biogeographic regions may vary across studies; this is for many reasons, such as variable dispersal among taxa, and many insect taxa have evolutionary histories that predate modern continents (Holt et al., 2013; Olson et al., 2001). As such, we chose modified Wallacean biogeographic regions which are not only hypothesized as being broad geographic ranges of *Somatochlora* (Allen et al., 1985; Walker, 1925; Walker & Corbet, 1975) but have been used previously in inferring biodiversity of Odonata in the Nearctic and Palearctic regions (Abbott et al., 2022; Kalkman et al., 2022). We used three geographic ranges, (A) IndoMalay region (referred to as IndoMalay in Olson et al., 2001 including the Chinese provinces of Sichuan, Hubei, Anhui and Jiangsu), (B) Eastern North Hemisphere (defined as Palearctic by Olson et al., 2001) and (C) Western North Hemisphere (defined as Nearctic by Olson et al., 2001), which does not include the three mountain ranges of northern and central Mexico.

We tested for statistical differences of constrained versus unconstrained models (e.g.: DIVA and DIVA + j), using the *p*-value of likelihood ratio test (LRT). We determined the most optimal model for explaining our data using the criterion of the highest negative log-likelihood (−LnL), and the lowest Akaike information criterion (AIC). Furthermore, we performed an unstratified analysis (without areas allowed/adjacency files, dispersal matrices, maximum number of areas and dispersal multipliers) due to the wide geographic range of *Somatochlora*.

RESULTS

Phylogenetic analysis

Within our *no-coded* analysis, we captured an average of 571 loci (range: 37–1049) across taxa in our dataset of de novo sequences. The concatenated alignment of these loci resulted in an alignment length of 216,393 basepairs with a total of 67,846 parsimony informative characters, 24,386 singleton sites and 124,161 constant sites. Within our outgroup taxa, nodal support was high across our tree, and the topology was relatively consistent among our SH-aLRT/UFBoot, and ASTRAL phylogenies (Figure 2).

We recover the monophyly of the superfamily Libelluloidea, as well as its constituent families including Libellulidae, Corduliidae, Synthemistidae and Macromiidae with high support. Synthemistidae is sister to the remaining Libelluloidea. Macromiidae is recovered as sister to Corduliidae and Libellulidae, with Corduliidae and Libellulidae being sister taxa. We recover all genera as monophyletic with strong support, with gCF and sCF values being high among species-level relationships, but low among genus- and family-level relationships (Figure 2) (see Supplemental information for ASTRAL analysis).

Throughout all analyses conducted (*no-coded*, *RY-all*, *RY-third*), we recover the monophyly of *Somatochlora* with high support (SH-alrt: 100, UFBoot: 100, LPP: 1.0), with *C. borisi* recovered as sister to the rest of *Somatochlora* with high support (SH-alrt: 100, UFBoot: 100, LPP: 1.0) (Figures 2 and 3). The remaining results pertain to species and clade-level relationships within *Somatochlora* for our *no-coded*, *RY-all*, *RY-third* analyses.

Within our *no-coded* ML tree, we recover one clade of mixed support (SH-alrt: 99.9, UFBoot: 64) and three highly supported clades (Figures 2 and 3). *Somatochlora ozarkensis* is recovered sister to Clades I and II with mixed support (SH-alrt: 96.7, UFBoot: 78). Clade I (mixed support) comprises eight species of IndoMalay (*S. daviesi*, *S. dido*) and Northern Hemisphere distribution including *S. georgiana*, which is sister to *S. daviesi* with high support. Clade II contains nine species of North Hemisphere distribution, including the circumpolar species *S. sahlbergi*. Clade III contains three species of IndoMalay (*Somatochlora shanxiensis*) and Eastern North Hemisphere (*S. graeseri*,

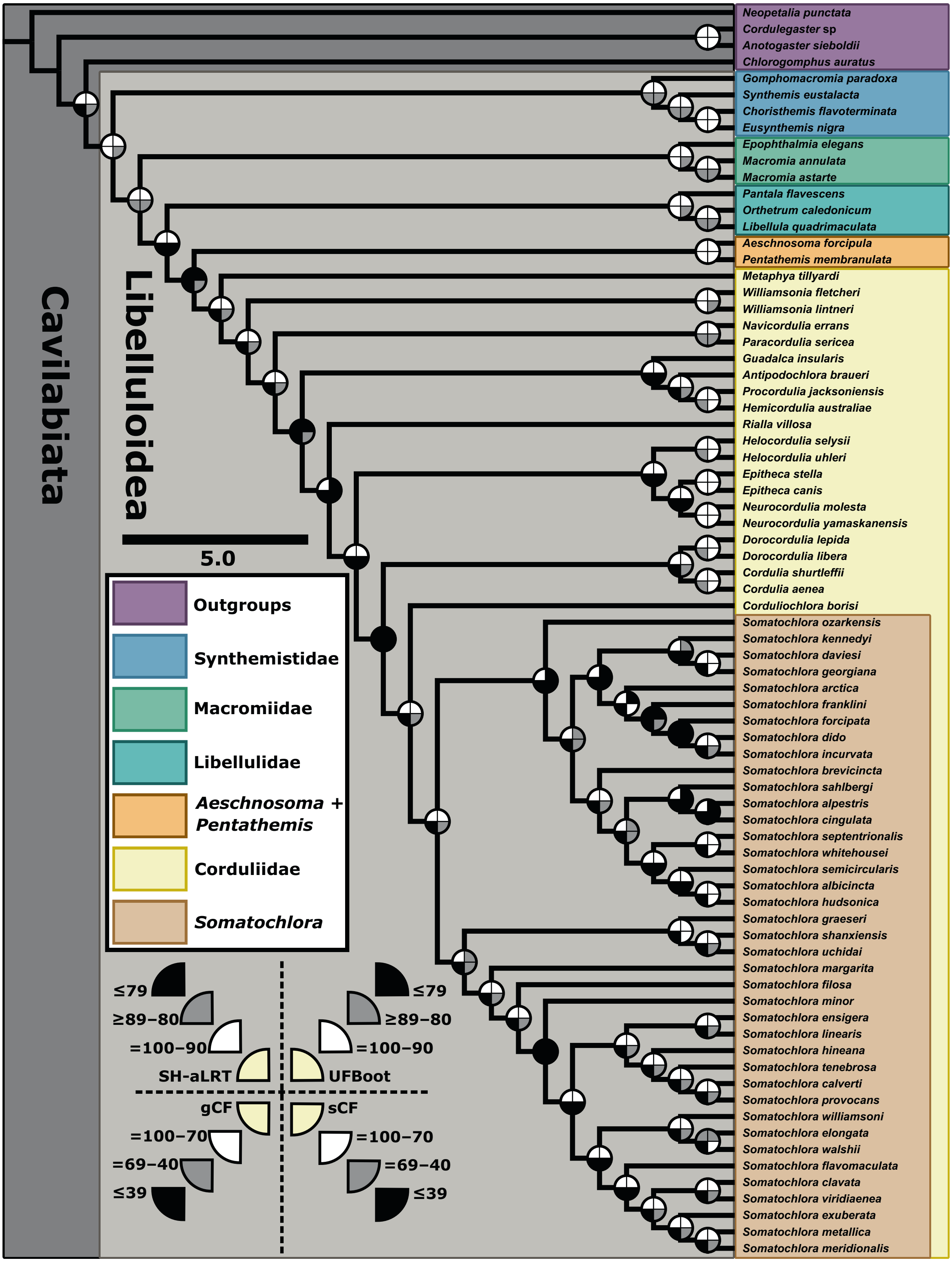

FIGURE 2 Molecular phylogeny of Cavilabiata using our *no-coded* maximum likelihood topology of best-scoring tree inferred in IQ-TREE. Loci were amplified using Anchored Hybrid Enrichment probes outlined in Bybee et al. (2021). We performed analyses using assemblies of the probe region of our loci. Nodal supports and metrics are expressed as circles, where the top left half represent SH-like approximate likelihood ratio tests (SH-alrt), the right half represent Ultrafast bootstrap support (UFBoot), the bottom left half represent gene concordance factors (gCFs), and the bottom left represent site concordance factors (sCFs). Vertical grey bars represent Cavilabiata (Neopetaliidae + Cordulegastridae + Chlorogomphidae + Libelluloidea), and the superfamily Libelluloidea (Synthemistidae + Macromiidae + Libellulidae + Corduliidae). Horizontal coloured regions represent odonate families as well as *Aeschnosoma* + *Pentathemis* and the genus *Somatochlora*.

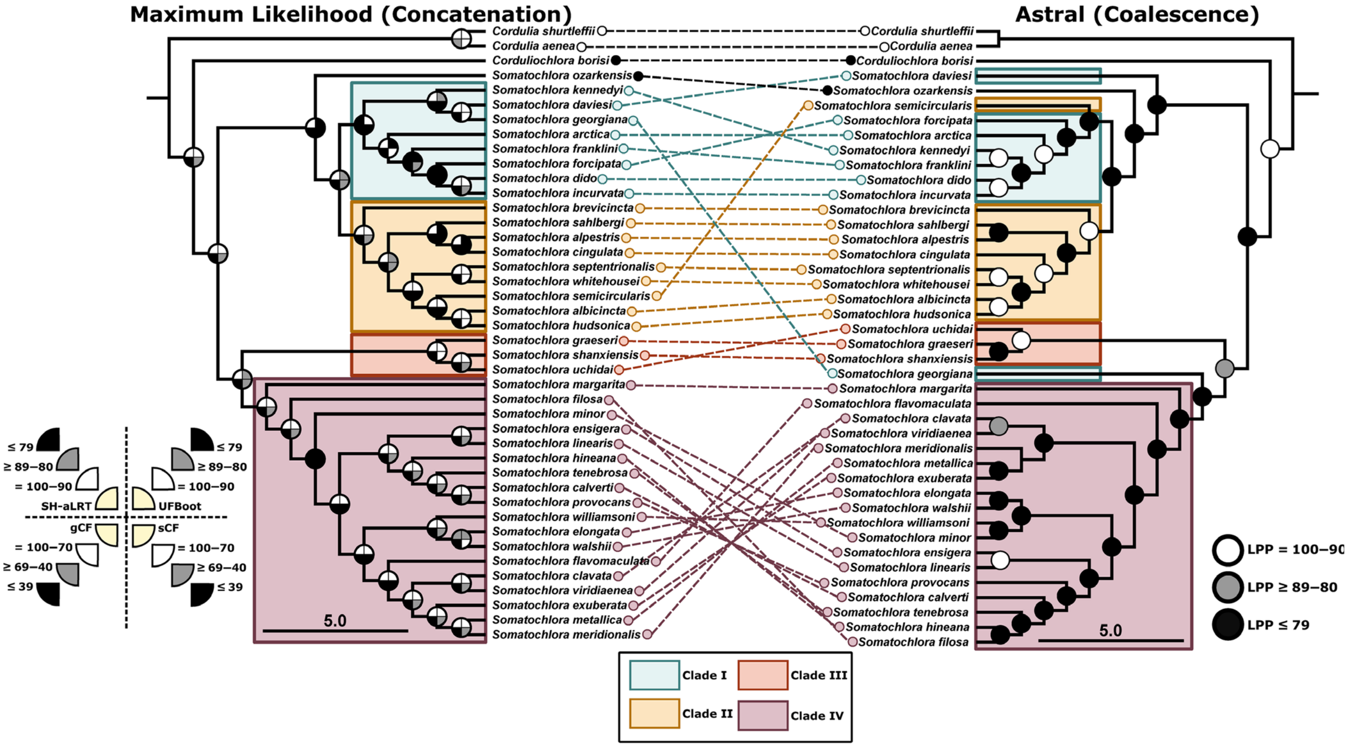

FIGURE 3 Molecular phylogeny of *Somatochlora* and *Corduliochlora* excluding outgroups, using concatenation-based maximum likelihood (ML) topology of best-scoring tree inferred in IQ-TREE (left), and coalescence-based topology of our tree using ASTRAL, derived from Ultrafast Bootstrap trees of each of our loci (right) of our *no-coded* dataset. Nodal support for our ML tree is the same as Figure 2, while nodal support for our ASTRAL tree is expressed as whole circles which represent local posterior probabilities (LPP) support. Horizontal coloured regions and circles represent recovered clades within *Somatochlora* within our ML tree, which are reflected within our ASTRAL tree. Black circles represent recovered outgroups as well as *Corduliochlora borisi* and *Somatochlora ozarkensis*. Dashed lines across phylogenies represent topological similarities differences of taxa between our ML and ASTRAL trees between clades. gCFs, gene concordance factor; sCF, site concordance factor; SH-alrt, SH-like approximate likelihood ratio tests; UFBoot, ultrafast bootstrap support.

S. uchidai) distribution. Finally, Clade IV contains 18 species of North Hemisphere distribution. Species-level relationships within clades possessed varying support (SH-alrt: 100–10.1, UFBoot: 100–33) Concordance factors were low within species-level and clade-level relationships within *Somatochlora* (gCF: 17–0, sCF: 73.2–25) (Figures 3 and 4).

Within our *no-coded* ASTRAL tree, we recover *S. daviesi* as sister to *S. ozarkensis* + Clade I + Clade II, all with low support (Figures 3 and 4). *Somatochlora semicircularis* jumps from Clade II (ML tree) to being sister to Clade I with low support, with the monophyly of Clade I still possessing low support. The monophyly of Clade II, Clade III and Clade IV are highly supported. As with our ML tree, species-level relationships within clades possess varying support (LPP: 1.0–0.07) (Figures 3 and 4).

Within our *RY-all* ML tree, we recover *S. ozarkensis* being sister to Clades I and II with mixed support (Figure 4). We recover the monophyly of Clade I with mixed and Clade II with high support. *Somatochlora georgiana* and *S. daviesi* are recovered as sister taxa with high support, sister to Clade III with mixed support. We recover the monophyly of Clades III and IV with high support. Concordance factors were extremely low across our *RY-third* ML tree (gCF: 0–21.1, sCF: 0) (Figure 4).

Within our *RY-all* ASTRAL tree, we recover *S. daviesi* sister to *S. semicircularis*, which is sister to *S. ozarkensis* which is sister to Clades I and II, all with low support (Figure 4). We recover Clades I and II with low support. *Somatochlora georgiana* recovers sister to Clade III with low support, and we recover the monophyly of Clade III and IV with low support (Figure 4).

Within our *RY-third* ML tree, we recover an identical topology to our *RY-all* ML analysis in which *S. ozarkensis* is sister to Clades I and II with mixed support (Figure 4). We recover the monophyly of Clade I with mixed support, and Clade II with high support. *Somatochlora georgiana* and *S. daviesi* are recovered as sister taxa with high support, sister to Clade III with mixed support. We recover the monophyly of Clades III and IV with high support. sCFs were higher compared with our *RY-all* analysis (gCF: 0–17.5, sCF: 99.3–21.3) (Figure 4).

Within our *RY-third* ASTRAL tree, we recover *S. georgiana* sister to the remaining *Somatochlora* with high support (Figure 4). We also recover *S. daviesi* which is sister to *S. semicircularis*, which is sister to *S. ozarkensis* + Clade I + Clade II, all with low support. We recover the monophyly of Clade I with low support, and Clades II, III and IV with high support (Figure 4).

Morphological analyses of fossil placement of *S. brisaci*

Scored morphologic traits revealed synapomorphies shared among and within families sampled within our phylogenetic analysis;

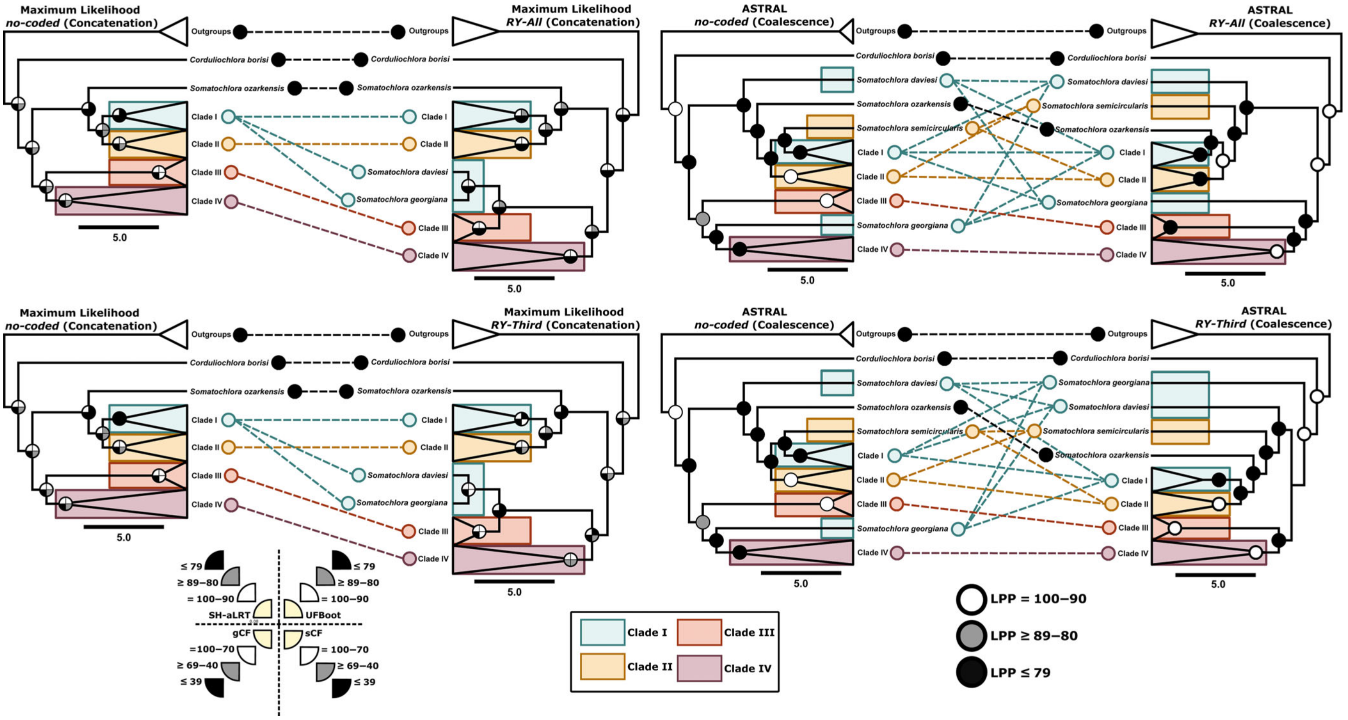

FIGURE 4 Molecular phylogeny of *Somatochlora* and *Corduliochlora* excluding outgroups, using concatenation-based maximum likelihood (ML) topology of best-scoring tree inferred in IQ-TREE (left), *RY-all* and *RY-third* ML analysis (top and bottom middle, respectively), and *RY-all* and *RY-third* coalescence-based topology of our trees using ASTRAL, derived from Ultrafast Bootstrap trees of each of our loci (top and bottom right, respectively). RY-coded phylogenies were generated by first changing the reading frame of our alignments to reduce the amount of stop codons. We then replaced the third-codon position to 'R' for purine nucleotides (A and C), and 'Y' for pyrimidines (C and T) for all our loci (*RY-third*) and recoded all basepairs as R's or Y's for all of our loci (*RY-all*). Nodal support graphics and coloration of clades are the same as Figure 3. Dashed lines across phylogenies represent topological differences of clades and taxa between our original ML tree (left) and our RY-coded ML trees (middle), and between our RY-coded ML trees (middle) and RY-coded ASTRAL trees (right). Horizontal coloured regions and circles represent recovered clades within *Somatochlora* within our original ML tree, which are reflected among our RY-coded ML and ASTRAL trees. Black circles represent recovered outgroups, as well as *Corduliochlora borisi*, and *Somatochlora ozarkensis*. gCF, gene concordance factor; LPP, local posterior probabilities; sCF, site concordance factor; SH-alrt, SH-like approximate likelihood ratio tests; UFBoot, ultrafast bootstrap support.

however, we found no wing traits which are synapomorphies of *Somatochlora*, as only plesiomorphies were found, shared among Corduliidae and, in some cases, Corduliidae + Libellulidae (see Supplemental information).

ML, Bayesian and parsimony-based phylogenetic analyses of our wing morphology data did not recover *S. brisaci* within *Somatochlora*. We did not recover a polytomy of *Antipodochlora*, *Guadalca* and *S. brisaci* as previously hypothesized (Nel et al., 1996), as all three analyses recovered large polytomies for the family Corduliidae with low phylogenetic support for any genus-level relationships. All three analyses recover the families Libellulidae, Macromiidae and Cordulegastridae with high support (ML: >70, BI: >0.70, Parsimony: >70) and in some cases Synthemistidae except for *Gomphomacromia* which recovered within Corduliidae with low support. Due to the results of our morphological phylogenetic analysis, we chose to exclude *S. brisaci* as a calibration point within our divergence analysis (Scenario I, 'no *S. brisaci*'), and all subsequent discussion will pertain to the results of Scenario I, with the remaining scenarios mentioned briefly to explore the degree of divergence time of *Somatochlora* when *S. brisaci* is placed at different nodes (see Supplemental information).

Time divergence analysis

Within Scenario I (*No Fossil*), we estimate the clade that contains *C. borisi* + *Somatochlora* to be 23 Ma (HPD: 11–35 Ma). We estimate *Somatochlora* to be 19 Ma (HPD: 9–29 Ma), *S. ozarkensis* to be 17 Ma (HPD: 8–27 Ma), Clade I to be 13 Ma (HPD: 6–21 Ma), Clade II to be 12 Ma (HPD 6–20 Ma), Clade III to be 7 Ma (HPD: 2–14 Ma) and Clade IV to be 16 Ma (HPD: 7–24 Ma) (Figure 5).

We estimate the most recently diverging species of *Somatochlora* (Clade II: *S. hudsonica* + *S. albicincta*) to have originated 1 Ma (0.3–4 Ma). We estimate the same divergence times for *Somatochlora* in Scenarios II and III (*Guadalca* and *Antipodochlora* nodes, respectively) at 19 Ma (HPD: 10–29 Ma). For Scenario II (*Somatochlora node*), we estimate *Somatochlora* at 18 Ma (HPD: 9–25 Ma), suggesting minimal divergence time variation for *Somatochlora* without the use of *S. brisaci* as a calibration point (see Supplemental information).

Biogeographic analysis

BioGeoBears ancestral range analysis revealed our constrained DEC + J model received the highest likelihood (LnL = −32.53,

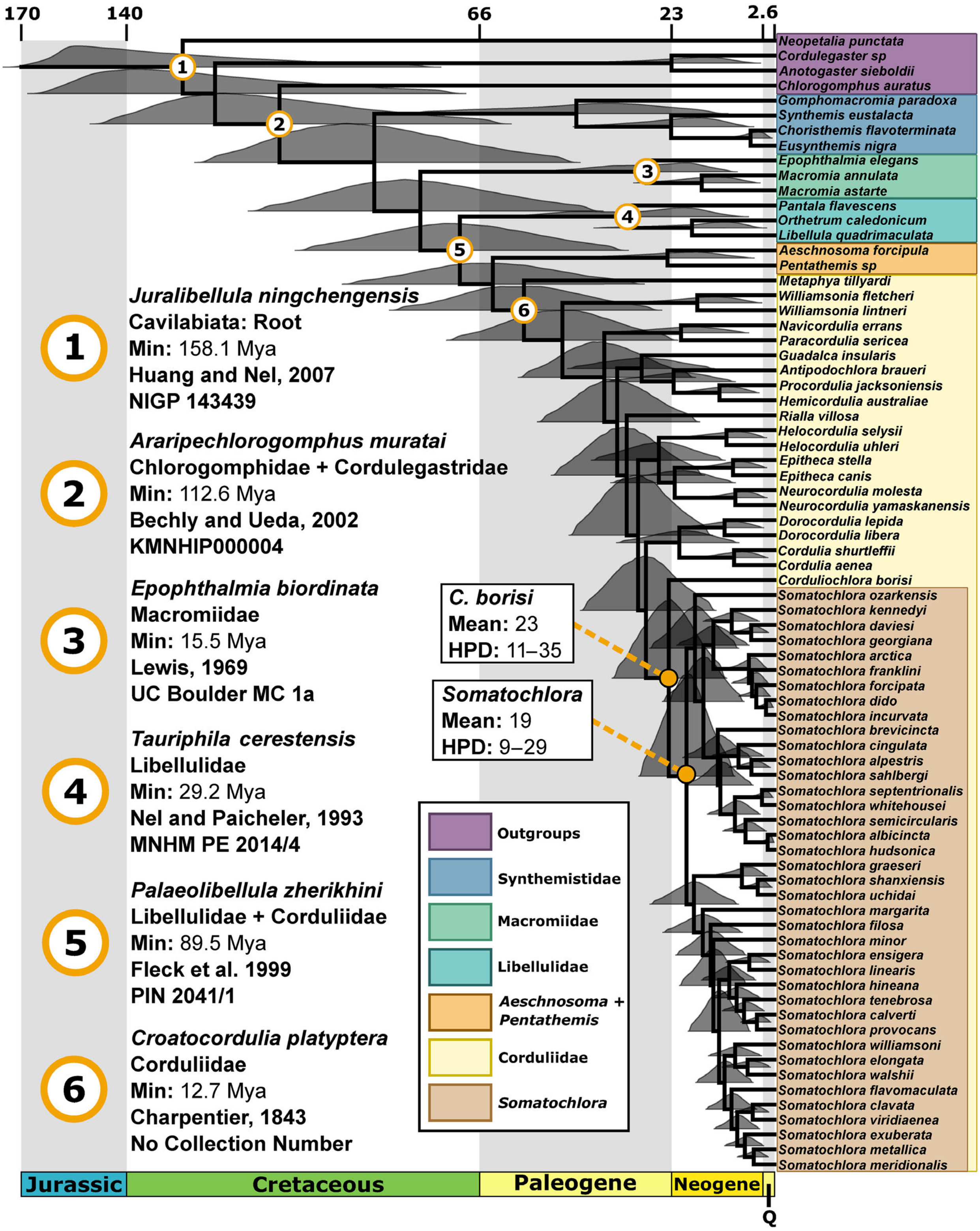

FIGURE 5 Bayesian-estimated time-calibrated phylogeny of Cavilabiata using an ultrametric (equal branch length) version of our *no-coded* ML tree without use of *S. brisaci* as a calibration node. Divergence time estimates were generated using MCMCtree as implemented in the software package *PAML v.4.7a* Yang (2007) using our full unpartitioned dataset. Numbered circles represent placement of fossil calibration points throughout the tree (Table 1), along with the name of the fossil taxa, the hypothesized family of the fossil (Kohli et al., 2016), the minimum age of the fossil from Kohli, Letsch, et al. (2021), publication which describes the fossil and museum/university catalogue number. Horizontal coloured regions are the same as Figure 3. White boxes indicate the mean divergence time, and highest probability density for *Corduliochlora* and *Somatochlora* Grey distributions represent full posterior distribution from MCMCtree run using the R package *MCMCtreeR v1.1* (Puttick, 2019).

$\text{AIC}_c = 71.73$) and was statistically different than our unconstrained DEC model ($p < 0.001$) (see Supplemental information). Under our DEC + J model, *C. borisi* possesses an ancestral Laurasian (North Hemisphere) distribution, with *Somatochlora* originating in the Western North Hemisphere (Figure 6).

We also estimate that *S. ozarkensis* + Clade I + Clade II originated and diversified in the Western North Hemisphere within the early Miocene, except for *S. alpestris* + *S. sahlbergi* which possess an ancestral Laurasian distribution. Although *S. dido*, and *S. daviesi* (both Clade I) are modern-day IndoMalay species, we estimate a Western North Hemisphere origination within the early Pliocene and Pleistocene, respectively. We also estimate two distinct dispersal events to the Eastern North Hemisphere within Clade III, and in a monophyletic group of six modern-day Eastern North Hemisphere species within Clade IV (*S. flavomaculata*, *Somatochlora clavata*, *Somatochlora viridiaene the Eastern and Westerna*, *Somatochlora exuberatea*, *S. metallica* and *S. meridionalis*) (Figure 6). We recover very similar biogeographic results within our DIVA + j model ($\text{LnL} = -32.77$, $\text{AIC}_c = 72.21$), as well as our BayArea-like + j model ($\text{LnL} = -34.59$, $\text{AIC}_c = 75.82$); both models were statistically different than their unconstrained models ($p < 0.001$) (see Supplemental information).

DISCUSSION

Phylogenetic analysis

Within all analyses conducted, we recover the monophyly of *Somatochlora* using both concatenation (ML) and coalescent-based (ASTRAL) approaches with high support, congruent with the conclusions of past single-locus, multi-locus, transcriptomic and AHE analyses (Bybee et al., 2021; Goodman et al., 2023; Kohli, Letsch, et al., 2021; Ware, 2008; Ware et al., 2007). We recover three well-resolved clades within *Somatochlora*, and one of mixed support. Although support for species-level relationships varies across our tree, with sister-species relationships differing between our ML and ASTRAL trees (Figures 3 and 4), we recovered similar relationships within smaller phylogenies of *Somatochlora* (Geiger et al., 2021; Hernandez & Cognato, 2024; Karube et al., 2012; Kohli et al., 2018; Pàmies-Harder et al., 2024).

The two most comprehensive ML-based phylogenetic analyses of *Somatochlora* are from Kohli et al. (2018) (18 species) and Pàmies-Harder et al. (2024) (31 species), both using cytochrome c oxidase subunit I (COI) of multiple individuals. Results from our *no-coded* analyses agree with their analyses for some species-level relationships, but deeper node relationships are incongruent. Although both phylogenies only possess a few Clade I taxa, Kohli et al. (2018) recover *S. arctica* (*S. franklini* + *S. semicircularis*), while Pàmies-Harder et al. (2024) recovers *S. incurvata* (*S. franklini* + *S. arctica*). Since we recover Clade I with mixed support within our ML and ASTRAL trees (Figure 3), we are unsure of the placement of *S. arctica*, *S. franklini* and *S. incurvata* in relation to the other clades recovered. Furthermore, *S. semicircularis* jumps from Clade II within our ML tree, to Clade I within our ASTRAL tree (Figure 3). *Somatochlora semicircularis* does possess very long branch lengths within our ML tree (see Supplemental information) due to the recovery of only 250 loci (500 kb specimen) causing noise in the placement of this species. *Somatochlora arctica*, *S. franklini* and *S. incurvata* all possess the shortest branch lengths of any *Somatochlora* species, diverging at the end of the Miocene (5 Ma) (Figures 4 and 5). Distributions for *S. incurvata* and *S. semicircularis* include the east and western coasts of the United States and Canada, respectively, *S. franklini* inhabits both coasts of United States and Northern Canada and *S. arctica* resides throughout the entire Eastern North Hemisphere; nymphs of all four species are recorded to reside in sphagnum/peat bogs, swamps and fens (Brunelle, 2020; Groenendijk & Bouwman, 2010; Kennedy, 1913; Shiffer, 2017; Walker, 1918, 1925; Walker & Corbet, 1975; Willey, 1974; Willey & Eiler, 1972). Finally, *S. semicircularis*, *S. arctica* and *S. franklini* are considered alpine/subalpine high-elevation species (Walker, 1925). Due to the similarity in range, habitat and elevation, we hypothesize insufficient time has occurred for all species to become genetically distinct from one another due to their rapid cladogenesis and hypothesized ancestral sympatry.

Within Clade II, we recover *S. albicincta* + *S. hudsonica* and *S. whitehousei* + *S. septentrionalis* which is supported in Pàmies-Harder et al. (2024). However, Kohli et al. (2018) recovers *S. hudsonica* (*S. septentrionalis* (*S. whitehousei* + *S. albicincta*)). Interestingly, Pàmies-Harder et al. (2024) note that roughly 40% of species groups in their analysis not identified based on COI were from the Nearctic, suggesting the inclusion of more loci within our phylogeny provides the more accurate result.

Although all three phylogenies recover the monophyly of Clade III with high support, Pàmies-Harder et al. (2024) recover Clade III within Clade II making it paraphyletic, while Kohli et al. (2018) recover Clade III sister to the remaining *Somatochlora* species. Our phylogeny is unique in that we add *S. shanxiensis* to this clade, which may affect the topology of *Somatochlora*, placing Clade III sister to Clade IV. Furthermore, support for the placement of Clade III is low both within Kohli et al. (2018) and Pàmies-Harder et al. (2024); a consequence of COI not recovering deep node relationships. Since we recover high nodal support for the separation of Clades II, III and IV within our ML and ASTRAL trees, we are confident in stating Clade III is sister to Clade IV. Finally, within Clade IV, all three phylogenies recover *S. metallica* + *S. exuberata*; however, all other species-level relationships vary within Kohli et al. (2018) and Pàmies-Harder et al. (2024). These discrepancies may be due to lack of sample size in species coverage for *Somatochlora* within both previous analyses.

There was incongruence in the species-level relationships and clade-level support within our concatenated ML and coalescent-based phylogenies, and thus, a question that arises is why is there such discordance between our ML and ASTRAL trees? Three possible phenomena may be driving this incongruence in our analyses (or a combination of thereof): ILS, noise due to third-codon position saturation, and wide amounts of hybridization and introgression among taxa.

Organisms which undergo reticulate evolution (which is observed more within high-throughput sequencing datasets) as opposed to

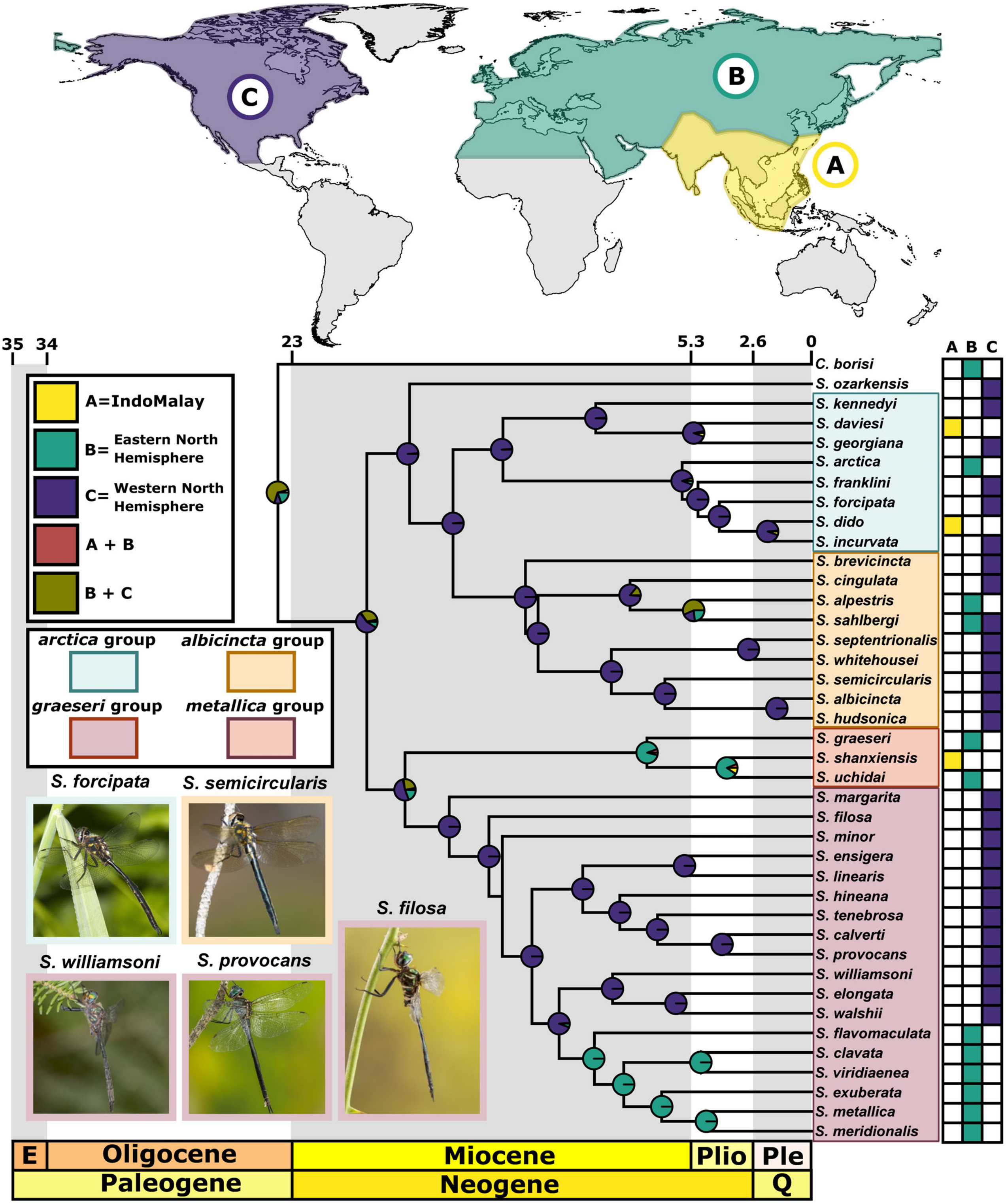

FIGURE 6 Ancestral ranges of *Somatochlora* and *Corduliochlora* excluding outgroups from our DEC + J model output from *BioGeoBears v1.1.2* analysis (Matzke, 2013), using our Bayesian-estimated time-calibrated *no-coded* phylogeny from MCMCtree. Map represents modified Wallacean biogeographic regions implemented in our analysis. Geographic ranges include (A) IndoMalay region (referred to as IndoMalay in Olson et al., 2001 including the Chinese provinces of Sichuan, Hubei, Anhui and Jiangsu), (B) Eastern North Hemisphere (defined as Palearctic by Olson et al., 2001), and (C) Western North Hemisphere (defined as Nearctic by Olson et al., 2001), which does not include the three mountain ranges of northern and central Mexico. Horizontal coloured regions at phylogeny tips represent recovered clades within *Somatochlora* from our original ML phylogeny (Figure 3). Coloured boxes represent modern-day distributions of *Somatochlora* species in accordance with our biogeographic regions (see Supplemental information). Pie charts at each node represent the most likely ancestral distribution of each *Somatochlora* species. Photographs of *Somatochlora* species provided by J. Abbott.

classic bifurcation leads to differences between gene and species trees (Mallet et al., 2016). This 'phylogenomic conflict' can be caused by a litany of factors including ILS, introgression (hybridization) and rapid evolution (Degnan & Rosenberg, 2009; W. P. Maddison & Knowles, 2006; Steenwyk et al., 2023). ILS is common among rapidly evolving lineages of insects, especially when populations are high, and branch lengths are short (Cicconardi et al., 2023; He et al., 2023; Herrig et al., 2024; Nabholz, 2024; Wang et al., 2023). The results of our ASTRAL tree suggest that the ILS is causing substantial noise within our concatenated ML phylogeny. The starkest example of this is within Clade IV, where species-level relationships completely reshuffle within our ASTRAL tree (Figure 3). Clade II seems to be the most stable (except for *S. semicircularis*) in which most species-level relationships were retained among both analyses, even though our ASTRAL support values were lower. Clades I and III seem to express an intermediate degree of ILS compared with Clades IV and II (Figure 3). Although diversification tests would have to be conducted to determine if *Somatochlora* is an example of a rapid radiation within Corduliidae, its short branch lengths, relatively recent cladogenesis, widespread Northern Hemisphere distribution, high degrees of morphological variation within male claspers and high degrees of ILS would suggest so.

Our concordance factors provide additional support for ILS within *Somatochlora*. Larger genomic datasets are less prone to variance but consequentially are more prone to inflated support values (Thomson & Brown, 2022). As such, traditional metrics of support (bootstrap, posterior probability) provide limited utility. Concordance factors attempt to describe heterogeneity among phylogenies, and the degree of concordance and discordance particular nodes possess (Baum, 2007). Concordance factors are not a metric of support but provide identification of branches in a species tree which possess the highest proportion of the genome for which a given clade is true (Lanfear & Hahn, 2024). Gene concordance factors provide the most comprehensive approach to genealogical variation, as it is the proportion of gene trees that support a clade in a species tree. However, this metric is prone to gene-tree estimation error; a consequence of limited data if genes possess short basepair length (Lanfear & Hahn, 2024). Site concordance factors are less affected by gene-tree estimation error as it calculates the proportion of sites supporting clades in species-tree topology. Within our analyses, we observed low gCF and sCF values across the phylogeny of *Somatochlora*. Low gCF values suggest high degrees of discordance among gene trees and the species tree, supporting ILS, as well as hybridization and introgression; gene-tree estimation error could be affecting gCF values since our loci vary between ~200 and 500 bp. Low sCF factors could also suggest sites undergoing multiple substitutions (saturation), obscuring evolutionary signal (Lanfear & Hahn, 2024).

When we account for third-codon site saturation, that is, our *RY-third* analyses, our results suggest substantial noise exists at the third-codon position of our loci that is otherwise causing disagreements in topology of *Somatochlora* between our *no-coded* ML and ASTRAL trees. Due to the evolutionary age of *Somatochlora*, among site variation at the third-codon position seems plausible, in which accumulation of silent mutations at the third position is obscuring phylogenetic signal at the species level (Bofkin & Goldman, 2007; Yang, 1996). Codon site saturation is becoming more apparent in next-generation high-throughput sequencing techniques because of higher amounts genes sampled (Breinholt & Kawahara, 2013; Kapli et al., 2021; Thode et al., 2020). Within our *RY-third* ML tree, we recover a similar topology to our *no-coded* ML tree, except *S. daviesi* and *S. georgiana* are recovered as sister to Clade III with mixed support (SH-alrt: 100, UFBoot: 65) (Figure 4). As a result, support for (*S. georgiana* + *S. daviesi*) + Clade III and support for Clades III + Clade IV are mixed, suggesting the position of *S. daviesi* and *S. georgiana* may be unstable and preventing resolution, which could be due to low loci count for *S. georgiana* (37 loci) (Figure 4). Our *RY-third* ASTRAL tree recovers a very dissimilar topology to our *no-coded* ASTRAL tree in which *S. georgiana* is sister to the remaining *Somatochlora* with high support, followed by *S. daviesi*, *S. semicircularis* and *S. ozarkensis*, which is sister to Clades I and II, all with low support. The monophyly of Clade I remains low, while support for Clades II and III and IV remains high (Figures 3 and 4).

Our *RY-all* tree considers transversions and transitions across all basepairs in our dataset. Within our *RY-all* tree, we recover an identical topology to our *RY-third* ML tree, further supporting hypothesis of among site rate variation and third-codon position site saturation within our analysis (Figure 4). We recover *S. georgiana* + *S. daviesi* as sister to Clade III with mixed support, suggesting there is not enough signal to resolve the relationships of these two taxa. Our RY-*all* ASTRAL also differs from our *no-coded* ASTRAL tree in which *S. daviesi* recovers sister to *S. semicircularis*, then *S. ozarkensis* which is sister to Clades I and II, all with low support. Support for Clades I, II and III are low, while support for Clade IV is high.

Hybridization is another possible driver of these incongruences among our concatenated and coalescent analysis of our original dataset. Studies of hybridization have been most focused on damselflies (Zygoptera) using microsatellite and polymorphic DNA, and genitalic data (Hayashi et al., 2005; Sánchez-Guillén et al., 2005; Solano et al., 2018; Tynkkynen et al., 2008). Hybridization among *Somatochlora* species has only been noted anecdotally (Bick & Bick, 1981; Monroe & Britten, 2014; Walker, 1925), with *S. albicincta* and *S. hudsonica* hybridizing with *S. sahlbergi* in the Northern Yukon (Cannings & Cannings, 1997). Although all three species are observed to have specialized habitat preferences (Cannings & Cannings, 1985; Catling, 2003), hybridization seems to occur whenever they come into contact causing difficulty in species-level identification. A recent paper by Hernandez and Cognato (2024) provides the first molecular evidence of heterospecific mating and introgression within *S. hineana* + *S. tenebrosa*, *S. kennedyi* + *S. forcipata* and *S. calverti* + *S. provocans* + *S. filosa*. Phylogenies generated using mitochondrial DNA recover polytomies among these taxa, yet nuclear DNA resolves species-level relationships. Hernandez and Cognato (2024) hypothesize that increased sexual pressure to mate with heterospecifics result in the dilution of genotypes and that prezygotic barriers (i.e., male cerci) are much less pertinent to reproductive isolation than previously thought.

More recent studies have elucidated the importance of introgression (repeated hybridization events) as dominant evolutionary forces within Odonata within the past and present (Suvorov et al., 2022). What is needed to assess hybridization in *Somatochlora* is more population level sampling and/or genomic datasets (Hernandez & Cognato, 2024). Advances in long-read sequencing have allowed cost-effective genome assemblies of Odonata (Grether et al., 2023; Liu et al., 2022; Patterson et al., 2023; Price et al., 2022, 2023). Research of such genomes have explored gene family expansion, chromosomal evolution and past population size (Tolman, Beatty, Bush, Kohli, Frandsen, et al., 2023; Tolman, Beatty, Bush, Kohli, Moreno, et al., 2023; Tolman, Beatty, Frandsen, Bush, Burchim, et al., 2023). Although the complete mitochondrial genome has been sequenced for *S. hineana* (Jackson et al., 2018), a complete genome assembly of any *Somatochlora* species has yet to be sequenced, presenting an opportunity to further explore the degree of hybridization and introgression events within the genus.

Validity of *Corduliochlora*

Overall, our phylogenetic tree recovers the monophyly of *Somatochlora*, with *C. borisi* recovered as sister to the remaining species (Figures 2 and 3). This consistent placement of *C. borisi* as sister to the other *Somatochlora* species across all analyses, including our RY-coding schemes, provides strong evidence for its genetic distinctiveness. Original descriptions of adult *C. borisi* justify its elevation to *Corduliochlora* based on 17 morphological characters, most notably the absence of mesotibial keels, a female vulvar scale very deeply or completely clefted, blunt male appendages and a medial notch on the occipital triangle (Marinov, 2001; Marinov & Seidenbusch, 2007) (Figure 7). However, previous descriptions of *C. borisi* only compare adults and nymphs with other Palaearctic and some Nearctic corduliid and *Somatochlora* species but does not consider the breadth of morphological variation found across the genus. As such, we scored all 40 species we sequenced of *Somatochlora* for a subset of characters within Marinov and Seidenbusch (2007) to evaluate the diagnostic characters used in the genus. Additionally, we assessed several characters from Ware (2008) pertaining to male and female secondary genitalia, nymphs, and coloration (see Supplemental information).

Starting with the absence of mesotibial keels, Marinov (2001) and Marinov and Seidenbusch (2007) noted their absence in *C. borisi* as a diagnostic character separating it from other corduliid genera including *Helocordulia* and *Neurocordulia*, and this trait was vestigial in *Cordulia* and *Dorocordulia* (Figure 7). Although we observe their absence in the majority of *Somatochlora* species scored, we observed vestigial mesotibial keels in the eastern North Hemisphere species *S. shanxiensis*, and the Western North Hemisphere species *S. williamsoni* and *S. elongata* (Garrison et al., 2006) and keels approximately half the length of the tibia in *S. linearis*; also observed in Garrison et al. (2006) and Marinov and Seidenbusch (2007). Marinov and Seidenbusch (2007) also indicate the female vulvar scale are deeply or completely clefted in *C. borisi*, while other *Somatochlora* species possess acute or slight notches. We found the species which have secondarily elongate vulvar scales (ovipositor) do not possess any cleft. Species which possessed reduced vulvar scales, *S. whitehousei* is the only species which do not possess a cleft, *S. alpestris*, *S. cingulata* and *S. hudsonica*, possess a rudimentary cleft (slight notch), while *S. shanxiensis*, *S. uchidai* and *S. graeseri* (Clade III), as well as *S. brevicincta*, *S. sahlbergi*, *S. septentrionalis*, *S. semicircularis* and *S. albicincta* all have acute clefts (Figure 8). *Somatochlora flavomaculata* possesses the deepest cleft but does not divide the vulvar scale such as *C. borisi*. Blunt male appendages are another trait which differentiated *C. borisi* from other *Somatochlora* species; however, we found *S. ozarkensis*, *S. margarita*, *S. provocans* and *S. calverti* to possess blunt superior appendages (Needham et al., 2000); we define blunt as appendages as possessing either rounded, or club-like terminal ends, not possessing narrowed or trailing ends (Figure 8). Finally, Marinov and Seidenbusch (2007) observe *C. borisi* as possessing a medial notch on the occipital triangle. We observe no other *Somatochlora* species exhibiting this trait.

Other minor differences of *C. borisi* noted by Marinov and Seidenbusch (2007) include coloration of the frons which they cite as being yellow on both sides with inner lower edges closing towards each other, while *Somatochlora* possess yellow spots on the transverse bar. We observe both these traits across our phylogeny, with *S. georgiana* and *S. hineana* possessing no distinctive marks, being singular in colour. Another trait includes a divided inferior appendage in *C. borisi*, while other *Somatochlora* species possess narrowing inferior appendages (Marinov & Seidenbusch, 2007) (Figure 7). While the majority of *Somatochlora* possess narrowing inferior appendages, we observe *S. tenebrosa* as possessing the same divided inferior appendage as *C. borisi*, while *S. cingulata* exhibits a very divided inferior appendage (Figure 8). Wing venational characters cited in Marinov and Seidenbusch (2007) which separate *C. borisi* from *Somatochlora* include a straight crossvein at the arculus, and arculus being distal to the triangle, while *Somatochlora* possess a 'kinked' crossvein and the arculus aligning to the triangle. However, we found both straight and kinked crossveins, as well as the arculus being distal or aligned to the triangle across *Somatochlora* species.

We do observe two traits which appear to be unique within *C. borisi*: the genital lobe possessing a rectangular projection at the distal end and with a distinctive spine-like lobe which is absent in other *Somatochlora* species (Figure 7), as well as a short hamulus with an acute end, while other *Somatochlora* species possess longer hamuli with the same acute end. Although the spine-like lobe does appear to be unique to *C. borisi*, variation in the genital lobe does appear among *Somatochlora* species, with morphologies ranging from spade shovel-like, rounded and rectangular. Furthermore, the shape of the hamule in *C. borisi* is the same as in all *Somatochlora* species, curving posteriorly and terminating acutely, but measurement of hamule length requires further study, as 'longer' and 'shorter' hamuli are not specified quantitatively in Marinov and Seidenbusch (2007), as only four individuals were sampled.

In conclusion, we find characters which were proposed to separate *C. borisi* from other corduliid genera to be variable, including the

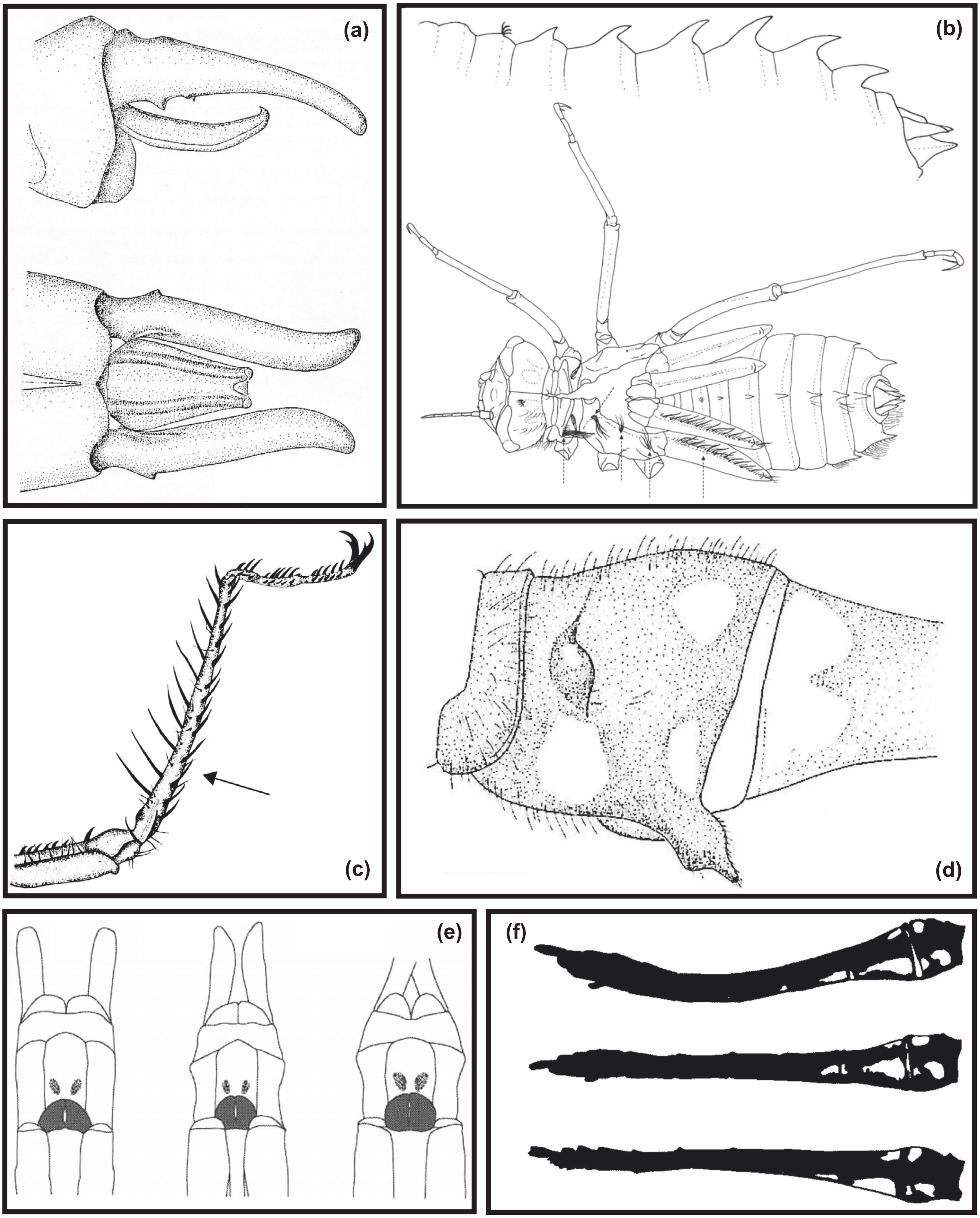

FIGURE 7 Morphological characters of adult and nymph *Corduliochlora borisi* used to differentiate it from *Somatochlora* and elevation to genus level. (a) Downcurved, blunt male appendages (cerci), (b) presence of middorsal spines on the nymphs, (c) absence of mesotibial keels, (d) Rectangular projection at the distal end of the genital lobe, and with a distinctive spine-like lobe (e) deeply clefted vulvar scale (subgenital plate), (f) reduced subgenital plate (ovipositor). Illustrations were modified from Boudot et al. (2004), Fleck et al. (2007) and Marinov (2001).

presence of two cubital-anal crossveins and the absence of mesotibial keels. Although we find the presence of two cubital-anal crossveins within our analysis (Garrison et al., 2006; Needham et al., 2000), we discover additional *Somatochlora* species which possess mesotibial keels, making this trait less stable from differentiating among other corduliid genera. Since *C. borisi* was most similar to *Somatochlora*, *Cordulia* and *Helocordulia* within Marinov and Seidenbusch (2007), they proposed the designation of *Corduliochlora* as opposed to lumping all three genera into one. However, our phylogenetic analysis recovers the monophyly *Cordulia*, *Neurocordulia*, *Dorocordulia* and *Helocordulia*, and they are found as distinct taxa from *Somatochlora* suggesting further morphological work is required to parse out intergeneric relationships (Figure 2) (Goodman et al., In Prep.). Thus, for *Somatochlora*, *C. borisi* is unique in possessing a medial notch on the occipital triangle, and a deep nearly divided cleft in the female vulvar scale, and a spine-like projection on the genital lobe. An ecological difference is an earlier flying season for *C. borisi* between mid-May and late June. Considering the morphological differences retained for *C. borisi*, as well as its phylogenetic placement, sister to the remaining *Somatochlora*, we argue that our data remain inconclusive in assigning *C. borisi* to *Somatochlora*, thereby maintaining the validity of *Corduliochlora*.

Taxonomy of *Somatochlora*

Considering the variation we see in our *RY-third* and *RY-all* analyses, we are unable to determine the position of *S. georgiana*, *S. daviesi* and *S. semicircularis* in our topology; we regard these taxa to be unresolved. We tentatively suggest the monophyly of Clade I of *Somatochlora* with or without the inclusion of all three species. Interestingly, our phylogeny does recover a *metallica* group of *Somatochlora* dragonflies, albeit greatly expanded, but refute the *arctica* group due to *S. arctica* being recovered within Clade I and *S. alpestris* recovered within Clade II. Acknowledging the uncertainties of our phylogeny, we assign Clade I as a modified *arctica* group, Clade II as the *albicincta* group, Clade III as the *graeseri* group and Clade IV as an expanded *metallica* group.

From the first classifications of *Somatochlora* by Walker (1925) to the recent field guides and classifications of Odonata (Garrison et al., 2006), the shape, size and ornamentations of the male cerci (appendages), are used for species-level differentiation among taxa (Garrison et al., 2006). Within our phylogeny, we do observe some similar patterns in cerci morphology among each of our clades (Figure 8).

The cerci of *S. ozarkensis* run parallel at the base and converge towards each other at the distal half. Tips of the cerci (apices) are blunt, rounded and slightly swollen. Within the *arctica* group, the cerci are ‘earwig-shaped’, in which they run parallel at the proximal half, curve outward at the distal half, and curve inward at the distal 2/3, converging at the tips (akin to the claspers of an earwig); the degree of downward bending in lateral profile or curvature of angular bend in dorsal profile, thickness of cerci, presence of ventrobasal teeth at the distal 2/3 (very pronounced in *S. arctica* and *S. forcipata*), and length of paraproct varies among species (Hernandez & Cognato, 2024; Walker, 1918, 1925; Walker & Corbet, 1975). Finally, the apices are pointed (Figure 8).

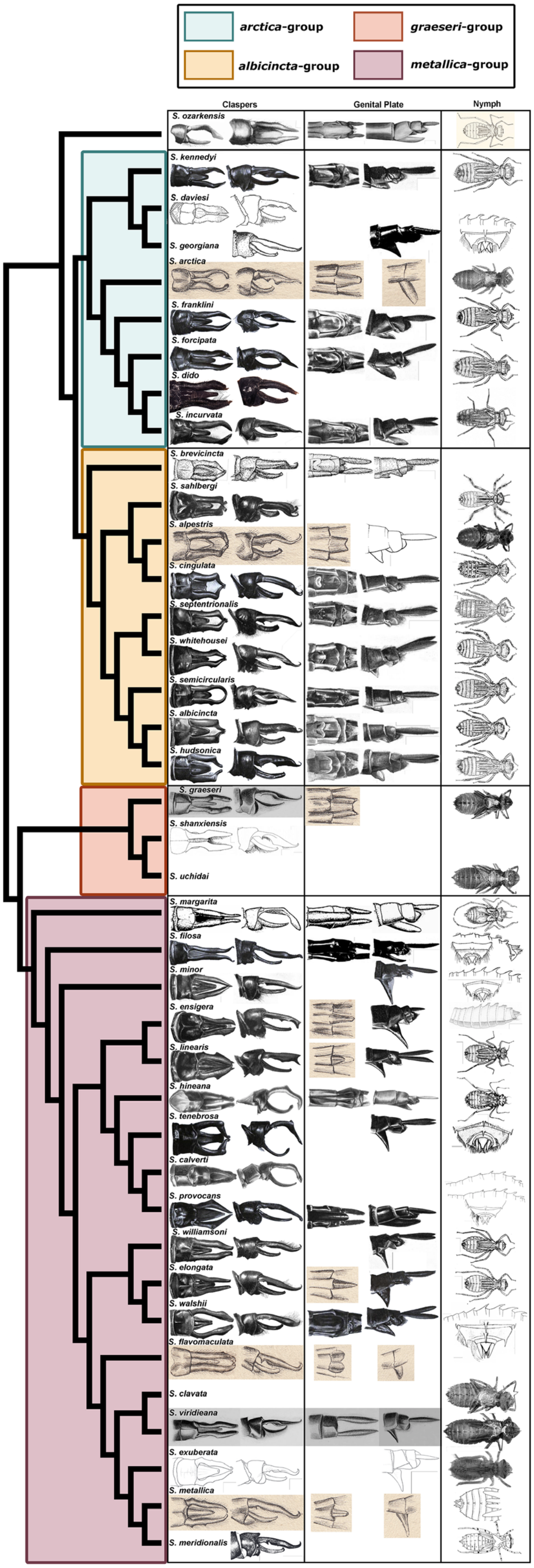

FIGURE 8 Legend on next page.

The *albicincta* group are similar to the *arctica* group except the cerci bend more angularly at the distal 2/3, and in some cases overlap each other at the tip (*S. sahlbergi*) (Figure 8). The apices possess curled, distally tapered, pointed ends (akin to elf-shoes), which curl completely backwards. Furthermore, some species possess a lateral spine, whose position along the length of the cerci varies among species, as well as presence of ventrobasal teeth at the base of the cerci (*S. alpestris*, *S. septentrionalis*, *S. whitehousei*, *S. albicincta*, *S. hudsonica*). Finally, in dorsal profile, there is variation in the degree of bending of the cerci, in which some species bend inward (*S. cingulata*, *S. septentrionalis*, *S. whitehousei*), outward (*S. brevicincta*) or are straight (*S. sahlbergi*, *S. albicincta*) (Figure 8).

The cerci of the *graeseri* group are parallel at the base and possess a bend outward at the distal half dorsally and bend inward at the distal half, but do not converge at the tip (Figure 8). Species possess a ventrobasal tooth at the base of the cerci, and the apices possess curled, distally tapered, pointed ends, which curve upwards.

Cerci variation is highest within the *metallica* group with clades within expressing similar morphologies (Figure 8). The cerci of *S. margarita* run convergently at the base, running parallel at the terminal ends; the apices are blunt and slightly swollen (same as *S. ozarkensis*). *Somatochlora* filosa are unique in which the claspers run convergently at the base, but curve outward at the distal half, terminating with pointed apices facing each other. *Somatochlora minor* is similar to *S. margarita* but are more broad in dorsal profile, possess lateral spines at the distal 1/3 of the cerci, with the cerci meeting at the end, with apices pointed and curled upward. *Somatochlora ensigera* and *S. linearis* are unique in possessing bifid paraprocts in lateral profile with each lobe pointed and possess two ventrobasal teeth and a pronounced lateral spine at the distal 2/3. *Somatochlora hineana*, *S. tenebrosa*, *S. calverti* and *S. provocans* all possess distinctive ‘clamp-like’ cerci, which are highly angulated in lateral profile (almost 90-degrees), as well as the inferior appendage (epiproct); *S. provocans* expressed the least amount of angulation. Furthermore, the apices can be either pointed (*S. hineana*, *S. tenebrosa*) or blunt/swollen (*S. calverti*, *S. provocans*). In dorsal profile, cerci can either be convergent at the base and narrow (*S. calverti*, *S. williamsoni*), or broad and widely divergent, and sharply angulate, converging in the distal half (*S. tenebrosa*, *S. provocans*). The remaining taxa within the *metallica* group, share similarities in their cerci, in which all possess 1–2 ventrobasal teeth and lateral spines at the base. Differences among these taxa include the cerci being broad in dorsal profile and divergent at the base (*S. walshii*, *S. exuberata*, *S. meridionalis* and *S. metallica*), narrow in dorsal profile and divergent at the base (*S. elongata and S. williamsoni*), or narrow in dorsal profile and parallel at base (*S. flavomaculata*) (Figure 8).

The genital plate is another trait which has been used in the past to group species of *Somatochlora*, due to variation in the degree of elongation, clefting and angulation (Needham et al., 2000; Paulson, 2011; Walker, 1925; Walker & Corbet, 1975). The genital plate of *S. ozarkensis* is secondarily elongate (ovipositor), but not projected ventrally (Figure 8). The *arctica* group possess secondarily elongate subgenital plates but are not projected ventrally. The *albicincta* group possess reduced subgenital plates, expressing variable degrees of clefting, with some ventrally projected taxa (*S. alpestris*, *S. whitehousei*). The *graeseri* group all possess reduced genital plates which are deeply clefted, and not projected ventrally. Finally, the *metallica* group possess secondarily elongate ovipositors and are ventrally projected except for *S. margarita* and *S. hineana* (Figure 8).

The presence of middorsal hooks in the nymphs of *Somatochlora* has been used previously to assign groupings among European species, with *S. metallica*, *S. meridionalis* and *S. flavomaculata* all possessing spines, while northern European species (*S. alpestris*, *S. arctica*) lacking hooks (Dijkstra & Schröter, 2020; Pinkert et al., 2018). Within our analysis, we find that the nymphs of the *arctica* group and *albicincta* group possess no middorsal spines, while *S. ozarkensis*, the *graeseri* group and *metallica* group do. Furthermore, we find that nymphs of the *graeseri* group and *metallica* group possess lateral spines on segments S8 and S9, while this trait is variable among the *arctica* group and *albicincta* group (*S. semicircularis* possess spines only on S9) (Figure 8).

In the case of taxa which we were unable to resolve (*S. georgiana*, *S. daviesi* and *S. semicircularis*), the morphology might provide some clues as to which *Somatochlora* clade they belong to (Figure 8). *Somatochlora georgiana* possess parallel cerci which converge at the tip, with the apices pointed and curled upward, the genital plate is secondarily elongate and ventrally projected, while the nymphs possess middorsal hooks, and lateral spines on S8 and S9. *Somatochlora daviesi* possess divergent cerci at the base, which are broad and run parallel at the distal 1/3, with pointed non-curled apices. *Somatochlora semicircularis* possess ‘earwig-like’ claspers with lateral spines on the distal ½, the genital plate is reduced and clefted, while the nymphs do not possess middorsal hooks. Due to the convergence noted among the morphological characters scored within our analyses (Carle, 1982, 1995; Carle et al., 2008; Dijkstra & Vick, 2006; Hovmöller, 2006; Hovmöller & Johansson, 2004; Pilgrim & Von Dohlen, 2008), we caution their definitive placement using morphology alone without additional molecular evidence.

Time divergence and biogeographic analysis

Time-calibrated estimates suggest *Somatochlora* originated 19 Ma (earliest Miocene), radiating predominantly within the Western North

FIGURE 8 Molecular phylogeny of *Somatochlora* using maximum likelihood (ML) topology of our best-scoring tree inferred in IQ-TREE from our *no-coded* dataset (left), with illustrations and photographs of *Somatochlora* males and females and morphology of their cerci, subgenital plate and nymphs discussed in this study. Coloured boxes represent newly designated species groups within *Somatochlora*. Illustrations and photographs were modified from Abbott and Mynhardt (2007), Cannings and Cannings (1985), Carchini (1983), Cashatt and Vogt (2001), Daigle (1994), Dijkstra and Schröter (2020), Donnelly (1962), Dunkle (1977), Huggins (1983), Martin (1906), Murányi (2007), Pritchard (1936), Robert (1954), Seidenbusch (1996), Steffens and Smith (2006), Tennessen (1975), Walker (1925, 1941), Williamson (1931), Williamson and Gloyd (1933) and Zhang et al. (2014). Additional illustrations were modified from watercolour paintings created Edmond de Sélys de Longchamp and Severin, provided by the Virtual Collections portal of the Royal Belgian Institute of Natural Sciences (RBINS). Black and white photographs of *Somatochlora* nymphs were modified with permission from Odonata.jp (Digital picture book Exuviae of Odonates of Japan) (odonata.jp).

Hemisphere. Our analysis shows dispersal events to the Eastern North Hemisphere followed by speciation, one event resulting in the *graeseri* group (7 Ma), one resulting in the *metallica* group (11 Ma), and one within the *albicincta* group (5 Ma). In addition, the less well-resolved *arctica* group includes one or more dispersal events from Western North Hemisphere to the Eastern North Hemisphere and the Indo Malayan region. Dispersal to the Eastern North Hemisphere most likely occurred when there were Holarctic connections across the NALB (Zhou et al., 2006) (Figure 6). Dispersal across the Holarctic for *Somatochlora* is highly plausible as the genus *Cordulia* also possesses Western North Hemisphere (*Cordulia shurtleffi*) and Eastern North Hemisphere (*Cordulia aenea*) sister species. Laurasian connections across the NALB have been well studied in many taxa, such as disjunct plant families, butterfly species and tapeworms (Brigham-Grette, 2001; DeChaine, 2008; Galbreath et al., 2020; Haas et al., 2020; Hopkins, 1973; Maguilla et al., 2018; Scholl et al., 1968; Tiffney, 1985; Toussaint, Ellis, et al., 2021; Wooller et al., 2018). Plausible NALB connections for *Somatochlora* during the Miocene only include the Beringian Land Bridge (Brikiatis, 2014). Additionally, one species *S. sahlbergi* is found in both the Eastern and Western North Hemisphere at the poles. Population structure analyses from Kohli et al. (2018) revealed low genetic diversity within *S. sahlbergi*, in which individuals collected within the Beringian region clustered closely to both Nearctic and Palearctic individuals, suggesting that the Beringian Land Bridge may have acted as a corridor and driver of speciation for *Somatochlora* throughout its evolutionary history (Kohli et al., 2018; Kohli, Djernæs, et al., 2021).

Within our analysis, *S. ozarkensis*, the *arctica* group and *albicincta* groups originate and diversify in the Western North Hemisphere, except for *S. alpestris* + *S. sahlbergi*. *Somatochlora alpestris* (Eastern North Hemisphere) lives in sympatry with *S. sahlbergi*. Kohli et al. (2018) hypothesized that *S. sahlbergi* diverged from *S. alpestris* within the Quaternary period (~1 Ma). Although we recover an older age for the divergence of *S. sahlbergi* + *S. alpestris* of 5 Ma (Figures 5 and 6), it is still possible that these two species diverged after the closing of the Bering Land Bridge. Due to most species already diversifying before the Pleistocene (~2 Ma), we hypothesize that cyclic glaciation did not contribute to the diversification of *Somatochlora* except possibly within *S. albicincta* + *S. hudsonica* as these two species diversified 1 Ma (HPD: 0.3–4 Ma), which may account for their propensity to hybridize when in sympatry; the same can be said for *S. whitehousei* + *S. septentrionalis* which diversified ~2 Ma (HPD: 0.7–6 Ma) (Figures 4 and 5). Both species groups reside within the Eastern United States, where glacial refugia are hypothesized to exist (Barnard-Kubow et al., 2015; Gonzales et al., 2008).

A recent biogeographic study of 14 Holarctic odonate genera (including *Somatochlora*) suggests no generalized pattern of species exchange and divergence between the eastern and western North Hemispheres and that biogeographic histories are more lineage specific (Pàmies-Harder et al., 2024). Our results are in partial agreement with Pàmies-Harder et al. (2024), as we also conclude not that only did *Somatochlora* utilize the Bering Land Bridge throughout its evolutionary history and underwent three dispersal events to and from the Nearctic and Palearctic, but also that most diversification of the genus occurred before the Pleistocene. However, our data disagree with Pàmies-Harder et al. (2024) in reference to the topology of *Somatochlora* and the divergence time of the genus (10.2 Ma). Pàmies-Harder et al. (2024) do acknowledge the shortfalls of their phylogeny due to the utilization of just COI as a marker and the increasing awareness of low genetic distances between Nearctic odonate taxa resulting in noise in species-level differentiation (Galimberti et al., 2021; Geiger et al., 2021). Furthermore, Pàmies-Harder et al. (2024) discuss in detail that the lack of fossil taxa and how the utilization of published mutation rates of insects in their divergence analyses might have contributed to younger ages of their phylogenies than previously published time-calibrated estimates of Odonata (Kohli, Letsch, et al., 2021; Letsch et al., 2016). As such, we provide a modified biogeographic hypothesis of *Somatochlora* which utilizes more loci, *Somatochlora* species, and fossil calibrations, allowing for a more robust interpretation of its evolutionary history.

CONCLUSION

Our study provides the first and most comprehensive phylogenetic analysis of *Somatochlora* to date and provides a well-resolved phylogenetic hypothesis of its classification, divergence time and subsequent evolutionary history. We recover the monophyly of *Somatochlora* and define four species groups within the genus. Our molecular data show strong support for *Corduliochlora* to be the sister of *Somatochlora* and some morphological support for the validity of *Corduliochlora* as a separate genus. We recover *Somatochlora* as originating within the Miocene, possessing a Western North Hemisphere distribution that dispersing to the Eastern North Hemisphere twice. Finally, we highlight the utility of RY-coding and concordance factors in high-throughput sequencing phylogenetics to elucidate codon position saturation and ILS in species-level relationships.

AUTHOR CONTRIBUTIONS

Aaron Goodman: Conceptualization; investigation; writing – original draft; methodology; validation; visualization; writing – review and editing; formal analysis; data curation. **John Abbott:** Funding acquisition; investigation; writing – review and editing; project administration; validation; visualization; resources. **Jesse W. Breinholt:** Methodology; software; writing – review and editing; resources. **Seth Bybee:** Conceptualization; investigation; funding acquisition; writing – review and editing; project administration; validation; supervision; resources. **Paul B. Frandsen:** Methodology; software; writing – review and editing; resources; validation. **Rob Guralnick:** Funding acquisition; validation; writing – review and editing; supervision; resources; project administration. **Vincent J. Kalkman:** Funding acquisition; writing – review and editing; validation; project administration; supervision; resources. **Manpreet Kohli:** Investigation;

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ACKNOWLEDGEMENTS

We thank Ruth Salas for her tireless efforts to curate the Odonata collection at the AMNH. We thank Max Caspers for his work to curate the Odonata collection at the RMNH. We thank Bill Mauffray for his work to curate the Odonata collection at the FSCA. We also thank the three anonymous reviewers to our manuscript. The primary author also gives special thanks to Edmund Murton Walker (1877–1969), whose comprehensive classification and illustrations of *Somatochlora* (*The North American Dragonflies of the genus Somatochlora*), proved instrumental in the formulation of our phylogenetic hypotheses.

FUNDING INFORMATION

This work was supported by the National Science Foundation (NSF) Awards (Abbott: 2002489; Ware: 2002473; Bybee: 2002432; Guralnick: 2002457): Genealogy of Odonata (GEODE): Dispersal and colour as drivers of 300 million years of global dragonfly evolution.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

Data are available at the permanent Dryad link: DOI: 10.5061/dryad.prr4xgxw5.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

Data S1. Supplemental results and discussion pertaining to the divergence time of Cavilabiata and Libelluloidea, as well as morphological scores from our Maximum Likelihood (ML), Bayesian (BI), and Parsimony based analyses. Datafiles can be obtained from Dryad Digital Repository Link here: https://datadryad.org/stash/landing/show?id=doi%3A10.5061%2Fdryad.prr4xgxw5.

Table S1. Specimen provenance data including locality, date, author, collector and determiner for taxa used in our phylogenetic analysis.

Table S2. Diagnostic characters derived from Nel et al., 1996 which distinguish the fossil taxa *Somatochlora brisaci* from the modern-day taxa *Antipodochlora* and *Guadalca*. The authors conclude that the *S. brisaci* does seem to be related to *Somatochlora* but forms an unresolved trichotomy with the genera *Antipodochlora* and *Guadalca*. *Somatochlora brisaci* was temporarily attributed to *Somatochlora* because the arculus is midway between the first two antenodal crossveins, but the authors acknowledge several key traits which separate it from recent species; the most noticeable being the triangle possessing four cells.

How to cite this article: Goodman, A., Abbott, J., Breinholt, J.W., Bybee, S., Frandsen, P.B., Guralnick, R. et al. (2025) Systematics and biogeography of the Holarctic dragonfly genus *Somatochlora* (Anisoptera: Corduliidae). *Systematic Entomology*, 1–26. Available from: https://doi.org/10.1111/syen.12672

APPENDIX

FOSSIL CALIBRATIONS

Calibrating node, Scenario 1: No fossil

Calibrating node, Scenario 2: *Somatochlora*

Calibrating node, Scenario 3: *Guadalca*

Calibrating node, Scenario 4: *Antipodochlora*

Fossil item: *Somatochlora brisaci*, Nel et al., 1996

Original description: Nel, A., A. Arillo that X. Martínez-Delclòs. 1996. New fossil Odonata (Insecta) from the Upper Miocene of France and Spain (Anisoptera and Zygoptera). Neues Jahrbuch für Geologie und Paläontologie-Abhandlungen 167-219.

Further descriptive accounts: None

Locality: Montagne d'Andance (Saint-Bauzile, Ardéche, France)

Criterion 1: MNHN-LP-R.10420

Criterion 2: Table S2

Criterion 3: Parsimony analysis by Nel et al. (1996) conclude that the *S. brisaci* does seem to be related to *Somatochlora* but forms an unresolved trichotomy to the genera *Antipodochlora* and *Guadalca*. *Somatochlora brisaci* is temporarily attributed to *Somatochlora* because the arculus is midway between the first two antenodal crossveins, but the authors acknowledge several key traits which separate it from recent species including (1) The arculus is slightly distal to the proximal side of the discoidal cell, (2) Only the two postnodal crossveins are incomplete, (3) The discoidal cell is divided into four cells and (4) Vein CuP is in a distal position, nearly upon Cuspl.

As such, we ran divergence time estimation under four different scenarios. The first scenario was designated as 'no *S. brisaci*'; we exclude *S. brisaci* from our analysis for a total of five fossil calibrations. The second scenario was designated as '*Somatochlora* node'; we place *S. brisaci* on the stem of the *Somatochlora* node. The third scenario was designated as '*Guadalca* node'; we place *S. brisaci* on the stem node of *Guadalca*. The fourth scenario was designated as '*Antipodochlora* node'; we place *S. brisaci* on the stem node of *Antipodochlora*.

Criterion 4: Miocene Konservate-Laggerstätte of Montagne d'Andance (Saint-Bauzile, Ardéche, France). The specimen was most likely deposited in a lake formed by a maar crater (Demarcq et al., 1989) with a warm humid climate (Brice, 1965). The lake was most likely shallow (Ehrlich, 1966) surrounded by heterogeneous forest with an undergrowth of ferns (Grangeon, 1958; Nel et al., 2022). The lake was filled with diatomite, forming a rock strata up to 30 m (Serieyssol & Gasse, 1991). Diatomite is a soft siliceous rock which preserved the insects which presumably drowned in the lake at the time, allowing for conservation of mummified organic matter (Uhl et al., 2022). The age of the rock corresponds to the Tortonian-Messinian boundary (7.30 ± 0.15 Ma) (Pastre et al., 2004).

Criterion 5: Radioisotopic age or numeric age references given and agreeing with Criterion 4.