Universidade Federal do Rio Grande
Instituto de Ciências Biológicas
Pós-graduação em Biologia de
Ambientes Aquáticos Continentais

Estrutura de assembleias de Trichoptera (Insecta) em riachos: integrando atributos funcionais e história evolutiva

Mariana Nunes Menegat

Orientador: Prof. Dr. Luiz Ubiratan Hepp
Co orientadora: Profa. Dra. Silvia Vendruscolo Milesi

Rio Grande
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ATA DE DEFESA DE TESE DE DOUTORADO EM BIOLOGIA DE AMBIENTES AQUÁTICOS CONTINENTAIS – Nº 003/2026

Às 08h30 (oito horas e trinta minutos) do dia 18 (dezoito) do mês de junho de 2026 (dois mil e vinte e seis) reuniram-se docentes, discentes e comunidade em geral, para a Defesa Pública da Tese de Doutorado da acadêmica Mariana Nunes Menegat. A Tese intitulada **"Estrutura de assembleias de Trichoptera (Insecta) em riachos: integrando atributos funcionais e história evolutiva"** foi avaliada pela Banca Examinadora composta pelo Prof. Dr. Luiz Ubiratan Hepp (Orientador, PPGBAC/UFMS), Profa. Dra. Cristina Stenert Garcia (PPGBAC/FURG), Prof. Dr. Manuel Graça (Universidade de Coimbra – UC, Portugal) e Prof. Dr. Victor Satoru Saito (UFSCar). Após a defesa e arguição pública, a Banca Examinadora reuniu-se, para deliberação final, e considerou a acadêmica APROVADA. Desta forma, a acadêmica concluiu mais uma das etapas necessárias para a obtenção do grau de **DOUTOR EM BIOLOGIA DE AMBIENTES AQUÁTICOS CONTINENTAIS**. Nada mais havendo a tratar, às 11h00h (onze horas) foi lavrada a presente ata, que lida e aprovada, foi assinada pelos membros da Banca Examinadora, pela Acadêmica e pela Coordenadora do Curso.

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RESUMO

Ecossistemas de água doce estão entre os ambientes mais biodiversos e ameaçados do planeta, tornando central a compreensão dos mecanismos que estruturam suas comunidades biológicas. Embora numerosos estudos demonstrem que gradientes ambientais influenciam a distribuição de atributos funcionais, ainda permanece pouco compreendido como e em que medida influenciam a relação entre atributos e história evolutiva, potencialmente promovendo desacoplamentos entre os componentes filogenético e funcional. Nesse contexto, a partir da integração de bases de dados taxonômicas, funcionais e filogenéticas, investigamos como atributos funcionais e história evolutiva interagem na resposta de assembleias de Trichoptera a gradientes ambientais. Em escala regional, realizamos dois estudos em riachos de cabeceira (≤ 3ª ordem), sendo o primeiro nos biomas Pampa e Mata Atlântica (coletando os organismos com um amostrador Surber) e o segundo em riachos do Cerrado (experimento de colonização com substrato artificial), com o objetivo de avaliar a resposta dos atributos funcionais e sua relação com a história evolutiva ao longo de gradientes ambientais. Em escala temporal, compilamos uma série de dados de 19 anos em riachos da Bacia do Rio Mondego (Portugal), permitindo investigar variações na estrutura das comunidades ao longo do tempo. De maneira geral, os resultados demonstraram que atributos funcionais diferem quanto à força de associação com a história evolutiva. Atributos como tamanho e formato corporal apresentaram baixa restrição filogenética e elevada sensibilidade a gradientes ambientais, respondendo de forma consistente a variáveis locais e regionais. Além disso, os componentes taxonômico, funcional e filogenético da diversidade responderam de forma complementar no espaço e no tempo, capturando dimensões distintas da reorganização das comunidades. Em conjunto, os resultados indicam que a resposta das assembleias às pressões ambientais não é uniformemente limitada pela história evolutiva, mas mediada pela interação entre atributos com diferentes graus de conservadorismo filogenético. Nesse contexto, atributos evolutivamente lábeis mostraram maior associação com a reorganização funcional das comunidades ao longo dos gradientes ambientais. Esta tese demonstra que a reorganização das assembleias de Trichoptera em riachos frente à gradientes ambientais depende, em grande parte, de atributos funcionais evolutivamente lábeis. Isso reforça a necessidade de integrar história evolutiva e ecologia funcional para compreender e prever respostas da biodiversidade em ambientes em rápida transformação.

Palavras-chave: atributos funcionais; conservadorismo; insetos aquáticos; labilidade; sinal filogenético.

ABSTRACT

Freshwater ecosystems are among the most biodiverse and threatened environments on the planet, making the understanding of the mechanisms structuring their biological communities a central issue. Although numerous studies have demonstrated that environmental gradients influence the distribution of functional traits, it remains poorly understood how and to what extent they influence the relationship between traits and evolutionary history, potentially promoting decoupling between the phylogenetic and functional components. In this context, through the integration of taxonomic, functional, and phylogenetic databases, we investigated how functional traits and evolutionary history interact in the response of Trichoptera assemblages to environmental gradients. At the regional scale, we conducted two studies in headwater streams (≤ 3rd order), the first in the Pampa and Atlantic Forest biomes (sampling organisms with a Surber sampler) and the second in Cerrado streams (colonization experiment using artificial substrates), aiming to evaluate the response of functional traits and their relationship with evolutionary history along environmental gradients. At the temporal scale, we compiled a 19-year dataset from streams in the Mondego River Basin (Portugal), allowing us to investigate variations in community structure over time. Overall, the results demonstrated that functional traits differ in the strength of their association with evolutionary history. Traits such as body size and body shape exhibited low phylogenetic constraint and high sensitivity to environmental gradients, responding consistently to local and regional variables. In addition, the taxonomic, functional, and phylogenetic components of diversity responded complementarily across space and time, capturing distinct dimensions of community reorganization. Together, the results indicate that the response of assemblages to environmental pressures is not uniformly constrained by evolutionary history but rather mediated by the interaction among traits exhibiting different degrees of phylogenetic conservatism. In this context, evolutionarily labile traits showed a stronger association with the functional reorganization of communities along environmental gradients. This thesis demonstrates that the reorganization of Trichoptera assemblages in streams in response to environmental gradients largely depends on evolutionarily labile functional traits. These findings reinforce the need to integrate evolutionary history and functional ecology to understand and predict biodiversity responses in rapidly changing environments.

Keywords: functional traits; conservatism; aquatic insects; lability; phylogenetic signal.

APRESENTAÇÃO

Esta Tese segue o modelo de formatação adotado pelo Programa de Pós-graduação em Biologia de Ambientes Aquáticos Continentais (PPGBAC) da Universidade Federal do Rio Grande (FURG). A **Introdução Geral** contém o arcabouço teórico do tema em estudo, que será investigado ao longo dos capítulos. O primeiro capítulo da tese, intitulado "*Lability versus conservatism: evolutionary patterns of traits in stream Trichoptera*" segue as normas de formatação e está publicado na revista Freshwater Biology (Qualis A1; Percentil: 85; IF: 2.7). Neste capítulo, utilizei o sinal filogenético para verificar padrões evolutivos (labilidade evolutiva e conservadorismo filogenético) em atributos funcionais de Trichoptera, utilizando uma árvore filogenética reconstruída, a partir de uma hipótese publicada na literatura, abrangendo todos os gêneros que ocorrem no Brasil segundo o Catálogo Taxonômico da Fauna do Brasil. O segundo capítulo "*Trait lability mediates how environmental variation overrides phylogenetic constraints*" segue as normas de formatação e será submetido ao periódico Marine and Freshwater Research (Qualis A2; Percentil: 71; IF: 1.5). Neste capítulo, explorei a capacidade de atributos funcionais lábeis responderem às pressões ambientais. Avaliei como atributos lábeis e conservados respondem ao ambiente, considerando a escala de paisagem (biomas: Pampa *vs* Mata Atlântica), impacto antrópico (riachos impactados *vs* minimamente impactados) e condições limnológicas locais. O terceiro capítulo, intitulado "*Evolutionary constraint and local limnological drivers of stream Trichoptera body size*" segue as normas de formatação e está submetido ao periódico Hydrobiologia (Qualis A2; Percentil: 83; IF: 2.5). Neste capítulo, utilizei um único atributo lábil (i.e. tamanho do corpo), mensurado individualmente em cada indivíduo coletado. Avaliei como o tamanho corporal de Trichoptera responde às condições limnológicas locais quantificando a contribuição das restrições evolutivas sobre esse atributo. O quarto capítulo, intitulado "*Time and space as predictors: taxonomic, functional and phylogenetic structure of Trichoptera assemblage in Temperate streams*" segue as normas de formatação e será submetido ao periódico Hydrobiologia (Qualis A2; Percentil: 83; IF: 2.5). Neste capítulo, avaliei uma série temporal de 19 anos na Bacia Hidrográfica do Rio Mondego (Portugal) para compreender as dinâmicas espaço-temporais em relação as três dimensões da diversidade (i.e, taxonômica, funcional e filogenética) em resposta a alterações ambientais recentes. Por fim, apresento a seção de Considerações Finais e Perspectivas obtidas com o desenvolvimento da Tese. Nesta última seção, e na Introdução Geral, as citações e referências bibliográficas seguem as normas da Associação Brasileira de Normas Técnicas (ABNT).

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INTRODUÇÃO GERAL

Os ecossistemas aquáticos continentais estão entre os ambientes mais biodiversos e, simultaneamente, mais ameaçados do planeta (Albert et al., 2021; Dudgeon et al., 2006; Tickner et al., 2020). Os riachos de pequena ordem (≤ 3ª ordem; Strahler,1957) representam a maior parte da extensão das redes de drenagem e refletem diretamente a integridade ecológica de suas bacias hidrográficas (Allan, 2004; Wohl, 2017). Alterações na paisagem atuam como filtros ambientais capazes de modificar variáveis limnológicas e a heterogeneidade de habitats, influenciando a estrutura das comunidades aquáticas (Petsch et al., 2021; Poff, 1997). Desde o estudo pioneiro sobre o contínuo fluvial (Vannote, 1980) até abordagens recentes que integram múltiplas escalas espaciais (por exemplo, Liu et al., 2023), os riachos são reconhecidos como integradores da paisagem, conectando processos terrestres e aquáticos ao longo do tempo e do espaço.

Ao longo do tempo evolutivo, os ecossistemas aquáticos atuaram como cenários centrais da diversificação biológica, moldando linhagens intimamente associadas à água doce (Dijkstra, Monaghan e Pauls, 2014). A colonização desses ambientes ocorreu por meio de múltiplas transições independentes a partir de linhagens terrestres, frequentemente acompanhadas por convergência adaptativa em resposta a fatores ambientais, como gradientes de heterogeneidade ambiental, dinâmica hidrológica e conectividade entre habitats aquáticos (Dijkstra, Monaghan e Pauls, 2014; Lancaster e Downes, 2013). Em regiões tropicais, a complexidade histórica das paisagens e extensas redes de drenagem promovem elevados níveis de endemismo e radiação evolutiva (Dijkstra, Monaghan e Pauls, 2014; Naka et al., 2022). Como resultado, houve diversificação de linhagens aquáticas com ampla variedade de estratégias ecológicas, morfológicas e funcionais (Lancaster e Downes, 2013).

Nesse contexto, os insetos aquáticos se destacam como um dos grupos mais diversos, resultantes de múltiplas colonizações independentes de habitats de água doce e de repetidas transições adaptativas ligadas à colonização de diferentes tipos de ambientes aquáticos, de riachos a lagos e zonas de transição (Dijkstra, Monaghan e Pauls, 2014). Enquanto linhas paleópteras, como Ephemeroptera e Odonata, retêm caracteres ancestrais de grupos que colonizaram as águas continentais ainda no Paleozoico, grupos de Neoptera, como Plecoptera e Trichoptera, exibem radiações mais recentes associadas à ocupação de novos nichos e à reorganização das paisagens fluviais durante o Mesozoico e Cenozoico (García-Girón et al., 2024; Letsch et al., 2021; Misof et al., 2014). Além da elevada diversidade, esses organismos desempenham funções ecológicas essenciais para o funcionamento dos ecossistemas, atuando, por exemplo, na decomposição e processamento da matéria orgânica e na transferência de energia ao longo das cadeias tróficas (Oester et al., 2026; Wallace e Webster, 1996). Além disso, a forte dependência desses organismos

em relação à integridade física e química dos habitats os torna particularmente sensíveis a alterações antrópicas (Breda et al., 2018; Hepp, Landeiro e Melo, 2012; Rosenberg e Resh, 1993). Essa sensibilidade, aliada à elevada diversidade, ampla distribuição e importância para o funcionamento ecossistêmico, consolidou os insetos aquáticos como modelos indispensáveis para avaliar a integridade dos ecossistemas aquáticos, sendo amplamente utilizados no biomonitoramento de riachos (Bonada et al., 2006; Hepp e Melo, 2013; Rosenberg e Resh, 1993). Como consequência, as respostas desses organismos às pressões ambientais resultam em padrões informativos sobre a organização das comunidades, refletidos nos componentes taxonômico, funcional e filogenético (Cianciaruso, Silva e Batalha, 2009; Garcia-Raventós, Viza e Múrria, 2021; Milesi, Dolédec e Melo, 2016; Mouillot et al., 2013).

Componentes da Diversidade: Taxonômico, Funcional e Filogenético

A biodiversidade pode ser descrita a partir de diferentes componentes da diversidade, cada um enfatizando dimensões específicas da organização das comunidades biológicas. O reconhecimento de que diferentes processos atuam em múltiplas escalas levou à consolidação de abordagens que integram três componentes da diversidade: taxonômico, funcional e filogenético (Cianciaruso, Silva e Batalha, 2009). Estudos em gradientes espaciais demonstraram que esses componentes capturam mecanismos distintos de reorganização comunitária, com a diversidade taxonômica refletindo substituições de espécies (Baselga, 2010), enquanto as dimensões funcional e filogenética revelam alterações na ocupação do espaço de atributos e na composição das linhagens, respectivamente (Devictor et al., 2010). Trabalhos recentes mostram que alterações hidrológicas e no uso do solo podem afetar a diversidade funcional e filogenética de forma não paralela (Roa-Fuentes et al., 2019). Essa diferença na resposta entre componentes indica que mudanças na composição de espécies nem sempre se traduzem em alterações equivalentes nas estratégias ecológicas ou na história evolutiva representada nas comunidades. Essa perspectiva integrativa estende-se também à escala temporal, uma vez que evidências baseadas em séries temporais indicam que a estrutura funcional pode responder rapidamente a mudanças ambientais, enquanto a estrutura filogenética tende a refletir respostas mais lentas e não lineares, associadas ao conservadorismo evolutivo das linhagens (Wiens e Graham, 2005). Dessa forma, isoladamente, nenhum componente da diversidade é capaz de capturar toda a complexidade das respostas das comunidades aquáticas às pressões ambientais.

Tradicionalmente, o componente taxonômico representa a abordagem mais clássica, ao descrever a composição, riqueza e abundância de espécies em um determinado ambiente ou ao longo de gradientes ambientais, sendo historicamente consolidado como a principal métrica de

avaliação da diversidade biológica (Magurran, 2004). Embora fundamental, essa abordagem não captura totalmente alterações na ocupação do espaço funcional ou na história evolutiva representada nas comunidades, especialmente quando substituições de espécies não implicam mudanças equivalentes em atributos dos organismos ou linhagens evolutivas (Devictor et al., 2010; Purvis e Hector, 2000). Como resposta a essas limitações, o componente funcional surgiu como uma abordagem capaz de fornecer uma base mecanicista para melhorar a compreensão de como gradientes ambientais moldam a estrutura das comunidades por meio das estratégias ecológicas das espécies (Violle et al., 2007). Em insetos aquáticos, perspectivas funcionais começaram a ser incorporadas a partir dos trabalhos de Cummins (1973), que propôs a classificação dos organismos em grupos funcionais de alimentação, estabelecendo uma ligação entre a estrutura das comunidades, o uso de recursos e o funcionamento dos ecossistemas aquáticos (Cummins, 1973; Cummins e Klug, 1979). Nesse sentido, Violle et al. (2007) definem atributos (*trait*) como "qualquer atributo morfológico, fisiológico ou fenológico identificaveis ou mensuráveis no nível individual, desde a célula até o organismo como um todo, sem referência ao ambiente ou a qualquer outro nível de organização"; e distinguem atributos funcionais (*functional trait*) como "qualquer atributo que impacta a aptidão indiretamente por meio de seus efeitos no crescimento, reprodução e sobrevivência". Portanto, os atributos representam caracteres mensuráveis dos organismos que expressam estratégias ecológicas resultantes de adaptações a condições ambientais específicas, constituindo a interface entre os organismos e o ambiente. Ao integrar atributos à biologia dos organismos, a abordagem funcional permite relacionar diretamente a composição das comunidades aos processos ecológicos subjacentes e ao funcionamento dos ecossistemas (McGill et al., 2006; Petchey e Gaston, 2006). Por essa razão, abordagens funcionais têm sido amplamente aplicadas em ecossistemas aquáticos para compreender como pressões ambientais se traduzem em alterações funcionais das comunidades (por exemplo, Garcia-Raventós, Viza e Múrria, 2021; Hepp et al., 2023; Menegat et al., 2025; Milesi, Dolédec e Melo, 2016).

Além da identidade taxonômica e funcional, a estrutura das comunidades também é fortemente condicionada pela história evolutiva das espécies, incorporada pelo componente filogenético. A diversidade filogenética (PD), formalizada por Faith (1992), quantifica o comprimento total dos ramos da árvore evolutiva que conectam as espécies presentes em uma comunidade, expressando quanta história evolutiva está representada em um determinado conjunto de táxons. Essa abordagem foi posteriormente expandida para incorporar padrões de agrupamento e dispersão filogenética em comunidades, permitindo inferir sobre processos ecológicos com base no grau de parentesco entre as espécies (Cadotte, Albert e Walker, 2013; Tucker et al., 2018; Webb et al., 2002). A interpretação ecológica desses padrões baseia-se na premissa de que espécies

filogeneticamente próximas tendem a compartilhar atributos ecológicos semelhantes devido ao conservadorismo filogenético (Webb et al., 2002; Wiens e Graham, 2005). Particularmente, o agrupamento filogenético é frequentemente associado à ação de filtros ambientais que selecionam linhagens com atributos semelhantes, enquanto a dispersão filogenética pode refletir processos como exclusão competitiva ou divergência de nicho (Cavender-Bares et al., 2009; Mayfield e Levine 2010, Kraft et al 2015). Ao integrar informação evolutiva, o componente filogenético fornece uma perspectiva histórica sobre a organização das comunidades e permite avaliar se mudanças na composição taxonômica e funcional, implicam também perda de linhagens únicas ou de porções desproporcionalmente grandes da história evolutiva (Cadotte, Albert e Walker, 2013).

Sinal Filogenético, Conservadorismo e Labilidade de Atributos

Desde *A origem das espécies* (Darwin, 1859) reconhecemos que a similaridade entre organismos não é aleatória, mas resultado da história evolutiva compartilhada, nos quais caracteres derivam de ancestrais comuns. O reconhecimento de que espécies compartilham história evolutiva levou à compreensão de que elas não constituem observações estatisticamente independentes, um problema explicitado no contexto da biologia comparativa a partir dos trabalhos de Felsenstein (1985) e posteriormente consolidado no método comparativo (Harvey e Pagel, 1991). Desde então, a integração explicita da filogenia tornou-se importante para interpretar padrões ecológicos e evolutivos, especialmente no estudo de atributos funcionais e da estrutura das comunidades (Cavender-Bares et al., 2009; Webb et al., 2002). Nesse contexto, o desenvolvimento de métricas voltadas à quantificação da dependência filogenética permitiu reconhecer e descrever padrões de estrutura filogenética nos dados (Blomberg, Garland e Ives, 2003; Keck et al., 2016; Münkemüller et al., 2012; Pagel, 1999). Blomberg e Garland, 2002 definiram sinal filogenético como a "tendência de espécies relacionadas se assemelharem mais umas às outras do que a espécies escolhidas aleatoriamente da árvore", enfatizando que se trata "simplesmente do reconhecimento de padrões", sem implicar diretamente processos subjacentes. Ou seja, o sinal filogenético não fornece evidência direta sobre taxas evolutivas (Ackerly, 2009) nem sobre os mecanismos subjacentes, como deriva genética ou seleção estabilizadora (Blomberg, Garland e Ives, 2003), mas expressa o grau em que a variação de atributos acompanha a estrutura da filogenia. Como destacado por Revell, Harmon e Collar (2008), embora o sinal filogenético esteja intrinsecamente relacionado aos processos evolutivos, sua interpretação deve ser feita como uma propriedade estatística dos dados, e não como evidência direta dos mecanismos que os geraram.

Nesse sentido, a quantificação do sinal filogenético é realizada em relação a um modelo nulo de evolução ao longo da árvore filogenética, sendo o modelo de movimento Browniano (MB)

o mais amplamente utilizado (Blomberg, Garland e Ives, 2003; Diniz-Filho et al., 2012; Freckleton, Harvey e Pagel, 2002). Nesse modelo, a evolução dos atributos é descrita como um processo estocástico de deriva acumulativa, no qual a variância entre espécies aumenta proporcionalmente ao tempo desde a divergência, resultando na expectativa de maior similaridade entre espécies proximamente aparentadas (Felsenstein, 1985; Pagel, 1999). A partir disso, diferentes métricas foram desenvolvidas para quantificar o desvio em relação ao padrão previsto, incluindo o K de Blomberg, o λ de Pagel e o D de Fritz e Purvis, os quais permitem avaliar a presença e a intensidade do sinal filogenético em diferentes tipos de dados (Blomberg, Garland e Ives, 2003; Diniz-Filho et al., 2012; Fritz e Purvis, 2010; Pagel, 1999). Entre essas abordagens, a extensão multivariada da estatística K, proposta por Adams (2014), permite avaliar simultaneamente conjuntos de atributos, sendo particularmente adequada para análises que integram múltiplas dimensões do fenótipo. Assim como o K, K*mult* ≈ 1 indica padrão compatível com MB, valores < 1 sugerem sinal mais fraco (parentes menos similares que o esperado), e valores > 1 indicam sinal mais forte (parentes mais similares que o previsto) (Adams, 2014; Blomberg, Garland e Ives, 2003). A presença de sinal é avaliada por testes de permutação, nos quais os valores dos atributos são aleatorizados nas pontas (tips) da árvore filogenética. O valor observado de K*mult* é comparado à distribuição nula; um valor de p significativo indica que a semelhança entre espécies próximas é maior que a esperada ao acaso, caracterizando presença de sinal filogenético (Adams, 2014; Blomberg, Garland e Ives, 2003; Diniz-Filho et al., 2012; Kamilar e Cooper, 2013; Revell, Harmon e Collar, 2008). Dessa forma, enquanto o teste de permutação responde se há sinal filogenético, o valor de K*mult* quantifica quão forte é esse sinal em relação à expectativa sob MB. A distinção entre presença e intensidade do sinal é fundamental para a interpretação dos padrões, referindo-se, respectivamente, à existência de estrutura filogenética e ao grau em que ela se manifesta nos dados (Münkemüller et al., 2012). Em relação aos dados de atributos, a aplicação de métricas multivariadas é particularmente relevante quando os atributos são descritos por meio da codificação difusa (*fuzzy coding*), abordagem amplamente utilizada em ecologia funcional para representar a afinidade das espécies com múltiplas modalidades de um mesmo atributo (Chevenet, Dolédec e Chessel, 1994; Pavoine et al., 2009). Ou seja, cada táxon é representado por um vetor contínuo de afinidades (variando entre 0 e 3, ou entre 0 e 5; Tachet et al., 2010) para diferentes modalidades de um atributo. Essa abordagem permite representar estados não mutuamente exclusivos, como o uso de múltiplos habitats ou recursos, preservando a complexidade dos atributos (Chevenet, Dolédec e Chessel, 1994). Assim, a estatística K*mult* incorpora a natureza multivariada e contínua desses dados, permitindo avaliar como a variação conjunta das modalidades dos atributos se estrutura ao longo da filogenia.

Paralelamente aos estudos sobre sinal filogenético, os conceitos de conservadorismo

filogenético e labilidade evolutiva foram inicialmente formulados no contexto da evolução de nicho por Harvey e Pagel (1991) e amplamente desenvolvidos em estudos posteriores sobre conservadorismo de nicho e atributos funcionais (Ackerly, 2009; Kozak e Wiens, 2010; Wiens e Graham, 2005). O conservadorismo filogenético descreve a tendência de linhagens manterem ao longo do tempo atributos semelhantes, de modo que táxons aparentados exibem combinações de atributos similares ao longo da filogenia (Ackerly, 2009; Wiens et al., 2010), como exemplificado na Figura 1. Esse padrão é geralmente associado a processos como seleção estabilizadora, restrições fisiológicas e de desenvolvimento, baixo fluxo gênico e limitações históricas (Crisp e Cook, 2012). Por sua vez, a labilidade evolutiva refere-se a atributos com baixa estrutura filogenética (Figura 1), que mudam rápida ou repetidamente ao longo da árvore filogenética. Cenários de convergência evolutiva, radiação adaptativa e elevadas taxas de diversificação fenotípica estão associadas a este processo (Ackerly, 2009; Wiens et al., 2010). Em vez de categorias mutuamente exclusivas, conservadorismo e labilidade representam um continuum, no qual o mesmo atributo pode ser relativamente mais conservado em determinados clados, escalas espaciais ou contextos ambientais, e mais lábil em outros. Além disso, diferentes atributos dentro de um mesmo grupo podem apresentar graus distintos de conservadorismo, refletindo a ação combinada de múltiplos processos ecológicos e evolutivos (Harmon et al., 2010). Assim, o forte sinal filogenético pode ser interpretado como indicativo de conservadorismo, enquanto fraco sinal filogenético pode ser interpretado como labilidade.

A labilidade de um atributo pode ser compreendida a partir de duas dimensões (ou eixos interpretativos) complementares. A primeira refere-se à sua baixa estruturação filogenética (Blomberg, Garland e Ives, 2003; Revell, Harmon e Collar, 2008), expressa por sinal filogenético fraco, como exemplificado na Figura 1 e discutido no parágrafo anterior. A segunda relaciona-se à capacidade de resposta dos atributos aos gradientes ambientais. Nesse contexto, as diferenças entre táxons tendem a se alinhar a filtros ambientais, entendidos como a seleção de atributos mediada por condições ambientais, incluindo variáveis limnológicas (e.g., temperatura, oxigênio dissolvido e sólidos dissolvidos totais), a estrutura e integridade do habitat e o contexto de paisagem (Poff, 1997; Mouillot et al., 2013). Em síntese, um atributo pode ser interpretado como lábil quando (i) apresenta baixa estruturação filogenética e (ii) responde de forma consistente a gradiente ambientes. A integração entre abordagens filogenéticas e funcionais permite avaliar empiricamente essas duas dimensões, ao relacionar padrões de similaridade evolutiva com a variação de atributos ao longo dos gradientes ambientais (Faith, 1992; Mouillot et al., 2013; Webb et al., 2002).

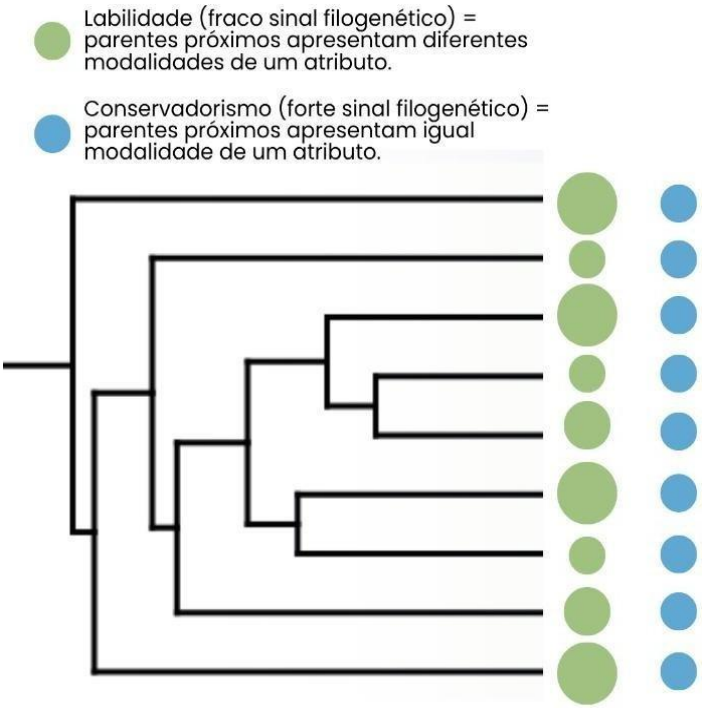


Figura 1. Representação conceitual dos padrões de labilidade e conservadorismo filogenético em atributos funcionais.

Ordem Trichoptera (Kirby, 1813): modelo de estudo

Diante desse cenário, organismos que combinam elevada diversidade, forte associação com gradientes ambientais e sensibilidade a alterações no habitat representam modelos particularmente informativos para investigar os mecanismos que estruturam a biodiversidade em riachos. A Ordem Trichoptera destaca-se como uma das mais representativas dentre os insetos aquáticos, constituindo a mais diversa entre os insetos holometábolos de água doce (Morse et al., 2019). Trichoptera compreende mais de 16.000 espécies descritas, distribuídas em cerca de 600 gêneros e mais de 50 famílias, com ocorrência em todos os continentes, exceto a Antártica (Frandsen et al., 2024; Morse et al., 2019). No Brasil, o esforço taxonômico tem sido catalogado em uma fonte nacional de referência para taxonomia e distribuição da fauna, o Catálogo Taxonômico da Fauna do Brasil (CTFB). O CTFB lista 969 espécies de Trichoptera para o país, distribuídas em 68 gêneros válidos e 16 famílias (CTFB; acessado em setembro 2024). A riqueza taxonômica, associada à estrutura filogenética, evidencia a ampla variação morfológica e ecológica entre linhagens; conforme ilustrado na Figura 2.

Funcionalmente, Trichoptera exibem ampla diversidade de estratégias associadas à exploração de habitat, aquisição de recursos e resposta às condições hidrodinâmicas (Merritt, Cummins e Berg, 2008; Wiggins, 2005). Um dos atributos mais marcantes do grupo é o hábito de construção, possível devido a secreção de seda que é utilizada na construção de redes, tubos e abrigos, com implicações diretas para a ocupação de microhabitats, fixação ao substrato e

exploração de recursos alimentares (Morse et al., 2019; Thomas et al., 2020; Wiggins, 2005). Esse comportamento fundamenta a distinção entre as subordens Annulipalpia, cujas larvas constroem redes e estruturas fixas ao substrato; e Integripalpia, cujas larvas constroem abrigos portáteis (Figura 2). Além disso, Trichoptera são classificadas em diferentes grupos tróficos funcionais, por exemplo, desempenhando funções na fragmentação e processamento de matéria orgânica nos riachos (Cummins, 1973; Merritt, Cummins e Berg, 2008; Wallace e Webster, 1996). Diversos estudos demonstram que a composição funcional das comunidades aquáticas está intimamente associada à estrutura e heterogeneidade do habitat, uma vez que ambientes mais complexos oferecem maior diversidade de microhabitats, recursos alimentares e refúgios (Heino et al., 2007; Milesi et al., 2016). Nesse contexto, ambientes heterogêneos e minimamente impactados tendem a sustentar maior diversidade funcional (Milesi et al., 2016; Castro et al., 2017), enquanto ambientes homogêneos ou degradados favorecem subconjuntos mais restritos de estratégias ecológicas. Em riachos submetidos a distúrbios ambientais, é comum a predominância de organismos com atributos associados à resistência e tolerância, como pequeno tamanho corporal, ciclos de vida mais curtos e construção de redes fixas de seda (Milesi et al., 2016; Poff et al., 2006). Esses padrões evidenciam a forte associação entre atributos funcionais e filtros ambientais, considerando que a literatura demonstra consistentemente que tais atributos respondem às variações ambientais ao longo de gradientes ecológicos. Esses atributos frequentemente ocorrem de maneira integrada, formando conjuntos funcionalmente correlacionados associados a estratégias ecológicas específicas, descritos na literatura como *trait syndromes* (Poff et al., 2006). Esses padrões evidenciam a forte associação entre atributos funcionais e filtros ambientais, considerando que a literatura demonstra consistentemente que tais atributos respondem às variações ambientais ao longo de gradientes ecológicos. Nesse cenário, condições locais selecionam estratégias ecológicas específicas, frequentemente sob restrições associadas à história evolutiva das linhagens. Como consequência, parte das respostas observadas pode ocorrer de maneira indireta, refletindo de forma mais fraca a ação dos filtros ambientais, uma vez que determinados atributos podem estar associados a características evolutivamente conservadas e funcionalmente correlacionadas.

Do ponto de vista evolutivo, Trichoptera compõem, juntamente com Lepidoptera, a superordem Amphiesmenoptera, com origem no Permiano e diversificação acentuada ao longo do Mesozoico, associada à expansão de nichos aquáticos e à inovação em estratégias de construção e uso do habitat (Frandsen et al., 2024; Ge et al., 2024; Malm, Johanson e Wahlberg, 2013; Thomas et al., 2020). Nas últimas décadas, a incorporação de dados moleculares e, mais recentemente, filogenômicos, transformou a compreensão das relações evolutivas dentro da ordem. Os primeiros estudos multilocus conduzidos por Kjer, Blahnik e Holzenthal (2001, 2002) forneceram evidências

robustas para a monofilia de Annulipalpia e Integripalpia, além de indicar que a tradicional subordem Spicipalpia não constituía um grupo natural. Posteriormente, análises com maior cobertura taxonômica e molecular ampliaram a resolução das relações entre famílias e reforçaram o posicionamento parafilético de Spicipalpia na base de Integripalpia (Holzenthal et al., 2007; Malm, Johanson e Wahlberg, 2013). Mais recentemente, abordagens filogenômicas em larga escala, utilizando centenas de loci, permitiram resolver relações profundas e historicamente instáveis dentro da ordem, refinando hipóteses sobre diversificação e evolução de atributos em Trichoptera (Thomas et al., 2020; Frandsen et al., 2024). Paralelamente, sínteses filogenéticas baseadas em sequências disponíveis em bases públicas, como a proposta por García-Girón et al. (2024), ampliaram significativamente a representatividade taxonômica em nível de gêneros, favorecendo abordagens comparativas e macroecológicas em larga escala. Em conjunto, esses avanços não apenas ampliaram a compreensão da história evolutiva do grupo, mas também estabeleceram bases importantes para investigações ecológicas e macroevolutivas envolvendo a evolução de atributos, estratégias funcionais e respostas ambientais.

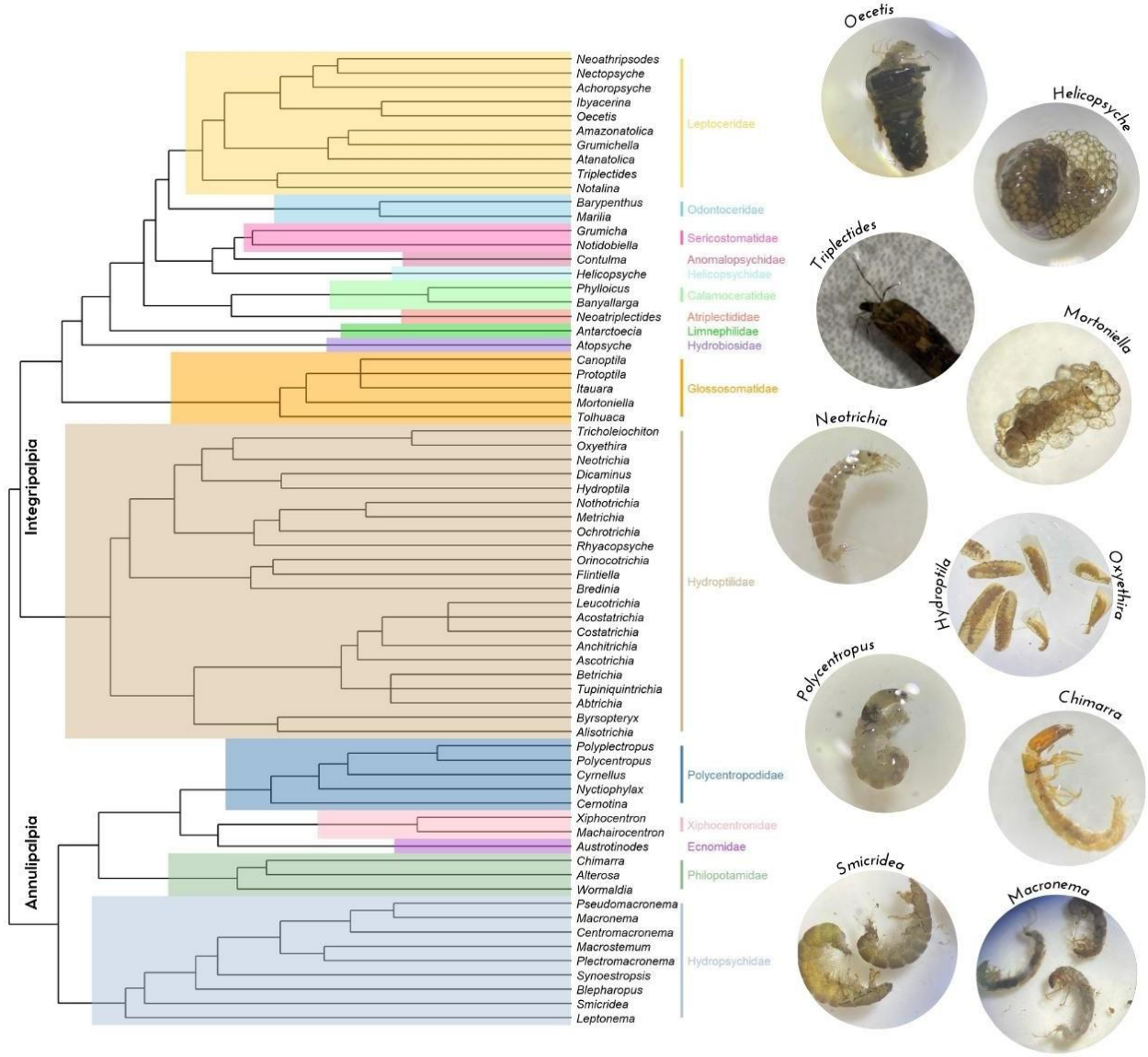


Figura 2. Figura 2. Árvore filogenética dos gêneros de Trichoptera com ocorrência no Brasil, baseada na reconstrução filogenética apresentada no Capítulo 1 desta tese. Árvore construída no R utilizando o pacote *ggtree*. Fotos: Mariana Nunes Menegat.

Nesse contexto, a combinação entre elevada diversidade taxonômica, ampla variação funcional e filogenias cada vez mais resolvidas, torna Trichoptera um importante modelo para investigar como gradientes ambientais modulam a relação entre atributos funcionais e história evolutiva em ecossistemas de água doce. Entretanto, os ecossistemas aquáticos continentais, onde essas linhagens se diversificaram, vêm sendo rapidamente modificados por alterações antrópicas. A crise ambiental global avança em ritmo mais rápido do que a capacidade das espécies, paisagens e processos ecológicos absorverem essas mudanças (Isbell et al., 2023; Nolan et al., 2018; Tonkin et al., 2026). A intensificação de eventos climáticos extremos, associada à conversão contínua da vegetação nativa, à fragmentação da paisagem e ao uso intensivo do solo, impõe pressões múltiplas e simultâneas sobre os ecossistemas (Jones et al., 2024; Reid et al., 2019). No Brasil, esse cenário assume um caráter estrutural: nas últimas décadas, todos os

biomas apresentaram aumento do desmatamento, com impactos cumulativos sobre biodiversidade e funcionamento dos ecossistemas (Rajão et al., 2020). Florestas, savanas e campos vêm sendo substituídos por matrizes agrícolas, pastagens e áreas urbanas, o que promove uma ruptura na conectividade estrutural e funcional da paisagem (Haddad et al., 2015). Essa desarticulação altera fluxos hidrológicos, regimes de fogo, ciclos biogeoquímicos e a estabilidade climática regional (Hou, 2023; Spera et al., 2016). Consequentemente, perdas de biodiversidade são registradas para diversos grupos terrestres e aquáticos (Albert et al., 2021; Isbell et al., 2023).

Diante desse cenário, torna-se evidente que alterações ambientais antropogênicas atuam como filtros ambientais capazes de modificar a estrutura das comunidades biológicas. Pressões ambientais operam sobre os atributos funcionais dos organismos, influenciando seu desempenho, sobrevivência e, consequentemente, o funcionamento dos ecossistemas (Castro et al., 2017; Milesi, Dolédec e Melo, 2016; Mouillot et al., 2013). Além disso, atributos funcionais tendem a exibir algum grau de conservadorismo filogenético (Ackerly, 2009; Wiens e Graham, 2005), especialmente em grupos próximos filogeneticamente, como dentro de uma única ordem (Trichoptera). Nesse contexto, o **objetivo geral** desta Tese foi investigar como a história evolutiva influencia a resposta de atributos funcionais de assembleias de Trichoptera em gradientes ambientais, integrando abordagens taxonômicas, funcionais e filogenéticas ao longo de escalas espaciais e temporais. Especificamente, investiguei: (i) os padrões evolutivos dos atributos funcionais, distinguindo entre conservadorismo e labilidade; (ii) se atributos evolutivamente lábeis são mais sensíveis a gradientes ambientais do que atributos conservados filogeneticamente; (iii) como atributos-chave, como tamanho corporal, respondem a variáveis limnológicas em escala local; e (iv) como a diversidade taxonômica, funcional e filogenética variam ao longo do tempo e gradientes ambientais.

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CAPÍTULO 1

From lability to conservatism: evolutionary patterns in the traits of stream Trichoptera



Polyplectropus (Polycentropodidae), coletado no córrego da Lagoa em Três Lagoas, Mato Grosso do Sul, Brasil (52°17'18.52"S 20°42'54.45"O). Foto: Mariana Nunes Menegat.

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From lability to conservatism: evolutionary patterns in the traits of stream Trichoptera

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Keywords

Phylogenetic Signal; Caddisfly; Trait-Based; Evolutionary History.

Abstract

The ability to predict biodiversity responses to ongoing environmental change depends on understanding the extent to which traits are conserved across evolutionary history. Examining the relationship between traits and phylogeny provides key insights into how evolutionary history constrains and structures the traits. We quantify the strength of the phylogenetic signal (PS) in Trichoptera traits, as an indicator of evolutionary patterns from phylogenetic conservatism to evolutionary lability. We hypothesized that construction behavior, feeding group, respiration, locomotion, and body flexibility would exhibit strong PS, consistent with phylogenetic conservatism. In contrast, we hypothesized that body size, body shape, and voltinism would exhibit weak PS, consistent with evolutionary lability. We compiled data on Brazilian genera of Trichoptera from the Taxonomic Catalog of the Fauna of Brazil and data on eight traits distributed across 28 categories associated with these genera. We reconstructed a phylogenetic tree of Brazilian Trichoptera genera based on a published hypothesis. We estimated the PS in traits of Trichoptera using K*mult* statistics. Voltinism, body size, and body shape exhibited more evolutionary lability than construction behavior, body flexibility, locomotion, respiration, and feeding group traits, with families and suborders overlapping in their trait composition. We concluded that Trichoptera traits span a continuum from phylogenetic conservatism to evolutionary lability, and that recognizing the strength of evolutionary constraints on these traits could inform prediction of their responses to ongoing environmental change. By integrating phylogenetic and trait-based perspectives, this study reveals that the strength of evolutionary constraints can vary markedly even within a single insect order. This underscores the importance of evolutionary history for understanding trait distributions and predicting how freshwater assemblages may respond to environmental changes.

Introduction

The interaction between ecological processes (e.g. predation, competition, energy flow) and evolutionary processes (e.g., natural selection, gene flow, and speciation), shapes the structure of biological communities (Siepielski et al. 2018; Beal-Neves et al. 2024). Ecological processes often drive evolutionary changes, and the phylogenetic history of a species thus depends, if not entirely then decisively, on the interplay of eco-evolutionary dynamics (Thompson 1998). Exemplifying this interaction, the complex geological and climatic history of the Neotropical region has promoted prolonged habitat isolation, diversification of lineages, and endemic species (Hazzi et al. 2018; Naka et al. 2022).

Trichoptera constitute an ancient insect lineage, with fossil evidence dating to the Permian (c. 275 Ma), followed by extensive diversification (Thomas et al. 2020), a long evolutionary trajectory that parallels the Neotropical region's complex history. In freshwater ecosystems, trichopteran larvae exhibit remarkable taxonomic and functional diversity, occupying a wide range of ecological roles and contributing to key ecosystem processes that include organic matter processing, energy transfer across trophic levels, and nutrient cycling within stream networks (Wallace and Webster 1996; Bonada et al. 2007; Smith and Storey 2018). These ecological roles emerge from suites of morphological, physiological, behavioral, and life-history traits (Resh et al. 1994; Poff et al. 2006; Violle et al. 2007), whose distribution reflects a balance between environmental influences and constraints imposed by shared ancestry (Ackerly 2009; Beal-Neves et al. 2024). This combination of ancient origin and extensive diversification makes Trichoptera an effective model for examining how evolutionary history constrains the structure and distribution of traits.

Traits such as construction behavior, including case building and net spinning, are the defining features of the suborders Integripalpia and Annulipalpia, respectively (Frandsen et al. 2019; Thomas et al. 2020; Ge et al. 2024). Although construction behavior can be modulated by local environmental conditions through trade-offs involving material availability, energetic costs, and resistance to disturbance, they remain strongly associated with phylogenetic lineages (Malm et al., 2013; Rouhová et al., 2024; Deng et al., 2025). Similarly, feeding groups and respiration mode depend on specialized mouthparts and tracheal or gill systems, whose evolutionary modifications are shaped by developmental integration and morphological complexity (Labandeira 2019). Body flexibility and locomotion are linked to thoracic and abdominal morphology, which in turn relates to construction behavior; consequently, similar morphologies can arise across lineages occupying similar ecological contexts (Kunz et al. 2024). In contrast, substrate characteristics and hydraulic conditions (e.g., water velocity) can act as environmental filters that favor streamlined or flattened body shapes that reduce drag and enhance attachment or

station-holding, leading to convergence within habitats (Orlofske and Baird 2014; Edwards and Moore 2017; Ditsche et al. 2022). Body size integrates metabolic rate, growth, and developmental time (Nijhout and Callier 2015) and responds to environmental gradients such as water temperature, resource availability, and habitat stability (Bonada et al. 2007; Verbeck et al. 2020), often varying within and among closely related taxa (Zeuss et al. 2016). Likewise, voltinism is closely linked to season length and thermal regimes and commonly varies along climatic and latitudinal gradients, even among congeners (Bonada et al. 2007; Zeuss et al. 2016; Bonada and Dolédec 2018).

Closely related taxa often have similar trait profiles due to their shared evolutionary history (Kamilar & Cooper, 2013). Phylogenetic signal (PS) measures phylogenetic relatedness, defined as the "tendency for related species to resemble each other more than they resemble species drawn at random from the tree" (Blomberg and Garland, 2002). Although PS is expected for all traits within an order, the strength of this signal varies according to evolutionary constraints. Patterns of trait evolution can be described along a continuum from phylogenetic conservatism, where traits are maintained by stabilizing selection or genetic constraints, to evolutionary lability, where traits are less constrained, thus allowing for rapid adaptive changes (Blomberg et al. 2003; Ackerly 2003, 2009; Sanchez-Martinez et al. 2024). Quantifying phylogenetic constraints along this continuum is necessary to account for shared ancestry that can bias trait-based ecological inferences, leading to overestimation of trait-environment relationships (Ackerley 2009).

Herein, we quantified the strength of the PS in Trichoptera traits, as an indicator of evolutionary patterns from phylogenetic conservatism to evolutionary lability. We hypothesized that construction behavior, feeding group, respiration, locomotion, and body flexibility would exhibit strong PS, consistent with phylogenetic conservatism. In contrast, we hypothesized that body size, body shape, and voltinism would exhibit weak PS, consistent with evolutionary lability.

Methods

Taxonomic and trait databases

We created a database of Trichoptera genera occurring in Brazil, based on the *Catálogo Taxonômico da Fauna do Brasil* (CTFB) (Santos et al. 2020; Boeger et al. 2024; Table S1). The CTFB is an important resource for Brazilian biodiversity, providing a curated list of taxa and their distribution and nomenclature (Boeger et al. 2024). Based on the taxonomic database, we constructed a matrix of larval traits of trichopteran genera. We considered 28 trait categories distributed across eight traits (i.e., body size, voltinism, respiration, construction behavior, feeding

group, locomotion, body shape, and body flexibility; Table 1). We used fuzzy coding to represent intrataxon trait variability (Chevenet et al. 1994). Fuzzy coding quantifies each taxon's affinity to each trait category, with whole number from 0 to 3 representing no, weak, moderate, and strong affinity, respectively (Chevenet et al. 1994). We primarily obtained fuzzy coding information from Santos et al. (2024). When information was unavailable, we consulted studies from Neotropical streams (Tomanova and Usseglio-Polatera 2007; Castro et al. 2017). For one trait-genus combination lacking Neotropical data, we used information from Palearctic streams (Tachet et al. 2010). In four cases, we assigned a maximum affinity value (3) based on taxon-specific studies (Holzenthal and Ríos-Touma 2012; Saito et al. 2015; Casari and Constantino 2024). For genera lacking trait information, we assigned affinity values based on those reported at the family level. Full details of trait assignments are provided in Table S2.

Table 1. Traits and their respective categories.

Traits	Categories
Body size (cm)	≤ 0.25
	0.25 - 0.5
	0.5 - 1.0
	> 1.0
Voltinism	Univoltine
	Multivoltine
Respiration	Tegument
	Gill
Construction behavior	Free living
	Net spinning
	Case building
Feeding group	Collector
	Shredder
	Scraper
	Filterer
	Predator
Locomotion	Swimmer
	Burrower
	Clinger
	Climber
	Sprawler
Body shape	Streamlined
	Flattened
	Cylindrical
	Spherical
Body flexibility	< 10°
	≥ 10° - ≤ 45°
	> 45°

Phylogenetic tree reconstruction

We reconstructed a phylogenetic tree of Brazilian Trichoptera genera, based on that hypothesized by García-Girón et al. (2024), which provides near complete family-level coverage of global Trichoptera diversity. This time-calibrated phylogenetic backbone was generated using DNA sequences of nine independent genes obtained from GenBank (Sayers et al. 2019). Building on this backbone, we grafted genera in our dataset but absent from the published tree as a polytomy at the base of their corresponding families using the *bind.tip* function, then pruned genera not in our dataset using the *drop.tip* function, both implemented in the **ape** package (Paradis et al. 2024).

Data analysis

We conducted all analyses in R software (version 4.4.1; R Core Team 2024). We quantified the PS across the full set of traits and for each trait individually. For this, we prepared and normalized fuzzy-coded trait data by category using the *prep.fuzzy.var* function from the **ade4** package (Dray et al. 2025). We computed a Gower's distance matrix (**ade4**::*dist.ktab*) and performed a principal coordinate analysis (PCoA) (**ape**::*pcoa*). PCoA scores were combined with the phylogeny to estimate the PS using the *physignal* function in the **geomorph** package (Adams et al. 2024). We used the PCoA score matrix (genera × axes) as input and retained all axes with positive eigenvalues (no negative eigenvalues were detected). The significance of PS was assessed with 1,000 permutations ($\alpha = 0.05$).

We measured the PS with the K*mult* statistic (Adams 2014), a multivariate extension of Blomberg's K (Blomberg et al. 2003). K*mult* compares the observed resemblance among genera to that expected under a Brownian motion (BM) model of trait evolution, in which trait variance accumulates proportionally to time since divergence (Blomberg et al. 2003; Freckleton and Harvey 2006; Adams 2014). Values of K*mult* range from 0 to ∞, with an expected value of 1.0 under BM. Values of K*mult* < 1 and > 1 indicate that close relatives are less and more similar than expected under a BM, respectively. We interpreted (i) genera with K*mult* > 1 as exhibiting phylogenetic conservatism (i.e., PS stronger than expected under BM) and (ii) genera with K*mult* < 1 as exhibiting evolutionary lability (i.e., PS weaker than expected under BM). We use these terms solely to describe relative evolutionary patterns without assuming specific underlying evolutionary processes or rates (Blomberg *et al*. 2003; Adams 2014).

We performed fuzzy correspondence analysis (FCA) to explore patterns of trait composition. We ran two FCA, one including only phylogenetically conserved traits (i.e. K*mult* >1) and one including only evolutionarily labile traits (K*mult* <1). For this, we prepared fuzzy-

coded data with *prep.fuzzy.var* and conducted an FCA using **ade4**::*dudi.fca* (Dray et al. 2025).

We visualized the phylogenetic trees from all analyses with the *dotTree* function in the **phytools** package (Revell 2024) and exported it to Adobe Illustrator (v29.5; Adobe Inc. 2025) for aesthetic visual adjustments to improve clarity and layout.

To evaluate the robustness of our results, we conducted sensitivity analysis, i.e., repeating analyses excluding genera lacking trait information. Because missing information varied among traits, exclusions were performed separately for each trait, removing only the genera lacking information for that specific trait (see Table S2 for details). Removing these taxa reduced K*mult* values (inflating estimates of evolutionary lability), although the overall pattern remained consistent, except for the feeding group trait, for which K*mult* decreased from 1.119 to 0.999 (K*mult* <1; Table 2). We based our interpretation on the analyses including all genera, because excluding taxa with incomplete trait data can bias PS estimates when missing values are non-random (Penone et al. 2014). Trait imputation methods that incorporate phylogenetic information can preserve trait relationships and reduce bias relative to complete-case deletion, especially when traits exhibit PS and inter-trait correlation (Penone et al. 2014; Johnson et al. 2020; Debastiani et al. 2021). Excluding taxa a priori based on missing trait data can disproportionately remove rare or distinct lineages and distort ecological patterns of diversity and trait evolution (Kim et al. 2018).

Results

We compiled trait information for 68 genera of Trichoptera distributed across 16 families (Figure 1). For voltinism, genera within the Glossosomatidae, Philopotamidae, and Polycentropodidae shared similar trait categories, whereas Hydropsychidae genera were more variable. For example, *Macrostemum* was multivoltine whereas most of its congeners were univoltine. Within Hydroptilidae, which comprises 22 genera, voltinism categories were highly variable. Body shape was relatively coherent among genera in the Leptoceridae, Polycentropodidae, Philopotamidae, and most Hydropsychidae (except *Blepharopus*), whereas Hydroptilidae exhibited substantial within-family variation. Body size was only consistent within the Hydroptilidae, in which genera predominantly fell into smaller size categories (< 0.25 mm and 0.25–0.5 mm). Construction behavior clearly separated the suborders. Integripalpia genera occupied a wide range of categories, whereas most Annulipalpia genera were net-spinning, with exceptions for *Pseudomacronema* (Hydropsychidae; free living) and *Centromacronema* (Hydropsychidae; case building). For respiration, genera within families generally shared the same respiratory mode, with few exceptions. For example, *Grumichela* (Leptoceridae) and *Barypenthus* (Odontoceridae) exhibited integument respiration, differing from the gill respiration

observed in most genera within their respective families. Finally, for feeding groups, Hydroptilidae genera were scrapers, with exceptions such as *Tricholeiochiton* (shredder) and *Ochrotrichia* and *Rhyacopsyche* (collectors). In contrast, Leptoceridae and Odontoceridae genera were predominantly shredders, whereas Philopotamidae and Hydropsychidae were predominantly collectors-filterers. Polycentropodidae exhibited greater variability, with genera spanning predators and collectors.

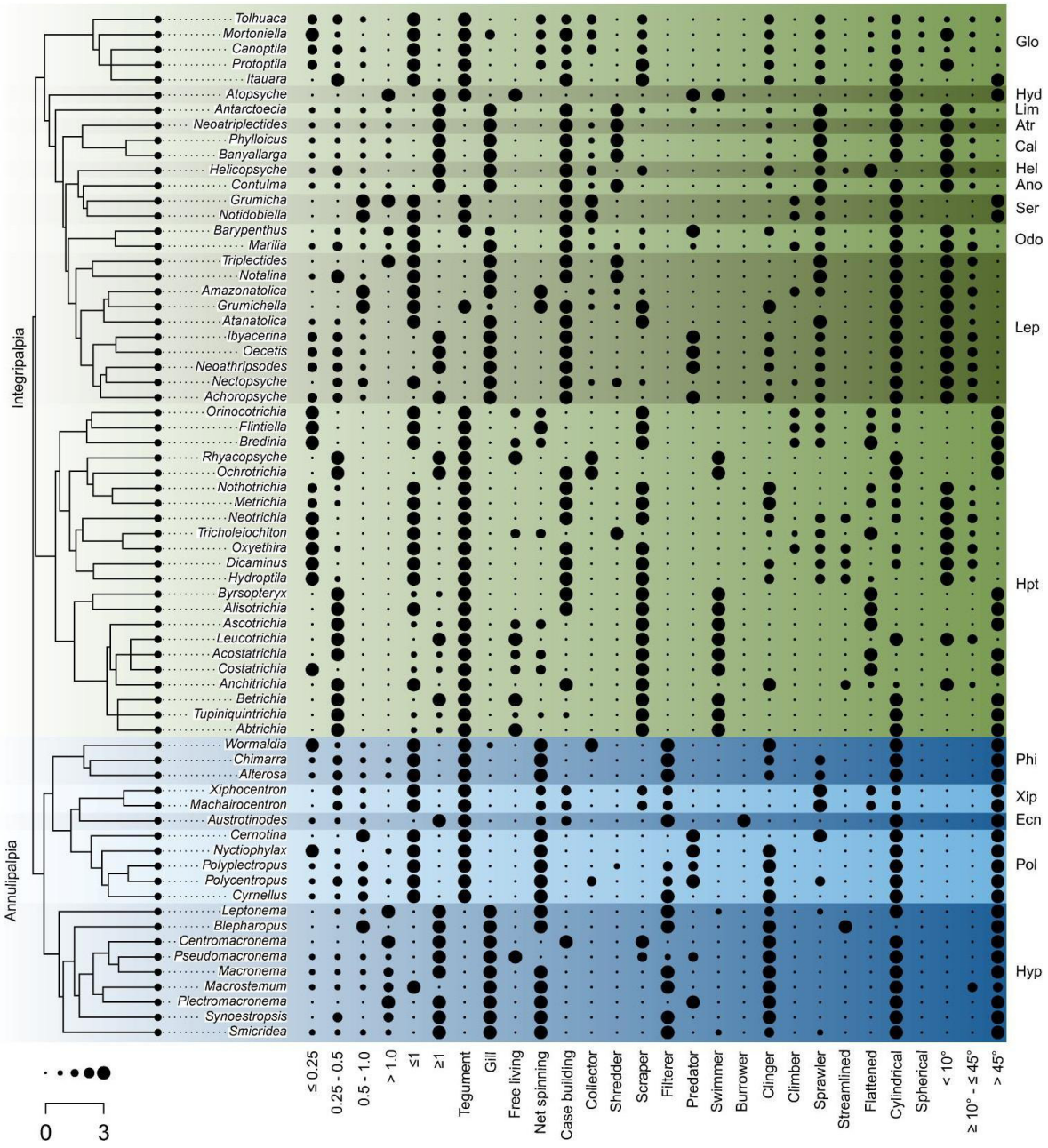


FIGURE 1. Phylogenetic tree of the genera and their respective families of the order Trichoptera occurring in Brazil and a heatmap with the affinities of each of the 28 categories of traits for each genus of Trichoptera. Abbreviations: Glo = Glossosomatidae; Hyd = Hydrobiosidae; Lim = Limnephilidae; Atr = Atriplectididae; Cal = Calamoceratidae; Hel = Helicopsychidae; Ano = Anomalopsychidae; Ser = Sericostomatidae; Odo = Odontoceridae; Lep = Leptoceridae; Hpt = Hydroptilidae; Phi = Philopotamidae; Xip = Xiphocentronidae; Ecn = Ecnomidae; Pol =

Polycentropodidae; Hyp = Hydropsychidae.

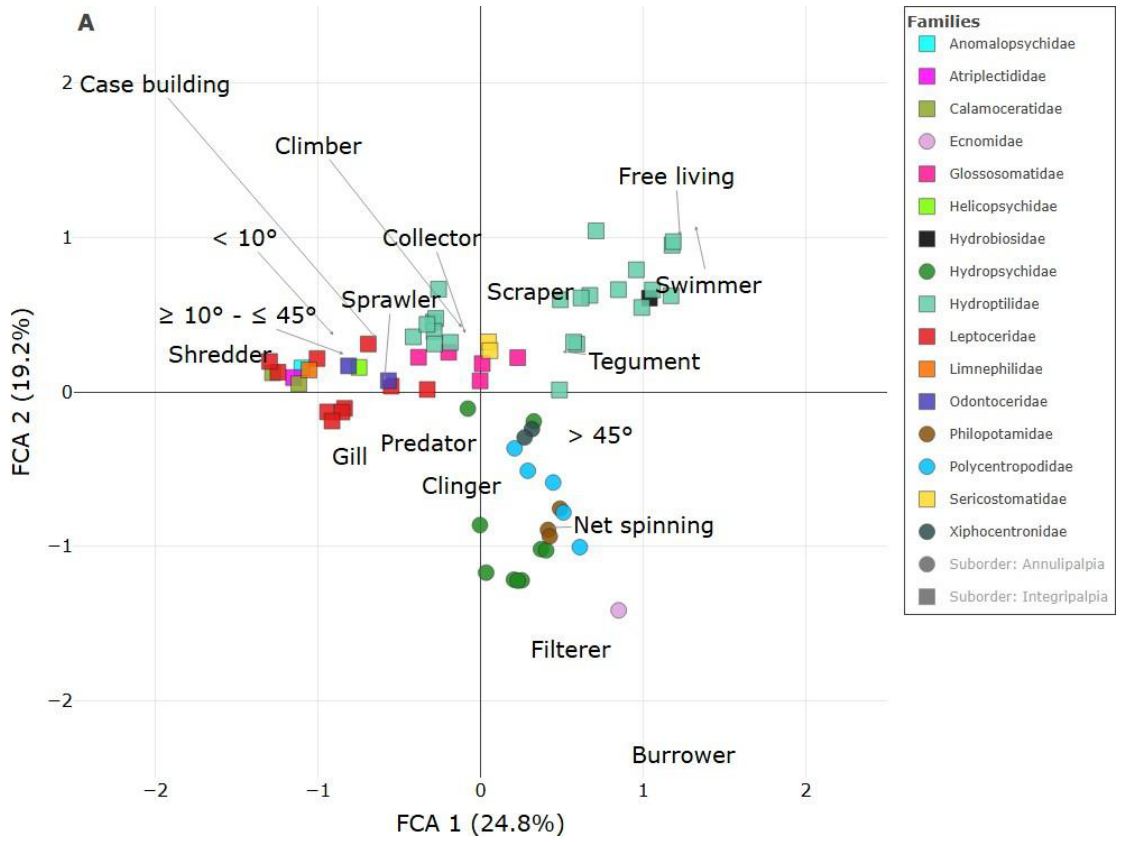
Considering all traits, we observed a significant and strong PS (*Kmult* = 1.112; $p < 0.01$; Table 2). For each trait, we observed a significant PS ($p < 0.01$; Table 2), however, the strength of PS varied among traits As hypothesized, indicating phylogenetic conservatism, construction behavior, respiration, feeding group, body flexibility, and locomotion traits showed higher K*mult* values (K*mult* > 1; Table 2); in contrast, indicating evolutionary lability, body size, voltinism, and body shape traits showed lower K*mult* values (K*mult* < 1; Table 2). Body shape and respiration represented the extremes of this continuum, with the lowest (0.785) and highest (2.104) K*mult* values among all traits, respectively (Figure 2A).

Table 2. Phylogenetic signal (K*mult*) for Trichoptera traits, including effect size (z-score) and p value. Results are presented for all genera and for sensitivity analyses excluding genera lacking trait information.

	All genera			**Sensitivity analyses**		
	K*mult*	**z-score**	**p value**	**K*mult***	**z-score**	**p value**
All traits	1.112	21.3	0.001	0.997	15.5	0.001
Body shape	0.785	3.9	0.003	0.735	2.1	0.032
Body size	0.949	10.9	0.001	0.854	7.04	0.001
Voltinism	0.974	7.4	0.001	0.934	4.93	0.002
Construction behavior	1.064	11.6	0.001	1.036	6.5	0.001
Feeding group	1.119	16.7	0.001	0.998	11.1	0.001
Locomotion	1.160	15.1	0.001	1.021	10.09	0.001
Body flexibility	1.204	12.1	0.001	1.005	12.08	0.001
Respiration	2.104	21.7	0.001	1.803	14.4	0.001

The first two FCA axes for the phylogenetically conserved trait ordination explained 44% of the total variance in the trait composition (Figure 2A). The first FCA axis (24.8% of explained variance) was primarily structured by a set of traits associated with free-living habits, including swimmer and burrowing locomotion, scraper and filterer feeding groups, tegument respiration, and net-spinning construction behavior, which were predominantly related to genera of Annulipalpia, as well as some genera of Hydroptilidae. In contrast, genera associated with case-building construction behavior, gill respiration, the shredder feeding group, and low body flexibility, as well as climber and sprawler locomotion, were positioned on the opposite side of this gradient and were mainly represented by Integripalpia. The second axis (19.2%) further reinforced this suborder separation, highlighting associations between burrower and filterer categories and genera of Annulipalpia, particularly within Hydropsychidae and Polycentropodidae, while categories such as case building and low body flexibility remained linked to Integripalpia.

The first two FCA axes of the evolutionary labile traits explained 50% of the total variance in the trait composition (Figure 2B). The first FCA axis (31.0% of explained variance) was primarily structured by traits related to life cycle and body size, including multivoltinism and larger body size (0.5 - 1.0 and > 1.0), which were associated with several genera across different families, but mainly with most Hydropsychidae. In contrast, univoltinism, smaller body size (≤ 0.25 and 0.25 - 0.5), and body shapes such as streamlined and spherical were associated with genera distributed on the opposite side of this gradient, particularly Glossosomatidae and most Hydroptilidae. The second axis (19.3%) was mainly defined by body shape, with flattened shape associated with one end of the gradient, while cylindrical and streamlined body shapes, along with intermediate body size (0.5 - 1.0), were associated with the opposite end. Overall, genera were distributed widely across the ordination space, with no clear separation between suborders and overlap among families.



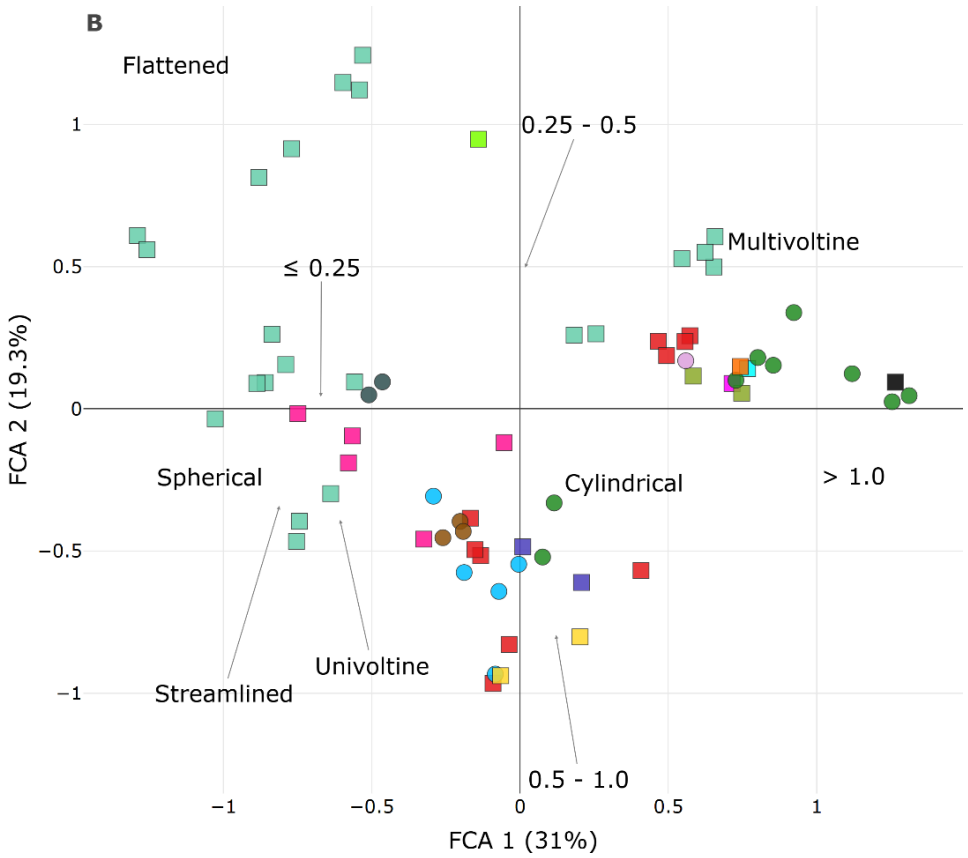


FIGURE 2. Fuzzy correspondence analysis (FCA) summarizing the patterns of trait composition across Trichoptera genera. (A) Phylogenetically conserved, and (B) evolutionarily labile traits. These FCA are interactive and available at https://maarianan.github.io/interactive-figures/conserved.html and https://maarianan.github.io/interactive-figures/labile.html. Hovering over a symbol displays the taxonomic details.

Discussion

In agreement with our hypothesis, the traits voltinism, body size, and body shape showed evolutionary lability, in contrast to construction behavior, feeding group, locomotion, body flexibility, and respiration that showed phylogenetic conservatism. These results suggest that these traits showed low phylogenetic constrained. The low K*mult* values for traits such as body size and voltinism should be interpreted in light of the restricted phylogenetic scope of the analysis, which includes genera within a single order. Under these conditions, phylogenetic distances are essentially reduced, and K*mult* values are expected to approach those predicted by a BM (i.e., K*mult* ≈ 1) (Revell et al., 2008; Harmon et al., 2010; Pennell et al., 2015). Despite this, values below 1 indicate a weak phylogenetic structure, consistent with evolutionary lability (Losos, 2008; Münkemüller et al., 2012). By focusing on a single order of aquatic insects (Trichoptera) and using fuzzy-coded traits, we captured within-taxon variation and worked at a phylogenetic scale at which trait differences are more likely to reflect recent ecological adaptations than deep evolutionary divergences (McGill et al., 2006; Violle et al., 2007). This pattern is consistent with other aquatic

insect groups, for which trait congruence weakens at finer taxonomic scales (Picazo et al., 2012).

Phylogenetic Conservatism

Supporting our hypothesis, a strong PS was observed for construction behavior, feeding group, respiration, locomotion, and body flexibility, indicating phylogenetic conservatism. Traits associated with complex morphological and functional structures are often constrained by deep phylogenetic history, due to their developmental complexity and slow rates of evolutionary change (e.g., Blomberg and Garland 2002; Poff et al., 2006). For example, Saito et al. (2016) found that behavioral traits related to habitat use exhibited significant PS in aquatic insects. Buchwalter et al. (2008) showed that physiological and respiratory traits were strongly conserved across insect families, and Cano-Barbacil et al. (2022) reported pronounced phylogenetic conservatism in complex morphological traits related to locomotion and body shape in fishes.

Among the traits evaluated, respiration showed the highest K*mult* value, standing out as the most conserved trait. The transition from terrestrial ancestors shared with Lepidoptera to aquatic larval and pupal stages in Trichoptera required profound physiological innovations for submerged gas exchange (Thomas et al. 2020; Ge et al. 2022; Matthews 2024). In aquatic insects, respiratory structures and gas-exchange modes are often conserved at higher taxonomic levels, reflecting strong phylogenetic constraints and the high developmental and physiological costs of shifting between respiratory strategies (Matthews and Terblanche 2015; Harrison et al. 2023). In Trichoptera, respiration is tightly coupled with silk production and construction behavior (Wiggins, 2005). Larvae in different lineages build shelters, the porosity and structure of which modulate water flow over the body and gills, and abdominal undulations actively ventilate these structures (Okano et al. 2010). This integration of traits likely places them under strong stabilizing selection, consistent with the high K*mult* values observed.

Although shelter structure varies among taxa and may reflect adaptations to different aquatic microhabitats, the construction behavior itself reflects stabilizing selection acting on structural efficiency and performance (Kingsolver and Buckley 2017; Clarck et al. 2020). Moreover, construction behavior may impose phylogenetic constraints by limiting diversification of associated traits, such as locomotion and body flexibility (Wake and Larson 1987; Cianciaruso et al. 2009). The conservatism of these traits can reflect selective pressures to maintain structural efficiency, because major modifications could compromise shelter performance and larval survival (Wiggins, 2005). Additionally, free-living genera (except *Leucotrichia*) exhibited greater body flexibility, which enhances agility for obtaining food and escaping predators (Segre et al. 2019). Because these individuals are more exposed to disturbances, greater flexibility may represent a

trade-off, investing in one trait at the expense of another (Verberk et al. 2013). This pattern illustrates how, even within a general framework of conservatism, adaptive adjustments to the environment can occur.

For feeding groups, phylogenetic conservatism may reflect the close relationship among trophic strategies, specialized mouth morphology and digestive system adaptations to specific types of resources, which limit the rate of evolutionary change (Merritt et al. 2017; Tomczyk et al. 2022). Traits linked to resource use, including feeding group, often reflect niche conservatism, resulting in aggregated trait distributions despite there being no strong phylogenetic overdispersion at the local level (Saito et al. 2016; Céréghino et al. 2018). Specialization in specific food resources favors the occupation of distinct ecological niches, reducing interspecific competition and promoting the coexistence of multiple species in the same habitat (Relyea and Ricklefs 2021).

Evolutionary Lability

Supporting our hypothesis, weak PS was observed for voltinism, body size, and body shape indicating evolutionary lability. Traits linked to life history, such as voltinism, are often less phylogenetically constrained and more responsive to environmental conditions such as water flow, temperature, and disturbance regimes (e.g., Blomberg et al. 2003; Schmitz et al. 2023; Seifert et al. 2025). Under similar environmental conditions, life-history traits can converge repeatedly among unrelated taxa, reflecting filtering and adaptive convergence rather than deep phylogenetic constraints (Pavoine et al. 2014; Bower and Winemiller 2019; McNeil et al. 2025).

Body shape, which had the lowest K*mult* value among the traits, mediates interactions with hydraulic conditions (e.g., current velocity) and substrate characteristics (Brouwer et al. 2019; Godoy et al. 2022). In aquatic insects, flattened or streamlined morphologies evolved repeatedly in fast flows to reduce drag, enhance stability, and facilitate inhabitation of interstices (Brouwer et al. 2019; Godoy et al. 2022). Body size aligns closely with body shape, mediating metabolic demands, predation risk, dispersal, and microhabitat use along gradients of flow and disturbance (Tolonen et al. 2003; Amarillo-Suárez et al. 2011; Lövei and Magurra 2022). In aquatic macroinvertebrates, maximal size often varies strongly among genera and shifts with hydrological regime and habitat stability (Yan et al. 2021; García-Girón et al. 2022; Mittag et al. 2025). In parallel, multivoltinism is often favored in warm environments due to accelerated developmental rates and in unstable or disturbed environments, where it promotes rapid life cycle completion, whereas univoltine or semivoltine strategies prevail in colder or more seasonal systems with constrained growth windows (Vieira et al., 2004; Altermatt et al. 2010; Gayatán et al. 2022). Unlike traits exhibiting strong phylogenetic conservatism, body shape, body size, and voltinism

did not clearly separate Annulipalpia and Integripalpia, indicating similar ecological strategies may have arisen repeatedly across phylogenetically distinct lineages within the Trichoptera. The selection imposed by variability in environmental conditions including flow and temperature operates at shorter evolutionary timescales, promoting the repeated emergence of similar trait combinations rather than phylogenetically conserved trait patterns (Münkemüller et al. 2012).

Phylogenetic and Trait-Based Insights

Our integrated approach provides phylogenetic and trait-based insights into evolutionary patterns of traits, contributing to better understanding of the trait-based ecology of aquatic insects. Trichoptera traits were phylogenetically constrained to different extents. Traits that are labile are more likely to respond to changes in environmental conditions, and therefore may respond earlier, potentially contributing to cascading effects (such as body size, e.g., Lövei and Magura 2022; Bernardino et al. 2024; Milesi and Hepp 2025) within trait syndromes. In contrast, phylogenetically conserved traits tend to respond more slowly and/or only indirectly to environmental conditions, reflecting deeper evolutionary conservatism and making them useful for comparative studies and trait-based extrapolations across taxa (Ackerley 2009; Martiny et al. 2013). Recognizing this continuum, from labile traits that enable rapid biological responses to conserved traits constrained by evolutionary history, helps explain why different traits, and even different taxonomic groups, respond differently to environmental conditions. As such, directly measuring labile traits could enable characterization of context-dependent biological responses, such as in biomonitoring applications.

Limitations

Although we used a taxonomically robust database, our study was constrained by limited availability of trait and phylogenetic information. To reconstruct the phylogenetic tree (García-Girón *et al*. 2024), we included 14 genera as polytomies. For six of these genera, i.e., *Contulma* (Anomalopsychidae), *Amazonatolica* (Leptoceridae), *Neoatriplectides* (Atriplectididae), and *Dicaminus*, *Tupiniquintrichia*, and *Orinocotrichia* (Hydroptilidae), no molecular data (e.g., GenBank sequences) were available. Furthermore, several genera are endemic to Brazil (*Tupiniquintrichia* and *Amazonatolica*) and South America (*Contulma*, *Dicaminus*, *Orinocotrichia*, and *Neoatriplectides*) (Pes et al. 2018; Thomson et al. 2022), thus highlighting a broader shortfall in phylogenetic data for South America. These gaps are common challenges in the field and highlight the need to generate missing genetic sequence data (e.g., DNA barcoding

and phylogenetic markers) as a research priority. In addition, although traits are relatively well studied for aquatic insects (e.g., Merritt and Cummins 1998; Poff et al. 2006; Tachet et al. 2010), notable gaps persist in Neotropical systems. As well as *Dicaminus*, *Tupiniquintrichia* and *Orinocotrichia* the larvae of *Achoropsyche* and *Neoathripsodes* (Leptoceridae), *Alterosa* (Philopotamidae), *Tolhuaca* and *Canoptila* (Glossosomatidae), and *Nothotrichia* (Hydroptilidae) are completely undocumented (Pes et al. 2018), explaining poor characterization of their traits. Consequently, as in this study, researchers resort to databases from the Nearctic and Palearctic regions (e.g., Tachet et al. 2010) to fill these gaps for Brazilian taxa (e.g., Milesi et al. 2016). However, this can introduce inaccuracies because these assignments may not reflect the traits of Neotropical lineages, which have evolved separately for millions of years and under distinct climatic, vegetational, and wider environmental conditions (Pinto-Junior et al. 2024).

Conclusions

A continuum from phylogenetic conservatism to evolutionary lability was observed among traits of Brazilian Trichoptera. The traits voltinism, body size, and body shape showed more evolutionary lability than construction behavior, feeding group, locomotion, body flexibility, and respiration. These findings highlight the role of lineage-specific evolutionary history in shaping trait diversification in freshwater insects. Recognizing the evolutionary dimension of traits could therefore enhance understanding and prediction of biodiversity responses in freshwater ecosystems facing climate change and increasing anthropogenic pressures, reinforcing the relevance of integrated trait-based and phylogenetic approaches.

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draft of the manuscript. After using this tool, we carefully reviewed and revised the content and take full responsibility for the final version.

Author contributions

Conceptualization: MNM; SVM; LUH. Developing methods: MNM; SVM. Data analysis: MNM. Preparation figures and tables: MNM. Conducting research, data interpretation, writing: MNM; SVM; LUH.

Data Availability Statement: Data supporting this study will be made available upon request.

Conflict of Interest Statement: The authors declare no conflict of interest.

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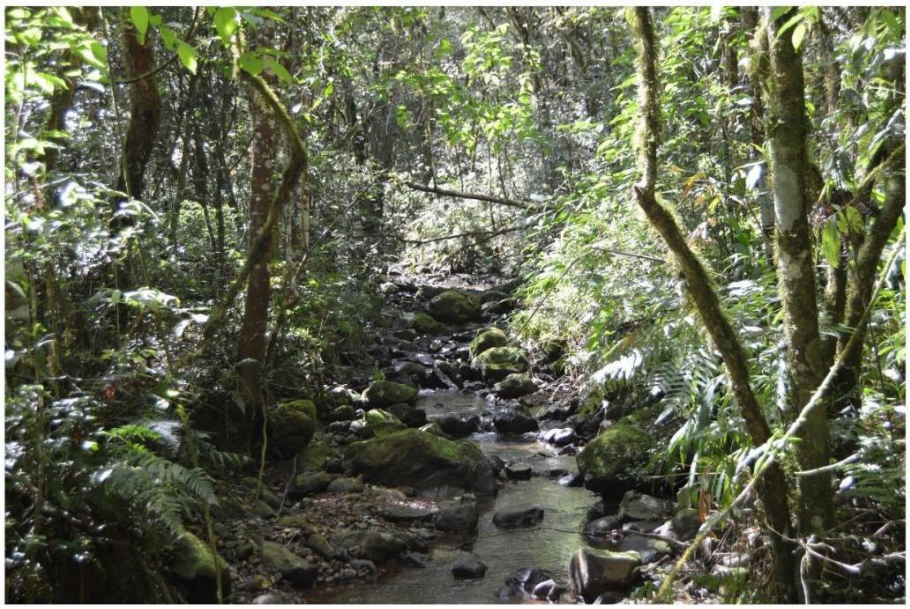
Supplementary Material

Table S1. Occurrence of order Trichoptera genera in Brazil and their respective suborder, family and subfamily, based on data from the *Catálogo Taxônomico da Fauna do Brasil* (accessed in September 2024). The reconstruction of phylogeny follows phylogenetic hypothesis proposed by García-Girón et al. (2024); Genus highlight in pink refer to the 14 genera insert as polytomies.

Table S2. Traits of Trichoptera genera occurring in Brazil according to our database. The values represent the fuzzy code, which indicates the affinity of each genus with each trait category, where: 0 = no affinity, 1 = weak affinity, 2 = moderate affinity, and 3 = strong affinity. Genera for which fuzzy code values were obtained for all or most of their traits from Santos et al. (2024) are highlighted in orange. The highlighted fuzzy code values correspond to the information source for each trait, as follows: Tomanova and Usseglio-Polatera (2007) (green), Tachet et al. (2010) (brown), Holzenthal and Ríos-Touma (2012) (aqua green), Saito et al. (2015) (pink), Castro et al. (2017), Pes et al. (2015) (red), Papockri and Silva (2024) (blue), and family-level values (grey).

CAPÍTULO 2

Trait lability mediates how environmental variation overrides phylogenetic constraints





A primeira foto foi registrada na Mata Atlântica, no Riacho Dourado, Erechim – RS (27º36’29.18”S e 52º16’30.84”O); Foto: Luiz Ubiratan Hepp. A segunda foto exemplifica um riacho no bioma Pampa; Imagem gerada por IA: Gemini.

Manuscrito nas normas da *Marine and Freshwater Research*.

Trait lability mediates how environmental variation overrides phylogenetic constraints

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Abstract

Context: Understanding how evolutionary patterns of functional traits shape taxa responses to environmental variation is essential for predicting community reorganization in freshwater ecosystems under anthropogenic pressure. **Aims:** We investigated how labile traits (body size, voltinism, body shape) and conserved traits (feeding habit, shelter construction, respiration) of Trichoptera assemblages respond to landscape context (forest vs. grassland), anthropogenic impact, and local limnological conditions in stream ecosystems. We hypothesized that functional composition would vary strongly with environmental impact and limnological gradients, particularly for labile traits, than between landscapes. **Methods:** We sampled Trichoptera assemblages in 16 low-order streams across forest and grassland landscapes in southern Brazil, including minimally impacted and impacted sites. Trait-environment relationships were evaluated using RLQ and fourth-corner analyses, complemented by two-way ANOVA. **Key results:** Functional composition based on labile traits showed a significant interaction between landscape and impact, whereas conserved traits did not respond to these predictors. At the individual level, only body size and body shape exhibited significant responses, both driven by landscape-impact interactions. Minimally impacted streams were associated with cylindrical body shapes, while impacted streams were associated with flattened forms. RLQ analysis revealed that trait variation was strongly structured along environmental gradients, particularly current velocity, which was consistently associated with body shape and size. Fourth-corner analysis confirmed that higher current velocity favored flattened body shapes and disfavored cylindrical forms and intermediate body sizes. **Conclusions:** Labile traits exhibited more sensitivity to environmental gradients than conserved traits. **Implications:** Accounting for evolutionary lability improves the detection of functional changes in stream communities and highlights the importance of specific environmental conditions, such as flow conditions, in structure trait distributions.

Keywords: Biomonitoring; Caddisfly; Evolutionary patterns; Functional traits; Forest; Grassland; Landscape; Subtropical streams.

Introduction

Aquatic ecosystems rank among the most biodiverse and equally the most threatened environments on Earth (Dudgeon et al., 2006; Yang et al., 2021). In headwater streams, the conversion of riparian zones into agroecosystems contributes to habitat degradation, diffuse pollution, and altered energy and nutrient dynamics (Finoto-Viana et al., 2023; Gu et al., 2025). The removal of riparian vegetation increases contaminant input, raises nutrient loads, and reduces structural complexity, impairing water quality and stream functioning (Liu et al., 2023; Bega et al., 2024). Consequently, shifts in key limnological variables (e.g., dissolved oxygen, turbidity, and water temperature) further drive biodiversity loss and functional simplification in aquatic communities (Tonkin et al., 2018).

The functional traits of aquatic communities respond to environmental filters operating across multiple spatial scales, from broad landscape-level factors to local limnological conditions (Poff, 1997; Bonada et al., 2007; Kunz et al., 2024). Although landscape context (e.g., forest versus grassland) can shape the availability of organic resources and physical refuges, studies suggest that anthropic disturbance often overriding landscape-related differences in functional composition (Bonada et al., 2007; Ferreira et al., 2017; Tonkin et al., 2018). For example, in streams subjected to disturbance, aquatic insects often converge toward generalist feeding habits, smaller body sizes, and a higher prevalence of skin respiration, strategies which are commonly associated with increased resistance to habitat homogenization, thermal stress, and flow disturbance (Castro et al., 2018; Hepp et al., 2023; Lemes Da Silva et al., 2024; Menegat et al., 2025). Consistent with this pattern, recent studies have demonstrated that limnological variables such as increased turbidity and nutrient concentrations have been linked to higher dominance of small-bodied filter-feeders (Milesi et al., 2024). That is, the local environmental conditions act as stronger selective filters, promoting convergence in functional strategies that confer resistance and resilience, ensuring survival under adverse conditions (Godoy et al., 2022; Kunz et al., 2024), although not all functional traits respond with the same intensity to these filters.

Although numerous studies show that functional traits of taxa are structured by environmental conditions (Castro et al., 2018; Milesi et al., 2024; Menegat et al., 2025), it remains unclear how different evolutionary patterns affect their sensitivity to environmental change. More evolutionarily labile traits tend to experience weaker constraints imposed by shared phylogenetic history, which is reflected in weaker phylogenetic signal and greater divergence across the phylogeny (Blomberg et al., 2003; Sánchez-Martínez et al., 2024). Reduced phylogenetic constraint expands the range of possible variation, which may translate into greater ecological sensitivity when environmental filters are imposed (Poff et al., 2006; Sanchez-Martinez et al., 2024). In turn, more phylogenetically conserved traits remain more similar among closely related

taxa, reflecting deep evolutionary constraints and long-term adaptations shaped by lineage history, which tend to limit their capacity to respond to rapid environmental changes (Blomberg et al., 2003; Sanchez-Martinez et al., 2024). Here, these concepts are addressed in a contrasting framework to understand how the degree of phylogenetic constraint of traits relates to their environmental sensitivity. Although treated contrastively for analytical purposes, phylogenetic lability and conservatism represent extremes along a continuum of evolutionary constraint, associated with the strength of phylogenetic signal. This distinction is fundamental for understanding the functional composition of aquatic communities. In this context, Schmera et al., (2022) demonstrated that the occupation of functional space is simultaneously limited by phylogenetic and ecological constraints, reinforcing the need to consider the alignment between evolutionary history and functional traits when evaluating trait-based responses to environmental change.

We used Trichoptera as a model group due to their ecological diversity, broad distribution in freshwater systems, wide range of functional traits and ecological roles, and well-established role as bioindicators (Pes et al., 2018; Morse et al., 2019; Menegat et al., 2025). We investigated how traits are characterized by contrasting degrees of phylogenetic restriction, here classified as labile (body size, voltinism, body shape) or conserved (feeding habit, shelter, respiration), respond to landscape context (forest vs. grassland), anthropic impact (minimally impacted vs. impacted), and local limnological conditions. We hypothesized that the functional composition and structure of Trichoptera assemblages would vary more strongly in response to environmental impact and limnological conditions than to landscape context, particularly for more labile traits. By jointly considering labile and conserved traits, this study advances trait-based approaches by explicitly linking evolutionary constraint to environmental sensitivity, contributing to the assessment of aquatic ecosystem functioning under rapid environmental change.

Materials and methods

Study area and sampling design

We conducted the study in low-order streams (<3rd order) located in southern Brazil, encompassing two distinct landscape physiognomies: forest and grassland. The forest streams are situated within the Atlantic Forest biome, in a mosaic of transitional vegetation between the Semideciduous Seasonal Forest and the Mixed Ombrophilous Forest (Oliveira-Filho et al., 2015). This landscape is characterized by dense, multi-strata forests, with a continuous and heterogeneous canopy formed by a mix of deciduous and evergreen species, including *Araucaria angustifolia*. In contrast, the grassland streams are in the Pampa biome, flowing through a matrix of natural

grasslands dominated by perennial grasses and herbaceous species, interspersed with small patches of woody vegetation, such as gallery forests and shrub-tree clusters (Overbeck et al., 2022).

We selected 16 streams, eight located in the forest landscape (Atlantic Forest biome) and eight in the grassland landscape (Pampa biome) (Figure 1; Table S1). Within each landscape, we classified four streams as "minimally impacted" and four as "impacted" based on the Rapid Assessment Protocol (Protocolo de Avaliação Rápida - PAR; Silva et al., 2020). In this protocol, the ecological condition of riparian zones was assessed based on land use and vegetation cover within a 30-meter buffer on both stream banks, as well as the physical structure of the habitat (e.g., mesohabitats, heterogeneity) (Silva et al., 2020). As both landscapes are embedded in an agricultural matrix, minimally impacted streams were defined as those with well-preserved and continuous riparian vegetation, showing no visible signs of agricultural intrusion, deforestation, or channel modification within the assessed buffer. In contrast, impacted streams showed marked alteration of the riparian zone, usually characterized by reduced or absent native vegetation and the presence of adjacent agricultural fields or pastures.

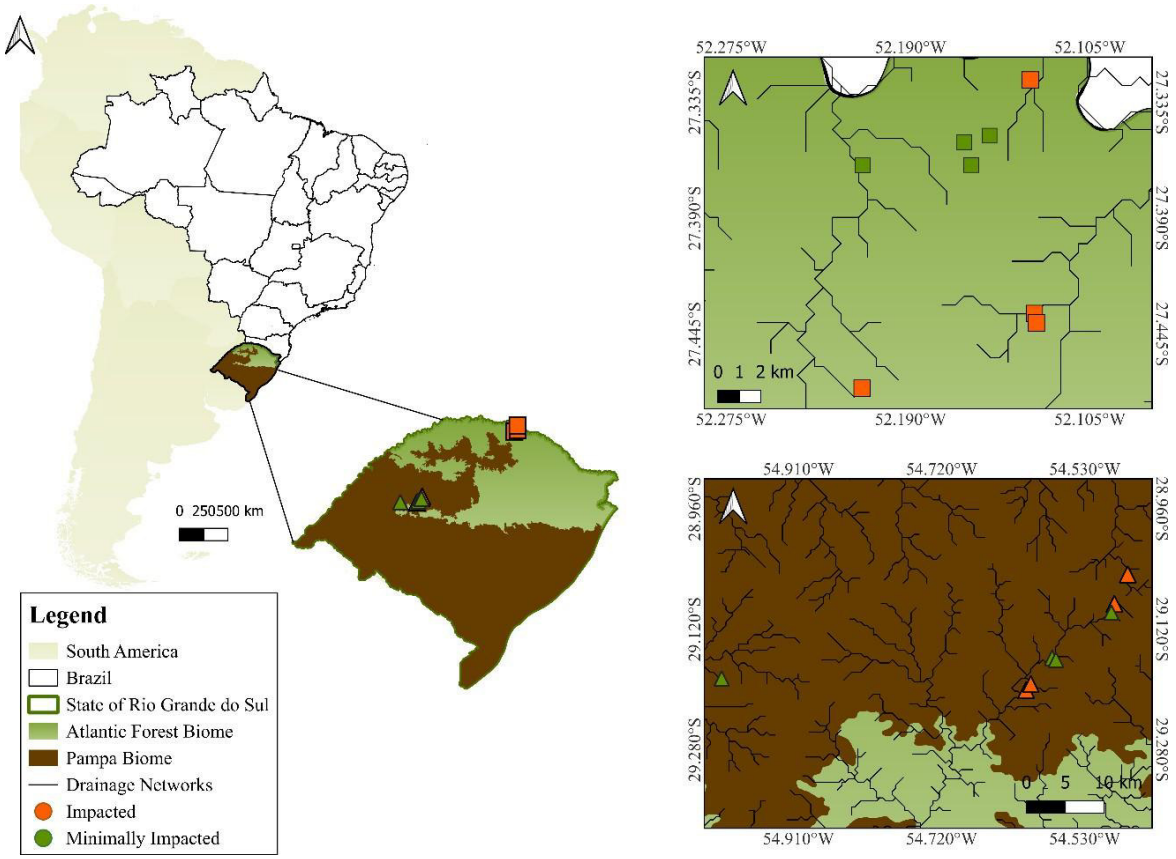


Figure 1. Study area in southern Brazil showing sampling in impacted and minimally impacted streams across Atlantic Forest and Pampa biomes in state of Rio Grande do Sul.

Limnological variables

We measured water temperature, electrical conductivity, total dissolved solids (TDS), dissolved oxygen, pH, and turbidity using a HORIBA® U50 multiparameter probe and recorded current velocity with a flow meter. We also collected water samples for laboratory analysis of total nitrogen (TN) and dissolved organic carbon (DOC). We prefiltered samples through 0.22 μm Millipore® filters and quantified TN and DOC concentrations with a Shimadzu® TOC analyzer (APHA, 2012).

Biological sampling

We collected six benthic subsamples per stream using a Surber net (250 μm mesh, 0.09 m^2), with three samples taken from leaf litter and three from rocks substrates to account for microhabitat heterogeneity. All subsamples were preserved in 80% ethanol in the field. In the laboratory, samples were sorted and Trichoptera were identified to genus level following (2005; 2018). We deposited all specimens in the Aquatic Invertebrates Collection at the Alto Uruguai Regional Museum, Universidade Regional Integrada do Alto Uruguai e das Missões.

Functional traits

We selected six functional traits, distributed across 19 categories (Table S2). We quantified functional traits using a fuzzy coding approach, which assigns each taxon an affinity score for each trait category, ranging from zero (no affinity) to three (strong affinity) (Chevenet et al., 1994). Most trait information was sourced from Santos et al., (2024) and Castro et al. (2017) (Table S2). When data were unavailable in these sources, we consulted Neotropical literature (Tomanova and Usseglio-Polatera, 2007) or, when necessary, Palearctic literature (Tachet et al., 2010). In addition, when only qualitative descriptions were available, we assigned a maximum affinity value (3) based from specialized taxon-focused studies (Pes et al., 2018; Saito et al., 2015). For the genus *Grumicha*, which lacked genus-specific information, we assigned trait affinities based on its family (i.e., Sericostomatidae). We provide full details of trait assignments in Table S2. These functional traits were classified into two evolutionary subsets: conserved traits, including respiration, construction behavior, and feeding group; and labile traits, including body size, voltinism, and body shape. This classification was based on the relative magnitude of phylogenetic signal estimated in Menegat et al. (2026).

Statistical analysis

We performed all statistical analyses in R (version 4.4.1; R Core Team, 2025). Prior to analyses, we standardized limnological variables using *scale* function. We explored multivariate

limnological gradients among streams using Principal Coordinates Analysis (PCoA) based on a Euclidean distance matrix calculated from standardized environmental variables. We tested differences in environmental conditions among landscapes (forest vs. grassland), impact levels (minimally impacted vs. impacted), and their interaction using permutational multivariate analysis of variance (PerMANOVA) with 999 permutations, implemented with the *adonis2* function in the "vegan" package (v. 2.7-1; Oksanen et al., 2022). To aid interpretation of the ordination, we fitted limnological vectors onto the PCoA space using the *envfit* function (999 permutations), identifying variables most strongly associated with site separation in the environmental dissimilarity space.

We considered three trait datasets: labile, conserved and all traits. We standardized the fuzzy-coded trait matrix using the *prep.fuzzy.var* function from the "ade4" package (v. 1.7.23; Dray & Dufour, 2007) to account for differences in the number of categories per trait. We then quantified community-level functional structure by multiplying the standardized trait matrix by the community composition matrix and normalizing values to obtain proportional representation of each trait category at each site. For each trait dataset, we calculated a Gower dissimilarity matrix and evaluated the differences in functional composition across landscapes, impact levels, and their interaction were subsequently tested with separate permutational multivariate analyses of variance (PERMANOVA) for each trait dataset, using 999 permutations and the *adonis2* function from the "vegan" package (v. 2.7-1; Oksanen et al., 2022).

We further examined individual trait categories by using the proportional representation of each category at each site as response variables in two-way ANOVAs to test the effects of categorical factors landscape, impact, and interaction.

We assessed relationships between environmental variables and functional traits using RLQ analysis (Dolédec et al., 1996) and Fourth-corner analysis with model type 6 and 999 permutations (Legendre et al., 1997), following the framework proposed by Dray et al. (2014), using the package ade4 R package (v. 1.7.23; Dray & Dufour, 2007). We used RLQ analysis to identify the joint structure linking environmental gradients and functional traits through community composition. We evaluated the significance of the RLQ model using Monte Carlo permutation tests with 999 permutations.

Results

The PCoA analysis showed that the sowed that the two first axis of ordination using limnological variables explained 64% of the total variation (Figure 2). PERMANOVA results showed that limnological variables differed between landscape ($F_{(1,12)} = 15.0$, $p < 0.01$) and impact ($F_{(1,12)} = 2.5$, $p = 0.05$), with a interaction between both ($F_{(1,12)} = 3.6$, $p = 0.01$). Impacted forest streams exhibited higher electrical conductivity (0.11 ± 0.01 mS cm^{-1}) and TN (2.07 ± 0.30

mg L^{-1}), while minimally impacted forest streams showed higher dissolved oxygen concentrations (13.61 ± 3.60 mg L^{-1}) (Table 1). In contrast, impacted grassland streams showed higher turbidity (46.42 ± 22.02 NTU) (Table 1). Results of envfit showed that, except for water temperature, all variables significantly correlated with the first axis, highlighting differences in limnological conditions across landscape and impact (Table 2).

Table 1. Mean ± standard deviation of limnological variables in forest and grassland streams under minimally impacted (MI) and impacted (I) conditions.

Limnological Variables	Forest		Grassland	
	MI	I	MI	I
Water temperature (°C)	16.11±2.29	13.02±0.51	16.77±2.92	18.09±3.94
Electric Conductivity (mS cm^{-1})	0.09±0.00	0.11±0.01	0.04±0.00	0.04±0.01
Total dissolved solids (g L^{-1})	0.06±0.00	0.07±0.01	0.02±0.00	0.02±0.00
Turbidity (NTU)	0.48±0.51	2.04±1.58	9.58±6.72	46.42±22.02
pH	7.32±0.41	7.12±0.41	6.93±0.41	6.01±0.50
Dissolved oxygen (mg L^{-1})	13.61±3.60	9.59±1.15	7.18±1.19	7.23±1.32
Total nitrogen (mg L^{-1})	1.67±0.43	2.07±0.30	0.89±0.12	0.95±0.14
Dissolved organic carbon (mg L^{-1})	12.15±1.36	13.30±0.97	12.25±6.44	13.12±3.77
Current velocity (m s^{-1})	0.13±0.03	0.27±0.04	0.16±0.07	0.11±0.07

Table 2. Results of environmental vector fitting (envfit) onto the Principal Coordinates Analysis (PCoA) ordination based on Euclidean distances of standardized limnological variables. The table presents the direction cosines of each variable on the first two PCoA axes (PCoA1 and PCoA2), the coefficient of determination (r^2), and the significance level (p-value) based on 999 permutations. Statistically significant variables ($p < 0.05$) are highlighted in bold.

Limnological Variables	PCoA1	PCoA2	r^2	p-value
Water temperature	0.977	0.208	0.333	0.071
Electric Conductivity	-0.998	0.057	0.900	**0.001**
Total dissolved solids	-0.996	0.087	0.896	**0.001**
Turbidity	-0.606	0.795	0.653	**0.002**
pH	-0.739	-0.673	0.592	**0.003**
Dissolved oxygen	0.976	0.215	0.612	**0.001**
Total nitrogen	-0.625	-0.780	0.441	**0.029**
Dissolved organic carbon	-0.981	0.189	0.743	**0.001**
Current velocity	-0.059	0.998	0.759	**0.001**

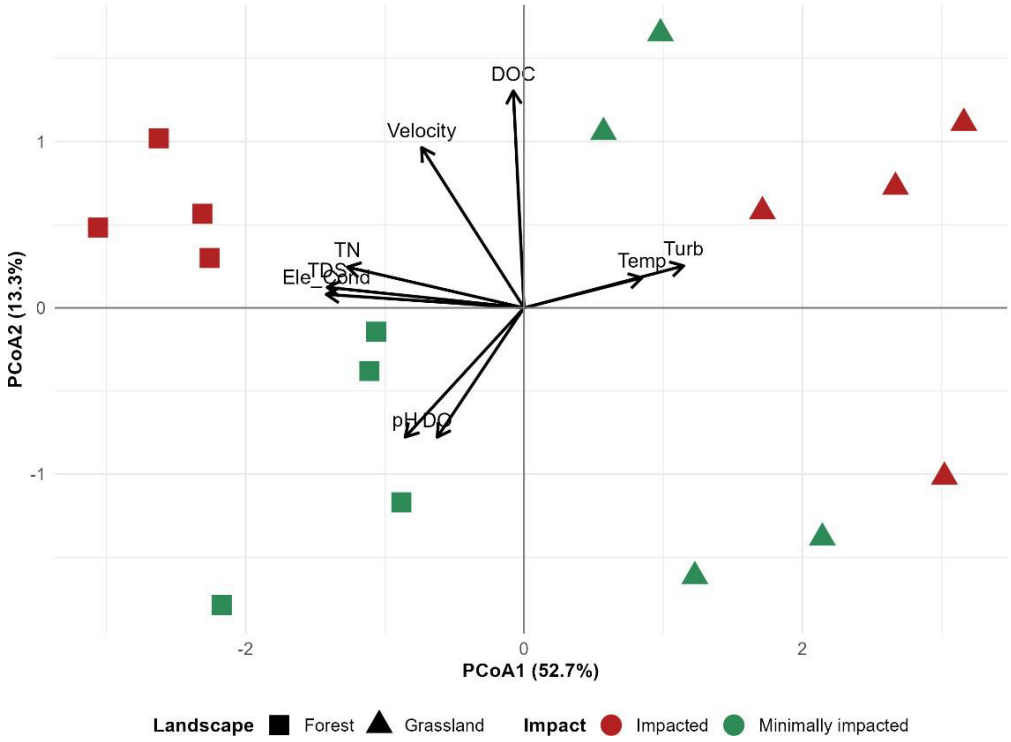


Figure 2. Principal Coordinates Analysis (PCoA) of sampling streams based on Euclidean distances of standardized limnological variables. Point colors indicate impact level (red = impacted; green = minimally impacted), and shapes represent landscape type (circles = forest; triangles = grassland). Arrows indicate the correlation vectors of limnological variables with the ordination (see Table 2). Variables include water temperature (Temp), electrical conductivity (Ele_Cond), total dissolved solids (TDS), pH, dissolved oxygen (DO), total nitrogen (TN), dissolved organic carbon (DOC), current velocity (Velocity), and turbidity (Turb).

We collected a total of 1,975 Trichoptera, distributed in 9 families and 16 genera (Table 3). In forest streams, we recorded 13 genera, minimally impacted streams hosted all 13 genera, while impacted streams hosted 10 (Table 3). In grassland streams, we recorded 11 genera in total; both minimally impacted and impacted streams hosted 9 genera (Table 3). The genera *Marilia*, *Cyrnellus*, *Wormaldia*, *Helicopsyche*, and *Chimarra* occurred only in forest streams, while *Barypenthus*, *Hydroptila*, and *Oxyethira* occurred only in grassland streams. Regarding impact, *Hydroptila* and *Barypenthus* occurred only in impacted streams, and *Wormaldia, Oxyethira* and *Helicopsyche* occurred only in minimally impacted streams.

Overall, minimally impacted streams accounted for 68.5% of all individuals, whereas impacted streams accounted for 31.5%. Considering landscape, grassland streams concentrated 66.0% of the individuals, while forest streams accounted for 34.0% of the total abundance (Table 3).

Table 3. Abundance of Trichoptera genera in forest and grassland streams under minimally impacted (MI) and impacted (I) conditions, collected in southern Brazil.

		Forest		Grassland	
Family	**Genera**	**MI**	**I**	**MI**	**I**
Calamoceratidae	*Phylloicus* Müller, 1880	30	24	5	2
	Mortoniella Ulmer, 1906	4	124	147	4
Helicopsychidae	*Helicopsyche* von Siebold, 1856	1	0	0	0
	Smicridea McLachlan, 1871	241	143	604	164
Hydroptilidae	*Hydroptila* Dalman, 1819	0	0	0	7
	Alisotrichia Flint, 1964	1	2	1	0
	Metrichia Ross, 1938	3	21	164	37
	Neotrichia Morton, 1905	10	32	113	5
	Oxyethira Eaton, 1873	0	0	2	0
Odontoceridae	*Marilia* Müller, 1880	5	1	0	0
	Barypenthus Burmeister, 1839	0	0	0	1
Philopotamidae	*Chimarra* Stephens, 1829	5	7	0	0
	Wormaldia McLachlan, 1865	8	0	0	0
Polycentropodidae	*Cernotina* Ross, 1938	2	2	2	10
	Cyrnellus Banks, 1913	3	2	0	0
Sericostomatidae	*Grumicha* Schmid, 1958	1	0	1	1

The PERMANOVA showed a significant interaction between landscape and impact ($F_{(1,12)} = 3.2$, $p = 0.049$) for labile traits. In contrast, the conserved and all traits did not yield significant results ($p > 0.05$) (Table 4).

Table 4. Results of PERMANOVA analyses assessing the effects of landscape, impact, and interaction on the datasets of functional traits of Trichoptera. Reported metrics include degrees of freedom (Df), coefficient of determination (R^2), F-statistic, and the significance level (p-value). Statistically significant variables ($p < 0.05$) are highlighted in bold and *.

	Df	**R^2**	**F**	**p-value**
Full				
Landscape	1	0.02	0.4	0.609
Impact	1	0.13	2.3	0.123
Landscape: Impact	1	0.15	2.6	0.092
Residual	12	0.68		
Total	15	1.00		
Labile				
Landscape	1	0.01	0.2	0.860
Impact	1	0.14	2.6	0.103
Landscape: Impact	1	0.18	3.2	**0.049***
Residual	12	0.66		
Total	15	1.00		
Conserved				
Landscape	1	0.04	0.6	0.516
Impact	1	0.11	1.7	0.167
Landscape: Impact	1	0.09	1.5	0.227
Residual	12	0.75		
Total	15	1.00		

The ANOVA analysis of individual traits showed that only body size and body shape responded to categorical predictors (Table S4). Minimally impacted streams predominantly exhibited insect with cylindrical shapes ($F_{(1,12)} = 6.1$, $p = 0.02$), while impacted streams predominantly exhibited insects with flattened shapes ($F_{(1,12)} = 5.8$, $p = 0.03$), with significant

interactions between landscape and impact for both traits ($F_{(1,12)} = 6.8$, $p = 0.02$; $F_{(1,12)} = 4.4$, $p = 0.05$, respectively). The larger body size categories showed significant interaction between landscape and impact ($F_{(1,12)} = 5.6$, $p = 0.03$; $F_{(1,12)} = 4.4$, $p = 0.05$, respectively). All other functional traits did not respond to landscape or impact (Table S4).

The first RLQ axis accounted for 47.1% of the total co-inertia, while the second axis explained 42.8%, together representing 89.9% of the variation in the trait-environment relationship (Fig. 3). The first RLQ axis represented a gradient mainly associated with variation in current velocity, temperature, pH, and DOC. Toward the positive side of RLQ1, taxa were characterized by flattened and streamlined body shapes, scraper feeding group, case builder behavior, tegument respiration, and univoltine life cycles. In contrast, the negative side of the axis was associated with taxa characterized by filter-feeding, multivoltine, larger body size, and gill respiration. In addition, the space ordination showed that impacted sites located in forest landscapes were predominantly positioned on the positive side of RLQ1. The second RLQ axis was mainly associated with variation in turbidity and DOC. The ordination space showed that grassland sites were predominantly positioned on the positive side of RLQ2, where predators were more strongly represented. In contrast, forest sites occurred predominantly toward the negative side of RLQ2, where shredders and collectors were more frequent and environmental conditions were associated with lower turbidity and DOC.

The fourth-corner analysis showed that current velocity was significantly associated with traits, being negatively associated with intermediate body size (Size3; $r = -0.236$; $p = 0.013$) and cylindrical body shape ($r = -0.204$; $p = 0.036$) and positively associated with flattened body shape ($r = 0.202$; $p = 0.031$).

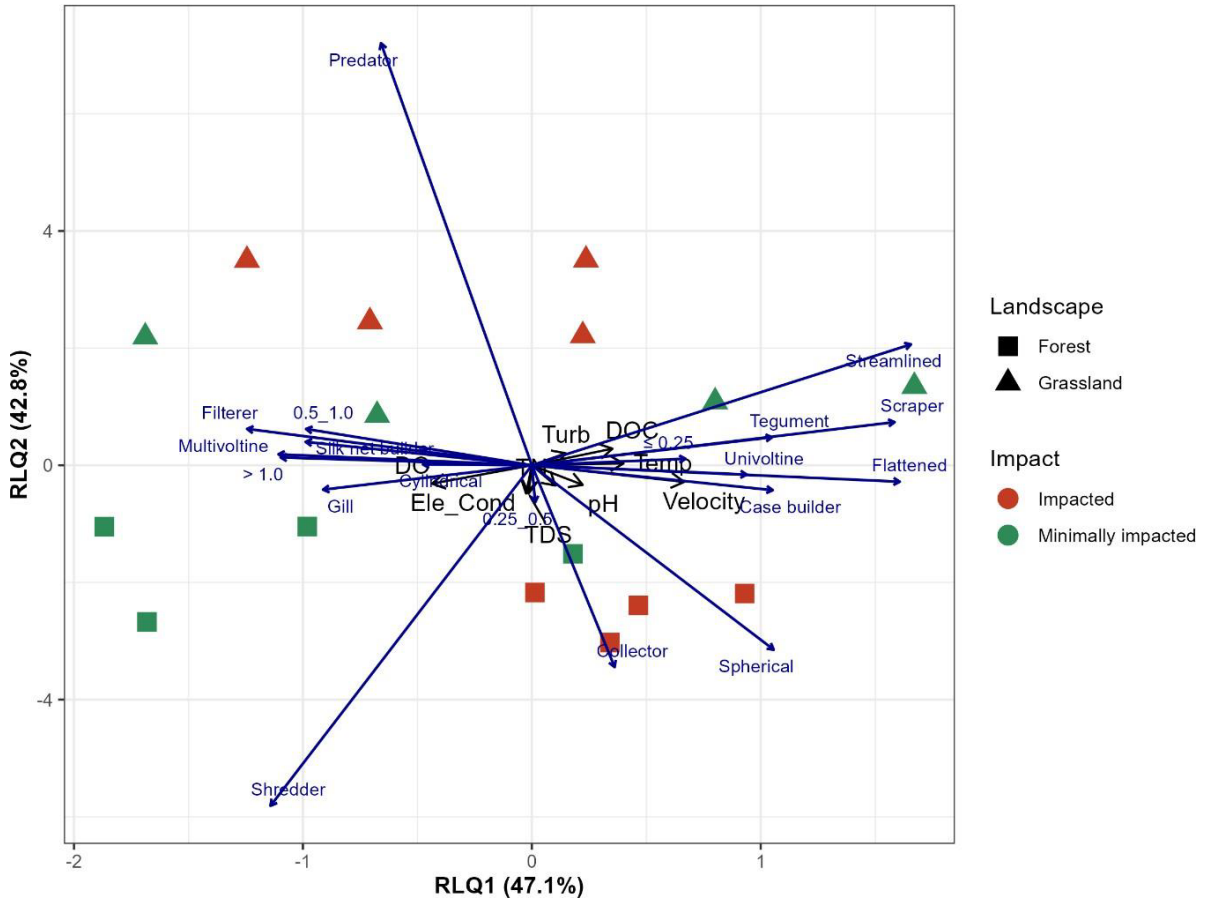


Figure 3. RLQ analysis results showing relationships between limnological variables and functional trait categories of Trichoptera. Environmental variables include water temperature (Temp), electrical conductivity (EC), total dissolved solids (TDS), pH, dissolved oxygen (DO), total nitrogen (TN), dissolved organic carbon (DOC), current velocity (Velocity), and turbidity (Turb). Functional trait categories are described in Table S1.

Discussion

We observed clear differences in limnological conditions driven by both landscape and impact level, indicating distinct environmental gradients shaping stream environments. Grassland streams formed a more homogeneous group, regardless of anthropogenic impact, likely due to the naturally open structure of riparian zones in this landscape (Iganci et al., 2011; Overbeck et al., 2022). Reduced canopy cover in these systems contributes to increased water temperatures (Luke et al., 2017) and, when associated with conversion to agroecosystems, leads to lower soil stability, favoring fine erosion and sediment suspension, and promoting increased turbidity (Mello et al., 2018). In contrast, forest streams showed greater variation, with clear separation between minimally impacted and impacted sites. Minimally impacted forest streams were primarily associated with higher dissolved oxygen concentrations, which is expected given the presence of dense riparian vegetation that reduces temperature, contributes coarse organic matter, and limits nutrient and sediment inputs. On the other hand, impacted forest streams exhibited elevated TN, EC, and TDS; patterns commonly linked to riparian degradation and adjacent agricultural land use

(Kadir et al., 2021). Altogether, these findings indicate that the effects of anthropogenic impact on stream conditions are strongly mediated by landscape context, with forest streams exhibiting greater environmental differentiation between impact levels than grassland. This environmental structuring on a regional scale is also reflected in the functional organization of Trichoptera assemblages, linking environmental gradients to biological responses.

This pattern becomes particularly evident when considering labile traits, which showed a significant interaction between landscape and impact. On the one hand, supports the idea that evolutionarily labile traits are more sensitive to environmental variation (Blomberg et al., 2003; Losos, 2008; Poff et al., 2006). On the other hand, it highlights an important caveat: when combining large sets of functional traits into multivariate metrics without considering their evolutionary patterns, we may mask important functional responses. When we examine singly trait categories, the sensitivity of labile functional traits becomes especially evident, with only body size and shape responding significantly to impact and landscape, e in local scale, to limnological conditions (i. e., current velocity). The lack of a significant response in other functional traits (e.g., feeding habits, respiration, and shelter construction) supports the idea that labile traits are the first to signal environmental change. Sánchez-Martínez et al. (2024), which suggests that evolutionarily labile traits exhibit high responsiveness to subtle environmental gradients, whereas more phylogenetically conserved traits respond only under stronger or prolonged environmental pressures. Moreover, recent findings by Li et al. (2023) demonstrate that incorporating both phylogenetic conservatism and trait intercorrelations into machine learning models enhances the detection and prediction of functional responses in stream invertebrates, reinforcing the need for trait-based approaches sensitive to evolutionary structure. These findings highlight the importance of assessing labile traits (Blomberg et al., 2003; Ackerly, 2009; Sánchez-Martínez et al., 2024), as they tend to exhibit faster and more detectable responses to anthropogenic disturbances, serving as early indicators of environmental change.

The RLQ analysis revealed that functional traits were not independently associated with environmental conditions but rather organized into coherent trait syndromes structured along major environmental gradients. The first RLQ axis described a gradient primarily associated with current velocity, temperature, pH, and DOC, separating two contrasting functional strategies. On one side of this axis, taxa were characterized by flattened and streamlined body shapes, scraper feeding habits, case-building behavior, tegument respiration, and univoltine life cycles. On the opposite side, taxa exhibited larger body sizes, cylindrical shapes, filter-feeding habits, multivoltine cycles, and gill respiration.

This pattern indicates strong hydrodynamic filtering, in which current velocity acts as a central driver structuring functional organization, particularly for more labile traits. Under higher

flow conditions, typically observed in impacted streams, increased drag forces select for traits that enhance resistance to hydraulic stress, such as flattened and streamlined body shapes, tegument respiration, and case-building behavior, allowing organisms to maintain attachment and exploit microhabitats within the substrate (Callisto et al., 2023). These conditions are often associated with habitat simplification and reduced channel roughness due to riparian degradation (Liu et al., 2017; Yang et al., 2018). In contrast, environments with lower current velocity and greater habitat stability favor taxa with larger body sizes and cylindrical shapes, reflecting reduced hydrodynamic constraints and increased mobility (Poff et al., 2006).

Although individual traits may respond to environmental filters, organisms typically exhibit coordinated sets of functionally related traits, known as trait syndromes, which reflect integrated responses to ecological constraints (Poff et al., 2006; Villéger et al., 2010; Winemiller et al., 2015). In our study, the interaction between landscape context and current velocity, particularly in forest streams, suggests the emergence of trait syndromes that integrate morphology, physiology, and behavior in response to hydrodynamic and structural conditions. Traits such as flattened and smaller body size, tegument respiration, and scraper feeding habit form a functional syndrome that enhances resistance to drag while maintaining efficient resource acquisition under physically stressful conditions. This coordinated trait expression likely maximizes organismal performance in environments characterized by increased flow and habitat simplification, where both mechanical stability and physiological efficiency are critical. In contrast, more stable environments with lower hydrodynamic stress favor alternative syndromes associated with larger body size, cylindrical shapes, and filter-feeding strategies, reflecting reduced physical constraints and greater ecological opportunity. Together, these results highlight that community responses are not driven by isolated traits but by integrated functional strategies shaped by interacting environmental gradients.

Conclusion

We conclude that labile traits tended to respond more strongly and consistently to environmental gradients. These traits may be directly filtered by local conditions, effectively "pulling" certain taxa into favorable positions within environmental filters. In contrast, conserved traits may be indirectly "co-selected" due to their phylogenetic association with labile traits under direct selection. This dynamic can generate contrasting patterns in response to impact, such as the absence of response in the full trait dataset and a negative response in the labile trait subset. Consequently, multivariate approaches or aggregated trait metrics that overlook the lability of traits may fail to detect key aspects of how communities respond to environmental change.

By reinforcing the concept that environmental filters act on syndromes rather than on isolated traits, our study contributes to a growing body of evidence advocating for more integrative, trait-based frameworks in stream ecology. These patterns were especially evident in response to current velocity, which acted as the main environmental filter selecting for functional syndromes such as flattened body shape, skin respiration, and scraper feeding; traits that collectively optimize performance under high-flow, low-complexity conditions. In this context, integrating functional and phylogenetic approaches becomes especially important under global environmental change, where trait lability and functional convergence may determine not only how communities persist, but also how they reorganize and adapt. Understanding how trait syndromes evolve, restructure, and respond to local environmental pressures is essential for predicting the future trajectories of biodiversity under intensifying environmental stress.

Statements and Declarations

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Supplementary Material

Table S1. Location and environmental characterization of sampled stream sections across landscape (Forest and Grassland) and impact (Minimally Impacted – MI; Impacted – I). The table provides geographic coordinates, altitude (m) and mean ± standard deviation for stream width and depth.

Forest / MI	Geographic Coordinates	Altitude (m)	Width (m)	Depth (m)
S1	27º30'53"S; 52º19'53"W	699	2.84 ± 0.37	0.055 ± 0.025
S2	27°35'43.26"S; 52°16'39.67"W	620	1.50 ± 0.31	0.093 ± 0.011
S3	27º34'38"S; 52º14'47"W	626	2.33 ± 0.43	0.088 ± 0.051
S4	27º36'10.95"S; 52º16'19.76"W	661	2.38 ± 1.28	0.083 ± 0.023
Forest / I				
S5	27º47'5.93"S; 52º22'59.81"W	635	0.58 ± 0.10	0.037 ± 0.006
S6	27º43'49"S; 52º13'23"W	643	2.13 ± 0.91	0.073 ± 0.021
S7	27º43'51.5"S; 52º13'02.1"W	644	2.97 ± 0.15	0.177 ± 0.050
S8	27º42'47"S; 52º13'32.8"W	680	2.87 ± 0.78	0.084 ± 0.086
Grassland / MI				
S9	29º09'28.07"S; 54º34'23.82"W	353	1.26 ± 0.16	0.095 ± 0.015
S10	29º09'37.36"S; 54º34'05.59"W	326	2.63 ± 0.06	0.077 ± 0.058
S11	29º05'52"S; 54º29'40.57"W	409	1.88 ± 0.46	0.090 ± 0.026
S12	29º11'06.67"S; 55º00'42.86"W	308	3.13 ± 0.80	0.157 ± 0.052
Grassland / I				
S13	29º12'03.04"S; 54º36"28.60"W	402	0.87 ± 0.15	0.103 ± 0.058
S14	29º11'33.74"S; 54º36'05.96"W	407	1.67 ± 0.46	0.093 ± 0.041
S15	29º05'10.43"S; 54º29'26.36"W	408	1.46 ± 0.22	0.143 ± 0.056
S16	29º02'49.66"S; 54º28'22.53"W	310	1.00 ± 0.26	0.038 ± 0.025

Table S2. Functional traits of Trichoptera genera affinity described by fuzzy coding for 19 categories in six functional traits. The score for each taxon belonging to each trait category ranged from 0 to 3 (0 = none affinity; 1= affinity weak; 2 = affinity moderated; 3 = affinity strong). Genera for which fuzzy code values were obtained for all or most of their traits from Santos et al. (2024) are highlighted in orange, and from Castro et al., 2017 in yellow. The highlighted fuzzy code values correspond to the information source for each functional trait, as follows: Castro et al., 2017 (yellow); Tomanova & Usseglio-Polatera, 2007 (green); Saito et al. (2015) (pink), Tachet et al. 2010 (brown), Pes et al. (2018) (red), and extrapolated to the family level (gray).

Table S3. Results of two-way ANOVAs testing the effects of predictors: landscape (forest vs. grassland), impact (impacted vs. minimally impacted), and their interaction on Trichoptera trait categories proportion. Reported metrics include degrees of freedom (df), sum of squares (SS), mean square (MS), F-statistics, and the significance level (p-value). Statistically significant variables ($p < 0.05$) are highlighted in bold*.

Traits / Categories	Predictors	df	SS	MS	F-statistic	p-value
Body Size						
≤ 0.25	Landscape	1	0.006	0.006	0.42	0.528
	Impact	1	0.010	0.010	0.69	0.422
	Landscape: Impact	1	0.029	0.029	1.94	0.189
	Residual	12	0.179	0.015		
0.25 - 0.5	Landscape	1	0.002	0.002	0.73	0.407
	Impact	1	0.007	0.007	2.58	0.134
	Landscape: Impact	1	0.002	0.002	0.91	0.357
	Residual	12	0.034	0.002		
0.5 - 1.0	Landscape	1	< 0.001	< 0.001	0.10	0.751
	Impact	1	0.008	0.008	3.26	0.096
	Landscape: Impact	1	0.015	0.015	5.63	**0.035***
	Residual	12	0.032	0.002		
> 1.0	Landscape	1	< 0.001	< 0.001	0.12	0.732
	Impact	1	0.008	0.008	4.04	0.067
	Landscape: Impact	1	0.009	0.009	4.43	**0.057***
	Residual	12	0.026	0.002		
Body Shape						
Cylindrical	Landscape	1	< 0.001	< 0.001	0.01	0.897
	Impact	1	0.054	0.054	6.13	**0.029***
	Landscape: Impact	1	0.054	0.054	6.17	**0.028***
	Residual	12	0.106	0.008		
Flattened	Landscape	1	< 0.001	< 0.001	0.02	0.884
	Impact	1	0.033	0.033	5.76	**0.033***
	Landscape: Impact	1	0.025	0.025	4.40	**0.057***
	Residual	12	0.069	0.005		
Spherical	Landscape	1	0.001	0.001	0.71	0.416
	Impact	1	< 0.001	< 0.001	0.35	0.562
	Landscape: Impact	1	0.004	0.004	1.94	0.189
	Residual	12	0.028	0.002		
Streamlined	Landscape	1	0.001	0.001	0.61	0.447
	Impact	1	< 0.001	< 0.001	0.15	0.699
	Landscape: Impact	1	< 0.001	< 0.001	0.01	0.908
	Residual	12	0.0343	0.002		
Voltinism						
Multivoltine	Landscape	1	< 0.001	< 0.001	< 0.01	0.946
	Impact	1	0.119	0.119	3.19	0.099
	Landscape: Impact	1	0.120	0.120	3.20	0.098
	Residual	12	0.448	0.037		
Univoltine	Landscape	1	< 0.001	< 0.001	< 0.01	0.946
	Impact	1	0.119	0.119	3.19	0.099
	Landscape: Impact	1	0.120	0.120	3.20	0.098
	Residual	12	0.448	0.037		
Feeding Group						
Collector	Landscape	1	0.027	0.027	2.09	0.173
	Impact	1	< 0.001	< 0.001	< 0.01	0.937
	Landscape: Impact	1	< 0.001	0.005	0.37	0.551
	Residual	12	0.160	0.013		
Filterer	Landscape	1	0.015	0.015	0.22	0.643
	Impact	1	0.070	0.070	1.01	0.336
	Landscape: Impact	1	0.048	0.048	0.69	0.422
	Residual	12	0.836	0.069		
Predator	Landscape	1	< 0.001	< 0.001	0.18	0.672
	Impact	1	< 0.001	< 0.001	0.31	0.585
	Landscape: Impact	1	< 0.001	< 0.001	1.37	0.264
	Residual	12	0.005	< 0.001		

Scraper	Landscape	1	0.028	0.028	1.11	0.314
	Impact	1	0.097	0.097	3.81	0.074
	Landscape: Impact	1	0.061	0.061	2.39	0.148
	Residual	12	0.308	0.025		
Shredder	Landscape	1	0.018	0.018	3.78	0.075
	Impact	1	0.004	0.004	0.95	0.347
	Landscape: Impact	1	0.005	0.005	1.15	0.306
	Residual	12	0.058	0.004		
Construction Behavior						
Case builder	Landscape	1	0.004	0.004	0.07	0.784
	Impact	1	0.042	0.042	0.78	0.393
	Landscape: Impact	1	0.015	0.015	0.29	0.597
	Residual	12	0.643	0.053		
Silk net builder	Landscape	1	0.004	0.004	0.07	0.784
	Impact	1	0.042	0.042	0.78	0.393
	Landscape: Impact	1	0.015	0.015	0.29	0.597
	Residual	12	0.643	0.053		
Respiration						
Gill	Landscape	1	0.009	0.009	0.38	0.545
	Impact	1	0.100	0.100	3.92	0.071
	Landscape: Impact	1	0.067	0.067	2.65	0.13
	Residual	12	0.308	0.025		
Tegument	Landscape	1	0.009	0.009	0.38	0.545
	Impact	1	0.100	0.100	3.92	0.071
	Landscape: Impact	1	0.067	0.067	2.65	0.13
	Residual	12	0.308	0.025		

CAPÍTULO 3

Evolutionary constraint and local limnological drivers of stream Trichoptera body size



Um dos riachos estudados em Três Lagoas, Mato Grosso do Sul, Brasil (20º51’25.72” S 52º10’41.14” O). Foto: Mariana Nunes Menegat.

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Evolutionary constraint and local limnological drivers of stream Trichoptera body size

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Abstract

Anthropogenic impacts increasingly modify freshwater ecosystems, altering key organismal traits such as body size. We evaluated how body size responds to limnological gradients and how much of this variation is shaped by evolutionary constraint. We predicted that variation reflects taxonomic structure, and that dissolved oxygen, temperature, ammonium, and total phosphorus explain additional variation once evolutionary constraint is accounted for. We used two linear mixed-effects models, one with all individuals and another with up to five randomly selected individuals per genus per site, incorporating family and genus as random effects. We also fitted a linear model for a single genus to assess fine-scale responses. Body size varied among genera (44% of variance), whereas families explained only 8%. Body size responded negatively to dissolved oxygen, ammonium, total phosphorus, and alkalinity, and these patterns remained consistent in the reduced dataset. Within *Cernotina*, body size also decreased with temperature, dissolved oxygen, ammonium, and phosphorus, while increasing with conductivity and turbidity. Our results emphasize that body size can serve as both an ecological indicator and a trait-based metric, as it shows rapid and consistent responses to environmental gradients.

Keywords: Aquatic insects. Functional Traits. Caddisfly. Phosphorus. Ammonium. Organismal performance.

Introduction

Body size is a key functional trait that integrates multiple dimensions of organismal performance, including metabolism, growth rate, dispersal capacity, and trophic interactions (Chown & Gaston, 2010). This trait is a key trait because it scales predictably with energy use and resource demand (Brown et al., 2004), integrating resource availability and habitat quality with processes from individuals to ecosystems (Peters, 1983; Chown & Gaston, 2010). Variation in body size in response to environmental conditions has been widely documented across taxa and ecosystems (e.g., Daufresne et al., 2009; Forster et al., 2012; Sheridan & Bickford, 2011). In ectotherms, smaller body sizes tend to predominate under warmer conditions (Bergmann, 1847; Deutsch et al., 2022). In aquatic ecosystems, body size reductions have been associated with nutrient enrichment (e.g., benthic macroinvertebrates, Daufresne et al., 2009), oxygen limitation (e.g., aquatic insects, Verberk et al., 2011), and increases in water and air temperature (e.g., stream fishes and invertebrates, Atkinson, 1994; Forster et al., 2012). Therefore, body size arises as a sensitive trait for multiple environmental gradients, although the cause of variation in local scale

remains poorly understood.

Aquatic environments offer an important context for studying patterns of body size distribution because these ecosystems are highly heterogeneous, and both habitat structure and environmental conditions can vary substantially even at local scales (Townsend & Thompson, 2009; Heino, 2013). Alterations in physical and chemical conditions can drive rapid responses by inducing phenotypic plasticity or adaptive shifts in morphological and physiological traits (Verberk et al., 2013; Stoks et al., 2014). Elevated ammonium concentrations may impair physiological performance or reproductive success (Park et al., 2021; Zhang et al., 2023), while oxygen limitation constrains aerobic scope and growth efficiency (Verberk & Bilton, 2011). Similarly, turbidity and electrical conductivity may shape body-size patterns indirectly by modifying feeding efficiency, visual foraging, and habitat structure, given that suspended sediments alter optical conditions and benthic habitat complexity (Bilotta & Brazier, 2008; Wagenhoff et al., 2017) and that ionic stress associated with elevated conductivity affects macroinvertebrate performance, survival, and trait expression (Kefford et al., 2007, 2012; Abelho et al., 2021).

However, environmental drivers alone cannot fully account for body-size variation. Organisms do not respond to these conditions as evolutionarily unconstrained units; they do so within the limits set by their shared evolutionary history (Freckleton et al., 2002). Lineages inherit developmental pathways, trait correlations, and ecological strategies that can restrict or channel how body size evolves, even when strong environmental pressures act on populations (Hansen, 1997; Hansen et al., 2008). Here, we use taxonomic structure, that is, genus and family, as proxies for these evolutionary constraints. Closely related taxa tend to share morphological and functional traits because they inherit them from common ancestors (Blomberg et al., 2003; Pannell & Harmon, 2013). Such evolutionary constraint often emerges more strongly at lower taxonomic levels (e.g., genera), where ecological strategies remain relatively conserved, and weakens at broader ranks (Losos, 2008; Münkemüller et al., 2012). This rationale underlies the common practice of assigning functional traits of aquatic insects at the genus level (Tachet et al., 2010; Santos et al., 2024), where phylogenetic signal tends to be highest and ecological coherence remains meaningful (Poff et al., 2006; Saito et al., 2016). Despite this theoretical understanding, it remains unclear how strong these constraints are in practice and whether they are sufficient to limit or redirect body-size responses to local limnological gradients.

Given these evolutionary constraints, the question is whether limnological conditions still influence body size once shared ancestry is accounted for. To address this, we analyzed body size using three complementary response matrices. The first captures individual-level measurements for all individuals in our dataset, preserving the full range of intraspecific variation. The second

relies on a random selection of five individuals per genus at each site; this approach is common practices in functional ecology, where body size is estimated from a small set of measured individuals per taxon (Castro et al., 2017). In addition, this approach has applied implications because a reduced dataset makes body-size assessments operationally feasible in biomonitoring studies. Finally, the third response matrix consists of measurements of all individuals of a single genus. This genus-level analysis minimizes the influence of deeper evolutionary constraints, by reducing variation associated with divergence among lineages. Together, these approaches allow us not only to control evolutionary constraint, but also to evaluate how body size responds to limnological gradients and to explore its potential as a viable and sensitive indicator of local limnological variation.

A natural step, then, is to consider which freshwater taxa provides the most informative model to examine how evolutionary constraints and limnological conditions jointly shape body size. Trichoptera larvae offer an excellent model in this regard. The order encompasses high taxonomic and functional diversity across Neotropical streams, including lineages that differ markedly in life-history strategies, case-building behaviors, and feeding modes (Morse et al., 2019). These ecological strategies often reflect fine-scale responses to habitat structure and the physical and chemical properties of water, making Trichoptera particularly sensitive to limnological gradients (Morse, 2019; Bonada & Resh, 2013). Their well-established role as bioindicators in biomonitoring programs emphasizes applied relevance to evaluating how body size responds to environmental change. Previous work in Neotropical streams, including studies quantifying trait-environment relationships and functional diversity in Trichoptera (e.g., Menegat et al., 2025), further underscores their role as a model for linking body size trait variation with ecological gradients. Moreover, body size in the group also shows evolutionary lability among genera (Menegat et al., 2026), which allows both the detection of phylogenetically structured patterns and the identification of environmentally driven variation.

Freshwater ecosystems are increasingly threatened by multiple stressors, including nutrient enrichment, warming, and oxygen depletion, which can profoundly alter organismal traits and community structure. Understanding how organisms respond to such environmental changes is essential for developing robust ecological indicators for biomonitoring. From an eco-evolutionary perspective, trait variation reflects both local environmental filtering and evolutionary constraints, yet disentangling these influences remains a key challenge. Within this framework, we evaluate how body size responds to limnological gradients and quantify the extent to which this variation is shaped by evolutionary constraint. We tested the hypothesis that limnological conditions, such that dissolved oxygen, temperature, ammonium, and total phosphorus play a major role in explaining a variation in the body size of Trichoptera. We addressed this hypothesis using

individual-level measurements, a reduced subset per genus per site, and a single-genus approach. Overall, this framework allows us to disentangle the relative contributions of evolutionary constraints and local environmental variation to body size patterns.

Material and Methods

Study area

We conducted this study in headwater streams of the Paraná River basin, located in the state of Mato Grosso do Sul, Brazilian Cerrado (Fig. 1). The Cerrado (a Neotropical savanna biome) is a global biodiversity *hotspot*, characterized by a mosaic of forest formations, savanna-like physiognomies, and open grasslands (Klink & Machado, 2005). Despite its ecological importance, more than half of its original vegetation has been lost, mainly due to pasture expansion and large-scale agriculture (MapBiomas, 2023). The region lies in a transitional zone between the Cerrado and the Atlantic Forest. The climate is tropical savanna (Aw, Köppen classification), with two distinct seasons: a dry winter (May to October) and a rainy summer (November to April). Annual rainfall ranges from 1,200 to 1,800 mm and mean temperatures vary between 20 and 26 °C (Alvares et al., 2013).

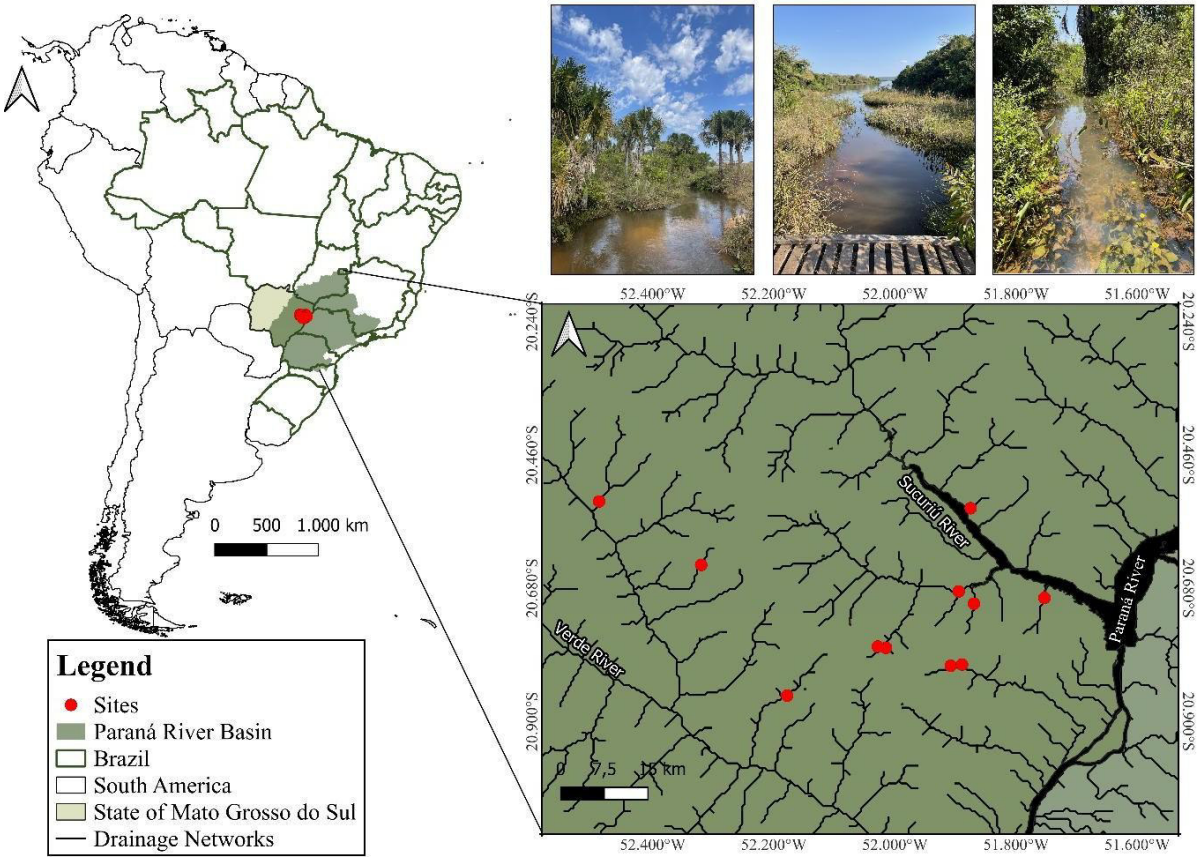


Fig. 1 Location of the headwater streams sampled in the Brazilian Cerrado (Mato Grosso do Sul)

Sampling design

We selected 11 third-order streams (Table S1) and placed six artificial substrates in each stream, totaling 66 sampling units. We constructed the artificial substrate (15 x 10 cm) from 10-mm mesh nylon bags and filled each one with a single loofah sponge (*Luffa cylindrica*, Cucurbitaceae) measuring approximately 8 × 5 × 1 cm, with a volume of ~40 cm³ (Fig. S1). We used a loofah sponge as a standardized organic substrate because its fibrous, porous network provides a heterogeneous microhabitat favorable for colonization by aquatic insect larvae. Previous freshwater colonization experiments and artificial-substrate protocols have successfully used loofah sponge to sample benthic macroinvertebrates (e.g., Leite-Rossi et al., 2015; Magossi et al., 2023). We placed the artificial substrate on the streambed and secured them with wooden stakes and nylon string to prevent displacement. The artificial substrate was incubated in the streams for 30 days. We conducted fieldwork during the dry season, from 21 August to 22 September 2023, when stable flow conditions favor aquatic insect sampling in Cerrado streams (Bispo et al., 2006). During this period, mean air temperature was 26.3 ± 6.01 °C and the mean precipitation was 0.05 ± 0.85 mm, based on data from the Brazilian National Institute of Meteorology (INMET).

Body Size

After the incubation period, we retrieved the artificial substrates and carefully washed each sponge in a fine-mesh sieve (0.125 mm) to remove attached macroinvertebrates. We identified Trichoptera individuals to the genus level using the key by Pes et al., (2018). We measured the body size of each individual under a stereomicroscope equipped with a Petri dish with a millimeter scale. We measured body length from the anterior margin of the head to the posterior end of the abdomen. We removed case-building individuals from their cases prior to measurement to ensure accurate body-length estimates. For the analysis, we excluded individuals clearly belonging to the earliest instars, identified by unsclerotized head capsules or translucent bodies, to avoid confounding ontogenetic differences with environmental effects on body size. This procedure retains biologically meaningful intraspecific variation and minimizes bias from extreme developmental stages.

Limnological variables

We measured *in situ* limnological variables using a Horiba U-50 multiparameter probe. The variables included water temperature (°C), electrical conductivity (mS/cm), pH, turbidity

(NTU), dissolved oxygen (mg/L), and total dissolved solids (g/L). In each stream, we took three measurements with the multiparameter probe at the beginning and at the retrieval of the 30-day incubation. The final value for each limnological variable was calculated as the mean of six measurements, three taken at installation and three at retrieval, in each stream.

Additionally, on the first day of incubation, in each stream, we collected three water samples for chemical analyses. We quantified total phosphorus (mg/L) and ammonium (NH_4^+, µg/L) using a UV/VIS spectrophotometer (Logen LS 7022). We determined total alkalinity (mg $CaCO_3$/L) by titrating the samples with 0.02 N sulfuric acid (H_2SO_4). We followed all analytical procedures according to the protocols described in Standard Methods for the Examination of Water and Wastewater (APHA, 2017).

Statistical Analyses

We assessed the influence of limnological variables on body size using a linear mixed-effects model (LMM; **lme4**::*lmer*). We log10-transformed body size and standardized all predictor variables using the scale function. The predictor set included water temperature, pH, dissolved oxygen, ammonium, total phosphorus, alkalinity, conductivity, total dissolved solids, and turbidity.

We fitted two complementary LMMs with the same predictors and random-effects structure. To account for taxonomic structure, used here as a proxy for evolutionary constraints, we included family and genus as random effects in all mixed models. We quantified the proportion of variance associated with each taxonomic level using Intraclass Correlation Coefficients (ICC). The ICC expresses how much of the total variation in body size reflects clustering within families and genera rather than individual-level variation; higher ICC values therefore indicate stronger evolutionary structuring of morphological traits. So, the first used all individual (N = 503) measurements and captured the full range of intraspecific variation. The second used a reduced dataset (N = 126) generated by randomly selecting up to five individuals per genus per site (**dplyr**::*slice_sample*), with a fixed seed for reproducibility. This subsampled model allows us to evaluate whether reduced sampling effort alters the estimated effects of limnological variables on body size.

Additionally, we constructed a dendrogram of Trichoptera genera based on a matrix of mean body size. For this, we computed a Euclidean distance matrix (**stats**::*dist*) and performed hierarchical clustering (**stats**::*hclust*) using the average linkage method.

Finally, we examined body-size responses within a single widespread genus, *Cernotina*, using a simple linear model (**stats**::*lm*) with the same set of limnological predictors. Occurring in

nine of the eleven streams, *Cernotina* provides a suitable focal group for testing whether environmental drivers detected at the community level also emerge within a single genus. All analyses were performed in R version 4.3 (R Core Team, 2023).

Results

Limnological variables ranged across sites (Table S2). Water temperature ranged from 24.2 to 27.1°C, and dissolved oxygen ranged from 4.2 to 6.4 mg/L. Electrical conductivity remained low, ranging from 0.034 to 0.039 mS/cm, and pH stayed near neutral, ranging from 6.3 to 7.2. Regarding nutrients, total phosphorus ranged from 0.015 to 0.025 mg/L, and ammonium concentrations ranged from 72.3 to 117.3 µg/L. Turbidity ranged from 20.8 to 60.2 NTU, and alkalinity ranged from 70 to 180 mg $CaCO_3$/L.

We collected a total of 503 Trichoptera larvae distributed across six families and 14 genera (Table S3). The most abundant genus was *Leptonema* (Hydropsychidae) (51.4%), followed by *Smicridea* (Hydropsychidae) (14.9%) and *Cernotina* (Polycentropodidae) (9.1 %). Body size varied widely among genera, from the smallest values for *Neotrichia* (1.63 ± 0.33 mm) to the largest for *Macronema* (10.12 ± 2.96 mm) (Fig. 2). This pattern was consistent at the family level, with Hydroptilidae exhibiting the smallest average body size (2.07 ± 0.37 mm) and Hydropsychidae the largest (5.71 ± 3.12 mm). Intermediate values were observed in Polycentropodidae (3.99 ± 0.84 mm), Leptoceridae (4.97 ± 2.03 mm), and Helicopsychidae (4.14 ± 0.00 mm).

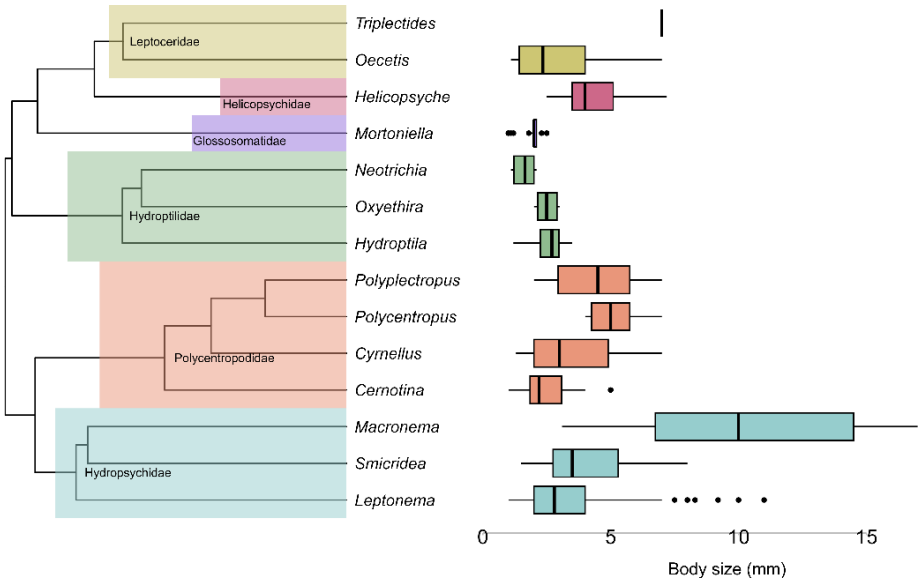


Fig. 2 Phylogenetic tree of Trichoptera genera collected in headwater streams in Brazilian Cerrado alongside boxplots showing body size distributions for each genus/family. The tree was constructed based on phylogenetic hypothesis by Gárcia-Girón et al. (2024)

The dendrogram means body size revealed that genera clustered independently of their phylogenetic affiliation at the family level (Fig. 3). Genera belonging to different families frequently grouped together. This result is consistent with the proportion of variance explained, where 44% of body size variation was explained by differences among genera (ICC = 0.44), while families explained only 8% (ICC = 0.08).

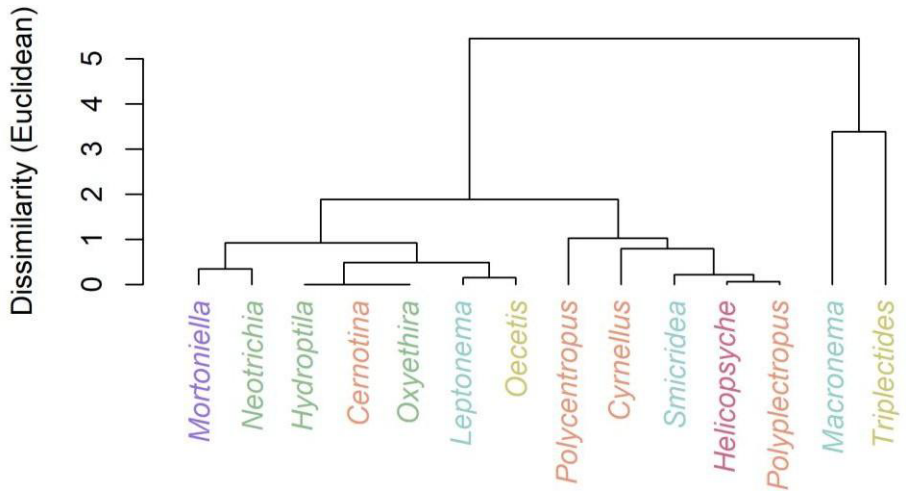


Fig. 3 Dendrogram of Trichoptera genera based on Euclidean dissimilarities of mean body size. Colors indicate families (yellow = Leptoceridae; pink = Helicopsychidae; purple = Glososomatidae; green = Hydroptilidae; orange = Polycentropodidae; blue = Hydropsychidae)

Regardless of the random effects, body size responded negatively to dissolved oxygen ($p = 0.02$), ammonium ($p = 0.03$), total phosphorus ($p = 0.04$), and alkalinity ($p = 0.03$) (Table 1). When we analyzed a reduced dataset with up to five individuals per genus per site, patterns remained consistent with the result of full dataset. Genera explained 43% of variation in body size among genera (ICC = 0.43), while families explained only 7% (ICC = 0.07). Regardless of the random effects, body size responded negatively to dissolved oxygen ($p = 0.01$), ammonium ($p = 0.01$), total phosphorus ($p = 0.02$), and alkalinity ($p = 0.01$) (Table 1). Finally, within the genus *Cernotina* ($F_{(8, 37)} = 3.3$; $p = 0.005$), body size responded negatively to temperature ($p = 0.02$), dissolved oxygen ($p < 0.01$), ammonium ($p < 0.01$), and total phosphorus ($p < 0.01$), while showing positive responses to electrical conductivity ($p = 0.04$) and turbidity ($p = 0.01$) (Table 1).

Table 1. Results of linear mixed-effects models (LMMs) testing the relationships between Trichoptera body size and limnological variables. The body size was response variable in all models. The first model "Size" includes all genera; and the second "Size_5" represents the reduced sampling. The third model included the results of the linear model (LM) evaluating the relationships between body size and limnological variables within the genus *Cernotina*. Significant predictors ($p < 0.05$) are highlighted in bold.

Response	Predictor	t value	p
Size ~	Water Temperature	-0.676	0.499
	Electrical conductivity	0.545	0.585
	pH	1.483	0.138
	Turbidity	-0.694	0.488
	Dissolved oxygen	-2.261	**0.024**
	Total dissolved solids	0.940	0.347
	Phosphorus total	-2.030	**0.042**
	Ammonium	-2.154	**0.031**
	Alkalinity	-2.066	**0.039**
Size_5 ~	Temperature	0.006	0.995
	Electrical conductivity	0.484	0.629
	pH	1.956	0.053
	Turbidity	-0.631	0.529
	Dissolved oxygen	-2.551	**0.012**
	Total dissolved solids	0.865	0.389
	Phosphorus total	-2.315	**0.022**
	Ammonium	-2.457	**0.015**
	Alkalinity	-2.478	**0.014**
Size_*Cernotina* ~	Temperature	-2.367	**0.023**
	Electrical conductivity	2.035	**0.049**
	pH	-1.536	0.133
	Turbidity	2.573	**0.014**
	Dissolved oxygen	-4.530	**<0.001**
	Phosphorus total	-3.213	**0.002**
	Ammonium	-3.538	**0.001**
	Alkalinity	1.484	0.146

Discussion

Our findings support our hypothesis that, although evolutionary constraints account for a portion of the variation, local limnological conditions play a major role in shaping Trichoptera body size. Evolutionary history acts as a baseline constraint on trait expression, establishing the morphological framework within which environmental factors operate. The consistent patterns using both the full and the reduced datasets reinforce that most of the variation is structured among genera rather than among individuals. At the same time, revealing that limnological conditions, particularly nutrient-related variables, strongly influence Trichoptera body size. Because larger body sizes tend to be associated with more stable habitat conditions (Liberal et al., 2025; Munyai et al., 2025), such as lower ammonium and phosphorus levels, they provide a meaningful signal of environmental quality. This reinforces the potential of body size for detecting subtle shifts in water chemistry. From an applied perspective, the reduced dataset proved equally informative compared with the full dataset, demonstrating practical ease and efficiency for application. Together, these findings underscore the potential of body size as a key functional trait to integrate

into biomonitoring approaches.

Nutrient enrichment emerges as the dominant environmental driver in our study. As observed in both large-scale studies, report shifts in macroinvertebrate body-size structure under nutrient enrichment (e.g., Cross et al., 2006; Nessel et al., 2021) and controlled laboratory experiments on growth responses to nitrogen compounds (e.g., Camargo & Alonso, 2006), ammonium and phosphorus can negatively influence the body size of aquatic insects. Anthropogenic activities are major sources of these nutrients in freshwater ecosystems, especially through agricultural expansion and wastewater inputs (Esteves, 2011; Ansari et al., 2014). Our study region is characterized by extensive monoculture (Bacani et al., 2024); these activities alter catchment-level nutrient dynamics, particularly increasing nitrogen and phosphorus export via soil disturbance and runoff (Esteves, 2011; Tanaka et al., 2016). Such nutrient inputs elevate ammonium and phosphorus concentrations in streams, driving eutrophication processes that degrade water quality and disrupt oxygen dynamics (Esteves, 2011; Ansari et al., 2014). These same nutrient patterns were the strongest predictors of body-size reduction in our models. Within this context, our results align with broader ecological findings: agricultural intensification in Amazonian streams reduces the prevalence of larger-bodied macroinvertebrates (Liberal et al., 2025) and elevated ammonium can directly inhibit growth and compromise respiratory structures (Camargo & Alonso, 2006), indirectly reducing body size by impairing feeding efficiency and increasing physiological stress. Smaller organisms thus tend to dominate under disturbed conditions (Hepp et al., 2023) as reduced investment in growth confers advantages associated with lower metabolic demand and shorter life cycles (Winemiller, 2005; Demi et al., 2019). This shift aligns with the size spectrum we observed across sites with higher nutrient loads.

Beyond nutrient effects, alkalinity and dissolved oxygen also exhibited negative associations with body size, indicating that larger individuals were less prevalent under conditions of higher buffering capacity and elevated oxygen availability. Although counterintuitive at first, given that low oxygen often constrains growth in aquatic insects (Verberk & Bilton, 2011; Hoefnagel & Verberk, 2015), this pattern suggests that the environmental context in our system may favor smaller-bodied individuals precisely where water quality is more stable. Streams with higher alkalinity and oxygen typically correspond to faster-flowing reaches with greater turbulence, increased substrate mobility, and higher energetic costs associated with maintaining position and foraging efficiency (Allan & Castillo, 2007; Pinheiro et al., 2021). Under these conditions, smaller individuals may benefit from reduced drag, shorter development times, and lower metabolic investment, aligning with predictions from life-history theory that rapid turnover is advantageous in hydrologically dynamic habitats (Winemiller, 2005). Conversely, larger individuals may be favored in more stable reaches where energetic costs are lower, and growth

can proceed with less disturbance (Townsend & Hildrew, 1994). Thus, the negative relationships with alkalinity and dissolved oxygen reinforce the idea that body size reflects not only chemical stressors but also physical habitat structure, integrating multiple dimensions of environmental filtering in Neotropical streams. At the same time, these results suggest that part of this response may also be shaped by evolutionary constraints, with lineages differing in their inherent sensitivity, illustrating environmentally mediated constraints interacting with evolutionary ones.

In parallel with environmental drivers, evolutionary constraint accounts for about half of the variation in body size. Taxonomic proxy for evolutionary constraint makes this pattern clear: body size variation was strongly structured at the genus level, indicating that individuals within the same genus tend to response similarly to environmental gradients. This suggests that trait evolution has occurred predominantly within, rather than among, genera. The clustering of genera by body size, irrespective of family affiliation, underscores that evolutionary constraints operate more strongly at finer taxonomic levels, where genera share developmental pathways and ecological strategies shaping their trait distributions (Freckleton et al., 2002; Poff et al., 2006). Consequently, certain lineages are more sensitive, while others remain more tolerant, to environmental change, a process that may alter both the functional structure and the taxonomic composition of local communities. Part of this evolutionary constraint, however, may reflect not only historical heritage but also the legacy of regional selective pressures. The lineages present in our study area (which encompasses a radius of ~100 km) have coexisted over long periods under broadly similar environmental conditions, including precipitation patterns, temperature, and the intensity of forestry and agriculture, as well as other regional factors that influence water quality and habitat structure. Communities subjected to similar regional conditions tend to converge on functional attributes over time, and this can leave a stronger evolutionary signature precisely at the genus level, where the sharing of evolutionary history and ecology is closest (Webb, 2000; Ackerly, 2009). Thus, the degree of evolutionary constraint tends to be stronger among communities long exposed to similar selective regimes. Nevertheless, within this scenario, local limnological conditions can influence variations in body size. This interaction reinforces the role of body size as a highly responsive trait within an evolutionary structured context.

Further reinforcing this role, zooming in on *Cernotina*, the negative associations between nutrient concentrations persisted, indicating a consistent response to conditions that described impacted environments. Notably, this similarity to the broader Trichoptera pattern highlights an important point: even under evolutionary constraints expressed at the genus level, body size still responds predictably to local stressors across lineages. Smaller individuals likely have an adaptive advantage under such stressful conditions because reduced body size lowers absolute metabolic demand and increases the relative surface-to-volume ratio, facilitating diffusive oxygen supply

under hypoxia (Brown et al., 2004; Verberk & Bilton, 2011). In disturbed or eutrophic habitats, nutrient-driven shifts in resource quality select for fast-life-history strategies (earlier reproduction, faster development) and tolerant taxa-traits commonly associated with smaller body size (Winemiller, 2005; Demi et al., 2019).

Beyond these shared patterns, *Cernotina* also displayed additional responses to electrical conductivity, turbidity, and temperature. These responses indicate that habitat-structure variables may operate through lineage-specific mechanisms, consistent with the idea that genera differ in how case-building, respiratory morphology, and foraging mode mediate exposure to suspended particles and ionic gradients. At the mechanistic level, in *Cernotina*, the absence of tracheal gills, structures that are particularly sensitive to ionic stress and prone to clogging by suspended particles, may reduce vulnerability to such conditions, which is consistent with the observed positive association with turbidity and conductivity. Furthermore, the negative responses to increased temperature are consistent with the widely documented temperature-size relationship, in which higher temperatures accelerate development rates and result in smaller adult body sizes (Forster et al., 2012). Taken together, these patterns suggest that while nutrient-related stressors generate consistent size reductions across Trichoptera, variables tied to habitat structure may reveal lineage-specific ecological strategies.

From an applied standpoint, largely due to the evolutionary constraint, our approach using up five individuals highlights applied implications for biomonitoring in freshwater ecosystems. Even with reduced sampling effort, body size consistently captured signals of limnological stress, reinforcing its utility as an efficient and accessible trait for monitoring environmental change, particularly under scenarios of climate warming and recurrent anthropogenic disturbances. The importance of body size as a functional trait is well established in the literature (Wonglersak et al., 2020; Lövei & Magura, 2022; Bernardino et al., 2024; Milesi & Hepp, 2025). Although our results show that body size retains a recognizable evolutionary imprint at the genus level, its strong responsiveness to environmental variation makes it a sensitive indicator of early ecological responses to habitat degradation and declining water quality. By integrating evolutionary constraint with local environmental filtering, body size emerges not only as an informative trait but as a practical tool for rapid assessments in regions where monitoring capacity is limited and environmental pressures are intensifying.

Conclusion

In summary, body size in Neotropical Trichoptera emerges from the combined influence of evolutionary constraint and local limnological conditions. Although half of the variation was due to the evolutionary constraint, body size remained strongly responsive to environmental

gradients after accounting for taxonomic structure. Ammonium and total phosphorus consistently showed negative effects on the body sizes across all analyses (the full dataset, the reduced dataset, and the *Cernotina* - only model), demonstrating that body size captures robust response to limnological conditions, even within an evolutionarily constrained framework. These results emphasize that body size can serve as both an ecological indicator and a trait-based metric for responding rapidly to environmental change.

Together, these findings position body size as a sensitive functional trait that integrates evolutionary history with local environmental filtering, offering both theoretical insight and practical utility for monitoring ecosystem change. Future studies should test whether these patterns hold across other taxonomic groups and ecological contexts. One immediate applied implication of our findings is that reliable ecological signals can be detected even when only a subset of individuals is measured, opening a concrete path for developing rapid, low-effort biomonitoring protocols. Building on this, future work could extend this approach to additional aquatic insect orders and to temporal comparisons using long-term datasets.

At the same time, integrating body size with complementary functional traits, such as respiration mode and feeding group, will be essential to strengthen mechanistic interpretation. From an applied perspective, refining these approaches may substantially enhance the feasibility of trait-based monitoring, particularly in regions with limited technical and financial resources, reinforcing the use of body size as an accessible and sensitive indicator of environmental change.

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Supplementary Material

Table S1. Geographical coordinates and physical characteristics of the sampled streams in Brazilian Cerrado, including depth and width (mean ± standard deviation).

Stream	Latitude	Longitude	Depth	Width
R1	20°48'21.34"S	51°53'21.52"W	33.3±18.9	0.6±0.4
R2	20°48'28.74"S	51°54'28.63"W	21.7±6.2	3.3±1.7
R3	20°42'19.76"S	51°52'10.80"W	58.6±8.6	4.6±0.8
R4	20°41'6.69"S	51°53'41.51"W	80.1±10.1	5.4±0.8
R5	20°33'5.82"S	51°52'44.60"W	11.1±1.1	1.3±0.4
R6	20°38'29.42"S	52°19'12.49"W	46.0±5.7	3.3±0.4
R7	20°46'38.52"S	52° 1'19.97"W	41.7±19.1	2.4±0.3
R8	20°46'38.38"S	52° 0'56.38"W	31.7±10.3	2.1±0.5
R9	20°51'25.85"S	52°10'41.09"W	48.0±13.4	3.5±0.6
R10	20°32'12.50"S	52°29'18.80"W	46.0±5.7	4.6±0.9
R11	20°41'45.50"S	51°45'11.60"W	27.6±2.6	1.4±0.3

Table S2. Mean (±SD) values of limnological variables measured in Brazilian Cerrado streams. Variables include depth (cm), width (m), dissolved oxygen (DO, mg/L), turbidity (Turb, NTU), water temperature (Temp, °C), pH, electrical conductivity (EC, mS/cm), total dissolved solids (TDS, g/L), ammonium (NH_4^+, µg/L), total phosphorus (PT, mg/L), and alkalinity (Alk, mg $CaCO_3$/L).

	Limnological variables								
Stream	**DO**	**Turb**	**Temp**	**pH**	**EC**	**TDS**	**NH_4^+**	**PT**	**Alk**
R1	4.2±1.1	33.8±5.7	24.5±0.1	6.3±0.1	0.050±0.002	0.032±0.001	117.25±4.08	0.024±0.001	10.0±0.0
R2	5.2±0.4	60.2±4.8	26.3±0.1	6.3±0.0	0.038±0.007	0.025±0.004	113.50±13.2	0.024±0.000	9.0±0.81
R3	5.7±0.4	24.9±0.4	24.3±0.1	6.6±0.0	0.059±0.000	0.038±0.000	91.00±7.10	0.025±0.001	11.0±0.81
R4	5.6±0.6	38.0±15.6	25.6±0.1	6.8±0.1	0.039±0.025	0.035±0.005	72.25±2.04	0.023±0.000	18.0±1.63
R5	6.1±0.0	26.0±3.1	27.1±0.1	7.0±0.2	0.036±0.003	0.019±0.001	106.00±1.02	0.017±0.000	7.0±0.81
R6	6.0±0.3	23.3±7.4	25.7±0.0	7.2±0.0	0.121±0.075	0.118±0.018	89.75±6.10	0.020±0.002	16.0±0.0
R7	6.4±0.5	27.4±7.6	25.2±0.3	6.7±0.0	0.073±0.003	0.039±0.002	93.50±1.02	0.015±0.001	10.0±3.26
R8	6.3±0.9	27.5±6.5	25.4±0.3	6.6±0.1	0.070±0.009	0.046±0.040	99.75±2.04	0.015±0.002	11.0±0.81
R9	6.2±0.2	40.1±3.1	24.2±0.1	6.6±0.2	0.034±0.007	0.065±0.004	106.00±1.02	0.018±0.001	8.0±1.63
R10	5.9±0.2	20.8±0.7	24.5±0.5	6.9±0.1	0.068±0.000	0.044±0.000	98.50±1.02	0.017±0.001	13.0±2.44
R11	5.6±0.2	25.8±0.1	25.7±0.1	6.3±0.0	0.063±0.000	0.040±0.000	92.25±12.20	0.020±0.001	11.0±0.81

Table S3. Abundance of Trichoptera genera in each stream collected in Brazilian Cerrado streams.

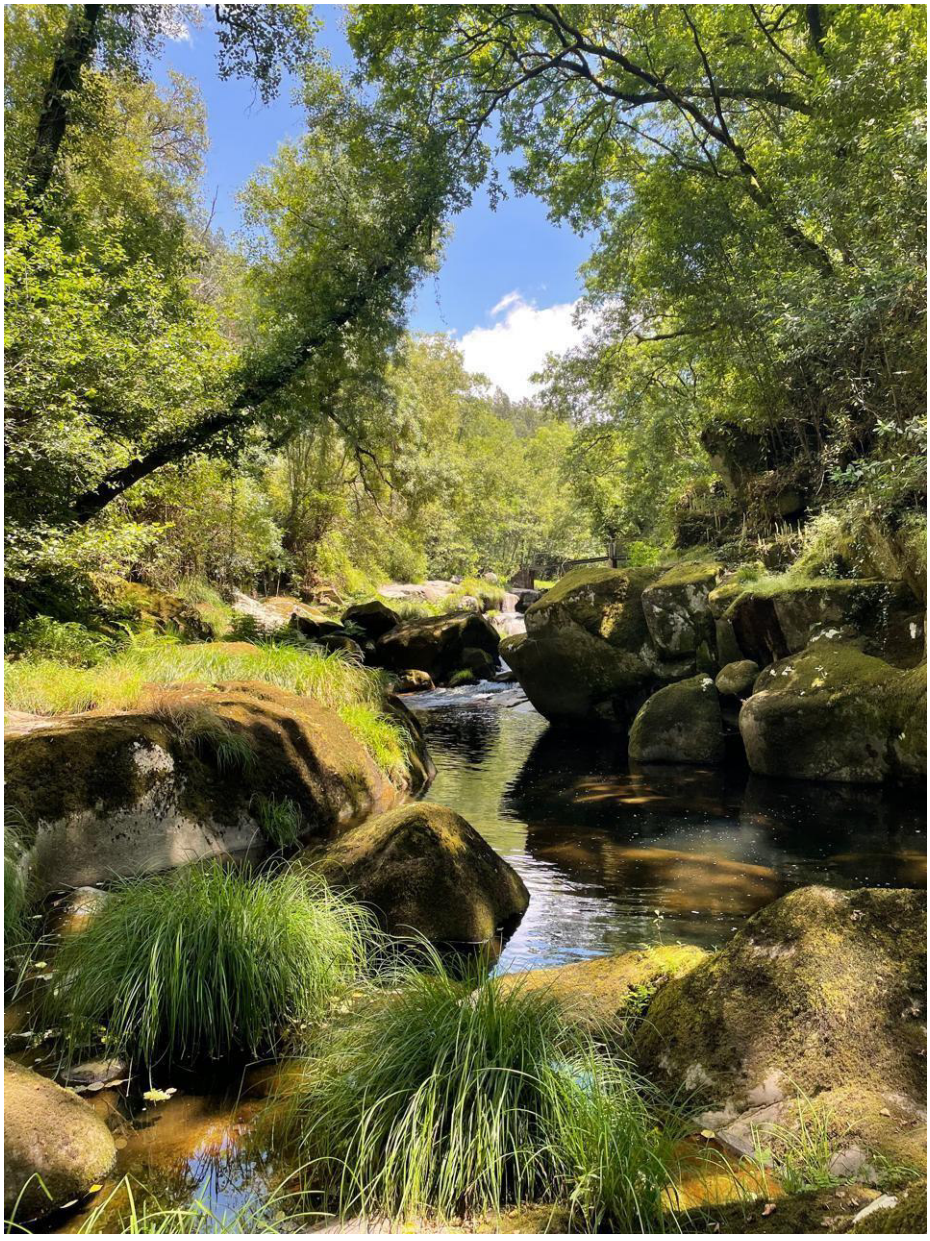
		Streams										
Family	**Genera**	R1	R2	R3	R4	R5	R6	R7	R8	R9	R10	R11
Glososomatidae	*Mortoniella* Ulmer, 1906	0	0	2	0	0	0	0	0	28	0	0
Helicopsychidae	*Helicopsyche* von Siebold, 1856	0	0	0	0	0	0	0	0	0	0	23
Hydropsychidae	*Leptonema* Guerin-Meneville, 1843	1	0	1	0	0	0	0	0	1	1	255
	Macronema Pictet, 1836	0	0	0	5	0	0	0	0	0	0	6
	Smicridea McLachlan, 1871	0	0	1	0	6	0	0	0	0	0	68
Hydroptilidae	*Hydroptila* Dalman, 1819	0	0	0	0	0	0	0	0	1	1	2
	Neotrichia Morton, 1905	0	0	1	2	1	0	0	1	1	1	3
	Oxyethira Eaton, 1873	0	0	0	0	0	2	0	2	0	0	3
Leptoceridae	*Triplectides* Kolenati, 1859	0	0	0	0	0	0	0	1	0	0	0
	Oecetis McLachlan, 1877	0	1	0	0	0	0	0	2	4	0	1
Polycentropodidae	*Cernotina* Ross, 1938	4	2	14	0	0	4	1	7	1	1	12
	Cyrnellus Banks, 1913	0	0	2	1	0	0	8	4	0	0	0
	Polycentropus Curtis, 1835	0	0	0	0	0	0	2	0	2	2	0
	Polyplectropus Ulmer, 1905	0	0	1	0	0	4	1	1	0	1	0



Fig. S1 Artificial substrate composed of a 15 × 10 cm nylon bag (10-mm mesh) filled with a loofah sponge (*Luffa cylindrica*) used for Trichoptera colonization.

CAPÍTULO 4

Time and space as predictors: taxonomic, functional, and phylogenetic structure of Trichoptera assemblage in Temperate streams



Coimbra, Portugal. Foto: Mariana Nunes Menegat.

Manuscrito nas normas da revista *Hydrobiologia.*

Time and space as predictors: taxonomic, functional, and phylogenetic structure of Trichoptera assemblage in Temperate streams

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Abstract

Understanding how freshwater biodiversity reorganizes across multiple dimensions over time is essential for predicting the impacts of global environmental change. We investigated the temporal (2001–2019) and environmental drivers of taxonomic (TD), functional (FD), and phylogenetic (PD) diversity of Trichoptera assemblages in the Mondego River Basin, Portugal. Using a long-term dataset from 32 stream sites, we applied linear mixed models, variation partitioning, and RLQ analysis to evaluate how these biodiversity facets respond to water quality, habitat structure, and hydroclimatic variability. Environmental gradients, particularly electrical conductivity and habitat quality (HQA), were the primary drivers of community structure across all dimensions. However, we observed a decoupling in sensitivity: TD responded more strongly and immediately to local habitat degradation and ionic stress, reflecting rapid species turnover. In contrast, FD and PD exhibited more buffered responses, suggesting that ecological roles and evolutionary lineages are maintained by conserved trait syndromes despite shifts in species identity. Our findings demonstrate that examining a single biodiversity dimension is insufficient to capture the complexity of community dynamics. We highlight the resilience of functional and evolutionary structures in the face of species loss and reinforce the need for integrative, multidimensional approaches in long-term biomonitoring to detect early signals of environmental erosion in lotic ecosystems.

Keywords: Caddisflies, Multiple stressors, Temporal dynamics, Functional traits, Evolutionary constraints, Stream ecology

Introduction

Aquatic ecosystems are among the most biodiverse and threatened environments in the world (Dudgeon et al., 2006; Young et al., 2016). Recent evidence shows that freshwater biodiversity is changing over time, but these changes are not unidirectional. On the one hand, several studies document biodiversity losses, including reductions in taxonomic richness (Stepanian et al., 2020; Houghton et al., 2021; Houghton et al., 2023), abundance (Baranov et al., 2020), and shifts in the functional and phylogenetic structure of aquatic assemblages (Greenop et al., 2021; Saito et al., 2016) as well as declines in biological quality as assessed by multimetric biotic indices (e. g., Feio et al., 2022). On the other hand, increases in richness and abundance have also been reported, particularly at local or regional scales (Haase et al., 2019; Pillotto et al., 2020; Haubrock et al., 2023). These contrasting trends highlight that biodiversity responses depend

strongly on environmental context and change over time, making broad generalizations difficult. Multiple drivers, including land-use intensification, hydromorphological alteration, pollution, and climate change, act simultaneously but heterogeneously across landscapes, driving temporal reorganization of communities (Baranov et al., 2020; Sinclair et al., 2024). This complexity underscores the need for integrative approaches that capture how communities change through time across multiple biodiversity dimensions.

Beyond changes in taxonomic composition, freshwater communities also reorganize functionally and phylogenetically over time and along environmental gradients. These biodiversity dimensions may respond independently of species richness and abundance (Webb et al., 2002; Cadotte et al., 2011; Mouillot et al., 2013). Early conceptual work revealed that many traits of stream insects exhibit phylogenetic signal, indicating that environmental filtering can simultaneously reshape trait composition and evolutionary structure (Poff et al., 2006). Functional traits reflect species' tolerance to habitat alteration, chemical stressors and land-use gradients, often capturing ecological adjustments that taxonomic metrics alone do not detect (Statzner & Bêche, 2010). Importantly, these dimensions differ in their temporal responsiveness: trait composition often responds rapidly to environmental variation, whereas phylogenetic structure reflects deeper evolutionary constraints (Silva et al., 2017; Tonkin et al., 2018; Sarremejane et al., 2020). Recent studies further show that functional diversity tends to track environmental change more closely over time, while phylogenetic diversity exhibits slower or non-linear dynamics (Zhang et al., 2023). Together, this suggests that different biodiversity dimensions capture complementary aspects of community reorganization under ongoing environmental change.

Despite these advances, long-term studies integrating taxonomic, functional, and phylogenetic dimensions remain scarce. Most research focuses either on spatial gradients or short-term compositional changes, leaving unresolved how these biodiversity facets interact through time under continuous environmental pressure (Brown et al., 2018; Tonkin et al., 2017). Evidence suggests that multidimensional responses can diverge, with functional shifts often preceding phylogenetic restructuring, while taxonomic patterns capture only part of the underlying ecological dynamics (Fontana et al., 2022; Mouillot et al., 2023). This gap is particularly critical in systems exposed to cumulative human impacts, where long-term approaches are necessary to detect subtle ecological changes and progressive lineage reorganization (Suding et al., 2008; Poff et al., 2010). For instance, communities may undergo substantial temporal reorganization without consistent declines in taxonomic richness (e.g., Dornelas et al., 2014; Hillebrand et al., 2017), masking important ecological changes when relying solely on traditional diversity metrics.

Aquatic insects, and particularly Trichoptera, provide a powerful model to investigate these multidimensional and temporal dynamics. Caddisflies are taxonomically diverse, functionally

heterogeneous, and sensitive to variation in temperature, habitat quality, oxygen availability, hydrological stability, and land use (Feio et al., 2005; Morse et al., 2019; Menegat et al., 2025). Their larval traits, including body size, feeding group, respiration mode, case-building behavior, and voltinism, respond to environmental gradients (Milesi et al., 2016), making them suitable for tracking ecological change over time. Similar patterns have been documented across aquatic insect groups, where functional traits respond rapidly to environmental disturbance while phylogenetic structure reflects deeper constraints (e.g., Poff et al., 2006; Statzner & Bêche, 2010). Because these traits exhibit varying degrees of phylogenetic signal (Menegat et al., 2026), Trichoptera offer a unique opportunity to link functional responses to their evolutionary context.

We investigate these patterns in the Mondego River Basin, a watershed that supports water supply, agriculture, and industry (Alves et al., 2002; APA, 2015) and has undergone substantial anthropogenic change over recent decades. These include the expansion of *Eucalyptus* plantations, urbanization, industrial development, and hydromorphological alteration (Feio et al., 2006; Feio et al., 2010). Together, these pressures have created a heterogeneous and dynamic environmental mosaic across space and time. Understanding how aquatic communities respond to this environmental heterogeneity is essential for predicting future impacts and improving biomonitoring strategies.

We assessed how the taxonomic, functional, and phylogenetic structure of Trichoptera assemblages varies over time (2001–2019) and along environmental gradients in streams of the Mondego River Basin. We expected contrasting responses among biodiversity dimensions. Specifically, we predicted that: (i) functional diversity would show stronger and more consistent associations with environmental gradients; (ii) phylogenetic structure would exhibit more constrained responses due to evolutionary limitations; and (iii) taxonomic patterns would capture broader variation but differ in sensitivity relative to functional and phylogenetic dimensions. Additionally, we expected environmental variables to explain more variation in functional diversity, whereas temporal dynamics would play a greater role in structuring taxonomic and phylogenetic patterns.

Methodology

Study area and sampling design

The Mondego River Basin (between 39°46′ and 40°48′ N, and between 7°14′ and 8°52′ W), located in central Portugal, is the largest river basin entirely within Portuguese territory, covering an area of approximately 6,670 km². The watershed stretches from the mountainous

regions of the upper and middle reaches, where its source is in the Serra da Estrela, with altitudes reaching up to 2,000 meters, to the vast alluvial plain where the river flows into the Atlantic Ocean at Figueira da Foz (Marques et al., 2002). Throughout the Mondego River Basin, diverse topography and climatic conditions support a wide range of environmental settings. Detailed information on the basin's geology, hydrology, and biology can be found in Pardal et al. (2002).

We conducted the study using a historical-contemporary dataset comprising 32 sites across the Mondego River Basin (Feio et al., 2005; Feio et al., 2006; Feio et al., 2007; Feio et al., 2009; Feio et al., 2010) (Fig. 1). The dataset spans a 19-year period, including the years 2001, 2004, 2005, 2007, 2011, 2012, 2017, and 2019. Although not all dates were available for all the sites, resulting in different time series for each site, ranging from 3 to 7 years, depending on the sampling site.

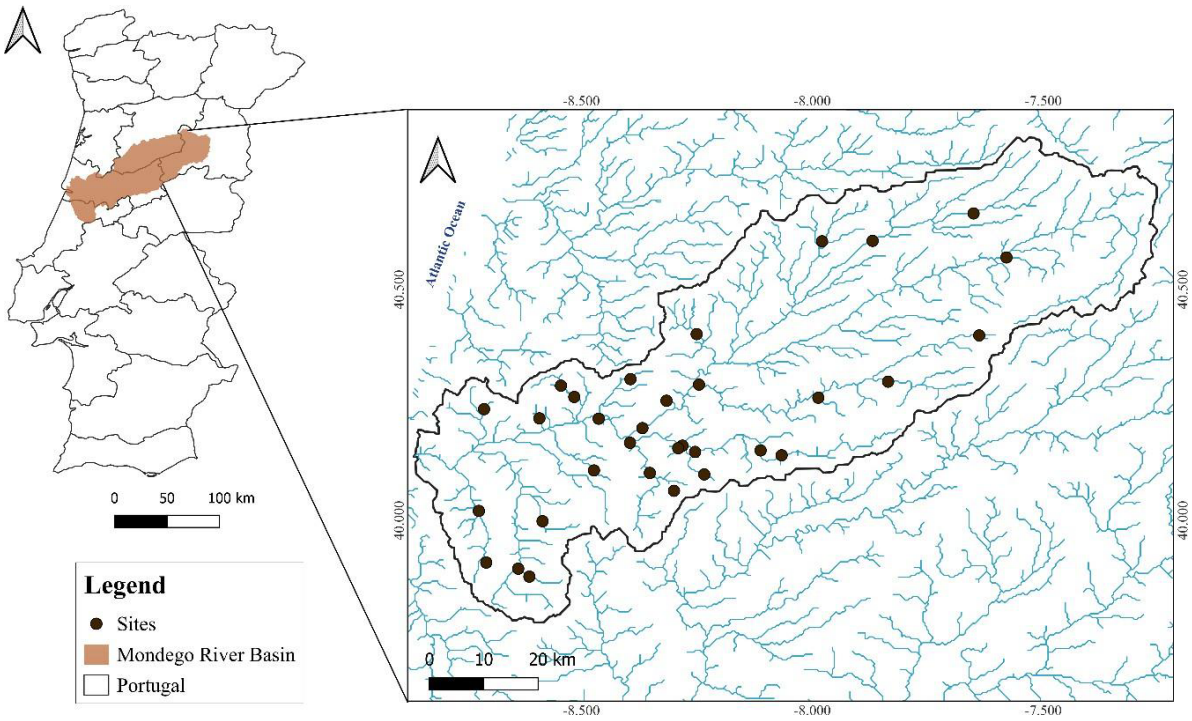


Fig. 1. Location of sites sampled in Mondego River Basin, Portugal.

Environmental variables

We measured water temperature (°C), electrical conductivity (µS/cm), dissolved oxygen (mg/L), pH in situ using portable meters (HANNA multiparameter probe HI 9812-5 and oxygen probe HI98193). We measured alkalinity (mg/L) using the volumetric method according to ISO 9963-1 (ISO, 1994; Feio et al., 2010). We extracted distance to source (km) along the stream network and altitude (m a.s.l.) for each sampling site from cartographic data (1:25,000 digital military maps – Instituto Geográfico do Exército, Portugal; Atlas do Ambiente; Corine Land Cover

1990). We classified the rivers according to their typology (INAG 2008). We assessed hydromorphological quality using the Habitat Modification Score (HMS) and the Habitat Quality Assessment (HQA), following the River Habitat Survey (RHS) methodology (Raven et al., 1997; 2002; Fox et al., 1998). HQA reflects habitat diversity and the presence of natural features, whereas HMS quantifies the extent of artificial modification and anthropogenic disturbance (Raven et al., 1998, 2002). We obtained mean annual and monthly (collection-month) air temperature (°C) and precipitation (mm) from the Instituto Português do Mar e da Atmosfera (IPMA).

Biological Sampling

We collected aquatic insects with a kick net (0.5 mm mesh size; 0.25 × 0.25 m opening). We sampled all representative habitats by kicking upstream over a 6 × 1 m area, following the official Portuguese protocol for benthic macroinvertebrates (INAG, 2008). We sampled blocks, stones, gravel, sand, rifles, pools, macrophyte and underwater roots or deposits of coarse organic matter. We preserved the insects in 70% ethanol and later sorted and identified them in the laboratory. We identified Trichoptera to the genus level using the key by Vieira-Lanero (2004).

Functional Traits

We used 12 functional traits (i.e., body size, voltinism, respiration, locomotion, feeding group, reproduction, construction behavior, dispersal strategy, adult life span, lifelong fecundity and propensity to drift) distributed across 44 trait categories (Table S1). The fuzzy code information was obtained on Tachet et al. (2010) and Sarremejane et al. (2020). Functional traits were quantified using a fuzzy coding approach, which assigns affinity of each taxon to each category for a trait, ranging from zero (no affinity) to five (strong affinity) (Chevenet et al., 1994).

Phylogeny

We based our reconstruction on the phylogenetic hypothesis of García-Girón et al. (2024) which provides near-complete family-level coverage of global Trichoptera diversity. This backbone was built from DNA sequences of nine independent genes obtained from GenBank (Sayers et al., 2019). Building on this backbone, we first grafted only one genus (*Calamoceras*) that present in our dataset but absent from the published tree as a polytomy at the base of its corresponding family using *bind.tip* function, and then pruned genera not in our dataset using *drop.tip* function in the 'ape' package (Paradis et al*.,* 2020). This process generated our phylogenetic tree of Trichoptera genera.

Diversity metrics

We calculated the Taxonomic Diversity (TD) through the Shannon-Wiener diversity index (H') (Log Base 2). The index considers the number of species in assemblage (richness) and their relative abundance (evenness), i.e., consider the proportional abundance of species when estimating diversity. We calculated TD using *diversity* function from R package 'vegan' (Oksanen, 2022).

We calculated the Phylogenetic Diversity (PD) of Trichoptera assemblages through Faith's PD, which considers the sum of branch lengths connecting species across the phylogenetic tree (Faith, 1992). For comparison purposes, we calculated the Functional Diversity (FD) using the total branch length of a functional dendrogram (Petchey and Gaston, 2002). For this, we used Euclidian distance to extract the functional distance matrix (dissimilarity between all pairs of species), then we transformed it into a dendrogram using the unweighted pair group method with arithmetic mean (UPGMA). We calculated FD and PD using the *pd* function from R package 'picante' (Kembel et al., 2020).

Statistical Analyses

We evaluated interannual variation in environmental conditions using Principal Component Analysis (PCA) based on standardized limnological, climatic, habitat, and spatial variables. PCA was performed using the *PCA*::FactoMineR, and the first two axes were retained for interpretation based on eigenvalues and the proportion of variance explained. To formally test whether multivariate environmental conditions differed among sampling years, we applied a permutational multivariate analysis of variance (PERMANOVA) using Euclidean distances and 999 permutations, implemented with the *adonis2* function in the 'vegan' package.

To evaluate how environmental and spatial predictors influence diversity metrics, that is, TD, FD e PD of Trichoptera assemblages, we fitted Linear Mixed Models (LMMs) using the *lmer* function from the 'lme4' package. Prior to modeling, we standardized all environmental predictors with *scale* function. We assessed variance inflation factors (VIF) and excluded alkalinity due to collinearity with electrical conductivity. Final models included spatial (altitude, distance from source, HQA and HMS), physical and chemical variables (water temperature, dissolved oxygen, pH and electrical conductivity), climatic (air temperature and precipitation) and time (year) as fixed effects. We included sampling sites as random intercepts in all models to account for the hierarchical structure of the data and for repeated measurements across space.

We evaluated the independent and shared contributions of environmental variables and sampling year to taxonomic, functional and phylogenetic diversity using variation partitioning. We grouped all physical, chemical, climatic and habitat descriptors as environmental predictors

and treated sampling year as a categorical temporal factor. For each diversity metric, we partitioned the explained variance into unique environmental, unique temporal and shared fractions using the function *varpart* from the 'vegan' package. We tested the statistical significance of the environmental and temporal components using permutation-based ANOVA (999 permutations) implemented with the *anova.cca* function, based on Redundancy Analysis (RDA). We used RLQ analysis to investigate the relationships between environmental variables, species composition, and functional traits of Trichoptera assemblages. This approach integrates three matrices: R (environmental variables), L (species abundance), and Q (species traits), through a double-inertia analysis (Dolédec et al., 1996). To test the significance of trait-environment associations at the level of individual variables, we performed a fourth-corner analysis using the *fourthcorner* function in the 'ade4' package. We applied model 6 (Dray et al., 2014), which combines permutations of sites (model 2) and species (model 4), providing a robust test of the relationship between trait categories and environmental variables. Statistical significance was assessed using 999 permutations.

Results

The environmental conditions differed significantly among sampling years ($F_{(7;103)} = 7.9$, $p = 0.001$, $R^2 = 0.35$). The ordination analysis summarized environmental variation across sampling years, with the first two principal components explaining 39.6% of the total variance (Fig. 2). The first principal component (PC1 = 24.3%) represented a gradient primarily driven by electrical conductivity, alkalinity, and pH, opposing altitude and distance to source. The second principal component (PC2 = 15.3%) captured a hydroclimatic gradient, positively associated with precipitation and water temperature, and secondarily with HQA, while negatively associated with dissolved oxygen. Later years (2012, 2017, and 2019) were predominantly distributed along positive values of PC1, reflecting conditions characterized by higher electrical conductivity, alkalinity, and pH. In contrast, early sampling years (2001 and 2004) were more associated with positive values of PC2, corresponding to periods of higher precipitation, water temperature and HQA.

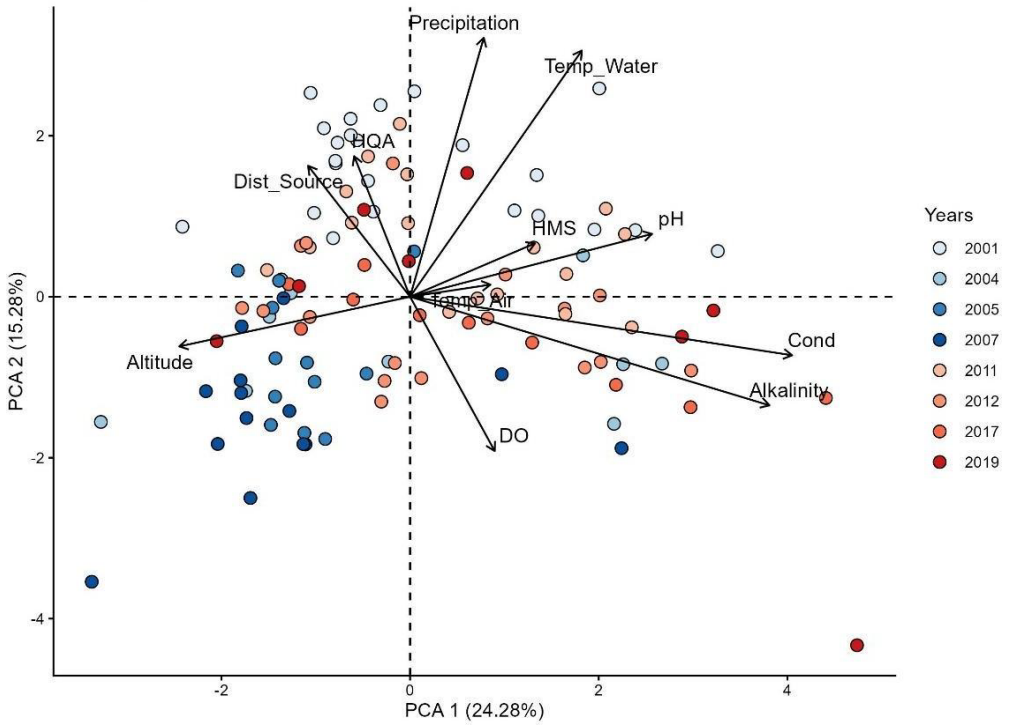


Fig. 2. Principal Component Analysis (PCA) of environmental variables across the temporal series (2001–2019) in streams of the Mondego River basin, Coimbra, Portugal. Points represent samples, colored by sampling year. Arrows indicate the variables, include precipitation, water temperature (Temp_Water), air temperature (Temp_Air), electrical conductivity (Cond), pH, dissolved oxygen (DO), Alkalinity, Habitat Modification Score (HMS), Habitat Quality Assessment (HQA), distance source (Dist_Source) and altitude.

A total of 7,441 individuals of Trichoptera, distributed across 19 families and 49 genera were collected over the years (Table S2). The genera *Hydropsyche* (34.6% of total) and *Chimarra* (24% of total) were the most abundant, with their presence consistently recorded across all sampling years. In addition to these, only *Rhyacophila* and *Agapetus* were observed in every year of the study. While *Allotrichia*, *Orthotrichia*, *Ptilocolepus, Trianodes*, *Helicopsyche*, *Notidobia*, *Baraeodes, Odontocerum, Goera, Philopotamus* and *Metalype* represent rare genera, with occurrences recorded with fewer than 10 individuals over the study period.

The TD was positively associated with dissolved oxygen ($p = 0.039$; Table 1) and habitat quality (HQA; $p = 0.007$) and negatively associated with electrical conductivity ($p = 0.011$; Table 1). FD e PD showing a significant negative association with conductivity ($p = 0.015$; $p = 0.011$, respectively; Table 1) and positively associated with HQA ($p = 0.057$; $p = 0.043$, respectively; Table 1).

Table 1. Results of Linear Mixed Models (LMM) evaluating the effects of environmental predictors on taxonomic (TD), functional (FD) and phylogenetic (PD) diversity. Estimates, standard errors (SE), t values and p values are shown. Significant effects are indicated in bold.

	Predictor	Estimate	t value	p value	Variance	Std. Dev
TD ~	(Intercept)	11.431	0.433	0.665		
	Year	-0.005	-0.400	0.689		
	Altitude	0.057	0.568	0.571		
	Dist_Source	-0.157	-1.685	0.095		
	Temp_ Water	0.070	0.891	0.374		
	DO	0.113	2.083	**0.039**		
	pH	0.009	0.130	0.896		
	Cond	-0.254	-2.587	**0.011**		
	Temp_Air	0.062	0.996	0.321		
	Precipitation	0.038	0.512	0.609		
	HQA	0.185	2.750	**0.007**		
	HMS	-0.044	-0.559	0.578		
	Sites				0.1337	0.3656
	Residual				0.2513	0.5013
FD ~						
	(Intercept)	14.794	0.616	0.538		
	Year	-0.006	-0.581	0.562		
	Altitude	0.035	0.390	0.697		
	Dist_Source	-0.131	-1.555	0.122		
	Temp_Water	0.045	0.641	0.522		
	DO	0.059	1.180	0.240		
	pH	-0.008	-0.133	0.894		
	Cond	-0.221	-2.468	**0.015**		
	Temp_Air	0.092	1.628	0.106		
	Precipitation	0.072	1.058	0.292		
	HQA	0.118	1.922	**0.057**		
	HMS	<0.001	-0.005	0.995		
	Sites				0.1120	0.3347
	Residual				0.2071	0.4550
PD ~						
	(Intercept)	12965.719	0.541	0.589		
	Year	-6.047	-0.506	0.613		
	Altitude	9.860	0.104	0.916		
	Dist_Source	-152.537	-1.755	0.082		
	Temp_Water	48.742	0.689	0.492		
	DO	52.945	1.070	0.287		
	pH	-14.995	-0.221	0.825		
	Cond	-234.231	-2.567	**0.011**		
	Temp_Air	84.023	1.494	0.138		
	Precipitation	66.252	0.967	0.335		
	HQA	126.177	2.048	**0.043**		
	HMS	4.376	0.059	0.952		
	Sites				123803	351.9
	Residual				203862	451.5

In relation to variation partitioning across taxonomic, functional, and phylogenetic diversity metrics, the pure environmental component explained the largest fraction of variation, accounting for 32.2% of taxonomic diversity, 26.7% of functional diversity, and 27.9% of phylogenetic diversity. In contrast, the unique temporal fraction explained substantially less variation (TD: 3.98%; FD: 6.25%; PD: 6.08%). The shared fraction between environmental variables and year was negligible in all cases (Adj. $R^2 \approx 0$), whereas a large proportion of variation remained unexplained (64.64 - 67.08%). Environmental models were significant for taxonomic

($F_{(10;100)}$ = 5.05, p = 0.001), functional ($F_{(10;100)}$ = 4.66, p = 0.001), and phylogenetic diversity ($F_{(10;100)}$ = 4.85, p = 0.001), whereas models including only year were not significant for any diversity dimension (TD: $F_{(1;109)}$ = 0.10, p = 0.759; FD: $F_{(1;109)}$ = 0.23, p = 0.639; PD: $F_{(1;109)}$ = 0.09, p = 0.732).

Trait-environment relationships showed consistent patterns across traits. Smaller-bodied organisms (<0.25–0.50 cm) were generally associated with higher dissolved oxygen, alkalinity and electrical conductivity, whereas larger size classes were more related to altitude and temperature gradients (Fig 3). Voltinism varied along these gradients, with univoltine organisms linked to higher altitude and lower temperature, and multivoltine organisms associated with warmer conditions (Fig. 3). Dispersal and life-history traits also varied, with passive aquatic dispersal and higher fecundity associated with warmer and downstream conditions, while other traits were related to dissolved oxygen and habitat integrity (Fig 3).

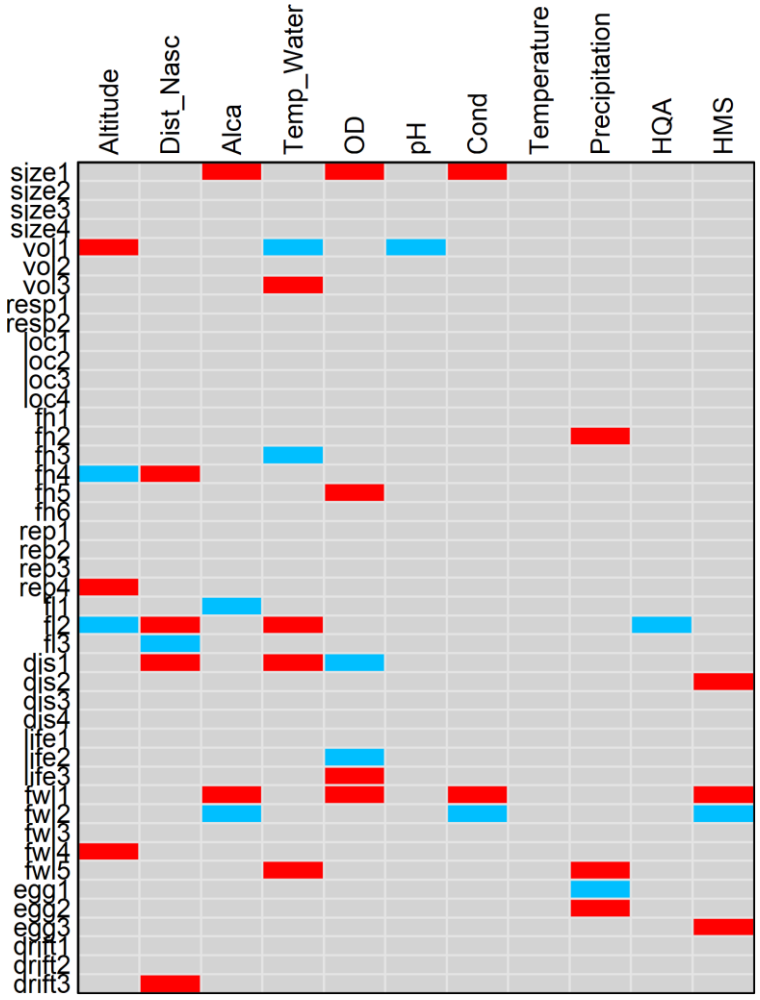


Fig. 3. Fourth-corner associations between environmental variables and functional traits (codes in table 1). Red cells indicate positive relationships, and blue cells indicate negative relationships. Only significant associations after permutation tests (n = 999) are shown.

Discussion

Our results show that environmental gradients related to water physical and habitat quality exert a dominant influence on taxonomic diversity, whereas temporal variability explains a larger share of functional and phylogenetic variation. This pattern directly does not support our hypothesis of consistency among diversity metrics. Local environmental filters, particularly habitat quality and physical conditions, act more strongly on species composition, while functional traits and evolutionary lineages respond more gradually to temporal processes and historical constraints. Importantly, these environmental gradients are themselves structured along both spatial (e.g., longitudinal position and catchment characteristics) and temporal axes, meaning that environmental filtering is intrinsically embedded within spatial and interannual variability rather than acting independently of them. This decoupling between taxonomic and higher-level diversity dimensions is consistent with previous long-term studies. For example, local environmental conditions primarily structure species occurrence, whereas trait and phylogenetic responses integrate temporal variability and legacy effects (Haase et al., 2019; Baranov et al., 2020; Haubrock et al., 2023). More broadly, studies in freshwater ecosystems indicate that community responses to environmental change are often complex and non-linear. These findings reinforce that taxonomic, functional, and phylogenetic patterns may diverge depending on the diversity metric and the temporal and spatial scale considered (e.g., Baranov et al., 2020; Tonkin et al., 2021).

Our results therefore do not contradict evidence of diversity decoupling, but rather demonstrate that, even within a single order and under an expectedly constrained phylogenetic and functional context, subtle divergence among taxonomic, functional, and phylogenetic responses can still be detected, particularly in relation to temporal and environmental filtering. Linked to this, the strong correlations observed among taxonomic, functional, and phylogenetic diversity in this study can be partially attributed to the taxonomic scope adopted. By focusing on single insect order (Trichoptera), taxa share a narrower evolutionary background, which promotes phenotypic similarity through the conservation of functional traits (Adams, 2014). Under such conditions, functional and phylogenetic diversity are expected to covary more tightly with species diversity, as conserved traits reinforce the linkage between lineage history and ecological strategies (Adams, 2014). This pattern contrasts with several long-term and large-scale studies reporting pronounced decoupling among diversity dimensions when taxonomic breadth increases, environmental gradients intensify, or anthropogenic pressures selectively filter traits and lineages (Mouchet et al., 2010; Villéger et al., 2013; Tonkin et al., 2017; Heino et al., 2020).

Functional trait-environment relationships showed that body size, voltinism, wing length and reproduction were primarily associated with short-term climatic and hydrological variables, particularly water temperature and precipitation. Multivoltine organisms and larger female wing

sizes were positively related to warmer and wetter conditions, while fecundity classes tracked precipitation gradients. In contrast, traits related to respiration, feeding strategy, construction behavior, dispersal mode and drift propensity were structured along more stable environmental axes, including channel position, water chemistry and habitat integrity (e.g., dissolved oxygen, alkalinity, electrical conductivity, distance from source, altitude, HQA and HMS). These patterns are consistent with the diversity responses observed, where taxonomic diversity showed stronger responses to environmental degradation, whereas functional and phylogenetic diversity appeared comparatively buffered. This suggests that species replacement may precede functional losses, with conserved traits and shared ancestry maintaining functional roles despite shifts in species composition (Webb et al., 2002; Cavender-Bares et al., 2009; Mouquet et al., 2012; Menegat et al., 2026). For example, variation in habitat quality and modification (HQA and HMS) was associated with shifts in traits related to construction behavior and dispersal, indicating that changes in physical habitat structure are accompanied by adjustments in how organisms exploit and move within the environment. This highlights how trait-environment relationships reflect coupled spatial and temporal gradients, while also revealing specific pathways through which habitat alteration influences community organization.

Among the environmental predictors, electrical conductivity consistently showed as a strong driver of community structure across diversity dimensions. Electrical conductivity is widely recognized as an integrative stressor, reflecting land-use intensification, altered hydrology and increased ionic loads, and has been repeatedly associated with reductions in macroinvertebrate richness and shifts toward tolerant trait syndromes (Fanelli et al., 2024). As such, conductivity integrates cumulative land-use effects that vary spatially across the catchment and manifest temporally through persistent or episodic changes in hydrological conditions (Allan, 2004; Bernhardt et al., 2017). Similar patterns have been reported in long-term studies, where taxonomic diversity responds strongly to persistent environmental stressors such as nutrient enrichment, temperature increase, or hydrological alteration, often showing directional changes in community composition over time (e.g., Haase et al., 2019; Baranov et al., 2020). In these cases, environmental variables act as integrative filters, even when different predictors are considered (Tonkin et al., 2018). The congruent response observed here across taxonomic, functional and phylogenetic metrics suggests that ionic stress acts as a pervasive environmental filter, simplifying assemblage structure while favoring conserved lineages and redundant trait combinations. This reinforces the interpretation that gradients associated with long-term catchment alteration structure communities through persistent filters acting on conserved ecological strategies, rather than inducing rapid reorganization of functional or evolutionary structure (Poff, 1997; Haddad et al., 2015; Zhang et al., 2023).

Taking together, our results demonstrate that temporal changes in stream insect communities cannot be fully understood by examining a single dimension of biodiversity only. Instead, the combined analysis of taxonomic, functional and phylogenetic facets reveals how environmental filtering, climatic variability and evolutionary constraints interact to shape community dynamics through time. Fundamentally, this interaction operates through environmental gradients that are spatially structured and temporally dynamic, highlighting the need to interpret environmental predictors as integrative drivers acting across scales. The stronger and more immediate responses of taxonomic diversity reflect species-level turnover driven by habitat degradation and water-quality gradients. Conversely, the buffered responses of functional and phylogenetic facets indicate that ecological roles and evolutionary lineages are maintained by conserved trait syndromes, even as species identities change. At the same time, the responsiveness of evolutionarily labile traits to climatic and hydrological variability suggests a pathway through which environmental signals may be first detected in trait composition, potentially before propagating to deeper functional or phylogenetic erosion (Mouillot et al., 2013; Zhang et al., 2023). By linking trait-environment relationships to phylogenetic structure, this study advances the understanding of how multiple dimensions of biodiversity respond to change, reinforcing the value of integrative and phylogenetically informed approaches in biomonitoring.

Conclusion

This study shows that environmental degradation and temporal variability affect biodiversity dimensions in distinct yet complementary ways in stream ecosystems. Taxonomic diversity responds strongly to local habitat quality and physicochemical stressors, whereas functional and phylogenetic diversity are more constrained by evolutionary history and vary more gradually over time The presence of both evolutionarily labile and conserved traits suggests that assemblages can partially track environmental fluctuations through species turnover. This turnover, however, does not necessarily translate into an immediate loss of broader functional roles or evolutionary lineages, indicating a degree of structural resilience in these communities. These findings highlight the importance of integrative frameworks that jointly consider taxonomic, functional and phylogenetic dimensions, particularly in long-term datasets. Such approaches improve ecological inference and help anticipate how freshwater communities respond to ongoing climatic and anthropogenic pressures. Long-term studies spanning multiple decades are essential for understanding the dynamics and resilience of aquatic biodiversity.

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Supplementary Materials

Table S1. Functional trait, categories and their respective code.

Trait	Trait category	Code
Body size (cm)	≤ 0.25_0.5	size1
	0.5_1.0	size2
	1.0_2.0	size3
	2.0_4.0	size4
Voltinism	≤1	vol1
	≥1	vol2
Respiration	Skin	resp1
	Branchial	resp2
Locomotion	Swimmer	loc1
	Crawler	loc2
	Burrower	loc3
	Fixation	loc4
Feeding groups	Collector	fh1
	Shredder	fh2
	Scraper	fh3
	Filterer	fh4
	Piercer	fh5
	Predator	fh6
Reproduction	Eggs	rep1
	Fixed clutches	rep2
	Free clutches	rep3
	Terrestrial clutches	rep4
Construction behavior	Free living	fl1
	Silk net builder	fl2
	Case builder	fl3
Dispersal strategy	Aquatic Passive	dis1
	Aquatic Active	dis2
	Aerial Passive	dis3
	Aerial Active	dis4
Adult life span	< 1 week	life1
	≥ 1 week – 1 month	life2
	≥ 1 month – 1 year	life3
Female wing length (mm)	< 5	fwl1
	≥ 5–10	fwl2
	≥ 10–15	fwl3
	≥ 15–20	fwl4
	≥ 20–30	fwl5
Lifelong fecundity	< 100	egg1
	≥ 100–1000	egg2
	≥ 1000–3000	egg3
Propensity to drift	Rare/catastrophic	drift1
	Occasional	drift2
	Frequent	drift3

Table S2. Total abundance of Trichoptera genera per year in the Mondego River Basin (2001-2019).

		Year							
Family	**Genera**	**2001**	**2004**	**2005**	**2007**	**2011**	**2012**	**2017**	**2019**
Baraeidae	*Beraea*	3	0	1	1	26	9	150	0
	Beraeodes	1	0	0	0	0	0	0	0
Brachycentridae	*Brachycentrus*	0	42	0	0	1	0	0	0
	Micrasema	0	1	0	25	0	0	0	6
Calamoceratidae	*Calamoceras*	4	4	3	7	28	14	0	0
Ecnomidae	*Ecnomus*	24	0	0	0	0	0	0	0
Glossosomatidae	*Agapetus*	1	9	8	23	8	54	66	29
	Catagapetus	0	1	2	20	2	13	0	0
	Glossosoma	32	1	31	7	12	1	16	0
	Synagapetus	0	11	0	40	104	10	2	0
Goeridae	*Goera*	4	0	0	0	0	0	0	4
	Larcasia	5	4	0	51	0	13	0	30
Helicopsychidae	*Helicopsyche*	4	0	0	1	0	0	0	0
Hydropsychidae	*Cheumatopsyche*	100	12	166	0	12	192	62	52
	Diplectrona	17	0	0	0	0	0	0	0
	Hydropsyche	850	133	146	155	387	500	345	86
Hydroptilidae	*Allotrichia*	0	0	0	0	0	1	0	0
	Hydroptila	4	124	10	0	72	57	48	38
	Orthotrichia	3	0	0	0	0	0	0	0
	Oxyethira	0	0	0	0	1	6	2	18
Ptilocolepidae	*Ptilocolepus*	0	2	0	0	0	0	0	0
Lepidostomatidae	*Lepidostoma*	72	20	26	12	20	28	44	0
Leptoceridae	*Adicella*	5	2	0	0	3	4	0	0
	Athripsodes	2	10	0	0	0	1	4	2
	Ceraclea	0	1	25	0	2	2	0	0
	Mystacides	12	0	0	0	3	0	0	0
	Oecetis	2	0	1	0	10	23	9	13
	Setodes	1	0	1	0	6	0	31	36
	Triaenodes	0	2	0	0	0	0	0	0
Limnephilidae	*Allogamus*	4	1	4	3	29	0	9	12
	Limnephilus	11	2	0	38	50	8	1	36
	Stenophylax	22	0	0	0	0	0	0	4
Odontoceridae	*Odontocerum*	0	1	0	0	0	0	0	2
Philopotamidae	*Chimarra*	148	56	144	3	31	580	111	11
	Philopotamus	0	0	0	1	6	0	0	0
	Wormaldia	2	0	2	0	13	4	3	0
Phryganeidae	*Phryganea*	22	0	0	0	0	0	0	0
Polycentropodidae	*Cyrnus*	15	0	0	0	0	0	0	0
	Plectrocnemia	12	28	8	0	38	0	13	2
	Polycentropus	59	81	24	0	11	54	46	2
Psychomyiidae	*Lype*	3	0	1	0	9	9	2	2
	Metalype	1	0	0	0	2	0	1	0
	Psychomyia	7	0	6	0	2	8	3	4
	Tinodes	27	0	0	0	0	0	1	1
Rhyacophilidae	*Rhyacophila*	35	28	21	12	33	48	57	34
Sericostomatidae	*Notidobia*	16	0	0	0	0	0	0	0
	Schizopelex	0	1	5	0	0	10	0	0
	Sericostoma	129	11	0	14	25	19	5	0
Uenoidae	*Thremma*	2	3	0	0	10	13	45	4

CONSIDERAÇÕES FINAIS E PERSPECTIVAS

Nesta tese, investiguei como atributos funcionais e história evolutiva interagem para moldar a resposta das comunidades às pressões ambientais. Ao integrar diferentes escalas, demonstrei que a resposta das comunidades às pressões ambientais não é limitada de forma uniforme pela história evolutiva; ela é resultado da interação entre atributos com diferentes graus de conservadorismo. Especificamente, a organização da estrutura funcional das comunidades é predominantemente conduzida por atributos evolutivamente lábeis, que respondem diretamente aos gradientes ambientais, enquanto atributos conservados permanecem associados à estrutura filogenética das assembleias.

No **Capítulo 1**, evidenciei que atributos como voltinismo, formato e tamanho do corpo são evolutivamente lábeis. Ao focar em uma única ordem, este resultado indica que, para Trichoptera, esses atributos são menos restringidos pela filogenia, sugerindo maior flexibilidade ao longo da história evolutiva. No **Capítulo 2**, mostrei que atributos lábeis, neste caso, tamanho e formato corporal, quando comparados a atributos filogeneticamente conservados, foram mais sensíveis aos gradientes ambientais, tanto em escala regional (paisagem e impacto) quanto local (velocidade da correnteza). Em conjunto, os capítulos 1 e 2 revelaram que, em Trichoptera, esses atributos sustentam simultaneamente as duas faces da labilidade (baixa restrição filogenética e alta sensibilidade ambiental). Isso sugere que, em um grupo reconhecidamente sensível, condições ambientais específicas podem atuar diretamente sobre atributos lábeis, moldando a estrutura funcional das assembleias. Em contraste, atributos filogeneticamente conservados tendem a responder de forma indireta, frequentemente associados a síndromes funcionais e à identidade taxonômica das assembleias. No **Capítulo 3**, ao mensurar diretamente o tamanho corporal dos organismos, demonstrei, de forma ainda mais clara, o potencial dos atributos lábeis como indicadores ecológicos. Ao evidenciar que o tamanho corporal de Trichoptera respondeu de forma consistente a variações sutis das concentrações de nutrientes inorgânicos, em escala geográfica restrita, este capítulo reforçou o papel de atributos relativamente simples de medir, mas altamente informativos, como indicadores precoces de reorganização funcional das comunidades. No **Capítulo 4**, por fim, apresentei um estudo considerando uma escala temporal de 19 anos. Demonstrei que os componentes da diversidade, taxonômico, funcional e filogenético, responderam de forma complementar ao espaço e tempo. Esses resultados ressaltam a importância de abordagens integrativas baseadas em séries temporais para compreender a resiliência e a estabilidade taxonômica, funcional e filogenética das comunidades aquáticas frente a pressões ambientais.

Em conjunto, esta Tese destaca:

i) Compreender sobre a labilidade e conservadorismo dos atributos é fundamental para interpretar respostas ecológicas e melhorar a compreensão sobre a organização das comunidades de insetos aquáticos. A integração entre atributos, história evolutiva e múltiplas abordagens de análise, não apenas avança o entendimento teórico sobre os mecanismos que estruturam comunidades biológicas, mas também oferece aplicações práticas relevantes para biomonitoramento de ecossistemas aquáticos. Em um cenário de mudanças climáticas e intensificação de impactos antrópicos, abordagens que considerem a biodiversidade de forma multidimensional são essenciais para a manutenção do funcionamento e serviços ecossistêmicos associados e, em última instância, a saúde integrada dos sistemas naturais e humanos.

ii) A escala local é extremamente importante para comunidades de insetos aquáticos, como evidenciamos, particularmente, para a ordem Trichoptera. Análises a partir de diferentes abordagens e contextos ambientais demonstraram que Trichoptera responde taxonômica, funcional e filogeneticamente a variáveis limnológicas, sobretudo aquelas relacionadas a contextos de perturbação e/ou stress ambiental, como amônia, fósforo, velocidade da correnteza e condutividade elétrica.

iii) Ao integrar atributos, história evolutiva e escalas espaciais e temporais em Trichoptera, esta Tese avança o estado da arte e explora uma lacuna importante ao investigar o sinal filogenético dentro de uma única ordem. Ao fazer isso, (i) explicito quais atributos são mais evolutivamente lábeis nesse grupo, evidenciando baixa restrição filogenética mesmo em um clado relativamente restrito; (ii) demonstro que tamanho e formato do corpo sustentam as duas faces da labilidade, sendo simultaneamente pouco conservados filogeneticamente e altamente responsivos a gradientes ambientais; e (iii) evidencio que componentes taxonômicos, funcionais e filogenéticos capturam dimensões distintas, porém complementares, da resposta das comunidades de insetos aquáticos a um ambiente em rápida transformação.

A partir desta Tese, algumas **perspectivas** para pesquisas futuras surgiram:

i) Em primeiro lugar, ampliar a análise dos padrões de evolução de atributos funcionais para outros grupos de insetos aquáticos e para diferentes regiões biogeográficas permitiria testar se o padrão de labilidade evolutiva ou

conservadorismo filogenético de determinados atributos constitui um padrão geral ou dependente do contexto histórico-ambiental. Comparações entre regiões com histórias evolutivas e regimes ambientais contrastantes, como Neotrópicos, Paleotrópicos e zonas temperadas, já demonstraram convergência de perfis funcionais em insetos de riachos e restrições filogenéticas variáveis entre atributos (Poff et al., 2006; Wilkes et al., 2020). Nesse cenário, estender a abordagem aqui adotada poderia revelar em que medida o conservadorismo e a labilidade funcional observados para Trichoptera são replicáveis em outros clados ou resultam de trajetórias históricas particulares.

ii) Em segundo lugar, incorporar a dimensão temporal representa importante passo para compreender a estabilidade e a reconfiguração de estratégias funcionais. Abordagens baseadas em atributos têm mostrado que a composição funcional pode responder de forma mais sensível e consistente aos gradientes de perturbação ambiental do que a composição puramente taxonômica (Hepp et al., 2023). Isso abre espaço para avaliar se certos atributos, em especial o tamanho corporal, frequentemente associado à resposta de insetos aquáticos frente a perturbações ambientais (Milesi et al., 2016; Hepp et al., 2023), funcionam como sinais precoces de alteração ambiental, variando antes que ocorram mudanças marcantes na composição taxonômica. A exploração de séries temporais, dados de monitoramento e coleções biológicas, em recortes hierárquicos (por exemplo, dentro de ordens, famílias ou gêneros), permitiria investigar se a distribuição de tamanho corporal, e de outros atributos (potencialmente lábeis), se antecipam ao turnover taxonômico e como essas respostas se acumulam ao longo do tempo (Milesi et al., 2019).

iii) Por fim, talvez a implicação mais abrangente desta tese seja ressaltar a importância de avanços no conhecimento filogenético e funcional dos insetos aquáticos neotropicais. A disponibilidade recente de filogenias calibradas no tempo para grandes clados de insetos aquáticos (Thomas et al., 2020; Garcia-Girón et al., 2024), que já têm sido usadas para integrar diversidade filogenética, história biogeográfica e evolução de atributos, mostra o potencial desse arcabouço. Investimentos em filogenias mais completas e bem resolvidas para linhagens neotropicais, aliados ao refinamento e à ampliação de bases de dados funcionais (por exemplo, Santos et al., 2024), são, portanto, essenciais para conectar, de forma robusta, sinal filogenético e respostas ecológicas a um ambiente em rápida transformação. Assim, ao propor a integração entre esforços ecológicos, evolutivos

e sistemáticos, esta tese aponta para pesquisas futuras que devem elucidar como a história evolutiva molda não apenas a diversidade que observamos hoje, mas também a capacidade das comunidades aquáticas de persistir, adaptar-se ou se reorganizar diante das mudanças globais.

Finalmente, a aplicação de abordagens multidimensionais nesta tese evidenciou que lacunas funcionais e filogenéticas são pronunciadas na região Neotropical. Ao reconstruir a árvore filogenética para a Bacia do Rio Mondego (região Temperada), foi necessário inserir apenas um único gênero como politomia. Em contraste, ao aplicar essa mesma abordagem ao contexto brasileiro (região Neotropical), a carência de dados moleculares para gêneros endêmicos da região Neotropical demandou a inserção de 14 politomias. Essa lacuna estende-se ao componente funcional: enquanto os gêneros europeus apresentam cobertura mais completa em bases de dados consolidadas, as informações disponíveis para a região Neotropical permanecem escassas e fragmentadas, apesar de esforços importantes na literatura. Portanto, o preenchimento dessas lacunas taxonômicas, funcionais e filogenéticas é imprescindível para que a ecologia avance em direção a uma ciência global e comparativa, permitindo avaliações mais precisas sobre a resiliência da biodiversidade aquática em um cenário de rápidas mudanças ambientais.

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ANEXOS

Artigo publicado que compõe a Tese:

1. **MENEGAT, MARIANA NUNES**; MILESI, SILVIA VENDRUSCOLO; HEPP, LUIZ UBIRATAN. From Lability to Conservatism: Evolutionary Patterns in the Traits of Stream Trichoptera. FRESHWATER BIOLOGY, v. 71: e70229, p. 1 – 11, 2026. Doi: https://doi.org/10.1111/fwb.70229



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From Lability to Conservatism: Evolutionary Patterns in the Traits of Stream Trichoptera

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Dear Ms. Menegat,

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Artigos publicados durante o processo de doutoramento que não compõem a Tese (abril/2022 - maio/2026):

1. **MENEGAT, MARIANA NUNES**; RESTELLO, ROZANE MARIA; MILESI, SILVIA VENDRUSCOLO; HEPP, LUIZ UBIRATAN. Which is more important for stream caddisfly colonization: Substrate heterogeneity or food resources? ECOHYDROLOGY & HYDROBIOLOGY, v. 25, p. 100657, 2025. Doi: https://doi.org/10.1016/j.ecohyd.2025.100657

Disponibilidade

Acesso para os scripts das análises de dados dos capítulos publicados referentes à tese.

https://github.com/maarianan/data-thesis

Declaração de Uso de Ferramentas de IA (Portaria CNPq 2.664/2026)

Em conformidade com a Portaria CNPq nº 2.664/2026, declara-se que ferramentas de Inteligência Artificial (IA) foram utilizadas como suporte técnico em etapas específicas desta Tese, compreendendo o auxílio na identificação de referências acadêmicas (Consensus), a revisão estilística e linguística para aprimoramento da fluidez textual (ChatGPT) e o suporte técnico para o refino de códigos em linguagem R aplicados na análise estatística e produção das figuras (GitHub Copilot). Ressalta-se que o uso dessas tecnologias se limitou ao suporte estrutural, analítico e linguístico, não substituindo a autoria humana em decisões intelectuais fundamentais, como a formulação de hipóteses e a interpretação de resultados. Todo o conteúdo gerado foi revisado, validado e conferido criticamente pela autora, que assume integral responsabilidade pela originalidade, veracidade e integridade ética do trabalho final.