UNIVERSIDADE FEDERAL DE SANTA MARIA
CENTRO DE CIÊNCIAS NATURAIS E EXATAS
PROGRAMA DE PÓS-GRADUAÇÃO EM BIODIVERSIDADE ANIMAL

Bruna Marmitt Braun

DIVERSIDADE E DISTRIBUIÇÃO ESPACIAL DE ELMIDAE (COLEOPTERA) NO ESTADO DO RIO GRANDE DO SUL

Santa Maria, RS
2018

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Tese apresentada ao curso de Pós-Graduação em Biodiversidade Animal- Área de Concentração em Bioecologia, da Universidade Federal de Santa Maria (UFSM, RS), como requisito parcial para obtenção do grau de **Doutora Biodiversidade Animal.**

Orientador: Dr. Sandro Santos
Coorientadora: Dra. Carla Bender Kotzian

Santa Maria, RS
2018

Braun, Bruna Marmitt
Diversidade e distribuição espacial de Elmidae (Coleoptera) no estado do Rio Grande do Sul / Bruna Marmitt Braun.- 2018.
107 p.; 30 cm

Orientador: Sandro Santos
Coorientadora: Carla Bender Kotzian
Tese (doutorado) - Universidade Federal de Santa Maria, Centro de Ciências Naturais e Exatas, Programa de Pós-Graduação em Biodiversidade Animal, RS, 2018

1. Mata Atlântica 2. Conversão 3. Insetos Aquáticos 4. Buffers Ripários 5. Vegetação Nativa I. Santos, Sandro II. Kotzian, Carla Bender III. Título.

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Santa Maria, RS
2018

A minha família, em especial ao meu amor Gabriel. Dedico também aos meus avós José Danilo Braun e Velida Weschenfelder Braun que infelizmente não estão mais entre nós para presenciar esse momento.

AGRADECIMENTOS

Agradeço primeiramente a minha orientadora Carla Bender Kotzian, pelos ensinamentos ao longo da minha carreira acadêmica. Agradeço por me fazer crescer como pesquisadora, cientista e pessoa. Obrigada pela paciência, estímulo e por sempre estar ao meu lado.

A Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) pela bolsa de estudo concedida.

Aos pesquisadores Cristina Stenert (UNISINOS), Luiz Ubiratan Hepp (URI), Marcia Regina Spies (UNIPAMPA) pelo empréstimo dos materiais de Elmidae das coleções de ensino.

Ao secretário do PPG Sidnei Cruz, pelo empenho e dedicação nas questões e atividades do PPG.

Ao meu querido amigo e colega Mateus Marques Pires, por todo auxílio durante a preparação da tese, correções e análises estatísticas, além das nossas conversas, cafezinhos e barras de chocolate para suprir o estresse do dia-a-dia.

A minha amiga e colega Luciane Santin, que sempre esteve ao meu lado, me auxiliando em tudo que estava ao seu alcance. Obrigada pela ajuda, pelas conversas e pela amizade

Ao pessoal do Laboratório de Sensoriamento Remoto da Vegetação (INPE) pela acolhida final, por me ajudar a dar o último gás na tese. A convivência com vocês fez com que meus dias fossem mais leves e com muita energia positiva.

À professora Tatiana Mora Kuplich, pela generosidade, empatia, amizade. Por ter me apresentado um ambiente de trabalho prazeroso e agradável. Obrigada pela oportunidade de conviver com vocês e utilizar seu laboratório.

Ao meu querido amigo Alberto Senra Gonçalves, me faltam palavras para agradecer a sua generosidade e amizade nessa etapa final. Sua ajuda foi de fundamental importância, nossas conversas e divagações me foram úteis para tentar ver o mundo e as pessoas de forma diferente. Obrigada pelos ensinamentos de modelagem e ArcGis, pelas discussões a respeito do mundo, da ciência. Obrigada por fazer acreditar que as pessoas são boas, e que no fundo tudo tem solução!

A minha querida amiga e colega Luíza Zuchetto Magnus, que me acompanha desde a graduação. Sem seu companheirismo e amizade a pós-graduação seria muito mais difícil. Obrigada por me ouvir, por me dar forças, por não me deixar desistir. Devo muito a nossa amizade.

A minha família, minha base, meu alicerce. Agradeço imensamente a força que vocês me deram nesses anos de doutorado. Agradeço aos meus pais Jair e Claudia, minha madrasta Carolina, aos meus irmãos Caio, Lucas e Leonardo por todo carinho e amor. Também quero agradecer aos meus avós Danilo e Velida (*in memoriam*), que sempre foram incansáveis, sem a ajuda de vocês eu não estaria aqui.

Aos meus queridos CÂOpanheiros Armani e Malu, o amor incondicional que vocês me dão todos os dias me fizeram chegar ao final dessa jornada. “Um cão é um anjo que vem ao mundo para ensinar amor. Quem mais pode dar amor incondicional, amizade sem pedir nada em troca. Afeição sem esperar retorno. Proteção sem ganhar nada. Fidelidade 24h por dia! Alguns anjos não possuem asas, possuem quatro patas, um corpo peludo, nariz de bolinha, orelhas de atenção, olhar de aflição e carência. Apesar dessa aparência, são tão anjos quanto os outros (aqueles com asas) e se dedicam aos seus humanos tanto quanto qualquer anjo costuma dedicar-se.”

Ao meu amor, meu querido companheiro Gabriel. Obrigada por sempre me incentivar, e a nunca me deixar desistir. Por sempre me dar forças nos momentos difíceis e ter paciência. Obrigada por estar sempre ao meu lado e me transformar numa pessoa melhor. Te amo incondicionalmente.

"...Nunca deixe que lhe digam que não vale a pena
acreditar no sonho que se tem
ou que seus planos nunca vão dar certo
ou que você nunca vai ser alguém
tem gente que machuca os outros
tem gente que não sabe amar

mas eu sei que um dia a gente aprende
se você quiser alguém em quem confiar
confie em si mesmo
quem acredita sempre alcança..."

Legião Urbana

RESUMO

Tese de doutorado
Programa de Pós-Graduação em Biodiversidade Animal

DIVERSIDADE E DISTRIBUIÇÃO ESPACIAL DE ELMIDAE (COLEOPTERA) NO ESTADO DO RIO GRANDE DO SUL

AUTORA: Bruna Marmitt Braun
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Santa Maria, 09 de março de 2018

Este estudo teve como objetivos, i) Analisar como as assembleias de larvas e adultos de Elmidae respondem a conversão florestal em uma área subtropical preservada de Floresta Atlântica semi-decídua; ii) Avaliar a influência de diferentes buffers de vegetação ripária (mais de 40 m de largura para menos de 5 m) e tipos de substrato (sedimentar e orgânico), bem como sua interação, na estrutura da comunidade de Elmidae; iii) Modelar a distribuição potencial de táxons de Elmidae no extremo sul do Brasil, em relação a preditores ambientais climáticos e paisagísticos (topografia, hidrologia e cobertura terrestre) usando modelos de distribuição de espécies (SDM). Além disso, estimamos a semelhança na distribuição prevista dos táxons modelados, a fim de investigar a semelhança de nicho entre os gêneros Elmidae e *Macrelmis.* A integridade florestal foi um importante fator para a estruturação das comunidades de Elmidae, onde em áreas convertidas foram encontradas as menores abundâncias de indivíduos de elmídeos. Os estágios larvais e adultos foram afetados pelo desmatamento, assim como a estrutura das comunidades foi distinta entre as áreas desmatadas e florestadas. Largura da vegetação ripária e tipo de substrato também foram fatores que influenciaram a estruturação das comunidades. Os resultados mostraram que as comunidades foram influenciadas por diferenças marcantes nas larguras de vegetação ripária. Somente a abundância relativa dos gêneros de Elmidae e seus estágios da vida foram diferentes entre os extremos de largura de vegetação. Por outro lado, a forte influência do tipo de substrato (orgânico e inorgânico) na estrutura da comunidade de macroinvertebrados neotropicais foi corroborada. A potencial distribuição de espécies mostrou relação de fatores paisagísticos e ambientais de larga escala com a estruturas de gêneros e espécies de *Macrelmis.* Fatores como declividade, precipitação, ecorregiões foram importantes para a estruturação das comunidades de gêneros e espécies, mas verificamos que pode haver uma interação entre os preditores em escala regional e local. É fundamental entender as relações complexas entre padrões de diversidade em larga escala e as características locais ecológicas das espécies. As técnicas de modelagem são uma ferramenta eficaz para indicar áreas para futuros esforços de amostragem e assim conhecer a diversidade da família Elmidae no sul do Brasil.

Palavras-chave: Áreas convertidas; Buffers ripários; Biologia da Conservação; Insetos Aquáticos; Modelos de Distribuição de Espécies.

ABSTRACT
Doctoral thesis
Graduate Program in Animal Biology
Federal University of Santa Maria

DIVERSITY AND DISTRIBUTION OF ELMIDAE (COLEOPTERA) IN THE STATE OF RIO GRANDE DO SUL

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ADVISOR: Sandro Santos
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Santa Maria, march 9, 2018

The objective of this study was to i) Analyze how the assemblages of larvae and adults of Elmidae respond to forest conversion in a subtropical area preserved in semi-deciduous Atlantic Forest; ii) Evaluate the influence of different buffers of riparian vegetation (more than 40 m wide to less than 5 m) and substrate types (sedimentary and organic), as well as their interaction in the community structure of Elmidae; iii) Model the potential distribution of Elmidae taxa in the extreme south of Brazil, in relation to climatic and landscape environmental predictors (topography, hydrology and land cover) using species distribution models (SDM). In addition, we estimated the similarity in the predicted distribution of the modeled taxa in order to investigate the niche similarity between the genera Elmidae and Macrelmis. Forest integrity was an important factor for the structuring of Elmidae communities, where in the converted areas the lowest abundances of elmidae individuals were found. The larvae and adult stages were affected by deforestation, just as the structure of the communities was distinct between deforested and forested areas. Width of riparian vegetation and type of substrate were also factors that influenced the structuring of communities. The results showed that the communities were influenced by marked differences in widths of riparian vegetation. Only the relative abundance of the genera of Elmidae and their stages of life were different between the wide widths of vegetation. On the other hand, the strong influence of the substrate type (organic and inorganic) on the structure of the neotropical macroinvertebrate community was corroborated. The potential distribution of species showed a relation of landscape and environmental factors of large scale with structures of genera and species of Macrelmis. Factors such as declivity, precipitation, and ecoregions were important for structuring the communities of genera and species, but we verified that there may be an interaction between the predictors on a regional and local scale. It is critical to understand the complex relationships between large-scale diversity patterns and the local ecological characteristics of species. The modeling techniques are an effective tool to indicate areas for future sampling efforts and thus to know the diversity of the Elmidae family in southern Brazil.

Keywords: Buffer zones, Conservation Biology, Converted areas, Species Distribution Model, Stream insects.

LISTA DE FIGURAS

CAPÍTULO 1:

CAPÍTULO 2:

CAPÍTULO 3:

LISTA DE TABELAS

CAPÍTULO 1:

CAPÍTULO 2:

CAPÍTULO 3:

Sumário

INTRODUÇÃO GERAL

Vegetação Ripária

A presença e a integridade da vegetação ripária são fatores importantes para a conservação das comunidades aquáticas (NESSIMIAN et al., 2008). A vegetação ripária contribui para a complexidade ambiental dos rios, possuindo um papel importante na manutenção dos processos naturais como estabilização do solo, evitando a erosão das margens; sombreamento, realizando a manutenção da temperatura d'água; filtragem de nutrientes lixiviados do solo, atuando como uma barreira entre o ecossistema terrestre e aquático; fonte de energia e regulação da produção primária (RIOS E BAILEY, 2006; LORION E KENNEDY, 2009; CONIGLAY et al., 2017). Também contribui com o *input* de matéria orgânica alóctone, que fornece alimento e habitat para os macroinvertebrados (SWEENEY, 1993), muitas vezes servindo como a mais importante fonte de energia em riachos de cabeceira (VANNOTE et al., 1980). As zonas ripárias também têm papel importante na ciclagem de nutrientes de riachos florestais, estando diretamente relacionadas com a dinâmica de energia de ecossistemas aquáticos (CUMMINS et al., 1973; VANNOTE et al., 1980).

Nas últimas décadas houve um aumento do desmatamento da vegetação ripária em decorrência de atividades antrópicas, como a silvicultura, agricultura, urbanização e mineração, as quais alteram as condições químicas e físicas dos rios, bem como a dinâmica, estrutura e funcionamento das comunidades aquáticas (ALLAN, 2004; NESSIMIAN et al., 2008; DOSSKEY et al., 2010). Em particular, o desmatamento pode afetar os habitats fluviais (WANTZEN, 2006), alterando os regimes de temperatura da água e a abundância e a diversidade de alimentos basais (BOJSEN E JACOBSEN, 2003) e, portanto, levando a grandes mudanças na estrutura das comunidades e declínio na diversidade de macroinvertebrados (BOJSEN E JACOBSEN, 2003; DUDGEON, 2006). Muitos estudos avaliaram as respostas de macroinvertebrados a alterações na vegetação ripária, como desmatamento e conversão do uso da terra. Alterações na mata ciliar podem influenciar diretamente os macroinvertebrados em decorrência de seus hábitos alimentares, pois eles são dependentes da qualidade e quantidade de matéria orgânica que entra nos córregos (MÁRQUEZ et al., 2015; GRAÇA et al., 2015). As respostas a essas alterações ambientais incluem mudanças na estrutura das assembleias de macroinvertebrados (composição e riqueza) (LORION E KENNEDY, 2009; CORBI et al., 2013). Adicionalmente, mudanças nas características da vegetação ripária também podem afetar a estrutura das assembleias de macroinvertebrados. Tais características incluem o tamanho (altura) da vegetação ciliar, largura, posição longitudinal (montante, jusante), composição (tropical, temperada, decídua) e distância de outros fragmentos florestais e áreas convertidas (NESSIMIAN et al., 2008; LORION E KENNEDY, 2009; CORBI et al., 2013; IÑIGUEZ-ARMIJOS et al., 2014). No entanto, respostas de assembleias de macroinvertebrados não são padronizadas (FEND E CARTER, 2005), pois outros fatores ambientais podem interagir com características da vegetação ciliar (por exemplo, geomorfologia regional e geologia superficial) e impedir a descoberta de padrões mais claros (SHEARER E YOUNG, 2011).

A largura da vegetação ripária é importante para políticas de conservação da biodiversidade (MARCZAK et al., 2010). Como a vegetação ripária controla a entrada de matéria orgânica nos sistemas aquáticos, ela também regula a distribuição dos grupos tróficos funcionais (FFG) ao longo dos cursos fluviais (VANNOTE et al., 1980). Assim, rios com *buffers* (faixas) de vegetação mais largas também recebem maior entrada de matéria orgânica, e mostram predominância de organismos trituradores (HEINO et al., 2004). Por outro lado, naqueles que apresentam pouca ou nenhuma vegetação ripária, predominam os organismos raspadores, pois a maior luminosidade favorece o aumento do aumento da matéria orgânica autóctone (LI E DUDGEON, 2008). A eficiência dos diferentes *buffers* na manutenção da integridade das comunidades aquáticas é pouco compreendida. Muitos estudos observaram que a redução das larguras ciliares pode levar à diminuição da riqueza e abundância de insetos aquáticos (NESSIMIAN et al., 2008, LORION E KENNEDY, 2009, IÑIGUEZ-ARMIJOS et al., 2014, MOARES et al., 2014; BRAUN et al., 2018). No entanto, o efeito de diferentes larguras de vegetação sobre as comunidades aquáticas pode ser muito variável de acordo com as particularidades ambientais dos rios. Largura, profundidade e declividade dos rios, proximidade a áreas urbanas e outras características de uso dos solos próximo aos rios, bem como o clima regional, podem interferir no papel amortecedor (*buffer* ecológico) da mata ciliar (LEE et al., 2004), dificultando encontrar padrões de efeito para as diferentes larguras de *buffer* (MARCZAK et al., 2010).

No Brasil, o novo Código Florestal (Código Florestal nº 12.651, 2012) reduziu a largura legal da mata ciliar em propriedades privadas de 30 a 15 m ou menos (até 5 m). O ponto crítico desta nova legislação é que aproximadamente 53% da vegetação nativa brasileira ocorre em propriedades privadas (SOARES-FILHO et al., 2014) e, portanto, que a conservação da mata ciliar não variará de acordo com a largura do rio, mas de acordo com o tamanho da propriedade rural. Os poucos estudos conduzidos no país, enfocando a influência de diferentes larguras de vegetação ripárias nas comunidade aquáticas, produziram dados insuficientes para políticas e programas conservacionistas (COUCEIRO et al., 2007; NESSIMIAN et al., 2008; MORAES et al., 2014; BRAUN et al., 2018). Entender como a redução da largura do *buffer* ripário afeta as comunidades aquáticas é uma contribuição importante para a proteção ambiental dos rios.

Família Elmidae

A família Elmidae Curtis, 1830 tem uma distribuição cosmopolita (JÄCH E BALKE, 2008), e contem cerca de 1.500 espécies, classificadas em 149 gêneros (SLIPINSKIET et al., 2011) e duas subfamílias, Elminae Curtis, 1830 e Larainae (LECONTE, 1861). Para o Brasil, são registradas 151 espécies, classificadas em 24 gêneros (SEGURA et al., 2013). Estudos sobre a ecologia das espécies são escassos (e.g., BROWN, 1987; PASSOS et al., 2003a,b; ELLIOTT, 2008; BRAUN et al., 2014; BRAUN et al., 2018), mas indicam que substrato com folhiço e/ou pedregoso favorecem a existência de uma fauna mais numerosa de Elmidae, e que fatores como disponibilidade de alimento, velocidade de correnteza, oxigênio dissolvido e presença de vegetação ripária bem preservada são importantes para o ciclo de vida das espécies. Além disso, a pouca informação existente sobre a biodiversidade dos elmídeos no Brasil (PASSOS et al., 2007; PASSOS et al., 2012), soma-se a concentração de estudos nas regiões Sudeste, Norte e Centro-Oeste, os quais têm enfoque predominantemente taxonômico (PASSOS E FELIX, 2004; PASSOS et al., 2007; PASSOS et al., 2009; PASSOS et al., 2010, FERNANDES et al., 2010a; FERNANDES et al., 2011; SAMPAIO et al., 2012; MIRANDA et al., 2012, SEGURA et al., 2011,2013, BARBOSA et al., 2013).

Os Elmidae são coleópteros aquáticos cujos os dois estágios de vida (larvas e adultos) vivem preferencialmente em ambientes lóticos com alta concentração de oxigênio (DOMÍNGUEZ E FERNÁNDEZ, 2009). Assim, eles são comuns em rios com fundo pedregoso e forte correnteza, ocorrendo também em locais com folhas, raízes e/ou troncos submersos de vegetação ripária (PASSOS et al., 2003). Os elmídeos possuem uma baixa tolerância a poluentes, principalmente substâncias surfactantes (e.g., detergentes), que diminuem a tensão superficial da água, impedindo a formação do plastrão, um delicado sistema composto por microestruturas cuticulares que retém bolhas de ar aderidas ao corpo

(BROWN, 1987). Por esse fato, e também pela pouca mobilidade de larvas e adultos, muitas espécies podem ser consideradas bioindicadoras de mudanças físicas e químicas no meio aquático (JÄCH E BALKE, 2008).

A alta densidade de Elmidae em ambientes lóticos (BROWN, 1987) reflete-se nas numerosas adaptações fisiológicas (respiração) e morfológicas (garras tarsais desenvolvidas; tamanho pequeno) (SEGURA et al., 2007) que lhes permitem ocupar este tipo de sistema aquático. Larvas e adultos possuem preferência pelos mesmos habitats e, portanto, o tipo de substrato, a temperatura da água e a quantidade de oxigênio dissolvido são características que afetam a distribuição das comunidades (PASSOS et al., 2003a,b; BRAUN et al., 2014; BRAUN et al., 2018). Larvas e adultos também possuem uma ampla plasticidade alimentar, e obtêm alimento raspando a superfície de rochas, fragmentos de madeira, raízes e folhas, e consumindo perifíton e detritos (SEAGLE JR, 1982). Desta forma, são considerados detritívoros-herbívoros e, como o hábito alimentar pode variar conforme a disponibilidade de alimento no habitat (TOMANOVA et al., 2006; BRAUN et al., 2018) também são classificados como raspadores, coletores-catadores e/ou retalhadores (SEAGLE JR, 1982; WHITE E BRIGHAM, 1996). O desenvolvimento larval dura de seis a 36 meses, e o número de instares varia de cinco a oito, de acordo com a temperatura e a disponibilidade de alimento. Após completar o desenvolvimento, a larva rasteja para fora da água e empupa próximo à margem. Quanto maior a temperatura e a disponibilidade de alimento, mais rápido é o desenvolvimento (BROWN, 1987).

O extremo sul do Brasil (estado do Rio Grande do Sul, RS) apresenta algumas características fisiográficas adequadas ao estabelecimento dos Elmidae, como o clima temperado úmido (Cfa, conforme classificação de Köppen; (MALUF, 2000)) que permite a existência de uma rica rede de drenagem. Além disso, ao longo da encosta do Planalto Meridional Brasileiro, que se estende de Leste a Oeste no RS, existem numerosos rios e riachos com fundos pedregosos (SIMÕES, 2002) e águas bem oxigenadas. Contudo, o conhecimento sobre a diversidade e a distribuição espacial da família no RS é escasso. Os primeiros e únicos estudos sobre a ocorrência de espécies para o estado do Rio Grande do Sul são de Howard E. Hinton que, em 1972, registrou e descreveu quatro espécies: *Neoelmis atys*, *Neoelmis mila*, *Neoelmis mormo* e *Neoelmis nicon* (HINTON, 1972). Desde então, os elmídeos tem sido registrados em inventários realizados em diversas regiões do estado, mas classificados apenas até família (e.g., KÖNIG et al., 2008, MILESI et al., 2008, NIN et al., 2009) ou gênero (*Austrolimnius* Carter & Zeck, 1929; *Heterelmis* Sharp, 1882;

Hexacylloepus Hinton, 1940; *Macrelmis* Motschulsky, 1859; *Microcylloepus* Hinton, 1935; *Neoelmis* Musgrave, 1935; *Phanocerus* Sharp, 1882; *Stegoelmis* Hinton, 1939) (BENETTI et al., 1998; SALVARREY et al., 2014; BRAUN et al., 2014; BRAUN et al., 2018).

Material estudado e estruturação da tese

Para desenvolvimento e elaboração da tese foram utilizados exemplares de Elmidae provenientes de diversas coleção científicas do estado do Rio Grande do Sul: Universidade Federal do Pampa- UNIPAMPA (São Gabriel), Universidade Vale dos Sinos-UNISINOS (São Leopoldo), Universidade Regional Integrada do Alto Uruguai e das Missões-URI (Erechim) e Universidade Federal de Santa Maria-UFSM, além de dados publicados na literatura científica a respeito da família Elmidae.

A tese foi elaborada em três capítulos, redigidos em formato de artigo científico, formatados nas normas das revistas em que estão (foram) submetidos/publicados, a saber:

1) Responses of riffle beetle assemblages to deforestation in a semi-deciduous Atlantic Forest remnant;
2) Effects of riparian vegetation width and substrate type on riffle beetle community structure;
3) Potential distribution of riffle beetles (Coleoptera: Elmidae) in southernmost Brazil.

OBJETIVO GERAL

O presente estudo tem como objetivo geral inventariar a biodiversidade e a distribuição espacial de Elmidae no Rio Grande do Sul, contribuindo para o conhecimento da família no Brasil.

OBJETIVOS ESPECÍFICOS

a) Analisar como as assembleias de larvas e adultos de Elmidae respondem a conversão florestal em uma área subtropical preservada de Floresta Atlântica semi-decídua;

b) Avaliar a influência de diferentes buffers de vegetação ripária (mais de 40 m de largura para menos de 5 m) e tipos de substrato (sedimentar e orgânico), bem como sua interação, na estrutura da comunidade de Elmidae;

c) Modelar a distribuição potencial de táxons de Elmidae no extremo sul do Brasil, em relação a preditores ambientais climáticos e paisagísticos (topografia, hidrologia e cobertura terrestre) usando modelos de distribuição de espécies (SDM). Além disso, estimamos a semelhança na distribuição prevista dos táxons modelados, a fim de investigar a semelhança de nicho entre os gêneros Elmidae e espécies de *Macrelmis*.

CAPÍTULO 1- RESPONSES OF RIFFLE BEETLE ASSEMBLAGES TO DEFORESTATION IN A SEMI-DECIDUOUS ATLANTIC FOREST REMNANT

Artigo aceito no periódico “Anais da Academia Brasileira de Ciências”
ISSN 1678-2690, Fator de Impacto (JCR) = 0.875, Qualis Biodiversidade
CAPES Estrato B2.

RESPONSES OF RIFFLE BEETLE ASSEMBLAGES TO DEFORESTATION IN A SEMI-DECIDUOUS ATLANTIC FOREST REMNANT

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Running title: Effect of deforestation on riffle beetles

Academy Section: Biological Sciences

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Abstract

Deforestation affects freshwater assemblages since aquatic organisms are dependent on the structure of the riparian vegetation. To investigate the responses of riffle beetles (Elmidae) to deforestation, this study assessed the structure of riffle beetle assemblages found in streams running through a large and well-preserved semi-deciduous Atlantic Forest remnant in Southern Brazil and in nearby deforested areas. Additionally, the effects of different substrate types (litter and stone) on the assemblages were assessed. Riffle beetle assemblages showed a remarkably distinct structure between forested and deforested areas. Lower abundance occurred in deforested streams. Indicator genera of forested streams were detected (e.g., *Macrelmis* and *Microcylloepus*), while a generalist genus (*Heterelmis*) predominated in deforested streams. Substrate type did not affect the composition of riffle beetle genera, although higher abundance was found in litter. Our results are likely associated with changes in the vegetation type and levels and quality of allochthonous material input between forested (native and higher levels) and deforested (exotic and lower levels) streams, which are directly linked to the feeding habits of riffle beetles. Thus, our study reveals an interesting potential of riffle beetles as indicators of stream ecological integrity in semi-deciduous areas of the Atlantic Forest biome.

Keywords: Coleoptera, Converted areas, Elmidae, Forest integrity, Riparian vegetation

INTRODUCTION

Forest ecological integrity plays an important role in the conservation of the stream fauna (Nessimian et al. 2008). Shading provided by riparian vegetation regulates water temperature, dissolved oxygen concentration and primary production in streams (Lorion and Kennedy 2009). Riparian vegetation acts as a buffer against the effects of terrestrial human activities, as it reduces bank erosion, siltation and flooding through the filtering of undesirable particles entering the water (Roy et al. 2003, Reid et al. 2010). In addition, the aquatic food web structure is influenced by riparian vegetation through the input of allochthonous organic matter (Weigel et al. 2003, Frady et al. 2007, Lorion and Kennedy 2009). Therefore, deforestation leads to changes in the water quality and trophic structure of streams, impacting its associated fauna such as macroinvertebrates (Lorion and Kennedy 2009, Reid et al. 2010, Iñiguez-Armijos et al. 2014, Márquez et al. 2015).

Many studies have assessed the responses of stream macroinvertebrates to alterations in riparian vegetation such as deforestation and land use conversion. Alterations in riparian vegetation can directly influence macroinvertebrates, because they are dependent on the quality of organic matter entering the streams for their feeding habits (Márquez et al. 2015, Graça et al. 2015). Responses to such environmental alterations include changes in macroinvertebrate assemblage structure (composition and richness) (Lorion and Kennedy 2009, Corbi et al. 2013). Additionally, changes in specific riparian vegetation features can also affect macroinvertebrate assemblage structure. Such features include riparian vegetation size, width (buffer effect), longitudinal position (upstream, downstream), composition (tropical, temperate deciduous) and distance from other forest fragments and converted areas (Roy et al. 2003, Fend and Carter 2005, Death and Collier 2010, Reid et al. 2010, Nessimian et al. 2008, Lorion and Kennedy 2009, Corbi et al. 2013, Iñiguez-Armijos et al. 2014). However, macroinvertebrate assemblage responses are not standardized (Fend and Carter 2005) since other environmental factors may interact with riparian vegetation features (e.g. regional geomorphology and superficial geology), and hinder the finding of clearer patterns (Shearer and Young 2011).

Riffle beetles (Elmidae) are coleopterans that inhabit lotic systems with well-oxygenated waters with strong currents during the larval and adult stages (Brown 1987, Passos et al. 2003a, Passos et al. 2003b, Couceiro et al. 2007, Elliott 2008, Braun et al. 2014a, 2018) and commonly found in well-preserved streams (Braun et al. 2014b). Some genera have been associated with streams running through well-preserved and sometimes large forested areas (Weigel et al. 2003, Iñiguez-Armijo et al. 2014), while others seem to be tolerant to forest conversion and can be abundant in deforested streams (Paula and Fonseca-Gessner 2010). Thus, the structure of riffle beetle assemblages could constitute an important environmental indicator of forest ecological integrity. The limited dispersal ability of adult and larval stages of riffle beetles (Elliott 2008) reinforces their potential role as environmental indicators because they should be sensitive to alterations in local environmental conditions.

The presence of leaves, macrophytes, and woody debris in the stream substrate is also an important driver of riffle beetle assemblages (Passos et al. 2003a, Passos et al. 2003b, Braun et al. 2014a), which suggests a direct relationship between riffle beetles and the quality of the vegetal remains arriving into streams. The relationship of riffle beetles with organic matter is related to their scraper or shredder feeding habits (White and Brigham 1996). However, subtropical streams with stony streambeds can also sustain rich and abundant riffle beetle assemblages (Braun et al. 2014a). Adult and larval riffle beetles feed on periphyton or scrape organic matter from rocks (e.g., roots, leaves, and wood) (Brown 1972, Seagle Jr. 1982). In fact, riffle beetle feeding habits can also differ according to the life stage in some genera (Tomanova et al. 2006).

This study assessed the responses of riffle beetle assemblages to deforestation in a well-preserved subtropical semi-deciduous Atlantic Forest remnant. Considering that the riparian vegetation influences the occurrence and composition of riffle beetles (Weigel et al. 2003, Paula and Fonseca-Gessner 2010, Iñiguez-Armijo et al. 2014), we hypothesized that riffle beetle assemblage structure will change between forested and deforested streams, because riffle beetles are sensitive to changes in structure of riparian vegetation such as land use (natural vs. converted) and composition (native vs. exotic), mostly through changes in the quality of the vegetal remains. In addition, due to the low richness of riffle beetle genera recorded in southern Brazilian streams (Braun et al. 2014ab), we expected to find (i) higher abundance in forested streams (rather than richness) and (ii) more tolerant genera to predominate in deforested streams. The effects of litter and stone substrates on riffle beetles were also assessed, as substrate type is an important driver of riffle beetle assemblage structure (Passos et al. 2003b; Braun et al. In press). Considering that riffle beetles have different feeding habits, we expected to find changes in the composition of riffle beetles between substrates, because they provide different feeding sources to stream insects. In addition, we investigated whether riffle beetle genera were indicative of forested and deforested streams, though previous studies that indicate plasticity in the feeding habits of some riffle beetle genera (Tomanova et al. 2006) prevented us from previous expectations.

MATERIALS AND METHODS

Study area

The study was conducted in the Parque Estadual do Turvo (PET) conservation unit and nearby areas, located on the Southern Plateau (Planalto Meridional, Rio Grande do Sul State; 27°14’34.08”S, 53°57’13.74”W; ca. 400 m a.s.l.; Fig. 1) (SEMA 2005). The PET is the largest environmentally protected area in Southern Brazil (17,491-ha area, 90-km-long perimeter). It comprises a significant portion of the preserved semi-deciduous Atlantic Forest in the region (Oliveira-Filho et al. 2015) and extends over 65,000 ha in Argentina (Iguazú National Park) (Martínez et al. 2009).

The land use near the conservation unit is characterized by soybean culture and pasture (SEMA 2005), and streams commonly have riparian vegetation. The northern boundary of the study area is delimited by the Uruguay River basin, into which the streams running through the PET and adjacent agriculture areas drain (Fig. 1). The climate in the region is subtropical and sub-humid with a dry summer (Maluf 2000), and the mean annual rainfall is ~1,665 mm, well distributed throughout the year (SEMA 2005). In general, the streams run through a geomorphologically dissected region composed of deep valleys carved on the basalt rocks of the Serra Geral Formation (SEMA 2005).

Sampling

Samplings were conducted during austral summer (between 23 and 25 January 2011) in six small-order streams (1st and 2nd order, according to Strahler’s classification) during a period of low precipitation. These climatic conditions are favorable for collecting riffle beetles in southern Brazilian streams (Braun et al. 2014a). Three streams (F1, F2, and F3) were located within the PET (forested streams), and the other three (C1, C2, and C3) were located outside the PET (deforested streams) in a deforested area that had been converted to agricultural land use near the edge of the PET (Fig. 1). In both areas, the streams had riparian vegetation and were similarly shadowed, but in the deforested areas, vegetation included exotic species such as *Eucalyptus* and Japanese raisin tree (*Hovenia dulcis*) (Bertaso et al. 2015).

Riffle beetles were collected using a Surber sampler (0.0361-m^2 area, 250-µm mesh size). Samplings were performed within a ~50-m reach in each stream. Ten subsamples were collected in each reach (five in litter substrate and five in stone substrate), which were posteriorly pooled according to substrate type. Sampling reaches had no wood or aquatic plants in order to assess the effect of litter and stone substrate on riffle beetle assemblage structure. Riffle beetles were identified at genus level since the diversity of Neotropical Elmidae in southern Brazil is poorly known at species level (Domínguez and Fernández 2009). Furthermore, many specimens occurred as larvae, which are identifiable at only genus level by the available taxonomic keys (Manzo 2005, Passos et al. 2007, Segura et al. 2012, Barbosa et al. 2013). Specimens were deposited in the Collection of Aquatic Insects at Federal University of Pampa (UNIPAMPA).

Some landscape-scale environmental descriptors were obtained for each sampling site, such as the distance from the forest edge (‘BorDist’; m) and riparian vegetation features. The latter include the length of riparian vegetation upstream (‘RipVegL’; m); percentages of native vegetation cover (‘NatVeg’) and converted area (‘Land use’), both of which were measured within an arbitrarily defined buffer of ~100 m around each sampling site in deforested areas; and riparian vegetation width (‘RipVegW’; m), which was measured considering the sum of both bank widths. Measures of RipVegW were standardized to 500 m in forested streams to avoid overestimation in data analysis. These measures were taken from Google Earth images (dated from February 2011), and followed similar methodology used in previous studies conducted in subtropical streams (Salvarrey et al. 2014, Moraes, et al. 2014).

The following local-scale environmental descriptors were taken at each sampling site: percentage of siltation (visually estimated), stream width, and stream depth (cm). In addition, water abiotic descriptors such as water temperature (WT; °C), electric conductivity (EC; µS/cm), dissolved oxygen (DO; mg/L), and turbidity (Turb; NTU) were measured with a multiparameter probe.

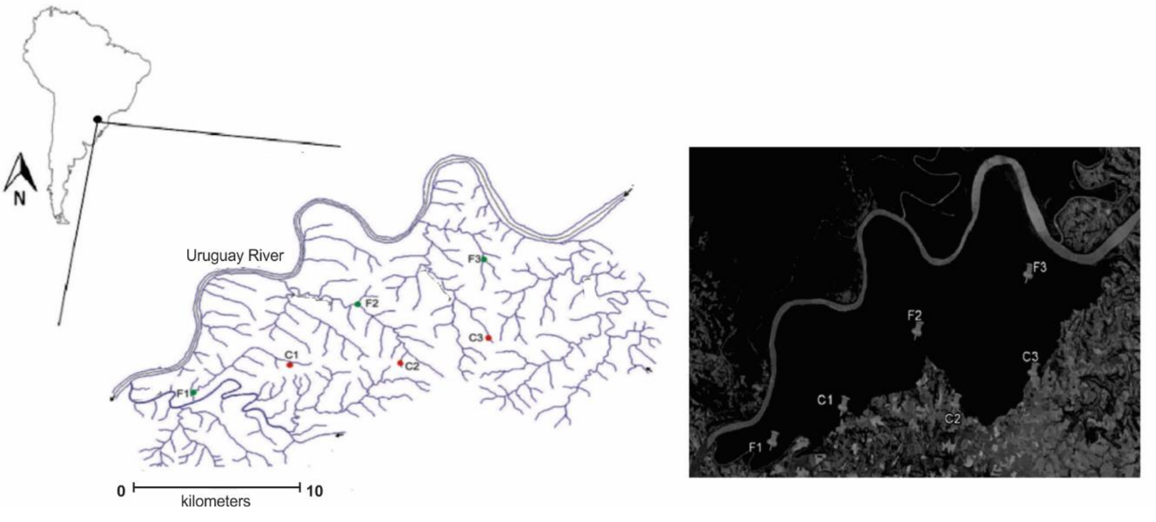

Figure 1. Location of the sampled streams in forested (F1, F2, and F3) and converted areas (C1, C2, and C3) at Parque Estadual do Turvo and adjacent areas, in Southern Brazil. Note. Adapted from: Bertaso et al.2015.

Data analysis

Riffle beetle assemblage structure was previously assessed through non-metric multidimensional scaling (NMDS) ordination diagrams. Differences in riffle beetle assemblage structure between forested and deforested streams were tested through a non-parametric MANOVA followed by 9,999 permutations (perMANOVA). In this analysis, the larval and adult stages of each genus were pooled (i.e., eight entries), because their feeding habits might not be strongly affected by differences in riparian vegetation quality. The influence of substrate type (litter *vs.* stone) on riffle beetle assemblage structure was also tested through a perMANOVA. The fauna matrix used in this analysis, however, kept the larval and adult stages separate (i.e., fourteen entries) in order to assess whether life stages respond differently to the substrate type according to previous studies (Tomanova et al. 2006). The NMDS and perMANOVA analyses were conducted with distance matrices (Bray-Curtis dissimilarity index) based on a log transformed matrix of the fauna. Both analyses were performed using the R *vegan* package.

Redundancy analysis (RDA) was used to assess the influence of environmental descriptors on the spatial distribution of riffle beetle genera and their life stages in the study area. Since percentages of land use, native vegetation cover and siltation were highly correlated, the model included only the distance from the forest edge, land use and stream width. This method was also efficient in removing the multicollinearity among the explanatory variables, since none of them showed a high inflation factor (VIF) (*sensu* ter Braak and Šmilauer 2002). A Monte Carlo test (999 randomizations) was used to test the significance of the canonical axes (ter Braak and Šmilauer 2002). The biotic data were Hellinger transformed. The environmental percentage descriptors were arcsine transformed, and the others were square-root transformed and standardized by standard deviation. RDA was performed using the statistical software CANOCO v. 5.0.

An indicator species analysis (ISA) based on the indicator value (Indval; (Dufrêne and Legendre 1997)) was performed to highlight the relationships between Elmidae genera and their life stages with forest integrity and substrate type. Indval takes into considers the taxa fidelity and relative abundance in groups established a priori to identify the indicator taxa of each group (Dufrêne and Legendre 1997). Significance values (p) were obtained after 1,000 randomizations, and the analysis was performed using the R *labdsv* package.

RESULTS

Similar values of water abiotic descriptors were found in forested and deforested streams. In turn, higher values of landscape-scale environmental predictors (BorDist, NatVeg, RipVegW, and RipVegL and land use) were found in forested streams, as expected. Siltation values were higher in deforested streams (Table I).

Table I - Values of environmental descriptors recorded at sampled streams in forested (F) and deforested (C) areas. Numbers 1-3 refer to each sampled stream. For abbreviation of environmental variables, see Material and methods section.

	Forested streams			**Deforested streams**		
Variables	**F1**	**F2**	**F3**	**C1**	**C2**	**C3**
Coordinates	27°16'13.3"S 54°01'04.2"W	27°12'56.4"S 53°55'34.7"W	27°11'15.8"S 53°51'22.4"W	27°15'11.03"S 53°57'50.01"W	27°15'07.1"S 53°54'09.46"W	27°14'11.2"S 53°51'13.55"W
WT (°C)	26.15	23.36	23.04	25.3	22.27	24.74
DO (mg/L)	8.42	9.13	9.34	9.14	10.39	9.41
EC (µS/cm)	50	91	79	45	53	58
Turb (NTU)	3.27	2.4	2.9	2.6	1.2	0.6
Width (m)	0.6	1.48	1.17	0.6	1.91	1.62
Depth (cm)	4.5	4.5	6.3	4.9	4.9	6.6
RipVegW (m)	500	500	500	40	70	10
RipVegL (m)	1.000	1.300	1.500	200	140	40
Land use (%)	0	0	0	70	60	90
NatVeg (%)	100	100	100	60	85	20
Siltation (%)	0	0	0	10	7	20
BorDist (m)	600	1.100	2.850	-220	-1,000	-100

We collected 1,400 individuals from eight genera and one unidentified taxon (Table II). Adult and larval stages of five genera were found. *Hexanchorus* Sharp, 1882 was found only as larvae, and *Cylloepus* Erichson, 1847 and *Phanocerus* Sharp, 1882, only as adults. Higher abundance of riffle beetles was found in forested streams (895) and litter substrate (956). The larval stage of *Heterelmis* Sharp, 1882 was dominant in both forested and deforested streams and in litter substrate, and its adult stage was predominant in deforested streams and in both substrate types.

Table II - Composition, richness and abundance of riffle beetle genera sampled in forested (F) and deforested (C) streams and substrate types (L = leaf litter; S = stone). Note. Letter ‘(A)’ at the end of genus name indicates adult life stage.

Genera	**Streams**		**Substrate type**		
	F	**C**	**L**	**S**	**Total**
Cylloepus(A)	0	1	1	0	**1**
Heterelmis	302	277	421	158	**579**
Heterelmis(A)	99	134	166	67	**233**
Hexacylloepus	2	1	0	3	**3**
Hexacylloepus(A)	18	10	9	19	**28**
Hexanchorus	2	0	1	1	**2**
Macrelmis	79	0	14	65	**79**
Macrelmis(A)	22	2	5	19	**24**
Microcylloepus	10	2	12	0	**12**
Microcylloepus(A)	191	13	153	51	**204**
Neoelmis	101	38	104	35	**139**
Neoelmis(A)	13	2	3	12	**15**
Phanocerus(A)	56	24	66	14	**80**
Gen Indet. A	0	1	1	0	**1**
Abundance	**895**	**505**	**956**	**444**	**1,400**
Richness	**7**	**9**	**8**	**7**	**9**

The NMDS ordination diagram showed a clear segregation between forested and deforested streams (Fig. 2a; stress = 0.07), but not between substrate types (Fig. 2b; stress = 0.06). Riffle beetle assemblage structure differed significantly between forested and deforested streams (pseudo-$F_{1,11}$ = 4.72; R_2 = 0.32; p = 0.003), but not between substrate types (pseudo-$F_{1,11}$ = 0.88; p = 0.3). The RDA axes were significantly different from chance (F = 2.047, p = 0.047). The first two axes summarized 67.1% of the variance in riffle beetle abundance data and explained 88.9% of their relationship with the environmental descriptors (Table III). The first RDA axis showed a strong positive correlation with the distance from the forest edge (0.7533) and a strong negative correlation with land use (0.9403) and stream width (0.8914) (Fig. 3). The second RDA axis showed a strong negative correlation with the distance from the forest edge (0.6103) (Fig. 3). In general, the first RDA axis segregated forested from deforested streams (Fig.3).

Table III - Eigenvalues, taxa-environment correlation coefficients, and cumulative percentage explained by the first four RDA axes of riffle beetle assemblages of streams in Parque Estadual do Turvo.

Axes	**Axis 1**	**Axis 2**	**Axis 3**	**Axis 4**	**Total variance**
Eigenvalues	0.454	0.217	0.084	0.190	1.000
Species-environment correlations	0.979	0.987	0.971	0.000	

Cumulative percentage variance					
of taxa data	45.4	67.1	75.4	94.4	
of taxa-environment relation	60.2	88.9	100.0	0.0	
Sum of all eigenvalues					1.000
Sum of all canonical eigenvalues					0.754

Some riffle beetle genera were influenced by the environmental gradient reflecting deforestation (Fig. 3). Adult and larval stages of *Neoelmis* Musgrave, 1935 and larvae of *Macrelmis* Motschulsky, 1859 showed close relationships with forested streams. There were no strong relationships with the distance from forest edge for both life stages of *Microcylloepus* Hinton, 1935 and *Hexacylloepus* Hinton, 1940, the larvae of *Hexanchorus* and *Phanocerus*, and adults of *Macrelmis*. However, they were associated with forested streams (F1 and F2). Both life stages of *Heterelmis* and adults of *Cylloepus* were positively associated with streams located in deforested areas, as well as stream width and land use.

The ISA showed significant indicator values of larval and adult stages of riffle beetles only in forested streams. Larvae of *Macrelmis* (IV = 100%, p = 0.004) and adults of *Microcylloepus* (IV = 94%, p = 0.029) had the highest indicator values for forested streams. Larvae of *Neoelmis* and adults of *Hexacylloepus*, *Macrelmis,* and *Neoelmis* had marginally significant indicator values. Regarding substrate type, only larvae of *Heterelmis* showed a high indicator value for litter substrate, although it was marginally significant (IV = 73%, p = 0.071).

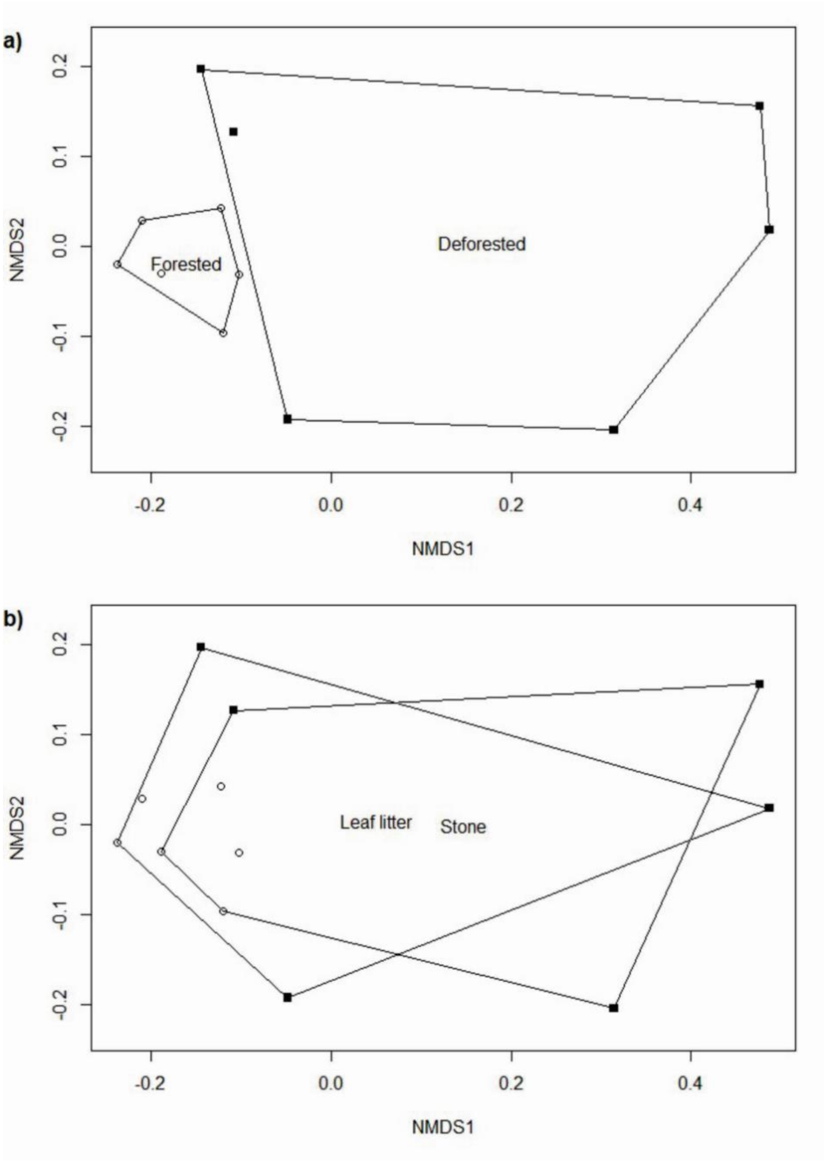

Figure 2. Non-metric multidimensional scaling (NMDS) ordination diagram of samples from a) forested (white squares) and deforested (filled squares) areas; b) substrate types.

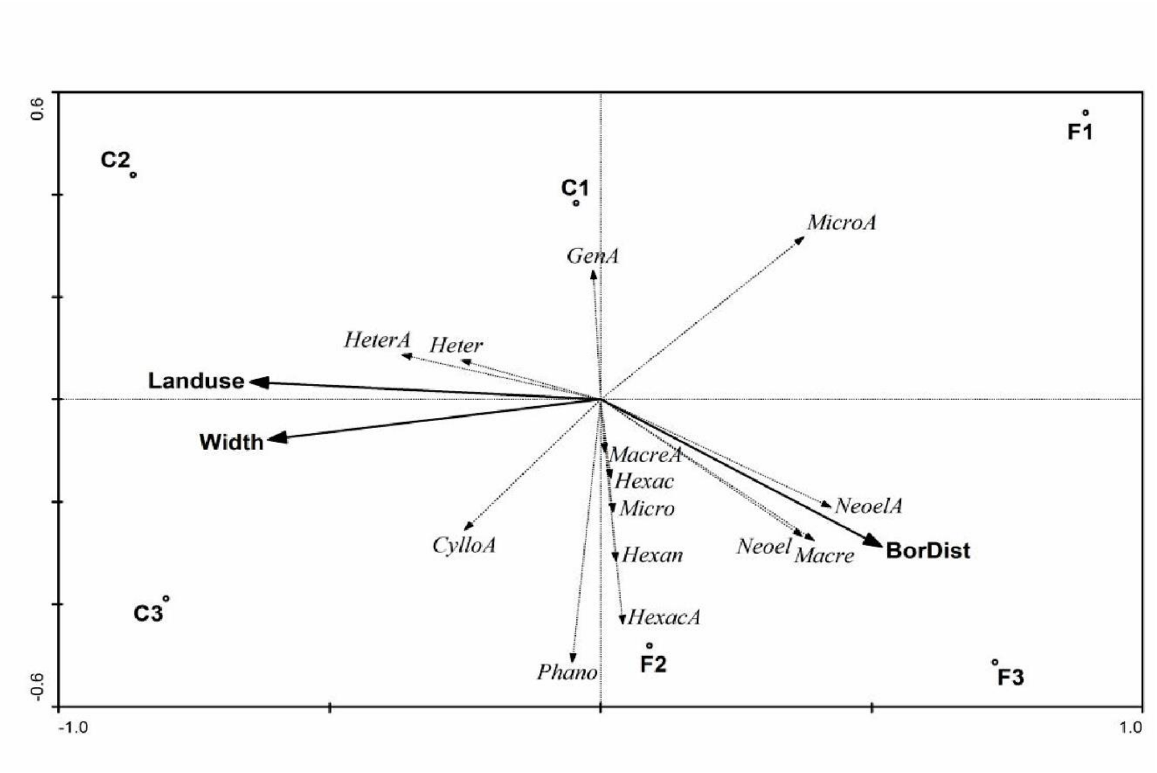

Figure 3 - Diagram of streams and riffle beetle genera assemblages of the Redundancy Analysis constrained by land use, distance from the forest edge and stream width in Parque Estadual do Turvo. Note. taxa abbreviation: Cyllo: Cylloepus, Gen: Genus indetermined; Heter: Herelmis, Hexac: Hexacylloepus, Hexan: Hexanchorus, Macre: Macrelmis; Micro: Microcylloepus; Neoel: Neoelmis; Pha: *Phanocerus*; letter A at the end of genus name refers to adult stage; and F1, F2, F3 and C1, C2, C3 refer to the streams studied in forested (F) and converted (C) areas.

DISCUSSION

Responses of riffle beetle assemblages to deforestation and the value of some genera as environmental indicators of forest integrity

Our results showed that assemblages of riffle beetles were sensitive to deforestation in the study area. Streams running through forested areas had assemblages with higher similarity and higher abundance of riffle beetles than those running through deforested and converted areas. Streams running through forested areas tend to be well shadowed with lower water temperature and higher dissolved oxygen values than those running through deforested areas. However, water abiotic descriptors had very similar values in forested and deforested streams in the study area. The presence of riparian vegetation in all streams (forested and deforested) should have prevented expressive changes in water quality. The buffer effect provided by riparian vegetation, even when it is narrow and short in length (Lorion and Kennedy 2009), should also have prevented expressive increases in water temperature. Thus, the explanation for the differences found between assemblages from forested and deforested streams in the PET region should be related to other environmental descriptors.

Higher abundance of riffle beetles occurred in forested streams. The size of the forested area, the vegetation type (semi-deciduous forest) and the preservation condition should have enabled the differences found in the PET region. Previous studies have demonstrated the influence of large-sized forest fragments, including wider riparian vegetation on macroinvertebrate assemblage structure (Couceiro et al. 2007, Iñiguez-Armijos et al. 2014, Moraes et al. 2014). This relationship has not been discussed so far, but it can be easily explained by the plasticity in the feeding habits of riffle beetles. Riffle beetles are shredders and grazers. Some genera feed on allochthonous vegetal matter, while others are xylophagous (Anderson and Sedell 1979, Elliott 2008). In fact, riffle beetles have been mainly considered shredders (Elliott 2008), and the abundance of this functional group is typically linked to the abundance of litter (Cummins et al. 1989), which has higher input in streams flanked by deciduous vegetation (Frady et al. 2007). Thus, the greater the size of the forest and the wider the riparian vegetation, the higher the abundance of shredders (Weigel 2003, Frady et al. 2007, Lorion and Kennedy 2009, Valle et al. 2010, Iñiguez-Armijos et al. 2014).

Riffle beetle assemblages were more similar within the PET area than in the nearby deforested areas. Previous studies have also recorded more similar macroinvertebrate assemblages in streams from forested areas than deforested ones (Death and Collier 2010, Corbi et al. 2013, Iñiguez-Armijos et al. 2014, Bertaso et al. 2015). Disturbances promoted by agricultural activities as well as environmental heterogeneity (Heino 2000) are important drivers of freshwater macroinvertebrate diversity. Thus, the occurrence of more dissimilar riffle beetle assemblages among deforested streams should have been determined by the greater variation in landscape-scale environmental descriptors, particularly the occurrence of different exotic species in the riparian vegetation (e.g., *Eucalyptus* and Japanese raisin). Previous studies have shown that exotic vegetation remains entering streams affect macroinvertebrate assemblages because their leaves have lower nutritional quality and reduced palatability (Graça et al. 2002, 2015, Márquez et al. 2015). Thus, exotic vegetation also affects shredder organisms (Death and Collier 2010), including riffle beetles (Huamantinco and Nessimian 1999).

Many riffle beetle genera and life stages showed high value as indicators of forest integrity in the PET region. Previous studies analyzing the influence of forest removal and conversion to agricultural uses on stream macroinvertebrate assemblages have recorded the occurrence of riffle beetles, especially (or exclusively) in streams running into native forest and grassland areas (Paula and Fonseca-Gessner 2010, Azevedo et al. 2012, Corbi et al. 2013, Iñiguez-Armijos et al. 2014). In this study, *Neoelmis*, *Hexacylloepus* and *Microcylloepus* were indicative of forested streams. These genera have been found in streams located in forested areas from other regions (Lorion and Kennedy 2009, Paula and Fonseca-Gessner 2010, Corbi et al. 2013, Iñiguez-Armijos et al. 2014). *Cylloepus* and *Phanocerus* showed weak relationships with deforested streams in the PET region and were previously associated with streams running into forested areas (Paula and Fonseca-Gessner 2010, Corbi et al. 2013, Iñiguez-Armijos et al. 2014). *Heterelmis* has been recorded in streams located in both impacted and protected areas (Paula and Fonseca-Gessner 2010). The larval stage of *Macrelmis* showed the highest indicative value for PET forested area, suggesting a relation to the presence of native vegetation leaves with good nutritional quality. The adult stage of *Microcylloepus*, which had the second highest indicative value for forested areas, was previously associated with preserved areas (Paula and Fonseca-Gessner 2010). In other words, except for the adult and larval stages of *Heterelmis*, many other genera and their life stages seem to be strongly associated with forested areas and constitute good environmental indicators of forest integrity.

Factors related to forest environmental integrity, such as land use, stream width, and distance from the forest edge, were important drivers of riffle beetle assemblage structure in the PET region. However, the influence of the distance from the forest edge is possibly related to the effects of isolation and dispersal ability of riffle beetles. Connectivity is a strong driver of stream insect assemblages, mostly due to dispersal limitation (Rosenberg and Resh 1993). Riffle beetles disperse via drift and flight (Elliott 2008), although their dispersal ability is limited because adults seldom leave the water after settling (Yee and Kehl 2010). In this study, sites that were more distant from each other had different compositions. Therefore, spatial isolation must have played an important role in structuring riffle beetle assemblages in the PET region.

Substrate influence on riffle beetle assemblages

Riffle beetle assemblages in leaf litter substrate had higher abundance than in stone substrate. Previous studies also recorded riffle beetle assemblages with higher richness and abundance in leafy substrates than in other substrate types (Tavares and Williams 1990, Passos et al. 2003a, Passos et al. 2003b, Azevedo et al. 2012, Graça et al. 2015). This relationship was attributed to the importance of vegetal remains to the life habits of riffle beetles. In contrast, richness was very similar between assemblages inhabiting both substrate types in the study area. Some previous studies also found rich and very abundant assemblages in stony streambeds, where litter does not accumulate (Brown 1987, Elliott 2008, Braun et al. 2014a). This result may be determined by the scraper feeding habit of riffle beetles over rocks (Elliot 2008), which lead to the occurrence of similar richness between both substrate types in the PET region.

Heterelmis was indicator of substrate type, although showing marginally significant value. Larvae of *Heterelmis*, which were associated with litter substrate, have been classified as generalist collectors (including the adult stage) in Neotropical streams (Chara-Serna et al. 2012). However, they have also been found in predominantly stony substrates (Braun et al. 2014a) and in very impacted areas (Corbi et al. 2013). Thus, species of *Heterelmis* should also be tolerant to differences in substrate type, as suggested by the marginal significance of its indicative value in the PET region.

CONCLUSIONS

The structure of riffle beetle assemblages was sensitive to forest integrity in the PET conservation unit and to land use in nearby agricultural converted areas. Riffle beetle assemblages had lower abundance and similarity in deforested streams. In addition, some genera such as *Macrelmis* and *Microcylloepus* showed indicator value of environmental integrity in streams from semi-deciduous Atlantic Forest remnants. Furthermore, the assemblages had higher abundance in litter than in stone substrate, reinforcing the role of substrate type in the structure of their assemblages. Our results could be related to specific features of the study area, such as the importance of the amount and quality of the allochthonous organic matter entering the streams, since the input of a broad array of vegetal remains (leaves, branches, twigs, and woody debris) is guaranteed in native forested streams (Marquéz et al. 2015). In contrast, streams running in converted areas contained exotic vegetation, which likely affected the establishment of shredder organisms such as riffle beetles. In summary, we suggest that the quality of the riparian vegetation (native) be as important as its buffer role in structuring riffle beetle assemblage structure.

Finally, we recommend that the role of temporal changes in the input of native allochthonous matter in structuring riffle beetle assemblages be further investigated. The input of organic matter from native deciduous to semi-deciduous vegetation is not seasonal in Neotropical streams (Gonçalves et al. 2006). Complementarily, many streams from temperate areas in the Holarctic Region also have riparian vegetation with seasonal (deciduous vegetation) input patterns, and show lower vegetation diversity than in subtropical areas of the Neotropical Region (Campbell et al. 1992). Thus, through similar mechanisms, the role of riparian vegetation in influencing riffle beetle assemblages can differ between the different vegetation types in the Atlantic Forest biome (e.g., subtropical semi-deciduous type covered in this study and tropical evergreen ones) and zoogeographical regions.

ACKNOWLEDGEMENTS

We thank biologists Marlon da Luz Soares and Viviane Souza Mirada for their inestimable help in the data collection in field. Thanks to Secretaria do Ambiente e Desemvolvimento Sustentável (SEMA-RS), for permission to conduct research in the Parque Estadual do Turvo, and Instituto Chico Mendes de Conservação da Biodiversidade (ICMBIO), for the collecting license (26085-1). Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) is thanked for granting a scholarship to the first author, T.R.N. Bertaso, and to M.M. Pires.

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CAPÍTULO 2- EFFECTS OF RIPARIAN VEGETATION WIDTH AND SUBSTRATE TYPE ON RIFFLE BEETLE COMMUNITY STRUCTURE

Artigo publicado no periódico “Entomological Science” ISSN 1343-8786, Fator de Impacto (JCR) = 1.262, Qualis Biodiversidade CAPES Estrato B1.

EFFECTS OF RIPARIAN VEGETATION WIDTH AND SUBSTRATE TYPE ON RIFFLE BEETLE COMMUNITY STRUCTURE

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Abstract

Riffle beetle community structure is influenced by the preservation condition of stream riparian vegetation. Though, the width of riparian vegetation required to ensure conservation of stream insect communities is still controversial. Effects of alterations in riparian vegetation widths on stream insect community structure can be overcame by other environmental variables, like substrate type, hindering accurate assessments. We tested the effects of different riparian vegetation widths (> 40 m, 30-15 m, 15-5 m and < 5 m) along with different substrate types (inorganic and organic) on riffle beetle community structure in southern Brazilian 4th- to 5th-order streams. Riparian buffer widths and substrate types influenced riffle beetle community structure, but no interaction between them was observed. Reduced riparian vegetation widths downstream were associated with changes in riffle beetle dominant genera (*Macrelmis* predominated only in streams with narrowest riparian widths). Additionally, communities in organic substrates had lower equitability and different dominant genera (*Hexacylloepus* and *Heterelmis*) than inorganic ones. Our results showed that reductions in riparian vegetation were associated with water pollution and changes in riffle beetle community structure, suggesting that buffer strips narrower than 5 m are not adequate to maintain environmental integrity of southern Brazilian streams. These results have special importance for the conservation of stream insects in Brazil, as reductions up to less than 5 m in stream banks of small properties are allowed by the new Brazilian Forest Code, independently of stream order.

Key words: buffer zones, conservation biology, Elmidae, shredders, stream insects.

INTRODUCTION

Riffle beetles (Elmidae Curtis, 1830) are common coleopterans in strong-current and well-oxygenated streams (Brown 1987; Elliott 2008; Domínguez & Fernández 2009). The preservation condition of forest streams has been related to riffle beetle community structure; the occurrence of many riffle beetle taxa or specific life stages has been related to streams with well-preserved riparian vegetation (Lorion & Kennedy 2009; Paula & Fonseca-Gessner 2010; Azevedo *et al*. 2012; Corbi *et al*. 2013; Iñiguez-Armijo *et al*. 2014). Changes in riffle beetle composition and community equitability were also detected in streams with narrower riparian vegetation widths or running through agricultural areas (Paula & Fonseca-Gessner 2010; Azevedo *et al*. 2012; Corbi *et al*. 2013). In fact, the family has been considered a potential good environmental indicator group (Elliott 2008).

Riparian vegetation has an important role in stream ecological integrity. It minimizes bank erosion and filters nutrient loading from converted areas, preventing water pollution (Rios & Bailey 2006; Nessimian *et al*. 2008; Lorion & Kennedy 2009). Allochthonous organic matter is the main energy source for benthic invertebrates in forested streams, and therefore has an important role in the trophic structure of aquatic communities (Vannote *et al*. 1980; Tank *et al.* 2010). Thus, removal or changes in riparian vegetation width can alter not only stream water quality, but also the structure of macroinvertebrate communities (Heino *et al*. 2004; Lee *et al*. 2004; Li & Dudgeon 2008; Siegloch *et al*. 2016). Decreases in overall richness (e.g. Kiffney *et al*. 2003; Couceiro *et al*. 2007) and changes in community equitability (increase in abundance of opportunistic taxa and reduction of environmentally sensitive ones) have been observed in macroinvertebrate communities after changes in riparian vegetation (Tomanova *et al*. 2006; Nessimian *et al*. 2008; Lorion & Kennedy 2009; Valle *et al*. 2013; Iñiguez–Armijos *et al*. 2014; Conigley *et al.* 2017). In this context, many studies focusing the required widths of riparian vegetation buffers to maintain the ecological integrity of stream macroinvertebrate communities were conducted (e.g. Rios & Bailey 2006; Lorion & Kennedy 2009; Iñiguez–Armijos *et al*. 2014). The results varied not only among targeted groups, but also among studies (e.g. Lee *et al*. 2004; Marczak *et al*. 2010; Moraes *et al*. 2014). Differences in regional (e.g. climate and land use types) or local (e.g. stream width, depth and slope, substrate) drivers of macroinvertebrate communities usually interact and co-variate, yielding contrasting results (Lee *et al*. 2004; Marczak *et al*. 2010).

In Brazil, the new Forest Code (Código Florestal no. 12.651, 2012) has reduced the width of riparian vegetation buffer that should be preserved along medium- to small-sized streams (≤10 m width) in private properties, from 30 to 15 m (both bank sides considered). However, reductions up to 5 m are legally authorized in small rural properties (article 61-A, law 2012; http://www.planalto.gov.br/ccivil_03/_ato2011-2014/2012/lei/l12651.htm), independently of stream size (width or order *sensu* Vannote *et al.* 1980) (Brancalion *et al*. 2016). The critical point of this new legislation is that approximately 53% of Brazilian native vegetation occurs on private properties (Soares-Filho *et al*. 2014). Additionally, studies focusing the effects of riparian vegetation reduction on aquatic communities are insufficient to support conservation policies (Couceiro *et al*. 2007; Nessimian *et al*. 2008; Moraes *et al*. 2014).

The influence of riparian vegetation on the occurrence of some genera and, consequently, on the riffle beetle community structure is certainly related to their detritivorous-herbivorous diets, and sensitivity to water pollution. Larval and adult stages of riffle beetles feed especially on organic material (e.g., algae, leaves, roots and woods; Seagle Jr. 1982). Thus, substrate type (inorganic *vs*. organic), which is directly related to riparian vegetation, is also a strong driver of riffle beetle communities (Brown 1987; Elliott 2008). On the other hand, depending on the availability of food items and particle size, larvae and adults can show different feeding habits, such as scrapers, shredders or collector-gatherers (Tomanova *et al*. 2006). Grain size of inorganic sediment (coarse *vs*. fine) is indirectly related to the size of particulate organic matter accumulated and to the abundance of encrusting microalgae occurrence (Vannote *et al*. 1980). Thus, not only sediment type but also organic particle size can also constitute a strong driver of riffle beetle communities. A scraper habit could explain the high abundance of certain riffle beetle genera and their life stages in montane streams with stony streambeds in southern Brazil (Braun *et al*. 2014), where leaf litter does not accumulate and microalgae grow. Finally, previous studies showed that some riffle beetle taxa are sensitive to water pollution (Paula & Fonseca-Gessner 2010; Corbi *et al*. 2013; Iñiguez-Armijos *et al*. 2014), which is directly affected by riparian vegetation removal (Rios & Bailey 2006; Lorion & Kennedy 2009; Iñiguez–Armijos *et al*. 2014).

In this study we tested the influence of different riparian vegetation widths (from more than 40 m to less than 5 m wide) and substrate types (inorganic *vs*. organic), as well as their interaction, on riffle beetle community structure. Based on the assumptions that riffle beetles are plastic in their feeding habits and sensitive to alterations in riparian vegetation and water quality, we hypothesize that narrower widths will favor more generalist taxa, while different substrate types will favor taxa with different feeding habits. Thus, we expect to find: i) changes in dominant taxa among riparian vegetation widths and between substrate types (inorganic *vs*. organic); and ii) lower equitability in narrower widths, as well as in organic rather than inorganic substrates.

MATERIAL AND METHODS

Study area

The study was conducted in the Sinos River basin, southern Brazil (51° W; 29° S; Fig. 1). The basin is located within the southernmost limits of the Atlantic Forest biome. The drainage area is of approximately 4,000 km^2, and is one of the main regions of urban development (IBGE 2010). Land use consists mainly of fields and pastures (48.4 %), forests (28.2 %), and agricultural areas (8.8 %) (Oliveira *et al*. 2013). The climate of the region is subtropical humid, and the average annual rainfall ranges from 1,200 to 2,000 mm (Maluf 2000). The Sinos River is approximately 190-km long and its headwaters are located at an elevation of 900 m, while its mouth, around 5 m a.s.l. (Rolon *et al*. 2003).

Four forest fragments were selected to conduct this study, comprising four different riparian vegetation buffer strip widths (hereafter 'buffer strips') in three permanent medium-order streams (one of 4th order and two of 5th order), totalizing twelve 400-m long fragments. We classified the buffer strips according to their width in: 1) wider than 40 m on both stream margins, 2) ranging from 15 to 30 m on both stream margins, 3) ranging from 5 to 15 m on both stream margins, and 4) narrower than 5 m on both margins (Fig. 1). Streams reaches and buffer strips selected to study were determined in order to allow discussing modifications proposed by the new Brazilian Forest Code (Brancalion *et al*. 2016).

In buffer strips wider than 40 m on both stream banks, the riparian vegetation can reach up to 1,000-m wide on one bank, and range from 210 to 440 m on the other bank. Although riparian vegetation widths followed a spatial gradient (narrower buffer widths were located downstream), environmental features such as stream order and surrounding land use, commonly associated with stream longitudinal gradient (Vanotte *et al*. 1980), which affect stream insects, did not change among the streams studied. In addition, the riparian vegetation fragments chosen for this study were non-contiguous, and the minimum distance between them was 1 km to increase the independence of the sampled areas. Some reduction of riparian forest occurred over the last 30 years to expanding areas for subsistence agriculture. However, native vegetation was relatively well preserved by the families inhabiting the adjacent areas to the studied streams. No forest reduction was observed during the sampling period. Tree richness was greater in fragments with wider buffer strips, but vegetal species composition was similar in all buffer strips (Oliveira *et al*. 2013).

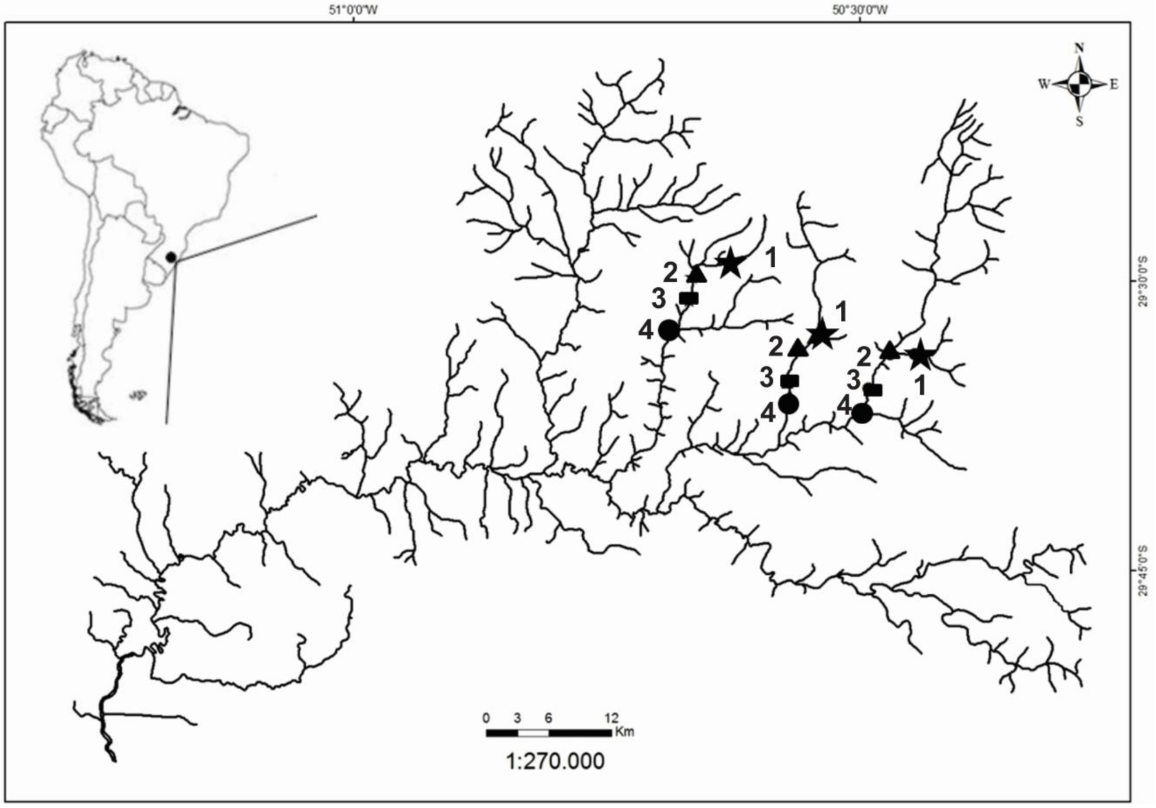

Figure 1 Location of the study area and sampling sites. Symbols indicate the categories of riparian vegetation widths selected for the study (stars, buffer strips 1 (>40 m); triangles, buffer strips 2 (30–15 m); rectangles, buffer strips 3 (15–5 m); circles, buffer strips 4 (<5 m)). Adapted from Viegas et al. (2014).

Sampling

Riffle beetles were collected in winter of 2010 and summer of 2011, during periods of low precipitation. A Surber sampler (30×30 cm frame; 250-µm mesh) was used to sampling in cobble (grain size: 20–28 cm, hereafter‘stone’) and gravel (grain size: 1–6.5 cm) substrates, in riffle/run areas. A dip net (30 cm diameter; 250-µm mesh) was used to collect the individuals from the leaf litter substrate in stream margin and pool habitats. Six subsamples were taken in each substrate (stone, gravel and litter), and pooled to form a single sampling unit. Two 1-m sweeps in the submersed litter constituted one subsample. Riffle beetles were stored in 80 % ethanol and taken to the laboratory, and samples were washed in a 0.25-mm mesh sieve. Samplings conducted in winter and summer seasons were pooled, according to each sampling unit. This procedure helped increase the number of individuals of each sampling unit as the influence of season on richness and abundance in the southern Brazilian riffle beetle communities is not yet clear (Braun *et al*. 2014). A total of 36 sampling units were used, corresponding to samplings conducted in each of the three substrate types (stone, gravel and litter), from each of the four buffer strips (1, 2, 3, 4), in each of the three streams studied.

Riffle beetles are poorly known at species level in Brazil (Domínguez & Fernández 2009; Segura *et al.* 2013), especially those inhabiting southern Brazilian rivers. Additionally, most individuals collected (> 66%) in Sinos' streams were larval stages, hindering to identify the individuals at species level. Thus, both riffle beetle life stages (adults/larvae) were identified here to genus level according to the taxonomic keys available to South America (Manzo 2005; Passos *et al*. 2007; Segura *et al*. 2011; Barbosa *et al*. 2013). Feeding habits of adult and larval life stages were assigned according to Merrit & Cummins (1996), Tomanova *et al.* (2006), and Oliveira & Nessimian (2010). Voucher specimens were deposited in the Collection of Aquatic Insects, Departament of Ecology and Evolution, of the Federal University of Santa Maria (UFSM), and in the Laboratory of Ecology and Conservation of Aquatic Ecosystems, of the Vale do Rio dos Sinos University (UNISINOS).

Environmental descriptors

Altitude, stream width, pH, water temperature, turbidity, electrical conductivity, an dissolved oxygen were measured *in situ* in the twelve streams segment studied. Physicochemical factors were analyzed with a multiparameter probe. The area (ha) of the land use classes concerning pasture, forest, agriculture and urbanization in each stream segment was calculated with help of the geographic information system ArcGis 10 (based on QuickBird images extracted from Google Earth Pro 4.2.1; images from April 2010). On each side of each stream, 50-m buffers were defined within concentric longitudinal 500-m windows over the sampling points and parallel to the water body, which were also used to calculate land use classes.

Data analysis

One-way analyses of variance (ANOVA) were used to test for differences in mean values of environmental descriptors, as well as mean areas of land use classes, among buffer strips, followed by a Tukey test. The level of significance used was 5%. For physicochemical factors, statistical analyses were performed using the mean values calculated for both winter and summer sampling periods.

Because the sampling method varied among substrate types, samples used in the multivariate analyses were standardized by dividing the abundance of each genus in the sample by the total abundance of the sample (*sensu* Moraes *et al*. 2014). Non-metric multidimensional scaling (NMDS) ordination diagrams were generated to assess the variation in larval and adult riffle beetle community structure among buffer strips and substrate types. Differences in riffle beetle community structure among buffer strips and substrate types were tested through a two-way non-parametric MANOVA followed by 9999 permutations (PERMANOVA) with streams as blocking factor. NMDS and PERMANOVA analyses were conducted with distance matrices (Bray-Curtis dissimilarity index) based on riffle beetle relative abundance matrix. Analyses were performed using the R *vegan* package (Oksanen *et al*. 2016).

Variation dominance of genera/life stages of riffle beetle communities among buffer strips and substrate types was tested through a factorial ANOVA, with streams as blocking factor. In order to meet the assumption of data homoscedasticity among substrate types, dominance values of litter samples were square-root transformed. Post-hoc Tukey tests were conducted for multiple comparisons among levels of buffer strips and substrate types. Simpson’s Diversity index was used as measure of dominance and calculated for each sample.

RESULTS

Environmental descriptors

No difference in mean values of the environmental descriptors measured (Table 1) among the different buffer strips were detected, except for electrical conductivity and forest area. Buffer strip 4 (< 5 m) showed higher values of electrical conductivity than other buffer strips ($F_{3,8}$ = 8,47; $P < 0.001$; Tukey test $P < 0.05$), and the area of the forest class was larger in buffer strip 1 (> 40 m) than in buffer strips 3 and 4 ($F_{3,8}$ = 9.701; $P = 0.004$; Tukey test $P < 0.05$).

Table 1. Mean and standard error values of the environmental descriptors taken in streams at each buffer strip

Buffer strips	**1**	**2**	**3**	**4**
Water temperature (°C)	20 (±0.15)	19.3 (±1.24)	19.6 (±0.8)	20.1 (±0.9)
pH	7.4 (±0.21)	7.4 (±0.18)	7.3 (±0.11)	7.5 (±0.2)

Dissolved oxygen (mg/L)	10.7 (±0.2)	10.5 (±0.63)	10 (±0.14)	9.9 (±0.27)
Electrical conductivity (ms/cm)	4.4 (±0.1)	4.7 (±0.2)	4.9 (±0.3)	5.4 (±0.5)
Turbidity (NTU)	10.2 (±1.2)	8.2 (±1.2)	7.6 (±1.3)	7.1 (±0.7)
Altitude (m)	152 (±18.5)	119 (±7)	73.6 (±4.7)	69.7 (±5.1)
Agriculture (ha)	1.4 (±0.8)	0.4 (±0.3)	1.74 (±0.6)	1.9 (±1.5)
Forest (ha)	9 (±0.6)	7 (±0.1)	4.9 (±0.7)	6.6 (±0.4)
Pasture (ha)	0.4 (±0.2)	3 (±1.5)	3.1 (±1.4)	2.9 (±1.5)
Urbanization (ha)	0.3 (±0.2)	1.5 (±0.2)	2.8 (±1.3)	1.2 (±0.1)

Riffle beetle community structure

We collected 6,785 individuals (2,270 adults; 4,515 larvae) over the sampling period. Eight riffle beetle genera were registered, all represented by both life stages. Two undetermined genera occurred only as larvae (Table 2).

Table 2. Composition, abundance, richness and Simpson's diversity index values of riffle beetle communities and number of individuals and functional feeding groups (FFG) of genera and their life stages to each buffer strip (1 = > 40 m, 2 = 30-15 m, 3 = 15-5 m, 4 = < 5 m) and substrate types (C = stone, G = gravel, L = leaf litter).

	FFG	Buffer strip				Substrate type			
		1	**2**	**3**	**4**	**C**	**G**	**L**	**Total**
Heterelmis(A)	CG,SC	164	72	75	74	209	99	77	**770**
Heterelmis	SH	225^{1}	353^{1}	350^{2}	220	577^{1}	228	343^{2}	**2296**
Hexacylloepus(A)	CG,SC	180^{3}	110	161	179	297	264^{4}	69^{4}	**1260**
Hexacylloepus	CG,SC	181^{2}	348^{2}	431^{1}	142	59	95	948^{1}	**2204**
Hexanchorus(A)	CG,SC	0	3	1	2	3	3	0	**12**
Hexanchorus	CG,SC	34	22	35	62	82	25	46	**306**
Macrelmis(A)	CG, SC	26	13	54	44	47	80	10	**274**
Macrelmis	CG,SC	41	73	128	477^{1}	375^{3}	336^{3}	8	**1438**
Microcylloepus(A)	CG, SC	141	35	87	47	181	69	60	**620**
Microcylloepus	CG	23	14	77	264^{3}	233	119	26	**756**
Neoelmis(A)	CG, SC	168^{4}	147^{3}	215^{4}	252^{4}	365^{4}	346^{2}	71^{3}	**1564**
Neoelmis	CG,SC	75	142^{4}	233^{3}	412^{2}	467^{2}	365^{1}	30	**1724**
Phanocerus(A)	CG, SC	3	2	4	4	6	3	4	**26**
Phanocerus	SH	18	29	33	29	39	14	56	**218**
Xenelmis(A)	CG, SC	3	1	2	1	4	2	1	**14**
Xenelmis	CG,SC	4	4	7	18	14	16	3	**66**
Genus A	-	0	2	0	1	0	3	0	**6**
Genus M	-	0	0	2	6	4	2	2	**16**
N		**1286**	**1370**	**1895**	**2234**	**2962**	**2069**	**1754**	**6875**

S	**15**	**17**	**17**	**18**	**17**	**18**	**16**	**18**
Simpson (1-D)	**0.71**	**0.71**	**0.71**	**0.67**	**0.8**	**0.79**	**0.51**	

Letter ‘(A)’ at the end of genus name indicates adult life stage. Superscript numbers following abundance values indicate the rank order of abundance (1 = 1st, 2 = 2nd, 3 = 3rd, 4 = 4th) of the four dominant genera/life stages (CG = collector-gatherers, SC = scrapers, SH = shredders, - = data unavailable).

The NMDS ordination diagrams displayed a clear structure among samples from different buffer strips and substrate types (stress = 0.12; Fig. 2). PERMANOVA tests showed that riffle beetle community structure significantly differed among different buffer strips (pseudo-$F_{3,24} = 3.63$; $R^2_{adj.} = 0.13$; $P < 0.001$), and especially among substrate types (pseudo-$F_{2,24} = 18.06$; $R^2_{adj.} = 0.45$; $P < 0.001$), although no interaction between buffer strips and substrate type was detected (pseudo-F = 1.47; $P = 0.13$). Some genera and their respective life stages were related to specific riparian buffer strips. For example, larvae of *Hexanchorus* Sharp, 1882, *Macrelmis* Motschulsky, 1859, *Microcylloepus* Hinton, 1935 and *Xenelmis* Hinton, 1936 were related to the narrower buffer strips (Fig. 2A). Other genera were also associated with different substrates. Larvae of *Heterelmis* Sharp, 1882, *Hexacylloepus* Hinton, 1940, *Hexanchorus* and *Phanocerus* Sharp, 1882 were related to leaf litter substrate. In turn, adults of *Heterelmis*, *Hexacylloepus*, and *Hexanchorus*, together with larvae and adults of *Microcylloepus, Neoelmis* Musgrave, 1935 and *Xenelmis*, as well as genera A and M, were associated with inorganic (gravel and stone) substrate (Fig. 2B).

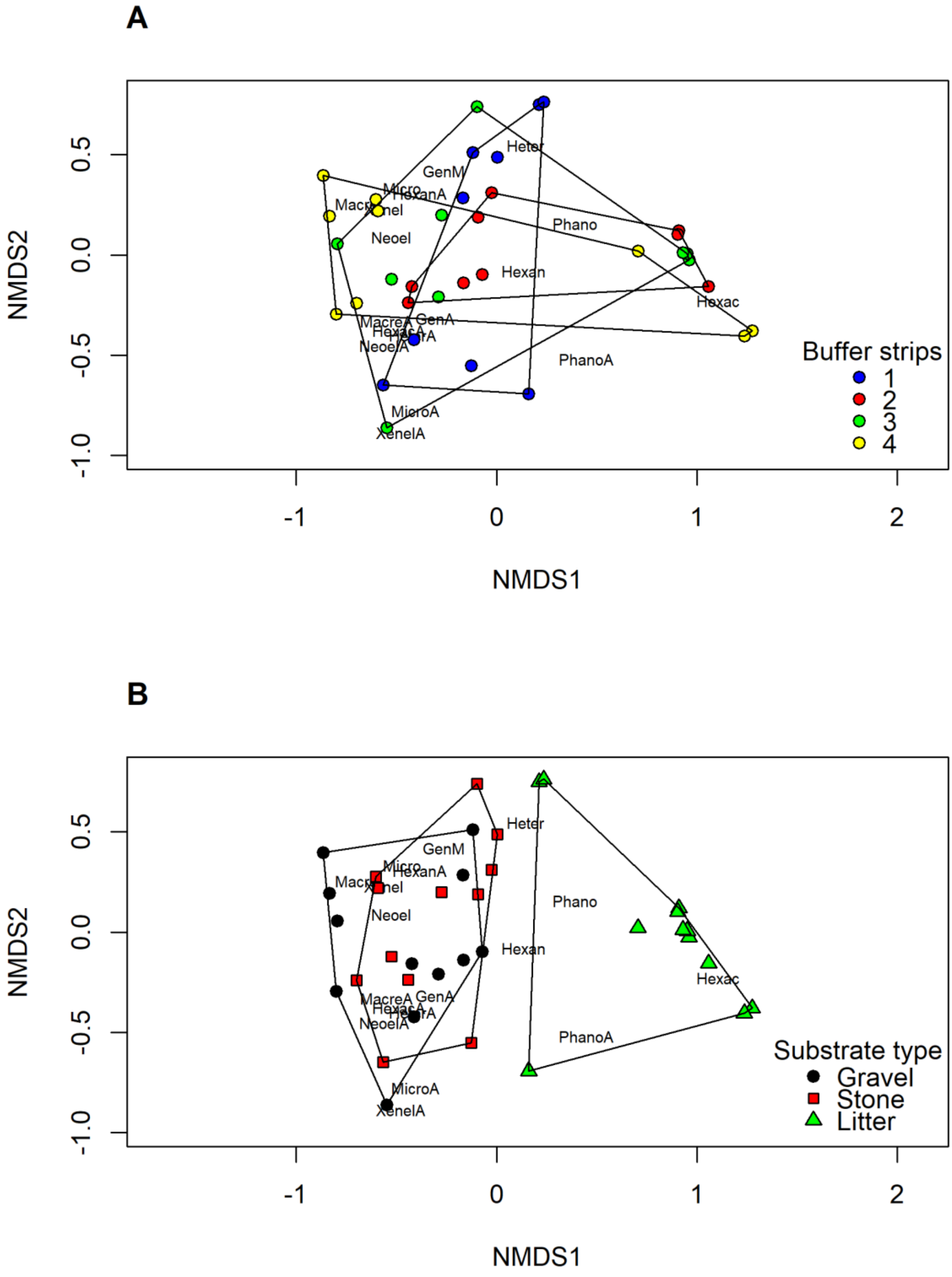

Figure 2 Non-metric multidimensional scaling (NMDS) ordination diagrams of samples according to: (a) buffer strips 1 (blue circle), 2 (red circle), 3 (green circle), and 4 (yellow circle) and: (b) substrate types Gravel (black circle), Stone (red square), and Litter (green triangle). GenA: Genus A; GenB: Genus B; Heter: Heterelmis; Hexac: Hexacylloepus; Hexan: Hexanchorus; Macre: Macrelmis; Micro: Microcylloepus; Neoel,: Neoelmis; Phano: Phanocerus; Xenel: Xenelmis. Note. Letter “A” at the end of genus name indicates adult life stage.

The dominant genera\life stage was similar among the three wider buffer strips (Table 2). Larvae of *Heterelmis*, as well as both life stages of *Neoelmis* and *Hexacylloepus*, dominated buffer strips 1, 2 and 3, despite variations in the order of their relative abundance. In turn, larvae of *Macrelmis* and *Microcylloepus* dominated buffer strip 4 (Table 2). The dominant genera changed among substrate types. Larvae of *Heterelmis* and *Macrelmis*, adults of *Hexacylloepus*, and both life stages of *Neoelmis* predominated and showed higher abundance in inorganic substrates (stone and gravel), in spite of different orders of relative abundance. In turn, larvae of *Hexacylloepus* and *Heterelmis* were dominant in litter substrate. *Hexacylloepus* also showed higher abundance in litter (Table 2).

A significant effect of substrate type was detected on dominance of riffle beetle genera/life stages ($F_{2,22} = 6.45$; $P < 0.001$). Dominance was higher in organic substrate than in inorganic substrate types (Tukey test < 0.01; Table 2). No effect of buffer strips was detected on dominance ($F_{3,22} = 0.43$; $P = 0.72$). The interaction between both factors was not significant either ($F_{6,22} = 1.69$; $P = 0.16$) (Fig. 3).

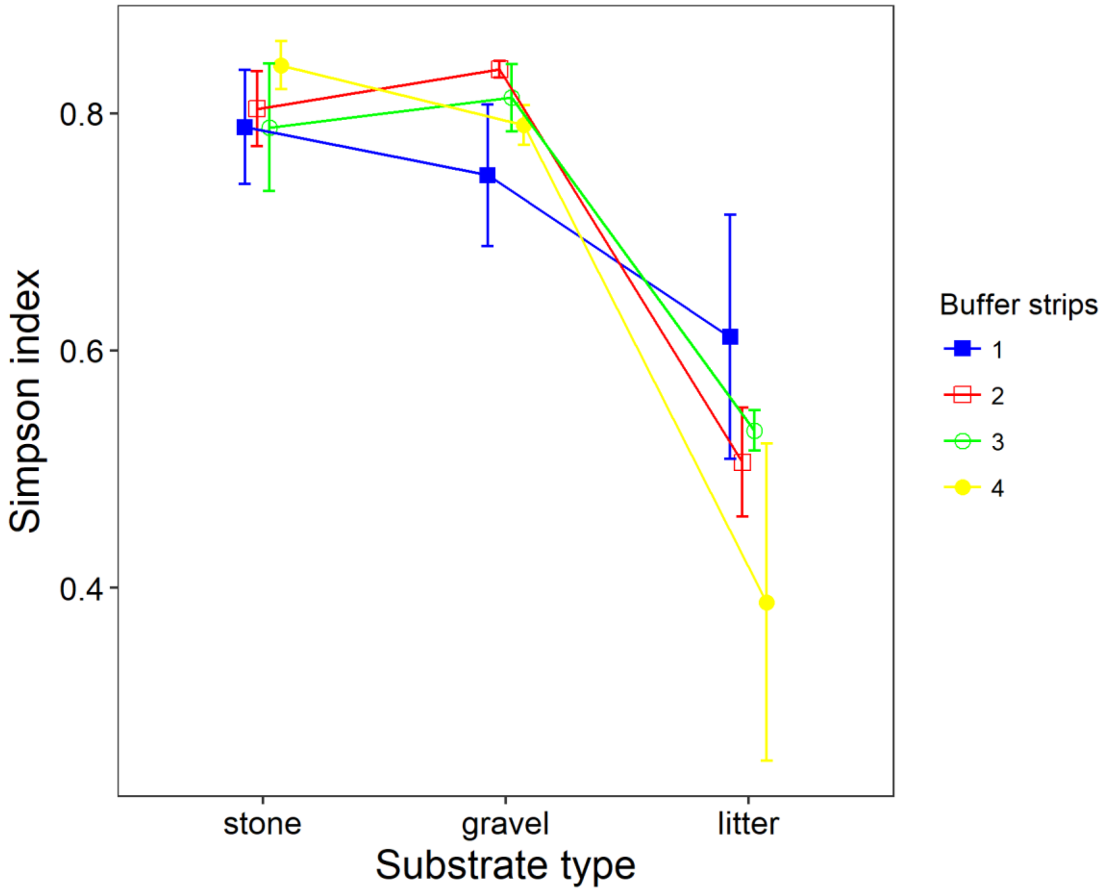

Figure 3 Interaction plot of Simpson' diversity index values among substrates types and buffer strips. Whiskers indicate standard error values. Buffer strips ( ) 1 (blue line), 2 (red line), 3 (green line), and 4. (yellow line).

DISCUSSION

Our study evidenced that changes in riparian vegetation width in streams of the Sinos River basin influenced riffle beetle community structure, supporting our main hypothesis. We also showed that the most extreme reductions in riparian vegetation width (up to 5 m wide) led to changes in dominant genera compared to wider ones. Thus, our results allow us to suggest that species of some riffle beetle genera could be important environmental indicators of stream ecological integrity. However, substrate type (inorganic *vs*. organic) had stronger influence on riffle beetle community structure than different riparian vegetation widths. The inorganic or organic nature of substrate determined differences not only on dominant genera but also in riffle beetle community equitability. In other words, at least along short longitudinal gradients (basin scale), substrate type may overcome the importance of other environmental variables that can influence the diversity of stream insect communities, such as riparian vegetation width (Moraes *et al*. 2014).

The role of the riparian vegetation in maintaining stream environmental integrity and conservation of aquatic communities is well documented (Rios & Bailey 2006; Couceiro *et al*. 2007; Nessimian *et al*. 2008; Lorion & Kennedy 2009; Iñiguez-Armijos *et al*. 2014). Previous studies show that larger riparian vegetation widths are required according to stream size in order to maintain their integrity (Rios & Bailey 2006; Lorion & Kennedy 2009, Iñiguez–Armijos *et al*. 2014; Moraes *et al*. 2014). The Brazilian Forest Code also ensures that high-order rivers have larger riparian vegetation widths, as in other countries (Lee *et al*. 2004). However, environmental laws have been historically neglected, and riparian vegetation, removed or converted throughout the Brazilian territory (Soares-Filho *et al*. 2014; Brancalion *et al*. 2016). In our study, reduction in riparian vegetation width affected environmental descriptors like electrical conductivity, changing the water quality of streams. Electrical conductivity was slightly higher in streams with the narrowest riparian vegetation width (< 5 m). Previous studies show that narrow riparian vegetation widths can have their buffer or filtering ecological role lost or minimized (Nessimian *et al*. 2008). They also show that reduced buffer strips favor water pollution by agricultural activities and domestic sewage (Sweeney 1993; Lorion & Kennedy 2009). Water pollution is associated with high electrical conductivity values (Stewart *et al*. 2000). In the studied area, only streams with riparian vegetation widths larger than 5 m were more protected against water pollution.

The most extreme reductions in riparian vegetation width were associated with changes in the dominant genera. Larvae of *Heterelmis* and *Hexacylloepus*, and larval and adult individuals of *Neoelmis* were dominant in streams protected by the widest riparian vegetation strips (buffer strips 1, 2 and 3). In turn, larvae of *Macrelmis* and *Microcylloepus* predominated in streams with the narrowest riparian vegetation (buffer strip 4). Changes in the composition of riffle beetle genera have also been recorded in previous studies. For instance, larvae of *Heterelmis* and *Hexacylloepus*, and adult individuals of *Neoelmis* have been found in streams with preserved riparian vegetation (Lorion & Kennedy 2009; Paula & Fonseca-Gessner 2010; Corbi *et al*. 2013; Iñiguez-Armijos *et al*. 2014). This result suggests that some species of *Macrelmis* and *Microcylloepus* could be tolerant to changes in water quality. Reduced riparian vegetation widths could favor the occurrence of more generalist macroinvertebrate taxa (Tomanova *et al*. 2006; Li & Dudgeon 2008) and affect the occurrence of sensitive aquatic insects (Conigley *et al.* 2017). In fact, m any riffle beetle taxa are sensitive to water pollution and high er levels of sedimentation, because these processes alter water quality and limit the offer of food resources such as epiphyton (Miserendino & Pizzolon 2004), and can be associated with reductions in riparian vegetation (Rios & Bailey 2006; Lorion & Kennedy 2009; Iñiguez–Armijos *et al*. 2014).

However, our prediction concerning equitability in riffle beetle communities among buffer strips was not corroborated. No difference in Simpson's index values was observed in communities inhabiting streams with different riparian vegetation widths. The width of riparian vegetation required to preserve macroinvertebrate community structure along medium- and low-order streams is still not clearly understood. A previous study conducted in the Sinos River basin, analyzing all macroinvertebrate families obtained a similar result. Changes in riparian vegetation width ranging from 30 to 5 m did not influence community descriptors, such as richness (Moraes *et al*. 2014). Thus, our results showed that riparian vegetation reductions do not determine differences in riffle beetle community equitability, although extremely narrow widths (< 5 m) can affect the composition of dominant genera/life stages.

Substrate type also affected riffle beetle community structure, supporting our hypothesis. In this study, substrate type had a stronger role than riparian vegetation width in driving riffle beetle communities. Dominant riffle beetle taxa changed between inorganic and organic substrates. *Heterelmis, Macrelmis,* and *Neoelmis* were dominant in inorganic substrates (stone and gravel), while larvae of *Hexacylloepus* corresponded to more than 50% of the total individuals found in organic substrate (litter). Larvae of *Heterelmis* and *Phanocerus* are considered shredders, while larvae of *Hexanchorus* and *Hexacylloepus*, scrapers and/or collector-gatherers (Tomanova *et al*. 2006). *Macrelmis* larvae have been found associated to stony substrates (Brown 1972). Leafy substrates usually show predominance of shredders (Graça *et al*. 2001). Additionally, non-dominant riffle beetles, such as larvae of *Phanocerus* and *Hexanchorus,* were more abundant in litter than in inorganic substrates. Differences concerning feeding functional groups can occur among species from a single riffle beetle genus and between life stages from some species (Tomanova *et al*. 2006; Elliott 2008). Plasticity in feeding habits of riffle beetles may also occur, according to geographic region and food availability (Tomanova *et al*. 2006). Thus, it is possible that feeding habits varied during the larval stages among species of *Hexanchorus* and *Hexacylloepus*.

Substrate type also influenced riffle beetle community equitability. Lower abundance and higher dominance were recorded in organic (litter) substrate (lower Simpson's index values). In turn, higher abundance and similar Simpson's index values were observed in stone and gravel substrates. In addition, abundance of riffle beetle and of dominant genera (*Macrelmis*, *Heterelmis* and *Neoelmis*) was higher in inorganic substrates than in organic ones (litter). The opposite was also evident (*Hexacylloepus* was more abundant in litter than in inorganic substrates). These results indicate that streambeds with coarse inorganic grain sizes provided more favorable environmental conditions for the occurrence of most riffle beetle genera than litter substrate. The higher equitability found in sedimentary substrates was possibly determined by the feeding habits of most riffle beetle genera and their life stages, which are mainly represented by scrapers and/or collector-gatherers in the studied region (Tomanova *et al*. 2006). Coarse inorganic substrates, such as cobbles and gravel, allow the growth of micro algae and periphyton (Príncipe *et al*. 2010), favoring the occurrence of scrapers (Merritt & Cummins 2006). Richer riffle beetle communities have also been recorded in other southern Brazilian streams characterized by cobble substrates (Braun *et al*. 2014).

No interaction was observed between riparian vegetation widths and substrate types in this study. The taxonomic composition of the riparian vegetation can determine the quality and the amount of allochthonous organic matter arriving into the streams, affecting substrate types (Graça *et al*. 2002; Hepp *et al*. 2008, Lacerf & Richardson 2010; Graça *et* *al*. 2015; Marquez *et al*. 2015). In this context, it is important to emphasize that the studied region is environmentally homogeneous. The drainage area of the Sinos River basin is well urbanized and many areas were converted into agricultural land use, with reduced forest fragments with riparian vegetation (Moraes *et al*. 2014). As observed by Oliveira et al. (2013), the species composition of the riparian vegetation is similar in the forest fragments of the studied area, although their extensions vary.

CONCLUSIONS

In this study we showed that the most pronounced reductions in the riparian vegetation width downstream (< 5 m) were associated with changes in riffle beetle community structure, especially dominant genera. Our results suggested that reduced riparian buffer widths occurred with higher levels of water pollution, affecting the occurrence of sensitive aquatic insects such as riffle beetles. Our findings have special importance for the conservation of stream insects in Brazil, as reductions in riparian vegetation width up to less than 5 m in each stream bank are allowed in small private rural properties by the new Forest Code (Código Florestal no. 12.651, 2012), independently of stream order. Thus we recommend that medium-order streams running in slope areas from Southern Brazil should be protected by riparian vegetation wider than 5 m, in order to preserve riffle beetle and other stream insect communities.

ACKNOWLEDGEMENTS

We thank Drs Ana Emília Siegloch (UNIPLAC), Luiz Ubiratan Hepp (URI), Maria Angélica Oliveira and Sandro Santos (UFSM) for their suggestions on an early version of the manuscript. This research was funded by PETROBRAS and UNISINOS. Cristina Stenert and Leonardo Maltchik hold CNPq Research Productivity grants. CAPES is thanked for granting scholarships to the first author and M.M. Pires.

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CAPÍTULO 3- POTENTIAL DISTRIBUTION OF RIFFLE BEETLES (COLEOPTERA: ELMIDAE) IN SOUTHERNMOST BRAZIL

Artigo formatado nas normas do periódico "Austral Entomology" ISSN 2052-1758, Fator de Impacto (JCR) = 1.341, Qualis Biodiversidade CAPES Estrato B1.

Potential distribution of riffle beetles (Coleoptera: Elmidae) in southernmost Brazil

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Running title: Potential distribution of Elmidae in south Brazil

Abstract

The knowledge on the diversity and occurrence/distribution of many freshwater insects is still insufficient in the Neotropical Region. Elmidae are aquatic Coleoptera that have been considered important indicators of environmental integrity. However, in the Brazilian territory, the country with the largest area and highest richness of Elmidae in the Neotropics, many areas remain poorly studied in relation to the knowledge of the diversity of the family. The southernmost region of Brazil (state of Rio Grande do Sul) is reported to hold elevated richness of stream insects. Thus, in this study we used species distribution models (SDMs) to estimate the potential distribution of Elmidae taxa in the region, in order to highlight areas that should deserve higher priority for future sampling efforts regarding the knowledge of Elmidae diversity. We predicted the potential distribution of the occurring Elmidae genera and *Macrelmis* species in the region in relation to climatic, topographic, hydrographic and land cover predictors. Our results suggested that montane regions and basins located within well-preserved forested regions showed the higher potential richness of Elmidae. In addition, the areas with higher predicted suitability for the occurrence of Elmidae genera overlapped with the suitable areas for the species of *Macrelmis*, suggesting an elevated ecological similarity among most of the modelled taxa. The most important environmental predictors related to the predicted distribution of Elmidae taxa were associated with climate (precipitation) landscape (forest cover) and topography. These predictors probably interact to provide suitable local environmental conditions for the occurrence of Elmidae genera and *Macrelmis* species. Freshwater ecoregions were also suggested as important drivers of Elmidae distribution, probably due to the low dispersal ability of both larva and adult individuals. Finally, we strongly recommend that future studies aiming at biodiversity inventories related to Elmidae diversity and distribution in South America consider with closer attention watersheds located in montane and forested regions.

Key words: aquatic insects, Neotropical Region, species distribution models, spatial distribution, *Macrelmis*

INTRODUCTION

Freshwater ecosystems hold a great diversity of species and high levels of endemism (Collen *et al*. 2014). On the other hand, there is growing evidence that freshwater biodiversity is under increasing threat, with many species facing global declines in their distribution and populations (Strayer & Dudgeon 2010). Most impacts on freshwater ecosystems are associated with the intensification of anthropic activities, which include land cover conversion, urbanization and pollution (Vörösmarty *et al*., 2010). Therefore, the conservation of aquatic biodiversity strongly relies on the protection of freshwater habitats (Dudgeon *et al.* 2006; Poff *et al.* 2012). However, the diversity and occurrence of several freshwater species remain unknown in many regions of the globe. In general, the knowledge on biodiversity is still insufficient due to two main factors: the majority of species remains to be formally described, and the actual distribution range is unknown for most species (Linnean and Wallacean shortfalls) (Bini *et al.* 2006; Diniz-Filho *et al.* 2010). The unavailability of such information hinders the understanding of large-scale diversity patterns and biogeographic relationships of species (Thomas *et al.* 2008). Consequently, it also hinders the development of adequate conservation policies, including the selection of preservation areas in the freshwater realm (Williams *et al.* 1996).

The understanding of species' distributions along environmental gradients, as well as the knowledge on the ecological predictors associated with their geographic range, is of high importance to biodiversity conservation and to the protection of threatened species (Peterson *et al.* 2001; Anderson *et al*. 2003). Species distribution models (SDM) have been employed to predict the distribution range of a species based on a combination of occurrence data and information on the environmental predictors from the known occurrence localities (Sánchez-Fernández *et al.* 2011). These modelling approaches identify 'optimal' environmental conditions, which assumedly describe the fundamental niche (Hutchinsonian niche) of the target species (Soberón & Peterson 2005; Phillips *et al.* 2006) and estimate the suitability of a geographic space for the occurrence of the modelled taxa (Phillips *et al.* 2006). Representations of SDMs show the potential geographic distribution range of the target species (Colwell & Rangel 2009). This framework has been extensively used for conservation purposes, such as the selection of preservation areas for threatened species and identification of priority areas for future sampling efforts (Soberón & Peterson 2005; Diniz-Filho *et al.* 2010).

Riffle beetles (Elmidae Curtis, 1830) are a cosmopolitan family of freshwater coleopterans that predominantly inhabit clean and well-oxygenated running waters, with both adult and larval stages showing aquatic habits (Brown 1987). Elmidae have been considered important indicators of environmental integrity, because they are sensitive to changes in the structure and physicochemical conditions of aquatic ecosystems (Brown 1987; Jäch & Balke 2008). In fact, the community structure and the occurrence of many genera are influenced by the presence of native vegetation, and water temperature and oxygen content values (Passos *et al.* 2003 a;b; Braun *et al.* 2014a, b; Braun *et al.* 2018a, b). Studies conducted at restricted spatial scales (i.e., stream-, within basin-level) show that the spatial distribution of Elmidae is influenced by environmental factors such as elevation, substrate type (organic; inorganic) and riparian vegetation structure (Site *et al.* 2003; Elliott 2008; Jäch & Blake 2008; Braun *et al.* 2014b; 2018b). However, few studies have focused the factors driving the spatial distribution of their assemblages (Passos *et al.* 2003 a;b; Braun *et al.* 2014b; 2018b), and the knowledge of the factors influencing Elmidae distribution at broader spatial scales (i.e., regional-level, biogeographic scales) are poorly understood. Reviews on the global distribution of aquatic Coleoptera indicate that temperature, altitude and forested areas are important drivers of the diversity of freshwater beetles (Ribera *et al.* 2003; Eyre 2006; Calosi *et al.* 2010; Boyero 2011; Bruno *et al.* 2012; Guareschi *et al.* 2012; Pérez-Bilbao *et al.* 2014). Thus, Elmidae assemblages should be also influenced by such drivers.

Studies on Elmidae diversity and distribution remain particularly scarce in the South American continent (Jäch & Balke 2008; Domínguez & Fernández, 2009). Approximately 435 species were assigned to the Neotropical Region, though many areas are still underrepresented (Manzo *et al.* 2013). For instance, in Brazil, which covers ca 40% of the region, only 151 species from 46 genera were recorded, and most inventories were conducted in the southeastern and the Amazon regions (Segura *et al.* 2013). In turn, in extreme southern Brazil (state of Rio Grande do Sul, RS), which concentrates most recent studies related to the ecological drivers of Elmidae assemblages (Braun *et al.* 2014b; 2018a, b), only four species (*Neoelmis atys*, *Neoelmis mila*, *Neoelmis mormo* and *Neoelmis nicon* (Hinton 1972) have been recorded (Segura *et al.* 2013). However, the 12 genera recorded by the ecological studies (Braun *et al.* 2014b; 2018b) suggest that the family could be well-diversified in this region. In fact, montane streams from southernmost Brazil are reported to hold elevated richness of stream insect groups (e.g., Floss *et al.* 2012; Salvarrey *et al.* 2014; Kotzian *et al.* 2015), also indicating that the southernmost Brazilian region is a potential interesting area for aquatic biodiversity conservation.

In this study, we used species distribution models (SDMs) to estimate the potential distribution of Elmidae taxa in the RS, in order to highlight areas of high diversity of this family and suggest areas that should deserve higher priority for future sampling efforts regarding the knowledge of Elmidae diversity. Considering the importance of local and regional environmental predictors in the spatial distribution of riffle beetles (Passos *et al.* 2003a; b; Site *et al.* 2003; Elliott 2008; Jäch & Blake 2008; Braun *et al.* 2014b; 2018b), we modelled their potential distribution in relation to large-scale climatic and landscape predictors (topographic, hydrological and land cover). We estimated the potential distribution of two taxonomic categories based on the availability of the distribution data in the target area and from the consulted datasets. In other words, we modelled the responses of each Elmidae genus and each species of *Macrelmis* Mostchulsky, 1859 occurring in RS. In addition, we estimated the resemblance in the predicted distribution of the modelled taxa in order to investigate niche similarity among Elmidae genera and *Macrelmis* species. Resemblance in SDM distribution provides useful information on the niche (ecological) similarity among Elmidae taxa. This approach allows further insights on the specific ecological factors that favor the occurrence and distribution of the studied species, contributing to increase the knowledge on their distribution.

MATERIAL AND METHODS

Study area and riffle beetle occurrence records

The target area of the modeling procedures was the state of Rio Grande do Sul (RS) (Fig. 1). However, we chose to expand the range of occurrence sources of the modelled taxa in order to cover a broader range of environmental conditions, what allows estimating more accurate predictions, according to the suggestions of Sánchez-Fernandez *et al*. (2011). Thus, we retrieved Elmidae records in South America ranging between parallels 20° and 50°S, and meridians 45° and 70°W. This range covered the south and southeastern regions of Brazil (states of São Paulo, Paraná, Santa Catarina and Rio Grande do Sul), Paraguay, Uruguay and part of the Argentinean territory (Fig 1). The Elmidae occurrence dataset for RS included records from published papers and the following scientific collections: UNIPAMPA (Universidade Federal do Pampa); URI (Universidade Regional Integrada do Alto Uruguai e das Missões); UNISINOS (Universidade do Vale do Rio dos Sinos) and UFSM (Universidade Federal de Santa Maria). These procedures allowed us to build SDM for the following taxa: *Austrolimnius*, *Cylloepus*, *Gyrelmis*, *Heterelmis, Hexacylloepus, Hexanchorus, Macrelmis, Microcylloepus, Neoelmis*, *Phanocerus*, *Stegoelmis*, *Xenelmis*, *Macrelmis aeolis*, *Macrelmis alea*, *Macrelmis amana*, *Macrelmis celsa*, *Macrelmis codris*, *Macrelmis isis*, *Macrelmis plaumanni*. Genera and *Macrelmis* species not recorded in RS were not included in the dataset. Overall, 684 occurrence records were used in the analyses (611 records of Elmidae genera; 73 records of *Macrelmis* species) (Supplementary material).

To build the dataset, we used geographic coordinates whenever possible, and in some cases, the coordinates were estimated based on site descriptions using Geographical Information System tools (GIS). Genera records included larval and adult individuals, while for *Macrelmis* species records, only records of adult individuals were used (Supplementary material).

Environmental predictors

The environmental predictors used in the modelling procedures were obtained from the following databases: climate (dataset of 19 bioclimatic variables) and altitude predictors were retrieved from the WorldClim (http://www.worldclim.org/; Hijmans *et al*., 2005). Hydrographic predictors (slope, drainage and flux) were obtained from the HYDRO 1K (https://lta.cr.usgs.gov/HYDRO1K). Land cover predictors comprised data on areas of forest and grassland from the Harmonized World Soil (HWSD: http://webarchive.iiasa.ac.at/Research/LUC/External-World-soil-database/HTML/). Topographic data comprised the Vector Ruggedness Measure (VRM), a measure of terrain ruggedness, and were downloaded from the software ArcGIS 10.1 (http://www.arcgis.com/home/item.html?id=9e4210b3ee7b413bbb1f98fb9c5b22d4). Ecoregions were also considered predictors of Elmidae distribution, and they were obtained from the Freshwater Ecoregions of the World database (FEOW; http://www.feow.org/). All environmental predictors were downloaded at a spatial resolution of 30 arcseconds (pixels of ~1km^2 area) and the description of the ecological importance of each predictor is given in Table 1.

With regards to the climatic predictors, the bioclimatic variables were subject to correlation prior to the modelling due to their intrinsic collinearity. The Pearson coefficient correlation (r) was used to retain the least correlated variables. Correlation values ranging between -0.8 and 0.8 were used to select the environmental variables used in the modelling. This procedure retained the following bioclimatic variables: Bio1, Bio2, Bio 12, Bio13, Bio14 and Bio 15 (for their description, see Table 1). Correlations were carried out in the ENMTools 1.3 software (Warren & Seifert 2011).

Species distribution models

Species distribution models (SDM) rely on abiotic and/or biotic predictors to estimate the potential distribution of any target species, using georeferenced occurrence localities of the former (Peterson 2001; Peterson *et al*. 2002). SDMs are built on algorithms that estimate the occurrence probabilities of finding the target species along the axis of each predictor, based on the set of predictors of each locality. The joint information are combined, and the final result is projected on a region of interest, allowing for the mapping of the environmentally suitable areas for the occurrence of the target species (Elith *et al.* 2006).

The maximum entropy algorithm was selected for the modelling procedures. This algorithm builds an envelope of suitable environments, which is extrapolated to the study area, creating a gradient of suitability for the target species (Philips *et al*., 2004). It is also considered the most efficient algorithm, when only presence records of target species are available (Elith *et al*. 2006). The software MaxEnt version 3.4.0 (Philips *et al*. 2017) was used to generate the SDMs.

Model assessment and validation

Model efficiency was assessed through the area under the Receiver Operating Characteristic (ROC) curve (AUC). The AUC is a method that assesses the specificity (lack of omission error, i.e. exclusion of correct presences) and the sensitivity (absence of omission error, i.e., inclusion of incorrect presence) of a diagnostic test (Fielding & Bell 1997). The AUC provides a measure of the fulfillment threshold, independent of the model performance (threshold-independent measure), in comparison to the null expectation (Fielding & Bell 1997), and is the most common statistic of assessment of SDM performance (Elith *et al*. 2006). An AUC value of 0.50 shows that the model performance is no better than chance. Very high AUC values indicate better models, and a perfect prediction would have a value of 1.0 (Hanley & McNeil 1982).

The models produced an environmental equation for each species, which varies according to the environmental variability. To obtain the Potential Richness Distribution maps (PRD), the raster files generated for each taxa by the MaxEnt software were first reclassified into binary files (suitable and non-suitable habitats). For this reclassification, the threshold value used to determine which pixels should be considered a suitable habitat was the mean of logistic test points produced in each model for each species in MaxEnt. This approach considers the prevalence model calculated by MaxEnt, and is an efficient way to select the adequate threshold of the SDM (Liu *et al.* 2005). The priority areas were identified using the maximum number of species in all study area. The PRD map was drawn at a spatial resolution of 30 arcseconds (pixel area 1 km^2).

In order to assess the resemblance in the ecological requirements and distribution among the modelled taxa (Elmidae genera and species of *Macrelmis*), the values of suitable habitat for each taxa produced by MaxEnt were subject to correlation (Pearson coefficient). We considered taxa with high resemblance in their ecological requirements whenever the correlation between two taxa was < 0.8. The values of the Pearson product-moment correlation were calculated in the software ENMTools version 1.3 (Warren & Seifert 2011).

RESULTS

The species distribution models yielded values of AUC ranging from 0.881 to 0.999, and indicated a high prediction capability for most taxa. The five variables that showed the greatest percentage association with the modelled taxa were: precipitation of driest month (Bio14) and precipitation seasonality (Bio15), Forest cover, Ecoregions and Slope (Table 2).

In relation to the potential richness of Elmidae genera in the state of RS, two areas were predicted to hold higher PRD: the northwestern and eastern regions of the state (Fig 2A). For species of *Macrelmis*, the regions predicted to hold higher PRD were found to be located in the northernmost and eastern regions of RS (Fig 2B).

Correlations between the outputs of the SDMs of Elmidae genera showed a higher ecological resemblance between *Hexacylloepus* and *Heterelmis* (r= 0.983), *Microcylloepus* and *Macrelmis* (r= 0.925), *Neoelmis* and *Microcylloepus* (r= 0.935), *Neoelmis* and *Heterelmis* (r= 0.918), and *Neoelmis* and *Hexacylloepus* (r= 0.911). For *Macrelmis* species, the highest resemblances were found between *Macrelmis codris* and *Macrelmis aeolis* (r= 0.884), and *Macrelmis amana* and *Macrelmis alea* (r= 0.764).

DISCUSSION

Potential distribution of riffle beetles in southern Brazil and Conservation implications

The high values of AUC show that the species distribution models (SDMs) represent an important tool for recognizing areas with elevated potential for the occurrence of rich Elmidae and *Macrelmis* assemblages. The outputs of the SDMs estimated higher potential richness and environmental suitability in three main regions in the state of Rio Grande do Sul: i) north- and northwestern, ii) central-western and iii) eastern regions. The north- and northwestern region, as well as the eastern region, comprise areas located within the

Atlantic Forest biome (Prado *et al.* 2000). Atlantic Forest remnants are still present in these areas, such as the Parque Estadual do Turvo (PET), despite the elevated conversion rate occurring in the state (Fundação SOS Mata Atlântica 2017). Forested biomes hold high elevated richness of Elmidae throughout many Brazilian regions, such as Amazon Rainforest and Atlantic Forest (Jäch & Blake 2008). The central-western region comprises slope landscapes between Southern Plateau and Central Depression (Rolon *et al*. 2003). This region holds elevated diversity of genera of Elmidae (Braun *et al.* 2018b) and other stream insects (Moraes *et al*. 2014; Kotzian *et al*. 2015).

Predictors of riffle beetle distribution

The species distribution models (SDMs) also allow the recognition of spatial large-scale environmental factors, or macrovariables, driving the potential distribution of Elmidae genera and species of *Macrelmis* in southernmost Brazil. The main variables were related to climate (precipitation of driest month (Bio 14) and precipitation seasonality (Bio 15)) and landscape (Forest cover, Ecoregions and Slope). Some of these predictors are closely associated with in-stream environmental conditions affecting the spatial distribution of Elmidae assemblages. For instance, the climatic predictors associated with precipitation (Bio 14 and Bio 15), and the landscape and topographic predictors related to terrain declivity (Slope) strongly influence water level and discharge. Higher precipitation and declivity increase water discharge and velocity in streams, which in turn influence water dissolved oxygen concentration and temperature (Allan & Calisto 2007).). Elmidae is an insect group with both adult and larval stages living in water. Thus, a permanent water supply in streams, throughout the seasons, especially during the driest months could guaranty the occurrence of more diversified assemblages. Montane regions with higher declivity, in turn, promote water oxygenation, an important environmental driver of Elmidae assemblage at local spatial scale (Braun *et* al. 2014a). Additionally, a permanent water current downstream is known for contributing to Elmidae dispersal, as well as to other aquatic insects (Bispo *et al*. 2004; Elliot 2008)

Forest cover was also an important predictor of richness of Elmidae taxa. Locally, forest cover affects Elmidae mainly through the influence of riparian vegetation on in-stream ecological processes. Riparian vegetation influences water temperature, and consequently water oxygenation, and also provides allochthonous material input, the main food source of riffle beetles (Lorion & Kennedy 2009; Tank *et al.* 2010). In addition, several studies showed that the conversion of forested habitats has important effects on Elmidae composition (Braun *et al.* 2018a, b). In a broader spatial perspective, forested regions (biomes) are also important for the occurrence of many riffle beetles species (Jäch & Blake 2008). Besides ensuring suitable local habitat conditions to the establishment of riffle beetles, the composition of riparian vegetation also influences the quality of allochthonous material, because it changes across biomes (e.g., Amazon Rainforest and Atlantic Forest; Graça *et al.* 2015). Previous studies have showed that the nature of vegetal remains arriving into the streams can influence the assemblage composition of Elmidae (Braun *et al.* 2018b).

Freshwater ecoregions were found to influence riffle beetles. The role of ecoregions in explaining large-scale diversity patterns of freshwater species was first proposed by Abbel *et al*. (2008), mainly based on the distribution of fish species. However, ecoregions comprise several features, such as climate, topography and vegetation type, that can influence the occurrence of other freshwater taxa too (Vasconcelos *et al.* 2013). Elmidae assemblage composition varies across different Brazilian regions, as shown by many studies (Passos *et al.* 2010; Segura *et al.* 2013; Braun *et al.* 2018b). In fact, remarkable differences in biogeographic predictors, such as climate and biomes, occur within the Brazilian territory. However, in narrower spatial scales, the influence of freshwater ecoregions should have also be related to ecological barriers to dispersal. Elmidae are insects that passively disperse through water drift (Elliott 2008). Thus, even short distances could hinder the exchange of species composition among streams from different ecoregions. Additionally, the high rates of forest conversion in the studied region should also affect important drivers of Elmidae diversity, such as presence of native and riparian vegetation. The removal of riparian vegetation along streams could increase the spatial disruption among different ecoregions (Muneepeerakul *et al.* 2008).

Ecological resemblance among Elmidae genera and Macrelmis species

The majority of Elmidae genera showed high resemblance in their predicted distributions, which suggests an elevated niche (i.e., ecological) similarity among them. The high ecological resemblance among the predicted distribution of the modelled taxa indicate that similar predictors drive the distribution of Elmidae in the study region. *Hexacylloepus* and *Heterelmis* were registered in sites showing large fragments of riparian vegetation, as well as the presence of both organic and inorganic substrates (Passos *et al.* 2003; Braun *et al.* 2014; Braun *et al.* 2018b). *Microcylloepus* and *Macrelmis* were also influenced by similar environmental drivers. They were recorded in streams showing high water velocity and narrower riparian vegetation buffers, which indicates that they can be tolerant to changes in water quality (Braun *et al.* 2014b; 2018b). *Neoelmis* showed high resemblance with

Microcylloepus and *Heterelmis*, and their individuals could be found in litter and inorganic substrates, living in shallow waters with high water velocity (Passos *et al.* 2003; Passos *et al.* 2007; Braun *et al.* 2014a, b).

Correlations among species distribution models (SDMs) showed a strong ecological similarity among some species of *Macrelmis*. Studies providing information about Elmidae distribution at broader spatial scales are scarce in the literature. The occurrence of Elmidae species in regions with temperate climate is commonly related to substrate type (organic; inorganic), macrophyte presence, and water temperature and dissolved oxygen values (Elliott 2008; Braun *et al.* 2014b; 2018). *Macrelmis codris* and *M. aeolis* showed a high niche similarity, and were recorded in the eastern region, in basins located in areas of Atlantic Forest (personal communication). These species were previously recorded in other Brazilian states located within the Atlantic Forest (Hinton 1946; Brown 1984), suggesting that they are closely related to areas with forested biomes. *Macrelmis amana* and *M. alea* also showed strong niche similarity, and were recorded in streams with strong water current and stony and gravel substrates, and high dissolved oxygen content (personal communication).

Conclusions

Our results suggested that montane regions and basins located within well-preserved forested regions showed the higher potential richness of Elmidae in southernmost Brazil (state of Rio Grande do Sul). More specifically, results from species distribution models showed an overlap between the areas with higher suitability for the occurrence of Elmidae genera and species of *Macrelmis* occurring in the state of Rio Grande do Sul. The most important environmental drivers related to the predicted distribution of Elmidae taxa in the study region were associated with climate (precipitation), landscape (forest cover) and topography (declivity), which probably interact to provide suitable local environmental conditions for the occurrence of Elmidae genera and species. In addition, freshwater ecoregions were also suggested as important drivers of Elmidae distribution, probably due to the low dispersal ability of Elmidae individuals. Nevertheless, we also point out that other local environmental drivers of Elmidae composition, such as substrate type and riparian vegetation structure should also be considered in order to predict the distribution of Elmidae taxa with higher accuracy. Finally, we strongly recommend that future studies aiming at biodiversity inventories related to Elmidae diversity and distribution in South

America should consider with closer attention watersheds located in montane and forested regions.

Acknowledgements

We are grateful to Marcia Regina Spies (UNIPAMPA), Luiz Ubiratan Hepp (URI) and Cristina Stenert (UNISINOS) for gently providing access to Elmidae specimens. We thank Ana Emília Siegloch (UNIPLAC), Brunno Henrique Lanzellotti Sampaio (UFRJ), Cristiane Biasi and Luiz Ubiratan Hepp for their comments on an original version of the manuscript. CAPES (Coordination for the Improvement of Higher Education Personnel) granted a PhD scholarship to the first author and a postdoctoral fellowship to MMP. ASG was granted an Institution Training Program postdoctoral fellowship by INPE (National Institute of Space Research).

Disclosure of interests

The authors declare no conflict of interest.

Contribution of authors

BMB and ASG conducted the modelling procedures. BMB, ASG, MMP and CBK participated in the drafting of the manuscript. All authors read and approved the final version of the manuscript.

Supporting Information

S1. Geographic coordinates (decimal degrees) of the occurrence localities of the Elmidae taxa (genera and species of *Macrelmis*) used the modelling procedures.

S2. Individual maps of suitable and unsuitable areas for the occurrence of Elmidae taxa (genera and species of *Macrelmis*).

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Table 1. Predictors variables used in the Species Distribution Models, their abbreviations, sources and description of their ecological importance.

Source	**Predictors**	**Abbreviation**	**Ecological Importance**
Worldclim	Annual mean temperature	Bio1	Temperature variation can affect genera and species of Elmidae distribution.
	Mean diurnal range	Bio2	Diary temperature tends to fluctuate significantly in headwaters, and affect water temperature and oxygen content.
	Annual Precipitation	Bio12	Annual precipitation is directly related with water supply, and indirectly with water velocity and oxygen content.
	Precipitation of Wettest Month	Bio13	High precipitation values can be related to very strong water current, affecting substrate and population stability, and species dispersion
	Precipitation of Driest Month	Bio14	High precipitation values in driest periods is related to water supply, and affect water current, temperature, and oxygen content.
	Precipitation Seasonality	Bio15	Similar precipitation values along years, with scarce periods of dryness, provide a more stable environment, ensuring permanent water supply, and good conditions of water temperature and oxygenation.
	Altitude	Alt	Temperature tends to be colder at higher elevations, influencing water dissolved oxygen values.
HWSD	Grassland cover	Grass	Grassland cover do not provides shadow to small streams, allowing water temperature elevation and decrease in water oxygen values. Allochthonous organic material arriving into the streams is also scarce, affecting the occurrence of many Elmidae.
	Forest cover	Forest	Forest cover blocks the entry of solar light into the small, providing stability in water temperature and oxygen content. It also provide allochthonous organic material, a very important food item for the Elmidae.
Hydro 1K	Aspect	Aspect	This predictor is related to the terrain declivity and stream course direction. Thus, it is related to stream luminosity, which affects water temperature and oxygen values, as well as to algae grow. Algae is an important food item for certain Elmidae.
	Slope	Slope	Declivity is related to water velocity, and consequently is related to water oxygen content.
	Flow direction	Drainage	Flow direction indicates the water route. Thus, it could be related substrate accumulation, favoring the occurrence of more heterogeneous habitats. It can also be related to luminosity.
	Flow accumulation	Flux	Sites showing small values of flow accumulation are related to headwaters. Stream order is na important predictor of macroinvertebrate diversity, as well as of Elmidae diversity. Stream order also affects water temperature and oxygen content values, and allochthonous and autochthonous organic material input
ArcGIS	Vector Ruggedness Measure	VRM	Important information on habitat heterogeneity, dispersal capacity, and therefore help to better understand the processes that shape the formation of communities
FEOW	Freshwater Ecoregions of the World	Ecoregions	Ecoregions comprise different watershed, which represent geographical barrier to aquatic adult insects, such as Elmidae, affcteing species distribution. It also comprised features, such as climate, topography and vegetation type.

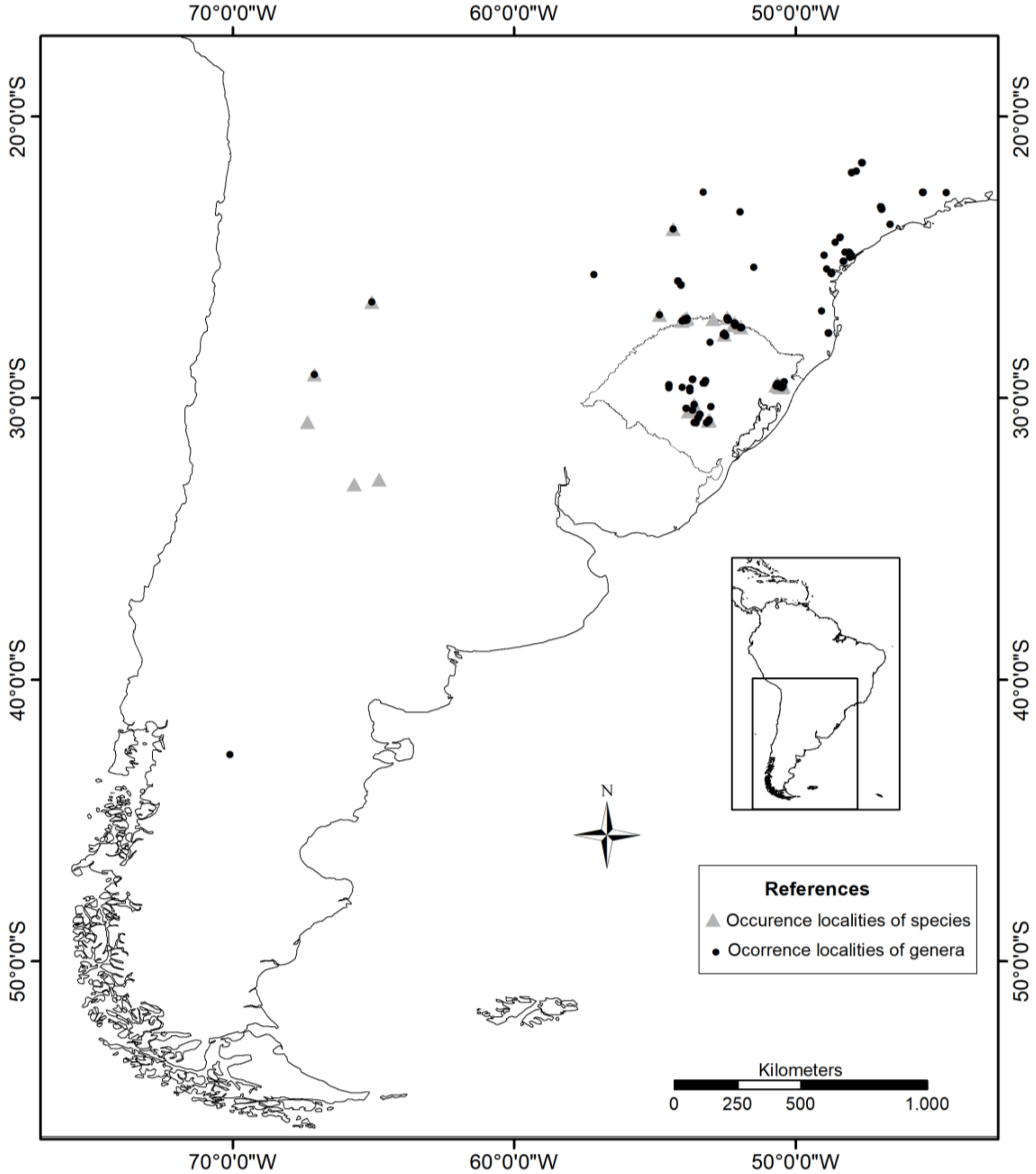
Table 2. Area Under the Curve (AUC), threshold of suitable habitat values and relative importance of the most important predictor to the distribution of genera of Elmidae and species of *Macrelmis*. (Bio 14 = precipitation of driest month; Bio 15 = precipitation seasonality)

Genera/Species	AUC	Threshold of suitable habitat	Predictor variable (%)
Austrolimnius Carter & Zeck, 1929	0.998	0.81	Forest (33.1)
Cylloepus Erichson, 1847	0.991	0.80	Ecoregions (20.2)
Gyrelmis Hinton, 1940	0.984	0.74	Forest (36.2)
Heterelmis Sharp, 1882	0.990	0.75	Ecoregions (41.4)
Hexacylloepus Hinton, 1940	0.992	0.77	Ecoregions (33.4)
Hexanchorus Sharp, 1882	0.998	0.85	Bio14 (23.5)
Macrelmis Mostchulsky, 1859	0.985	0.73	Bio14 (33.9)
Microcylloepus Hinton, 1935	0.992	0.77	Bio14 (16.0)
Neoelmis Musgrave, 1935	0.991	0.76	Bio14 (45.0)
Phanocerus Sharp, 1882	0.996	0.78	Forest (34.7)
Stegoelmis Hinton, 1939	0.932	0.78	Forest (62.2)
Xenelmis Hinton, 1936	0.994	0.76	Bio14 (20.6)
Macrelmis aeolis (Hinton, 1946)	0.999	0.84	Bio15 (41.9)
Macrelmis alea (Hinton, 1946)	0.999	0.98	Bio15 (40.6)
Macrelmis amana (Hinton, 1946)	0.996	0.79	Bio15 (34.7)
Macrelmis celsa (Hinton, 1946)	0.999	0.81	Bio15 (41.9)
Macrelmis codris (Hinton, 1946)	0.999	0.97	Bio15 (45.0)
Macrelmis isis (Hinton, 1946)	0.881	0.69	Slope (15.6)
Macrelmis plaumanni (Hinton, 1946)	0.997	0.80	Slope (45.0)

Figure legends

Fig. 1. Study area and occurrence localities of Elmidae genera and *Macrelmis* species used in the modelling procedures.

Fig. 2. Potential richness distribution of (A) Elmidae genera and (B) species of *Macrelmis*.


70°0'0"W
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50°0'0"W
20°0'0"S
30°0'0"S
40°0'0"S
50°0'0"S
N
References
Occurence localities of species
Ocorrence localities of genera
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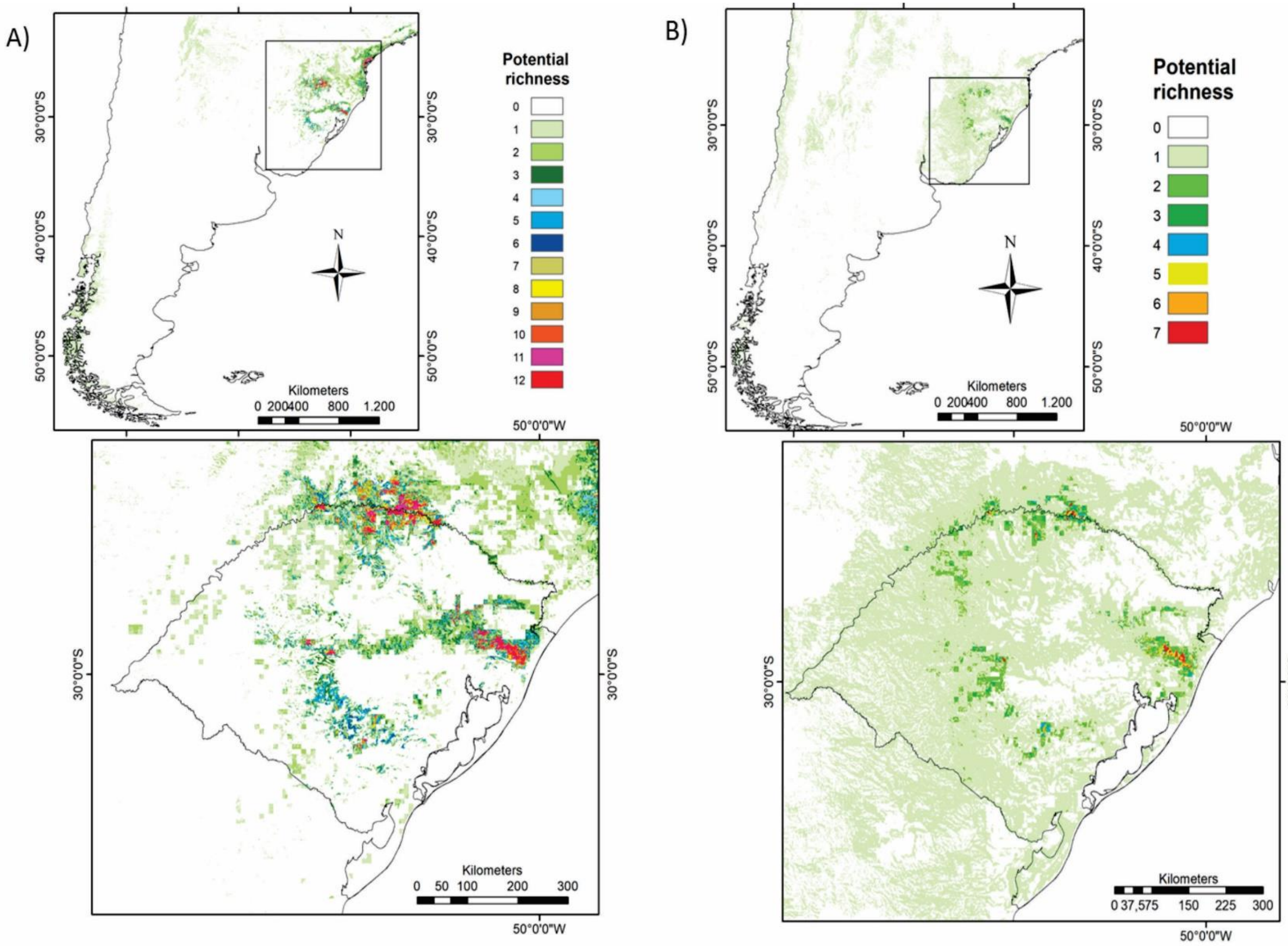
A)
Potential richness
0
1
2
3
4
5
6
7
8
9
10
11
12
30°0'0"S
40°0'0"S
50°0'0"S
N
Kilometers
0 200400 800 1.200
0 50 100 200 300
50°0'0"W
B)
Potential richness
0
1
2
3
4
5
6
7
Kilometers
0 200400 800 1.200
0 37,575 150 225 300
50°0'0"W

Supplementary material 1.

Taxa	Latitude	Longitude	Taxa	Latitude	Longitude
Austrolimnius	-29.4675	-53.2244	*Macrelmis*	-29.1792	-67.0800
Austrolimnius	-30.7897	-53.0646	*Macrelmis*	-30.8667	-67.3277
Austrolimnius	-30.2503	-53.5929	*Macrelmis*	-33.0826	-65.6628
Austrolimnius	-27.2050	-52.4030	*Macrelmis*	-25.6333	-57.1506
Austrolimnius	-27.2226	-52.3934	*Macrelmis*	-29.4295	-50.3931
Austrolimnius	-27.2319	-52.4004	*Macrelmis*	-25.5623	-48.7328
Austrolimnius	-27.2154	-52.4037	*Macrelmis*	-25.5539	-48.7374
Austrolimnius	-27.3609	-52.1612	*Macrelmis*	-25.5808	-48.7231
Austrolimnius	-27.3541	-52.1649	*Macrelmis*	-25.1592	-48.2971
Austrolimnius	-27.3634	-52.1607	*Macrelmis*	-23.4013	-51.9677
Austrolimnius	-27.6967	-48.8247	*Microcylloepus*	-29.6689	-53.7514
Austrolimnius	-24.8500	-48.0833	*Microcylloepus*	-27.2704	-54.0178
Austrolimnius	-25.0000	-48.0667	*Microcylloepus*	-27.2157	-53.9263
Austrolimnius	-22.7129	-44.6373	*Microcylloepus*	-27.1877	-53.8562
Austrolimnius	-25.8581	-54.1694	*Microcylloepus*	-27.2531	-53.9639
Austrolimnius	-21.6464	-47.6325	*Microcylloepus*	-30.8749	-53.5919
Austrolimnius	-22.6981	-45.4839	*Microcylloepus*	-30.8766	-53.5375
Austrolimnius	-24.2966	-48.4175	*Microcylloepus*	-30.7196	-53.4594
Austrolimnius	-23.2923	-46.9455	*Microcylloepus*	-30.8749	-53.1488
Austrolimnius	-23.2275	-46.9686	*Microcylloepus*	-30.8212	-53.0951
Austrolimnius	-26.0000	-54.0667	*Microcylloepus*	-30.7897	-53.0646
Austrolimnius	-23.0972	-64.7669	*Microcylloepus*	-30.2503	-53.5929
Austrolimnius	-42.6736	-70.0839	*Microcylloepus*	-30.4401	-53.6493
Austrolimnius	-25.6333	-57.1506	*Microcylloepus*	-30.5988	-53.3915
Austrolimnius	-22.0000	-48.0000	*Microcylloepus*	-30.3792	-53.8770
Cylloepus	-29.6689	-53.7514	*Microcylloepus*	-30.3257	-53.0028
Cylloepus	-27.2364	-53.8538	*Microcylloepus*	-29.5662	-50.5406
Cylloepus	-27.2050	-52.4030	*Microcylloepus*	-29.5791	-50.5518
Cylloepus	-27.4952	-51.9500	*Microcylloepus*	-29.6017	-50.5688
Cylloepus	-27.5020	-51.9266	*Microcylloepus*	-29.6130	-50.5676
Cylloepus	-27.3608	-52.1612	*Microcylloepus*	-29.5824	-50.4419
Cylloepus	-27.4247	-52.1332	*Microcylloepus*	-29.5824	-50.4666
Cylloepus	-27.7357	-52.5067	*Microcylloepus*	-29.6123	-50.4851
Cylloepus	-27.1611	-52.4167	*Microcylloepus*	-29.6316	-50.4948
Cylloepus	-27.6967	-48.8247	*Microcylloepus*	-29.5041	-50.6409
Cylloepus	-26.9194	-49.0661	*Microcylloepus*	-29.5154	-50.6623
Cylloepus	-24.8500	-48.0833	*Microcylloepus*	-29.5475	-50.6731
Cylloepus	-24.8333	-48.1000	*Microcylloepus*	-29.5680	-50.6860
Cylloepus	-25.0000	-48.0667	*Microcylloepus*	-27.2050	-52.4030
Cylloepus	-22.7130	-44.6373	*Microcylloepus*	-27.1947	-52.4007
Cylloepus	-21.9617	-47.8411	*Microcylloepus*	-27.2226	-52.3934
Cylloepus	-21.6464	-47.6325	*Microcylloepus*	-27.2319	-52.4004
Cylloepus	-22.6981	-45.4839	*Microcylloepus*	-27.2154	-52.4037
Cylloepus	-24.2967	-48.4175	*Microcylloepus*	-27.4952	-51.9500

Cylloepus	-25.6333	-57.1506	*Microcylloepus*	-27.5072	-51.9353
Cylloepus	-24.0200	-54.3400	*Microcylloepus*	-27.5020	-51.9266
Cylloepus	-25.5623	-48.7328	*Microcylloepus*	-27.5098	-51.9557
Cylloepus	-25.5539	-48.7374	*Microcylloepus*	-27.3608	-52.1612
Cylloepus	-25.5808	-48.7231	*Microcylloepus*	-27.3609	-52.1612
Gyrelmis	-27.2050	-52.4030	*Microcylloepus*	-27.3541	-52.1649
Gyrelmis	-27.1947	-52.4007	*Microcylloepus*	-27.4250	-52.1437
Gyrelmis	-27.2226	-52.3934	*Microcylloepus*	-27.4247	-52.1332
Gyrelmis	-27.2319	-52.4004	*Microcylloepus*	-27.4329	-52.1245
Gyrelmis	-27.3609	-52.1612	*Microcylloepus*	-27.4232	-52.1238
Gyrelmis	-27.7357	-52.5067	*Microcylloepus*	-27.4247	-52.1332
Gyrelmis	-27.6967	-48.8247	*Microcylloepus*	-27.4232	-52.1238
Gyrelmis	-22.7130	-44.6373	*Microcylloepus*	-27.3541	-52.1649
Heterelmis	-29.3422	-53.6611	*Microcylloepus*	-27.3958	-52.1635
Heterelmis	-29.3825	-53.2022	*Microcylloepus*	-27.7389	-52.5290
Heterelmis	-29.4675	-53.2244	*Microcylloepus*	-27.7357	-52.5067
Heterelmis	-29.4792	-53.2808	*Microcylloepus*	-27.1611	-52.4167
Heterelmis	-29.6689	-53.7514	*Microcylloepus*	-27.6967	-48.8247
Heterelmis	-29.7572	-53.7514	*Microcylloepus*	-22.7129	-44.6373
Heterelmis	-29.5447	-54.4906	*Microcylloepus*	-25.8581	-54.1694
Heterelmis	-29.5853	-54.4889	*Microcylloepus*	-21.9617	-47.8411
Heterelmis	-27.2704	-54.0178	*Microcylloepus*	-21.6464	-47.6325
Heterelmis	-27.2157	-53.9263	*Microcylloepus*	-22.6981	-45.4839
Heterelmis	-27.1877	-53.8562	*Microcylloepus*	-24.2966	-48.4175
Heterelmis	-27.2531	-53.9639	*Microcylloepus*	-26.0000	-54.0667
Heterelmis	-27.2520	-53.9026	*Microcylloepus*	-25.6333	-57.1506
Heterelmis	-27.2364	-53.8538	*Microcylloepus*	-24.0200	-54.3400
Heterelmis	-30.8749	-53.5919	*Microcylloepus*	-25.3833	-57.1333
Heterelmis	-30.8766	-53.5375	*Microcylloepus*	-25.5623	-48.7328
Heterelmis	-30.7196	-53.4594	*Microcylloepus*	-25.5539	-48.7374
Heterelmis	-30.8749	-53.1488	*Microcylloepus*	-25.5808	-48.7231
Heterelmis	-30.8212	-53.0951	*Microcylloepus*	-25.1592	-48.2971
Heterelmis	-30.7897	-53.0646	*Microcylloepus*	-22.0000	-48.0000
Heterelmis	-30.2503	-53.5929	*Neoelmis*	-29.3422	-53.6611
Heterelmis	-30.4401	-53.6493	*Neoelmis*	-29.3825	-53.2022
Heterelmis	-30.5988	-53.3915	*Neoelmis*	-29.4675	-53.2244
Heterelmis	-30.3792	-53.8770	*Neoelmis*	-29.4792	-53.2808
Heterelmis	-30.3257	-53.0028	*Neoelmis*	-29.6689	-53.7514
Heterelmis	-29.5662	-50.5406	*Neoelmis*	-29.7572	-53.7514
Heterelmis	-29.5791	-50.5518	*Neoelmis*	-29.6353	-54.0275
Heterelmis	-29.6017	-50.5688	*Neoelmis*	-29.5447	-54.4906
Heterelmis	-29.6130	-50.5676	*Neoelmis*	-29.5586	-54.4858
Heterelmis	-29.5824	-50.4419	*Neoelmis*	-27.2704	-54.0178
Heterelmis	-29.5824	-50.4666	*Neoelmis*	-27.2157	-53.9263
Heterelmis	-29.6123	-50.4851	*Neoelmis*	-27.1877	-53.8562
Heterelmis	-29.6316	-50.4948	*Neoelmis*	-27.2531	-53.9639

Heterelmis	-29.5041	-50.6409	*Neoelmis*	-27.2364	-53.8538
Heterelmis	-29.5154	-50.6623	*Neoelmis*	-30.8749	-53.5919
Heterelmis	-29.5475	-50.6731	*Neoelmis*	-30.8766	-53.5375
Heterelmis	-29.5680	-50.6860	*Neoelmis*	-30.7196	-53.4594
Heterelmis	-28.0337	-53.0337	*Neoelmis*	-30.8749	-53.1488
Heterelmis	-27.2050	-52.4030	*Neoelmis*	-30.8212	-53.0951
Heterelmis	-27.1947	-52.4007	*Neoelmis*	-30.7897	-53.0646
Heterelmis	-27.2226	-52.3934	*Neoelmis*	-30.2503	-53.5929
Heterelmis	-27.2319	-52.4004	*Neoelmis*	-30.4401	-53.6493
Heterelmis	-27.2154	-52.4037	*Neoelmis*	-30.5988	-53.3915
Heterelmis	-27.4952	-51.9500	*Neoelmis*	-30.3792	-53.8770
Heterelmis	-27.5072	-51.9353	*Neoelmis*	-30.3257	-53.0028
Heterelmis	-27.5020	-51.9266	*Neoelmis*	-29.5662	-50.5406
Heterelmis	-27.5098	-51.9557	*Neoelmis*	-29.5475	-50.6731
Heterelmis	-27.3608	-52.1612	*Neoelmis*	-29.6017	-50.5688
Heterelmis	-27.3609	-52.1612	*Neoelmis*	-29.6130	-50.5676
Heterelmis	-27.3608	-52.1612	*Neoelmis*	-29.5824	-50.4419
Heterelmis	-27.3609	-52.1612	*Neoelmis*	-29.5824	-50.4666
Heterelmis	-27.3541	-52.1649	*Neoelmis*	-29.6123	-50.4851
Heterelmis	-27.3634	-52.1607	*Neoelmis*	-29.6316	-50.4948
Heterelmis	-27.4250	-52.1437	*Neoelmis*	-29.5041	-50.6409
Heterelmis	-27.4247	-52.1332	*Neoelmis*	-29.5154	-50.6623
Heterelmis	-27.4329	-52.1245	*Neoelmis*	-29.5475	-50.6731
Heterelmis	-27.4232	-52.1238	*Neoelmis*	-29.5680	-50.6860
Heterelmis	-27.4247	-52.1332	*Neoelmis*	-27.1947	-52.4007
Heterelmis	-27.4329	-52.1245	*Neoelmis*	-27.2226	-52.3934
Heterelmis	-27.4232	-52.1238	*Neoelmis*	-27.2319	-52.4004
Heterelmis	-27.3541	-52.1649	*Neoelmis*	-27.2154	-52.4037
Heterelmis	-27.3958	-52.1635	*Neoelmis*	-27.4952	-51.9500
Heterelmis	-27.3456	-52.1607	*Neoelmis*	-27.5098	-51.9557
Heterelmis	-27.3644	-52.1348	*Neoelmis*	-27.5072	-51.9353
Heterelmis	-27.7716	-52.5147	*Neoelmis*	-27.5072	-51.9353
Heterelmis	-27.7751	-52.5262	*Neoelmis*	-27.5020	-51.9266
Heterelmis	-27.7540	-52.5297	*Neoelmis*	-27.5098	-51.9557
Heterelmis	-27.7389	-52.5290	*Neoelmis*	-27.3608	-52.1612
Heterelmis	-27.7357	-52.5067	*Neoelmis*	-27.3609	-52.1612
Heterelmis	-27.7329	-52.5327	*Neoelmis*	-27.3608	-52.1612
Heterelmis	-27.7428	-52.5538	*Neoelmis*	-27.3608	-52.1612
Heterelmis	-27.7231	-52.5352	*Neoelmis*	-27.3609	-52.1612
Heterelmis	-27.7202	-52.5318	*Neoelmis*	-27.3541	-52.1649
Heterelmis	-27.6967	-48.8247	*Neoelmis*	-27.4250	-52.1437
Heterelmis	-20.7793	-49.5812	*Neoelmis*	-27.4247	-52.1332
Heterelmis	-24.8500	-48.0833	*Neoelmis*	-27.4329	-52.1245
Heterelmis	-24.8333	-48.2333	*Neoelmis*	-27.4232	-52.1238
Heterelmis	-24.8333	-48.1000	*Neoelmis*	-27.4247	-52.1332
Heterelmis	-24.9833	-48.0333	*Neoelmis*	-27.4232	-52.1238

Heterelmis	-25.0000	-48.0667	*Neoelmis*	-27.3541	-52.1649
Heterelmis	-24.9500	-48.9833	*Neoelmis*	-27.3958	-52.1635
Heterelmis	-24.9500	-48.0000	*Neoelmis*	-27.3456	-52.1607
Heterelmis	-24.9667	-48.0000	*Neoelmis*	-27.7716	-52.5147
Heterelmis	-22.7129	-44.6373	*Neoelmis*	-27.7933	-52.4890
Heterelmis	-23.1016	-48.6158	*Neoelmis*	-27.7751	-52.5262
Heterelmis	-25.8581	-54.1694	*Neoelmis*	-27.7565	-52.5551
Heterelmis	-21.9617	-47.8411	*Neoelmis*	-27.7540	-52.5297
Heterelmis	-21.6464	-47.6325	*Neoelmis*	-27.7389	-52.5290
Heterelmis	-22.6981	-45.4839	*Neoelmis*	-27.7357	-52.5067
Heterelmis	-24.2966	-48.4175	*Neoelmis*	-27.7231	-52.5352
Heterelmis	-23.8412	-46.6279	*Neoelmis*	-27.7202	-52.5318
Heterelmis	-24.4833	-48.5833	*Neoelmis*	-27.1611	-52.4167
Heterelmis	-23.2923	-46.9455	*Neoelmis*	-25.4319	-48.8864
Heterelmis	-23.2275	-46.9686	*Neoelmis*	-25.3711	-51.4842
Heterelmis	-25.6333	-57.1506	*Neoelmis*	-20.7793	-49.5812
Heterelmis	-24.0200	-54.3400	*Neoelmis*	-22.7129	-44.6373
Heterelmis	-29.4295	-50.3931	*Neoelmis*	-25.8581	-54.1694
Heterelmis	-22.7105	-53.2759	*Neoelmis*	-21.6464	-47.6325
Heterelmis	-25.5623	-48.7328	*Neoelmis*	-22.6981	-45.4839
Heterelmis	-25.5539	-48.7374	*Neoelmis*	-24.2966	-48.4175
Heterelmis	-25.5808	-48.7231	*Neoelmis*	-23.8412	-46.6279
Heterelmis	-25.1592	-48.2971	*Neoelmis*	-23.2923	-46.9455
Heterelmis	-19.5758	-57.0142	*Neoelmis*	-23.2275	-46.9686
Heterelmis	-23.4013	-51.9677	*Neoelmis*	-41.2666	-71.2000
Heterelmis	-22.0000	-48.0000	*Neoelmis*	-25.6333	-57.1506
Hexacylloepus	-29.3422	-53.6611	*Neoelmis*	-24.0200	-54.3400
Hexacylloepus	-29.3825	-53.2022	*Neoelmis*	-25.3833	-57.1333
Hexacylloepus	-29.4792	-53.2808	*Neoelmis*	-29.4295	-50.3931
Hexacylloepus	-29.6689	-53.7514	*Neoelmis*	-25.5623	-48.7328
Hexacylloepus	-29.7572	-53.7514	*Neoelmis*	-25.5539	-48.7374
Hexacylloepus	-29.5447	-54.4906	*Neoelmis*	-25.5808	-48.7231
Hexacylloepus	-29.5853	-54.4889	*Neoelmis*	-25.1592	-48.2971
Hexacylloepus	-29.6433	-54.4936	*Phanocerus*	-29.4675	-53.2244
Hexacylloepus	-27.2704	-54.0178	*Phanocerus*	-27.2704	-54.0178
Hexacylloepus	-27.2157	-53.9263	*Phanocerus*	-27.2157	-53.9263
Hexacylloepus	-27.1877	-53.8562	*Phanocerus*	-27.1877	-53.8562
Hexacylloepus	-27.2531	-53.9639	*Phanocerus*	-27.2364	-53.8538
Hexacylloepus	-27.2364	-53.8538	*Phanocerus*	-29.5662	-50.5406
Hexacylloepus	-30.8749	-53.5919	*Phanocerus*	-29.5791	-50.5518
Hexacylloepus	-30.8766	-53.5375	*Phanocerus*	-29.6017	-50.5688
Hexacylloepus	-30.7196	-53.4594	*Phanocerus*	-29.6130	-50.5676
Hexacylloepus	-30.8749	-53.1488	*Phanocerus*	-29.5824	-50.4419
Hexacylloepus	-30.8212	-53.0951	*Phanocerus*	-29.5824	-50.4666
Hexacylloepus	-30.7897	-53.0646	*Phanocerus*	-29.6123	-50.4851
Hexacylloepus	-30.2503	-53.5929	*Phanocerus*	-29.6316	-50.4948

Hexacylloepus	-30.4401	-53.6493	*Phanocerus*	-29.5041	-50.6409
Hexacylloepus	-30.5988	-53.3915	*Phanocerus*	-29.5154	-50.6623
Hexacylloepus	-30.3792	-53.8770	*Phanocerus*	-29.5475	-50.6731
Hexacylloepus	-30.3257	-53.0028	*Phanocerus*	-29.5680	-50.6860
Hexacylloepus	-29.5662	-50.5406	*Phanocerus*	-27.1947	-52.4007
Hexacylloepus	-29.5791	-50.5518	*Phanocerus*	-27.2319	-52.4004
Hexacylloepus	-29.6017	-50.5688	*Phanocerus*	-27.3608	-52.1612
Hexacylloepus	-29.6130	-50.5676	*Phanocerus*	-27.3609	-52.1612
Hexacylloepus	-29.5824	-50.4419	*Phanocerus*	-27.3609	-52.1612
Hexacylloepus	-29.5824	-50.4666	*Phanocerus*	-27.3541	-52.1649
Hexacylloepus	-29.6316	-50.4948	*Phanocerus*	-27.3541	-52.1649
Hexacylloepus	-29.5041	-50.6409	*Phanocerus*	-27.1611	-52.4167
Hexacylloepus	-29.5154	-50.6623	*Phanocerus*	-25.0000	-48.0667
Hexacylloepus	-29.5475	-50.6731	*Phanocerus*	-22.7129	-44.6373
Hexacylloepus	-29.5680	-50.6860	*Phanocerus*	-23.1016	-48.6158
Hexacylloepus	-27.2050	-52.4030	*Phanocerus*	-25.8581	-54.1694
Hexacylloepus	-27.2226	-52.3934	*Phanocerus*	-21.9617	-47.8411
Hexacylloepus	-27.2319	-52.4004	*Phanocerus*	-21.6464	-47.6325
Hexacylloepus	-27.5098	-51.9557	*Phanocerus*	-22.6981	-45.4839
Hexacylloepus	-27.4952	-51.9500	*Phanocerus*	-24.2966	-48.4175
Hexacylloepus	-27.5072	-51.9353	*Phanocerus*	-23.2923	-46.9455
Hexacylloepus	-27.5020	-51.9266	*Phanocerus*	-23.2275	-46.9686
Hexacylloepus	-27.5098	-51.9557	*Phanocerus*	-29.4295	-50.3931
Hexacylloepus	-27.3608	-52.1612	*Phanocerus*	-25.5623	-48.7328
Hexacylloepus	-27.3609	-52.1612	*Phanocerus*	-25.5539	-48.7374
Hexacylloepus	-27.3609	-52.1612	*Phanocerus*	-25.5808	-48.7231
Hexacylloepus	-27.3541	-52.1649	*Phanocerus*	-25.1592	-48.2971
Hexacylloepus	-27.4250	-52.1437	*Stegoelmis*	-29.3422	-53.6611
Hexacylloepus	-27.4247	-52.1332	*Stegoelmis*	-29.3825	-53.2022
Hexacylloepus	-27.4329	-52.1245	*Stegoelmis*	-29.4675	-53.2244
Hexacylloepus	-27.4232	-52.1238	*Stegoelmis*	-21.6464	-47.6325
Hexacylloepus	-27.4247	-52.1332	*Stegoelmis*	-22.6981	-45.4839
Hexacylloepus	-27.4329	-52.1245	*Stegoelmis*	-24.2966	-48.4175
Hexacylloepus	-27.3541	-52.1649	*Stegoelmis*	-23.2923	-46.9455
Hexacylloepus	-27.3958	-52.1635	*Stegoelmis*	-23.2275	-46.9686
Hexacylloepus	-27.3456	-52.1607	*Stegoelmis*	-22.0000	-48.0000
Hexacylloepus	-27.7716	-52.5147	*Xenelmis*	-30.8766	-53.5375
Hexacylloepus	-27.7933	-52.4890	*Xenelmis*	-30.7196	-53.4594
Hexacylloepus	-27.7751	-52.5262	*Xenelmis*	-30.8749	-53.1488
Hexacylloepus	-27.7565	-52.5551	*Xenelmis*	-30.8212	-53.0951
Hexacylloepus	-27.7540	-52.5297	*Xenelmis*	-30.5988	-53.3915
Hexacylloepus	-27.7389	-52.5290	*Xenelmis*	-29.5662	-50.5406
Hexacylloepus	-27.7357	-52.5067	*Xenelmis*	-29.5791	-50.5518
Hexacylloepus	-27.7428	-52.5538	*Xenelmis*	-29.6017	-50.5688
Hexacylloepus	-27.7231	-52.5352	*Xenelmis*	-29.5824	-50.4419
Hexacylloepus	-27.7202	-52.5318	*Xenelmis*	-29.5824	-50.4666

Hexacylloepus	-27.1611	-52.4167	*Xenelmis*	-29.6123	-50.4851
Hexacylloepus	-27.6967	-48.8247	*Xenelmis*	-29.6316	-50.4948
Hexacylloepus	-20.79361	-49.5812	*Xenelmis*	-29.5154	-50.6623
Hexacylloepus	-24.8500	-48.0833	*Xenelmis*	-29.5475	-50.6731
Hexacylloepus	-24.8333	-48.2333	*Xenelmis*	-29.5680	-50.6860
Hexacylloepus	-24.8333	-48.1000	*Xenelmis*	-27.2050	-52.4030
Hexacylloepus	-24.9833	-48.0333	*Xenelmis*	-27.1947	-52.4007
Hexacylloepus	-25.0000	-48.0667	*Xenelmis*	-27.2226	-52.3934
Hexacylloepus	-24.9500	-48.9833	*Xenelmis*	-27.2319	-52.4004
Hexacylloepus	-24.9500	-48.0000	*Xenelmis*	-27.2154	-52.4037
Hexacylloepus	-24.9667	-48.0000	*Xenelmis*	-27.5020	-51.9266
Hexacylloepus	-22.7129	-44.6373	*Xenelmis*	-27.3609	-52.1612
Hexacylloepus	-25.8581	-54.1694	*Xenelmis*	-27.3609	-52.1612
Hexacylloepus	-21.9617	-47.8411	*Xenelmis*	-27.3541	-52.1649
Hexacylloepus	-21.6464	-47.6325	*Xenelmis*	-27.3634	-52.1607
Hexacylloepus	-22.6981	-45.4839	*Xenelmis*	-27.4250	-52.1437
Hexacylloepus	-24.2966	-48.4175	*Xenelmis*	-27.3541	-52.1649
Hexacylloepus	-23.2923	-46.9455	*Xenelmis*	-27.3958	-52.1635
Hexacylloepus	-23.2275	-46.9686	*Xenelmis*	-27.3456	-52.1607
Hexacylloepus	-25.6333	-57.1506	*Xenelmis*	-27.7933	-52.4890
Hexacylloepus	-24.0200	-54.3400	*Xenelmis*	-27.7540	-52.5297
Hexacylloepus	-25.5623	-48.7328	*Xenelmis*	-27.7389	-52.5290
Hexacylloepus	-25.5539	-48.7374	*Xenelmis*	-27.1611	-52.4167
Hexacylloepus	-25.5808	-48.7231	*Xenelmis*	-27.6967	-48.8247
Hexacylloepus	-25.1592	-48.2971	*Xenelmis*	-24.8500	-48.0833
Hexacylloepus	-23.4013	-51.9677	*Xenelmis*	-25.0000	-48.0667
Hexanchorus	-27.2704	-54.0178	*Xenelmis*	-22.7129	-44.6373
Hexanchorus	-27.2157	-53.9263	*Xenelmis*	-25.8581	-54.1694
Hexanchorus	-29.5662	-50.5406	*Xenelmis*	-21.6464	-47.6325
Hexanchorus	-29.5791	-50.5518	*Xenelmis*	-22.6981	-45.4839
Hexanchorus	-29.6017	-50.5688	*Xenelmis*	-24.9666	-48.4175
Hexanchorus	-29.5824	-50.4666	*Xenelmis*	-23.2923	-46.9455
Hexanchorus	-29.6123	-50.4851	*Xenelmis*	-23.2275	-46.9686
Hexanchorus	-29.6316	-50.4948	*Xenelmis*	-26.0000	-54.0667
Hexanchorus	-29.5154	-50.6623	*Xenelmis*	-25.6333	-57.1506
Hexanchorus	-29.5475	-50.6731	*Xenelmis*	-25.3833	-57.1333
Hexanchorus	-29.5680	-50.6860	*Xenelmis*	-25.5623	-48.7328
Hexanchorus	-27.5072	-51.9353	*Xenelmis*	-25.5539	-48.7374
Hexanchorus	-27.5098	-51.9557	*Xenelmis*	-25.5808	-48.7231
Hexanchorus	-27.1611	-52.4167	*Xenelmis*	-25.1592	-48.2971
Hexanchorus	-20.7793	-49.5812	*Xenelmis*	-22.0000	-48.0000
Hexanchorus	-22.7129	-44.6373	*Macrelmis aeolis*	-29.5662	-50.5406
Hexanchorus	-25.8581	-54.1694	*Macrelmis aeolis*	-29.5824	-50.4419
Hexanchorus	-24.4833	-48.5833	*Macrelmis aeolis*	-29.6123	-50.4851
Hexanchorus	-25.5623	-48.7328	*Macrelmis aeolis*	-29.6316	-50.4948
Hexanchorus	-25.5539	-48.7374	*Macrelmis aeolis*	-29.5475	-50.6731

Hexanchorus	-25.5808	-48.7231	*Macrelmis aeolis*	-27.1611	-52.4167
Hexanchorus	-25.1592	-48.2971	*Macrelmis alea*	-30.4917	-53.7984
Macrelmis	-29.3825	-53.2022	*Macrelmis alea*	-29.5791	-50.5518
Macrelmis	-29.4675	-53.2244	*Macrelmis alea*	-27.3608	-52.1612
Macrelmis	-27.2704	-54.0178	*Macrelmis alea*	-27.3541	-52.1649
Macrelmis	-27.2157	-53.9263	*Macrelmis alea*	-27.1611	-52.4167
Macrelmis	-27.1877	-53.8562	*Macrelmis amana*	-27.2704	-54.0178
Macrelmis	-27.2520	-53.9026	*Macrelmis amana*	-27.2157	-53.9263
Macrelmis	-27.2364	-53.8538	*Macrelmis amana*	-27.1877	-53.8562
Macrelmis	-30.8749	-53.5919	*Macrelmis amana*	-29.5662	-50.5406
Macrelmis	-30.7196	-53.4594	*Macrelmis amana*	-29.5791	-50.5518
Macrelmis	-30.8749	-53.1488	*Macrelmis amana*	-29.6017	-50.5688
Macrelmis	-30.8212	-53.0951	*Macrelmis amana*	-29.6123	-50.4851
Macrelmis	-30.7897	-53.0646	*Macrelmis amana*	-29.6316	-50.4948
Macrelmis	-30.2503	-53.5929	*Macrelmis amana*	-29.5475	-50.6731
Macrelmis	-30.4401	-53.6493	*Macrelmis amana*	-27.3608	-52.1612
Macrelmis	-30.5988	-53.3915	*Macrelmis amana*	-27.4250	-52.1437
Macrelmis	-30.3257	-53.0028	*Macrelmis amana*	-27.7540	-52.5299
Macrelmis	-29.5662	-50.5406	*Macrelmis amana*	-27.1611	-52.4167
Macrelmis	-29.5791	-50.5518	*Macrelmis amana*	-24.0200	-54.3400
Macrelmis	-29.6017	-50.5688	*Macrelmis celsa*	-30.4400	-53.6493
Macrelmis	-29.6130	-50.5676	*Macrelmis celsa*	-30.4917	-53.7984
Macrelmis	-29.5824	-50.4419	*Macrelmis celsa*	-30.8211	-53.0951
Macrelmis	-29.5824	-50.4666	*Macrelmis celsa*	-27.2157	-53.9263
Macrelmis	-29.6123	-50.4851	*Macrelmis celsa*	-29.5662	-50.5406
Macrelmis	-29.6316	-50.4948	*Macrelmis celsa*	-29.6017	-50.5688
Macrelmis	-29.5041	-50.6409	*Macrelmis celsa*	-29.6316	-50.4948
Macrelmis	-29.5154	-50.6623	*Macrelmis celsa*	-29.5041	-50.6409
Macrelmis	-29.5475	-50.6731	*Macrelmis celsa*	-29.6130	-50.5676
Macrelmis	-29.5680	-50.6860	*Macrelmis celsa*	-29.5475	-50.6731
Macrelmis	-27.2226	-52.3934	*Macrelmis celsa*	-27.1611	-52.4167
Macrelmis	-27.2319	-52.4004	*Macrelmis codris*	-29.6130	-50.5676
Macrelmis	-27.2319	-52.4004	*Macrelmis codris*	-29.6316	-50.4948
Macrelmis	-27.5098	-51.9557	*Macrelmis codris*	-27.1611	-52.4167
Macrelmis	-27.5072	-51.9353	*Macrelmis isis*	-29.6123	-50.4851
Macrelmis	-27.5020	-51.9266	*Macrelmis isis*	-29.6316	-50.4948
Macrelmis	-27.5098	-51.9557	*Macrelmis isis*	-29.5475	-50.6731
Macrelmis	-27.3608	-52.1612	*Macrelmis isis*	-29.5680	-50.6860
Macrelmis	-27.3609	-52.1612	*Macrelmis isis*	-29.6130	-50.5676
Macrelmis	-27.3608	-52.1612	*Macrelmis isis*	-27.1611	-52.4167
Macrelmis	-27.3541	-52.1649	*Macrelmis isis*	-24.0200	-54.3400
Macrelmis	-27.3634	-52.1607	*Macrelmis isis*	-22.3936	-42.3057
Macrelmis	-27.4250	-52.1437	*Macrelmis isis*	-22.2247	-69.6035
Macrelmis	-27.4247	-52.1332	*Macrelmis isis*	-27.0584	-54.8275
Macrelmis	-27.4232	-52.1238	*Macrelmis isis*	-32.9077	-64.7833
Macrelmis	-27.4247	-52.1332	*Macrelmis isis*	-26.6036	-65.0433

Macrelmis	-27.4232	-52.1238	*Macrelmis isis*	-29.1792	-67.0800
Macrelmis	-27.3541	-52.1649	*Macrelmis isis*	-30.8667	-67.3277
Macrelmis	-27.3456	-52.1607	*Macrelmis isis*	-33.0826	-65.6628
Macrelmis	-27.7389	-52.5290	*Macrelmis plaumani*	-30.2503	-53.5929
Macrelmis	-27.7357	-52.5067	*Macrelmis plaumani*	-30.4917	-53.7984
Macrelmis	-27.1611	-52.4167	*Macrelmis plaumani*	-30.7897	-53.0646
Macrelmis	-27.6967	-48.8247	*Macrelmis plaumani*	-27.2157	-53.9263
Macrelmis	-20.7793	-49.5812	*Macrelmis plaumani*	-27.1877	-53.8562
Macrelmis	-24.9833	-48.0333	*Macrelmis plaumani*	-29.5662	-50.5406
Macrelmis	-25.0000	-48.0667	*Macrelmis plaumani*	-29.5791	-50.5518
Macrelmis	-22.7129	-44.6373	*Macrelmis plaumani*	-29.6017	-50.5688
Macrelmis	-25.8581	-54.1694	*Macrelmis plaumani*	-29.5824	-50.4419
Macrelmis	-21.6464	-47.6325	*Macrelmis plaumani*	-29.5824	-50.4666
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Macrelmis	-24.2966	-48.4175	*Macrelmis plaumani*	-29.5041	-50.6409
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Macrelmis	-24.0200	-54.3400	*Macrelmis plaumani*	-29.5680	-50.6860
Macrelmis	-22.2247	-69.6035	*Macrelmis plaumani*	-27.4951	-51.9500
Macrelmis	-27.0584	-54.8275	*Macrelmis plaumani*	-27.4952	-51.9386
Macrelmis	-32.9077	-64.7833	*Macrelmis plaumani*	-27.1611	-52.4167
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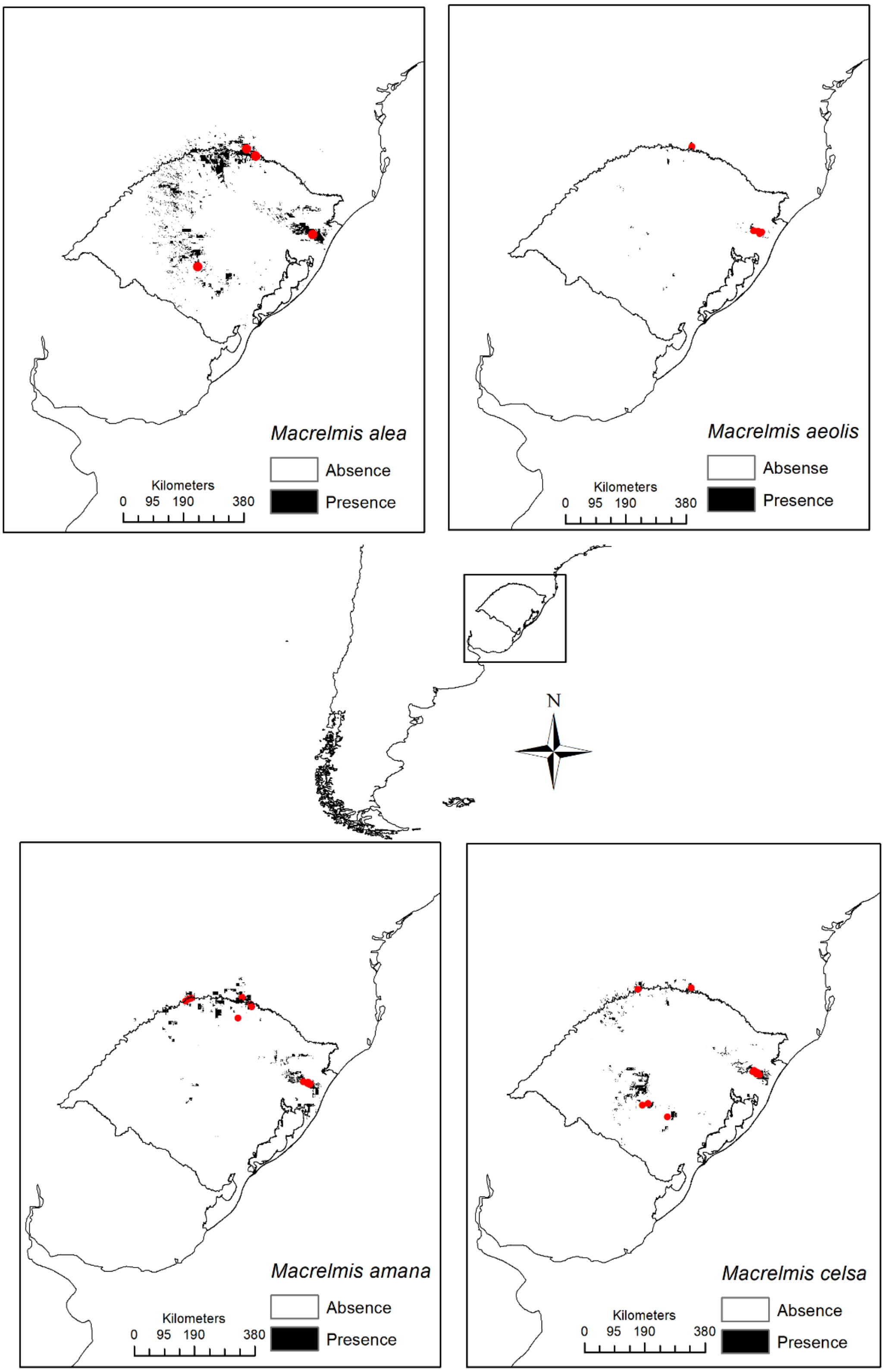
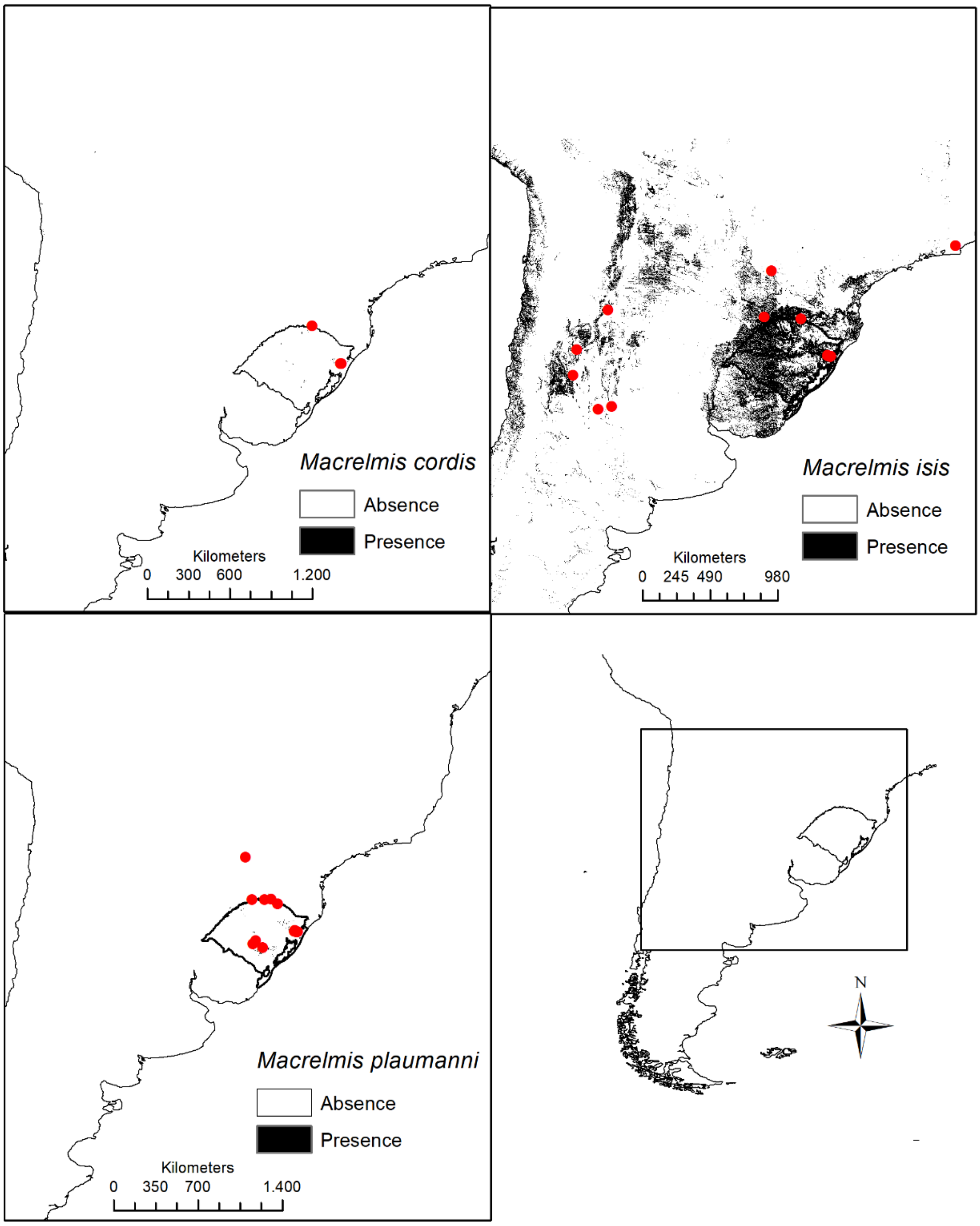
Supplementary material 2.

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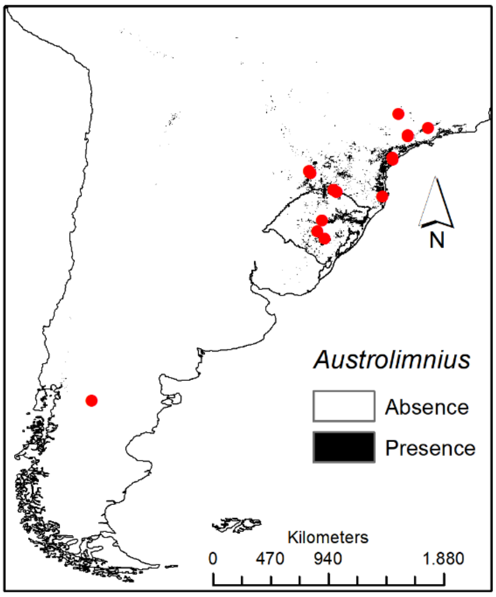
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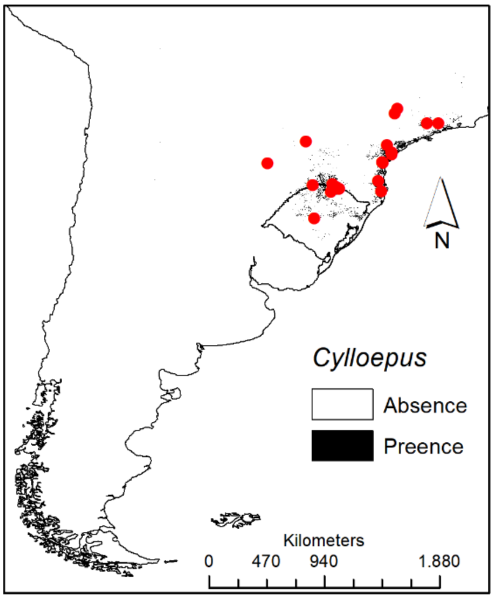
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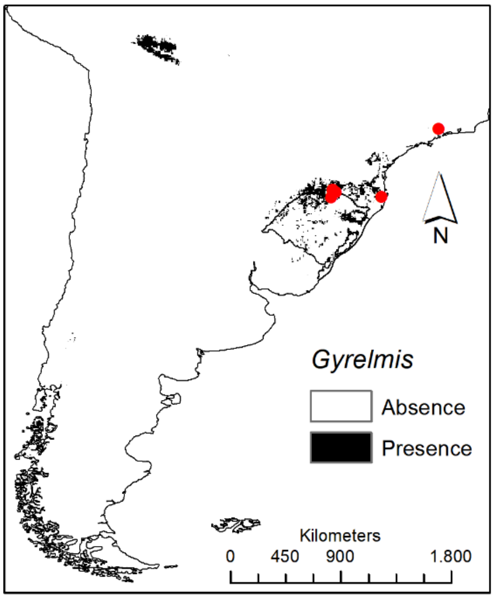
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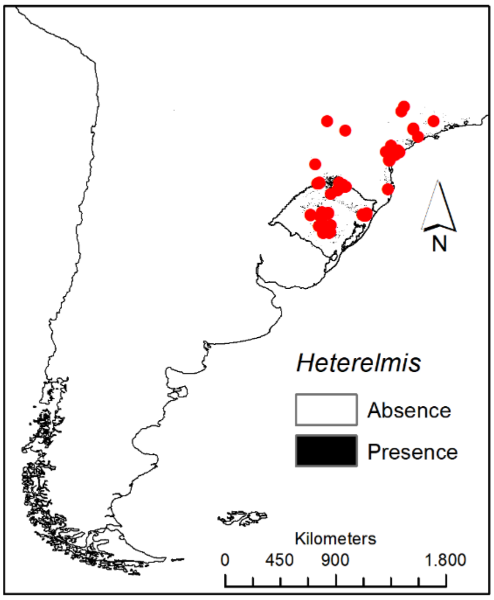
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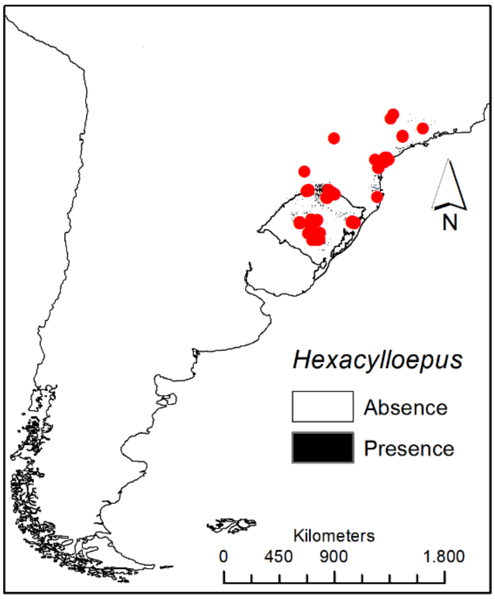
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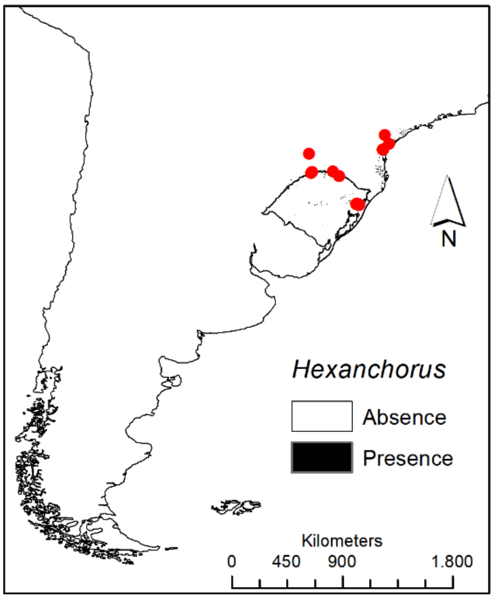
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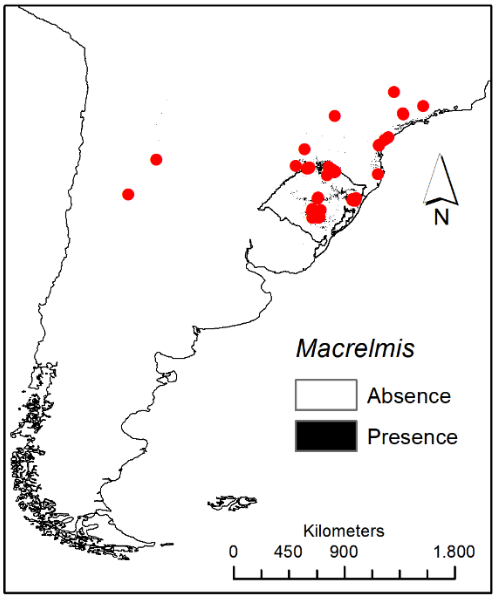
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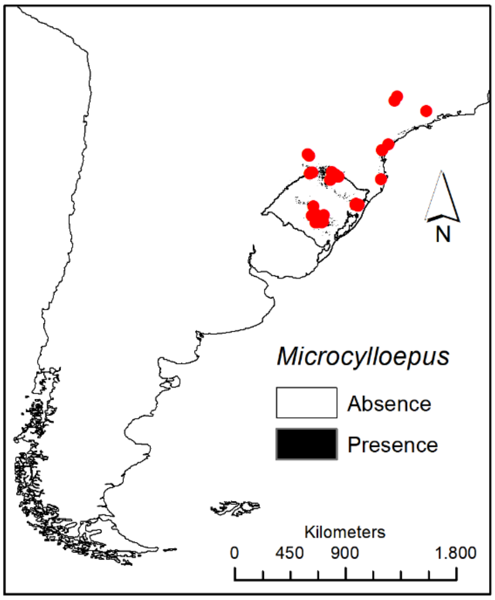
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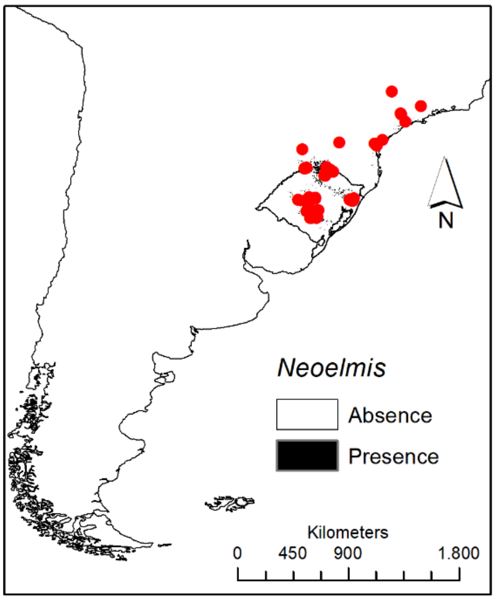
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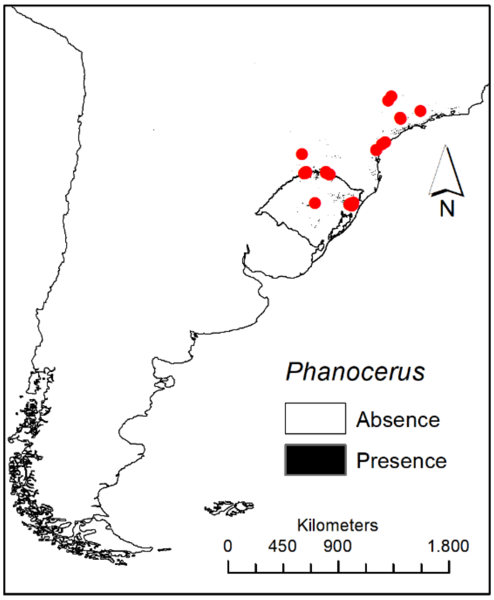
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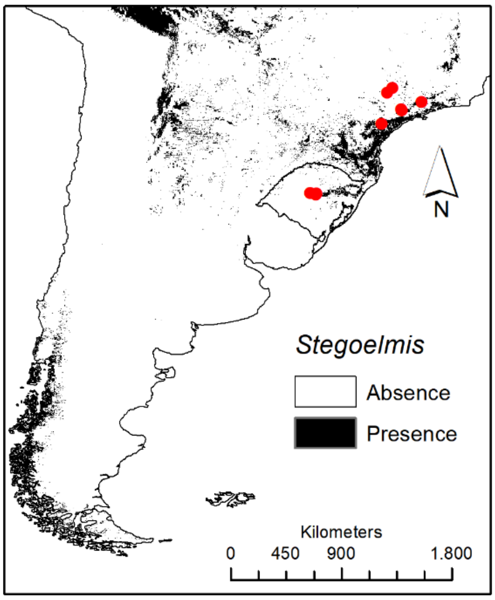
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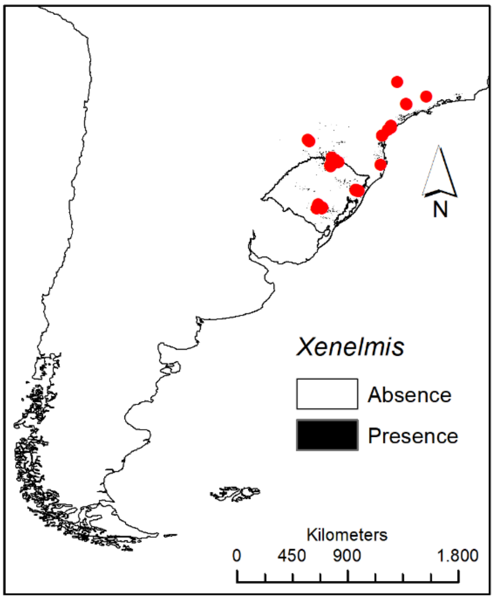
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CONSIDERAÇÕES FINAIS

Os resultados apresentados neste estudo sugerem que a presença e a integridade do Bioma Mata Atlântica são fatores determinantes para a estrutura das comunidades de elmídeos no Rio Grande do Sul. Características como largura de vegetação ripária e tipo de substrato dos riachos também são fatores importantes para a ocorrência do grupo nas áreas estudadas. Particularmente, nossos resultados mostram que a composição das comunidades, no que se refere aos gêneros e seus estágios de vida (adultos / larvas), é influenciada por diferenças marcantes nas larguras de vegetação ripária, mas não por pequenas diferenças entre elas. Desta forma, a manutenção de larguras razoáveis de vegetação ripária nas áreas estudadas (> 5m) são mais importantes para a diversidade taxonômica das comunidades do que outros fatores antrópicos, como as condições de uso da terra relativos à agricultura e urbanização, bem como outros fatores abióticos avaliados que não mostram resultados significativos. Contudo, riachos com larguras ripícolas mais estreitas (< 5m) podem apresentar riqueza similar às observadas em riachos melhor protegidos e, também, conter comunidades com grande abundância de indivíduos. Este resultado, possivelmente, está relacionado à numerosa presença de certos gêneros ambientalmente tolerantes à atividades antrópicas. Em outras palavras, as diferenças observadas entre riachos com largura de vegetação ripária muito estreita (< 5 m) e os demais (> 5 m) relacionam-se à diferenças na abundância relativa de gêneros e seus estágios de vida. Finalmente, os resultados obtidos neste estudo mostram, ainda que fatores ambientais analisados em escala regional também regulam a distribuição de gêneros de Elmidae, e de espécies de *Macrelmis* em particular, no sul do Brasil. Precipitação, declividade e ecorregiões são importantes preditores da ocorrência de gêneros de Elmidae e espécies de *Macrelmis*. Porém, pode haver interação entre preditores de escalas regional e local. Assim, considerando-se que os ecossistemas aquáticos estão sendo ameaçados por atividades antrópicas em todo o mundo, é importante incentivar a realização de mais estudos sobre a diversidade das comunidades de insetos de riachos no Brasil. Em relação aos Elmidae, é imprescindível aprofundar o conhecimento sobre a diversidade em nível de espécie, e conduzir mais estudos ecológicos sobre a distribuição espacial das espécies e de seus estágios de vida. Técnicas de modelagem poderão permitir melhor entendimento sobre a ocorrência dos Elmidae no Brasil, conforme aspectos ecológicos. Desta forma, será possível obter-se uma melhor compreensão sobre os processos ecológicos relacionados a estruturação das comunidades de Elmidae, bem como elaborar propostas mais adequadas para a conservação dos sistemas lóticos no país.

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