

Universidade Federal de São Carlos
Centro de Ciências Biológicas e da Saúde
Programa de Pós Graduação em Ecologia e Recursos Naturais

**Padrões de montagem de comunidades: investigando a
estrutura funcional e filogenética para inferir
processos em comunidades naturais**

Candidato: Victor Satoru Saito

Orientadora: Prof.^a Dr.^a Alaide Aparecida Fonseca Gessner

Co-orientador: Prof. Dr. Tadeu de Siqueira Barros

São Carlos - SP

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Tese apresentada ao Programa de Pós-
Graduação em Ecologia e Recursos Naturais do
Centro de Ciências Biológicas e da Saúde como
parte dos requisitos necessários para a obtenção
do título de doutor em Ciências, área de
concentração em Ecologia e Recursos Naturais

São Carlos - SP

2016

Ficha catalográfica elaborada pelo DePT da Biblioteca Comunitária UFSCar
Processamento Técnico
com os dados fornecidos pelo(a) autor(a)

S158p Saito, Victor Satoru
Padrões de montagem de comunidades: investigando a estrutura funcional e filogenética para inferir processos em comunidades naturais / Victor Satoru Saito. -- São Carlos : UFSCar, 2016.
141 p.

Tese (Doutorado) -- Universidade Federal de São Carlos, 2016.

1. Diversidade filogenética. 2. Diversidade funcional. 3. Montagem de comunidades. 4. Nicho Ecológico. 5. Teoria de coexistência. I. Título.



UNIVERSIDADE FEDERAL DE SÃO CARLOS

Centro de Ciências Biológicas e da Saúde
Programa de Pós-Graduação em Ecologia e Recursos Naturais

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*Dedico esta tese a Dri,
amor da minha vida*

Agradecimentos

Agradeço ao prof. Tadeu por me receber em seu laboratório. Agradeço por todo apoio, aprendizados, oportunidades e por acreditar que eu conseguiria fazer um doutorado de qualidade. Toda a minha gratidão não cabe nesta pequena seção e, por isso, escrevo para cada orientador individualmente na seção *Apresentação – Sobre meus orientadores*.

Agradeço à prof. Alaide por me orientar desde o mestrado. Obrigado por passar um pouco da experiência da senhora. As incertezas sempre foram menores sabendo que eu tinha todo o apoio necessário em São Carlos.

Agradeço à prof. Sandrine por me receber em Paris e me deixar aprender um pouco sobre análises, francês e programação.

Agradeço aos colegas do laboratório de Rio Claro, que não ousou nomear, para não esquecer ninguém.

Agradeço também aos colegas e professores do laboratório de São Carlos professora Susana, professor Rhainer e professora Lívia que, por mais que aparecesse esporadicamente, sempre fui bem recebido.

Agradeço ao prof. Antonio e todo seu laboratório pela possibilidade de participar do PELD-Itanhaém.

Agradeço ao Carlos e Amarílis pela ajuda nas coletas em Itanhaém.

Agradeço aos amigos brasileiros e franceses conhecidos na França, tudo foi mais fácil aí com vocês.

Agradeço a todos meus co-autores, pela boa colaboração e por enriquecer meu conhecimento.

Agradeço aos diversos revisores colegas voluntários que ajudaram a melhorar meus manuscritos. Cada um de vocês é lembrado ao final de cada capítulo.

Agradeço aos revisores e editores das revistas nas quais submeti meus manuscritos.

Agradeço aos funcionários do PPGERN pela colaboração durante meu mestrado e doutorado.

Agradeço ao prof. Irineu Bianchini Junior, atual coordenador do PPGERN, pela ajuda nessa reta final de defesa.

Agradeço à FAPESP pelo apoio financeiro.

Agradeço ao Raul e a Sayuri pela amizade e por toda a ajuda e apoio durante meu tempo em Rio Claro. Também os agradeço pelas discussões enriquecedoras e por cuidar de tudo em Rio Claro enquanto estive fora.

Agradeço ao prof. Fabio Cop e prof. Ursulla Souza pelas oportunidades que me foram dadas e pela amizade.

Agradeço ao prof. Gilmar Perbiche Neves pela amizade, incentivo e colaborações.

Agradeço ao Toshiro, Cintia e Carol pela ajuda durante minha estadia em Paris e também nos meus períodos na Bélgica.

Agradeço aos membros da banca por aceitarem participar desta defesa de tese e pelas contribuições para melhorá-la.

Agradeço a todos os amigos, presentes ou distantes que me ajudaram nessa caminhada.

Agradeço à família da Dri por ser minha segunda família. Nunca teria conseguido sem vocês.

Agradeço aos meus pais e irmãos por me apoiarem a vida toda e por permitirem que eu chegasse até aqui. Esta tese é dedicada a vocês também.

Agradeço à Dri, por tudo que faz por mim, pelo seu amor.

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Apresentação

Sobre o formato da tese

Esta tese está redigida conforme o Regimento Geral de 2012 do Programa de Pós-Graduação em Ecologia e Recursos Naturais da Universidade Federal de São Carlos. Nela constam uma Introdução Geral com o embasamento conceitual necessário para o entendimento de todos os capítulos; quatro capítulos referentes a estudos realizados e redigidos no formato para apreciação em periódicos científicos; além de Considerações Finais com um sumário desta tese e algumas opiniões minhas.

Apenas os capítulos de um a quatro estão em inglês, conforme normas dos periódicos científicos. Em comparação aos artigos publicados (Capítulos 1 a 3), realizei algumas mudanças para facilitar o entendimento do leitor. As figuras e tabelas foram inseridas no corpo do texto e alguns materiais suplementares foram trazidos ao texto, tornando-se figuras e tabelas. Um leitor atento também notará que alguns apêndices dos artigos publicados deixaram de ser citados, porém realizei isso apenas quando a omissão não comprometia o entendimento do estudo, ou quando o apêndice não tinha a minha participação.

Na Introdução Geral e Considerações Finais tentei me expressar de uma forma menos técnica que exigida para os artigos científicos, tendo em vista o entendimento dos leitores menos especializados, como alunos saindo da graduação e decidindo se vão se inscrever para a pós-graduação. A Introdução Geral contém resumos dos quatro capítulos científicos de modo que um leitor apressado possa lê-los e ter uma noção geral dos principais resultados e opiniões dos autores dos artigos.

Ainda sobre a linguagem da Introdução Geral e Considerações Finais, escrevi na primeira pessoa do singular quando estava expressando uma opinião ou decisão minha e

escrevi no plural quando as opiniões ou ações foram tomadas por todos os autores dos trabalhos. Acho a passagem do texto científico para a linguagem mais simples um grande desafio e na Introdução Geral e Considerações Finais busquei exercitar essa tarefa tão importante para o cientista moderno.

Sobre meus orientadores

Antes de falar de ecologia, insetos aquáticos e testes estatísticos, preciso aproveitar a oportunidade única de redigir uma tese para contar um pouco sobre coisas que não cabem e nem fazem sentido estar nos meus artigos científicos. Dentre esses assuntos, gostaria de contar um pouco sobre meu grande privilégio no quesito orientador(a).

Quando conto que nunca tive *um* problema com nenhum orientador as pessoas acham que é exagero, que é algo impossível, dado o tempo de convívio e a natureza da relação mestre-aprendiz. Porém, no meu caso isso é verdade. Desde a graduação tive a imensa oportunidade de ser orientado pela professora Maria Virgínia Urso Guimarães, a qual foi uma grande incentivadora dos meus estudos com invertebrados. Muito além disso, a professora não mediu esforços para acompanhar minhas três iniciações científicas e todas minhas ideias mirabolantes na época. Muito obrigado professora, hoje, se sou quase um doutor, muito se deve ao seu incentivo na graduação.

No mestrado, tive a grande felicidade de ser orientado pela professora Alaide Fonseca Gessner, hoje também minha orientadora de doutorado. Assim como a professora Virgínia, a professora Alaide jamais negou ou desestimulou minhas ideias. Com a ajuda da professora pude adentrar em um laboratório estimulante, onde conheci diversos amigos que hoje também são meus co-autores. A experiência da professora Alaide me deu sustentação para buscar o que o mestrado e o doutorado exigiam. Ainda

assim, com toda a liberdade para buscar o que precisasse, em nenhum momento estive desamparado. Muito obrigado, professora.

Todos meus orientadores foram fundamentais, mas não posso deixar de expressar a opinião de que o meu amigo e mestre, professor Tadeu de Siqueira Barros, é o meu espelho profissional e pessoal. No fim do mestrado gostaria de ser seu orientado, mas por motivos fora do nosso controle (a pós-graduação em Rio Claro não abriu a tempo), acabei sendo *apenas* seu co-orientado. Digo apenas, porque a orientação do Tadeu foi muito mais intensa e enriquecedora do que eu jamais ouvi alguém relatar. Não existiu um dia no laboratório em que eu não pude discutir ciência com o Tadeu. Não existiu uma vírgula dos meus manuscritos que não passou pela sua mente brilhante e exigente. Tudo isso moldou o profissional que sou e espero, um dia, retribuir tudo o que fez para a minha formação nessa caminhada. Por tudo isso, deixo aqui meu muito obrigado.

Por fim, realizei 10 meses de estágio sanduíche no Museu de História Natural de Paris, sob orientação da professora Sandrine Pavoine. A professora Sandrine foi muito enriquecedora para meu aprendizado em análise de dados e programação em R. Além disso, no seu laboratório, tive a chance de conviver com pesquisadores de diversas áreas, pois o laboratório englobava ecólogos, biólogos, sociólogos e matemáticos. Por toda essa experiência, reitero aqui a minha gratidão à professora. Espero que a parceria e o aprendizado continuem por um bom tempo durante a minha carreira.

A trajetória

Assim como muito biólogos, entrei na graduação querendo aprender sobre “bichos”. Isso me levou a procurar orientação para iniciação científica logo no primeiro ano. Inicialmente queria muito trabalhar com aranhas e com a ajuda e incentivo da prof. Virgínia passei dois anos estagiando no Instituto Butantã. Sempre gostei muito de aranhas, mas para o TCC, precisava de um professor da UFSCar e, mais uma vez, a professora Virgínia estava lá para me orientar. Realizei meu TCC com comunidades de insetos galhadores, que, com muito orgulho, resultou no meu primeiro artigo científico.

Ainda muito ligado aos invertebrados, no mestrado procurei a professora Alaíde para trabalhar com insetos aquáticos. Nessa época achava que poderia trabalhar em muitos lugares se soubesse sobre insetos aquáticos bioindicadores. Mal eu sabia a falta de recursos que nossos órgãos ambientais enfrentam e a falta de incentivos que qualquer empresa recebe para o monitoramento dos seus impactos na natureza. Durante o mestrado, a professora Alaíde e vários outros membros do laboratório (obrigado professora Susana) me ensinaram sobre os insetos aquáticos, grupo que considero fascinante. A amplitude filogenética e as diferenças nas formas e estratégias dos grupos, que podem ser encontrados apenas revirando algumas rochas nos riachos, ainda me impressionam. Durante o mestrado pude desenvolver um pouco meu pensamento científico, de modo que comecei a ter grande fascínio pela forma como a ciência é feita. Nessa época comecei a me interessar mais por métodos quantitativos e teorias mais complexas sobre comunidades. Muito desse interesse deve-se aos meus veteranos de laboratório, Francisco, Toshiro e outros amigos.

Com o fim do mestrado e com o interesse em ecologia bem mais aflorado, fui procurar o professor Tadeu, na UNESP de Rio Claro. No laboratório de São Carlos os

alunos consideravam o Tadeu um dos grandes *alumni* que o laboratório já teve. Já nas minhas primeiras conversas com ele, confirmei tudo isso. Em acordo com a professora Alaide, me mudei para Rio Claro onde comecei a frequentar o laboratório do Tadeu. Durante meus primeiros dois anos tive a oportunidade de participar do Programa de Pesquisa Ecológica de Longa Duração, intitulado Estrutura e Dinâmica de comunidades em rios e riachos costeiros da Mata Atlântica – Bacia do Rio Itanhaém, sob coordenação do prof. Antonio Camargo. Foi meu primeiro contato com projetos grandes, que envolviam muitas pessoas e diversas frentes científicas. Tive a oportunidade de coletar os dados para o Capítulo 2 desta tese e de coletar dados sobre invertebrados aquáticos para outros artigos que ainda estão por vir. Além disso, aprendi muito com a organização do projeto como um todo, da compra dos materiais até a redação dos relatórios.

Indo para o terceiro ano do doutorado, pedimos uma bolsa sanduíche para a França, onde decidi tentar uma vaga junto à professora Sandrine Pavoine no Museu de História Natural de Paris. Felizmente minha bolsa foi aceita rapidamente e a professora Sandrine, mais do que prontamente me aceitou em sua equipe. Na França tive uma das grandes experiências da vida, tanto pessoalmente como profissionalmente. Pessoalmente, fiz amigos de diversas nacionalidades e conheci muitas culturas e pessoas diferentes, tudo muito enriquecedor. Profissionalmente, estive em contato com uma pesquisadora aplicadíssima, com conhecimentos quantitativos impressionantes. Tentei absorver o máximo dessa experiência, apesar dos apenas 10 meses. Felizmente, sei que a parceria com a professora Sandrine irá continuar e espero continuar aprendendo com ela, mesmo à distância.

Agora no fim do doutorado meu interesse já mudou bastante, comparado ao da graduação. Se antes eu era um “*organism based researcher*” agora eu me considero um

“question based researcher”. Ou seja, antes minha motivação era baseada na curiosidade sobre os organismos, mas acho que hoje em dia meu interesse é mais em perguntas ecológicas, muitas vezes independente do grupo de estudo. Hoje, eu ainda tenho grande interesse em ecologia de insetos aquáticos, mas acho que caminho para uma abertura do meu leque. Daqui para frente quero responder perguntas sobre ecologia, ponto.

Resumo

A ecologia de comunidade visa separar a influência relativa de diferentes processos na montagem de comunidades naturais. Minha tese é composta por quatro capítulos com diferentes focos sobre os processos de montagem de comunidades naturais e simuladas. No primeiro capítulo, estudamos insetos aquáticos de riachos ao longo de 600 km. Descobrimos que a estrutura filogenética de comunidades tinha uma assinatura espacial, sugerindo limitação de dispersão. Descobrimos que grandes insetos, com capacidade de voo alta e com apenas um ciclo de vida ao ano foram os mais estruturados espacialmente. Sugerimos que o número de gerações por ano e, conseqüentemente, o número de eventos de dispersão é um forte fator para a distribuição de insetos aquáticos. No segundo capítulo, estudamos a montagem de insetos em uma escala menor, na bacia do rio Itanhaém-SP. Nosso interesse foi na competição e filtragem ambiental sobre a estrutura funcional e filogenética de insetos aquáticos. Se a filtragem ambiental fosse forte, atributos de respiração e sensibilidade à poluição deveriam ser mais semelhantes do que o esperado ao acaso, enquanto que, se a competição fosse forte deveríamos encontrar uma menor semelhança em estratégias de alimentação entre *taxa* coocorrentes. Encontramos evidências de filtragem ambiental, mas não de forte competição. Além disso, encontramos coocorrência de insetos aquáticos de clados antigos, provavelmente devido à estabilidade dos habitats de riachos ao longo da escala evolutiva. No terceiro capítulo, também investigamos a competição e a filtragem ambiental, mas usamos libélulas, um grupo com o comportamento agressivo contra espécies visualmente semelhantes. Isto acontece porque elas não identificam corretamente se um indivíduo é um competidor por parceiros da mesma espécie, ou não. No mesmo estudo, encontramos mais evidências de filtragem ambiental, provavelmente porque libélulas aparentadas têm estratégias reprodutivas semelhantes ligadas a características específicas dos rios, forçando estas espécies aparentadas a coocorrer. No quarto capítulo estávamos interessados novamente na competição, no entanto, usamos simulações computacionais para gerar comunidades influenciadas pela competição. Objetivamos identificar se a abordagem observacional poderia realmente identificar exclusão competitiva. Comunidades simuladas foram compostas por espécies semelhantes, porque as espécies com poucas diferenças competitivas não se excluem mutuamente. Surpreendentemente, nós comumente encontramos padrões aleatórios, devido à competição relaxada diluída entre múltiplas espécies. Concluindo, nós identificamos que a montagem de comunidades naturais e simuladas é influenciada pela dispersão e história na escala regional, e pela filtragem ambiental na escala local. A influência de competição ainda está aberta a perguntas, dada à falta de confiabilidade que encontramos na abordagem observacional tradicional.

Abstract

Community ecology aims to disentangle the relative influence of the drivers of community assembly. My thesis is composed of four chapters with different focus on the drivers of assembly in natural and simulated communities. In the first chapter we studied stream aquatic insects over 600 km extension. We found that the phylogenetic structure of communities had a spatial signature, suggesting dispersal limitation. We found that large insects, with high flight capacity and with univoltine life cycles were the most affected by spatial distance. We suggest that the number of generation per year and hence the number of dispersal events is a strong factor for the distribution of aquatic insects. In the second chapter we studied the assembly of stream insects over a smaller scale in the Itanhaém river basin. We were interested on the influence of competition and environmental filtering on the functional and phylogenetic structure of insects. If environmental filtering was strong, traits of respiration type and pollution sensitivity should be more similar than expected by chance, while if competition was strong we should find less similarity in feeding strategies among co-occurring taxa. We found evidence of environmental filtering but not of strong competition. Additionally, we found common co-occurrence of ancient clades of aquatic insect orders, likely due to the stability of stream habitats along evolutionary scale. In the third chapter we also investigated competition and environmental filtering, but we used odonates, a group with aggressive behavior against visually similar species. This happen because it cannot properly identify if a similar individual is a mate competitor or not. Even in this case, we found more evidence of environmental filtering, likely because related odonates have similar reproductive strategies linked to specific riverine characteristics, forcing these related species to co-occur. In the fourth chapter we were interested again on the signatures of competition, however we used computational simulations to generate communities assembled by competition. We aimed to identify if the observational approach could indeed identify competitive exclusion. Simulated communities were generally composed of similar species, because species with small competitive difference do not exclude each other. Strikingly, we commonly found random patterns owing to the relaxed competition diluted among multiple species. In conclusion, we found that the assembling of natural and simulated communities is influenced by dispersal and history on the regional scale, and by environmental filtering on the local scale. The influence of competition is still open to questioning given the lack of reliability of the traditional observational approach.

Introdução geral

A diversidade de vida na Terra é surpreendente. Por exemplo, em apenas 52 hectares de floresta um estudo contabilizou 1.200 espécies de vegetais, entre árvores, bromélias e arbustos (Lee et al. 2002). Talvez, mais surpreendente ainda, seja o fato de que, em apenas um hectare de floresta tropical, podemos encontrar até 41.000 espécies de artrópodes (Erwin 1982). A evolução e a manutenção de comunidades biológicas com tamanha variação de espécies, até mesmo em locais aparentemente inóspitos, são motivos de grande curiosidade e admiração, principalmente para aqueles que, como eu, decidiram ter como carreira estudar essa vasta diversidade de vida e seus processos adjacentes. A distribuição das comunidades biológicas é, em parte, reflexo da história da Terra. Pode explicar, por exemplo, por que a fauna de mamíferos africanos pouco se sobrepõe a dos mamíferos sul-americanos. Afunilando a escala espacial, interações biológicas de diferentes formas, como a predação, a competição e o mutualismo, moldam as espécies que podem ser mantidas em um mesmo local. Tudo isso ainda é influenciado pela dispersão das espécies nessas diferentes escalas, indo da dispersão de pulgões entre plantas até a dispersão de aves entre continentes, o que permitiria entender por que algumas espécies são mais amplamente distribuídas do que outras. Além disso, as comunidades ainda enfrentam inegáveis eventos imprevisíveis, como chances de nascimento e morte, os quais certamente influenciam a estruturação da vida na Terra. Entender a influência relativa desses processos na estruturação das comunidades naturais é o grande papel dos ecólogos de comunidade. Dentro desse objetivo geral, dou início à minha contribuição para o campo, com os capítulos redigidos nesta tese.

Começarei abordando os principais mecanismos que estruturam as comunidades naturais e geram os padrões de biodiversidade. Em seguida, explanarei como cada capítulo engloba os diferentes mecanismos e investiga diferentes facetas da estrutura das comunidades. Por estruturação de comunidades eu considero os padrões que observamos e os processos adjacentes que afetam a abundância das espécies que compõem uma ou mais comunidades de interesse. Apesar da incrível variedade de interações entre espécies e entre espécies e o ambiente, as quais acontecem em diferentes escalas do espaço e tempo, podemos agrupar todas as forças atuantes em apenas quatro grupos (Vellend 2010). São eles: processos de seleção, de especiação, de dispersão e processos estocásticos neutros.

Processos de seleção são processos determinísticos que resultam das diferenças entre espécies (Vellend 2010). Também chamados de processos de nicho na literatura (Chase e Leibold 2003), eles englobam, por exemplo, a competição entre microrganismos por recursos limitados que acabam resultando em exclusão competitiva (e.g. Gause 1934), ou também a coexistência indefinida de vários lagartos que se alimentam de diferentes presas em diferentes horários do dia (Case 1983). Apesar de alguns autores defenderem a ideia de que processos de seleção necessariamente envolvem diferenças adaptativas entre espécies (Vellend 2010), no segundo caso (dos lagartos) a diferença faz justamente o contrário: favorece a manutenção dessas espécies em conjunto, mesmo que a adaptação ao ambiente e a capacidade competitiva sejam equivalentes (Chesson 2000). Isso ocorre devido a processos que têm dinâmicas densidade-dependente. Por exemplo, as espécies podem ter uma autorregulação populacional sendo que, quanto mais indivíduos de uma mesma espécie, maior a competição entre esses indivíduos pelos recursos necessários para gerar proles. Nesse caso, essa elevada competição faz com que cada indivíduo consiga menos recurso e gere

menos prole, diminuindo a população da próxima geração. Essa geração no futuro enfrentará uma competição intraespecífica menor e a população voltará a crescer (Chase e Leibold 2003). Quando duas espécies competem, esse mecanismo densidade-dependente ocorre tanto entre espécies, como dentro das populações de cada espécie. A magnitude delas determina se as espécies podem ou não coexistir. Quando cada espécie regula mais a própria população do que a do concorrente, ambas conseguem crescer depois de serem levadas até baixas densidades, mantendo a coexistência estável por tempo indeterminado, enquanto o ambiente for estável (Chesson 2000). Resumindo, processos determinísticos, na minha concepção, são aqueles que podem ser preditos olhando-se as espécies em questão. Por exemplo, se duas espécies possuem diferenças nas habilidades de competição inerentes e precisam utilizar os mesmos recursos de formas similares, uma *sempre* vai excluir a outra porque o controle densidade-dependente de uma espécie será maior sobre a outra espécie do que nela mesma. Como vocês verão a seguir nos capítulos posteriores, grande parte da minha tese investiga processos de seleção, entre eles, a competição interespecífica e a interação espécie-ambiente (chamado filtro ambiental).

Em uma escala local é possível imaginar que o segundo processo, de especiação, não tenha muita importância na estruturação de comunidades. Um exemplo são pequenas comunidades fechadas de vegetais, nas quais a competição entre elas e não a especiação delas é que determina quem manterá uma população estável (Adler et al. 2010). Porém, a ecologia de comunidades já não lida apenas com escalas pequenas no tempo e no espaço e, nesses casos, a especiação pode ser um processo estruturador a ser considerado. Em uma escala temporal longa, não podemos ignorar a importância das especiações para gerar a diversidade específica de cada ambiente. Isso porque a especiação é a única maneira de se incluir novas formas de vida na face da Terra.

Ignorar a especiação numa escala de estudo mais ampla seria o mesmo que ignorar as mutações na estruturação genética das populações (Vellend 2010). Ambas são fontes de novidades e sem novidades não existe diversidade. Além disso, a especiação e o processo evolutivo como um todo têm uma assinatura importante na estruturação da diversidade que observamos. Por exemplo, a estrutura filogenética das comunidades pode nos indicar a quanto tempo há fauna de beija-flores de um lado de uma cordilheira está isolada da fauna do outro lado, observando a diferença filogenética entre os componentes das comunidades dos dois lados (Graham et al. 2009). Outro ponto importante das especiações é o fato de que espécies separadas por um menor intervalo tendem a ser mais parecidas em diversos aspectos (Darwin 1859, Wiens 2010) e isso nos dá abertura para inúmeras inferências ecológicas utilizando o parentesco das espécies de uma comunidade (e.g. Webb 2000). Nos meus capítulos eu não abordo a especiação em si, mas sim aspectos das assinaturas filogenéticas que as comunidades possuem e quais inferências podemos tomar quanto a isso.

A dispersão é um processo polêmico dentro da ecologia de comunidades. Apesar da sua importância relativa ter sido levantada desde estudos antigos, como o clássico modelo de Biogeografia de Ilhas de MacArthur e Wilson (1963), apenas recentemente observamos um crescente interesse na dispersão para a ecologia de comunidades, principalmente com a emergência da abordagem de metacomunidades (Leibold et al. 2004). Dentro da ecologia de metacomunidades, nenhuma comunidade é considerada sozinha no espaço, mas sim todas são conectadas pela possível dispersão das espécies entre elas (Leibold et al. 2004). Como essa dispersão pode mascarar a assinatura da relação espécie-específica com o ambiente, alguns autores trataram a dispersão como um processo antagônico ao de seleção, inclusive chamando de comunidades neutras aquelas sem assinatura de seleção e com alguma estruturação espacial (Smith e

Lundholm 2010). Porém, como a dispersão não é igual entre as espécies, sendo inclusive o fator principal da coexistência regional entre algumas espécies (Mouquet e Loreau 2002), considero errôneo nomear a dispersão como um processo contrário aos processos de seleção. Por exemplo, numa condição de trade-off competição-colonização, duas espécies podem coexistir regionalmente se uma é melhor dispersora, apesar de ser pior competidora (Cadotte 2007). Dessa forma, a dispersão é um dos grandes processos estruturadores de comunidades, não sendo nem um processo de seleção simples, nem um processo puramente estocástico. O papel da dispersão será melhor abordado no primeiro capítulo desta tese e retornará como um dos fatores importantes no último capítulo, inclusive com uma demonstração do trade-off competição-colonização.

Finalmente, processos neutros são aqueles nos quais a diferença entre espécies não importa e apenas a estocasticidade demográfica determina a estruturação das comunidades (Hubbell 2001). Por estocasticidade demográfica, considero os eventos de nascimento, morte e produção de novos indivíduos (Hubbell 2001). Apesar de existir um debate sobre a existência da estocasticidade como um todo na ciência (Vellend et al. 2014), aqui, tratarei como estocástico tudo aquilo que é imprevisível para a escala de interesse (Vellend et al. 2014). Por exemplo, estudando-se uma fêmea de algum mamífero, se considerarmos todo seu histórico genealógico, sua saúde e seus genes, podemos ter uma ideia de quantos filhotes essa fêmea terá por gravidez e qual a condição de saúde que eles terão. Porém, se pensarmos que não temos a informação necessária para todas as fêmeas e machos, a chance de nascimento e morte pode ser considerada estocástica na escala da população. Ou seja, na escala da população, todos possuem a mesma chance de ter filhos e de morrerem. Por isso, se considerarmos que em duas populações naturais as chances de nascimento e morte variam, mas não são

previsíveis, então podemos considerar esses processos como estocásticos na comunidade. Dessa forma, um desdobramento importante é que a chance de uma população ser extinta é relativa ao seu tamanho populacional, pois populações menores têm mais chances de que todos seus indivíduos morram sem deixar descendentes, enquanto nas populações maiores essas chances são menores (Chesson 2000, Hubbell 2001). Esse tipo de dinâmica neutra ocorre quando as espécies não possuem diferenças que geram seleção, ou seja, quando as espécies são equivalentes na habilidade competitiva, na utilização dos recursos e na adaptação ao ambiente (Chesson 2000, Adler 2007). A neutralidade não foi tema central em nenhum dos manuscritos que eu redigi nesta tese, porém todos os manuscritos abordam processos neutros, em maior ou menor escala. Especialmente no último capítulo, identificamos a emergência de padrões iguais aos gerados ao acaso (i.e. aleatórios), a partir de processos de seleção que envolvem competição entre múltiplas espécies.

Considerando o arcabouço teórico apresentado acima, estruturei minha tese para, ao longo de quatro capítulos, englobar os principais processos estruturadores de comunidades. Na figura 1 e nos parágrafos abaixo explico brevemente cada capítulo e a coesão entre eles.

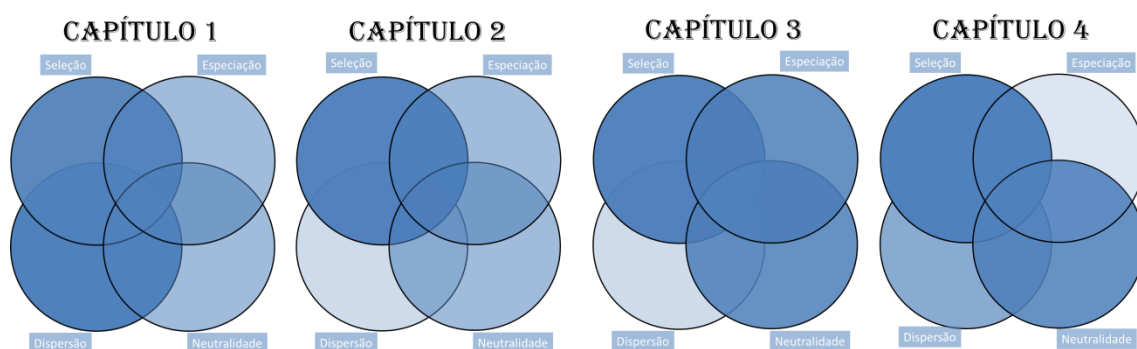


Figura 1. Diagramas de Venn mostrando a importância relativa de cada processo estruturador de comunidades em cada capítulo desta tese, conforme a gradação da cor.

A importância relativa é em relação à ênfase dos trabalhos e não dos processos que encontramos com maior influência na estruturação das comunidades.

No primeiro capítulo, investigamos padrões de comunidades na escala regional de todo o Estado de São Paulo. Neste capítulo estudamos como as comunidades de insetos aquáticos vão se diferenciando umas das outras, em relação à composição taxonômica e filogenética, ao longo do aumento na distância geográfica e ambiental entre elas. Nesse trabalho estávamos interessados em observar se a estruturação filogenética seguia um gradiente ambiental e, portanto, refletiria a relação espécie-específico com o ambiente, ou se seguia o gradiente de distância geográfica e, portanto, refletiria a dispersão limitada dos organismos. Dessa forma, abordamos primariamente a seleção e a dispersão como modeladores das comunidades em escala regional. Porém, como utilizamos informações filogenéticas, também pudemos discutir questões que englobam padrões de diversificação em insetos aquáticos. Além disso, nós consideramos nas nossas análises os atributos mais importantes para a dispersão dos insetos aquáticos e descobrimos que os grupos com maior assinatura de dispersão limitada são os insetos de tamanho grande, alta capacidade de dispersão e univoltinos (insetos com apenas um ciclo de vida completo por ano). Esses resultados contradizem estudos na região ártica (Astorga et al. 2012), em que encontraram maior assinatura de dispersão limitada em insetos pequenos e de pouco voo. Provavelmente isso ocorreu porque insetos tropicais variam muito na quantidade de gerações por ano, enquanto na região temperada e ártica os insetos possuem apenas um ciclo por ano. Isso faz com que a capacidade de voo seja muito importante para todos os insetos árticos dispersarem, enquanto nos trópicos o número de eventos de dispersão, que ocorrem a cada geração nova, pode ser mais importante, inclusive sobrepujando a importância da capacidade de voo em longa distância.

No capítulo dois investigamos comunidades de insetos aquáticos em uma escala espacial mais restrita, na bacia do Rio Itanhaém (SP). Neste trabalho separamos os atributos dos insetos que poderiam afetar a filtragem ambiental e a competição por recursos. Partimos do pressuposto de que, caso os filtros ambientais investigados fossem importantes, as espécies tenderiam a ter similaridades nesses atributos (Weiher e Keddy 1995), por exemplo, mesmo formato de corpo, tamanho e formas de respiração. Para os atributos de competição, caso a competição fosse limitante, então as espécies de um mesmo riacho deveriam ter atributos diferentes, para explorar diferentes recursos e diminuir a interferência das outras espécies (MacArthur e Levins 1967, Weiher e Keddy 1995). Portanto, nesse trabalho focamos em dois processos de seleção, a competição entre espécies e os filtros ambientais. Adicionalmente, nós também investigamos a estrutura filogenética das comunidades (i.e. quão aparentadas eram as espécies de cada comunidade), para identificar a congruência da estrutura funcional (de atributos) e filogenética. Isso porque espécies evolutivamente próximas devem, devido ao princípio da parcimônia evolutiva, ter maiores similaridades de fenótipos (Darwin 1859, Wiens 2010). Nós encontramos que as espécies coocorrentes comumente tinham atributos de filtros ambientais similares, mas não possuíam grandes diferenças nos atributos competitivos, indicando uma maior influência ambiental do que biótica na estruturação das comunidades de insetos aquáticos. A estrutura filogenética, em contrapartida, indicou que espécies com grande distância evolutiva comumente ocorrem juntas, não convergindo com o padrão fenotípico encontrado. Nós interpretamos isso como uma assinatura da comum coocorrência de clados antigos em riachos, um habitat museu que sofreu poucas extinções ao longo do tempo geológico.

No terceiro capítulo investigamos comunidades de libélulas adultas ao longo do gradiente longitudinal da bacia do Rio Betione (MS). Nesse capítulo nós estávamos

interessados na assinatura da competição e da filtragem ambiental, ou seja, novamente focamos em processos de seleção. Porém, utilizamos a taxonomia das espécies como substitutos das relações filogenéticas entre elas para investigar uma hipótese antiga. Darwin no seu livro ‘A origem das espécies’ de 1859, postulou que espécies próximas, de um mesmo gênero, devem competir mais intensamente por recursos, pois como elas divergiram a pouco tempo, seus fenótipos devem ser parecidos, forçando-as a explorar os mesmos recursos. Posteriormente a Darwin, toda uma linha de pensamento ecológico indicou que espécies muito similares deveriam ter dificuldade em coexistir, sendo que o resultado mais comum seria a exclusão competitiva de alguma das espécies (Gause 1934 e o princípio da exclusão competitiva, MacArthur e Levins 1967 e a similaridade limitante). Unindo Darwin e o princípio de exclusão competitiva, nós investigamos no capítulo três se libélulas mais aparentadas coocorriam menos nos trechos dos riachos devido à interferência entre as espécies aparentadas. De modo interessante, as libélulas possuem uma particularidade que nos instigou a utilizá-las nesse estudo. Elas possuem alta taxa de ataques agonísticos com indivíduos de espécies fenotipicamente similares (Anderson e Grether 2010); isso provavelmente devido à baixa capacidade cognitiva das libélulas de separar um indivíduo da mesma espécie de um de outra espécie, quando eles são parecidos (Anderson e Grether 2010). Apesar do embasamento teórico e das particularidades das libélulas, nós não encontramos sinais da exclusão competitiva entre espécies próximas, mas sim um forte sinal da influência da estrutura dos riachos na seleção das espécies. As espécies que coocorriam nas comunidades eram, geralmente, filogeneticamente próximas e com estratégias reprodutivas de oviposição similares. Essas estratégias talvez sejam mais relevantes que a competição, forçando as espécies mais similares a coocorrerem, mesmo que sob intensa interação negativa.

Como expus nos capítulos anteriores, um grande foco dos meus trabalhos foi encontrar assinaturas da exclusão competitiva na estruturação das comunidades. Nos capítulos dois e três utilizei abordagens observacionais, com dados de atributos e de parentesco entre grupos de insetos aquáticos. Como relatei, pouca influência foi constatada utilizando a abordagem tradicional (Weiher e Keddy 1995, Webb 2000) que inferem competição a partir da diferença elevada nos atributos das espécies coocorrentes, ou no seu parentesco. No quarto capítulo tomamos uma abordagem diferente, na qual investigamos o processo de competição entre espécies em uma simulação computacional e observamos como, quando e em qual intensidade a competição gera assinaturas que seriam captadas por métodos observacionais. Nós levamos em consideração uma sugestão importante que surgiu em 2010, de que a diferença fenotípica elevada nas espécies coocorrentes não é o único padrão possível quando a competição é forte (Mayfield e Levine 2010). Pois, quando as diferenças entre espécies indicam menor poder competitivo, a competição vai forçar as espécies coocorrentes a serem parecidas, mesmo que isso indique maior sobreposição no uso de recursos. Ou seja, no fim das contas, a competição poderia gerar comunidades com espécies com similaridades nos atributos muito baixas, ou muito elevadas (Mayfield e Levine 2010). Outro fator que não foi considerado até então na abordagem observacional tradicional (Weiher e Keddy 1995, Webb 2000) e inclusive nos outros capítulos desta tese, é que as espécies não competem em pares, sendo que geralmente múltiplas espécies estão competindo em uma rede complexa com efeitos negativos e também positivos entre elas, onde o “inimigo do meu inimigo é meu aliado”. Ou seja, quando múltiplas espécies competem, algumas espécies podem ser beneficiadas pela adição de mais espécies que evitam que um competidor forte as sobrepuje (Levine 1976, Lawlor 1979). Isso porque o efeito negativo dessa espécie mais forte será diluído entre

mais espécies. Simulando a competição nos termos descritos, nós encontramos que as comunidades são comumente compostas por espécies similares, porque essa similaridade garante uma habilidade competitiva igual entre elas e evita que se excluam. De modo inédito, encontramos comumente comunidades sem nenhuma estrutura diferente de uma estrutura gerada ao acaso, ou seja, uma estrutura aleatória de diferenças entre espécies, o que sugere falta de efeito competitivo. Essa aparente falta de efeito competitivo na estrutura de atributos das comunidades ocorre devido à diluição competitiva entre várias espécies, e não porque as comunidades são apenas influenciadas por processos neutros. Nesse caso, vários tipos de combinações entre espécies seriam possíveis e nenhuma assinatura aparente seria detectada utilizando os métodos tradicionais de se inferir competição. Essa aparente emergência de neutralidade com uma explicação completamente determinística é, na minha opinião, um dos destaques desta tese.

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Capítulo 1

Dispersal traits drive the phylogenetic distance decay of similarity in Neotropical stream metacommunities

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Este capítulo foi publicado na forma de artigo científico no periódico *Journal of Biogeography* em 2015.

Saito, V. S., Soininen, J., Fonseca-Gessner, A. A., & Siqueira, T. (2015). Dispersal traits drive the phylogenetic distance decay of similarity in neotropical stream metacommunities. *Journal of Biogeography*, 42(11), 2101-2111.

ABSTRACT

The drivers of phylogenetic beta diversity include both local processes (e.g. environmental filtering) and regional processes (e.g. dispersal limitation). The role of environmental filtering can be investigated more directly by analysing community—environment associations, but dispersal limitation is one of the most challenging processes to examine. We investigated the role of traits related to dispersal – flight capacity, body size and voltinism – as drivers of phylogenetic distance decay relationships in Neotropical stream insect communities. The study was conducted in headwater streams spread over 600 kilometres in southeastern Brazil. We compiled a dataset of aquatic insect communities inhabiting streams across the State of São Paulo (Brazil). Then, we investigated environmental and spatial signals on phylobetadiversity patterns of aquatic insects using Mantel tests, multiple regressions on distance matrices and variation partitioning. We employed null models to investigate if phylogenetic distance decay differed from pure compositional distance decay. We deconstructed the dataset based on dispersal related traits; we then ran distance decay analysis for these subsets separately. Geographical distance, rather than environmental distance, better explained the patterns of phylobetadiversity. We found that the phylogenetic decay relationship differed from the relationship expected for the null models only for univoltine, large-bodied genera with a high flight capacity. Dispersal limitation, rather than species sorting, was the main driver for phylogenetic beta diversity in the metacommunity that we studied. We suggest that life-history strategies and mainly voltinism drive the distance decay of similarity in the examined insect communities. We additionally discuss the role of dispersal events over time to explain differences in distance decay patterns among tropical and other regions.

Keywords aquatic insects, distance decay, dispersal, geographic distance, phylobetadiversity, Phylosor, southeastern Brazil, voltinism.

INTRODUCTION

Although Tobler's first law of geography – near things are more similar than distant ones (Tobler, 1970) – seems to be a useful guideline in community ecology (Nekola & White, 1999; Soininen *et al.*, 2007), studying this law involves disentangling many complex mechanisms related to the distance decay of similarity in ecological communities (Soininen *et al.*, 2007). The conceptual framework developed in Nekola and White's (1999) seminal paper on distance decay predicts two major processes driving the decay of community similarity – and the increase in beta diversity – with increasing geographical distance. The first process is based on species' niches and environmental sorting; communities become more dissimilar with geographical distance due to spatial variation in environmental features (“environmental filtering”; Weiher & Keddy, 1995). The second process is based on a neutral perspective where the spatial configuration of sampling sites and dispersal events irrespective of species identity determines community similarity among locations (Hubbell, 2006). As a result, investigations of the distance decay relationships using both environmental and geographical distances can shed light on niche and neutral processes as drivers of beta diversity (Nekola & White, 1999; Soininen *et al.*, 2007).

Given recent advances in phylogenetic methods applied to ecology, hypotheses related to historical factors driving beta diversity have been tested with the inclusion of evolutionary information. Phylogenetic beta diversity, or phylobetadiversity, can be defined as the sum of phylogenetic distances (branch lengths) of individuals or species between two communities (Bryant *et al.*, 2008; Graham *et al.*, 2009). Phylobetadiversity differs from classical taxonomic beta diversity – i.e. compositional differences between communities (Whittaker, 1960) – by including a temporal dimension that quantifies how evolutionary relatedness among species changes across space (Graham & Fine,

2008). For example, Graham *et al.* (2009) found that the Andean mountain chain separated entire clades of hummingbirds by comparing the phylobetadiversity of communities to a null expectation representing no influence of phylogenies (i.e., randomizing the “tip” of phylogenies and keeping alpha and beta taxonomic diversity constant). Therefore, studies of phylobetadiversity using the distance decay framework can provide evidence of historical imprints underlying how contemporary communities assemble. For example, if one finds a higher phylogenetic distance decay than expected by chance it is possible to infer that related species remain geographically close to one another due to long-term dispersal limitations (Graham & Fine, 2008).

Aquatic insects inhabiting streams are a useful target group to study phylogenetic distance decay relationships. Streams comprise network systems surrounded by terrestrial matrix. Such a structure provides the opportunity to study communities with explicit boundaries surrounded by an inhospitable matrix in that larvae must develop in water but adults fly overland to disperse. Aquatic insects possess several dispersal and life-history strategies that should have a direct influence on how they disperse through the terrestrial matrix (Bilton *et al.*, 2001; Shurin *et al.*, 2009). For example, several caddisfly species can direct their flight to track for optimal sites for oviposition while some groups of midges are not able to direct their flight and instead disperse mostly via the wind (Bilton *et al.*, 2001). Thus, our broad expectation here is that these differences in life-history strategies affect the distance decay relationships of aquatic insects.

Logistically, however, the study of dispersal among aquatic insects is very challenging due to difficulties gathering observational data (Bilton *et al.*, 2001). Aquatic insects are difficult to mark and recapture and only recently have molecular and isotopic techniques advanced (Bilton *et al.*, 2001). One way to overcome this challenge is to use

a deconstruction approach (Marquet *et al.*, 2004) to study biological traits that correlate with dispersal patterns or influence dispersal. Deconstructing biological communities into more homogeneous subsets can improve pattern searches since heterogeneous datasets may contain species that respond differently to ecological processes, which hides patterns that emerge only in some subsets (Marquet *et al.*, 2004). In this context, one could divide the entire assemblage dataset into subsets of species with similar ecological attributes such as body size, capacity to direct flight (e.g. Astorga *et al.*, 2012; Landeiro *et al.*, 2012; Heino, 2013; Grönroos *et al.*, 2013) and the number of generations per year (voltinism).

Body size and flight capacity are characteristics that have been explored in more detail recently, but no attention has been paid to the role of voltinism in the spatial distribution of species. Since voltinism in aquatic insects refers to the number of generations per year, this quantity is related to the number of dispersal events over time. Many aquatic insects have an aquatic larval stage and can only disperse through the terrestrial matrix in their winged adult phase. One can therefore expect that organisms that produce a larger number of generations per year will be more widely distributed due to larger number of dispersal events occurring each year. However, all dispersal-related traits are expected to be interrelated and cannot be treated as independent since they evolved under evolutionary constraints (“trait syndrome”; Poff *et al.*, 2006). As a result, dispersal-related traits should be interpreted in the context of an entire life-cycle strategy. For example, univoltine species should be large-bodied given that an entire year is used to grow to the final body size. Their large wings also might enable them to disperse directly with long distance flights. In this sense, species traits such as body size and voltinism are constrained by physiological and energetic limitations that determine several ecological patterns such as size-abundance distribution, range-abundance

distribution and rarity (Brown, 1995). Given the reasoning described above, we tested the following hypotheses (Fig. 1) using aquatic insect data from tropical and subtropical streams in southeastern Brazil.

Geographical versus environmental distance decay. We expected that dissimilarity in environmental characteristics would co-vary with geographical distance (**H1a**). Patterns of phylogenetic distance decay would accordingly be qualitatively similar when geographical and environmental distances were used since both represent imprints of dispersal limitation and species sorting. We additionally expected that a shared environmental and geographical component would better explain phylobetadiversity than ‘pure’ environmental and geographical distances because both distances would be correlated (**H1b**).

Phylobetadiversity versus null expectations. We expected that phylogenetic distance decay rates would be higher than expected under null models as a result of the turnover of clades (i.e. entire families) rather than the exchange of species drawn randomly from the species pool among distant communities (**H2**). This finding would demonstrate that closely related taxa remain geographically close because of a shared history of colonisation and dispersal limitation (in the case of geographical distance) or because of a similar habitat preference (in the case of environmental distance) (Graham & Fine, 2008; Morlon *et al.*, 2011; Swenson, 2011).

Directional flight capacity. We expected that groups with greater directional flight capacity – the ability to actively fly and search for suitable sites – would exhibit a lower distance decay rate (**H3**) because directional flight capacity enables an organism to disperse long distances in search for locations suitable to reproduction, which results in lower beta diversity (Bilton *et al.*, 2001; Astorga *et al.*, 2012; Heino, 2013; Grönroos *et al.*, 2013).

Body size. We expected that smaller insects would have lower initial similarity that indicated a strong response to fine-scale environmental features. This situation would result in high turnover rates at short distances (Soininen *et al.*, 2007; Shurin *et al.*, 2009) (**H4a**). We also expected that smaller insects would have lower decay rates (i.e. the slope of the distance decay regression) since they have greater chance of dispersal due to their larger population sizes and higher chance of traveling long distances carried by winds (Hillebrand *et al.*, 2001; Hillebrand, 2004) (**H4b**).

Voltinism. We expected that insects that produce a larger number of generations per year would have lower distance decay rates due to more dispersal events over time (**H5**). Multivoltine organisms should thus be able to colonise and recolonise more distant sites in shorter periods of time compared with univoltine organisms (Gillooly *et al.*, 2002).

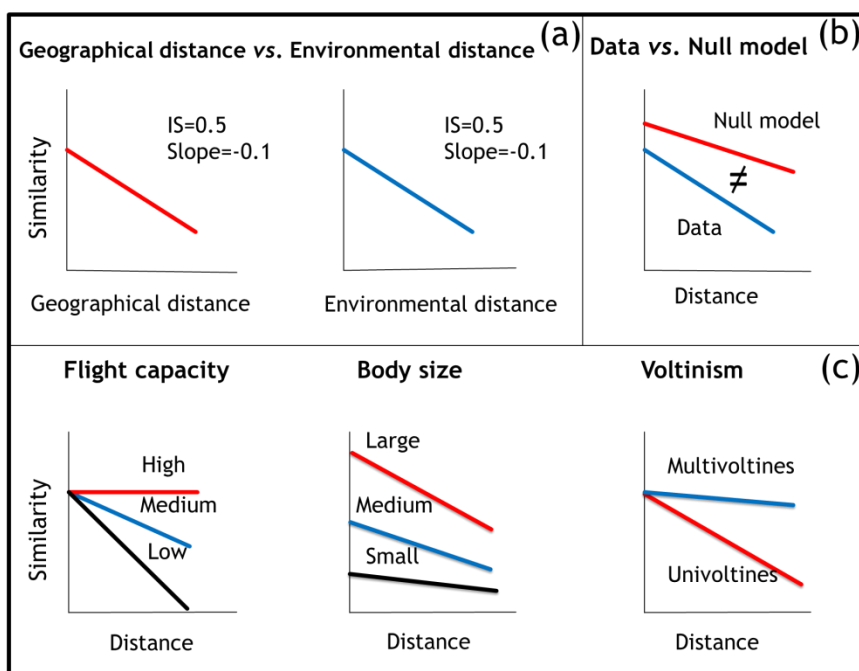


Figure 1. Predictions based on the distance decay framework. (a) The decay rates and initial similarity should be similar when using environmental and geographical distance. (b) The decay relationship should differ from that expected from the null model, which indicates the phylogeny of Neotropical stream aquatic insects structured in space. (c) The decay rate should be higher for species with a low flight capacity, large-bodied species and univoltine species.

MATERIALS AND METHODS

Dataset

Our dataset was composed of local communities of stream insects sampled at 32 sites selected from three different databases: the “macroinvertebrate database” (sampled from 2005–2008), the “cerrado macroinvertebrate database” (sampled in 2011) compiled by the aquatic entomology research group at the Universidade Federal de São Carlos in São Carlos city, Brazil (Roque *et al.*, 2010; Suriano *et al.*, 2011; Siqueira *et al.*, 2012; Saito *et al.*, 2015) and the “PELD Itanhaém macroinvertebrate database” (sampled in 2013) compiled by the research group in biodiversity and metacommunity ecology at the Universidade Estadual Paulista in Rio Claro city, Brazil (Saito *et al.*, 2015a).

The entire dataset comprises two major catchments along a spatial gradient of 600 kilometres in the State of São Paulo in southeastern Brazil (see Fig. 2). One major catchment includes streams that run along the interior part of the state; the other major catchment includes streams that run to the sea. The streams that we selected represent near-pristine conditions, and the sampling procedures were similar between the databases. The streams were located in micro-basins entirely covered by natural vegetation with water depths less than 50 cm. The tree canopy coverage exceeded 70% of the channel, and we recorded an absence of macrophytes, a high dissolved oxygen concentration, a low conductivity, and slightly alkaline-to-acid water. We have been continually visiting most of the sampling areas since 2001 and did not notice any drastic change in land use during this period; most streams are located within protected conservation areas.

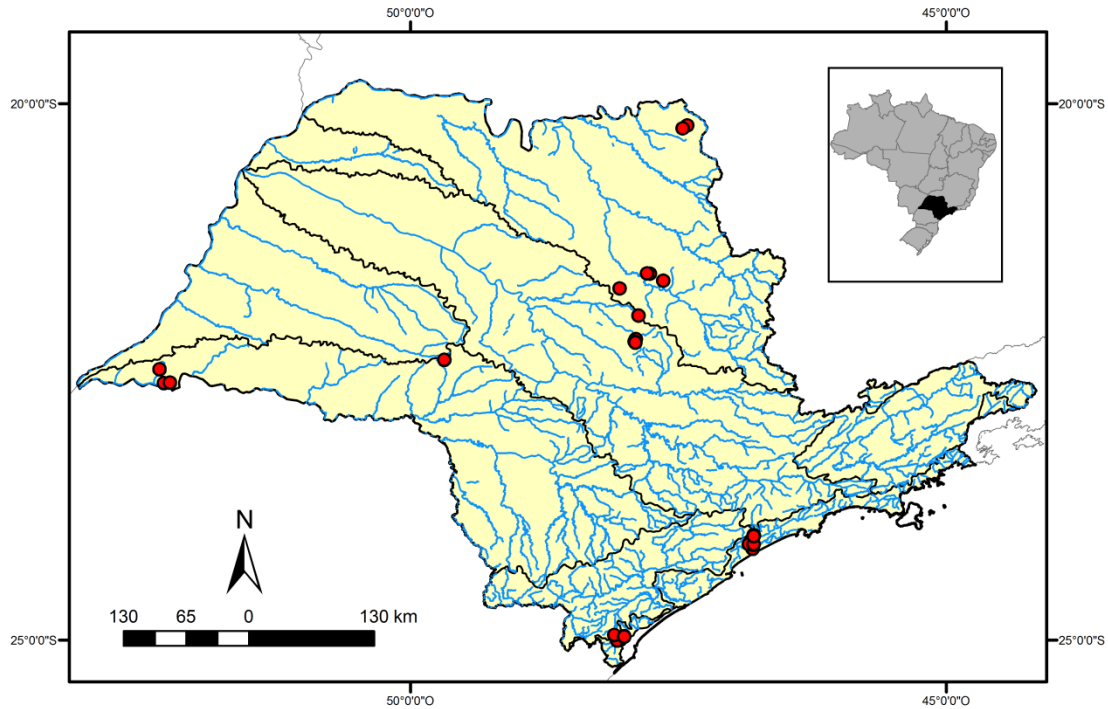


Figure 2. Map showing 32 sampled Neotropical stream aquatic insects communities in southeastern Brazil with main catchments and rivers.

We collected aquatic insects using a Surber sampler (0.3 m² area and 250 μm mesh size). Six to ten samples were collected randomly along a 100-m stretch of each stream. Although different institutions organised the sampling for each database, the same researchers helped with the sampling in the field (V.S.S. and T.S. participated in the field work of the three databases). The number of samples that we collected in the field does not influence substantially the community structure of these insects since we know that 5–6 Surber samples are sufficient to characterise reliably the macroinvertebrate community in these streams (Saito *et al.*, 2015a). The dataset that we compiled included abundance information on genus-level identifications, except for Hemiptera which was left on family level.

Phylogenetic information

We used the hypothetical supertree published in Saito *et al.* (2015b) that contains all the genera used in our analysis (139). To construct the supertree, we used information of ages nodes compiled from several molecular phylogenies (see Saito *et al.*, 2015b for details). We acknowledge that our supertree is not ideal for answering strict evolutionary questions of an individual genus, but we believe that our phylogenetic information is valid for large-scale community ecology studies requiring such information.

Environmental and geographical distance

We used a combination of local and regional environmental characteristics to estimate the environmental distance between stream sites. The local variables that we measured included pH, electrical conductivity, stream width, stream depth, and substrate composition (percentage of boulders, gravel, sand and mud); we used the mean of three values measured at three different sections of each stream. We estimated predominant substrata visually as the proportion of the stream bottom covered by boulders and cobble (>256 mm), gravel (2–255 mm), sand (0.125–2 mm) and mud (<0.125 mm). The regional variables included altitude (estimated using a GPS device), mean annual precipitation and mean annual temperature (obtained from WorldClim; Hijmans *et al.*, 2005).

We calculated the environmental distances between the pairs of sites using Euclidian distances on standardised values. We transformed the percentages to arcsine square root; the other numerical continuous variables were log-transformed prior to calculating environmental distances (except for pH). We calculated geographical distances using Euclidian distances between the pairs of sites based on geographical coordinates. Standardisation and distance calculations were conducted using the ‘vegdist’ function of the package VEGAN in R (Oksanen *et al.*, 2013). We did not use

watercourse distances in our analyses for a number of reasons. First, the majority of the organisms that we studied dispersed in air actively in their adult winged phase. Second, some of the sampled sites were located in catchments that ran directly to the sea without any water connections to sites located in different drainages. Third, there were several large rivers and lakes in the water routes; we do not believe that headwater species would inhabit these bodies (Heino & Mykrä, 2008). Therefore, we maintain that overland flying in the adult phase is the principal mechanism of dispersal. Fourth, several studies have investigated possible differences of using watercourse and overland distances for macroinvertebrates; these studies have found that these distances are typically highly correlated and provide largely similar community patterns (Landeiro *et al.*, 2011; Maloney & Munguia, 2011; Grönroos *et al.*, 2013).

Phylogenetic beta diversity

We quantified phylobetadiversity using the incidence-based Phylosor index (Bryant *et al.*, 2008). Phylosor, a pairwise metric based on the Sorensen index, estimates the total shared phylogenetic branch length of taxa between two sampled communities divided by the average branch length of each sampled community (Morlon *et al.*, 2011). Since we expected that environmental distances would co-vary with geographical distances, we tested the correlation between these two matrices using a Mantel test (**H1a**). Because we expected that phylobetadiversity would be better explained by shared components of environmental and geographical distance, we used multiple regressions on distance matrices (MRM; Lichstein, 2007) (**H1b**). We used the phylobetadiversity as the response distance matrix and environmental and geographical distances as the explanatory matrices. MRM is thought to be more flexible test than the often-used Mantel test since it can cope with linear, non-linear and non-parametric relationships between matrices. We tested the significance of R^2 values with 10,000

permutations. To tease apart the ‘pure’ and shared variation due to environmental and geographical distances, we used a variation partitioning technique (Borcard *et al.*, 1992). Variation partitioning measures the amount of variation in the response matrix that can be attributed exclusively to a focal matrix and shared by two or more explanatory matrices.

Phylogenetic distance decay

To relate the patterns of phylogenetic community structure with environmental and geographical distances, we used linear regression models with phylobetadiversity values among sites as the response variable. We used the initial similarity (the intercept of the linear model; Soininen *et al.*, 2007) and the slope to characterise the decay relationship. The intercept or initial similarity reflects the turnover at small geographical extents (e.g. the similarity at a distance of 1 km in Soininen *et al.*, 2007). A statistically significant intercept indicates that the community structure is affected by fine-scale heterogeneity in the environment or that organisms exhibit dispersal limitation at fine spatial scales (Nekola & White, 1999; Soininen *et al.*, 2007). The slope indicates the distance decay rate in space or along environmental gradients.

To test whether the phylogenetic similarity between two communities was solely a consequence of taxonomic similarity or if it was also caused by a non-random structure of shared and unshared lineages, we compared the initial similarity and slopes with null expectations (**H2**). To do so, we compared the values in the decay of phylogenetic similarity to values obtained by reshuffling the sampling location of genera along the tips of the phylogenetic tree 1,000 times (“taxa.labels”) (“taxashuffle”; Kembel, 2009). Following the conceptual approach of null models – holding all data patterns constant except for the one under study (Gotelli & Graves, 1996) – we chose the taxa shuffle model because it randomises only the location of the taxa in the

phylogeny. The taxa shuffle model holds the richness, occupancy and the alpha and beta diversity of samples constant and considers only the effect of phylogeny on the observed pattern (Swenson, 2011, 2014; Zhang *et al.*, 2013). The observed values of initial similarity and slope were considered to be significantly different from the null expectation if they fell into the lower or upper 2.5th percentile, which corresponds to a two-tailed test at $P=0.05$ (Bryant *et al.*, 2008).

Phylogenetic signal

In order for ecological inferences drawn from phylogenetic structure it is important to test whether there is a phylogenetic signal (*sensu* Losos, 2008) in the data. Therefore, we tested whether closely related genera exhibited more similar niches than was expected by chance using the Mantel correlogram analysis; similarity in environmental preferences were used as a measure of niche similarity (Diniz-Filho *et al.*, 2010). We calculated similarity in environmental preferences using the Euclidian distance among pairs of genera. We used the abundance-weighted mean value of all environmental characteristics for the calculations – i.e. the difference in the mean value of dissolved oxygen, turbidity, and temperature among the pairs of genera for the sites where they occurred (Stegen *et al.*, 2012; Wang *et al.*, 2013).

Deconstruction by biological traits

We created subsets of aquatic insects by dividing the entire dataset into groups according to the three biological traits that we expected to be linked to dispersal: directional flight capacity, body size and voltinism (Table 1). This methodology followed recent trait-based studies (Poff *et al.*, 2006; Colzani *et al.*, 2013; Heino, 2013, Table 1). Next, we investigated phylogenetic distance decay relationships within these subsets separately (**H3**, **H4a**, **H4b**, **H5**). We separated genera with (i) low directional flight capacity, (ii) medium directional flight capacity and (iii) high directional flight

capacity to analyse the influence of directional flight capacity on phylobetadiversity. For the body size analysis we separated the genera into three categories: > 1.5 cm in length (large-bodied subset), 1–1.5 cm (medium-bodied subset) and < 1 cm (small-bodied subset). Finally, for the voltinism analysis we separated univoltine genera from multivoltine genera. Although we inferred some information of trait states for the family level, we analysed traits that were phylogenetically conserved enough to reliably estimate the missing values (e.g. multivoltine, univoltine). For example, Dytiscidae species are all large predatory beetles with a high flight capacity and univoltine life cycles. To investigate trait syndromes, i.e., traits correlated with a whole life-history strategy, we prepared a contingency table to quantitatively show how many genera had the same combinations of trait states. To test for the significance in phylogenetic distance decay relationships relative to pure compositional turnover, we used the null model approach described above. All analyses were run in R using the packages PICANTE (Kembel *et al.*, 2010) and VEGAN (Oksanen *et al.*, 2013).

Table 1. Species traits of Neotropical aquatic insects related to dispersal capacity.

Order	Taxon	Body size	Dispersal capacity	Voltinism		
Coleoptera	<i>Dryops</i>	1	1	1	Body Size	
Coleoptera	<i>Helichus</i>	1	1	1	1	<1cm
Coleoptera	<i>Bidessonotus</i>	3	1	1	2	>1cm<1.5cm
Coleoptera	<i>Celina</i>	3	3	1	3	>1.5cm
Coleoptera	<i>Desmopachria</i>	3	3	1	Flight capacity	
Coleoptera	<i>Hydaticus</i>	3	3	1	1	low
Coleoptera	<i>Laccophilus</i>	3	3	1	2	medium
Coleoptera	<i>Liodessus</i>	3	3	1	3	high
Coleoptera	<i>Ranthus</i>	3	3	1	Voltinism	
Coleoptera	<i>Austrolimnius</i>	2	1	1	1	Univoltines
Coleoptera	<i>Cylloepus</i>	2	1	1	2	Multivoltines
Coleoptera	<i>Heterelmis</i>	2	1	1		
Coleoptera	<i>Hexacylloepus</i>	2	1	1		
Coleoptera	<i>Lara</i>	2	1	1		
Coleoptera	<i>Macrelmis</i>	2	1	1		

Coleoptera	<i>Microcylloepus</i>	2	1	1
Coleoptera	<i>Neoelmis</i>	2	1	1
Coleoptera	<i>Phanocerus</i>	2	1	1
Coleoptera	<i>Promoresia</i>	2	1	1
Coleoptera	<i>Stegoelmis</i>	2	1	1
Coleoptera	<i>Xenelmis</i>	2	1	1
Coleoptera	<i>Zaitzevia</i>	2	1	1
Coleoptera	<i>Gyretes</i>	1	3	1
Coleoptera	<i>Gyrinus</i>	2	3	1
Coleoptera	<i>Anacaena</i>	3	3	1
Coleoptera	<i>Berosus</i>	3	3	1
Coleoptera	<i>Derallus</i>	3	3	1
Coleoptera	<i>Helocombus</i>	2	3	1
Coleoptera	<i>Hydrochus</i>	3	3	1
Coleoptera	<i>Laccobius</i>	3	3	1
Coleoptera	<i>Tropisternus</i>	3	3	1
Coleoptera	<i>Lutrochus</i>	1	1	1
Coleoptera	<i>Hydrocanthus</i>	1	3	1
Coleoptera	<i>Suphisellus</i>	1	3	1
Coleoptera	<i>Ectopria</i>	1	1	1
Coleoptera	<i>Psephenus</i>	1	1	1
Coleoptera	<i>Anchytarsus</i>	2	2	1
Diptera	<i>Ceratopogonidae_sp</i>	1	1	2
Diptera	<i>Culicidae_sp</i>	1	1	2
Diptera	<i>Dixidae_sp</i>	1	1	2
Diptera	<i>Dolichopodidae_sp</i>	1	2	2
Diptera	<i>Empididae_sp</i>	2	2	2
Diptera	<i>Ephydriidae_sp</i>	1	2	2
Diptera	<i>Psychodidae_sp</i>	1	2	2
Diptera	<i>Simuliidae_sp</i>	1	2	2
Diptera	<i>Stratiomyidae_sp</i>	1	2	2
Diptera	<i>Tabanidae_sp</i>	2	2	2
Diptera	<i>Tipulidae_sp</i>	2	2	2
Ephemeroptera	<i>Campylocia</i>	3	2	1
Ephemeroptera	<i>Americabaetis</i>	2	2	2
Ephemeroptera	<i>Apobaetis</i>	2	2	2
Ephemeroptera	<i>Baetodes</i>	2	2	2
Ephemeroptera	<i>Callibaetis</i>	2	2	2
Ephemeroptera	<i>Cloeodes</i>	2	2	2
Ephemeroptera	<i>Caenis</i>	2	2	2
Ephemeroptera	<i>Leptohyphes</i>	2	2	2
Ephemeroptera	<i>Leptohyphodes</i>	2	2	2
Ephemeroptera	<i>Traverhyphes</i>	2	2	2
Ephemeroptera	<i>Tricorythodes</i>	2	2	2
Ephemeroptera	<i>Tricorythopsis</i>	2	2	2
Ephemeroptera	<i>Farrodes</i>	2	2	2

Ephemeroptera	<i>Hagenulopsis</i>	2	2	2
Ephemeroptera	<i>Hermanella</i>	2	2	2
Ephemeroptera	<i>Hylite</i>	2	2	2
Ephemeroptera	<i>Massartella</i>	2	2	2
Ephemeroptera	<i>Miroculis</i>	2	2	2
Ephemeroptera	<i>Needhamella</i>	2	2	2
Ephemeroptera	<i>Thraulodes</i>	2	2	2
Ephemeroptera	<i>Melanemerella</i>	2	2	2
Hemiptera	Belostomatidae_sp	3	3	1
Hemiptera	Gerridae_sp	2	3	1
Hemiptera	Helotrephidae_sp	1	2	1
Hemiptera	Naucoridae_sp	2	2	1
Hemiptera	Pleidae_sp	1	2	1
Megaloptera	<i>Archichauliodes</i>	3	3	1
Megaloptera	<i>Corydalus</i>	3	3	1
Odonata	<i>Castoraeschna</i>	3	3	1
Odonata	<i>Limnetron</i>	3	3	1
Odonata	<i>Neuraeschna</i>	3	3	1
Odonata	<i>Calopterygidae_sp</i>	3	3	1
Odonata	<i>Navicordulia</i>	3	3	1
Odonata	<i>Neocordulia</i>	3	3	1
Odonata	<i>Agriogomphus</i>	3	3	1
Odonata	<i>Aphylla</i>	3	3	1
Odonata	<i>Gomphoides</i>	3	3	1
Odonata	<i>Perigomphus</i>	3	3	1
Odonata	<i>Phyllocycla</i>	3	3	1
Odonata	<i>Progomphus</i>	3	3	1
Odonata	<i>Tibiogomphus</i>	3	3	1
Odonata	<i>Cannaphila</i>	3	3	1
Odonata	<i>Brechmorhoga</i>	3	3	1
Odonata	<i>Dasythemis</i>	3	3	1
Odonata	<i>Dythemis</i>	3	3	1
Odonata	<i>Erythrodiplax</i>	3	3	1
Odonata	<i>Macrothemis</i>	3	3	1
Odonata	<i>Orthemis</i>	3	3	1
Odonata	<i>Tramea</i>	3	3	1
Odonata	<i>Oxystygma</i>	3	3	1
Odonata	<i>Argia</i>	3	3	2
Odonata	<i>Cyanallagma</i>	3	3	2
Odonata	<i>Oxyagrion</i>	3	3	2
Plecoptera	<i>Gripopteryx</i>	2	2	1
Plecoptera	<i>Paragripopteryx</i>	2	2	1
Plecoptera	<i>Kempnyia</i>	3	2	1
Plecoptera	<i>Macrogynoplax</i>	3	2	1
Plecoptera	<i>Anacroneuria</i>	3	2	2
Trichoptera	<i>Protoptilinae</i>	2	1	1

Trichoptera	<i>Atopsyche</i>	2	2	1
Trichoptera	<i>Leucotrichinii</i>	2	1	1
Trichoptera	<i>Metrichia</i>	2	1	1
Trichoptera	<i>Neotrichia</i>	2	1	1
Trichoptera	<i>Ochrotrichia</i>	2	1	1
Trichoptera	<i>Oxyethira</i>	2	1	1
Trichoptera	<i>Atanatolica</i>	2	3	1
Trichoptera	<i>Grumichella</i>	2	3	1
Trichoptera	<i>Nectopsyche</i>	2	3	1
Trichoptera	<i>Notalina</i>	2	3	1
Trichoptera	<i>Triplectides</i>	2	3	1
Trichoptera	<i>Barypenthus</i>	2	2	1
Trichoptera	<i>Marilia</i>	2	2	1
Trichoptera	<i>Chimarra</i>	2	2	1
Trichoptera	<i>Cernotina</i>	2	1	1
Trichoptera	<i>Polyplectropus</i>	2	1	1
Trichoptera	<i>Grumicha</i>	2	2	1
Trichoptera	<i>Phylloicus</i>	3	2	2
Trichoptera	<i>Austrotinodes</i>	2	2	2
Trichoptera	<i>Helicopsyche</i>	2	1	2
Trichoptera	<i>Blepharopus</i>	2	2	2
Trichoptera	<i>Hydropsyche</i>	2	2	2
Trichoptera	<i>Leptonema</i>	3	2	2
Trichoptera	<i>Macronema</i>	3	2	2
Trichoptera	<i>Smicridea</i>	3	2	2
Trichoptera	<i>Oecetis</i>	2	3	2

RESULTS

We found a positive Mantel correlation between environmental and geographical distances as expected (Mantel statistic $r=0.48$, $P<0.01$; **H1a**). The variation partitioning of the MRM analysis revealed that we could explain 34% ($P<0.01$) of the total variation in phylobetadiversity. Contrary to our hypothesis (**H1b**), the shared component between environmental and geographical variables accounted for 12% of the variation ($P<0.01$); the pure geographical distance explained up to 21% of the variation ($P<0.01$). The pure environmental distance explained only 1% of the variation ($P<0.01$).

Since our use of environmental and geographical distances resulted in largely similar distance decay patterns (see Fig. 3), hereafter we report only the geographical distance decay relationships. The phylogenetic decay in similarity using the entire dataset was negative (slope= -0.038) but did not deviate from what was expected from the null model (**H2**) (Table 2).

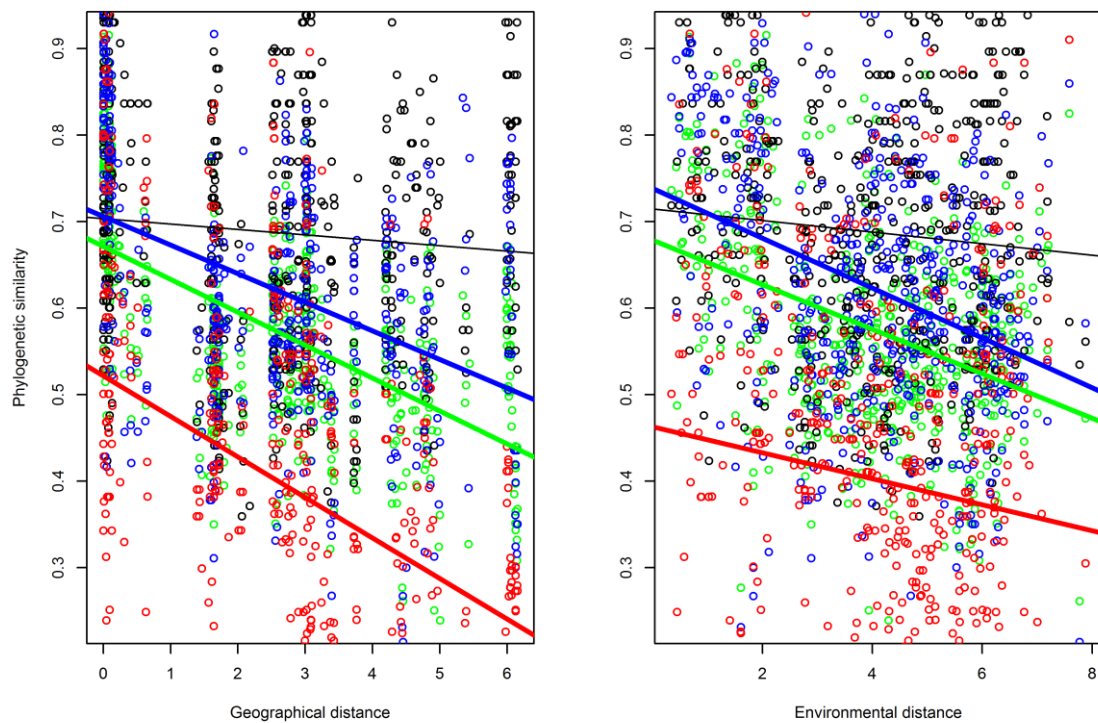


Figure 3. Phylogenetic distance decay of Neotropical stream aquatic insects relationships using geographical and environmental distance. Green line = complete dataset, black line = low-flight-capacity subset; blue line = medium-flight-capacity subset, red line = high-flight-capacity subset. The thinner line indicates a non-significant distance decay slope.

Table 2. Summary of results of phylogenetic distance decay analysis in Neotropical stream aquatic insects. The subsets represent biological traits related to dispersal capacity. IS = Initial similarity (fine scale phylobetadiversity; similarity at a distance of 1 km); Slope = Rate of decay in similarity (changes in phylobetadiversity as a function of distance). Bold values indicate significant phylogenetic distance decay relationships.

Analysed dataset	Subset	IS	Slope	Regression <i>P</i> value	Null model's (IS) <i>P</i> value	Null model's (slope) <i>P</i> value
Total dataset		0.67	-0.038	<0.01	0.469	0.144
Directional flight capacity	low capacity	0.70	-0.006	Ns	-	-
	medium capacity	0.70	-0.033	<0.01	0.374	0.427
	high capacity	0.52	-0.046	<0.01	0.052	0.025
Body size	small size	0.85	-0.035	<0.01	0.097	0.097
	medium size	0.63	-0.038	<0.01	0.385	0.165
	large size	0.65	-0.039	<0.01	0.024	0.025
Voltinism	multivoltine	0.72	-0.020	<0.01	0.067	0.189
	univoltine	0.49	-0.051	<0.01	0.019	0.023

The Mantel correlogram showed that taxonomically closely related genera did not have more similar environmental preferences than were expected by chance (Fig. 4). This finding implies that genera could be correctly treated as independent in the context of this study and that patterns and drivers of phylobetadiversity revealed using geographical distances are not related to the fact that closely related species have similar environmental preferences.

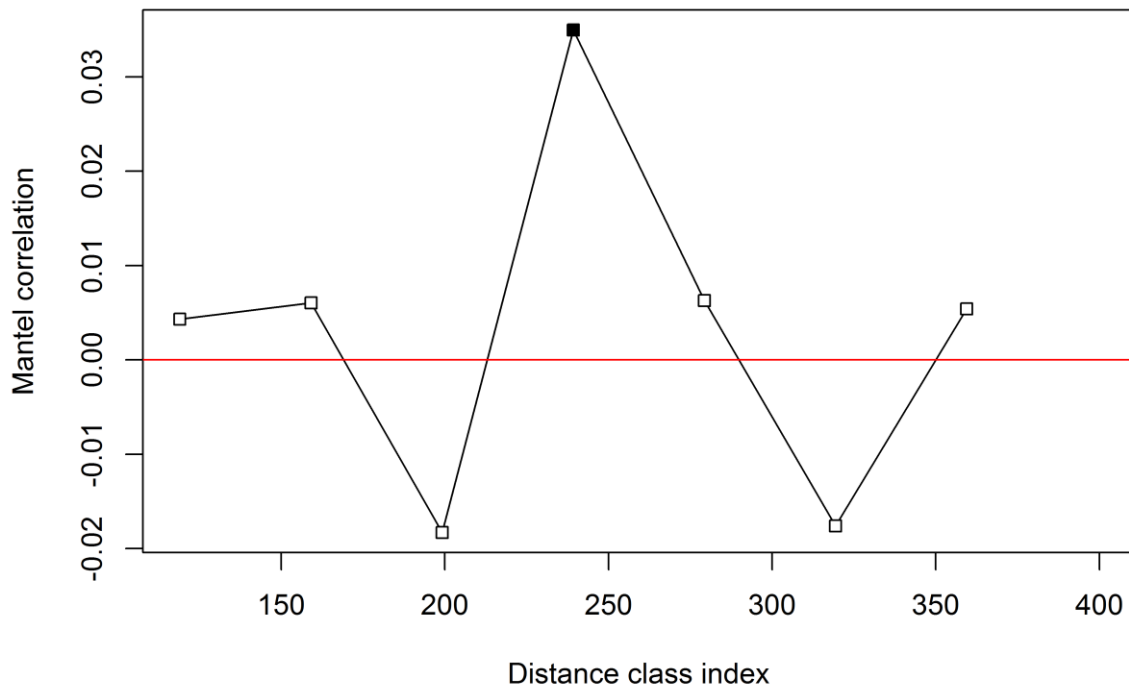


Figure 4. Correlation values of the Mantel correlogram computed between phylogenetic distances and niche distances (abundance-weighted species environmental affinity) of Neotropical stream aquatic insects. The solid square indicates a significant correlation ($P < 0.05$).

Contrary to our hypothesis, the low-flight-capacity subset did not exhibit a decay rate in phylogenetic similarity that differed from zero (Table 2); the slopes for the medium- and high-flight-capacity subsets were negative (**H3**). Furthermore, the low-flight-capacity subset had a larger initial similarity than the other subsets. Surprisingly, the subset with a higher flight capacity exhibited a higher phylogenetic decay rate compared with the null expectations (Table 2).

Contrary to our hypothesis, the small-bodied subset had higher initial similarity than the other groups (**H4a**). All body size groups exhibited distance decay relationships in phylogenetic similarity (i.e. phylogenetic similarity decreased with distance) but only for large-bodied insects was the decay higher than what was expected from the null

model (**H4b**) (Table 2). We note that the slopes were very similar across all body size categories (Table 2 and Fig. 5).

The multivoltine and univoltine subsets both exhibited a significant distance decay relationship in phylogenetic similarities, but only univoltines had higher decay rates than the null model expectations (Table 2). As we hypothesised (**H5**), multivoltines had much lower slopes than univoltines, which suggested notably lower decay in phylogenetic similarity with distance (Table 2 and Fig. 5). Multivoltines also had higher initial similarity.

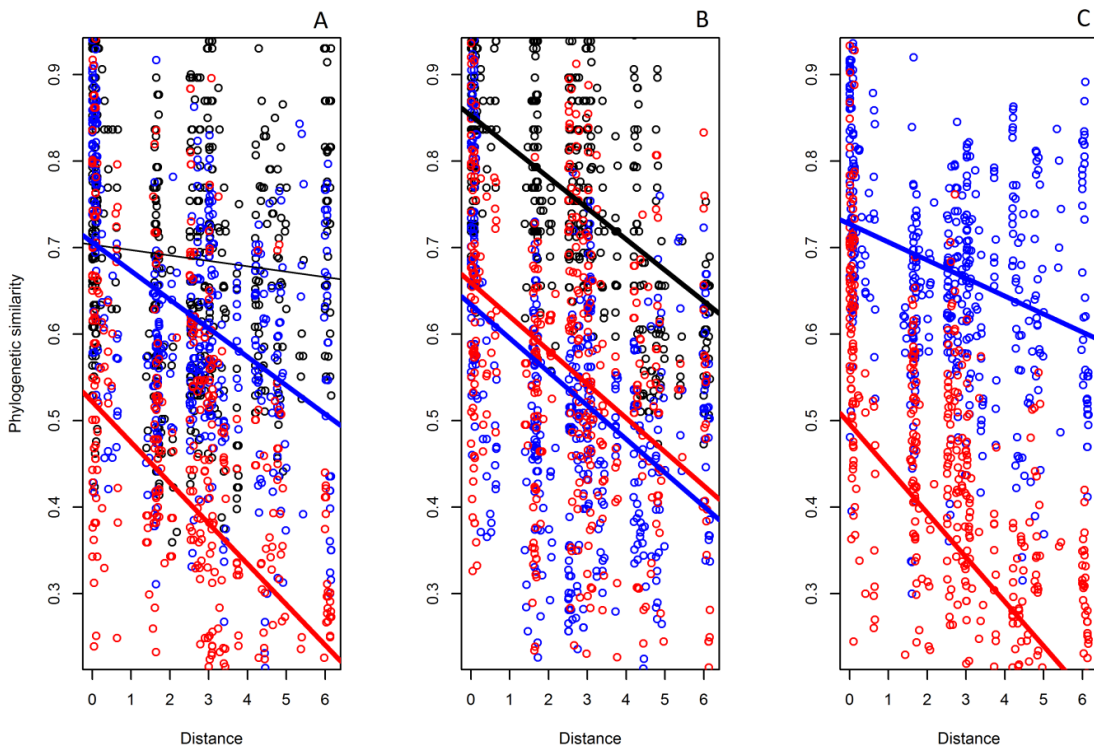


Figure 5. Distance decay in phylogenetic similarity of Neotropical stream aquatic insects using three traits linked to dispersal. (a) Decay patterns of subsets with different flight capacities. Black line = low flight capacity (not significant), blue line = medium flight capacity, red line = high flight capacity. (b) Decay patterns of subsets with different body sizes. Black line = small size genera, blue line = medium size genera, red line = large size genera. (c) Decay patterns of subsets with different numbers of life cycles per year. Blue line = multivoltines, red line = univoltines. The thinner line indicates a non-significant distance decay slope.

The majority of the univoltine genera were characterised by a large body size and a high flight capacity; most multivoltine genera were of medium size with a medium flight capacity (Table 3), which suggests linkages between the traits.

Table 3. Contingency table showing the correlation between trait state for flight capacity, body size and voltinism. Values indicate the number of genera with the same trait states.

Body size	Flight capacity	Voltinism	
		univoltine	multivoltine
small	low	5	3
	medium	1	5
	high	3	0
medium	low	21	1
	medium	10	26
	high	8	1
large	low	1	0
	medium	3	5
	high	37	3

DISCUSSION

The decay in similarity among communities might be a consequence of environmental control or dispersal limitation (Soininen *et al.*, 2007; Astorga *et al.*, 2012). We found evidence that the phylogenetic distance decay patterns varied among subsets of stream insects having different dispersal-related traits. We suggest that this variation is not due to closely related species having similar niches but rather to a historical imprint caused by dispersal limitation over time. The significant phylogenetic distance decay relationship that we found only for large-bodied univoltine genera with high flight capacity indicates that trait syndromes (Poff *et al.*, 2006) linked to life-history strategies determine phylobetadiversity in aquatic insects. These large-bodied univoltine genera likely faced dispersal limitations over time and were not able to colonise the studied area entirely (Graham *et al.*, 2009). A general explanation for these findings may be that *r*-strategists – abundant and small-bodied species with more

generations per year (MacArthur & Wilson, 1967; Pianka, 1970; Townsend & Hildrew, 1994) – have weak historical imprints in their spatial distribution due to intense dispersal between neighbouring sites (i.e. mass-effects), less time needed to complete a life cycle and a higher probability of traveling long distances by the wind.

Our results suggest that whereas individuals with less ability to direct flight are those that disperse farther, those that can direct flight likely prefer to stay close to where they emerged. Indeed, good flyers are typically able to actively find suitable patches at finer spatial scales (Bilton *et al.*, 2001). For example, dragonfly and caddisfly females are known for selecting the best microhabitat to increase egg survival and larval development (Michiels & Dhondt, 1990; Lancaster *et al.*, 2010). Because the environmental gradient that we investigated is correlated with geographical distance, it seems reasonable to infer that good fliers prefer closer and similar environments (to where they emerged) to lay their eggs. By actively searching for a closer site, an individual has a higher chance of finding a suitable similar patch to colonise, which avoids long-distance flights that require high consumption of energy (Harrison & Roberts, 2000). As a result, one would expect to find higher decay rates in phylogenetic similarity within this group. On the other hand, the subset with low flight directionality did not demonstrate significant distance decay. These organisms in fact most benefit from dispersal by wind (Kovats *et al.*, 1996; Hillebrand *et al.*, 2001; Rundle *et al.*, 2007; Heino, 2013), which may explain the occurrence of phylogenetically closely related groups at distant sites. Dispersal by wind can be a useful strategy for individuals with low flight directionality to disperse long distances, which would not be possible only with active dispersal. Wind dispersal is generally associated with body size since smaller insects with a limited ability to direct their flight are more frequently found at heights higher than 60 m, which makes them more prone to being dispersed by the wind

(Kovats *et al.*, 1996). Dispersal by the wind also involves a possible trade-off: although the wind can be an effective way to travel long distances it involves the risk of individuals being dispersed into unsuitable environments the individual or its offspring might not survive (Rundle *et al.*, 2007). This risk, however, could be compensated for if a species produces multiple generations per year (Gillooly *et al.*, 2002) and abundant offspring (Hillebrand *et al.*, 2001; Rundle *et al.*, 2007). In this sense, species with more individuals being carried by winds have a higher chance of colonising neighbouring patches (i.e. species sorting with high dispersal; *sensu* Winegardner *et al.*, 2012).

In this respect, voltinism could play a key role in the colonisation of new habitat patches since multivoltinism may also reduce the effects of environmental filtering. An individual with a shorter life cycle requires biological necessities within a shorter period of time to produce offspring. An unstable habitat patch that satisfies the individual's requirements in short intervals will therefore be sufficient for a multivoltine species to complete its life cycle but will unlikely be sufficient for a univoltine species. This reasoning is consistent with recent findings showing that European butterflies in disturbed fragments are those with fast life cycles, high mobility and high reproductive rates (Pavoine *et al.*, 2014). These authors argued that species with such characteristics are the only ones able to complete their life cycles before new disturbances occur in the environment. Thus, the ability to maintain viable populations in unstable habitat patches seems to be central to the widespread distribution of multivoltine insect groups.

Voltinism can also explain the differences between our results and those found by studies focusing on temperate and sub-arctic regions. In the tropics, multivoltine species are more abundant than in high-latitude regions because multivoltinism is the result of higher environmental temperatures (among other factors), which affect several life-history variables such as fecundity, growth and emergence (Ward & Stanford, 1982;

Altermatt, 2010). For example, Astorga *et al.* (2012) reported that species with a higher flight ability had lower decay rates in taxonomical similarity than species with a low ability to fly – exactly the opposite of what we found. Because the Finnish dataset used by Astorga *et al.* (2012) was likely composed of a large proportion of univoltine species, their subsets with different flight capacity included insects with a similar number of dispersal events over time, which may have caused flight capacity to be a decisive factor in the distance decay rate in temperate and sub-arctic regions. Since small size and low flight capacity are biologically linked to multivoltinism in the tropics (Ward & Stanford, 1982; Townsend & Hildrew, 1994), voltinism should determine the number of dispersal events per year and result in a more homogeneous distribution of multivoltine species throughout the tropics.

We have documented here how phylogenetic distance decay analysis can shed light on processes driving spatial patterns of phylogenetic similarity. Dispersal is one of the most difficult processes to measure at large scales but it is also one of the most fundamental processes driving biodiversity patterns (Bilton *et al.*, 2001; Vellend *et al.*, 2014). Thus, it is imperative that one finds ways to overcome this challenge such as including information about proxies (e.g. species traits) that likely represent dispersal ability. Using a deconstructive approach based on species traits, we found that some traits better explain spatial phylogenetic structure than do others. Furthermore, dispersal can be a crucial factor affecting the relative roles of deterministic versus stochastic processes in driving biodiversity patterns (Vellend *et al.*, 2014). Most ecological processes are complex enough to have some amount of stochasticity and determinism depending on the temporal and spatial scale of the study (Thompson & Townsend, 2006; Adler *et al.*, 2007; Clark, 2009; Chase & Myers, 2011). Although dispersal can be unpredictable at the level of individuals some species traits seem to drive dispersal

strategies at the population level, which makes dispersal patterns more predictable (Thompson & Townsend, 2006; Clark, 2009).

We conclude that historical constraints affect the phylogenetic distance decay relationships in aquatic insects and that this relationship can be analysed more deeply if one considers the life-history strategies of species since dispersal events seem to be affected by the identity and evolutionary history of different species. We demonstrated that both the number of dispersal events over time and the dispersal distances could affect the dispersal outcome and the resulting phylobetadiversity patterns. The number of dispersal events over time varies between geographical regions globally, which possibly explains the differences in distance decay relationships between temperate and tropical regions. Future work on the distance decay relationship must therefore ask not only "how far an organism can disperse" but also "how many times it disperses" to assess patterns in beta diversity.

ACKNOWLEDGMENTS

We are thankful to Luis M. Bini, Fabio O. Roque, and three anonymous reviewers for their suggestions on several versions of this manuscript. We are also thankful to Larissa Sayuri Sugai for elaborating the map. This study was partially funded by grant #2013/50424-1, #12/51511-2, and #2013/20540-0, São Paulo Research Foundation (FAPESP) and by grant #480933/2012-0 and #403723/2012-4, Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq).

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Capítulo 2

Phylogenies and traits provide distinct insights about the historical and contemporary assembly of aquatic insect communities

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Este capítulo foi publicado na forma de artigo científico no periódico *Ecology and Evolution* em 2016.

Saito, V. S., Cianciaruso, M. V., Siqueira, T., Fonseca-Gessner, A. A., & Pavoine, S. (2016). Phylogenies and traits provide distinct insights about the historical and contemporary assembly of aquatic insect communities. *Ecology and evolution*, 6(9), 2925-2937.

ABSTRACT

The assumption that traits and phylogenies can be used as proxies of species niche has faced criticisms. Evidence suggested that phylogenetic relatedness is a weak proxy of trait similarity. Moreover, different processes can select different traits, giving opposing signals in null model analyses. To circumvent these criticisms, we separated traits of stream insects based on the concept of α and β niches, which should give clues about assembling pressures expected to act independently of each other. We investigated the congruence between the phylogenetic structure and trait structure of communities using all available traits and all possible combinations of traits (4095 combinations). To account for hierarchical assembling processes we analyzed patterns on two spatial scales with three pools of genera. Beta niche traits selected a priori – i.e. traits related to environmental variation (e.g. respiration type) - were consistently clustered on the smaller scale, suggesting environmental filtering, while α niche traits – i.e. traits related to resource use (e.g. trophic position) - did not display the expected overdispersion, suggesting a weak role of competition. Using all traits together provided random patterns and the analysis of all possible combinations of traits provided scenarios ranging from strong clustering to overdispersion. Communities were phylogenetically overdispersed, a pattern previously interpreted as phylogenetic limiting similarity. However, our results likely reflect the co-occurrence of ancient clades due to the stability of stream habitats along the evolutionary scale. We advise ecologists to avoid using combinations of all available traits but rather carefully traits based on the objective under consideration. Both trait and phylogenetic approaches should be kept in the ecologist toolbox, but phylogenetic distances should not be used as proxies of traits differences. Although the phylogenetic structure revealed processes operating at the evolutionary scale, only specific traits explained local processes operating in our communities.

Keywords Assembly rules, community assembly, habitat filtering, niche complementarity, ecophylogenetics, trait structure.

INTRODUCTION

The understanding of local community assembly advanced substantially when ecologists began to change their focus from pure compositional approaches to those that consider differences among species (Cadotte, Albert & Walker 2013). Phylogenetic and trait-based approaches have been proposed as means to provide insights on whether environmental filtering and/or limiting similarity are the main drivers of community assembly (Weiher & Keddy 1995; Webb *et al.* 2002). The logic of these approaches is to compare observed trait and phylogenetic structures of communities with those expected under null models. When species with similar realized niches co-occur more than expected by chance, a trait- (or phylogenetic) clustered pattern would indicate the prevalence of environmental filtering (considering niche conservatism in the phylogenetic approach). In the opposite scenario, when species with similar niches co-occur less than expected by chance, a trait- (or phylogenetic) overdispersed pattern would be inferred as limiting similarity excluding similar competing species.

A possible advantage of the phylogenetic approach is that one does not need to select and measure the traits that are important to community assembly (Mason & Pavoine 2013). Instead it is assumed that these traits are conserved through evolution, and their signal should appear in the phylogenetic structure of local communities (Webb *et al.* 2002). However, after a plethora of studies, ecologists started debating whether phylogenies are useful to tackle community assembly questions (Mayfield & Levine 2010; Pavoine & Bonsall 2011; Cadotte *et al.* 2013; Mason & Pavoine 2013; Gerhold *et al.* 2015). In several scenarios, the phylogenetic structure of a community provides limited power to infer assembly processes even when traits have strong phylogenetic signal (Mason & Pavoine 2013). For example, greater competitive asymmetry among distant relatives (Mayfield & Levine 2010) and facilitation among close relatives

(Sargent & Ackerly 2008) can also cause phylogenetic clustering. The phylogenetic structure could thus be better used to tackle other questions rather than used as a proxy of species ecological similarity (Swenson 2013; Gerhold *et al.* 2015). Phylogenies could give clues about the dispersal limitation of clades (Saito *et al.* 2015) or reveal the colonization history of habitats (Gerhold *et al.* 2015; Lososová *et al.* 2015; Sobral & Cianciaruso 2015). For example, high levels of phylogenetic diversity within communities in comparison to the regional species pool could be interpreted not as a limiting similarity, but as an efficient colonization of distantly related clades from the species pool (Swenson *et al.* 2012).

Similarly, although the trait-based approach repeatedly proved its strength to predict local assembly processes (Weiher *et al.* 2011; Swenson *et al.* 2012; Kraft, Godoy & Levine 2015), a number of concerns have also been raised. First, some traits are related to competitive interactions while others are more related to habitat filtering providing opposite signals in analyses (Colwell & Winkler 1984). Second, similar to the problem in the phylogenetic approach, competitive exclusion can result in a clustered pattern if assembling traits are related to competitive asymmetry among species (Mayfield & Levine 2010). In this sense, the correct interpretation of clustering or overdispersion is a fundamental part of community assembly studies and requires detailed knowledge of systems and organisms (Mayfield & Levine 2010; Cadotte *et al.* 2013).

One way to tackle these problems is to search for key traits that are more reasonably linked to specific processes (Ingram & Shurin 2009; Pavoine & Bonsall 2011; Mason & Pavoine 2013; Winemiller *et al.* 2015). The concepts of the α niche and β niche (Ackerly & Cornwell 2007) can be used to separate traits that could be expected to respond independently of each other in community assembly. Indeed, α niche traits

and β niche traits should provide opposite signals when tested together against the same null model. Alpha niche traits would be those related to resource use within a community and thus expected to be evenly spaced if competition is a strong driver; and β niche traits would be those related to the environment that a species could inhabit and thus expected to be clustered if environmental filters are important.

One way to integrate these approaches is by explicitly recognizing that assembling processes act hierarchically on different spatial scales. During community assembling, environmental filtering is expected to act first and at large spatial levels (Cavender-Bares, Keen & Miles 2006; Ackerly & Cornwell 2007) while potential competitors are only those who have already passed this filtering acting thus locally (Götzenberger *et al.* 2012). The use of different scales and their associated pools of taxa can thus give clues about dispersal limitations that inhibit lineages to co-occur (Mittelbach & Schemske 2015; Sobral & Cianciaruso 2015). Based on this reasoning, community assembly studies should account for different scales and species pools to properly detect assembling processes.

Inspired by this, we studied stream insect communities because their trait and phylogenetic structure has potential to reveal signals of hierarchical ecological and evolutionary assembling. For example, channel structure and water chemistry are well-known to be strong forces acting over traits and filtering species in streams (Poff 1997). There is also evidence that competition may be an important driver of insect communities in streams mainly due to exploitative competition for food and space (Miyasaka *et al.* 2003). Moreover, the idea of hierarchical filters acting subsequently in streams is supported by other studies (Poff 1997). This provides sufficient evidence for us to expect this phenomenon in our system. In addition, the phylogenetic structure of

aquatic insects can complement our understanding by shedding light on processes like dispersal limitation of lineages from the species pool (Saito *et al.* 2015).

Based on this, we tested the following hypotheses related to the community assembly of aquatic insects. **(H1)** Limiting similarity and environmental filtering are both important drivers of community assembling but are only detectable when analyzing α and β niche traits, respectively. We predicted that overall trait similarity would show random patterns, while α niche traits would be overdispersed within communities, supporting limiting similarity, and β niche traits would be clustered, revealing habitat filtering. If limiting similarity and environmental filtering are not strong drivers, then α and β niche traits should not show patterns different from those expected under the null model. **(H2)** Assembly processes act hierarchically with environmental filtering acting first and on a larger community scale than competition. We predicted that α niche traits would be overdispersed only when communities are considered on the riffle micro-scale, while β niche traits would be clustered only when communities are considered on the stream scale. **(H3)** Aquatic insects are hypothesized to have colonization limitation with increasing spatial extent. We predicted that communities would have random phylogenetic structures on local community scales, but would show increasing clustering over increasing scales and increasing size of the pool of taxa.

MATERIAL AND METHODS

Sampling and study design

The study area is located in the Itanhaém river basin in southeastern Brazil (24°10'58" S, 46°47'20" W). This is a region with subtropical weather with hot summers (28°C average) and mild winters (17°C average). This catchment is located within the littoral

of São Paulo State and is characterized by headwaters that range from near pristine to slightly disturbed by banana plantations. The headwaters are slightly acid (pH: 5.1 – 7.4) with low conductivity (0.023 – 0.039 $\mu\text{S}/\text{cm}$). The water is formed by many parts of riffles with gravel (65 mm – 500 mm) and boulders (> 500 mm). There are a few pools with sand and litter. The maximum depth in these headwaters is less than 100 cm and the width ranges from 55 to 363 cm.

We selected 13 headwater streams and collected 10 samples per stream using a Surber sampler (net mesh size 0.025 mm and 900 cm^2 area) in riffles for a total of 130 riffles. Our riffle micro-scale communities were each Surber samples ($n=130$), and the communities on the stream scale were composed of the sum of 10 samples in the stream ($n=13$) (see Fig. 1 for a schematic view of sampling design). The insects were screened *in vivo* using illuminated trays and were preserved in 70% alcohol solution. Most of the insects were identified to the genus level, but some Lepidoptera and Diptera were left at the family level, i.e., Pyralidae, Dyxidae, Chironomidae.

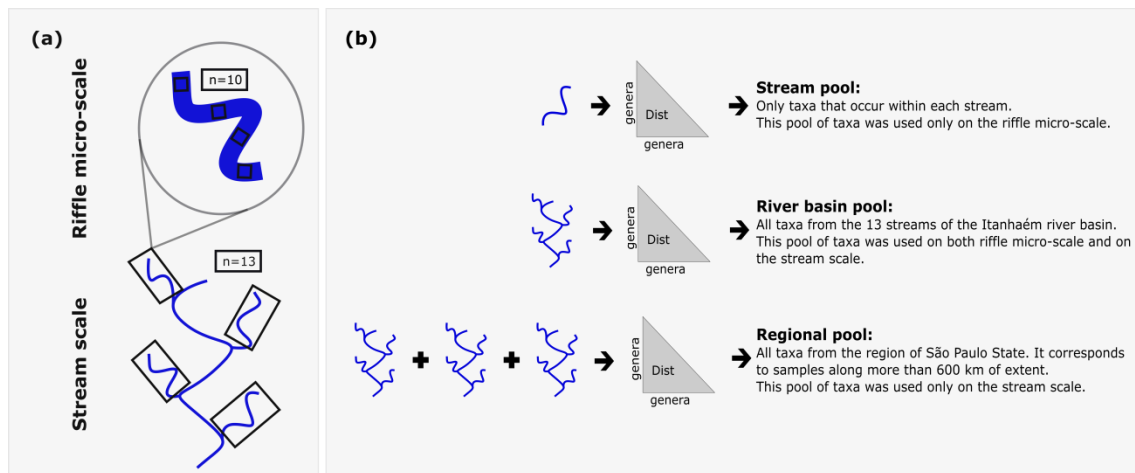


Figure 1. Schematic view of study design and pools of genera used in different null models. (a) On riffle micro-scale, each sample (30 x 30 cm) was considered an entire community, while, on the stream scale each community was the sum of abundances over the 10 samples collected. (b) We used three different pools of genera in our study. The stream pool is composed of all genera from the same stream as the analyzed sample. It thus considers that interacting genera are only those that inhabit the same stream. The river basin pool considers all genera from the Itanhaém river basin. It does not consider dispersal limitation on the river basin scale. The regional pool considers

taxa sampled in the whole São Paulo State region. This last pool is more prone to reveal patterns due to large scale processes.

Aquatic insects supertree

We used the supertree published in Saito et al. (2015a) that contains almost all genera used in our analysis. Genera not contemplated in Saito et al. (2015a) were included as new polytomies because all of their families were present in the former supertree. The original supertree used information on the age of 32 nodes compiled with reference to several recently proposed phylogenies constructed with both morphological and molecular information (see Saito et al. 2015a for details). Branch length were assigned using BLADJ algorithm that spaces undated nodes evenly between dated nodes using and adjuster algorithm (Kembel *et al.* 2010). To construct a phylogenetic distance matrix, we used the cophenetic distances among genera on the supertree.

Traits of aquatic insects

To construct trait distance matrices, we used traits available for tropical aquatic insects in the literature (Poff *et al.* 2006; Tomanova, Goitia & Helešić 2006; Colzani *et al.* 2013). The overall trait distance was calculated with the following traits: voltinism and life span (life cycle traits); exoskeleton, body shape, respiration, BMWP index (Biological Monitoring Working Party), body size, flight capacity and shelter (morphological/physiological traits); and reophily, microhabitat preference and trophic position (behavioral traits) (for a complete description of trait states, see Colzani *et al.* 2013). Trait values were assigned at the genus level for all traits except for the BMWP index, which is calculated per family as a level of tolerance to pollution. In this latter case, all genera within families were assigned the same BMWP score. To construct trait

distances, we used the modified Gower distance because it can handle numerical, categorical and ordinal data (Gower 1971; Pavoine *et al.* 2009).

α niche traits

The selection *a priori* of α niche traits was based on the four niche axes that determines the strength of species competition (Amarasekare 2003). Species may differ in terms of the resource used (nutrients, food), where they use the resource (space) and when they use this resource (time). A weaker interspecific competition is expected when species differ in the traits related to these niche axes. Thus, if competition is a strong driver in our communities, we expected to find overdispersed patterns in local α niche traits when compared to distributions generated at random. For aquatic insects, those selected to compose the α niche distance were “reophily”, “microhabitat preference” and “trophic position”. Reophily and microhabitat preferences represent the spatial niche. They indicate in which water velocity and stream substrate the species usually occur. Trophic position is related to the resource use axis because it indicates the foraging strategy of species.

In riverine landscapes, higher environmental heterogeneity is expected within streams (compared to among streams), as indicated by the variation in substrate types, the amount of organic material, depth and width (Heino 2005). This heterogeneity should provide all kinds of microhabitats within each stream (Heino 2005). Each microhabitat can be considered a competing resource, i.e., it can be expected to show overdispersed patterns if competition for microhabitat is intense. For example, hydropsychid congeners can have a segregated distribution at the micro-scale due to aggressive competition for food and net supplies (Harding 1997).

β niche traits

We selected β niche traits *a priori* as phenotypic traits expected to be linked to physiological limitations in individuals. For example, low levels of dissolved oxygen may limit the establishment of insect larvae in a gradient of river pollution. Following this reasoning, we selected “BMWP index” and “respiration” to compose the β niche traits. Thus, if environmental filters were the determinants of community structure, we should find clustered patterns compared to the distribution of traits drawn from a null model.

The BMWP is a biotic index that gives each family a score of tolerance to organic pollution ranging from 1 (very tolerant) to 10 (very sensitive) (Hawkes 1998). The “respiration” trait is composed of three different strategies of oxygen uptake: “tegumental respiration”, “gill respiration” and “aerial respiration”. A transition from taxa with gill respiration through taxa with cutaneous respiration to taxa with air respiration relying on spiracles, plastrons, or tracheae is expected with increasing environmental harshness (Saito *et al.* 2015a).

Phylogenetic signal

We investigated if there was a phylogenetic signal – i.e., the tendency of related genera to resemble one another more than they resemble genera drawn at random from the phylogenetic tree – in different ways. First, we measured phylogenetic signals using Mantel test between the square root of the phylogenetic distances (Hardy & Pavoine 2012) and the trait distances created with each individual trait (12 traits), with combined α and β traits, and with all traits combined. Phylogenetic signal in nominal traits (shelter, exoskeleton, body shape, reophily, microhabitat preference, trophic position and respiration) was also tested using Maddison and Slatkin (1999) method, which compares the minimum number of trait changes to a distribution of changes drawn from a null model (Maddison & Slatkin 1991). Ordinal (voltinism, body size and flight

capacity) and quantitative (BMWP index) traits were also tested for phylogenetic signal using Blomberg, Garland and Ives (2003) K , K^* , and Pavoine and Ricotta (2013) K_w statistics.

Community structure analysis

We calculated metrics of phylogenetic and trait community structure on the two scales (riffle micro-scale and stream scale) using Mean Nearest Neighbor Distance (MNND), and Mean Pairwise Distance (MPD) (Webb *et al.* 2002). The MNND metric is calculated as the mean distance to the closest relative individual (or genus when using incidence data) between all individuals (or genus) in a community (Webb *et al.* 2002). The MPD metric is calculated as the mean phylogenetic or trait distance among all individuals (or genus) in a community. To test whether the phylogenetic and trait structure of the communities were more clustered or dispersed than expected by chance, we used minus the standardized effect size of MNND and MPD, which are called the Nearest Taxon Index (NTI) and Net Relatedness Index (NRI), respectively (Webb *et al.* 2002). NTI and NRI compare the observed values to null values of MNND and MPD, respectively. Null values were obtained using the null model “taxa shuffle” that randomizes the rows and columns of the matrix of phylogenetic or trait distances among genera 1,000 times (Kembel 2009). Following the conceptual approach of null models – fixing all data patterns except the one of interest – we selected the taxa shuffle model because it randomizes only the locations of the taxa in the distance matrix. The model thus constrains the richness and abundance patterns of samples, and allows only the effect of distances to vary (Kembel 2009). We decided to use both NTI and NRI indices because NTI is less influenced by higher levels of phylogenetic and trait structure and is expected to have more power to show overdispersion, while NRI captures the whole structure of assemblages and is more robust to detect clustering (Kraft *et al.* 2007). All

analyses were run using incidence data and abundance data because competition is expected to be density-dependent, while some physiological constraints could act at the species, here genus, level (Swenson *et al.* 2012). All analyses were run with phylogeny, all combined traits, α niche traits and β niche traits. To test for clustering versus overdispersion of communities, we applied the two-tailed Wilcoxon tests (assuming significance for $P < 0.01$) in NTI and NRI results. If NTI or NRI were lower than zero we inferred an overall tendency to overdispersion. The opposite would mean an overall tendency to clustering (Webb *et al.* 2002).

We used three different pools of aquatic insect genera in null model randomizations that represent distinct hypothetical scenarios. (1) The river basin pool was composed of taxa found in the 13 streams of the Itanhaém river basin. The null model using the river basin pool of taxa assumes that the trait and phylogenetic structure of the 13 communities are not influenced and cannot be colonized by genera from outside the Itanhaém river basin. It also assumes that there is no dispersal limitation among streams of the Itanhaém basin. (2) The regional pool encompasses additional taxa ($n=160$) found in the region of São Paulo State (samples from the whole State, Suriano *et al.* 2011) and considers that genera in the Itanhaém river basin could already be a subsample of the regional pool due to large scale processes (Mittelbach & Schemske 2015 and see Discussion). (3) The stream pool considers only genera from the same stream. This pool assumes short dispersal limitations and assumes that possible interacting genera are only those occupying the same stream (Fig. 1). The river basin pool (1) was used for analyses on both the riffle micro-scale and the stream scale. The regional pool (2) was used only on the stream scale, and the stream pool (3) was used only on the riffle micro-scale.

To investigate the influence of trait selection in the output of NTI and NRI we ran analyses using all possible combinations of the 12 selected traits (4095 combinations). For brevity, in these investigations we ran 200 randomizations for NTI and NRI. The influence of trait selection was investigated using the river basin pool of taxa for both the riffle micro-scale and stream scale, with both incidence and abundance data. To summarize the results using all combinations of traits we used a redundancy analysis (RDA) with the results of all indices as response matrix and the composition of each of the 4095 combinations of traits as explanatory matrix. The RDA shows which traits are positively or negatively associated with the results of NTI and NRI using all possible combinations of traits. Analysis of NRI and NTI using individual traits were also ran, but it provided similar patterns as those explored through the RDA, thus for brevity these results are presented in Appendix.

All analyses were run in R using packages *ade4* (Chessel, Dufour & Thioulouse 2004) and *picante* (Kembel *et al.* 2010).

RESULTS

Alpha and β niche traits, and all traits together showed significant phylogenetic signal according to the Mantel test (Table 1). However, besides the phylogenetic signal in all traits together (Mantel correlation: $r=0.63$, $P=0.001$), the phylogenetic signal in α , β and individual traits were low (between $r=0.38$, $P=0.001$ and $r=0.17$, $P=0.005$). Among the individual traits, most of them presented phylogenetic signal according to Mantel test, unless respiration (β niche trait), shelter and reophily (α niche traits) (Table 1).

Blomberg's K , K^* and K_w presented similar results; all resulted in significant phylogenetic signal in ordinal and quantitative traits. For brevity, we present here only the results of K^* (Table 1).

Table 1. Testing the phylogenetic signal of aquatic-insect traits using three tests: Mantel test between the square root of the phylogenetic distance and the trait-based distance, Blomberg et al. (2003) K^* for ordinal (rank-transformed) and quantitative traits, and Maddison and Slatkin (1991) method for nominal traits. Alpha niche traits are reophily, micro-habitat preference and trophic position; β niche traits are respiration and the BMWP index.

	Mantel r	Mantel P	$K^* P$	Maddison and Slatkin P	Data type	Trait group
All traits	0.63	0.001	-	-	Multiple traits	Group of traits
α niche traits	0.38	0.001	-	-	Multiple traits	Group of traits
β niche traits	0.17	0.005	-	-	Multiple traits	Group of traits
Voltinism	0.25	0.001	0.001	-	Ordinal	Life cycle
Adult life span	0.40	0.001	-	0.001	Nominal	Life cycle
Exoskeleton	0.27	0.001	-	0.001	Nominal	Morphology/physiology
Body shape	0.26	0.001	-	0.001	Nominal	Morphology/physiology
Respiration	0.09	0.060	-	0.001	Nominal	Morphology/physiology
BMWP	0.14	0.001	0.001	-	Quantitative	Morphology/physiology
Body size	0.27	0.001	0.001	-	Ordinal	Morphology/physiology
Flight capacity	0.21	0.001	0.001	-	Ordinal	Morphology/physiology
Shelter	0.05	0.110	-	0.001	Nominal	Behavioral
Reophily	0.09	0.034	-	0.199	Nominal	Behavioral
Micro habitat	0.27	0.001	-	0.001	Nominal	Behavioral
Trophic position	0.20	0.001	-	0.001	Nominal	Behavioral

For the riffle micro-scale, using the river basin pool, NTI and NRI computed with all traits and α niche traits indicated random patterns – i.e., the results were not different from those expected under random assembly. The β niche traits showed clustering in NTI and NRI indicating that local communities are composed of a subset of β niche trait states different from one drawn at random (Wilcoxon test, $P < 0.01$, except for NTI with incidence data where Wilcoxon test was marginally significant, $P < 0.02$) (Fig. 2). So, our hypothesis H1 was only partially supported since we found evidences for environmental filtering (clustering in β niche traits) but not of competition (random α niche traits) structuring our communities. In contrast, the phylogenetic structure was significantly overdispersed in both NTI and NRI analyses indicating that co-occurring genera have distinct evolutionary history (Wilcoxon test, $P < 0.01$). These

results were consistent for both incidence and abundance data (Fig. 2). The results using the stream pool also showed qualitatively similar patterns (Fig. 3). The exception was for phylogenetic NRI (incidence data) that showed clustering; but NTI instead confirmed the overdispersion trend (Fig. 3).

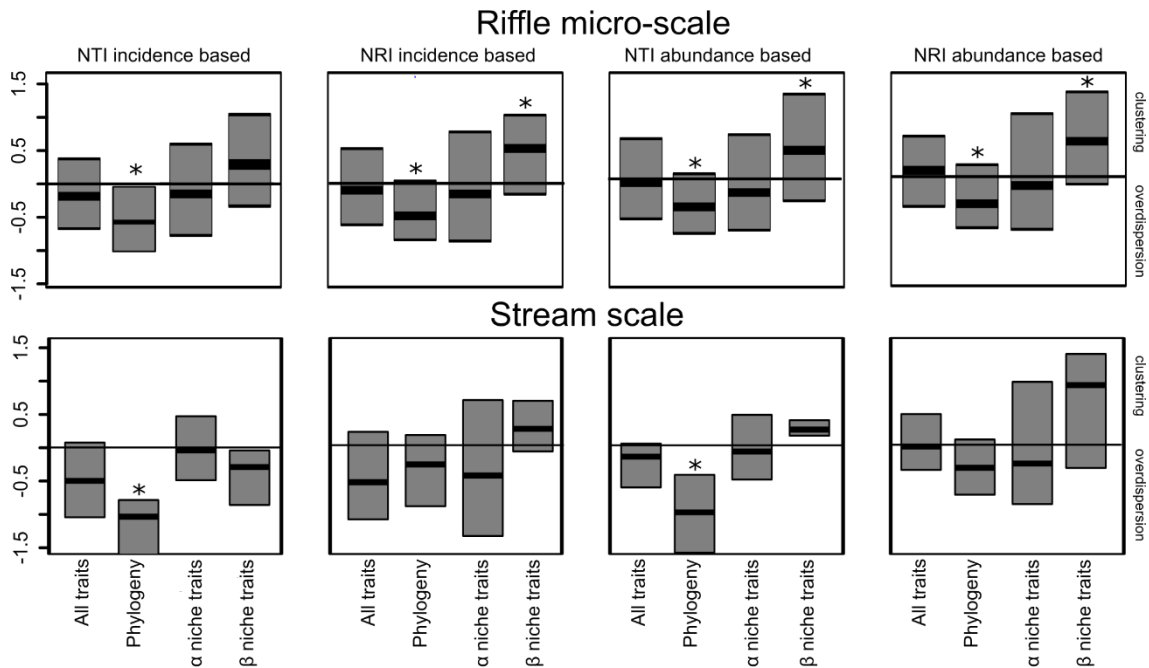


Figure 2. Box plots of values of Nearest taxon index (NTI) and Net relatedness index (NRI) on riffle micro-scale and on stream scale calculated with trait and phylogenetic distances. The pool of genera used in the null model was composed of taxa found in the 13 streams of the Itanhaém river basin. Trait distances were calculated in three different ways: using all traits, using α niche traits and using β niche traits. Median values significantly different from zero according to two-tailed Wilcoxon test have “*” for $P < 0.01$.

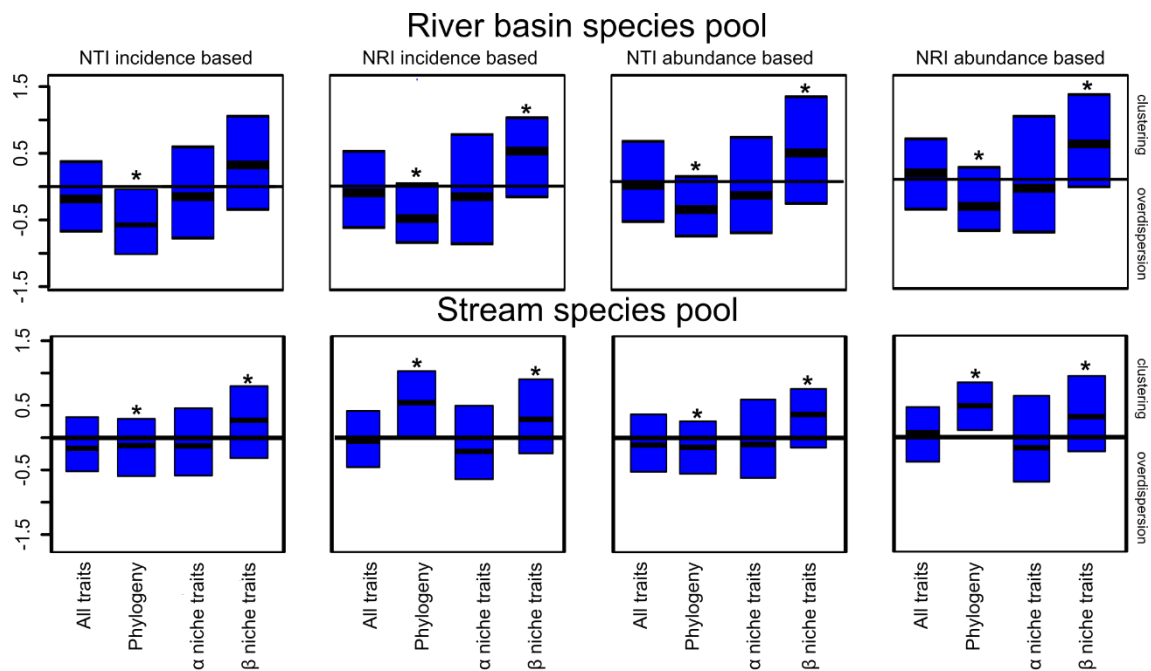


Figure 3. NTI and NRI results on riffle micro-scale using the two distinct null models. Box plots of values of Nearest taxon index (NTI) and Net relatedness index (NRI) on riffle micro-scale ($n=130$) calculated with trait and phylogenetic distances using two different pools of genera. The river basin pool considers all genera present in the Itanhaém river basin. The stream pool instead considers only the genera of each stream. Trait distances were calculated in three different ways: using all traits, using α niche traits and using β niche traits. Median values significantly different from zero according to two-tailed Wilcoxon test have “*” for $P<0.01$.

For the stream scale, using the river basin pool of genera, NTI and NRI indicated random patterns for overall trait distance and α and β niche traits (Fig. 2). Thus, our results do not support our hypothesis H2 that predicted a hierarchical assembly with clustering in β niche traits on the stream scale. The phylogenetic structure was consistent with the results found on the riffle micro-scale, showing overdispersion in NTI and NRI with both incidence and abundance data (Fig. 2). We found similar trends with the regional pool (Fig. 4). The absence of increasing clustering in the phylogenetic structure of communities suggests that phylogenetic dispersal limitation is not acting (refuting our hypothesis H3). One distinct result was a significant clustering in α niche traits in NRI with incidence data (Wilcoxon test, $P=0.01$). This reinforces the lack of

limiting similarity for competitive traits. However, β niche traits did not show consistent clustering even when considering all taxa in the region.

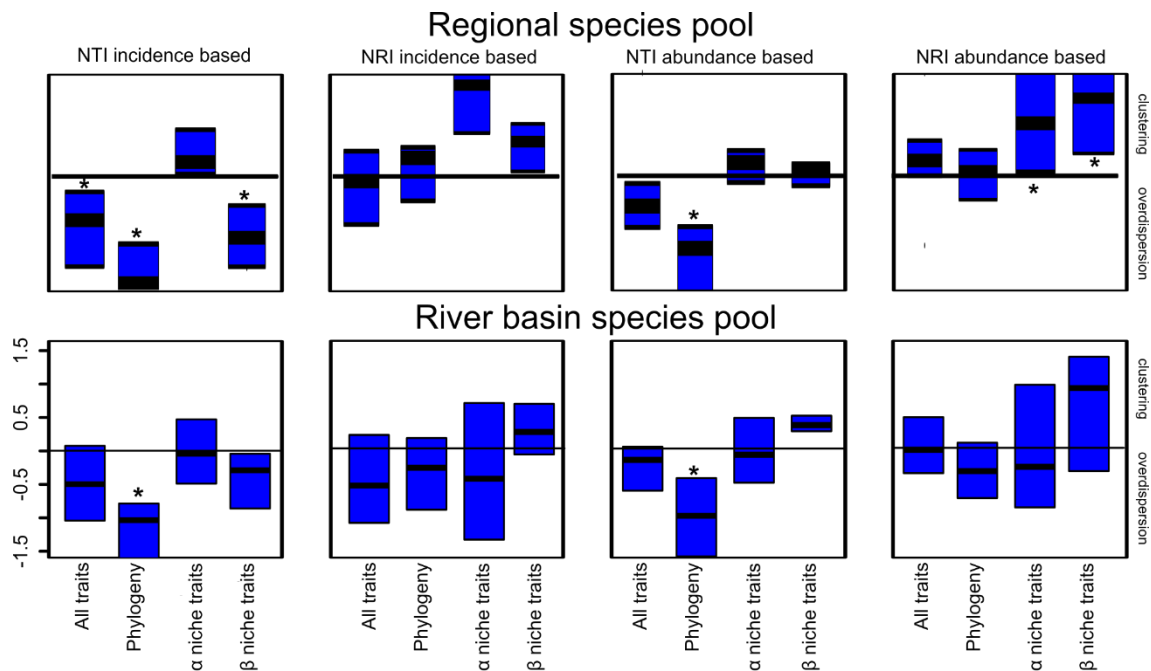


Figure 4. NTI and NRI results on stream scale using the two distinct null models. Box plots of values of Nearest taxon index (NTI) and Net relatedness index (NRI) on stream scale ($n=13$) calculated with trait and phylogenetic distances using two different pools of genera. The regional pool considers all genera sampled in the State of São Paulo (Suriano *et al.* 2011). The river basin pool instead considers only the genera of the Itanhaém river basin. Trait distances were calculated in three different ways: using all traits, using α niche traits and using β niche traits. Median values significantly different from zero according to two-tailed Wilcoxon test have “*” for $P < 0.01$.

Against our predictions, calculations of NTI and NRI using all possible combinations of traits did not result in random patterns. Rather, it resulted in values ranging from strong overdispersion to strong clustering depending on the trait combination (Fig. 5). We found that the first axis of RDA summarized a large proportion of variation in the response matrix (87%). This axis showed that exoskeleton, life span, reophily (α niche trait) and respiration (β niche trait) were associated to clustering results in most of indices (NRI and NTI on the two scales), while microhabitat preference (α niche trait), flight capacity and voltinism were associated to overdispersion results (Fig. 6). We did not find consistent evidence for the

idea that traits associated to clustering or overdispersion were those related to the α and β niche of aquatic insects. We found that traits not expected *a priori* to be structured by competition or environmental filtering were the most related to clustering (exoskeleton) or overdispersion (voltinism) patterns.

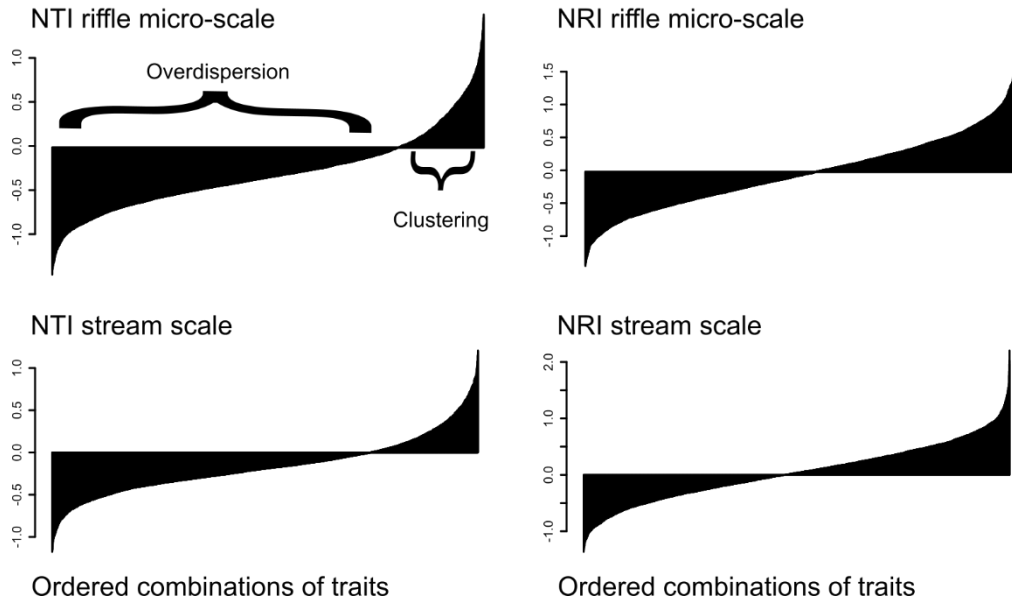


Figure 5. Barplot showing the ordered median value of Nearest taxon index (NTI) and Net relatedness index (NRI) on the riffle micro-scale and on stream scale using all combinations of traits (n=4095 combinations) and abundance data and considering all genera from the data set as the pool of taxa for the null model. NTI and NRI results are ordered from highest overdispersion to highest clustering.

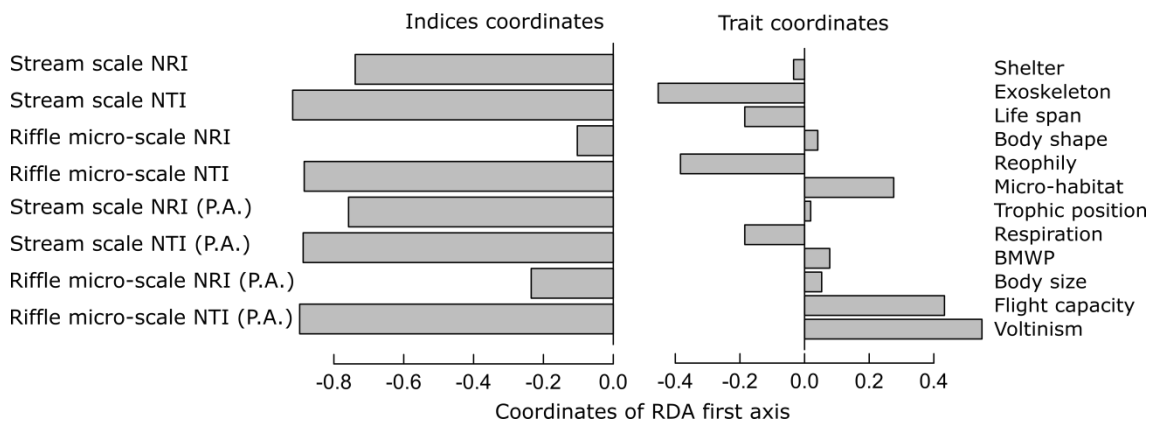


Figure 6. Coordinates in the first axis of redundancy analysis. The response matrix is the result of all indices using all combinations of traits (P.A. means presence-absence data). The explanatory variables were the identities of the traits in the 4095 combinations. The first axis summarizes 87% of variation in the data. Negative

coordinates of NRI and NTI on the first axis correspond to high NRI and NTI values and were thus interpreted as trait clustering.

DISCUSSION

Species in a given habitat must share similar traits that enable them to support the same abiotic and biotic pressures including environmental gradients and predators, but they should also be dissimilar in other traits to lower interspecific interference and avoid competitive exclusion (Chesson 2000). However, identifying which trait has strong net effects in which assembly process is not a simple task. The concepts of the α and β niches (Ackerly & Cornwell 2007) were useful here because they provided a guideline to select traits that are reasonably related to different assembly processes. Using this approach we found signals that only environmental filtering (clustering in β niche traits), but not limiting similarity (consistent random patterns in α niche traits), is an important process acting on studied communities. Previous studies that did not separate α from β niche traits could not disentangle interacting processes. For example, trait-clustering using all traits could be due to environmental filtering or asymmetric competition. Moreover, analyses using all possible combinations of traits demonstrated how several, distinct combinations could provide strong clustering or overdispersion, even if they were composed by traits not expected *a priori* to be related to any assembly process. Our results suggest that using traits without prior expectation can give uninformative results and lead researchers to erroneous inferences.

We expected that β niche traits would be clustered within the streams if environmental filtering was strong enough to exclude taxa with inappropriate trait state to occur in a given community. We found random and opposed patterns on the stream scale using the two pools of genera, but consistent clustering on the riffle micro-scale. This indicates that environmental filtering is a strong driver on the riffle micro-scale,

but not on the (a priori expected) stream scale in the studied communities. The environmental pressures over β niche traits were probably not strong enough on the stream scale because we did not design our study along an environmental gradient. Rather, each stream represented a replicate of each other. For the riffle micro-scale, however, we found that genera with the same respiration strategy and with the same BMWP score tended to co-occur in small patches of habitat. The BMWP index was created to represent the degree of resistance of aquatic insect families to organic pollution based on the occurrence of taxa along a gradient of impact (Hawkes 1998). This means that families that co-occur in environments with similar levels of pollution would share the same BMWP score. A gradient of pollution generally follows environmental changes along the entire stream. In the Atlantic Forest biome, a common environmental gradient starts with forested shaded streams with fast waters and rocky streambed. It then passes to streams with lower velocity and with sandier substrate, finally ending in muddy streams with open forest canopy. This suggests that genera with similar BMWP scores should also share strategies to inhabit similar riffle environments. For example, *Gripopteryx* (Plecoptera) and *Farrodes* (Ephemeroptera) have the same score and are dorsoventrally flattened with a similar body size and take oxygen by gills (Pastuchová, Lehotský & Grešková 2008). In this sense, clustering in the β niche traits could be found when species co-occur in microsites with high oxygenation and without large accumulation of organic matters, as in the small cascades formed by boulders. The results of β niche traits support the body of evidence that suggests environmental variation within streams as more important than the variation among streams for structuring aquatic insect communities in non-impacted streams (Heino, Louhi & Muotka 2004; Costa & Melo 2007). Together with results of α niche traits, we have indications that aquatic insects with similar micro-scale preferences

usually co-occur without competitive exclusions. A simple explanation for a relaxed or even clustered co-occurrence of competition traits (using the regional pool) is that high levels of productivity in a given habitat can maintain high levels of niche overlap without strong competition (Safi *et al.* 2011). Probably, the high amount of detritus continually entering tropical stream systems over the year enables high feeding overlap among aquatic insects without strong competition among them (Tomanova *et al.* 2006).

Our findings using all possible combinations of traits suggest that statistically significant clustering or overdispersion can be found using different combinations of traits, with different numbers of traits (see Appendix, Fig. S3). In many cases, traits strongly associated with clustering or overdispersion were not those related to what we defined *a priori* as related to the α niche and β niche of aquatic insects. Some non-exclusive explanations for these results are: (1) we know little about the ecology of aquatic insects in such a way that we did not select all traits that are indeed related to α and β niche. In this case, the association of some traits, as exoskeleton and voltinism, with clustering and overdispersion are due to ecological processes poorly understood. Although this view looks appealing, it relies on the strong assumption that environmental filtering and competition are always acting over communities; hence one just need to find which traits are responding to these processes. (2) The traits related to clustering or overdispersion actually reflect assembly processes that act in other traits which they are correlated to (e.g. due to phylogenetic or physiological constraints). However, we did not find strong associations between pairs of traits we considered (Mantel $r < 0.52$, Appendix), and thus we do not have evidence to support this reasoning. (3) Due to the large number of traits which resulted in an even larger number of trait combinations, there is an increased probability to find patterns of clustering or overdispersion in traits by chance but with few or no possible biological interpretations.

Due to the unexpected patterns in several traits (e.g. clustering in exoskeleton and life span and overdispersion in flight capacity and voltinism), we point out this explanation as the most likely in our case study. Nevertheless, these explanations are non-exclusive and we would greatly benefit from further studies using the α and β niche approach in other ecosystems.

Regarding the phylogenetic structure of aquatic insects, we found consistently overdispersed patterns within communities; a pattern that was commonly used to infer phylogenetic limiting similarity when species present conserved niches (Webb *et al.* 2002; Cavender-Bares *et al.* 2004; Violle *et al.* 2011). However, the phylogenetic limiting similarity logic can be biased because niche conservatism at the species pool level cannot predict the phylogenetic signal at the community and metacommunity levels (Mason & Pavoine 2013). Simulations showed that local communities composed of close related species are not necessarily composed of ecologically similar species, even when evolution of traits is highly conserved at the species pool level (Mason & Pavoine 2013). Although these authors did not make a definitive conclusion, no other study has yet shown opposite results. Consequently, local phylogenetic overdispersion coupled with phylogenetic signal at the pool of taxa level are likely weak evidence of limiting similarity structuring communities.

Following this, alternative explanations for non-random phylogenetic structures have recently been developed without relying on the phylogenetic-patterns-as-proxy-of-traits approach (Lososová *et al.* 2015; Gerhold *et al.* 2015). The phylogenetic structure of local communities is likely influenced by the diversification and dispersal history of lineages as well as by the stability and geological age of the habitat under study (Lessard *et al.* 2012). A long period of diversification in a given habitat can make contemporary communities share species from very distantly related lineages. Short-

term adaptation and diversification would make co-occurring species only share a small amount of history (Lososová *et al.* 2015). In this sense, although local contemporary communities are ephemeral, habitat types, such as streams, are available for colonization and evolution for a long time (Pauls, Lumbsch & Haase 2006). This makes local communities a result of lineage-diversification over millions of years (Gerhold *et al.* 2015). Due to physical and chemical characteristics of water, fast flowing streams were more stable environments for diversification and less prone to entire clade extinctions than terrestrial habitats (Ross 1967). For example, stream-dwelling insects used fast flowing mountainous streams (that were not frozen) as refuge during glaciations, avoiding regional extinctions (Pauls *et al.* 2006). Also, an initial colonization and diversification of several aquatic insect orders in oxygen-rich, cool-water streams was hypothesized (Ross 1967), which would explain the presence of ancient families in these habitats. Moreover, tropical lineages of aquatic insects probably suffered less extinction events due to less severe effects of glaciation in the Pleistocene. This enables survivorship of relictual taxa of some orders (De Moor & Ivanov 2008). Some of these relictual lineages have widespread and common co-occurring genera in our study region such as *Chimarra* (Philopotamidae, Trichoptera) and *Beatis* (Baetidae, Ephemeroptera) (De Moor & Ivanov 2008). In this way, the co-occurrence of these genera would generate high values of phylogenetic diversity within a community because they diverged close to the root of our supertree. This can explain the phylogenetic overdispersion in local communities and corroborate findings of high phylogenetic diversity within and among streams for several groups of aquatic insects (Saito *et al.* 2015b). Thus, phylogenetic overdispersion and high local phylogenetic diversity—combined with low phylogenetic signal in most traits—are likely the result

of the widespread distribution and co-occurrence of species from groups with long divergence times.

In conclusion, we found signals of assembly processes using an *a priori* selection of α niche and β niche traits that were not found using all traits together or using phylogenetic information. We suggest ecologists to avoid using combinations of traits without careful selection based on α and β niche concepts or any other grouping that make sense for the objective under consideration (e.g., Winemiller *et al.* 2015). Although assembly processes are difficult to predict they are more likely to be revealed if they are important drivers and if the selection of traits for an analysis relies on robust theory. Previous studies suggested that adding more traits likely increase the way in which a species could be ecologically different from another one, strengthening the power of null model analysis to detect assembly processes when community membership is determined by multiple traits (Kraft *et al.* 2007). However, our analysis of all combinations of traits shows that the conclusions depend on the selected traits. Adding a trait may change conclusions from overdispersion to randomness or clustering. Thus, ecologists cannot avoid the challenge of trait selection to properly identify assembly mechanisms. In addition, the trait and phylogenetic approaches should be kept together in the toolbox of ecologists because they offer complementary information about community assembling. While trait approaches provide insights about local processes such as habitat filtering, the phylogenetic structure of communities can reveal the signature of processes that work on an evolutionary scale including diversification in ancient habitats.

ACKNOWLEDGMENTS

We are thankful to the editors and reviewers who provided comments and suggestions that improved this manuscript. The writing of this study was partially funded by grants #2013/50424-1, #2013/20540-0, #2014/24532-4 São Paulo Research Foundation (FAPESP) and by grant 403723/2012-4, Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq). We are grateful to Leandro S. Duarte and Paulo I. Prado who provided insightful comments on an early version of this manuscript. Marcus Cianciaruso and Alaide Fonseca-Gessner have a productivity grant awarded by CNPq.

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APPENDIX

Additional analyses exploring the combination of traits

First analysis: We ran NRI and NTI for each individual trait using incidence and abundance data in the riffle micro-scale and in the stream scale.

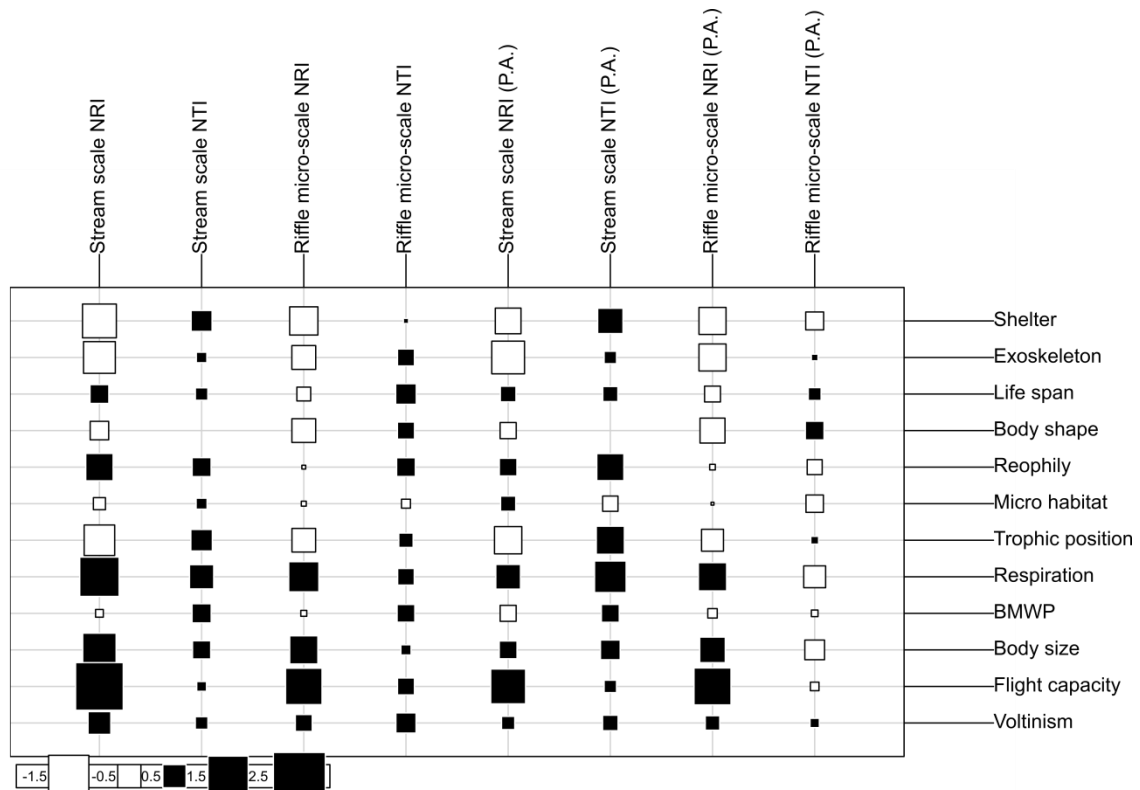


Figure S1. Results of NRI and NTI for each individual trait. Size and color of squares are related to the mean value of the indices. White squares are interpreted as mean tendency to overdispersion and black squares as mean tendency to clustering.

Similar to what we found using the redundancy analysis, we also found that the traits that resulted in strongest clustering (flight capacity) and overdispersion (exoskeleton) were not those expected to be related to the alpha and beta niche of aquatic insects. This could be explained if these traits were actually reflecting response of other linked traits (e.g. physiological or phylogenetic constraints).

Second analysis: We tested the correlation among traits using pairwise Mantel correlations.

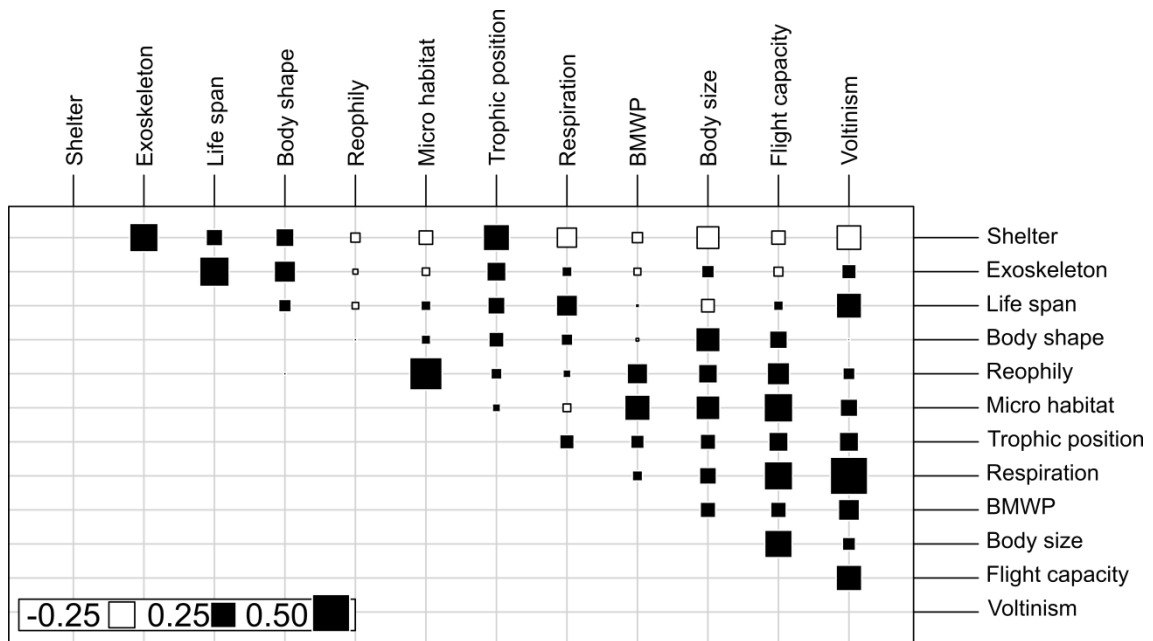


Figure S2. The correlations among traits were tested using Mantel correlations.

Square size and color indicates the value and direction of correlation. Voltinism and respiration presented moderated correlation ($r=0.52$), but other traits were independent of each other ($r<0.32$).

These results suggest that strongest clustering and overdispersion in traits not expected to be related to the alpha and beta niche of aquatic insects were not due to linkage with other traits.

Third analysis: We explored the relationship between the number of traits used and the results of dispersion analysis.

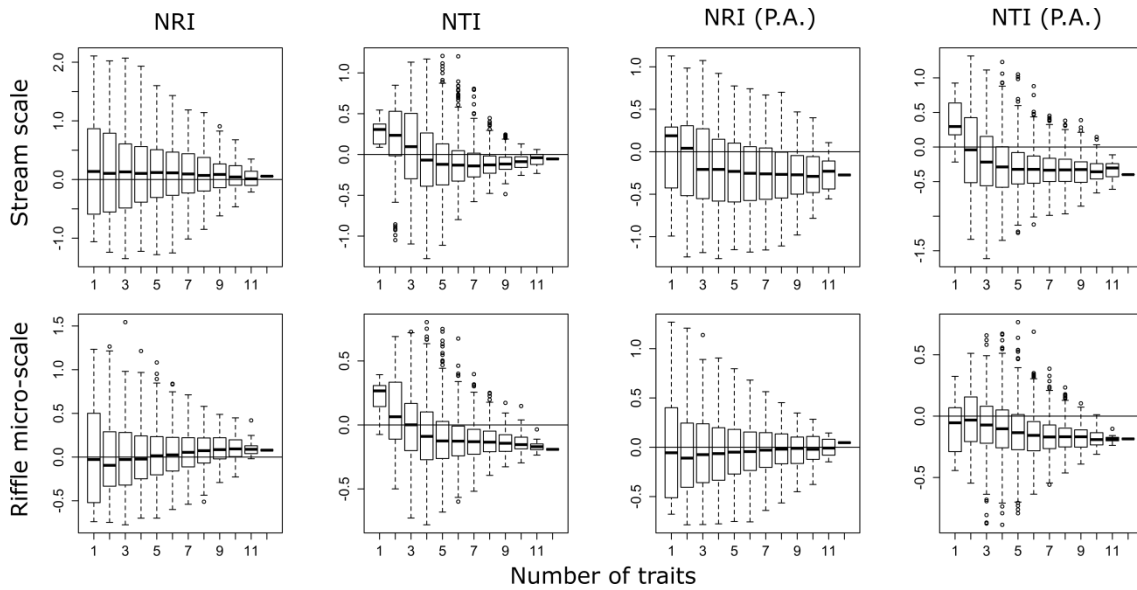


Figure S3. Results of NRI and NTI with a varying number of traits used in distance calculations. NRI have a decreasing variation with increasing number of traits. NTI have an increased variation with increasing number of traits but it decreased after 4-5 traits. P.A. are analysis using presence-absence data

Capítulo 3

Phylogenetic clustering among aggressive competitors: evidence from odonate assemblages along a riverine gradient

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Este capítulo foi publicado na forma de artigo científico no periódico *Oecologia* em 2016.

Saito, V. S., Valente-Neto, F., Rodrigues, M. E., de Oliveira Roque, F., & Siqueira, T. (2016). Phylogenetic clustering among aggressive competitors: evidence from odonate assemblages along a riverine gradient. *Oecologia*, 1-11.

ABSTRACT

Studies on phylogenetic community ecology usually infer habitat filtering when communities are phylogenetically clustered or competitive exclusion when communities are over dispersed. This logic is based on strong competition and niche similarity among closely related species; a less common phenomenon than previously expected. Dragonflies and damselflies are good models for testing predictions based on this logic because they behave aggressively against related species due to mistaken identification of conspecifics by odonates. This mistake might drive communities toward phylogenetic over dispersion if closely related species frequently exclude each other. However, phylogenetically clustered communities could also be the observed pattern if habitat filtering and/or competitive asymmetry among distantly related species are major drivers of community assembling. We investigated the phylogenetic structure of odonate assemblages in central Brazil in a watershed characterized by variations in stream width, vegetation cover, aquatic vegetation and luminosity. We found a general clustering in communities using two indices of phylogenetic structure. Phylogenetic beta diversity coupled with Mantel tests and RLQ analysis evidenced a correlation between the riverine gradient and phylogenetic structure. Larger rivers with aquatic vegetation were characterized by Anisopterans, while most Zygopterans stayed in small and shaded streams. These results indicate niche conservatism in Odonata habitat occupancy and that the environment plays a major role in driving the phylogenetic structure of these communities. We suggest this is due to clade-specific eco-physiological requirements, and because closely related species may also have competitive advantages and dominate certain preferred habitats.

Keywords community assembly, ecophylogenetic, eco-physiological hypothesis, dragonfly, damselfly.

INTRODUCTION

The competition-relatedness hypothesis predicts that congeners will experience stronger competition because they share numerous phenotypic characters, so they should exploit the environment in a similar fashion (Cahill et al 2008). Recently, along with the concept of limiting similarity and niche conservatism, the competition-relatedness hypothesis influenced the field of phylogenetic community ecology (Webb 2000; Cavender-Bares et al 2009). This new field uses the probability of co-occurrence of closely related species to understand contemporary community assembly (Webb et al 2002; Cavender-Bares et al 2009). Based on niche conservatism, phylogenetic community ecology assumes higher relatedness among species occurring simultaneously (i.e., phylogenetic clustering) as habitat filtering, since species with similar niche are co-occurring more than expected by chance. The opposite pattern, lower relatedness among co-occurring species, could be inferred as competition excluding closely related species with a significant niche overlap.

Although successful results have been reported (Cavender-Bares et al 2004; Vamosi and Vamosi 2007), phylogenetic community ecology has recently faced criticism owing to pairs of closely related species experiencing lower competitive interference than pairs that are only distantly related (Mayfield and Levine 2010; Godoy et al 2014). For example, greater competitive asymmetry among distant relatives can also drive phylogenetic clustering (Mayfield and Levine 2010). We therefore we suggest that predictions based on this field's logic could be tested more efficiently in systems wherein strong competition among closely related species is strongly documented and expected.

In this sense, Odonata are strong candidates for testing phylogenetic community ecology predictions, whereby previous evidence indicates intense aggression among

closely related species (i.e. Anderson and Grether 2011). Two factors explain such aggressive behavior: first, closely related and similar Odonata species can experience exploitative and interference competition for food, space and mating sites, leading individuals to attack others they deem as competitors (Singer 1989; Corbet 1999; Worthen and Patrick 2004). Second, the mistaken-identity-hypothesis (Singer 1989) states that imperfectly recognizing conspecifics can cause individuals to attack similar species (Tynkkynen et al 2006; Anderson and Grether 2011). According to this hypothesis, odonates will attack similar individuals with or without correct recognition as a conspecific or a heterospecific. This is because, otherwise, possible competition for mates would be energetically disadvantageous (Singer 1989; Schultz and Switzer 2001). Mistaken aggression may also occur because Odonata brains cannot quickly distinguish conspecific from other similar species (Grether 2011). This means that we can expect that Odonata assemblages have over dispersed phylogenetic structure owing to intense aggression driving less co-occurrence of closely related species than expected under random assembly.

From a different perspective, evidence exists for strong habitat filtering and ecological partitioning that enable the coexistence of closely related odonates (Siepielski et al 2011; Siepielski and McPeck 2013). Environmental filtering along a longitudinal riverine gradient can select species with different preferences for stream width and forest cover because species have varying eco-physiological requirements and thermoregulation behaviors (the Eco-physiological hypothesis, De Marco Jr. et al 2015). For example, sunlit streams and rivers favor large ectothermic species with direct exposure to the sun, while small ectothermic odonates prefer shaded streams, which maintain constant and steady temperatures (De Marco Jr. et al 2015). Consequently, if environmental drivers are important here, local odonate assemblages should be

phylogenetically clustered because closely related species tend to share similar environmental requirements. Based on that, we should also expect variations in the phylogenetic structure of assemblages (i.e., phylogenetic beta diversity) according to the riverine landscape. Thus, phylogenetic beta diversity might indicate whether clades are randomly distributed in the watershed or if environmental variation determines their spatial distribution.

On the basis of these contrasting expectations, we investigated the phylogenetic structure of adult odonate assemblages along a riverine system that varies significantly in altitude, width, luminosity, aquatic vegetation and forest cover. Our study aims to shed light on whether assemblages are phylogenetically over dispersed, which would suggest intense aggression among closely related species is a major driver, or if they are phylogenetically clustered along the riverine gradient because of similar eco-physiological requirements (Fig. 1).

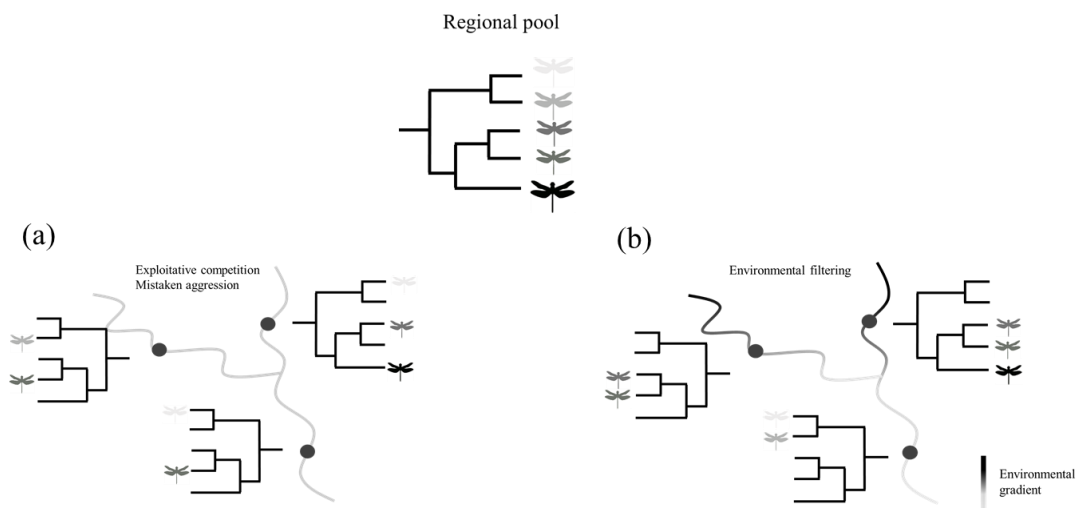


Figure 1. Hypothesis explaining the phylogenetic structure of Odonata assemblages in riverine habitats. (a) Close related species may co-occur less than expected by chance due to exploitative competition and mistaken aggression, or (b) close related species may co-occur more often than expected by chance due to similar environmental preference of related species

MATERIAL AND METHODS

Study area and sampling

We carried out the study in a riverine network on the Bodoquena Plateau, located in the southwest of Mato Grosso do Sul, Brazil (Fig. 2). The Betione river watershed belongs to the Miranda River basin, a subunit of the Upper Paraguay River which also holds part of the Pantanal wetlands. The region is characterized by the transition between the Cerrado and Atlantic Rainforest forests. The landscape of the region is a mosaic of remaining native vegetation patches due to cattle ranching. We selected 44 unique segments of the Betione watershed, from headwaters to the mainstream. In these segments we actively collected Odonata adults over the course of an hour using an entomological net along a 100 m transect parallel to the stream banks. We conducted sampling once at each site on sunny days between 10:00 and 15:00 h due to known thermal restrictions in odonates (sampling details in Valente-Neto et al 2016; Rodrigues et al 2016). This method has been effectively used in other studies (e.g., Juen and De Marco Jr. 2011). Moreover, we followed taxonomic keys and original descriptions to identify the specimens (Lencioni 2005, 2006; Garrison et al 2006, 2010; Heckman 2006, 2008). Voucher specimens are deposited in Federal University of Mato Grosso do Sul's Zoological Collection (ZUFMS).

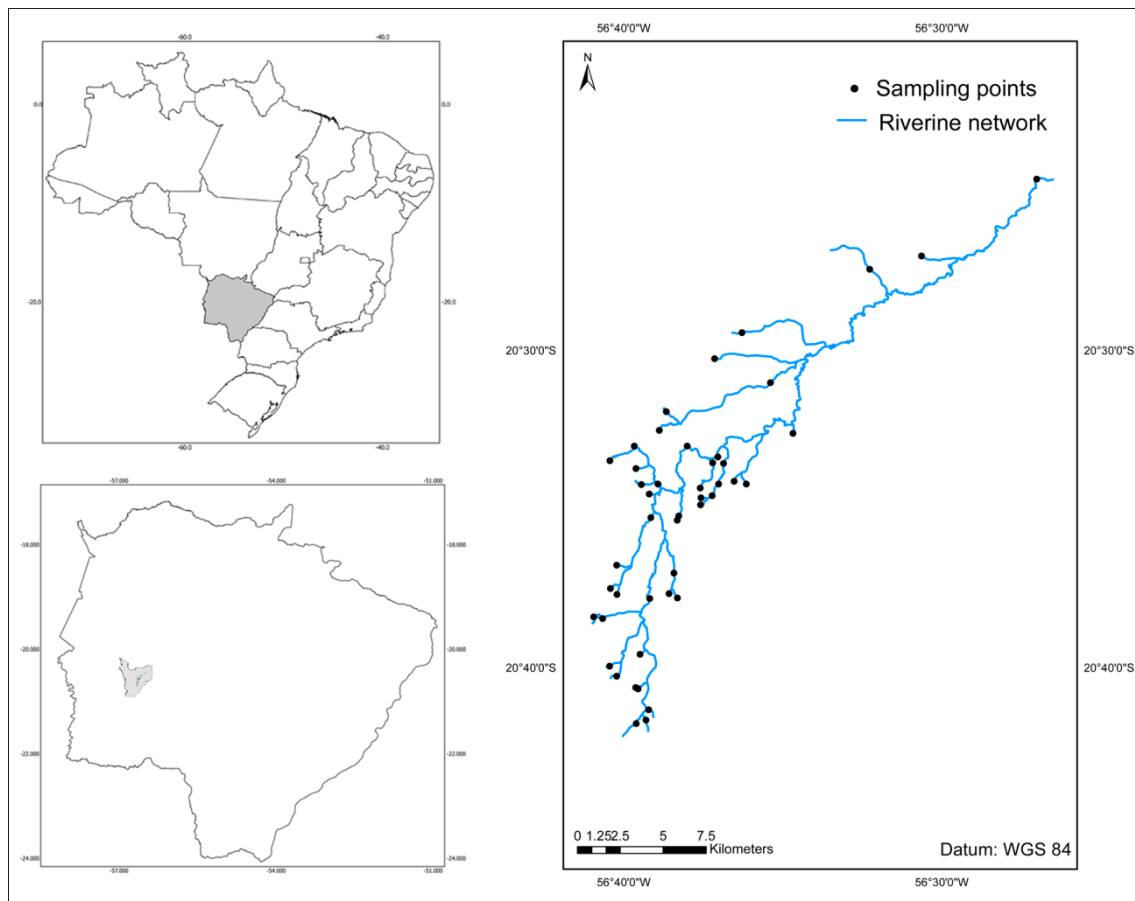


Figure 2. Sampling location of the 44 Odonata assemblages in the Betione watershed (modified from Valente-Neto et al 2016). Samples were taken from headwaters to mainstem. In each site we sampled adults and took measures of altitude, width, percentage of shading, percentage of forest cover and measures of aquatic vegetation

Environmental variables

Aiming to characterize the riverine system, we measured stream altitude, width, and luminosity, as well as evaluated forest cover and proportion of aquatic vegetation for all sample sites. A previous study used the same dataset and showed that these variables were the most important ones for structuring both larval and adult odonates communities (Valente-Neto et al 2016). We measured altitude from a GPS device and established stream width once every six meters along 30 meters totalizing five measures. We also estimated luminosity using three digital photographs of the canopy taken from the middle of the stream/river. The images were analyzed with Image J

(version 1.47, Wayne Rasband, National Institute of Health, USA). We first transformed the image to black and white scale, and then measured the white pixel area of each photograph, which represented the area that light passes through the canopy. The percentage of forest cover was calculated using ArcGis (version 10.1 ESRI, Redlands, California, USA), based on a digital map of forest coverage with 30 meters of resolution provided by the Environmental Institute of Mato Grosso do Sul state. We established a buffer of 250 meters from the middle of each sampling point as a means of estimating the percentage of forest cover. This distance was important considering that some odonates adults disperse in short distances (≤ 500 meters) (Keller and Holderegger 2013). The proportion of aquatic vegetation was visually estimated in three reaches of 10 meters.

We created an environmental distance matrix among streams using Euclidian distance on standardized values of altitude, width, luminosity, forest cover and aquatic vegetation. This environmental distance matrix represented the riverine gradient, where headwater streams had higher altitude, smaller width, and lower proportions of aquatic vegetation and luminosity than larger rivers. We transformed the percentages of the aquatic vegetation and forest cover to arcsine square root and the numerical continuous variables to logarithms before calculating the distance matrix.

Phylogeny

We created a topological phylogeny of Odonata from our species pool (Sanderson et al 1998) as a surrogate for phylogenetic relatedness. We used topological taxonomic distances because topological distances or estimated branch lengths (using molecular distance or divergence time) usually show similar community patterns (Webb et al 2002; Ives and Helmus 2010). We used six taxonomic levels (suborder, superfamily, family, subfamily, genus, and species) to create the tree based on recent

systematic reviews of Odonata phylogeny (Rehn 2003; Dumont et al 2010; Dijkstra et al 2014). Although the phylogeny of Odonata is still debated, we used the most comprehensive and recent data available (Dijkstra et al 2014). We calculated the phylogenetic distance matrix with the cophenetic distances among species in the supertree (Webb 2000).

Data analysis

We calculated the metrics of the phylogenetic community structure using Mean Nearest Neighbor Distance (MNND) and Mean Pairwise Distance (MPD) (Webb 2000). MNND is calculated as the mean distance to the closest relative individual (or species when using only presence/absence data) between all individuals (or species) in a community (Webb 2000). The MNND metric is similar to the species-to-genus ratio and influenced less by higher levels of phylogenetic structure (Webb 2000). MPD is calculated as the mean phylogenetic distance among all individuals (or species) in a community, thereby capturing the assemblages' whole phylogenetic structure. To test whether the phylogenetic structure of the examined assemblages was more clustered or dispersed than expected by chance, we used the Nearest Taxon Index (NTI) and Net Relatedness Index (NRI). These are the standardized effect size of MNND and MPD, respectively. NTI and NRI compare the observed values of MNND and MPD to null values obtained using the null model "taxa shuffle"; the model randomizes 1,000 times the rows and columns of the matrix of phylogenetic distance among species (Kembel 2009). The "taxa shuffle" null model was most appropriate because it randomizes only the location of the taxa in the distance matrix, thereby constraining the richness and the abundance of samples (Swenson 2014). We used both NTI and NRI because NTI is more prone to detect over dispersion while NRI is more keen to show clustering (Kraft

et al 2007). We used incidence and abundance data in all our analyses because density can affect competition and habitat filtering can occur at the species level (Swenson et al 2012). The overall clustering or over dispersion were confirmed with two-tailed Wilcoxon tests in NTI and NRI results. If NTI or NRI were higher than zero we would infer an overall over dispersion. Alternatively, indices significantly lower than zero indicates clustering (Swenson and Enquist 2009).

We calculated phylogenetic beta diversity among assemblages with the betaMPD and betaMNND indices. These indices are analogous to MPD and MNND. In this way, betaMPD estimates the mean phylogenetic distance among individuals (or species) among pairs of assemblages, while betaMNND estimates the phylogenetic distance among the closest related individual (or species) among pairs of assemblages (Swenson 2014). Both measures of phylogenetic beta diversity were calculated using incidence and abundance data. To observe how the phylogenetic structure affected the beta diversity in Odonata assemblages we ran 1,000 random betaMPD and betaMNND using the “taxa shuffle” null model. We then compared the mean values of the observed betaMPD and betaMNND to values generated at random. We considered significantly lower or higher beta diversity than expected at random if the observed results were lower or higher than 975 random values.

We established a phylogenetic test for the eco-physiological hypothesis by comparing whether changes in the phylogenetic structure of assemblages followed the riverine gradient. So, we used Mantel tests between the environmental distance matrix and betaMPD and betaMNND using incidence and abundance data. As a means of identifying whether the resulting correlation was dependent on the phylogenetic structure or if we could find it irrespective of species relatedness we ran 1,000 null Mantel correlations using assemblages with random phylogenetic structures (taxa

shuffle). Significant phylogenetic correlations were considered if observed correlations (Mantel statistic R) were lower or higher than 975 random mantel correlations.

To identify how the phylogenetic structure of assemblages had been changing along the riverine gradient we performed a RLQ analysis. RLQ analysis is an extension of co-inertia analysis, and produces a simultaneous ordination of three tables (Dolédec et al 1996; Dray et al 2014). Originally, RLQ analysis was a means to find the combination of traits that co-vary with environmental characteristics, but recently a variation was proposed to fit phylogenetic information instead (or together) of trait information (Pavoine et al 2011). The analysis in this case maximizes the covariance between a linear combination of the columns of an environmental matrix and the columns of a phylogenetic distance matrix, weighted by abundance of species (Pavoine et al 2011). The analysis starts with the ordination of the following matrices: R for a matrix of environmental characteristics, L for a matrix of species abundance and Q for a matrix of pairwise phylogenetic distance of species. The environmental matrix R is standardized in the same way as used for Mantel tests (see *Environmental variables* section) and analyzed through a PCA. We analyzed matrix L by a correspondence analysis and matrix Q by a PCoA. Matrix Q is a species distance matrix calculated using the square root of the sum of branch lengths of the supertree connecting the two species (Pavoine et al 2011). The RLQ analysis uses the axis of these ordinations to discover any relationships between R and Q weighted by abundances in matrix L ; it does this in a way that maximizes their squared cross-covariance (see Dolédec et al. 1996 and Dray et al. 2014 for mathematical details). The graphical observation RLQ is done by an ordination in a PCA fashion coupled with a dot plot next to a phylogenetic tree (Pavoine et al 2011).

RESULTS

We collected 1226 Odonata adults, representing six families (Calopterygidae, Coenagrionidae, Protoneuridae, Aeshnidae, Gomphidae and Libellulidae), 21 genera and 38 species (Table 1). The suborder Zygoptera was represented by 21 species and Anisoptera by 17. From the 21 genera found, 10 of them have two or more congeneric species. The two genera with more congeneric species were *Argia* and *Perithemis*, with four species each. On average in each segment of the Betione watershed we sampled eight species and 28 individuals.

Table 1. Taxonomic structure of odonates found in the Betione watershed, Brazil. We used six taxonomic levels based on recent systematic reviews of Odonata phylogeny (Rehn 2003; Dumont et al 2010; Dijkstra et al 2014).

Order	Infraorder	Superfamily	Family	Subfamily	Genus	Species	
Odonata	Anisoptera	Aeshnoidea	Aeshnidae	Aeshninae	<i>Staurophlebia</i>	<i>reticulata</i>	
		Gomphoidea	Gomphidae	Gomphoidinae	<i>Progomphus</i>	sp1	
		Libelluloidea	Libellulidae	Brachydiplacinae	<i>Elga</i>	<i>newsantosi</i>	
					<i>Micrathyria</i>	<i>pseudhyppodidyma</i>	
					<i>stawiarskii</i>		
					<i>Orthemis</i>	<i>cultriformis</i>	
					<i>discolor</i>		
				Palpopleurinae	<i>Perithemis</i>	<i>electra</i>	
				<i>lais</i>			
				<i>mooma</i>			
				<i>thais</i>			
				<i>fusca</i>			
		Sympetrinae	<i>Erythrodiplax</i>	<i>lativittata</i>			
		Trithemistinae	<i>Dythemis</i>	<i>multipunctata</i>			
			<i>Macrothemis</i>	<i>flavencens</i>			
		Zygoptera	Calopterygoidea	Calopterygidae	Hetaerinae	<i>Elasmothemis</i>	<i>cannacrioides</i>
						<i>Hetaerina</i>	<i>mortua</i>
	<i>rosea</i>						
	<i>Argia</i>					<i>chapadae</i>	
	<i>croceipennis</i>						
	<i>hasemani</i>						
<i>mollis</i>							
Coenagrionoidea	Coenagrionidae		Argiinae	Ischnurinae	<i>Acanthagrion</i>	<i>reclusa</i>	
					<i>aepiolum</i>		
					<i>gracile</i>		
					<i>Enallagma</i>	<i>novaehispaniae</i>	
					<i>Oxyagrion</i>	<i>sulmatogrossense</i>	
					<i>terminale</i>		
					<i>Tigriagrion</i>	<i>aurantinigrum</i>	
Protoneuridae	Protoneurinae	<i>Metaleptobasis</i>	<i>lillianae</i>				
		<i>Aeolagrion</i>	<i>dorsale</i>				
		<i>Epipleoneura</i>	<i>venezuelensis</i>				
		<i>Neoneura</i>	<i>bilinearis</i>				
		<i>ethela</i>					
		<i>rubriventris</i>					
		<i>sylvatica</i>					
<i>Peristicta</i>	<i>aeneoviridis</i>						

We found negative values of NTI in 32 analyzed assemblages (out of 44) using incidence and abundance data (Fig. 3). Therein, we found significant phylogenetic clustering in four of them (three in abundance data) – i.e., the observed MNND value was lower than 975 null values (Table 2); the remaining 12 assemblages showed positive values of NTI, and none of them presented significant phylogenetic overdispersion (see Table 2 for exact P values). As expected, signals of phylogenetic clustering were more evident in NRI. Using incidence and abundance data, 40 assemblages presented negative values and only four presented positive values (Fig. 3). We found significant phylogenetic clustering in 33 assemblages with incidence data and in 39 assemblages with abundance data ($P < 0.025$, see Table 2). Similar to NTI results, we did not find significant phylogenetic overdispersion using NRI. Wilcoxon tests confirmed NTI and NRI's overall tendency of phylogenetic clustering using incidence and abundance data ($P = 0.001$) (Fig. 3). Results show that odonates are not randomly distributed in local communities, but rather that closely related odonates commonly co-occurred in river stretches.

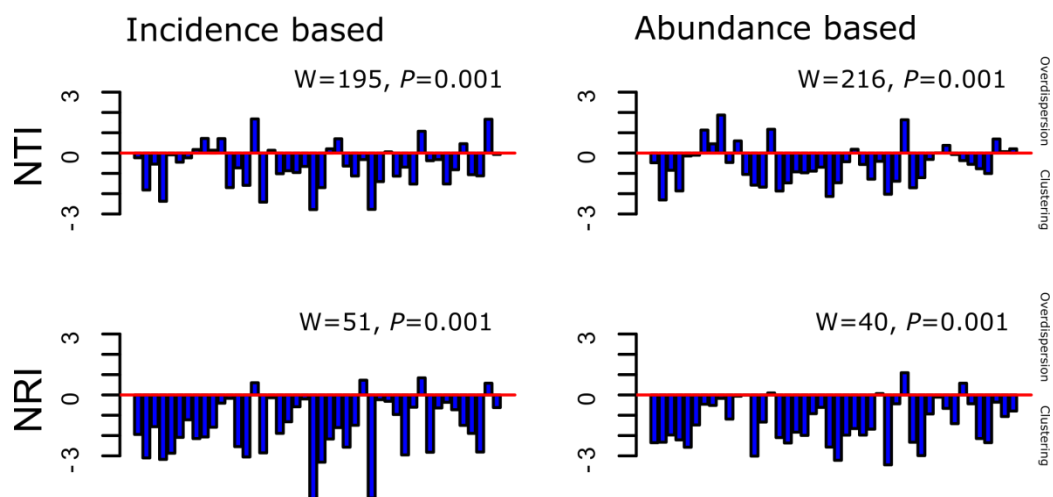


Figure 3. Results of nearest taxon index (NTI) and net relatedness index (NRI) using the phylogenetic structure of 44 Odonata assemblages from the Betione watershed. Positive values means communities with overdispersed phylogenetic structure, whereas

negative values mean phylogenetic clustering. *P* values are related to two-tailed Wilcoxon test

Table 2. Values of the nearest taxon index (NTI) and the net relatedness index (NRI) for Odonata assemblages of the Betione watershed calculated with incidence and abundance data. These two indices correspond to the standardized effect size of the metrics mean nearest neighbor distance (MNND) and mean pairwise distance (MDP), respectively. Positive values means communities with overdispersed phylogenetic structure and negative values means phylogenetic clustering. *P* values are related to comparisons against null models using communities with random phylogenetic structures.

Site	NTI incidence based		NTI abundance based		NRI incidence based		NRI abundance based	
	<i>P</i> value	<i>P</i> value	<i>P</i> value	<i>P</i> value	<i>P</i> value	<i>P</i> value	<i>P</i> value	
1	-0.232	0.400	-0.477	0.337	-1.945	0.041	-2.352	0.029
2	-1.818	0.035	-2.309	0.008	-3.102	0.010	-2.327	0.035
3	-0.544	0.310	-0.847	0.219	-1.563	0.070	-1.972	0.048
4	-2.373	0.011	-1.863	0.028	-3.175	0.014	-2.214	0.044
5	-0.084	0.474	-0.142	0.448	-2.869	0.017	-2.575	0.026
6	-0.448	0.333	-0.103	0.434	-2.091	0.057	-1.479	0.100
7	-0.234	0.404	1.133	0.875	-1.225	0.096	-0.457	0.267
8	0.174	0.568	0.454	0.666	-2.149	0.037	-0.515	0.276
9	0.720	0.753	1.874	0.963	-2.069	0.034	-0.169	0.376
10	0.141	0.558	-0.462	0.354	-1.590	0.067	-1.188	0.127
11	0.710	0.751	0.598	0.720	-0.405	0.271	-0.060	0.382
12	-1.700	0.044	-1.051	0.168	-0.166	0.373	-0.010	0.393
13	-0.729	0.238	-1.578	0.063	-2.546	0.027	-3.012	0.013
14	-1.588	0.063	-1.676	0.043	-3.054	0.009	-1.335	0.104
15	1.680	0.970	1.171	0.873	0.599	0.724	0.096	0.413
16	-2.414	0.018	-1.868	0.057	-2.853	0.015	-2.100	0.044
17	0.138	0.556	-1.468	0.074	-0.138	0.407	-2.366	0.028
18	-1.016	0.166	-0.925	0.154	-1.891	0.079	-1.834	0.077
19	-0.870	0.201	-0.971	0.186	-1.321	0.085	-1.993	0.053
20	-0.959	0.180	-0.888	0.200	-0.582	0.227	-0.921	0.145
21	-0.659	0.265	-0.697	0.266	-0.196	0.333	-0.613	0.212
22	-2.782	0.004	-2.137	0.002	-5.197	0.001	-2.567	0.017
23	-1.702	0.051	-1.462	0.066	-3.312	0.008	-3.218	0.011
24	0.208	0.568	-0.426	0.335	-2.170	0.037	-1.979	0.050
25	0.703	0.754	0.186	0.566	-1.609	0.075	-1.656	0.068
26	-0.639	0.262	-0.561	0.310	-2.572	0.028	-1.977	0.053
27	-1.128	0.136	-1.285	0.105	-1.494	0.078	-1.685	0.062
28	-0.320	0.375	-0.406	0.316	0.726	0.764	0.067	0.478
29	-2.773	0.006	-2.027	0.022	-5.076	0.001	-3.441	0.006
30	-1.405	0.090	-1.387	0.100	-0.233	0.316	-0.438	0.254
31	0.060	0.517	1.640	0.938	-0.311	0.284	1.094	0.913
32	-1.134	0.140	-1.709	0.037	-0.963	0.148	-2.328	0.035

33	-0.693	0.256	-1.211	0.130	-2.953	0.018	-2.988	0.020
34	-1.525	0.064	-0.313	0.382	-0.604	0.241	-0.935	0.166
35	1.074	0.860	0.007	0.484	0.841	0.830	-0.110	0.384
36	-0.375	0.364	0.380	0.629	-2.822	0.021	-0.663	0.201
37	-0.320	0.380	-0.069	0.481	-0.641	0.215	-1.414	0.087
38	-1.522	0.069	-0.370	0.343	-0.362	0.297	0.577	0.700
39	-0.820	0.214	-0.553	0.307	-0.729	0.199	-0.435	0.255
40	0.459	0.656	-0.772	0.241	-1.499	0.069	-2.147	0.054
41	-1.064	0.149	-1.007	0.171	-1.892	0.041	-2.350	0.032
42	-1.122	0.130	0.692	0.776	-2.811	0.018	-0.355	0.306
43	1.665	0.961	0.070	0.555	0.577	0.721	-1.063	0.189
44	-0.067	0.468	0.209	0.592	-0.622	0.221	-0.792	0.162

Observed mean values of phylobetadiversity were lower than expected using incidence and abundance data in both betaMNND and betaMPD ($P < 0.02$). These comparisons among observed phylobetadiversity and null models evidenced a significant effect of phylogenetic relatedness in the beta diversity of Odonata assemblages. Values of phylobetadiversity lower than expected indicate how certain sites generally share more phylogenetic information than expected. These results suggest that deterministic processes should be driving the phylobetadiversity of odonates assemblages, rather than random driven variation.

Mantel tests showed no correlation among phylobetadiversity of Odonata assemblages and the riverine gradient in betaMNND or betaMPD using incidence data (Table 3). However, abundance data revealed marginal correlation using betaMNND ($P = 0.067$) and significant correlation using betaMPD ($P = 0.006$ and see Table 3). This indicates that the abundance of closely related species changes depending on riverine gradient. Similarly, tests of the phylogenetic effect in Mantel correlation against null models showed a significant effect of phylogeny only in betaMPD using abundance data ($P = 0.023$ and see Table 3).

Table 3. Results of the Mantel test between phylogenetic beta diversity and the riverine gradient of the Betione watershed. Beta diversity was calculated using beta mean nearest neighbor distance (betaMNND) and beta mean pairwise distance (betaMPD), using incidence and abundance data. The riverine gradient was calculated using data of altitude, stream width, vegetation structure, aquatic vegetation and luminosity. The phylogenetic effect on Mantel correlation was calculated by comparing the observed Mantel correlation to 1,000 correlations generated under random phylogenetic distances among species.

	Mantel R	<i>P</i> value	Phylogenetic <i>P</i> value
betaMNND incidence	0.09	0.195	0.159
betaMNND abundance	0.16	0.067	0.217
betaMPD incidence	0.09	0.201	0.321
betaMPD abundance	0.27	0.006	0.023

The first two RLQ axes (with 66.4 and 21.5% of the cross-covariance between phylogenetic information and the environment for axis 1 and 2, respectively) summarized the connections between phylogenetic relatedness, species abundance and environmental variables. The negative (left) part of the RLQ first axis indicates all Anisoptera species and a few Zygoptera species related to channel width but also to luminosity and aquatic vegetation (Fig. 4). The positive (right) part of the first axis demonstrates that the majority of Zygoptera are negatively related to stream width. Anisoptera species tend toward large rivers with higher luminosity, whereas most Zygoptera related to shaded and small streams (Fig. 4). The first axis of RLQ therefore summarized the main environmental gradient of the riverine system. Some Zygoptera species (e.g., *Neoneura sylvatica*) did not follow negative association with stream width, but these species also demonstrated phylogenetic conservatism in this preference (e.g., all *Neoneura* species were positively related to stream width, see Fig. 4). The second axis indicates *Argia* species related to sites in higher altitude and some species of Anisoptera (e.g., species from genus *Micrathyria*) related to luminosity and aquatic vegetation (Fig. 4).

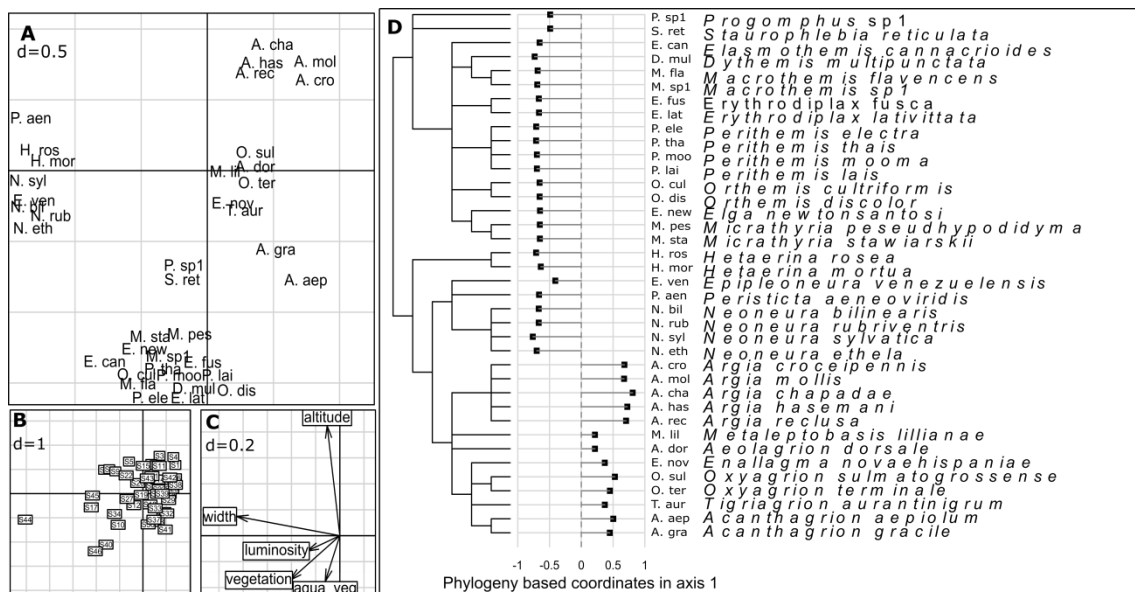


Figure 4. Results of the first two axes of RLQ analysis. The first two axes summarize 66.4 and 21.5% of the cross-covariance between the phylogenetic information and the environmental variables, all weighted by species abundance. (A) Is the score of species in the first two axes; (B) the score of sites; (C) the coefficients of environmental variables; (D) a dot plot of coefficients of phylogenetic information in the first axis next to the phylogenetic tree. The values of (d) give the grid size.

DISCUSSION

We expected that damselfly and dragonfly assemblages would be phylogenetically over dispersed because their phenotypic similarity is strong enough to make closely related species to often attack each other (Anderson and Grether 2010; Anderson and Grether 2011). Despite expectations, results indicate that competition among closely related species is not the major driver of the phylogenetic structure of these assemblages. In face of phylogenetic community ecology, our results are not surprising given how phylogenetic clustering is the most common result found and that habitat filtering is a common inference made in other studies (Vamosi et al 2009; Cavender-Bares et al 2009). However, the novelty of our study is in assessing a group overlooked by ecophylogenetics (Vamosi et al 2009) but known to compete through highly aggressive behavior and among closely related species. Below, we discuss how similar life strategies should influence a marked habitat filtering structuring the spatial

distribution of clades along the riverine longitudinal gradient. However, considering recent criticisms of the phylogenetic community ecology approach (Mayfield and Levine 2010), we also considered the possibility that closely related and competitive species could dominate the preferred habitat in some cases.

The phylogenetic structure found along the riverine gradient shed light on a plausible niche conservatism in habitat preference of odonates (Wiens et al 2010). For example, all Anisoptera species were strictly related to larger stretches, all *Argia* species were found in abundance in headwater streams, and the two *Micrathyria* species occurred specifically in waters with aquatic vegetation. The Betione watershed had a marked gradient in stream width, forest cover, shading and aquatic vegetation that became evident by the RLQ analysis. This gradient marks different sites for reproduction, feeding, and thermoregulation for species that exhibit distinct life strategies, including eco-physiological requirements and behaviors for thermoregulation. The eco-physiological hypothesis (De Marco Jr. et al 2015) predicts changes from narrow and shaded streams composed of small perching species dependent on air temperature and able to thermoregulate in shaded areas (Corbet 1999) to large rivers with high incidences of sunlight composed of larger species (e.g., *Hetaerina* and *Perithemis* species). Moreover, some odonates prefer specific macrophytes on which to perch and lay eggs (Butler and deMaynadier 2007), suggesting that availability of oviposition sites should have clustered closely related species that share the same oviposition strategy. This would include those that oviposit on macrophytes (i.e., *Acanthagrion* and Aeshnidae species, Bentes et al 2014), or drop them into the water (some *Orthemis* species, Corbet 1999). Altogether, we found that these life strategies are likely similar among closely related species and generally

constrain these species to inhabit similar environments, even if it implies strong aggression among them.

The association between the phylogenetic structure of odonate assemblages and the riverine gradient was always stronger when using abundance data. Variation in species abundance provides clues to identifying gradual changes in beta diversity patterns, which may not be possible when exclusively using incidence data (Siqueira et al 2015). The variation in abundance found in this study was also expected by the eco-physiological hypothesis (De Marco Jr. et al 2015); it predicts a decrease in the abundance of Zygoptera from headwaters to large rivers and the opposite pattern for Anisoptera. It is possible that changes in abundance provided more insightful results because the environmental gradient affecting the distribution of odonates does not follow abrupt changes and is not severe enough to eliminate individuals occurring outside their preferred habitat, such as an individual of Anisoptera that prefers large rivers but can fly over smaller streams.

The explanation above helps us to understand how closely related species prefer similar environments because of phylogenetically constrained and similar physiological responses, but it does not explain how these similar species share the same environment, often in the context of strong interference. In this way, coexistence among close related odonates could be facilitated, for example, because competitive differences among close related species are low and neutral dynamics control the total abundance of Odonata independent of species identity (Siepielski et al 2010). However, our assemblages do not support neutral dynamics because we found a marked phylogenetic structure along the landscape. We instead suggest that fine scale ecological partitioning exists in Odonata assemblages (Siepielski et al 2011; Siepielski and McPeck 2013). In this view, niche partitioning among closely related species could decrease interspecific

interference at levels that facilitate coexistence (Michiels and Dhondt 1987; Dudgeon 1989). For example, congeneric species can have non-overlapping times of activity during the day, decreasing the number of antagonistic encounters among closely related species (Michiels and Dhondt 1987). It is also evident that species sometimes also have different preferences in perching heights along the same patch of vegetation, decreasing contested territory (Worthen and Patrick 2004). Specific and fine-scale niche partitioning among close related species are therefore likely important stabilizing factors that make easier the co-occurrence of closely related species in odonate assemblages (Siepielski and McPeck 2013).

The phylogenetic community ecology approach faced recent criticism because some overlooked processes like asymmetric competition among distant related species can also drive phylogenetic clustering as in the case of the habitat filtering effect (Mayfield and Levine 2010). Here, our first assumption was that interference between closely related species should be stronger than between distantly related species, as previous findings demonstrated that congenics attack each other more than distantly related (and morphologically distinct) species (Anderson and Grether 2011). However, mistaken attacks do not discount the possibility that some closely related species may have competitive advantages over other clades according to the environment. This may have resulted in more aggressive or stronger competitors dominating preferred environments in some cases, pushing the distribution of weaker competitors or non-territorial species. For instance, experimental studies found that a larger species always won territorial contests against a smaller species when it involved floating vegetation in a river stretch (Tynkkynen et al 2004), and that the most aggressive species among groups with species of similar size excluded the others from their territories in pond systems (De Marco and Resende 2004). Under this perspective, we hypothesize that in

some cases where physiological constraints are not expected to be strong, asymmetric competition may drive phylogenetic clustering and structure of odonate assemblages. Larger stretches with high luminosity would be more prone to co-occurrence of both Anisoptera and Zygoptera species, but competitive asymmetry would affect the abundance of different species and drive the phylogenetic clustering. Zygoptera species may not frequently inhabit larger stretches with high luminosity; this is not necessarily because they are not adapted to thermoregulating there, but because they are likely competitively disadvantaged compared to larger species that can only thermoregulate in these sites.

Although competitive asymmetries can occur in some cases, habitat filtering should still be the major driver of community assembly and phylogenetic clustering. This is because some life strategies are strictly related to the environment and should constrain the distribution of some clades irrespectively of competitors. For example: large Anisoptera species are not expected to inhabit shaded streams because of their thermoregulation constraints (De Marco Jr. et al 2015), while some species cannot lay their eggs in a macrophyte that is not preferred (Butler and deMaynadier 2007), and several Gomphidae species must lay eggs close to sand substrates to enable larval development (McPeck 2008). Consequently, we conclude here that habitat filtering should be the stronger driver of odonate assemblages in most parts of the riverine system. Moreover, some cases of competitive asymmetry should reinforce the relationship between the phylogenetic structure of odonate assemblages and riverine characteristics.

ACKNOWLEDGMENTS

The authors are thankful to Dr. Scott Peacor and two anonymous reviewers for comments and suggestions on this manuscript. We are also thankful to Sandrine Pavoine for helping with the RLQ analysis. VSS was supported by grant #2013/20540-0, São Paulo Research Foundation (FAPESP). FVN was supported by grant #99999.009654/2014-03 and #1073627, and MER was supported by grant # 1171579, Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES). TS was supported by grant #2013/50424-1, São Paulo Research Foundation (FAPESP) and by grant #480933/2012-0, Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq). FOR received a productivity grant #09908/2013-2 from CNPq. The study was partially funded by the Fundect.

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Capítulo 4

Inferring competitive exclusion from trait-based community assembly: trait clustering and randomness emerge due to multispecies diffuse competition

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ABSTRACT

Coexistence theory challenged the assumption that competition only results in trait overdispersion by explaining that if species similarity translates into fitness equality, competition can also result in trait clustering. However, rigorous evaluations of how and when a process like competition can be detected from diversity patterns are lacking. To fill this gap, we simulated multispecies diffuse competition using modern coexistence theory, where indirect facilitation was possible. Simulated communities were generally composed of similar species, with low trait diversity, because species with small fitness differences need only small niche differences to coexist. However, random trait dispersion patterns were also common owing to the relaxed condition for coexistence when competition is diluted among multiple species, depending on community composition through arrival order. Our results emphasize the complexity and historical contingencies of competitive networks hindering the use of trait dispersion metrics for inferring competitive processes from patterns.

Keywords assembly rules, diffuse competition, functional diversity, indirect facilitation, limiting similarity, mechanistic simulations, niche theory, trait clustering, trait overdispersion

INTRODUCTION

Ecologists commonly make inferences about community assembly processes by analyzing richness, abundance, phylogenetic and trait structural patterns (e.g. Weiher & Keddy 1995; Webb 2000; Mayfield & Levine 2010). For example, it has become common practice to assume that communities composed of more dissimilar species (in terms of traits or relatedness) than communities assembled at random (using a null modeling approach) are dominated by the impacts of interspecific competition (Weiher & Keddy 1995; Webb 2000; HilleRisLambers *et al.* 2012). This approach (hereafter trait dispersion approach) is grounded on MacArthur and Levins (1967)'s limiting similarity hypothesis, which claims the existence of a limit to the resource use overlap among species for their stable coexistence. Within this approach, communities composed of species with similar traits are seen as evidence for relaxed competition for resources and strong abiotic filtering selecting phenotypes well adapted to the environment (Weiher & Keddy 1995). After years of intense application, Mayfield and Levine (2010), grounded on modern coexistence theory (*sensu* Chesson 2000), challenged the trait dispersion approach by showing that trait clustering can also be the result of interspecific competition if the measured traits are linked to the competitive hierarchy of species (Mayfield & Levine 2010, and see also HilleRisLambers *et al.* 2012; Adler *et al.* 2013). Besides their strong arguments, in general ecologists are still seldom considering trait clustering as a product of competition (e.g. Götzenberger *et al.* 2012; Purschke *et al.* 2013; Trisos *et al.* 2014; Mazel *et al.* 2016), likely because few studies have actually tested when and how often trait dispersion approaches give reliable results.

Mayfield and Levine (2010) based their study on the idea that species coexistence and diversity depend on two kinds of interspecific differences (Chesson

2000). First, niche differences favor coexistence by reducing the overlap in resource use among species in time and/or space, making intraspecific competition more intense than interspecific competition (Chesson 1991). Second, fitness differences determine which species will outcompete others in absence of niche differences – i.e. it relates to the competitive hierarchy among species (Chesson 2000). Stable coexistence among species is determined by a balance between niche and fitness differences, and the coexistence condition is that niche differences must overcome fitness differences (Chesson 2000). Understanding how traits are translated into these niche and fitness differences among species is a complex challenge (Godoy *et al.* 2014; Herben & Goldberg 2014; Kraft *et al.* 2015) as traits likely impact both niche differentiation and fitness advantage even if the net impact on one is higher than on the other (Adler *et al.* 2013; Kraft *et al.* 2015, but see Snyder *et al.* [2005] for an example of habitat preference that strictly relates to niche differences). For example, two animal species competing for different preys can have distinct preferences for prey size and taxonomic identity of prey (e.g. lizards from Sea of Cortez, Case 1983). These preferences could favor coexistence through resource partitioning but eventually lead to competitive asymmetry if preys differ in nutritional contents and make the species that feed on the best prey to outcompete the other for a second resource, e.g. sites for reproduction. Thus, prey preference would be a trait that contributes simultaneously to niche and fitness differences. One could, thus, expect that coexisting species would differ in some traits leading to niche differentiation and would be similar in other traits leading to fitness equality. But how trait similarity balance and shape communities composed of species with higher or lower trait similarities is largely unknown.

Beyond the importance of niche and fitness differences for competitive outcomes, an overlooked characteristic of competition is that it generally happens

among several species in nature and not only in pairwise situations, as commonly considered in conceptual examples. The addition of a third (or more) species to a community composed of only two species increases the complexity of the competitive network. This happens because three species can experience indirect facilitation where “the enemy of my enemy is my ally” (Levine 1976, Lawlor 1979). For example, considering two species A and B in a community in which species C is added, species C can negatively impact species B but can indirectly facilitate B by suppressing also species A (Davidson 1985). In that case, all pairwise coexistence conditions that species must have niche differences that exceed fitness differences to coexist do not need to be satisfied if a third species decreases the average fitness differences among the stronger and the weaker competitor (Lawlor 1979). Despite this direct influence of diffuse competition on determining species coexistence, its consequences have, as far as we are aware, never been considered in studies trying to infer competitive exclusion from trait dispersion patterns.

Based on the potential impact that multispecies diffuse competition may have on species coexistence and community assembly, we investigated the power of commonly used metrics (e.g. species richness, beta diversity and trait dispersion metrics) to detect signatures of competition under a mechanistic model of diffuse competition. We simulated scenarios under priority effects and colonization-competition trade-offs, and under different levels of colonization intensity from species pools of different sizes to observe when predicted patterns would be observed in natural communities. We had a general expectation to find higher species richness in communities under local coexistence mechanisms (e.g. density-dependent predation, Chase *et al.* 2002) and higher taxonomic beta diversity among communities under coexistence mechanisms operating at regional scales (e.g. colonization competition trade-off, Cadotte 2007). We

expected to find higher niche differences and smaller fitness differences than expected by chance in our simulated communities (HilleRisLambers *et al.* 2012). Ultimately, we expected to shed light on how trait dispersion patterns should be when among-species dissimilarities reflect both niche and fitness differences and when communities are embedded in multispecies diffuse competition. To accomplish that, we created six simulation scenarios:

Scenario 1=Trait types: We simulated studies gathering traits more related to niche or fitness differences among species. We aimed to reveal how the number of niche versus fitness traits affects the outcomes of trait dispersion metrics.

Scenario 2=Strength of stabilization: We varied a model parameter to show how stabilizing mechanisms such as density-dependent predation could affect community structure patterns.

Scenario 3=Colonization intensity: We varied the number of colonization events to show how dispersal could affect patterns of community structure.

Scenario 4=Colonization-competition trade-off: We included a colonization-competition trade-off to observe how regional mechanisms of coexistence could affect the outcomes of diversity metrics.

Scenario 5=Priority effect: We included a controlled priority effect among residents and invaders to observe how this contingency would change analyzed metrics.

Scenario 6=Species pool size: We varied the size of the species pools to observe how this could impact the detection of trait structure in our simulations.

In the end, analyzing all results we would see how often and under what circumstance diffuse competition leads to distinct patterns of species diversity and trait dispersion.

METHODS

Model description

We used a computational model to simulate community assembly based on coexistence theory and under diffuse multispecies competition, by first creating a regional species pool with 100 species (but see our Scenario 6) with simulated niche positions and fitness values for each species. Niche position (x) and fitness values (k) were both simulated from a discretized normal distribution and scaled between 1 and 100. Our selection of niche position considers that all species can use all the niche space and have the same niche width, but niche position represents the optimal position. In real communities this could represent the case when all species can use all available resources but with different preferences.

We then used the following conditions based on theoretical studies to determine if two or more species could coexist (eqs 3 and 4 in Chesson 2000). The mathematical proof of our equations was done by the senior author of this manuscript and will be on the Supplementary Material of the published version of this chapter. However, as it was done entirely by Sandrine Pavoine, I decided to not include it here. A conceptual explanation for our model selection can be found on the Appendix of this chapter. Considering eqs 3 and 4 in Chesson (2000) the resulting condition for coexistence is:

1. if the community is composed of one species (the resident s), the condition for an invader (i) to coexist with s is (eq. 1)

$$\begin{cases} (k_s - k_i) < (1 - \rho)k_s D & \text{if } k_i < k_s \\ (k_i - k_s) < (1 - \rho)k_i D & \text{if } k_i > k_s \end{cases}$$

where the difference in niche $(1-\rho)$ among the two species is defined as $|x_i - x_s|/99$ (eq. 2)

and ρ is niche overlap; D is a positive parameter which will be used to adjust the relative importance given to the stabilizing and equalizing mechanisms. When pairs of

species do not fit the coexistence condition, the species with the highest fitness (k) outcompetes the other.

2. if the community has more than one resident, the condition for an invader to enter the community and coexist with all residents is (eq. 3)

$$\left| \min_u \left(k_u - \frac{\sum_{v \neq u} k_v}{n-1} \right) \right| < \frac{(1-\rho)D}{n-1}$$

where n is the number of species (residents + invader), the difference in fitness is measured in the set of all species (resident + invader), $k_u - \frac{\sum_{v \neq u} k_v}{n-1}$ thus is the difference between the fitness of a species u in the set and the average fitness of all other species; D is again the positive parameter which will be used to adjust the relative importance given to the stabilizing and equalizing mechanisms. Let y_1, \dots, y_n be the niche values ordered from the lowest to the highest. The difference in niche positions for the whole community (residents + invader) is measured as (eq. 4)

$$1 - \rho = \begin{cases} 0 & \text{if } y_1 = \dots = y_n \\ \left| \frac{y_n - y_1}{99} \right| \frac{1}{(n-1) \sum_{i=1}^{n-1} \left(\frac{y_{i+1} - y_i}{y_n - y_1} \right)^2} & \text{if } y_1 \neq y_n \end{cases}$$

The first term of the equation ($|y_n - y_1|/99$) varies between 0 and 1 and measures the portion of the whole gradient axis occupied by the n species; in the second part,

$\sum_{i=1}^{n-1} \left(\frac{y_{i+1} - y_i}{y_n - y_1} \right)^2$ is Simpson's index of concentration (Simpson 1949) applied to the

segment lengths $(y_{i+1} - y_i)$ for all i ; $\frac{1}{(n-1) \sum_{i=1}^{n-1} \left(\frac{y_{i+1} - y_i}{y_n - y_1} \right)^2}$ varies from $1/(n-1)$ (when for any

species i , y_i either equals y_1 or y_n) to 1 (when the niche values are evenly distributed from y_1 to y_n); $1 - \rho$ thus varies between 0 (all niche values are equal) and 1 (niche values are evenly distributed from the minimum to the maximum of the gradient). In the particular case of two species only, eq. 4 reduces to eq. 2.

In this multispecies model, when the coexistence condition is not satisfied and the fitness of the invader is lower than the average fitness of the residents, then the invader is excluded. If the coexistence is not satisfied and the fitness of the invader is higher than the average fitness of the residents, then the species with the species minimizing $k_u - \frac{\sum_{v \neq u} k_v}{n-1}$ over u (having the most negative difference in fitness) is excluded and the coexistence condition is tested in the new resulting community. This process is repeated until the coexistence condition is satisfied.

Most simulations were done with D equal to unity. However, to simulate mechanisms promoting the coexistence of competitors we increased the values of D . Indeed, when $D > 1$, the fitness differences are relatively less important than niche differences in the coexistence function and smaller niche differences are necessary for species to coexist. For example, communities of preys under density-dependent predation can maintain more species than communities without predators because predators decrease the density of the dominant species and inhibit competitive exclusions (Holt 1977; Chesson & Kuang 2008). Given the parameter D , eq. 1 and eq. 3 simulate a realistic scenario when species are not stably coexisting, but are only co-occurring (i.e. one species will eventually exclude the other) in our communities (also called unstable coexistence, Chesson 2000). In this case, large niche differences and small fitness differences increase the time for competitive exclusions (Adler et al. 2007) enabling species to co-occur for a longer period of time. In this case, with larger niche differences and smaller fitness differences, the time for competitive exclusions increases (Adler *et al.* 2007), enabling species to co-occur for a longer period of time.

We then built a mechanistic model to simulate the assembly of communities using these coexistence functions. The model requires a given a priori number of colonization events to occupy a community. The first species is randomly selected from

the regional pool and occupies a local community (but see the Simulation scenarios for details). The species pool is unchanged so that a single species can invade a community several times during the simulations. The model thus assumes that all species from the species pool have the proper traits to inhabit the environment and abiotic filtering plays no role in our simulations. This scenario is similar to that described by the concept of functional species pool (de Bello et al. 2012). Then, the model randomly selects a second species to invade the community and applies the coexistence condition to decide whether the first and the second species can coexist in that community. If these species can coexist, the next (third) invader would be tested using the multispecies coexistence condition. Subsequent colonizing species are tested for the multispecies coexistence condition with all species that already coexist there. The end of the community assembly is when all the colonization events defined *a priori* were done. This provides a realistic possibility to create communities with different final number of species.

We defined a simulation scheme as each round starting from simulating the species pool to the assembling of 100 communities. A simulation scheme thus ends with a set of 100 communities assembled under the same conditions. Finally, we considered six different scenarios (see below in Section *Simulation scenarios*) each created with at least 100 simulation schemes.

Community metrics

To analyze the simulated communities, we calculated metrics often used to investigate community assembly in observational studies (Pavoine & Bonsall 2011). Species richness is expected to be higher when strong stabilizing or equalizing mechanisms are operating. For example, strong competition decreases species richness because dominant species outcompete weaker ones, but this effect can be damped by density-dependent predation acting as stabilizing force and enabling more species to

coexist (Chase *et al.* 2002). The Jaccard coefficient of dissimilarity among communities can depict coexistence mechanisms at the regional scale, since higher beta diversity and more species coexisting at the regional scale are expected when mechanisms like competition-colonization trade-off exist (Cadotte 2007). For example, weaker competitors can stably coexist regionally with stronger species if the best competitor is the worst colonizer and does not colonize all patches at the same time (Chesson 2000). Among trait dispersion metrics, the standardized effect size of mean pairwise distances (SES.MPD) and that of the mean distance to the most similar species (SES.MNTD) within communities (Webb 2000) are among the most used and explored metrics to infer assembly processes (Pavoine & Bonsall 2011). A standardized effect-size different from zero is usually used to infer deterministic assembly processes, although clustering or dispersion of species trait can be interpreted in many ways. The distances among species used in SES.MNTD, SES.MPD were calculated with the Euclidean distance applied to niche position (niche differences), fitness (fitness differences) and both (overall among-species distances).

Simulation scenarios

Scenario I = Trait types. This scenario shows how the model performs in 100 simulation schemes with 100 invasions per community and without any mechanisms promoting local or regional coexistence ($D=1$; the first species is randomly selected and occupies a community without any other criterion than randomness). To simulate studies gathering traits more related to niche or fitness differences among species (see Kraft *et al.* 2015), we added replicate data to give greater weight to specific factors (niche or fitness) before calculating the Euclidean distance among species. For example, to simulate a dataset with twice as many traits related to niche than traits related to

fitness we applied the Euclidean distance to a table with the niche trait replicated twice (two identical columns) and the fitness values in one column (species as rows).

Scenario 2 = Strength of stabilization. We ran several sub-scenarios (100 simulation schemes and 100 invasions in each community) with D values of 1, 2, 4, 6, 8, 10, 15, 20, 25, 30, 35, 40, respectively. This scenario would show how stabilizing mechanisms such as density-dependent predation could affect community structure for a given species pool.

Scenario 3 = Colonization intensity. Here we simulated schemes assembled with 30, 50, 100, 300 and 500 colonization events ($D=1$). This would show how limited, sufficient and intense dispersal could affect patterns of species coexistence and community structure.

Scenario 4 = Colonization-competition trade-off. We created simulation schemes with 10, 50, 100, 300 and 500 colonization events ($D=1$); but, compared to scenario 3, we added a colonization-competition trade-off: the chance of colonization for each species (be the colonization successful or not) was inversely proportional to their fitness, providing to the worst competitor the highest chance to colonize each community. Colonization-competition trade-off does not avoid competitive exclusion, but weaker competitors should occur in some communities when the dominant competitor does not colonize all communities (Chesson 2000). We expected to find higher beta diversity in the trade-off scheme than in scenario 3 where species had a random chance of invasion. However, with intense dispersal, we expected a decrease in beta diversity, since the strongest competitors should dominate most communities.

Scenario 5 = Priority effects. We considered schemes with 10, 50, 100, 300 and 500 colonization events ($D=1$), but compared to scenario 3, priority effects were simulated by increasing by 10 units the fitness values of residents compared to the invader in the

coexistence function, i.e. residents always have a small advantage over the invader. This creates a historical contingency that modifies competition among species depending on arrival sequence (Chase 2003). We expected to find higher beta diversity in this scenario compared to scenario 3 because more alternative community compositions would be possible (Chase 2010, Fukami 2015).

Scenario 6 = Species pool size. We created five groups of simulation schemes varying the size of the species pool. We used species pools with 30, 50, 100, 300 and 500 species. Our expectation was to find stronger trait overdispersion in simulations with smaller species pools while clustering should be more evident with larger species pools (Kraft *et al.* 2007). Each species pool size was analyzed under 10, 50, 100, 300 and 500 invasion events ($D=1$) to observe the interaction between species pool size and dispersal intensity.

All simulations and analyzes were performed in R (R Core Team 2016) with packages *ade4*, *picante* (Kembel *et al.* 2010; Chessel *et al.* 2012).

RESULTS

Simulation scenarios

Scenario 1 = Trait types. SES.MPD and SES.MNTD values for overall species distance were lower than expected by chance (trait clustering) when simulating studies that gather more species traits that translate into fitness than niche differences. When niche and fitness had the same importance in overall species distance calculation, we also found a tendency to trait clustering (Fig. 1). When niche differences were more influential than fitness differences in the calculation of among-species distances we found random patterns with SES.MPD, but still trait clustering using SES.MNTD (Fig. 1).

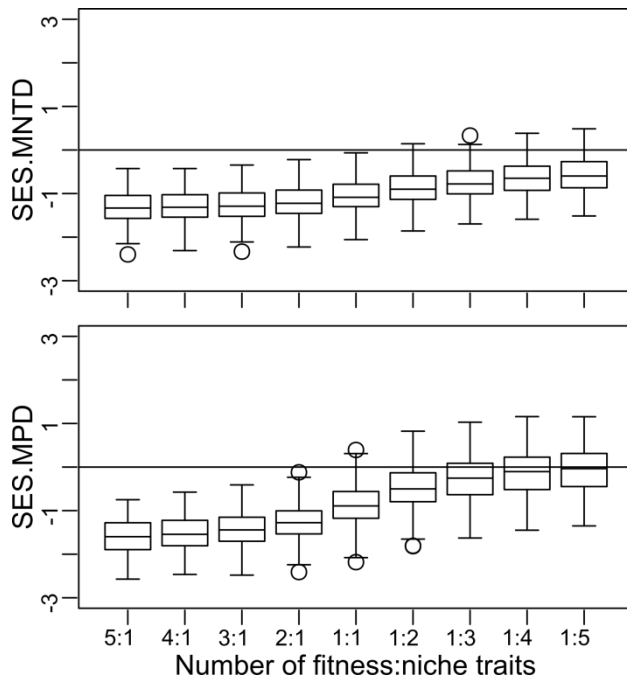


Figure 1. Boxplots showing results of scenario type 1 ("Trait types"). Each plot results from 100 simulation schemes assembled with 100 colonization events. Boxplots represent the mean value per simulation scheme. To simulate studies gathering traits more related to niche or fitness differences among species, we calculated the overall distances among species using distinct weights for niche versus fitness differences. We did that by multiplying the columns of niche positions or fitness values (from one to five) in our table of species traits (with species as rows and niche and fitness as columns) before calculating the Euclidean distance among species.

Scenario 2 = Strength of stabilization. Variation in D strongly impacted species richness but hardly beta diversity (Fig. 2). As predicted, we found more species coexisting under stronger stabilizing processes. Lower fitness differences than expected at random were the common pattern in SES.MPD and SES.MNTD. Niche differences tended to overdispersion, but were not clearly different from random. We found consistent clustering when SES.MNTD and SES.MPD was calculated with overall distances (mixing niche and fitness traits). When D became exaggeratedly high (tending to infinity), SES values all tended to zero as the stabilizing forces were so important that all species could co-exist.

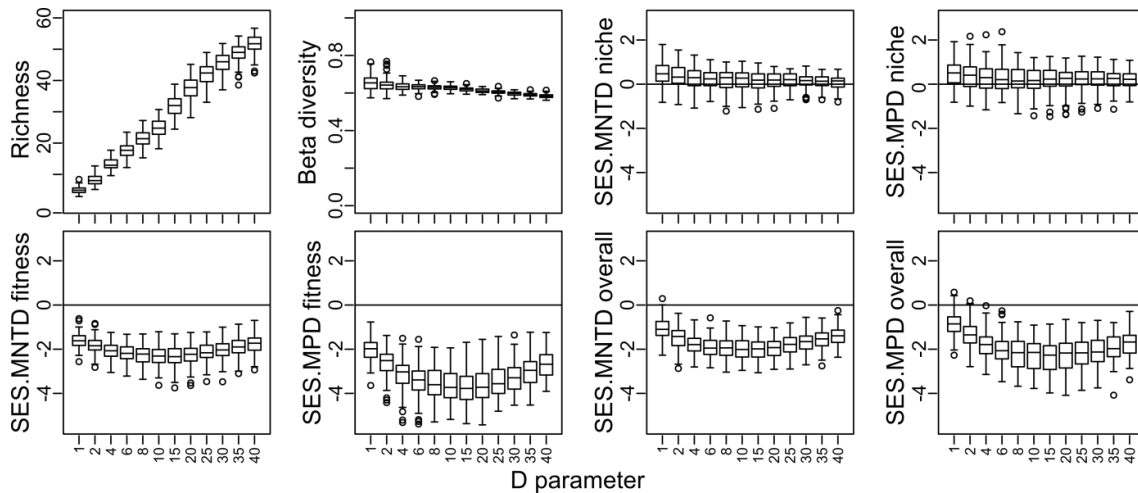


Figure 2. Boxplots showing results of scenario type 2 ("Strength of stabilization"). Each boxplot results from 100 simulation schemes assembled with 100 colonization events. Each plot within each graphic represents results for simulations when D was 1, 2, 4, 6, 8, 10, 15, 20, 25, 30, 35, 40. The higher D , the smaller the niche differences among species need to be for species to coexist. Codes for the trait-based distances among species used in indices SES-MNTD and SES-MPD are 'Niche' = Euclidean distance applied to the niche trait, 'Fitness' = Euclidean distance applied to the fitness trait, 'Overall' = Euclidean distance applied to niche and fitness with equal weight for both traits.

Scenario 3= Colonization intensity. Species richness saturated after a number of colonization events but the threshold varied considerably depending on the species pool (increased variation with increasing number of colonization events) (Fig. 3). Beta diversity strongly decreased with the number of colonization events. Variation in SES.MNTD and SES.MPD increased with the number of colonization events. These results show that increased colonization events lead communities to compositional and trait homogenization, but that this final community structure is dependent on the species pool.

Scenario 4=Colonization-competition trade-off. Species richness and trait dispersion metrics performed similarly under random invasions and when species had competition-colonization trade-offs (Fig. 3, second block). As predicted, the effect of colonization-competition trade-off was clearer in taxonomic beta diversity, as there was

more compositional variation among communities in this scenario even after a large number of invasions (Fig. 3).

Scenario 5=Priority effect. As in scenario 4, priority effects increased taxonomic beta diversity (Fig. 3, third block). It also slightly decreased species richness. Although the absolute values of the SES metrics decreased, trait overdispersion (with niche distances) and trait clustering (with fitness and overall distances) were still detectable.

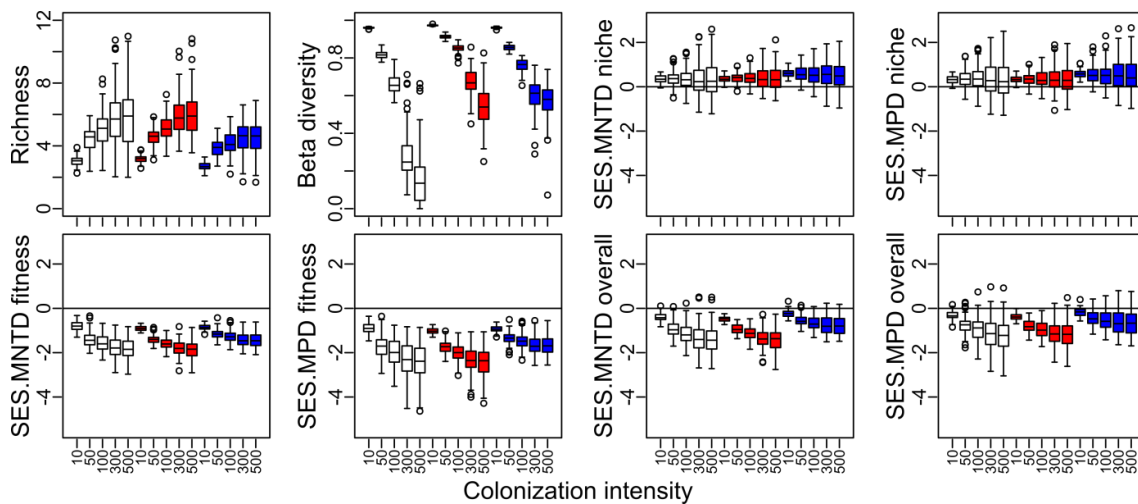


Figure 3. Boxplots showing results of scenario types 3 ("Colonization intensity"), 4 ("Colonization-competition trade-off") and 5 ("Priority effect"). Each boxplot results from 100 simulation schemes. Each boxplot within each graphic represent results when the number of colonization events was 10, 50, 100, 300 or 500, respectively. White plots represent results with random colonization events (Scenario type 3). Red boxplots represent results with colonization-competition trade-off in species arrival (Scenario type 4) and blue plots represent the priority effect scenario (Scenario type 5) when resident species have a small advantage over the invader in our model. Codes for the trait-based distances among species used in indices SES-MNTD and SES-MPD are 'Niche' = Euclidean distance applied to the niche trait, 'Fitness' = Euclidean distance applied to the fitness trait, 'Overall' = Euclidean distance applied to niche and fitness with equal weight for both traits.

Scenario type 6=Size of the species pool. The size of the species pool largely affected the outcomes of analyzed metrics. We found an increased species richness in communities simulated with larger species pools (Fig. 4). As predicted, beta diversity was also higher in simulations using larger species pools. SES.MPD and especially

SES.MNTD using overall species similarity showed an increased clustering with larger species pools.

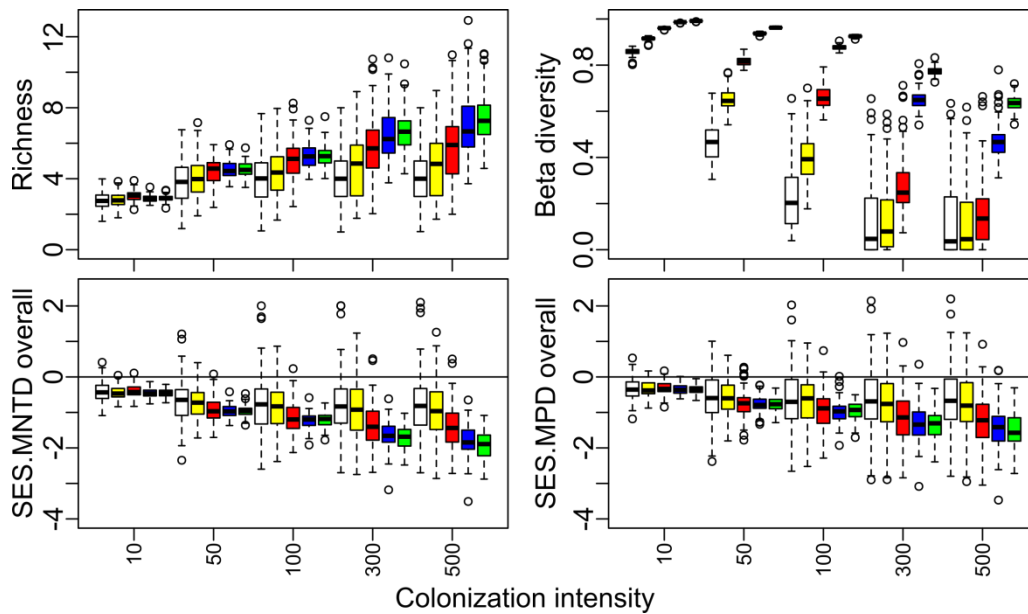


Figure 4. Boxplots showing results of scenario type 6 ("Species pool size"). Each boxplot results from 100 simulation schemes. Each boxplot within each graphic represents results when the number of colonization events was 10, 50, 100, 300 or 500, respectively. Different colors mean different sizes of species pool when running simulations (white=30 species, yellow=50 species, red=100 species, blue=300 species, green=500 species). Codes for the trait-based distances among species used in indices SES-MNTD and SES-MPD are 'Niche' = Euclidean distance applied to the niche trait, 'Fitness' = Euclidean distance applied to the fitness trait, 'Overall' = Euclidean distance applied to niche and fitness with equal weight for both traits.

DISCUSSION

The community patterns we observed from simulations are predicted by different ecological theories: more species coexisted locally with strong stabilizing mechanisms; inter-community variation increased and more species coexisted regionally under colonization-competition trade-offs; intense colonization from the regional species pool enabled strong competitors to dominate most communities, decreasing beta diversity (Chesson 2000; Chase 2003). The ability of our model to simulate these phenomena suggests that the model is a reliable tool for discussing the emerged patterns of trait dispersion metrics. Community assembly studies using the

trait/phylogenetic dispersion approach have the ambitious aim of inferring processes by looking to the number and characteristics of species in a given community (Weiher & Keddy 1995; Webb 2000). However, we found that the two Achilles' heels of linking patterns to processes apply to the trait dispersion approach. The first is when two distinct processes result in the same indistinguishable pattern. We found that communities composed of species more similar than expected at random - a pattern called to infer environmental filtering (Weiher & Keddy 1995; Webb 2000) - can also be the result of competition alone (Mayfield & Levine 2010) (see Figs 3, 4). The second is when one process can lead to different patterns. We found that competition based on a diffuse competition model can commonly result in communities with similar species (as predicted by Mayfield & Levine 2010), but can also result in communities with random or even overdispersed trait structure (see the full range of variation in results from Figs 1 to 4). Although these results bring concerns to the use of the trait dispersion approach to infer community assembly mechanisms, they also shed light into interesting issues that we discuss below.

Under several simulated scenarios, trait dispersion metrics showed overdispersion in niche differences, but clustering in fitness differences, suggesting that stable communities are more commonly achieved when species have large niche differences overcoming small fitness differences; a pattern similarly found by others. For example, Case (1990) and Kokkoris *et al.* (1999) using simulations found weak interaction strength (the per capita effect of one species on the growth rate of another) within communities assembled after sequential colonization – what equals large niche differences overcoming small fitness differences in Chesson (2000) framework. These results support empirical evidence that coexisting species commonly have way larger niche differences than the minimum necessary and that species tend to competitive

equivalence (Chu & Adler 2015), highlighting the importance of niche differences in stable communities. Moreover, our findings indicate that empirical studies able to separate niche- from fitness-related traits (e.g. Kraft *et al.* 2015) could have found overdispersion on niche related-traits but clustering on fitness-related traits. However, for a likely scenario in which traits impact both fitness and niche differences (Adler *et al.* 2013; Kraft *et al.* 2015), our results suggest that trait-based metrics will tend to clustering, rather than the previously expected overdispersion pattern when competition rules the assembling. Mayfield and Levine (2010) conceptually exposed how trait and phylogenetic clustering could arise as a product of competition, but rigorous evaluations of their ideas were lacking (Adler *et al.* 2013). Here we performed such an evaluation while making consideration of the complexity of multispecies competition. Previous empirical studies indeed found clustering and overdispersion in different traits with an overall clustering in among-species similarity, but the interpretation relied on the opposing effects of environmental filtering and limiting similarity (e.g. Swenson & Enquist 2009). Here, we demonstrated how these same patterns can arise only because of competition if some measured traits drive niche differentiation and others fitness equality. Notwithstanding, we are not disregarding environmental filtering *per se* as a driver of trait clustering, but it is evident that competition plays a role and should be also considered as a driver of this pattern in natural communities.

We found that an increased colonization from the same species pool can lead communities to compositional homogenization (decreasing beta diversity with increasing colonization in Fig. 3). However, whether these final communities would present overdispersed or clustered trait pattern depends on the species pool (e.g. we found clustering more commonly, but also random patterns using overall species similarity, see Fig. 3, and overdispersion if niche traits largely dominate the overall

among-species distances, Fig. 1). It is worth noting that the variation in simulation outcomes was high even when species pools were always simulated in the same way (i.e. sorting niche position and fitness values from a discretized normal distribution and scaled between 1 and 100). The explanation for this variation is that different species pools can assemble different types of communities after a large number of colonization events. These stable communities (i.e. communities that cannot be colonized by any other species from the species pool) can be composed by species coexisting in many different ways. Indeed, species can coexist by: 1) being sufficiently different in their niche traits, so that fitness differences are meaningless; 2) being sufficiently similar in their fitness, so that small niche differences enable coexistence (both ways already recognized by theory, Adler *et al.* 2007); 3) relying on indirect facilitation, which was not considered in trait dispersion approaches. For example, the net effect of including a third species to a community of two species is the sum of direct (negative) effects and indirect (positive) effects (by suppressing the other competitor) and coexistence among all species is possible when the net effect is positive (i.e. what would result in positive growth rate for all species, Davidson 1985). In our model the third possibility for species coexistence represents the situation when two competitors A and B, A having a higher fitness than B, can coexist if a third species C decrease the fitness differences among them - i.e. by diluting the competition of A (stronger), with B and C. This dilution effect is likely the cause for the common random patterns we have found for overall species similarity, since the coexistence condition is relaxed and species with different levels of niche and fitness differences can coexist depending on species combination. This dependency on the species pool and species combination increases with community richness, with more possible combinations of traits and more cases of indirect facilitation, giving the final community structure a strong contingency of

species arrival order (e.g. as a function of colonization-competition trade-off or priority effects giving advantage to the residents to the detriment of invaders). The possibility to assemble stable communities with distinct patterns but governed by the same process (i.e., competition) hinders also comparisons between results using trait dispersion metrics among areas or studies (e.g. Algar *et al.* 2011), even if comparisons control the null model and species pools size. For example, the final community structure varied from clustering and overdispersion depending on the pool and trait types; what a comparative study could misinterpret as communities assembled by distinct processes. Therefore, interpretation, such as, “communities of taxonomic group A are structured by competition given their trait overdispersion, but communities of B are not, given their random patterns”, should be taken with caution, since both groups could be assembled by diffuse competition, but with different levels of indirect facilitation.

An important finding in our study is that SES.MNTD (a nearest neighbor metric) was more prone to detect clustering than SES.MPD (a mean distance metric), contrasting previous studies that found nearest neighbor metrics more prone to detect overdispersion (Kraft *et al.* 2007). The explanation for this mismatch lies on the fact that previous models simulated competition relying on limiting similarity (Kraft *et al.* 2007; Münkemüller & Gallien 2015) – e.g., directly removing the most similar species within communities (Kraft *et al.* 2007) – or using asymmetrical and hierarchical competition (Münkemüller & Gallien 2015) – e.g., ranking competitive ability as a function of niche position differences. None of these previous models made possible the coexistence of similar and dissimilar species in the same community governed by competition. By allowing both niche and fitness differences, we assembled communities composed of species coexisting due to small differences in their fitness and niche, what would result in clustered SES.MNTD. However, species could also coexist due to

indirect facilitation, where trait structure is relaxed by diluted competition; a condition that could result in SES.MPD values not different from expected at random. This corroborates the prediction that there is hardly a single limiting similarity value that matches all species within a community, not only because this limiting value changes among species pairs (Chesson 2000; Adler *et al.* 2007), but also because the inclusion of more species changes the coexistence conditions in particular ways.

For a long time, ecologists have invested efforts finding evidence of competition based on the limiting similarity idea proposed by MacArthur and Levins (1967). However, our results add new support for a growing body of theoretical (Chesson 2000) and empirical evidence (Chu & Adler 2015) that the coexistence of similar species documented for several systems and taxonomic groups is not a paradox if we consider multispecies competition in light of modern coexistence theory. Moreover, the complexity of competitive networks found here is likely common in natural communities, especially those encompassing rich assemblages, where the dilution effect should be strong and community assembly less predictable in terms of trait dispersion patterns. Thus by simulating complex multispecies communities, we demonstrate how much we need to improve our methods to properly detect competitive processes from diversity patterns.

ACKNOWLEDGEMENTS

We are thankful to Raul Costa Pereira for providing insightful discussions about coexistence theory that fueled the elaboration of this manuscript. We also thank Andros Gianuca and three anonymous reviewers for commentaries on an earlier version of this manuscript. The writing of this study was funded by grants #2013/50424-1, #2013/20540-0, #2014/24532-4 São Paulo Research Foundation (FAPESP) and by

grant 403723/2012-4, Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq). VSS thanks the members of the Centre d'Ecologie et de Sciences de la Conservation (CESCO) from the Muséum National d'Histoire Naturelle in Paris for kindly receiving him during the elaboration of this manuscript.

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APPENDIX

Considerations about our simulation approach

First, we would like to emphasize that there is no simple way to model multispecies competition, nor a single theoretical model that fits all taxa and systems. The straightforward way we can analyze and model two-species competition is not easily transferable for multiple species because they can have distinct types of interaction depending on model premises and derivations (Barabás et al. 2016).

Few theoretical models consider that competition among multiple species equal the competition among all possible individual pairs. For instance, only when competition is symmetric and each species affects each other in the same magnitude - varying only by their degree of niche overlap - multiple competition may be modelled by pairwise competition. This is because symmetric competition can be explored by symmetric matrices of Lotka-Volterra competition coefficients in which positive eigenvalues (positive growth rate for all species) are only possible if all its minor components are positive (positive growth rate for all individual pairs). This is simply the application of Sylvester's criterion in matrix algebra; see Barabás et al. (2016) for the full exploration. In other words, when all species have the same fitness, multispecies coexistence is possible when all pairwise species coexist individually. However, as we were also interested in asymmetric competition, when species competition is determined not only by their degree of niche overlap but also by their fitness differences, we developed a multispecies competition approach. This approach accounts for an indirect dilution effect, where invasiveness is not strictly related to community richness.

Additive models of diffuse competition (where invasiveness is related to community richness), as developed by Pianka (1974) and further explored by Case (1990, 1991), Kokkoris (1999) and Steiner and Leibold (2004) relies on the most commonly applied

variation of Lotka-Volterra competition model, where species abundance of competitors is determined by an interspecific coefficient of competition and the carrying capacity (Chesson 2013). However, this traditional Lotka-Volterra model is phenomenological and does not give a mechanistic explanation for the carrying capacity, nor does it have a mechanism of density-dependency growth rate – a corollary of niche theory (Chesson 2000, 2013). Based on that, we decided to construct a diffuse competition model that accounts for nonadditive effect of competition (i.e. competition strength do not increase additively with species addition, Levine 1976). This model was developed upon the MacArthur's consumer resource model (1972) and includes direct and indirect interactions among all species within a community, enabling for indirect facilitation in some cases where “the enemy of my enemy is my ally”. This nonadditive diffuse competition is supported by theoretical (Levine 1976) and empirical studies, both observational (e.g. Lawlor [1979] exploring MacArthur's warblers) and experimental (e.g. Davidson [1985] with Chihuahuan Desert harvester ants).

Different from the traditional Lotka-Volterra formulation, Chesson (1994) used the variation of it, the MacArthur consumer-resource model (1972) to rework it in a mechanistic way. Instead of carrying capacity, in Chesson's formula species growth is determined by negative intra and interspecific density dependency. In this form, the Lotka-Volterra is mechanistic and species limit themselves according to their densities rather than according to an arbitrary carrying capacity. Finally, this formulation enables the simple conclusion that species coexist when each species limits itself more than it limits other species – i.e. species coexist when niche differences overcome fitness differences and every species can recover from low density (Chesson 2000).

Following the idea of nonadditive diffuse competition, Chesson (1994, 2000) also derived the approximate growth rate of species embedded in multispecies competition.

Multiple species can coexist when the stabilizing term (average niche difference among species) is larger than average fitness inequality (Chesson 2000). Thus, multispecies competition in Chesson (2000) indirectly accounts for nonadditive competition, since weak competitors can have stable coexistence with stronger ones when a third species decreases the average fitness difference within a community (i.e. controlling the negative effect of the stronger species on the weaker one). Chesson (1994, 2000) variation of the Lotka-Volterra therefore accounts for a mechanistic way to model intra and interspecific effect. In this case, coexistence among multiple species is achieved when average niche differences overcome average fitness differences, because diffuse competition would make interspecific effects diluted among coexisting species.

Considerações finais

Realizei meu doutorado na forma de estudos independentes, porém eles seguem e refletem a mudança e evolução dos tópicos investigados, além da mudança do meu próprio pensamento científico.

Dentro da ecologia de comunidades, a consideração de diferentes escalas espaciais é hoje essencial para entender como diferentes processos podem afetar a abundância das espécies (Levin 1992). Até pouco tempo atrás, processos determinísticos e estocásticos eram colocados frente a frente como antagônicos (Smith e Lundholm 2010), onde uma comunidade governada por um, seria menos influenciada pelo outro. O melhor exemplo talvez seja o paradoxo de MacArthur, que teve contribuição fundamental para a Teoria de Biogeografia de Ilhas (MacArthur e Wilson 1967), focada na dispersão e que explica padrões de riqueza sem invocar nenhuma diferenciação entre espécies e a Teoria de Similaridade Limitante (MacArthur e Levins 1967), que invoca diferenças interespecíficas como essenciais para diminuir a interferência competitiva e garantir a manutenção da coexistência estável. Apesar de chamado de paradoxo, MacArthur provavelmente já sabia que a solução do seu pensamento, aparentemente contraditório, era a diferença nas escalas de cada teoria. Enquanto a Biogeografia de Ilhas foca na escala regional, incluindo diversas comunidades, a Similaridade Limitante ocorre na escala local, de cada comunidade individualmente. Aqui nesta tese, observei o mesmo padrão já decifrado por MacArthur 50 anos atrás. No primeiro capítulo concluí que na escala regional de todo o Estado de São Paulo a estrutura filogenética dos insetos aquáticos é influenciada pela dispersão limitada, provavelmente em uma escala de tempo longo, de modo mais evidente do que o controle ambiental. No segundo e terceiro capítulo, na escala de bacias e micro-bacias, observei que insetos aquáticos são

fortemente selecionados pelo ambiente, estruturando os padrões funcionais e também filogenéticos. Isso só confirma o quanto MacArthur estava à frente da sua época. Ele construiu as bases teóricas 50 anos atrás e ainda hoje estamos tentando confirmar suas ideias.

Felizmente, nem tudo o que estamos fazendo (ecólogos de modo geral) é confirmar ideias antigas de grandes mentes. As próprias ideias da Teoria da Similaridade Limitante já não são absolutas (Chesson 2000). Nos capítulos dois, três e quatro tivemos grande interesse na competição como estruturador das comunidades naturais e simuladas. Nossa base conceitual era o framework de montagem de comunidades de Weiher e Keddy (1995), utilizando atributos funcionais e de Webb (2000), utilizando a estrutura filogenética de comunidades. Dentro desse framework, comunidades com menor similaridade em atributos ou parentesco, do que o esperado ao acaso em uma comparação com modelos nulos, seriam consideradas como evidências da similaridade limitante excluindo espécies muito parecidas entre si (Weiher e Keddy 1995, Webb 2000). Porém, no início do meu doutorado a teoria moderna de coexistência começou a ganhar destaque a partir da publicação do trabalho de Mayfield e Levine (2010). Eles destacavam o fato da similaridade limitante já ter sido rebatida pelos mais recentes avanços em teoria ecológica. Usando como base a teoria moderna de coexistência (Chesson 2000), eles indicaram que comunidades compostas por espécies muito similares também poderiam ser a assinatura da exclusão competitiva. Isso porque, se as diferenças fenotípicas aumentam a diferença competitiva das espécies, a competição moldaria comunidades com espécies similares, pois a igualdade na habilidade competitiva de todas as espécies garantiria que nenhuma espécie conseguisse excluir a outra (Mayfield e Levine 2010). Porém, se a diferença nas espécies refletisse em diferenças de nicho que favorecessem a diminuição na sobreposição no uso de recursos,

então as comunidades poderiam também ter um padrão de baixa similaridade entre espécies coexistentes. Nos capítulos desta tese sempre considerei a teoria de coexistência na interpretação dos resultados e, como vocês podem notar, foi difícil concluir a importância da competição utilizando o framework de montagem de comunidades nos capítulos dois e três.

Não satisfeito com a investigação observacional utilizando o framework de montagem de comunidades, no capítulo quatro, decidimos simular a competição nos termos da teoria moderna de coexistência (Chesson 2000) para verificar em quais circunstâncias a competição gera padrões de comunidades com espécies similares ou distintas. Graças à sugestão de um revisor também incluímos um novo complicador nas simulações. Enquanto boa parte da teoria de competição é baseada em interações entre duas espécies, na natureza a competição ocorre entre todos os componentes da comunidade. Incluímos essa complexidade e simulamos a competição como difusa, ou seja, a interferência de cada espécie é diluída entre todas as espécies da comunidade. Neste caso, alguns pares “proibidos” de coexistir podem coexistir quando uma terceira espécie dilui o efeito negativo entre elas (Levine 1976). O resultado mais interessante foi que comunidades montadas tinham comumente estruturas funcionais similares daquelas geradas ao acaso, ou seja, não eram nem formadas por espécies muito similares, como exposto por Mayfield e Levine (2010), nem por espécies muito diferentes, como sugerido pelo framework tradicional (Weiher e Keddy 1995). Isso ocorre porque a diluição da competição entre múltiplas espécies relaxa qualquer estruturação nas comunidades. Ou seja, estudos de montagem de comunidades que observaram padrões similares aos modelos nulos e inferiram um fraco efeito da competição, podem estar enviesados pela possibilidade da competição ser difusa nas comunidades estudadas.

No meu entendimento, esta tese contribui para mostrar exemplos do uso do framework de montagem de comunidades e como este possui pouco poder para identificar a estruturação por competição. A filtragem ambiental e a estruturação espacial são mais fáceis de serem identificadas nos estudos observacionais, como mostrado nos capítulos um, dois e três, porém, tendo em mente toda a discussão do capítulo quatro, considero que pouco sabemos sobre o papel da competição na estruturação e manutenção dos padrões em comunidades.

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Artigos adicionais que publiquei durante meu período de doutoramento (2013-2016).

- 1- Perbiche-Neves, G., **Saito, V. S.**, Previattelli, D., da Rocha, C. E., & Nogueira, M. G. (2016). Cyclopoid copepods as bioindicators of eutrophication in reservoirs: Do patterns hold for large spatial extents?. *Ecological Indicators*, 70, 340-347.
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