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**MACROINVERTEBRADOS AQUÁTICOS DA REGIÃO ANDINO- AMAZÔNICA:  
PAISAGEM FLUVIAL, DIVERSIDADE TAXONÔMICA E DIVERSIDADE  
FUNCIONAL**

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FUNCIONAL**

Tese apresentada ao Programa de Pós-graduação em Ecologia e Recursos Naturais, do Centro de Ciências Biológicas e da Saúde, da Universidade Federal de São Carlos, como parte dos requisitos para a obtenção do título de Doutor em Ciências, área de concentração em Ecologia e Recursos Naturais.

Orientador: Profa. Dra. Odete Rocha

Co-orientador: Prof. Dr. Marlon Peláez Rodríguez

SÃO CARLOS – SP

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*Dedicada à memória do meu pai e àqueles que continuam nesta vida comigo, minha mãe, meus irmãos, meus filhos e sobrinhos....*



**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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# 1. Resumo

A região Andino-Amazônica do departamento de Caquetá é conhecida por abrigar uma grande biodiversidade de organismos, tanto terrestres quanto aquáticos. No entanto, nos últimos anos, esta região sofreu diferentes impactos antropogênicos, associados ao desmatamento, evidenciados principalmente nas terras baixas, gerando grande preocupação sobre a perda de conectividade entre a Amazônia colombiana e a cordilheira oriental dos Andes. Isso nos levou a desenvolver um estudo sobre a paisagem fluvial associada a diferentes ecossistemas lóticos, com o propósito de entender sua dinâmica regional e como isso pode influenciar o conjunto de macroinvertebrados aquáticos. Estabelecemos três regiões de estudo: Andes, Transição e Amazônia, definindo em cada uma, dez estações de amostragem. Em cada local de trabalho, caracterizamos os diferentes componentes da paisagem fluvial. Além de isso, coletamos amostras de macroinvertebrados aquáticos, utilizando uma metodologia multihabitat. Para a análise de dados, estimamos a diversidade alfa e beta taxonômica e funcional. Implementamos uma Análise de Redundância, buscando estabelecer variáveis ambientais preditoras sobre a composição e beta diversidade de macroinvertebrados aquáticos, e um modelo linear generalizado, relacionando a riqueza e abundância da ordem Odonata com a altitude, latitude e longitude (escala regional). Nossos resultados mostraram um processo sucessional das diferentes variáveis ambientais, da região dos Andes em direção ao Amazonas, evidenciando também um processo de deterioração ambiental que aumenta das partes altas da região para as terras baixas. Além de isso, as variáveis atuam como filtros ambientais determinantes no estabelecimento da assembleia de macroinvertebrados aquáticos e ordem Ephemeroptera em uma escala local, e a partir de uma análise regional, na ordem Odonata, que também é um grupo bioindicador de fragmentação de floresta ribeirinha. Nossos resultados fornecem informações valiosas sobre a dinâmica da paisagem fluvial entre os Andes e a Amazonia de Caquetá. Além disso, foram feitos novos registros taxonômicos e estimativas de diversidade que podem ser muito importantes para o uso e manejo adequado dos recursos naturais na região, sendo um referencial futuro para uma análise de vulnerabilidade relacionada à perda de conectividade entre as regiões de estudo.

**Palavras-chave:** alfa diversidade, beta diversidade, composição, filtros ambientais, conservação.

## 2. General abstract

The Andean-Amazonian region of the Caquetá department is renowned for hosting a vast biodiversity of terrestrial and aquatic organisms. However, in recent years, this region has experienced various anthropogenic impacts, primarily associated with deforestation, most evident in the lowlands. Additionally, this has led to significant concerns over the loss of connectivity between the Colombian Amazon and the eastern Andes Mountain range. This prompted us to study the riverscape associated with different lotic ecosystems to understand their regional dynamics and how this may influence the assembly of aquatic macroinvertebrates. We established three study zones: the Andes, the Transition, and the Amazonia, defining ten sampling stations. At each study site, we characterized the different components of the riverscape. In addition, we collected samples of aquatic macroinvertebrates using a multi-habitat methodology. For data analysis, we estimated taxonomic and functional alpha and beta diversity. We implemented a Redundancy Analysis to identify environmental variables that predict the composition and beta diversity of aquatic macroinvertebrates, and we developed a generalized linear model linking the richness and abundance of the Odonata order to altitude, latitude, and longitude (regional scale). Our results revealed a successional process of various environmental variables from the Andean region towards the Amazon, also highlighting a pattern of environmental deterioration that increases from the higher parts of the region to the lowlands. Moreover, these variables act as crucial environmental filters in the establishment of the community of aquatic macroinvertebrates and the Ephemeroptera order on a local scale and, from a regional perspective, in the Odonata order, which also serves as a bioindicator group for the fragmentation of riparian forests. Our findings provide valuable insights into the dynamics of the riverscape between the Andes and the Amazonia in Caquetá. Additionally, we have established new taxonomic records and diversity estimates, which could be vital for adequately using and managing natural resources in the region. These will serve as a future reference for vulnerability analysis related to the loss of connectivity between the study areas.

**Keywords:** alpha diversity, beta diversity, composition, environmental filters, conservation.

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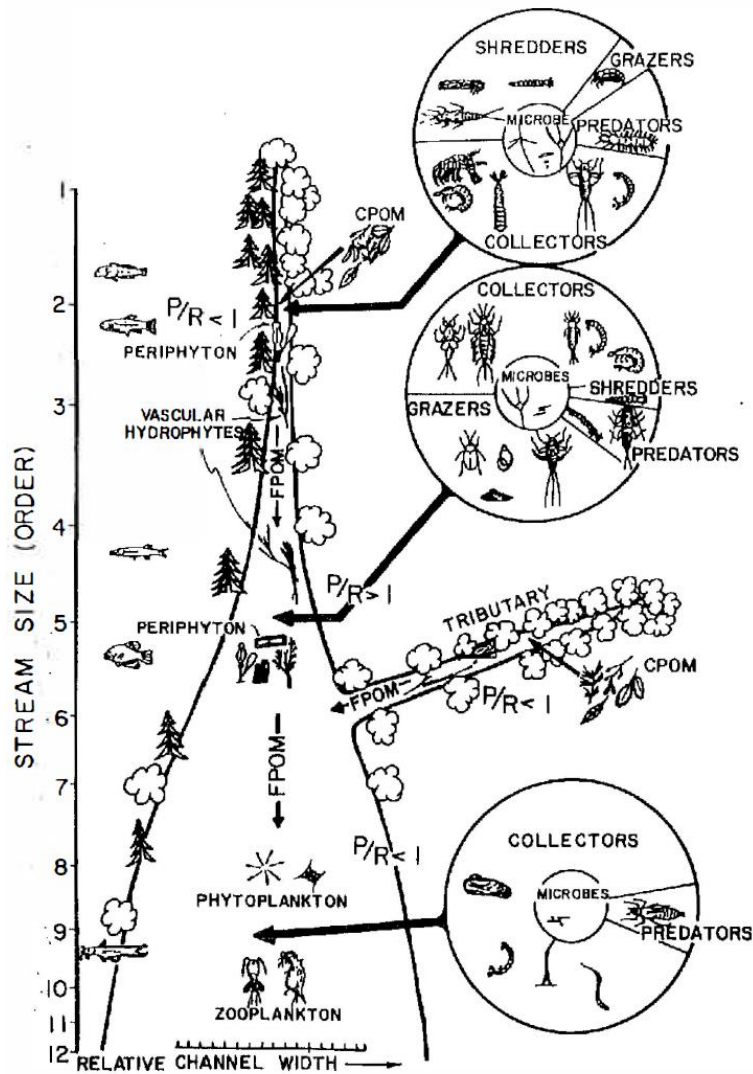
April 2022 in the Andean-Amazonian region, Caquetá, Colombia. dense forest (B.D), fragmented forest with secondary vegetation (B.F.V.S), secondary vegetation (V.S), grasslands (P.S), and miscellaneous with pastures and natural spaces (M.P.E.N).....171

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## 5. Introdução geral

A paisagem fluvial pode ser definida como a zona de interação entre elementos abióticos e bióticos, incluindo o ser humano, que dá origem a habitats dinâmicos e ecossistemas produtivos, integrando os componentes terrestres e aquáticos em um sistema coesivo (Torgersen et al., 2022; Graziano et al., 2022). No entanto, essas interações têm sido drasticamente modificadas devido a diversos fatores antropogênicos, como urbanização (Ren et al., 2014; Khirsachmansyah et al., 2020; Xu et al., 2022), agricultura intensiva (Gagliardi et al., 2013; Li et al., 2015; Foubert et al., 2020), pecuária extensiva (Bichsel et al., 2016; Lasanta et al., 2016) e industrialização (Sarker et al., 2021), entre outros. Além disso, as mudanças no uso e ocupação da terra tiveram consequências significativas na estrutura e função dos ecossistemas, afetando os serviços ecossistêmicos e a biodiversidade (Tschardt et al., 2005; Rey & Bullock, 2012; Su et al., 2012; Firbank et al., 2013; McDonald et al., 2013; Duru et al., 2015; Landis, 2016; Liu et al., 2019).

Existem diferentes conceitos ecológicos propostos que nos aproximam do entendimento da dinâmica da paisagem fluvial. Vannote et al. (1980) unificam diferentes trabalhos, estabelecendo o conceito do Rio Contínuo, como uma teoria geral para compreender sua dinâmica e evolução (**Figura 1**). Eles indicam que, sob uma perspectiva longitudinal, de águas de nascente ou cabeceira para águas de desembocadura, os diferentes componentes do rio mudam, como por exemplo, nas partes altas o substrato dominante é de origem rochosa (blocos, pedras), e nas partes baixas muda para substratos macios (lodos, argilas) ou, se analisado a partir dos grupos funcionais de macroinvertebrados aquáticos, na parte alta e média existe uma maior variedade de categorias funcionais (raspadores, coletores, predadores, trituradores) enquanto que na parte baixa estas são reduzidas (predadores, coletores).



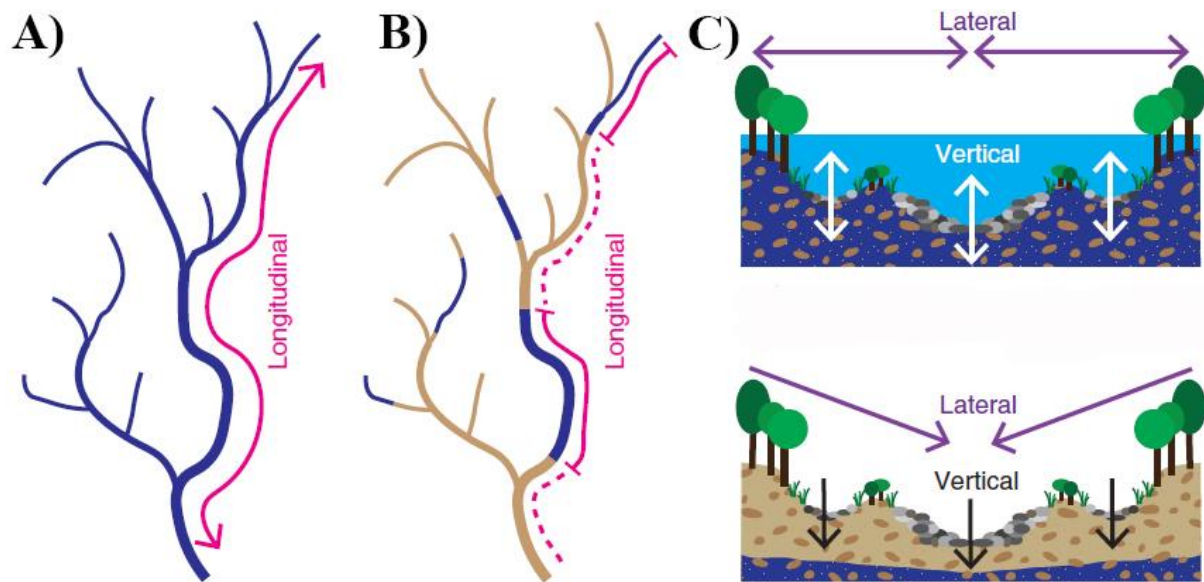
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No entanto, o conceito do Rio Contínuo tem sido debatido ao longo do tempo, propondo-se novas abordagens, como por exemplo a proposta de Poole (2002) que mostra que os rios apresentam uma descontinuidade em sua dinâmica e evolução, definida por segmentos geológicos alternados, fazendo com que cada rio seja um sistema único e irregular desde a nascente até a foz (hierarquia dinâmica de manchas) (**Figura 2**). Estabelecendo assim, um esquema conceitual que ressalta a importância ecológica de estudar individualmente cada rio, para entender as transições dos diferentes componentes da paisagem fluvial, não só em uma escala longitudinal, mas também lateral e vertical.



**Figura 2.** Ecossistemas lóticos de terras baixas, com altitudes menores que 250 m.a.n.m. Associados ao afloramento rochoso do escudo da Guiana (A, B, retirado de González-Trujillo, 2020), estação 18 associada à região de transição (C).

Focando-se no estudo da paisagem fluvial desde uma escala regional, existem propostas de análise, como por exemplo a de Lee et al. (2004), que propõem estudar a relação entre as redes fluviais hierárquicas e ramificadas com as diferentes perturbações estocásticas que se apresentam na dinâmica da bacia, e as interações que definem a organização espacial e temporal dos diferentes habitats fluviais. Além disso, seguindo esta proposta, o trabalho de Tonkin et al. (2018) propõe que os rios estão organizados espacialmente em redes dendríticas hierárquicas que podem ser determinantes na estruturação da biodiversidade e sua dispersão.



**Figura 3.** Comparação entre os três conceitos relacionados à paisagem fluvial. Conceito do rio contínuo (A), conceito do rio descontínuo e rede de drenagem (B), influência lateral e vertical do conceito do rio descontínuo (C). Modificado de Allen et al. (2020).

Esses três conceitos são considerados suportes-chave para entender a dinâmica da paisagem fluvial na região Andino-Amazônica do departamento de Caquetá. No entanto, é necessário integrar elementos locais ambientais que permitam identificar e, ao mesmo tempo, diferenciar as regiões de estudo, a partir do registro de informações abióticas, isto é variáveis ambientais como o pH, condutividade, temperatura, oxigênio dissolvido (Álvarez-Cabria et al., 2016; Yung-Chul et al., 2016; Kuzmanovic et al., 2017), composição do substrato (Vannote et al. 1980; Poole, 2002; Mishra & Nautiyal, 2016) ou cobertura vegetal (Strayer et al., 2003; Rios & Bailey, 2006; Wasson et al., 2010). Estas variáveis, entre outras, permitem diagnosticar as condições de qualidade da água que apresenta um ecossistema lótico, e relacioná-las com atividades antrópicas (Allan, 2004; Stammel et al., 2021), ou a influência geológica como determinantes nas características da paisagem fluvial (González-Trujillo et al., 2020). No entanto, essas variáveis também podem ser analisadas como filtros em uma escala de micro-habitat que podem ser determinantes no estabelecimento de um conjunto biológico (Poff, 1997; Colzani et al., 2013), sendo um componente ecológico explicativo importante para entender a composição e diversidade presente em qualquer estação de estudo.

Além disso, retomando a escala de análise regional, variáveis como altitude, latitude ou longitude podem ser relacionadas com processos de dispersão de espécies (González-Trujillo et al., 2019) ou valores de diversidade biológica (Hof et al., 2008; Al-Samhi et al., 2013; Heino et al., 2018). Por exemplo, Jacobsen (2003, 2004), Kubendran et al. (2017) e Castro et al. (2019), encontram uma diminuição na riqueza de espécies à medida que o gradiente altitudinal aumentou, resultados que podem ser relacionados com o efeito Rapoport proposto por Steven (1992), que também utiliza o gradiente latitudinal para explicar essa relação inversa com a diversidade biológica.

O conceito de diversidade biológica pode ser analisado em diferentes escalas, incluindo a genética, funcional, taxonômica, filogenética e ecossistêmica (Gotelli & Chao, 2013). Focando em duas dessas escalas, a diversidade taxonômica está centrada em analisar o número de espécies em uma área de estudo e como se distribuem considerando diferentes categorias taxonômicas (Magurran, 2004). Por outro lado, a diversidade funcional estuda a variabilidade e abundância de traços funcionais presentes em uma comunidade biológica (Violle et al., 2007; Biswas et al., 2015; Gross et al., 2017; Dawson et al., 2021), os quais podem ser características morfológicas, fisiológicas, fenológicas ou relacionadas à alimentação.

Whittaker (1972) propõe três medidas para sua avaliação: a diversidade alfa ( $\alpha$ -diversidade) que se relaciona com uma escala local, a diversidade beta ( $\beta$ -diversidade) que analisa a similaridade ou dissimilaridade entre estações, comunidades ou pontos de interesse, e a diversidade gama ( $\gamma$ -diversidade) que se foca em uma análise regional da diversidade. Focado taxonomicamente, para a diversidade alfa é importante estimar a diversidade efetiva ou números de Hill (Jost, 2006), e com relação à diversidade beta, identificar e analisar qual dos componentes, seja substituição ou aninhamento (Baselga, 2010) sobressai em um rio, bacia ou região de estudo. Igualmente, na diversidade beta, podemos identificar as contribuições locais de estações de amostragem (LCBD) e contribuição de espécies (SCBD), como insumos de análise para propostas de priorização ou conservação (Legendre et al., 2013; Valente-Neto et al., 2020), para o uso e manejo dos recursos naturais.

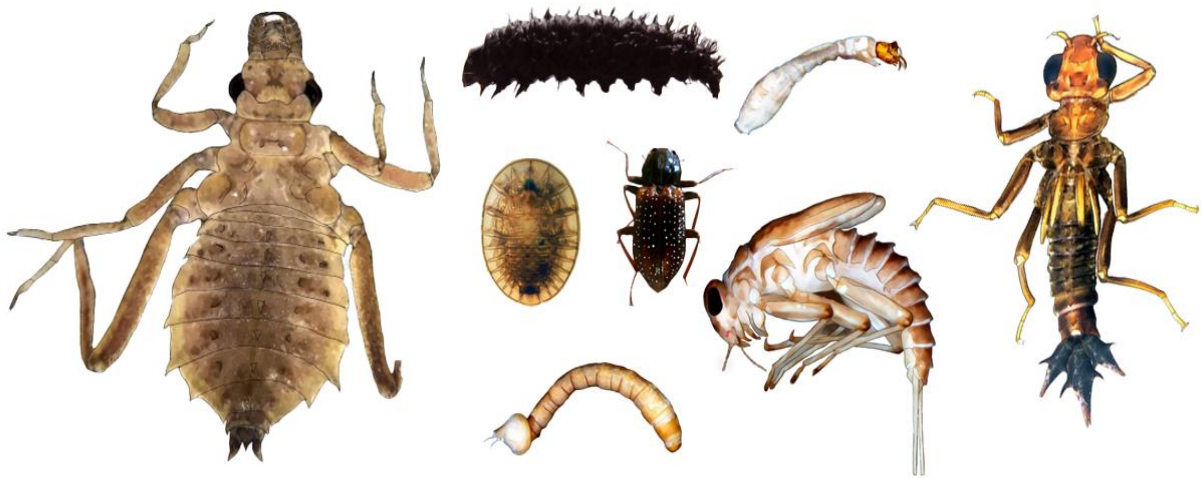
Ao integrar todos esses elementos no estudo da dinâmica da paisagem fluvial, podemos esquematizar uma proposta de uso e manejo adequado dos recursos naturais, integrando um novo elemento que se relaciona com a história evolutiva de um rio. Nesse aspecto, pode-se destacar o trabalho de Brierley & Fryirs (2022) que propõem quatro verdades a considerar na paisagem fluvial. A primeira fala sobre o respeito à natureza, o qual é um elemento fundamental, onde o ser humano deve se reconhecer dentro dessa matriz e não fora dela, como propõem Maturana & Varela (1972). A segunda enfatiza nas práticas de gestão sobre os recursos hídricos. A terceira enfatiza em estudar mais as causas que os efeitos em um processo de degradação ambiental. E a quarta analisa a história evolutiva da paisagem fluvial, considerando que esta última pode se apoiar a partir da caracterização dos diferentes componentes ambientais.

Para poder relacionar os componentes biológicos com o físico, químico, antrópico, geológico entre outros fatores da paisagem fluvial, é necessário focar no estudo das diferentes comunidades aquáticas. Entre elas, pode-se mencionar as bactérias, fungos filamentosos, algas, macrófitas aquáticas, peixes e macroinvertebrados aquáticos (Tundisi & Tundisi, 2008; Roldán & Ramírez, 2022), as quais desempenham um papel importante dentro do metabolismo dos rios.

No contexto das comunidades mencionadas anteriormente, neste estudo focamos nos macroinvertebrados aquáticos, que segundo Roldán & Ramírez (2008), são identificados como aqueles indivíduos com um tamanho maior que 0,5 mm de comprimento. Esses organismos desempenham um papel crucial na dinâmica dos ecossistemas, seja como produtores secundários ou consumidores primários (Allan & Castillo, 2007; Tamaris-Turizo, 2018), e são abundantes em qualquer ecossistema lótico ou lêntico (Tamaris-Turizo, 2018), ocupando uma grande variedade de micro-habitats disponíveis (Gutiérrez et al., 2014). Além disso, muitos dos táxons são utilizados como organismos bioindicadores da qualidade biológica da água (Roldán, 1996), ou podem ser indicadores de fragmentação vegetal ribeirinha como a ordem Odonata (de Paiva et al., 2010; da Silva et al., 2013; Berquier et al., 2016).

Quanto às categorias taxonômicas, os macroinvertebrados aquáticos em águas doces podem ser representantes dos filos Porifera, Cnidaria, Mollusca, Annelida, Nematomorpha, Platyhelminthes e Arthropoda (Roldán & Ramírez, 2008), sendo este último o que apresenta a

maior riqueza de táxons, em especial da classe Insecta sendo na maioria a estagios imaturos, com exceção de alguns gêneros de Coleoptera (por exemplo, *Macrelmis* spp., *Heterelmis* spp., *Gyretes* spp., *Derallus* spp., *Laccophilus* spp.; *Driops* spp.) ou Hemiptera (por exemplo, *Ambrysus* spp., *Limnocoris* spp., *Buenoa* spp., *Martarega* spp.) que passam toda a sua vida no ecossistema aquático (Domínguez & Fernández, 2009; González-Córdoba et al., 2020) (**Figura 4**).



**Figura 4.** Ilustração de alguns dos macroinvertebrados aquáticos coletados na região Andino-Amazonica durante as campanhas de amostragem, 2021 e 2022. Autor: Gutiérrez-Garaviz (2024).

## 5.1 Objetivos e estrutura da tese

### Objetivo Geral

Caracterizar a composição, diversidade taxonômica e diversidade funcional de macroinvertebrados aquáticos presentes em diferentes condições da paisagem fluvial na região Andino-Amazônica do departamento do Caquetá.

### Objetivos específicos

- Analisar a dinâmica da paisagem fluvial em diferentes ecossistemas lóticos associados à região Andino-Amazônica do departamento de Caquetá, Colômbia.
- Identificar a composição e diversidade da assembleia de macroinvertebrados aquáticos associados à região Andino- Amazônica.
- Relacionar as condições ambientais da paisagem fluvial com o estabelecimento da assembleia de macroinvertebrados aquáticos em escalas local e regional.

### Estrutura da tese

Esta tese está estruturada em quatro capítulos:

O primeiro capítulo intitula-se “**Riverscape dynamics in the Andean-Amazon Region, Caquetá, Colombia: Key elements for effective environmental management**”. Nossa hipótese de pesquisa indica que as condições ambientais da paisagem fluvial apresentam um processo sucessional regional desde a cordilheira dos Andes até a região Amazônica, apresentando também um aumento na degradação da paisagem das terras altas para as terras

baixas. Foram avaliadas variáveis físicas, químicas e componentes de paisagem, incluindo atividades antrópicas como o uso do solo. Além disso, essa caracterização permitiu identificar e integrar elementos para a elaboração de uma proposta esquemática que levaria a duas rotas de manejo ambiental em escala regional, a partir de um cenário rural.

O segundo capítulo é intitulado **“Taxonomic and functional diversity of aquatic macroinvertebrates under different environmental conditions in the Andean-Amazonian region, Caquetá-Colombia”**. Nossa hipótese de pesquisa propõe que entre as regiões existe uma diferenciação quanto à composição, diversidade taxonômica e diversidade funcional de macroinvertebrados aquáticos, com influência de filtros ambientais. Para isso, determinou-se a composição de macroinvertebrados aquáticos, calcularam-se índices de diversidade taxonômica e diversidade funcional. Posteriormente, a informação biológica foi relacionada com as variáveis físicas, químicas, componentes de paisagem, além do uso do solo, buscando fatores preditoras que podem influenciar sobre o estabelecimento de macroinvertebrados aquáticos na região Andino-Amazônica.

No terceiro e quarto capítulo, os estudos foram focados em duas ordens de interesse, Ephemeroptera e Odonata, buscando limitar a variabilidade na informação e ver as respostas que têm em relação aos objetivos específicos.

O terceiro capítulo se intitula **“Diversity and Distribution of Ephemeroptera along the Colombia Andes-Amazonian Transition”**. Nossa hipótese de pesquisa indica que existe uma substituição de táxons entre a cordilheira dos Andes e a planície Amazônica, influenciada por processos sucessionais das variáveis ambientais. Foram estimados índices de diversidade alfa, beta, além da influência que as variáveis ambientais locais podem ter sobre a composição e diversidade beta da ordem Ephemeroptera. Também foram determinadas as contribuições locais e de espécie à beta diversidade como referência para pesquisas futuras.

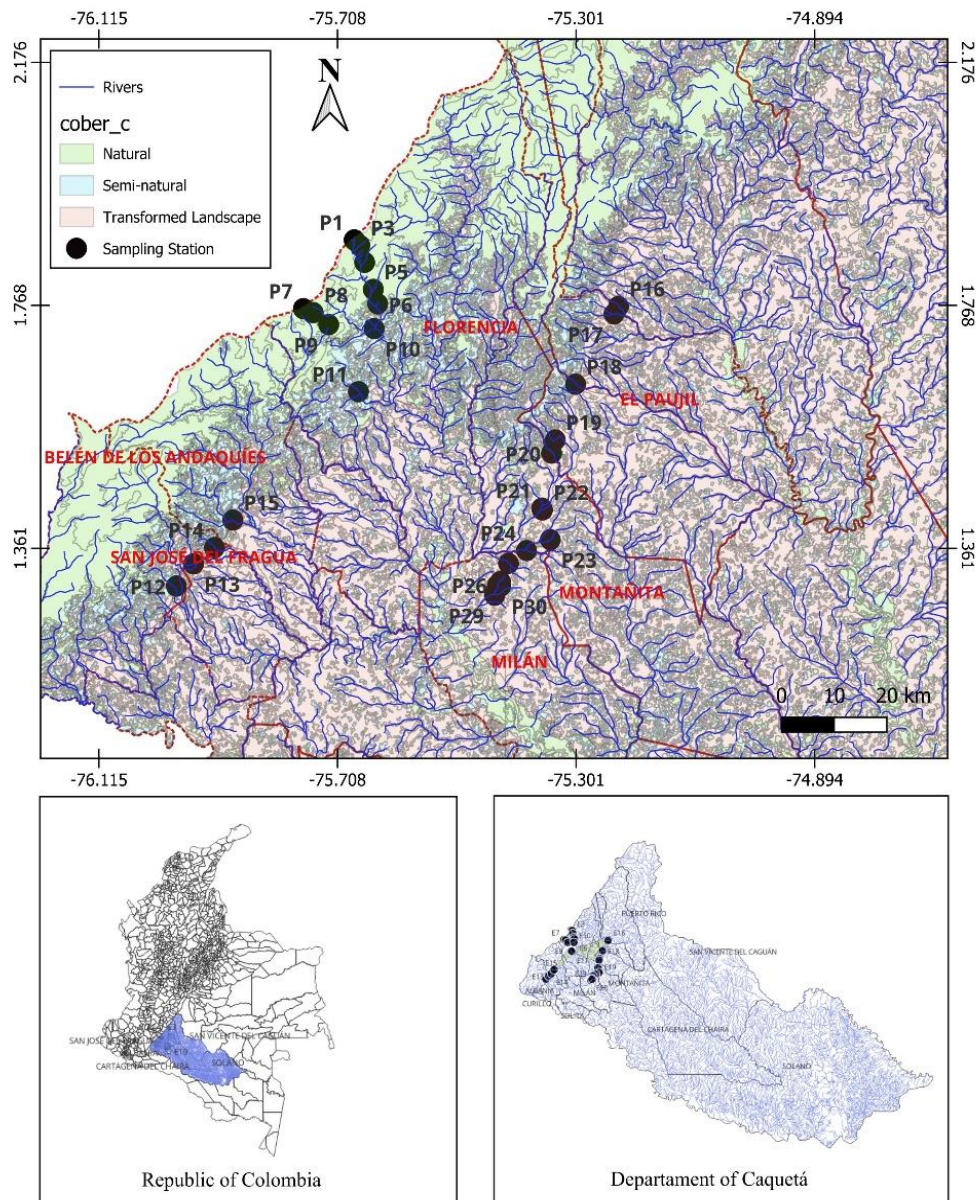
O quarto capítulo se intitula **“Odonata (Insecta) in the Andean-Amazonian Corridor, Caquetá-Colombia: Composition, Richness, and Regional Distribution”**. Nossa hipótese de pesquisa indica que a altitude, latitude e longitude são fatores determinantes na composição e

riqueza taxonômica para a ordem Odonata na região Andino-Amazônica. Foram estabelecidos os intervalos altitudinais de distribuição dos diferentes táxons de Odonata, e suas relações com as abundâncias, riqueza taxonômica com altitude, latitude, longitude, além da cobertura vegetal.

## **5.2 Descrição da área de estudo e amostragem**

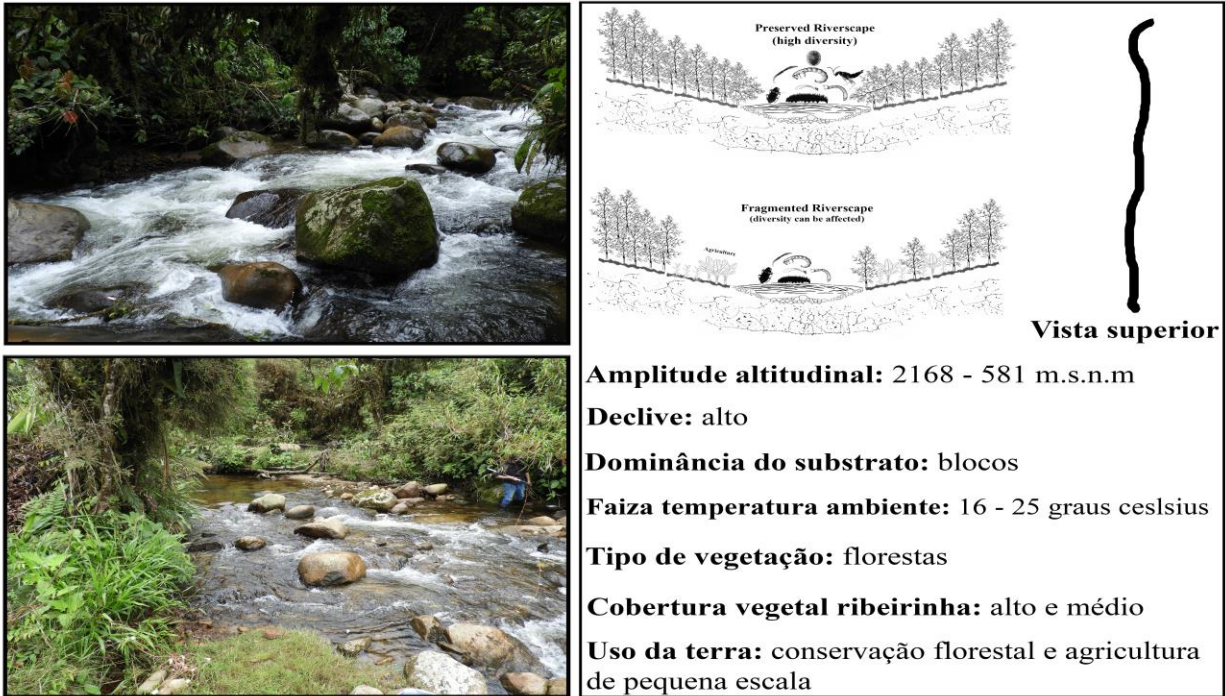
### *Área de estudo*

A área de estudo localiza-se no corredor Andino-Amazônico do departamento de Caquetá, abrangendo os municípios de Florencia, Belén de los Andaquíes, San José del Fragua, El Doncello, El Paujil, La Montañita e Milán (**Figura 5**). A zona Andina é composta por coberturas vegetais dominadas por florestas naturais, além de pequenas atividades agrícolas. À medida que diminui a altitude, a degradação da paisagem fluvial aumenta, influenciada principalmente pela atividade pecuária extensiva. No **Tabela S1** do material suplementar estão listadas as coordenadas geográficas para cada estação de amostragem.

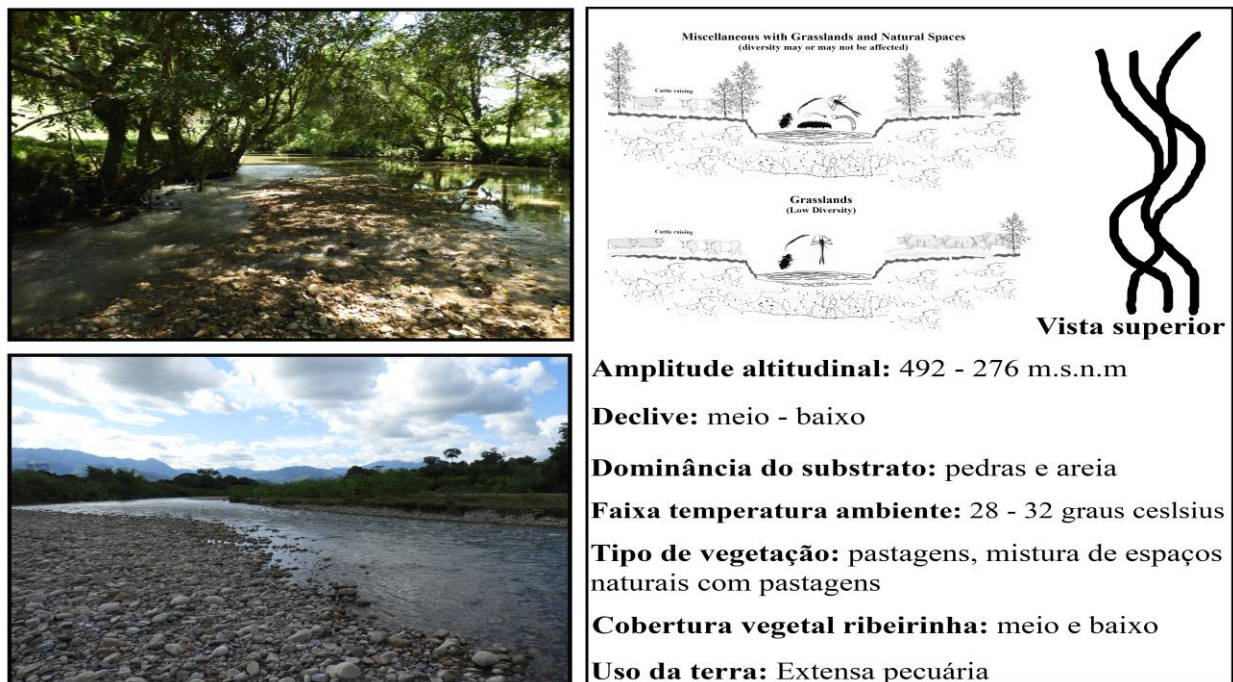


**Figura 5.** Localização das 30 estações de amostragem associadas às três regiões deste estudo (Andes, Transição, Amazônia). A caracterização dos diferentes componentes da paisagem fluvial foi realizada no período de outubro de 2021 a abril de 2022. Fonte: elaborado no Software QGIS v 3.32.3.

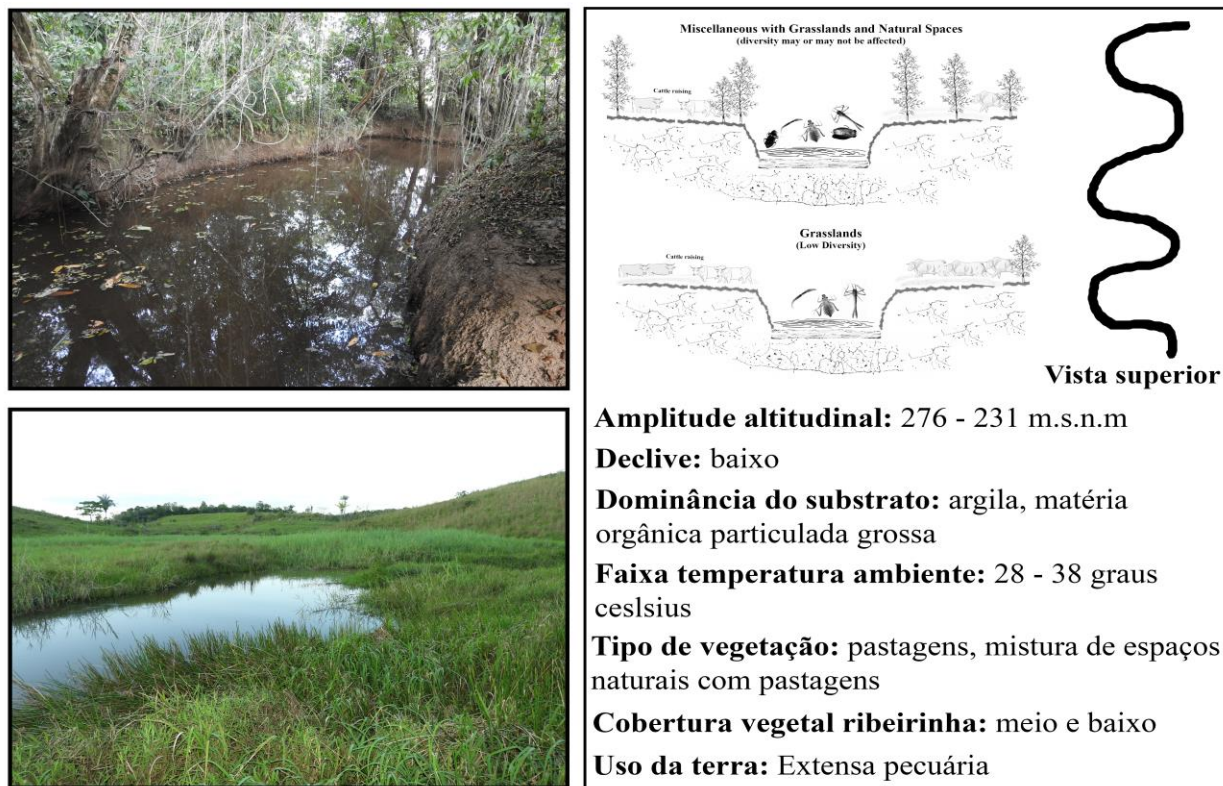
A seguir, descrevemos as três regiões de estudos definidas (Andes, Transição e Amazônia), em ordem altitudinal decrescente:



**Figura 6.** Descrição geral das estações de amostragem associadas à região dos Andes. Autor Gutiérrez-Garaviz (2024).



**Figura 7.** Descrição geral das estações de amostragem associadas à região de Transição. Autor Gutiérrez-Garaviz (2024).



**Figura 8.** Descrição geral das estações de amostragem associadas à região Amazônia. Autor Gutiérrez-Garaviz (2024).

### ***Amostragem***

Foram realizadas quatro campanhas de campo bimestrais (outubro e dezembro de 2021, fevereiro e abril de 2022), sobre 30 estações de amostragem. Para a coleta de dados biológicos, implementou-se a metodologia de amostragem composta, multihabitat para macroinvertebrados aquáticos (Roldán & Ramírez, 2008; Rodríguez-Capítulo et al., 2009; Wantzen & Rueda-Delgado, 2009), utilizando uma rede Surber com uma abertura de 0,3x0,3 metros e uma malha de 250 micra. Licença de coleta, resolução 01216, de 31 de julho de 2018 (ANLA), concedida à Universidade da Amazônia, que integra o Grupo de Pesquisa Qualidade e Preservação de Ecossistemas Aquáticos (CAPREA).

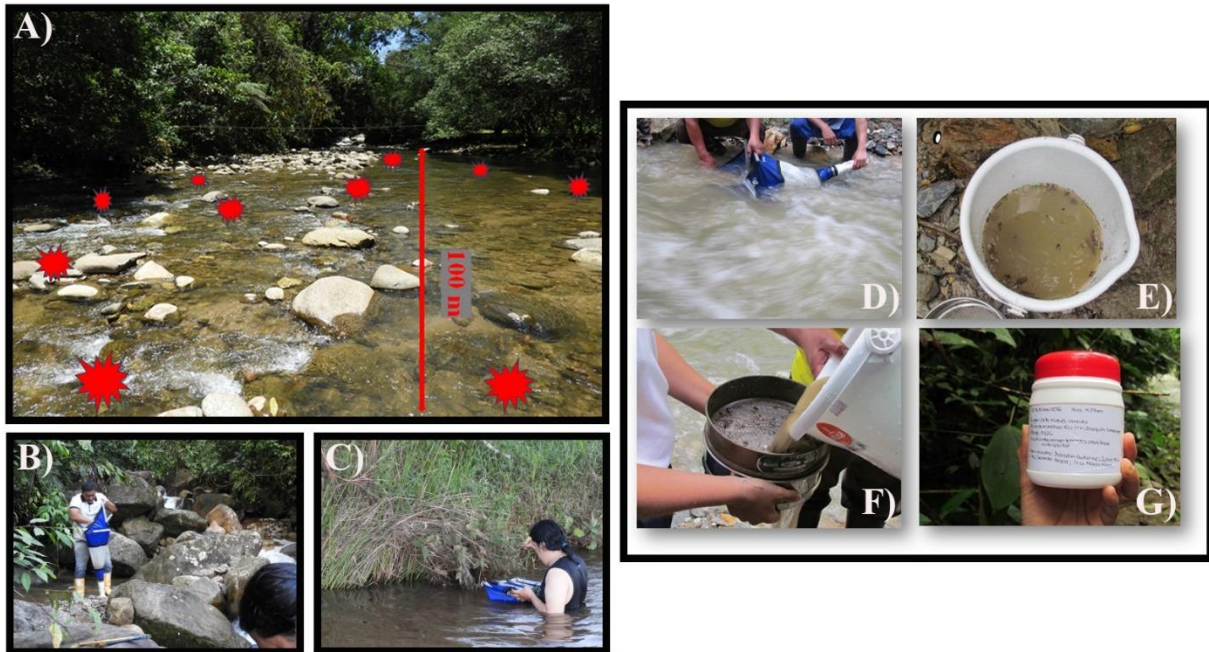
As seções de amostragem tiveram um comprimento de 100 metros e foram realizadas dez coletas contra a corrente, totalizando uma área de coleta de 0,9 m<sup>2</sup> em cada local (cinco varreduras sobre o substrato dominante, duas sobre o segundo substrato e três sobre o substrato

restante disponível). Posteriormente, homogeneizamos as amostras e embalamos todo o material coletado em garrafas plásticas de boca larga de 500 mililitros, fixadas com álcool a 75%. É importante destacar que, com base na experiência adquirida na área de estudo, para a região Amazônica, a maior parte das capturas é realizada sobre substratos associados a raízes, macrófitas aquáticas e material vegetal, que podem abrigar uma maior composição de organismos, em contraste com o leito do rio onde predominam as argilas (**Figura 9**).

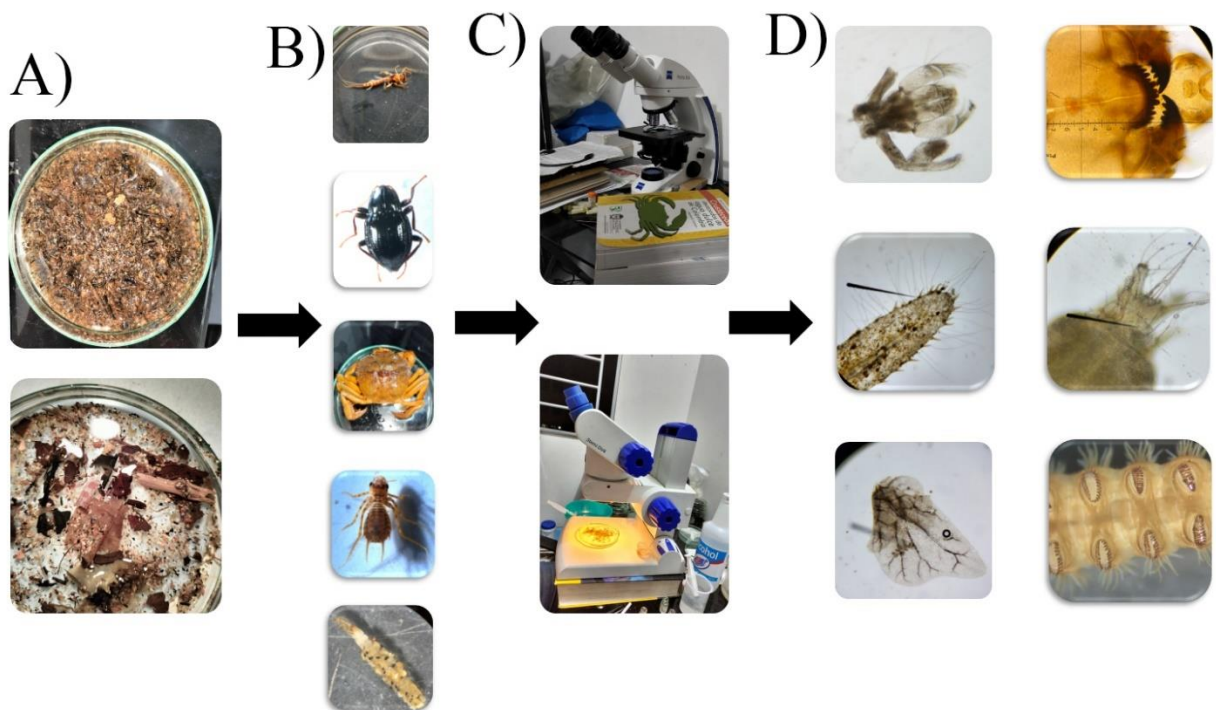
Utilizamos um estereoscópio Zeiss Stemi DV4 e um microscópio Zeiss Primo Star para classificar e identificar o material coletado (**Figura 10**) com base nos guias e chaves taxonômicas de Merritt et al. (2008), Domínguez & Fernández (2009), Hamada et al. (2014), Hamada et al. (2018), Roldán (1996), Springer et al. (2010), and Trivinho-Strixino (2011). Para a família Chironomidae, os indivíduos foram dissecados para montagem em lâmina (Trivinho, 2011), separando-se a cápsula cefálica do corpo. Posteriormente, foram fixados em uma lamínula com Hoyer, deixando secar por oito dias à temperatura ambiente. Após esse período, as bordas das montagens foram seladas com Euparal.

Foram visualmente estimadas a porcentagem de cobertura vegetal ribeirinha (com suporte de informações geográficas), a porcentagem de sombra, o tipo de vegetação (árvores, arbustos, gramíneas e plantas herbáceas) e a atividade de uso do solo (conservação de florestas, agricultura, recreação, assentamentos e pastoreio). Além disso, determinamos a composição do substrato considerando as seguintes categorias: rochas arredondadas (>25 cm de diâmetro), pedregulhos (6 cm-25 cm), cascalho (2 cm-6 cm), areia (0,06 cm-2 cm), lodo (<0.06 cm), argila (<0.06 cm) e matéria orgânica particulada grossa. Por fim, avaliamos a dinâmica do rio, estabelecendo a porcentagem de corredeiras, fluxo e poços.

Quanto às variáveis físicas e químicas, foram medidos os valores de oxigênio dissolvido ( $\text{mg L}^{-1}$ ), potencial de íons de hidrogênio (pH), condutividade ( $\text{uS cm}^{-1}$ ), sólidos dissolvidos totais (ppm) e temperatura da água ( $^{\circ}\text{C}$ ) usando uma sonda multiparamétrica profissional AZ86031. Além disso, medimos a largura da superfície em cada estação de amostragem e foram medidas a velocidade e profundidade do fluxo utilizando um fluxômetro da Global Water.



**Figura 9.** Protocolo de amostragem multihabitat para coleta de macroinvertebrados aquáticos, implementado durante as jornadas de campo, de outubro de 2021 a abril de 2022. Trecho longitudinal (A), coleta de amostras biológicas (B, C, D), homogeneização de arrastos (E), filtragem da amostra homogeneizada (F), embalagem da amostra (G). Autor Gutiérrez-Garaviz (2024).



**Figura 10.** Vista geral de atividades de laboratório relacionadas aos macroinvertebrados aquáticos coletados de outubro de 2021 a abril de 2022. Separação de indivíduos em material coletado (A), indivíduos separados para identificação (B), equipamentos utilizados para identificação (C), características morfológicas utilizadas para identificação (D). autor Gutiérrez-Garaviz (2024).

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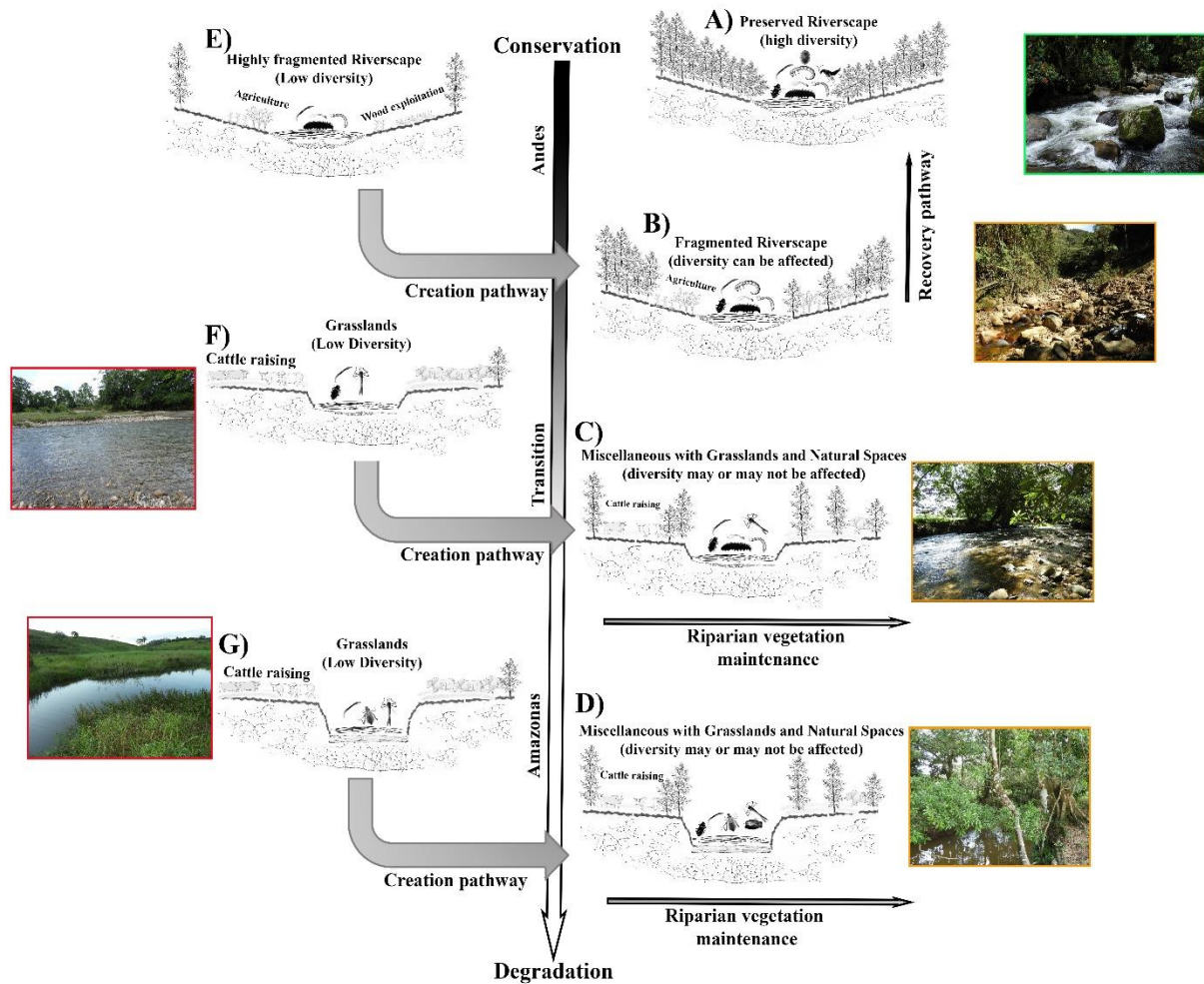
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## Material suplementar

**Tabela S1.** Informação geográfica detalhada em relação às estações em que foi realizado o presente estudo durante as campanhas de amostragem entre 2021 e 2022.

Região	Estação	Altitude	XCorrd	YCoord
Andes	P1	2205	-75,6796	1,8781
Andes	P2	2202	-75,6786	1,8773
Andes	P3	2070	-75,6700	1,8690
Andes	P4	1540	-75,6618	1,8404
Andes	P5	1000	-75,6470	1,7951
Andes	P6	957	-75,6394	1,7708
Andes	P7	2168	-75,7663	1,7627
Andes	P8	2041	-75,7496	1,7562
Andes	P9	1697	-75,7231	1,7359
Andes	P10	581	-75,6456	1,7294
Transição	P11	396	-75,6723	1,6236
Transição	P12	492	-75,9832	1,2977
Transição	P13	321	-75,9538	1,3350
Transição	P14	300	-75,9181	1,3622
Transição	P15	308	-75,8866	1,4083
Transição	P16	285	-75,2284	1,7665
Transição	P17	285	-75,2361	1,7539
Transição	P18	378	-75,3017	1,6362
Transição	P19	293	-75,3363	1,5427
Transição	P20	276	-75,3426	1,5205
Amazônia	P21	247	-75,3594	1,4283
Amazônia	P22	252	-75,3582	1,4253
Amazônia	P23	231	-75,3448	1,3754
Amazônia	P24	237	-75,3853	1,3572
Amazônia	P25	245	-75,4162	1,3358
Amazônia	P26	243	-75,4346	1,2999
Amazônia	P27	233	-75,4300	1,3052
Amazônia	P28	235	-75,4356	1,2954
Amazônia	P29	266	-75,4396	1,2821
Amazônia	P30	234	-75,4295	1,2973

## 6. Capítulo 1: Riverscape dynamics in the Andean-Amazon Region, Caquetá, Colombia: Key elements for effective environmental management



Graphical abstract

## **Riverscape dynamics in the Andean-Amazon Region, Caquetá, Colombia: Key elements for effective environmental management**

### **Abstract**

Regional analysis of riverscapes is key to understanding successional changes linked to different local components. This research aimed to examine the dynamics of the river landscape in various lotic ecosystems of the Andean-Amazon region of Caquetá and to develop a framework based on the historical evolution of rivers to suggest management strategies for using and conserving the river landscape. At each sampling point, multiple parameters were characterized, including vegetation cover, substrate composition, hydrodynamics, pH, electrical conductivity, dissolved oxygen, water temperature, total dissolved solids, and depth. We applied multivariate principal component analysis techniques, box plots, and the nonparametric Kruskal-Wallis test for data analysis. Our results reveal successional patterns in the local environmental variables studied, showing a marked distinction between the Andean and Amazonian regions, reflected in the substrate composition, the riverine vegetation cover, and hydrodynamics. Most physical and chemical variables showed significant differences between the analyzed areas. Regarding management strategies, we recommend that the Andes follow routes of occupation changes and restoration of the river landscape, given its favorable conservation conditions and lower human impact. In contrast, the Transition and Amazon regions, affected by degradation mainly due to livestock farming, require a strategy of change focused on the reconstruction of lotic ecosystems with better conditions, prioritizing the conservation of natural spaces and the implementation of sustainable agricultural practices.

**Keywords:** Physics, Chemistry, Substrate, Altitude, Vegetation Cover, Hydrodynamics

### **Introduction**

We can understand a fluvial landscape as a geographical space where environmental and ecological parameters interact, potentially defining biodiversity, with human activities being one

more of the elements in its configuration. To comprehend its structure and function from a general perspective, we highlight Vannote et al. (1980), who propose that riverine ecosystems exhibit continuous longitudinal changes from the headwaters to the mouth. These changes can be linked to water flow (Larsen et al., 2019), substrate composition (Doretto et al., 2022), organic matter (Roebuck et al., 2019), light availability (Erdozain et al., 2022), functional groups (Doretto et al., 2020; Jacquet et al., 2022; La Ode et al., 2023), vegetation cover, productivity (autotrophic, heterotrophic) (Sánchez-Hernández, 2023), and water quality (Larsen et al., 2019), among others.

However, this concept named as The River Continuity (RCC) has been challenged by Poole (2002), who indicates that fluvial landscapes are defined by the interaction of terrestrial elements (e.g., riparian vegetation) and aquatic elements (e.g., substrate composition in the river channel), proposing a spatially discontinuous configuration (patch) within the river network. Furthermore, in the comprehensive review conducted by Allen et al. (2020), other theories, such as the river wave concept, stream biome gradient concept, or flood pulse concept, are explored to determine which one better fits the study in non-perennial rivers.

Using the information gathered from the study of the riverscape, we can it is possible to establish management and restoration objectives for its future preservation. That encompasses not only an ecological perspective but also considers social, cultural, and aesthetic viewpoints (Berrocal & Molina, 2014; Sabater & Elosegui, 2014; Dufour et al., 2015; Molina et al., 2020; Graziano et al., 2022). This is particularly important in regions such as the Andean-Amazonian corridor of Caquetá, where environmental degradation caused by various human activities may lead to biodiversity loss and deterioration of ecosystem services. Additionally, this region is in a strategic area, which, according to Mesa et al. (2016), is part of the freshwater ecoregions of the Caquetá foothills (Andean region, eastern mountain range) and middle Caquetá (Amazonian region).

With the elements above, we can cite the work of Brierley & Fryirs (2022), who present us with four truths to consider for proper use and management of the river landscape: 1) Respect for nature. 2) Management practices that understand river behavior. 3) River evaluation based on

causes rather than effects. 4) Knowledge of the evolutionary trajectory of a river (river landscape dynamics) to determine what may be achievable within the management of a river system. We believe These truths are necessary to address current government policies in the department of Caquetá, and where we consider this research as a reference, providing elements for the third and fourth truths.

Furthermore, several studies in this region highlight its variety of ecosystems, which contribute to maintaining significant biological diversity in the country (Gutiérrez-Garaviz et al., 2016; Bogotá-Gregory et al., 2020; Castaño et al., 2020; Chica & Espinosa, 2020; Godoy-Losada & Peláez-Rodríguez, 2020; González et al., 2020; Gutiérrez et al., 2020; Marín et al., 2020; Plata-Díaz & Núñez-Avellaneda, 2020); some even consider it a biodiversity hotspot (Atuesta-Dimian et al., 2020). The region has undergone a negative natural transformation due to high deforestation rates (Bautista-Cespedes et al., 2021), affecting terrestrial and aquatic ecosystems.

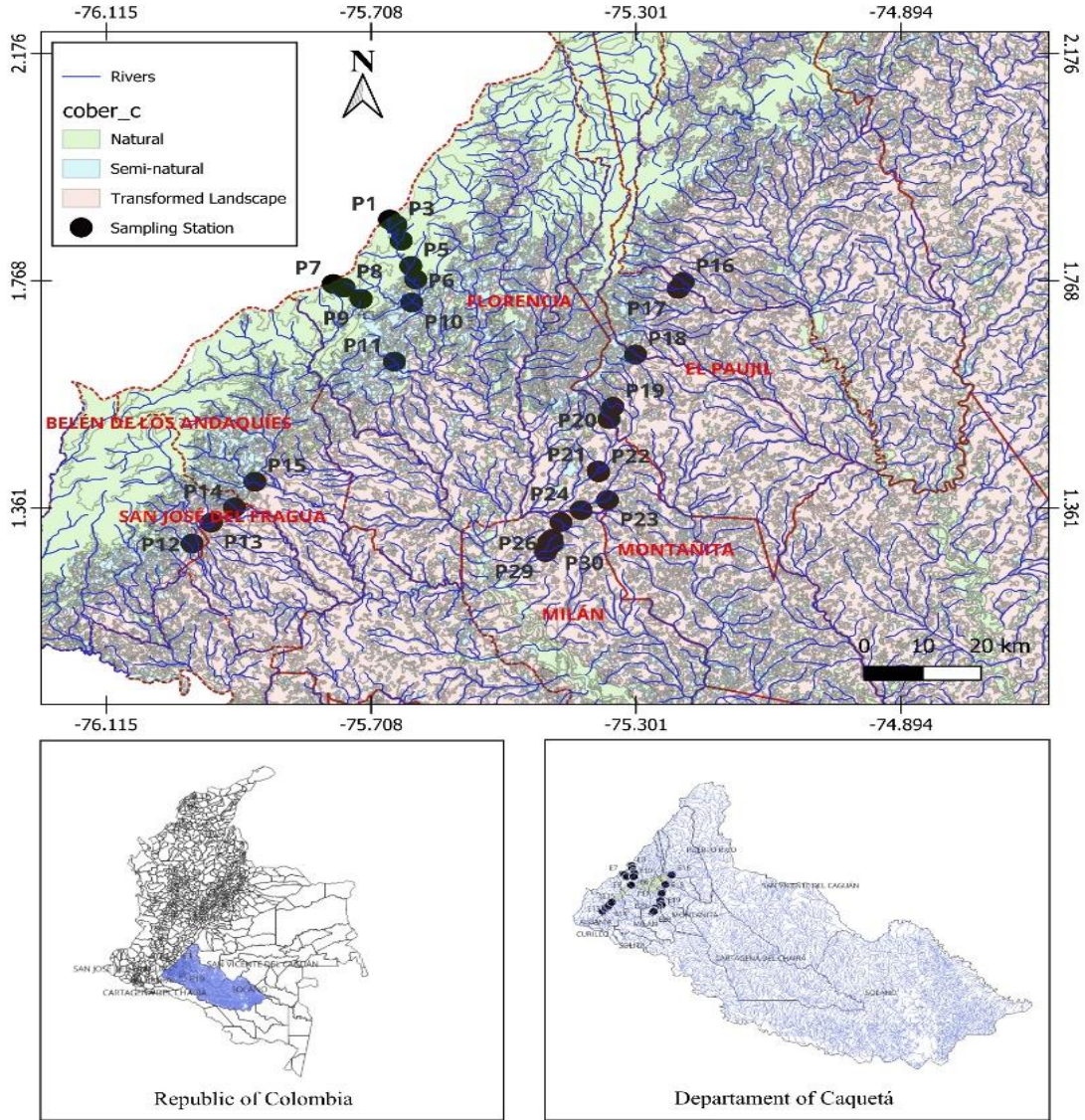
As the main objective of the research, we analyze the dynamics of the riverscape in various lotic ecosystems within the Andean-Amazon region of the department of Caquetá, considering that their local environmental conditions change between regions from the mountain range to the plain.

## **Material and Methods**

### *Study area*

Three sampling regions (Andes, Transition, and Amazon) were defined, considering a coverage map from the SINCHI Institute (Open Data - SINCHI Institute, 2023). In each of them, we selected ten stations randomly, seeking to locate each point in a different water body (**Figure 1**), covering an altitudinal range from 2205 meters above sea level to 231 meters above sea level. In **Table S1** of the supplemental material, we specify geographic coordinates spanning from 1.361°N to -76.115°W and 1.768°N to -75.301°W. Furthermore, for the selection of sampling

stations, we also consider variations in substrate composition, vegetation cover, river hydrodynamics, and physical and chemical properties of water.



**Figure 1.** Distribution of the 30 sampling points in the Andes-Amazon region, Caquetá-Colombia, sampled from October 2021 to April 2022. Source: from the Land Cover of the Colombian Amazon shapefile for the year 2022, Scale: 1:100,000 (Open data - SINCHI Institute, 2023). autor Gutiérrez-Garaviz (2024).

## *Field Phase*

We conducted field samplings bimonthly between October and December 2021 and February and April 2022. For the riverscape variables, we considered the work of Barbour et al. (1999), Elosegui & Sabater (2009), Hauer & Lamberti (2011), and Saito et al. (2020). We visually estimated the percentage of riparian vegetation cover (supported with geographic information), shadow percentage, vegetation type (trees (ARB), shrubs (ARBU), grasses (PA), and herbaceous plants (HER)), and land use activity (forest conservation (BD), agriculture (AGR), recreation (RECR), settlements (ASEN), and grazing (PAST)). Additionally, we determined substrate composition considering the following categories: boulders (BLO.= >25 cm in diameter), cobbles (CANT.= 6 cm-25 cm), gravels (GRAV.= 2 cm-6 cm), sands (ARE.= 0.06mm-2mm), silt (LIM.= <0.06 mm), clay (ARC.= <0.06 mm), and coarse particulate organic matter (MOPG). Finally, we evaluated river dynamics, establishing the percentage of rapids (RA), normal flow (COR), and pools (RE).

Regarding the physical and chemical variables, we recorded the values of dissolved oxygen ( $\text{DO-mg L}^{-1}$ ), hydrogen ionic potential (pH), conductivity ( $\text{CO-uS cm}^{-1}$ ), total dissolved solids (TDS-ppm), and water temperature ( $\text{TE-}^{\circ}\text{C}$ ) using a multiparameter Professional AZ86031 probe. Additionally, we measured the surface width at each sampling station and noted flow velocity and depth using a Global Water flowmeter. **Tables S2** and **S3** list all the information on the different riverscape components characterized in the study region.

## *Data Analysis*

Using the statistical software QGIS v. 3.30.2 (QGIS Development Team 2023) and the geoprocessing tool "Union," a shapefile coverage file from the SINCHI Institute (Open Data - SINCHI Institute, 2023) was overlaid onto the sampling stations. This allowed us to identify five types of coverage associated with the three conditions of the river riverscape: Dense Forest (B.D), Fragmented Forest with Secondary Vegetation (B.F.V.S), Secondary Vegetation (V.S), Grasslands (PS), and Miscellaneous with Grasslands and Natural Spaces (M.P.E.N).

We normalized the data of the variables related to land use, substrate, vegetation cover, and river dynamics using the arcsine function (function=  $\text{asin}(\sqrt{\cdot})$ ), which is suitable for proportional data (Warton & Huin, 2011). Before conducting the multivariate analysis, we applied a Mardia test to validate multiple normality based on the dataset's non-significance of skewness and kurtosis (Székeli & Rizzo, 2005). Subsequently, we utilized the `factoextra` (Kassambara, 2021) and `ggrepel` (Slowikowski, 2021) libraries, along with the base function `prcomp` (R Core Team 2021), to perform a Principal Component Analysis (PCA), relating the variables of the riverscape to the sampling stations. PCA is a multivariate technique that reduces the dimensionality of data by transforming variables into components (Guisande et al., 2011). Next, the riverscape condition data obtained through QGIS was incorporated into the PCA, and the regions were included as categorical variables, allowing for cluster analysis.

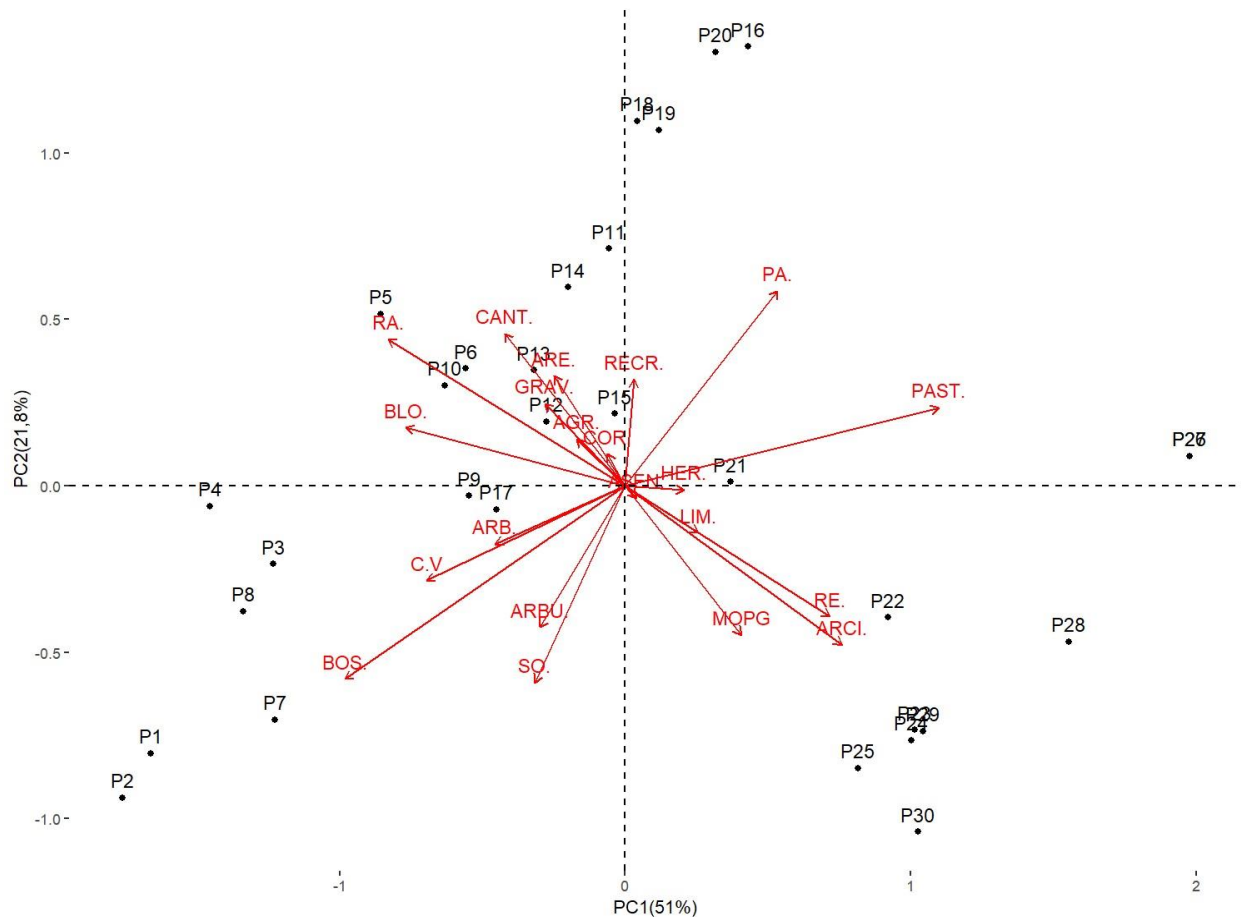
We employed the non-parametric Kruskal-Wallis test, based on the range of observations within each group, with a Chi-squared distribution (Guisande et al., 2011). Subsequently, we conducted a post-hoc Mann-Whitney comparison test (Guisande et al., 2011) with the Holm significance correction method (Dinno, 2015). Additionally, we graphically evaluated the behavior of each variable in the three regions, using the `ggplot2` library (Gregoire, 2021) to create box plots. We performed all the above analyses using the statistical software RStudio version 2023.03.1.

To highlight the importance of characterizing the dynamics of the riverscape in the Andean-Amazon region, we developed a regional-scale analysis framework based on Brierley & Fryirs (2022) proposal, defining potential management pathways. Our framework selected vegetation coverage percentage, land use, and aquatic macroinvertebrate diversity as key variables, considering that the evaluated stations are within a rural riverscape with an agricultural impact matrix.

## **Results**

### ***Substrate, Riparian Vegetation, and River Hydrodynamics***

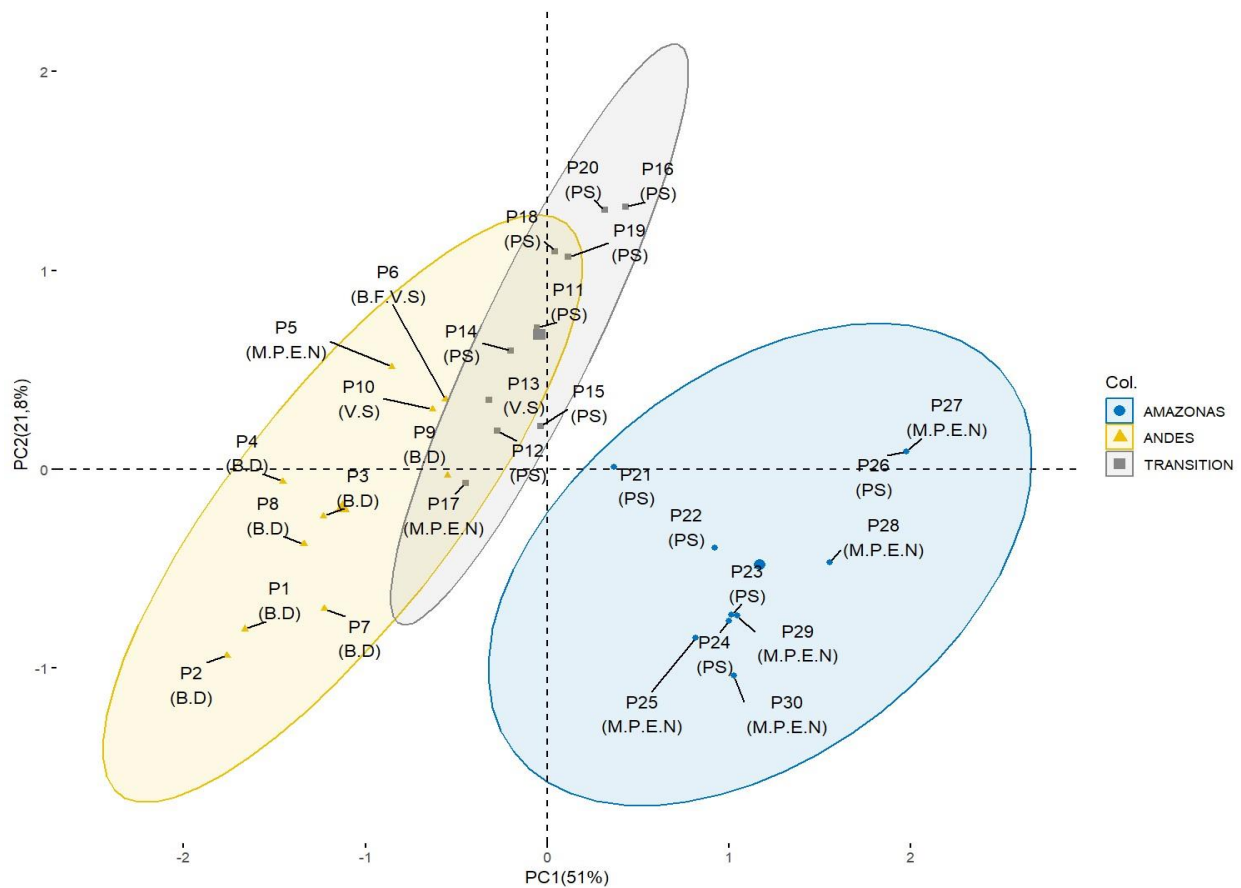
For the first PCA model (**Figure 2**), the first two axes captured 72.8% of the total variance. On the first positive axis (51%), variables such as grass (PAST.= 0.43), clay (ARC.= 0.30), and pools (RE.= 0.28) stood out, present in the stations of the Amazon region (stations P20 to P30). On the same axis, we observed a negative relationship with forest conservation activity (BOS.= -0.38), the percentage of riparian vegetation cover (C.V.= -0.27), and tree vegetation cover (-0.18) present in the higher-altitude stations (stations P1, P2, P3, P4, P7, P8, in the Andean Ecoregion). Additionally, rapids (RA.= -0.32) and boulders (-0.30) also showed an inverse association with this first axis, indicating a relationship with the Andean and Transition regions.



**Figure 2.** Principal Component Analysis (First model), applied to data from streams of the Andean-Amazonian Region of the Caquetá Department, Colombia, sampled from October 2021 to April 2022. Blackheads, stations 1 to 20 (E), red vectors, variables. Trees (ARB), shrubs

(ARBU), grasses (PA), herbaceous plants (HER), forest conservation (BD), agriculture (AGR), recreation (RECR), settlements (ASEN), grazing (PAST), boulders (BLO), cobbles (CANT), gravels (GRAV), sands (ARE), silt (LIM), clay (ARC), coarse particulate organic matter (MOPG), rapids (RA), normal flow (COR), pools (RE).

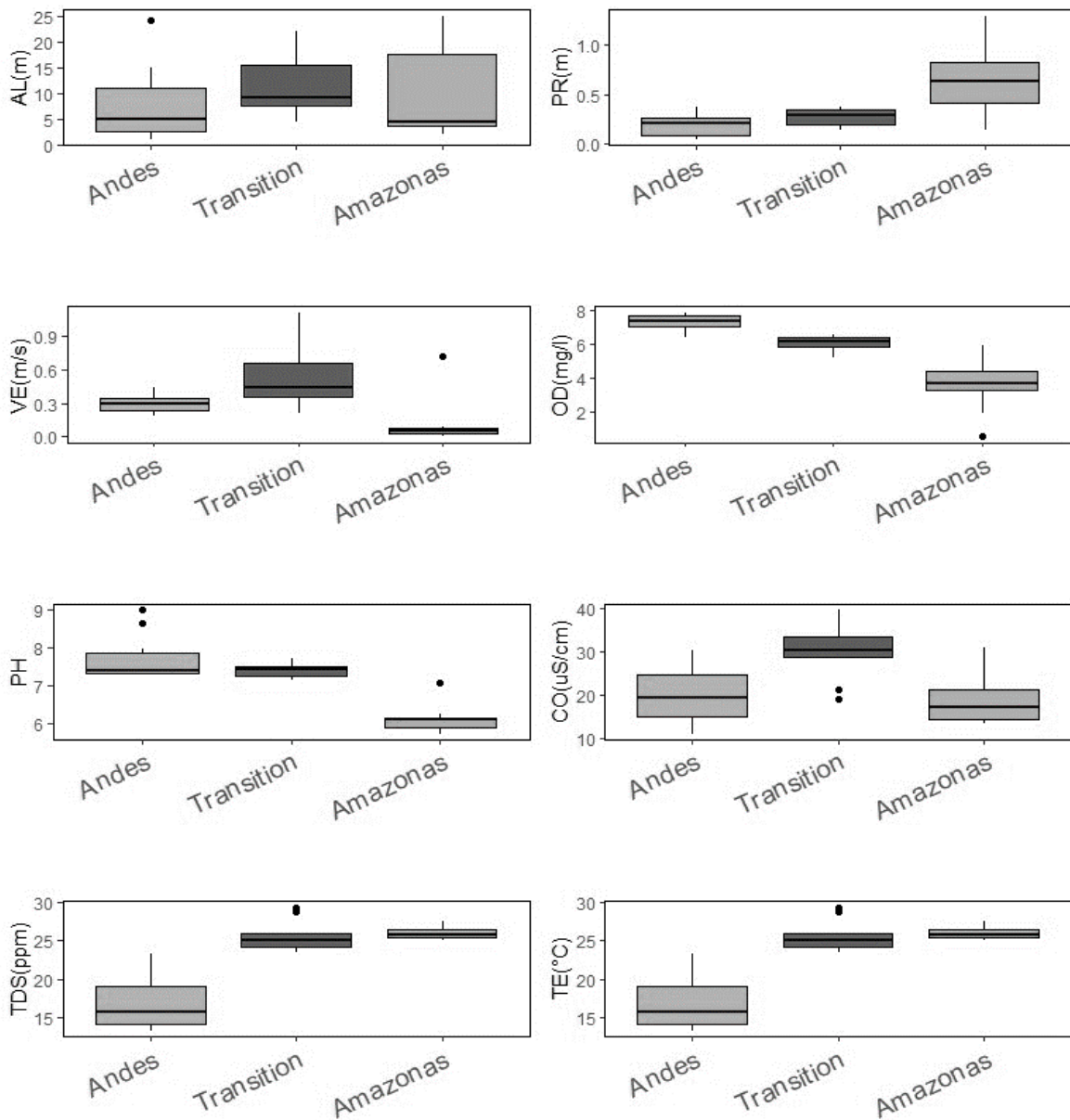
For the second PCA model (**Figure 3**), where we compared the regions and vegetation cover types, the first two components captured 72% of the total variance. We observed two groupings where Transition shares stations with both the Andes and the Amazon. Regarding the types of vegetation cover analyzed through QGIS, dense forests (BD) prevail in the first group, while grasslands (PS) and mixed grasslands with natural spaces (M.P.E.N) stand out in the second group (**Figure 3**).



**Figure 3.** Principal Component Analysis (Second model) was applied in streams of the Andean-Amazonian Region of the Caquetá Department, Colombia, which was sampled from October 2021 to April 2022. Dense forest (B.D), fragmented forest with secondary vegetation (B.F.V.S), secondary vegetation (V.S), grasslands (P.S), and miscellaneous with pastures and natural spaces (M.P.E.N).

## Physical and Chemical Variables

In the boxplot graph (**Figure 4**), we depict the behavior of the different physical and chemical variables recorded at the sampling stations. Surface width's values are very similar, while depth (PR), total dissolved solids (TDS), and water temperature (TE) increased from the Andes to the Amazon region. Conversely, dissolved oxygen (OD) and pH decreased. Regarding flow velocity (VE) and conductivity, their highest values were recorded in the Transition region.



**Figure 4.** Boxplot of the physical and chemical variables in the three study regions applied in streams of the Andean-Amazonian Region of the Caquetá Department, Colombia, sampled from October 2021 to April 2022.

When conducting the non-parametric test and subsequent post-hoc comparison (**Table 1**), surface width (AL) was the only variable that did not show significant differences. For depth (PR) and pH, there is a difference between the Andes and Transition ecoregions with the Amazon. Flow velocity (VE) differs between the Andes and Transition. Dissolved oxygen (OD) differs among all three ecoregions. Total dissolved solids (TDS) and temperature (TE) differ between the Andes and Transition with the Amazon region. Conductivity (CO) does not differ between the Andes and Amazon, but it does differ between these two and the Transition.

**Table 1.** Kruskal-Wallis non-parametric test results and Mann-Whitney post hoc test applied in streams of the Andean-Amazonian Region of the Caquetá Department, Colombia, sampled from October 2021 to April 2022.

<b>Variable</b>	<b>Kruskal-Wallis (p-value)</b>	<b>Mann-Whitney-Test</b>
Water sheet width (AL)	0.228	--- An-Tran= 0.104
Depth (PR)	0.003	<b>An-Am= 0.012</b> <b>Tran-Am= 0.038</b> <b>An-Tran= 0.025</b>
Flow velocity (VE)	0.001	<b>An-Am= 0.005</b> <b>Tran-Am= 0.005</b> <b>An-Tran= 0.001</b>
Dissolved oxygen (OD)	4.96E <sup>-03</sup>	<b>An-Am= 3.2e<sup>-05</sup></b> <b>Tran-Am= 0.001</b> An-Tran= 0.472
pH	5.49E <sup>-05</sup>	<b>An-Am= 0.001</b> <b>Tran-Am= 0.001</b> <b>An-Tran= 0.007</b>
Conductivity (CO)	0.003	An-Am= 0.795

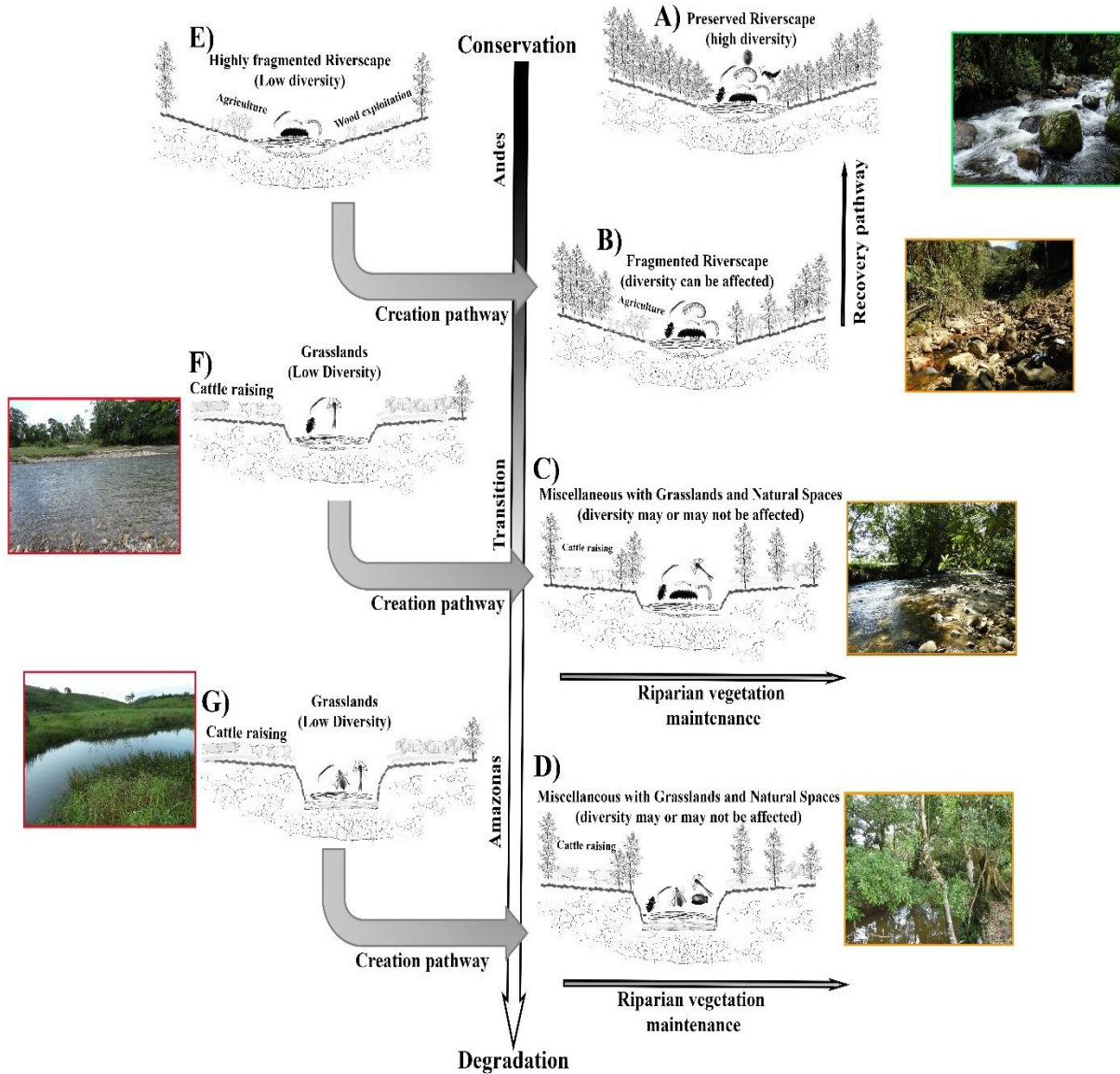
**Tran-Am= 0.004**

Total dissolved solids (TDS)	4.12E <sup>-05</sup>	<b>An-Tran= 3.2e<sup>-05</sup></b> <b>An-Am= 0.001</b> Tran-Am= 0.18
Temperature (T)	4.12E <sup>-05</sup>	<b>An-Tran= 3.2e<sup>-05</sup></b> <b>An-Am= 0.001</b> Tran-Am= 0.18

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### *Environmental analysis and management references*

From the above information, we developed a scheme to identify the reference conditions of the fluvial riverscape in the three study regions concerning riparian vegetation coverage and land use (Figure 5). The Andes stations exhibit the best conservation conditions (**Figure 5-A**), with some points showing riverscape fragmentation (**Figure 5-B**) and the absence of high transformation (Figure 5-E). In contrast, the Transition and Amazon regions' stations show more significant degradation (**Figure 5-F, G**), with some points featuring natural spaces (**Figure 5-C, D**). Additionally, we consider that the Andes station may present two natural resource management pathways (creation and recovery), while in the Transition and Amazon regions, we define only one (creation). Similarly, we illustrate the behavior of aquatic macroinvertebrate diversity, which would be inversely related to environmental degradation.



**Figure 5.** Management scheme region applied in streams of the Andean-Amazonian Region of the Caquetá Department, Colombia, sampled from October 2021 to April 2022. Stations with exemplary conservation of the river riverscape (A), Stations with agricultural fragmentation (B), stations with high fragmentation of the river riverscape (E, F, G), and stations with natural spaces and livestock activities (C, D). Scheme adapted from the work of Brierley & Fryirs (2022).

## Discussion

### *Substrate, Riparian Vegetation, and River Hydrodynamics*

Our findings demonstrate changes in fluvial riverscape conditions across the study area, starting from the Andes region and extending towards the Amazonas, as altitude decreases. These evaluated variables also validate the Transition region, as it shares characteristics of the mountain range and the lowlands.

We transited from stations with rigid substrates (boulders, cobbles, gravels) and higher flow velocities to soft substrates (clays) with lower flow velocities and more significant riparian vegetation fragmentation. These conditions can be attributed to the geomorphological history of each station within the fluvial riverscape (Poole et al., 2002; Fisher et al., 2007) at the ecoregional level (González-Trujillo et al., 2020). For instance, considering the work of Val et al. (2021), we can associate the Andes region and some Transition stations with Western Amazonia, which encompasses the Eastern Andes Mountain range and its foothills, featuring Cenozoic sedimentary basins and restricted Precambrian shields, with high relief topography, as evidenced in most stations (P1 to P20). On the other hand, the Amazonas region is closer to Eastern Amazonia, characterized by lower relief topography (<250 m.a.s.l) and a predominance of easily erodible Paleozoic sedimentary basins, which in our study are associated with stations P21 to P30.

Similarly, analyzing the behavior of vegetation cover type also reveals a successional process among the sampling stations. However, in this case, anthropogenic activity associated with land use is the main driving force behind the observed change in the two established regional groupings, transitioning from natural riverscapes in the Andes to transformed riverscapes primarily associated with extensive cattle farming.

From a riverscape perspective, these successional changes in substrate, riparian vegetation, and river hydrodynamics can also be broadly supported by the concept of the continuous river (Vannote et al., 1980; Doreto et al., 2020) or the concept of the discontinuous river (Poole, 2002) from a longitudinal variation dimension. However, we must note that our study analyzes several water bodies associated with different watersheds. Therefore, we consider that regional scale could be a significant spatial variable in these changes, which we support with the work of Tonkin et al. (2018), who analyze the importance of river network structuring and its

connectivity at a spatial scale, which can be decisive in the establishment of different biological communities (metacommunities) in the Andean-Amazonian region.

Referring to research from the Caquetá department, Tobes et al. (2016) studied the spatial distribution of fish and aquatic macroinvertebrates in different environmental conditions in the Andean Piedmont rivers. They found changes in substrate composition, river habitat quality (IHF), and vegetation cover (QBR) influenced by geomorphology and anthropogenic activities. They even defined the marked existence of two regions, the Andes and the Amazonian Plain, which aligns with our results obtained in the second PCA regarding the formation of the two groups.

Similarly, Gutiérrez-Garaviz et al. (2016) and Godoy-Losada & Peláez-Rodríguez (2020) also identified these successional changes in the Hacha River basin, highlighting that stations in the Andean region present the best conditions of vegetation cover, similar to the result of the second PCA where dense forests (BD) dominate in most stations; this vegetation cover decreases towards the Amazon, as evidenced in Transition and Amazonas where there are higher coverages of grasslands (PS), although there is a critical cover condition represented by a mixture of grasses with natural spaces (M.P.E.N), highlighting lotic ecosystems with significant riparian vegetation for the conservation and ecological maintenance of the fluvial riverscape.

### ***Physical and Chemical Variables***

The physical and chemical variables reveal longitudinal successional patterns at the regional level in the study area. However, the width of the water does not show a marked differentiation since the dimensions of most of the evaluated water bodies are comparable, preventing significant discrimination of this variable. Regarding other relevant variables, we proceed to analyze the following.

The depth of the river links intrinsically to the slope (Twidale, 2004) water flow (Evette et al., 2009; Avendaño & García, 2013) and the relative resistance of the substrate (Hauer & Lamberti, 2011), determining factors in riverbed erosion. This relationship manifests in the

Amazon region, where soft riverbeds predominate, dominated by clays, a material with lower erosion resistance (Roldán & Ramírez, 2022). In contrast, riverbeds with shallower depths, steeper slopes, and rocky substrates are observed in the Andean and Transition regions, potentially increasing resistance to riverbed erosion.

The speed of the water current can also be related to the slope, presenting higher values in the Andes and lower values in the Amazon. However, the highest values identified in Transition can be related to some rivers with a greater sheet width, in addition to the presence of large rocky substrates that can generate waterfalls, being part of the discontinuity of the river landscape as proposed by Poole (2002) (e.g., station E8).

Regarding Total Dissolved Solids (TDS), this metric encompasses both mineral and organic components (organic matter of various sizes) (Chacón, 2016; Roldán & Ramírez, 2022). We observe an increase in TDS in the Amazon region, possibly due to the accumulation of organic matter (leaves, branches, logs) in the riverbed, favored by the slow water velocity and increasing suspended material. This accumulation could also contribute to the reduced pH in the region, with the lowest value (pH = 5.74) recorded at station P30, indicative of an increase in organic decomposition and consequent water acidification. The findings of Roldán & Ramírez (2022), indicating a pH range of 6.5 to 7.5 in the upper part of the Amazon region and 4.5 to 6.9 in the lower areas, are consistent with our data and could be explained by the geological history of the region (Val et al., 2021). Similarly, Tobes et al. (2016) reported a decrease in pH from the Andes to the Amazon in the Caquetá department.

Water temperature exhibits an inverse correlation with the altitudinal gradient, as confirmed by Roldán and Ramírez (2022), and this relationship has been corroborated in our study region by Tobes et al. (2016) and Godoy-Losada & Peláez-Rodríguez (2020), thus reinforcing the results of our research. Additionally, temperature is significantly related to dissolved oxygen (Hauer & Lamberti, 2011), a key indicator in water quality assessment (Manrique-Losada & Peláez-Rodríguez, 2010), whose levels tend to decrease in the Amazon region. Temperature and dissolved oxygen are determinants for the configuration of aquatic communities (Fathi et al., 2022; Öztürk et al., 2022), and the latter is especially relevant as a

marker of anthropogenic pollution (Gutiérrez-Garaviz et al., 2014). In our study, we attribute the lower dissolved oxygen concentration recorded at station P30 (0.52 mg L<sup>-1</sup>) to temperature, the altitudinal gradient, and the intensified decomposition of organic matter prevalent in the Amazon region.

Conductivity, which measures the concentration of dissolved ions in a body of water (Galvin, 2008), does not exceed 50  $\mu\text{S}/\text{cm}$  in the study area. Higher values are associated with the Transition region, attributable to increased lateral erosion due to reduced riparian vegetation, which promotes more significant vegetative fragmentation and facilitates the dissolution of minerals of rocky origin. In contrast, the Andean region, also characterized by rocky substrates, exhibits riparian integrity with dense vegetative cover at most of its stations, contributing to the stabilization of the riparian soil. This factor acts as a natural barrier mitigating soil erosion. Studies by Hubble et al. (2010) and Hould-Gosselin et al. (2016) confirm the effectiveness of riparian vegetation as a natural filter in retaining sediments on the shores of aquatic ecosystems.

In the Amazon region, observed conductivity values are consistently low, a distinctive characteristic of Type II white waters (González et al., 2006; Ríos-Villamizar et al., 2014), and in our study, these did not exceed 22  $\mu\text{S}/\text{cm}$ . The geology of the sedimentary basins in the region (Val et al., 2021), livestock activity, and the loss of riparian vegetation observed at certain stations may influence these values. Although some studies in the region associate increased conductivity with residual contamination (Manrique-Losada & Peláez-Rodríguez, 2010; Tobes et al., 2016), in our analysis, this factor appears to have a minor influence, as the sampling sites are in rural areas far from urban centers.

### ***Environmental analysis and management references***

The proposed framework allows us to ask: What can be done with this fluvial riverscape characterization data? Are they sufficient to serve as a reference in proposals for the proper use and management of natural resources?

In Andrades work (2011), he coins the term "protected river" to refer to a conservation strategy for river ecosystems in Colombia, emphasizing the need to integrate physical dynamics, water quality, and knowledge of biological communities with socio-ecosystemic aspects. In our study, we examine physical, chemical, and anthropogenic components from the perspective of the fluvial riverscape. However, these elements must consider the historical evolution of rivers, one of the truths proposed by Brierley & Fryirs (2022), which would allow for a realistic scope within river restoration and conservation strategies, measurable through aquatic diversity as illustrated in the framework.

The Andean region would be the only one offering the possibility of a recovery pathway in some of the stations with small-scale agricultural activity (P5, P6, P9). This is because land use for the conservation of natural forests predominates, as identified in the study area map and the second PCA model. However, there is the possibility of an increase in rural human settlements, which could lead to a future scenario showing high riverscape fragmentation, as already demonstrated over time in the work of Torres & Castro (2015), positioning those rivers in a scenario of change of the pathway if their conditions are to be improved. Therefore, it is vital in this region to invest in creating civil society reserves (Decree 1076 of 2015) as strategic ecosystems for biodiversity (Carvajal et al., 2017; Orjuela-Salazar et al., 2018). This would allow us to embrace Brierley & Fryirs (2022) first truth regarding recognition and respect for nature.

The increase in fluvial riverscape degradation observed in the Transition and Amazon regions is primarily attributed to extensive livestock farming, an anthropogenic driver previously mentioned in this study and reported in the works of Murad & Pierce (2018) and Landholm et al. (2019). We have determined that the most appropriate management strategy involves change, focusing on restoring riparian vegetation, which we anticipate will enhance ecosystem services and biodiversity. This conservation management is imperative, particularly in the Amazon, a region with a relatively high legacy of deforestation (Mendez & Valánszki, 2019; Bautista-Cespedes et al., 2021). The situation has worsened since the 2016 peace agreement between the government of Juan Manuel Santos and the FARC, with a notable increase in deforestation evidence (Clerici et al., 2019; Agudelo et al., 2023). Additionally, to extensive livestock farming as an anthropogenic driver of deforestation, the increase in illicit crops such as coca production

(Murad & Pearse, 2018; Ganzenmüller & Castro-Nuñez, 2019) is added, something that was not evidenced in our results but does exist in the Caquetá department.

In these two regions, we consider that agroforestry or silvopastoral systems can be established, which have given excellent results for biodiversity in other places (Chará & Murgueitio, 2005; Delgado-Lemus & Morena-Calle, 2022; François et al., 2023; Matocha et al., 2012; Rodriguez et al., 2021), can be established. These systems would allow us to recover stations with high fragmentation towards stations made up of natural spaces with pastures or crops. This would improve local conditions (heterogeneity of habitats, non-native sources) to maintain biodiversity and improve ecosystem services without requiring resignation for current agricultural activities.

## **Conclusions**

Our study identifies the dynamics of the river riverscape, observing successional processes at a regional scale among the three study areas analyzed in the Andean-Amazonian corridor of Caquetá. Based on the local environmental variables, a separation is identified between the Andes and Amazon regions. Furthermore, we validate the transition region as an area that shares conditions with the two mentioned areas.

The Andes region has a rocky substrate, higher water flow speeds, relatively shallower depths, and riparian vegetation cover, with exemplary conservation of riparian vegetation in most seasons. In contrast, the Amazon region has a softer substrate, lower current flow velocities, and greater depth, with high or low riparian vegetation fragmentation.

Significant differences are observed in most of the physical and chemical variables analyzed. Depth, total dissolved solids (TDS), and water temperature (TE) show a progressive increase from the Andes region to the Amazon region. On the contrary, dissolved oxygen (DO) and pH levels decrease in the same direction. Regarding electrical conductivity (EC) and flow velocity (FV), their highest values are associated with the Transition region.

We consider that local environmental variables are reference analyses for a management proposal. Our results focus on the percentage of riparian vegetation cover and land use to establish two management routes that can improve the conditions of the river riverscape and its biological diversity, considering the historical evolution of the rivers. For the Andes, it is possible to direct those stations with small-scale agricultural activities that have fragmented part of the riparian forest towards a recovery path in the current scenario. However, we also propose a future scenario with more significant degradation of the river riverscape, which would lead to taking the path of creation. For Transition and Amazonas, the possible path would be to recover affected rivers and maintain those that integrate natural spaces and agricultural systems.

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## Supplementary Material

**Table S1.** Geographical information for each of the study stations worked on during the sampling campaigns in 2021 and 2022.

<b>Estação</b>	<b>Altitude</b>	<b>XCorrd</b>	<b>YCoord</b>
P1	2205	-75.6796	1.8781
P2	2202	-75.6786	1.8773
P3	2070	-75.6700	1.8690
P4	1540	-75.6618	1.8404
P5	1000	-75.6470	1.7951
P6	957	-75.6394	1.7708
P7	2168	-75.7663	1.7627
P8	2041	-75.7496	1.7562
P9	1697	-75.7231	1.7359
P10	581	-75.6456	1.7294
P11	396	-75.6723	1.6236
P12	492	-75.9832	1.2977
P13	321	-75.9538	1.3350
P14	300	-75.9181	1.3622
P15	308	-75.8866	1.4083
P16	285	-75.2284	1.7665
P17	285	-75.2361	1.7539
P18	378	-75.3017	1.6362
P19	293	-75.3363	1.5427
P20	276	-75.3426	1.5205
P21	247	-75.3594	1.4283
P22	252	-75.3582	1.4253
P23	231	-75.3448	1.3754
P24	237	-75.3853	1.3572
P25	245	-75.4162	1.3358

E26	243	-75.4346	1.2999
E27	233	-75.4300	1.3052
E28	235	-75.4356	1.2954
E29	266	-75.4396	1.2821
E30	234	-75.4295	1.2973

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**Table S2.** Substrate, Riparian Vegetation, and River Hydrodynamics related to the sampling stations worked during the 2021 and 2022 campaigns. Percentage of vegetation cover (C.V), shade (SO), trees (ARB.), shrubs (ARBU.), herbaceous (HER.), grasses (PA.), forest (BOS.), agriculture (AGR.), recreation (RECR.), settlement (ASEN.), grazing (PAST.), rapids (RA.) , water pool (RE.), normal flow (COR.), blocks (BLO.), stones (CANT.), gravel (GRAV.), sand (ARE.), silt (LIM.), clay (ARC.), coarse particulate organic matter (MOPG), type of vegetation cover (C.V.S). Dense Forest (B.D), Fragmented Forest with Secondary Vegetation (B.F.V.S), Secondary Vegetation (V.S), Grasslands (PS), and Miscellaneous with Grasslands and Natural Spaces (M.P.E.N).

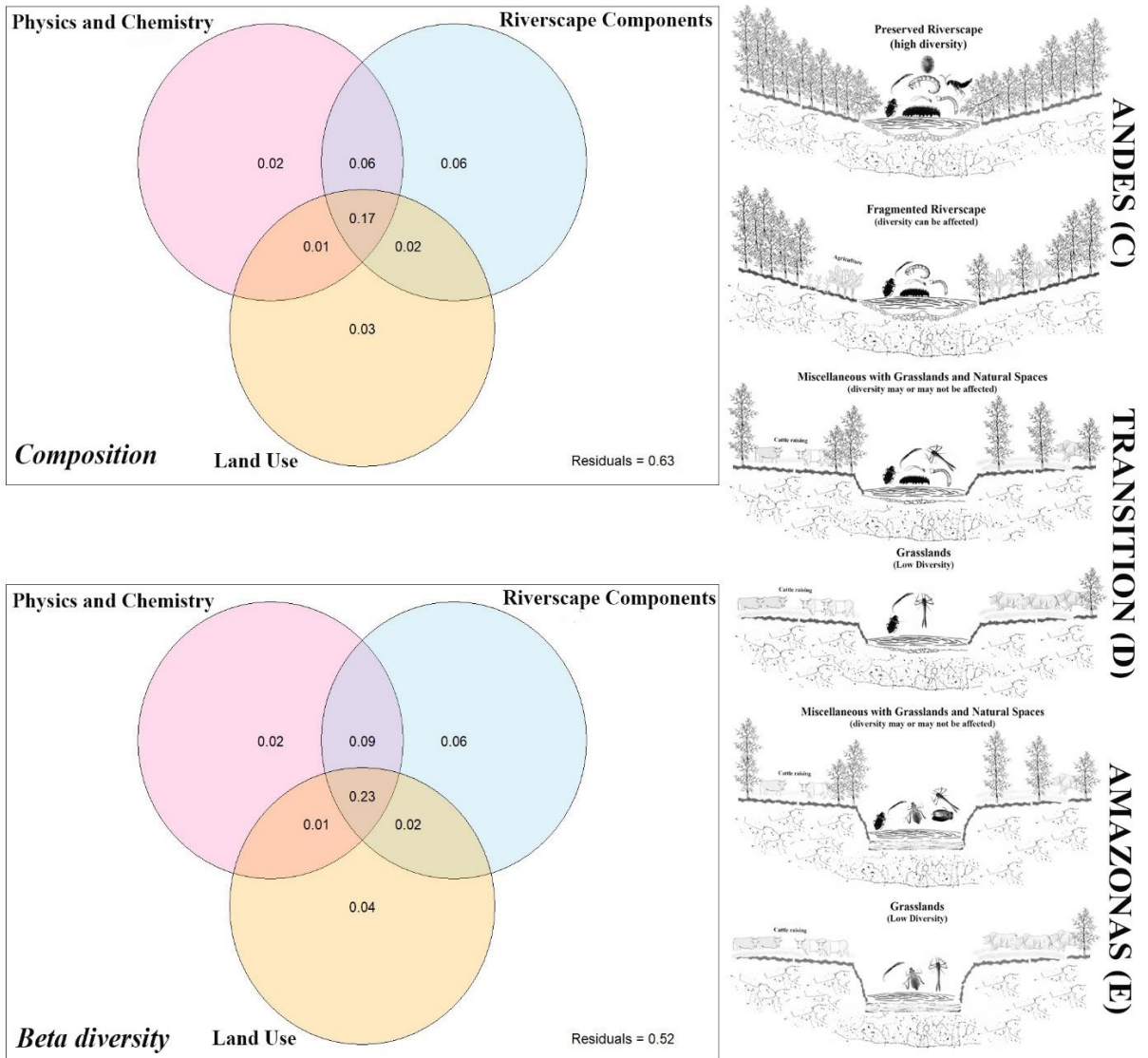
REGION	STATION	C.V	SO.	ARB	ARBU	PA.	HER	BOS	AGR	RECR	ASEN	PAST	RA.	RE.	COR	BLO.	CANT	GRAV	ARE	LIM.	ARCI	MOPG	C.V.S
ANDES	P1	0.95	0.95	0.7	0.3	0	0	1	0	0	0	0	0.7	0.1	0.2	0.7	0.1	0.05	0.1	0	0	0.05	B.D
ANDES	P2	1	1	0.7	0.3	0	0	1	0	0	0	0	0.7	0.1	0.2	0.75	0.1	0.05	0.05	0	0	0.05	B.D
ANDES	P3	0.9	0.2	0.2	0.4	0	0.4	0.8	0.2	0	0	0	0.7	0.1	0.2	0.65	0.1	0.1	0.1	0	0	0.05	B.D
ANDES	P4	0.9	0.3	0.4	0.6	0.1	0.1	0.8	0.2	0	0	0	0.8	0	0.2	0.7	0.1	0.1	0.1	0	0	0	B.D
ANDES	P5	0.6	0.2	0.4	0.2	0.2	0.2	0.3	0.5	0	0	0.2	0.8	0	0.3	0.6	0.2	0.1	0.1	0	0	0	M.P.E.N
ANDES	P6	0.6	0.2	0.4	0.2	0.3	0.1	0.3	0.4	0	0	0.3	0.6	0.1	0.3	0.55	0.1	0.1	0.2	0	0	0.05	B.F.V.S
ANDES	P7	0.9	0.6	0.6	0.3	0	0.1	1	0	0	0	0	0.2	0.3	0.5	0.2	0.35	0.2	0.2	0	0	0.05	B.D
ANDES	P8	0.6	0.1	0.4	0.6	0	0	1	0	0	0	0	0.6	0.1	0.3	0.5	0.25	0.1	0.1	0	0	0.05	B.D
ANDES	P9	0.7	0.4	0.2	0.6	0	0.2	0.2	0.6	0	0	0.2	0.4	0.3	0.3	0.1	0.55	0.2	0.1	0	0	0.05	B.D
ANDES	P10	0.8	0.2	0.7	0.1	0.1	0.1	0.3	0.2	0.1	0.1	0.3	0.5	0.1	0.4	0.3	0.4	0.05	0.2	0	0	0.05	V.S
TRANSITION	P11	0.5	0.1	0.3	0.1	0.4	0.2	0.1	0.1	0.1	0	0.7	0.4	0.2	0.4	0.4	0.2	0.1	0.3	0	0	0	PS
TRANSITION	P12	0.6	0.6	0.4	0.3	0.1	0.2	0.15	0.1	0.1	0.05	0.6	0.4	0.2	0.4	0.2	0.4	0.1	0.2	0	0	0.05	PS
TRANSITION	P13	0.6	0.4	0.4	0.2	0.2	0.2	0.2	0.2	0	0.05	0.55	0.4	0.2	0.4	0.2	0.4	0.2	0.2	0	0	0	V.S
TRANSITION	P14	0.4	0.6	0.2	0.2	0.6	0	0.15	0.2	0	0.05	0.6	0.5	0.1	0.4	0.1	0.6	0.1	0.2	0	0	0	PS
TRANSITION	P15	0.5	0.8	0.3	0.2	0.5	0	0.15	0.2	0.1	0.05	0.5	0	0.3	0.7	0.2	0.4	0.3	0.1	0	0	0	PS
TRANSITION	P16	0.3	0	0.1	0	0.8	0.1	0	0	0.2	0	0.8	0.5	0.2	0.3	0.1	0.4	0.2	0.3	0	0	0	PS
TRANSITION	P17	0.8	0.8	0.6	0.2	0.1	0.1	0.4	0.2	0.1	0	0.3	0.1	0.3	0.6	0	0.5	0.2	0.3	0	0	0	M.P.E.N
TRANSITION	P18	0.3	0.3	0.2	0	0.8	0	0	0	0.4	0	0.6	0.6	0.1	0.3	0.6	0.2	0.1	0.1	0	0	0	PS
TRANSITION	P19	0.3	0.1	0.2	0	0.8	0	0.1	0	0.3	0	0.6	0.5	0.2	0.3	0.1	0.4	0.2	0.3	0	0	0	PS

TRANSITION	P20	0.4	0	0.1	0	0.8	0.1	0	0.2	0.1	0	0.7	0.5	0.2	0.3	0.1	0.5	0.1	0.3	0	0	0	PS
AMAZONAS	P21	0.4	0.6	0.4	0.2	0.4	0	0.1	0.2	0.1	0	0.6	0	0.4	0.6	0	0	0.2	0.6	0	0	0.2	PS
AMAZONAS	P22	0.4	0.4	0.1	0.4	0.4	0.1	0.1	0.1	0	0	0.8	0	0.7	0.3	0	0	0	0.3	0.2	0.1	0.4	PS
AMAZONAS	P23	0.4	0.6	0.2	0.4	0.4	0	0.1	0.1	0	0.05	0.75	0	0.7	0.3	0	0	0	0	0.1	0.6	0.3	PS
AMAZONAS	P24	0.3	0.6	0.3	0.4	0.3	0	0.1	0.1	0	0.05	0.75	0	0.7	0.3	0	0	0	0	0.1	0.6	0.3	PS
AMAZONAS	P25	0.5	0.5	0.1	0.4	0.4	0.1	0.35	0.1	0	0.05	0.5	0	0.7	0.3	0	0	0	0	0.1	0.6	0.3	M.P.E.N
AMAZONAS	P26	0	0	0	0	0.5	0.5	0	0	0	0	1	0	0.8	0.2	0	0	0	0	0.1	0.6	0.3	PS
AMAZONAS	P27	0	0	0	0	0.5	0.5	0	0	0	0	1	0	0.8	0.2	0	0	0	0	0.1	0.6	0.3	M.P.E.N
AMAZONAS	P28	0.4	0.5	0.1	0.2	0.5	0.2	0	0	0	0	1	0	0.8	0.2	0	0	0	0	0.05	0.65	0.3	M.P.E.N
AMAZONAS	P29	0.5	0.6	0.5	0.1	0.3	0.1	0.1	0	0.1	0.05	0.75	0	0.8	0.2	0	0	0	0	0.05	0.65	0.3	M.P.E.N
AMAZONAS	P30	0.6	0.6	0.3	0.2	0	0.5	0.1	0	0	0	0.9	0	0.9	0.1	0	0	0	0	0	0.4	0.6	M.P.E.N

**Table S3.** Physical and chemical variables related to the sampling stations of the Andean-Amazon region of Caquetá were recorded during the 2021 and 2022 sampling campaigns. Current velocity (VE), dissolved oxygen (OD), hydrogen ion potential (pH), conductivity (CO), total dissolved solids (TDS), water temperature (TE), water sheet width (AL), Depth (PR).

REGION	STATION	AL	PR	VE	OD	PH	CO	TDS	TE
ANDES	P1	2.6	0.05	0.22	7.5	7.363	28.85	14.4	14.4
ANDES	P2	1.08	0.1	0.31	7.65	7.423	18.2	13.5	13.5
ANDES	P3	8	0.2	0.29	7.775	7.393	14.875	13.875	13.875
ANDES	P4	12	0.26	0.44	7.2	7.363	15.975	16.75	16.75
ANDES	P5	15	0.26	0.41	7.075	7.273	20.475	19.7	19.7
ANDES	P6	5.8	0.28	0.24	6.45	7.295	24.975	20.7	20.7
ANDES	P7	2.5	0.07	0.36	7.08	8.998	12.475	13.225	13.225
ANDES	P8	4	0.22	0.19	7.863	8.638	11.125	14.45	14.45
ANDES	P9	1.2	0.04	0.23	7.693	7.325	23.975	17	17
ANDES	P10	24	0.37	0.32	6.885	7.98	30.475	23.3	23.3
TRANSITION	P11	7	0.28	0.21	6.425	7.72	36.5	24.1	24.1
TRANSITION	P12	22	0.3	0.53	6.25	7.378	39.65	24.2	24.2
TRANSITION	P13	16	0.37	0.39	6.3	7.26	28.7	24.375	24.375
TRANSITION	P14	5	0.23	0.85	6.45	7.135	29.925	24.325	24.325
TRANSITION	P15	13.5	0.35	0.71	6.6	7.505	32.775	23.55	23.55
TRANSITION	P16	9	0.29	1.11	5.875	7.615	33.875	28.75	28.75
TRANSITION	P17	18	0.14	0.44	5.175	7.26	30.625	29.368	29.368
TRANSITION	P18	9	0.36	0.29	6.05	7.498	29.025	25.65	25.65
TRANSITION	P19	9.5	0.15	0.45	5.825	7.223	19.025	25.675	25.675
TRANSITION	P20	4.5	0.18	0.35	5.875	7.485	21.2	25.95	25.95
AMAZONAS	P21	22	0.36	0.72	5.925	7.07	18.9	25.775	25.775
AMAZONAS	P22	20	0.77	0.08	4.2	6.235	15.85	25.8	25.8
AMAZONAS	P23	5	0.84	0.08	3.95	6.115	18.75	24.975	24.975
AMAZONAS	P24	4	0.67	0.06	4.5	6.128	13.975	25.4	25.4
AMAZONAS	P25	3	0.58	0.06	3.25	6.108	13.6	25.2	25.2
AMAZONAS	P26	3.5	1.3	0.02	3.3	5.713	22.15	26.575	26.575
AMAZONAS	P27	25	0.22	0.02	1.875	6.06	25.15	27.575	27.575
AMAZONAS	P28	2	0.6	0.09	4.685	5.798	15.75	27.475	27.475
AMAZONAS	P29	10	0.91	0.06	3.45	6.098	13.675	26.5	26.5
AMAZONAS	P30	4	0.13	0.003	0.525	5.743	31.1	25.775	25.775

## 7. Capítulo 2: Taxonomic and functional diversity of aquatic macroinvertebrates under different environmental conditions in the Andean-Amazonian region, Caquetá-Colombia



Graphical abstract

# **Taxonomic and functional diversity of aquatic macroinvertebrates under different environmental conditions in the Andean-Amazonian region, Caquetá-Colombia**

## **Abstract**

Lotic ecosystems within the Andean-Amazonian corridor are home to a high diversity of aquatic macroinvertebrate assemblages. Therefore, we investigated this area associated with the Caquetá department, which has a marked difference in the composition and diversity of the macroinvertebrate assemblage, proposing the hypothesis that environmental variables may have great influence in determining their components. We defined three study regions: Andes, Transition, and Amazon. We established ten sampling stations in each, collected biological material, and characterized the riverscape. We estimated both taxonomic and functional alpha and beta diversity. Additionally, we related physical and chemical variables, riverscape components, and land use to aquatic macroinvertebrates' composition and beta diversity. Our results indicated a difference in composition, yielding a dissimilarity value of 0.49 compared to previous studies in the study area. This difference was also evident in taxonomic and functional alpha diversity, with the Amazon region standing out. Regarding taxonomic and functional beta diversity, the component of species replacement was more significant than the difference in richness. We identified variables such as dissolved oxygen, conductivity, total dissolved solids, substrate, grasslands, and forest conservation as predictors. Our research adds new elements to understanding the dynamics of aquatic macroinvertebrate assemblages in the Andean-Amazonian region, which could subsidize future studies integrating dispersion and phylogeny analysis.

**Keywords:** alpha diversity, beta diversity, physical variables, chemical variables, riverscape.

## **Introduction**

The Andean-Amazonian region of the Caquetá department is considered an area of high value in terms of biodiversity (Atuesta-Dimian et al., 2020; Carrillo & Capera, 2020; Morales-Martínez & Díaz, 2020; Peña-Venegas et al., 2020). However, this region has been significantly transformed in recent years, especially the Amazon, due to an increase in the deforestation rate, a

result of extensive cattle ranching (Murad & Pearse, 2018; Landholm et al., 2019), illicit crops such as coca (Murad & Pearse, 2018; Ganzenmüller & Castro-Nuñez, 2019), and the lack of significant political and social commitment to the proper use and management of natural resources. This has led us to the need to study the different components of the river riverscape to evaluate the historical extent of these anthropogenic activities on a current primary concern related to the loss of connectivity between the Andes Mountain range and the Amazonian plains (Anderson et al., 2018; Clerici et al., 2018; Murillo-Sandoval et al., 2022; Caldas et al., 2023).

Within these components of the riverscape, we can study the different aquatic assemblages, considering that they can provide spatial or temporal information regarding the structure and functioning of an ecosystem (Tundisi & Tundisi, 2008; Roldán & Ramírez, 2022). Among these assemblages, aquatic macroinvertebrates hold significant ecological value, whose roles can contribute to an ecosystem's metabolism and matter transformation (Benson et al., 2013; Yates et al., 2018), the energy flow (Estévez et al., 2019; Allam et al., 2021), the interaction between aquatic and terrestrial ecosystems (Bailey et al., 2003; Nash et al., 2021), and to the use of many of its individuals from a bioindicator perspective related to the biological quality of water (Akay & Dalkiran, 2020; Sumudumali & Jayawardana, 2021; Tampo et al., 2021).

Aquatic macroinvertebrates are defined as invertebrates with a length greater than 0.5 mm (Roldán, 1996) or visible to the naked eye (Gutiérrez-Garaviz et al., 2014). Most belong to the class Insect of the Phylum Arthropoda, primarily associated with immature stages (Domínguez & Fernández, 2009), with some exceptions like genera from the Elmidae family that may spend some moments of their adult life outside water for dispersion (Silva et al., 2007). However, within this community, we can also find taxa associated with the Phyla Annelida, Porifera, Mollusca, Nematoda, Nematomorpha, and Platyhelminthes (Roldán & Ramírez, 2022).

Some researchers have already studied these organisms in the Andean-Amazonian region of the Caquetá department (López-Erazo et al., 2012; Cortés & Hernández, 2016; Gutiérrez et al., 2016; Bravo & Restrepo, 2021). However, most authors have focused on characterization studies and have not delved into ecological aspects such as true diversity, beta diversity, or

functional diversity. These aspects could provide more information about how these organisms establish themselves in any water body and how environmental filters on a local and regional scale determine their establishment.

Effective diversity, also known as Hill numbers, allows us to make comparisons between community scales, ecosystems, regions, or stations based on estimates of taxonomic alpha diversity, according to Jost (2006). As for beta diversity, which seeks to identify dissimilarities (Rodríguez da Silva et al., 2022) at the mentioned scales, from a taxonomic perspective, Baselga (2010) and Legendre et al. (2013) propose studying the components of replacement (species substitution) or difference in richness (also known as nesting). Applying these two approaches can provide statistical support to understanding ecological dynamics within the Andean-Amazonian region. However, we consider the scope of our research to be useful for future integration of other analyses, such as dispersion and phylogeny.

Furthermore, functional diversity is based on studying functional traits related to biological morphological characteristics, life histories, reproduction, or survival (Violle et al., 2007). We can also analyze alpha diversity (Schmera et al., 2017) and beta diversity (Cardoso et al., 2015). In this case, we aim to understand how organisms develop different adaptive strategies to overcome various environmental filters (Poff, 1997), evident in the Andean-Amazonian corridor.

Many researchers recognize that altitudinal gradient influences the distribution and diversity of aquatic macroinvertebrates within these environmental filters (Dudgeon, 2012; Arana-Maestre et al., 2021; Masina et al., 2023). However, we consider that local environmental variables can provide us with more information, thus enabling us to understand, for example, existing functional traits. Physical and chemical water characteristics such as temperature, pH, dissolved oxygen, and electrical conductivity can be related to the presence of certain taxa and its their abundance (Jerves-Cobo et al., 2017; Fathi et al., 2022; Öztürk et al., 2022). The composition of the substrate provides microhabitats (Cummins, 2019; Quesada-Alvarado et al., 2020), riparian vegetation cover sources of allochthonous inputs (Lima et al., 2022; Luisa-Andrade et al., 2020) and corridors for adult dispersion (Peredo Arce et al., 2023) and stream

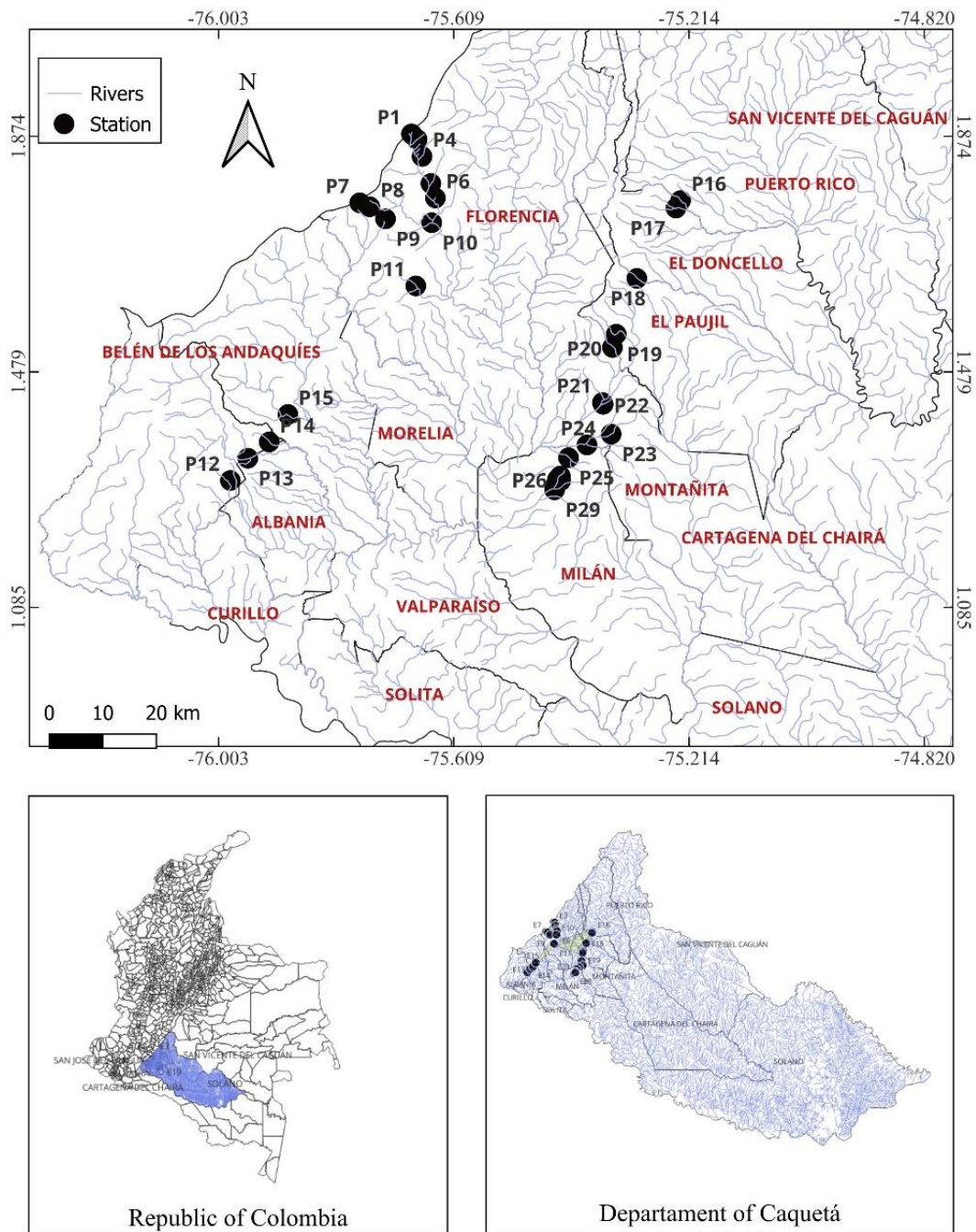
velocity (Tagliaferro & Pascual, 2017; Guellaf et al., 2021) can influence the availability of food resources.

Based on the above, our research hypothesis proposes that there is differentiation among the regions of the Andean-Amazonian corridor in terms of composition, taxonomic diversity, and functional diversity of aquatic macroinvertebrates influenced by environmental filters. To test this hypothesis, we identified the different taxa associated with each region, calculated estimates of taxonomic and functional diversity, and correlated biological information with physical and chemical variables, riverscape components, and land use, aiming to identify predictive factors that may influence the establishment of aquatic macroinvertebrates in the Andean-Amazonian region.

## **Material and Methods**

### *Study area*

Based on coverage map from the SINCHI Institute (Open Data - SINCHI Institute, 2023) and a preliminary visit, we defined three study regions along the Andean-Amazonian corridor of the Caquetá department, considering changes in substrate composition, current speed, slope, and physical and chemical properties of the water (**Figure 1**; see **Table S1** of the supplementary material where all geographical coordinates are listed). The first region was related to the eastern range of the Andes, comprising stations P1 to P10. As a transition area between the Andes and the Amazon, the second region includes stations P11 to P20. We located the last region in the Amazon, defining points P21 to P30.



**Figure 1.** Sampling stations in the Andean-Amazonian region of the Caquetá department. Created using the QGIS program V.3.32.3. Above a close view of the hydrography and all sampling stations. Below, at the left, the map of Colombia with Caquetá Department location highlighted in blue, and at the right the Caquetá Department showing the location of all sampling points.

## *Field Phase*

The sampling campaigns were bimonthly, covering the months of October-December 2021 and February-April 2022. For the collection of aquatic macroinvertebrates, we adapted a multi-habitat methodology from bibliographic references (Rodríguez-Capítulo et al., 2009; Wantzen & Rueda-Delgado, 2009; Roldán & Ramírez, 2022). We implemented a Surber net of 0.3x0.3 meters with a 250-micron mesh, conducting ten sweeps over a study stretch of 100 m in length (collection area 0.9 m<sup>2</sup>). The sweeps were homogenized in a container, sieved at 500 and 250 microns (to remove oversized material), and packed in wide-mouth plastic jars of 500 cm<sup>3</sup> with 75% alcohol. We collected on a vegetal origin substrate in the Amazon region, including tree roots, macrophytes, and coarse particulate organic material (M.O.P.G). This type of substrate harbors a higher richness of aquatic macroinvertebrates.

The samples were taken to the laboratory for identification and counting. We sorted the organisms by morphotype using a Zeiss Stemi DV4 stereoscope and a Zeiss Primo Star microscope. We then defined the associated taxonomic category (mainly at the genus level) using the guides and taxonomic keys of Roldán (1996), Merrit et al. (2008), Domínguez & Fernández (2009), Springer et al. (2010), Trivinho-Strixino (2011), Hamada et al. (2014), and Hamada et al. (2018).

Based on the identified taxa, we constructed **Table 1** of functional traits, considering the works of Tomanova (2007), Merrit et al. (2008), Chará et al. (2010), Henriques de Oliveira & Nessimian (2010), Rodríguez-Barros et al. (2011), Brasil et al. (2013), González-Trujillo et al. (2020), and Barros et al. (2024). It is important to mention that we only worked with the class Insecta, considering the availability of secondary information for most of the established functional traits.

**Table 1.** Biological functional traits and their representative states associated with the community of aquatic macroinvertebrates collected in the Andean-Amazonian region during the field sampling campaigns from October 2021 to April 2022.

<b>Trait</b>	<b>State</b>
1. Feeding strategy	Shredders (sh), collectors (cll), scrapers (sc),

	piercers (pi), predators (pr), parasites (pa), filterers (fi).
2.Respiration mode	Atmospheric breathers (ab), plant breathers (pb), temporary air store (ta), plastrons (pl), spiracular gills (sp), tracheal gills (tg), cutaneous (cu), hemoglobin (he).
3.Specific adaptations to flow constraints	Suckers (su), silk glands (sg), abdominal claws (ac), tarsal claws (gt), Anal Hooks (ah), case (cs), No Adaptation (na).
4.Locomotion	Skater (sk), planktonic (pl), divers (di), swimmers (sw), clingers (cl), sprawlers (de) climbers (cb), burrowers (bu).
5.Body size	<2.5 mm, 2.5-5 mm, 5-10 mm, 10-20 mm, 20-40 mm, 40-80 mm.
6.Body form	Cylindrical (cy), spherical (sph), flattened (fl), streamlined (st).

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As complementary information, we carried out a characterization of the river riverscape at each of the sampling stations, considering Barbour et al. (1999), Elosegui & Sabater (2009), Hauer & Lamberti (2011), and Saito et al. (2020). For the physical and chemical components, we recorded dissolved oxygen (OD-mg L<sup>-1</sup>), pH, conductivity (CO-μS cm<sup>-1</sup>), total dissolved solids (TDS-ppm), and water temperature (T-°C), implementing a multiparametric probe AZ86031. Additionally, we recorded current speed and depth using a Global Water flowmeter. For the riverscape components, we recorded the composition of the substrate (blocks (BLO) = >25 cm in diameter; cobbles (CANT) = 6 cm - 25 cm; gravel (GRA) = 2 cm - 6 cm; sand (ARE) = 0.06 mm – 2 mm; vegetal origin = tree roots, aquatic macrophytes, M.O.P.G. (O.VEG), percentage of vegetal cover, and type of vegetation (trees, shrubs, grasses, herbaceous plants). Regarding the third component that relates to land use, we defined the categories for forest conservation (BOS), agriculture (AGR), livestock grazing (PAST), settlements (ASEN), and recreational activities (REC).

## *Data Analysis*

Based on the identified composition of aquatic macroinvertebrates, we assessed the samples representativeness using a rarefaction analysis, comparing the number of individuals to richness (Rodriguez da Silva et al., 2022). Subsequently, we estimated the taxonomic alpha diversity following the recommendations of Jost (2006), calculating Hill numbers where  $q=0$  indicates richness,  $q=1$  the Shannon-Wiener diversity, and  $q=2$  the inverse of Simpson's index. We utilized the *ggthemes*, *iNEXT* and *vegan* libraries for these analyses within the statistical program RStudio V.2023.12.1+402.

Regarding taxonomic beta diversity, we implemented a non-metric multidimensional scaling (NMDS) analysis with Bray-Curtis distance and group selection based on the elbow method, considering the sampling stations and composition of the assembly of interest associated with each study region. We performed an ANOSIM with 999 permutations and Bray-Curtis distance, which statistically validated the separation of groups (Maiolini et al., 2011). In addition to that, we calculated the IndVal index to identify indicator species in each study region. We used the *vegan*, *ggplot2*, *stats*, and *indicspecies* libraries.

About functional diversity, we applied a fuzzy coding procedure to each trait to establish affinity scores for the evaluated taxa (Chevenet et al., 1994): 0 (no affinity), 1 (weak affinity), 2 (moderate affinity), 3 (strong affinity). Subsequently, we referred to the publication by Mammola and Cardoso (2020), who propose working with metrics using n-dimensional kernel density hypervolumes. The calculated alpha estimates were functional diversity from *kernel.alpha*, which calculates the functional richness of traits in space (Mammola & Cardoso, 2020); *kernel.dispersion*, which calculates the average distance of each species to the community centroid in trait space (Laliberté & Legendre, 2010); and *kernel.evenness*, which measures the uniformity of the hypervolumes or distribution of functional elements within the functional space (Mason et al., 2005). Subsequently, an analysis of variance (ANOVA) and a Tukey multiple comparison test were applied to the results of each estimate to compare means at a 95% level, aiming to identify significant differences between the study regions.

Concerning functional beta diversity, we employed the kernel.beta function (Mammola & Cardoso, 2020), calculating the components of replacement between hypervolumes ( $\beta_{\text{replacement}}$ ), the difference in richness between hypervolumes ( $\beta_{\text{richness}}$ ), and the total functional beta diversity ( $\beta_{\text{total}} = \beta_{\text{replacement}} + \beta_{\text{richness}}$ ). It is important to remember that replacement is associated with the substitution of species between communities or sampling stations. At the same time, the difference in richness is understood as a subset (secondary communities) that is part of a more extensive set (central community), differentiating in species number (de Cáceres et al., 2013; Legendre, 2014; Schmera et al., 2020). For functional diversity, we used the libraries BAT, FD, ggplot2, gridExtra, hypervolume, psych, and StatMatch, using the same statistical program, RStudio.

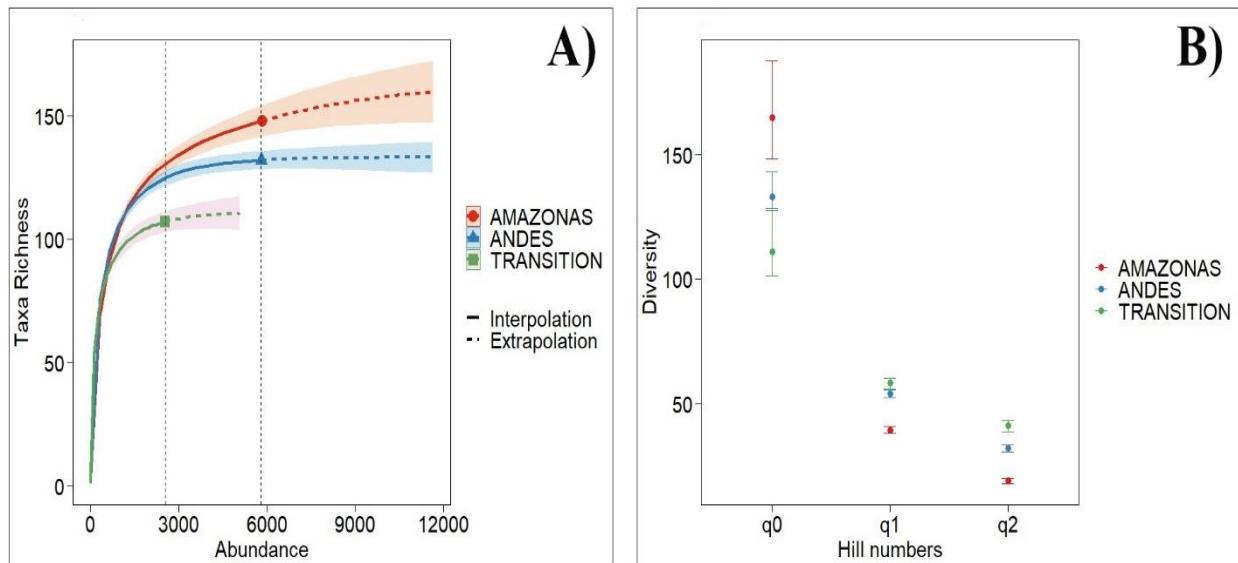
Finally, considering the environmental variables (see **Table S2, S3, S4** in the supplementary material), we conducted two ordination analyses to determine their impact on aquatic macroinvertebrates composition and beta diversity. These analyses classify objects according to predictor variables (Rodríguez da Silva et al., 2022). To prepare the data, we converted the abundances using the Hellinger scale (as the dependent variable). We selected environmental variables (as independent variables) with low correlation among them using the forward.sel function; then, we standardized them with the decostand function. In the first analysis, we employed a Redundancy Analysis (RDA) that accepts any independent variables and continuous dependent variables to examine the effect of the selected environmental variables on the composition of aquatic macroinvertebrates. In the second analysis, we used a dbRDA (Distance-based Redundancy Analysis for beta diversity, which accepts any independent variables and a distance matrix based on the studied dependent variables) to explore how the independent variables might influence beta diversity, using the Bray-Curtis measure (for more details, see the book by Rodríguez da Silva et al., 2022). With the anova.cca function we identified the environmental variables that can influence the composition and beta diversity of aquatic macroinvertebrates. In addition, we made a Veen diagram to relate the partitioned variance of the different environmental variables concerning the composition and beta diversity of aquatic macroinvertebrates. We used the varpart, adespatial, spdep, mvabund, reshape, vegan libraries.

## Results

### *Taxonomic and Functional Diversity*

We identified five phyla, eleven classes, 19 orders, 81 families, and 246 taxa (in most cases down to genus) in 14,173 individuals associated with the 30 sampling stations in the Andean-Amazonian region (**Table S5, supplementary material**). Regarding the sampling's representativeness, in **Figure 2-A**, we observed a stable trend in the rarefaction curves for the Andes and Transition; however, a greater richness of taxa is estimated for Amazon.

Regarding the effective diversity reflected in the Hill numbers (**Figure 2-B**), we observe variations in richness, with the Amazon region being particularly notable. The Simpson index recorded the highest value in the Transition zone. The Shannon-Wiener diversity simultaneously reveals an overlap between the Andean and Transition regions, which stands out from Amazonia.

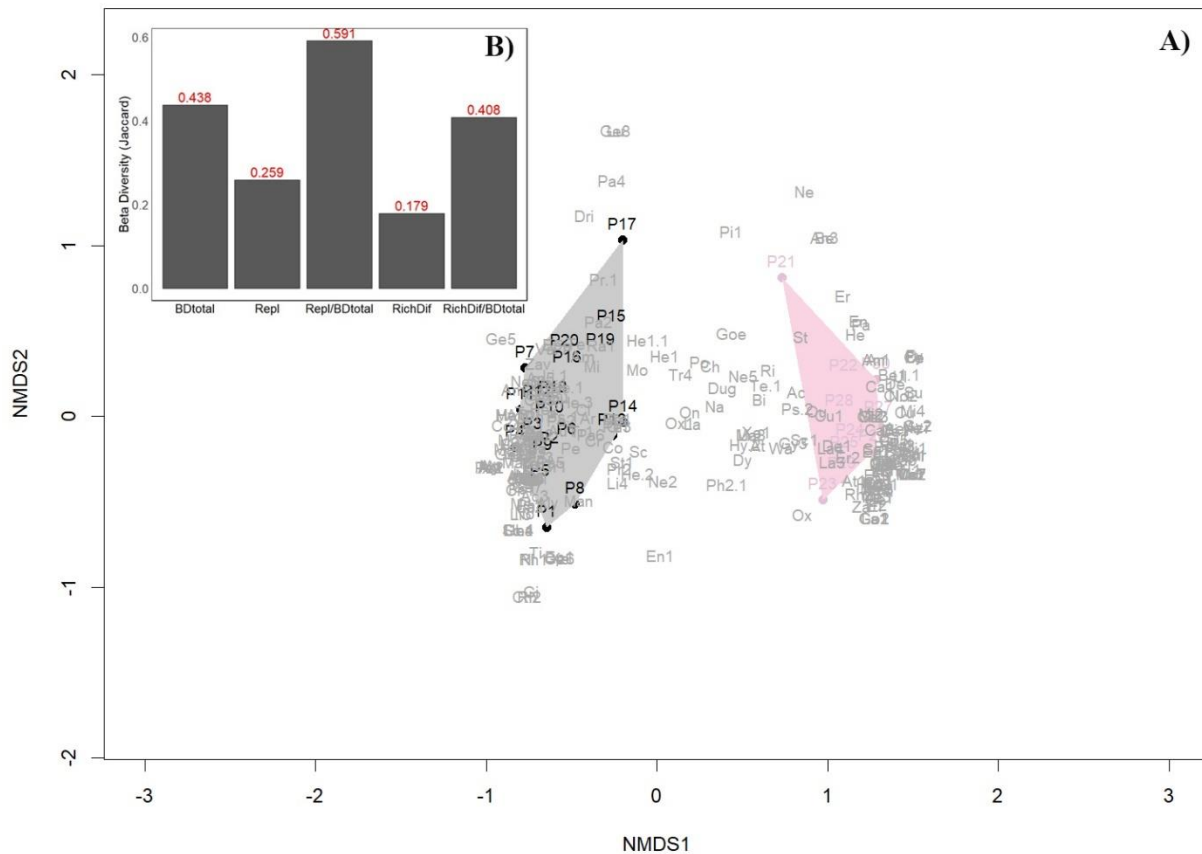


**Figure 2.** Sampling representativeness and estimated true diversity for the assembly of aquatic macroinvertebrates collected during field trips from October 2021 to April 2022 in the Andean-Amazonian region of the Caquetá department. Rarefaction curves (A) and Hill numbers (B) are shown.

When analyzing beta diversity, the NMDS method revealed two distinct clusters: a Stress value of 0.128 and a non-metric  $R^2$  of 0.98 (**Figure 3A**). Using ANOSIM, we obtained a p-value of 0.001 and an R of 0.8015, indicating statistically significant differences between regions based

on the composition of aquatic macroinvertebrates. The first cluster primarily grouped organisms from the Andean and Transition regions (Stations P1 to P20), while the second was associated with the Amazon region (Stations P21 to P30). As for the results of the taxonomic beta diversity components, calculated using the Bray-Curtis index (**Figure 3B**), taxon replacement was highlighted as the predominant factor, with a value of 0.591.

**Table S6** illustrates the results of the indicator taxa, determined by the IndVal association function. It selected 66 distinctive taxa, thirteen of which were significant for the Andes region, two for the Transition zone, and thirty for the Amazon. Thus, we observed differentiation related to significant taxa differing between regions, which is associated with beta diversity.

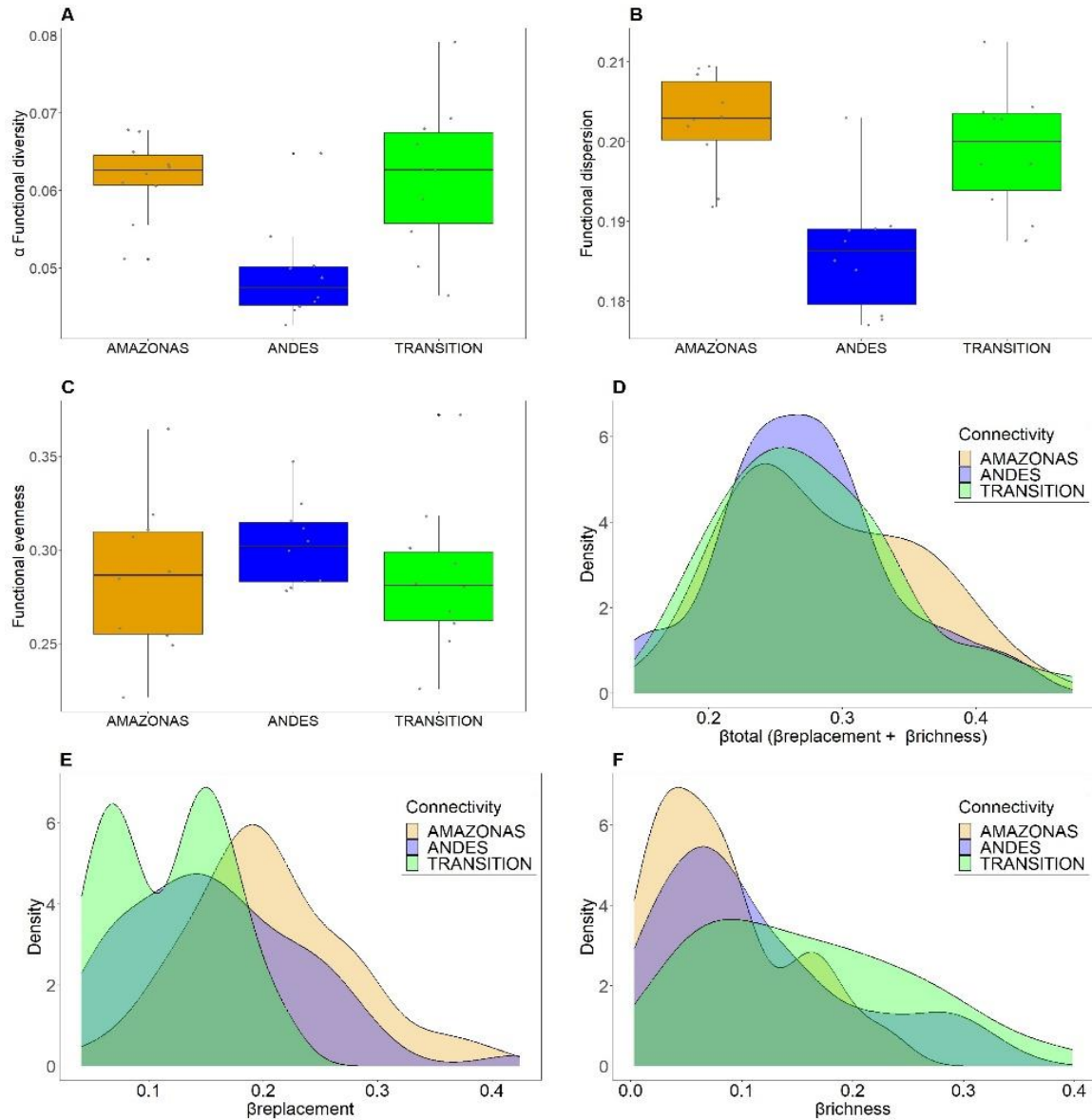


**Figure 3.** Results of the Non-metric Multidimensional Scaling (NMDS) analysis applied to the spatial distribution data of aquatic macroinvertebrate taxa collected during field trips from October 2021 to April 2022 in the Andean-Amazonian region of the Caquetá Department. The black dots represent stations associated with the Andes and Transition, and the pale red dot represents stations from the Amazonas region (A). The grey abbreviation identifies aquatic

macroinvertebrate taxa (see **Table S5** of the supplementary material). Histogram associating the components of taxonomic beta diversity (B).

Regarding the estimates of functional diversity presented in **Figure 4**, we identified that both functional diversity (**Figure 4A; Table S7**) and functional dispersion (**Figure 4B; Table S7**) are significant. On the other hand, functional evenness did not show statistically relevant differences between the regions (**Figure 4C; Table S7**). According to the results obtained from the Tukey multiple comparisons (**Table S7**), we identified that the comparisons between Andes – Transition and Andes – Amazon are statistically significant for functional diversity and dispersion, while the Transition – Amazon comparison is not.

**Figure 4** illustrates the components of functional beta diversity. Our results show that the total functional beta diversity exceeds 0.5, with more pronounced regional peaks around 0.3 (**Figure 4D**). As for the individual components, functional beta diversity due to replacement (**Figure 4E**) predominated over functional beta diversity attributed to differences in species richness (**Figure 4F**).

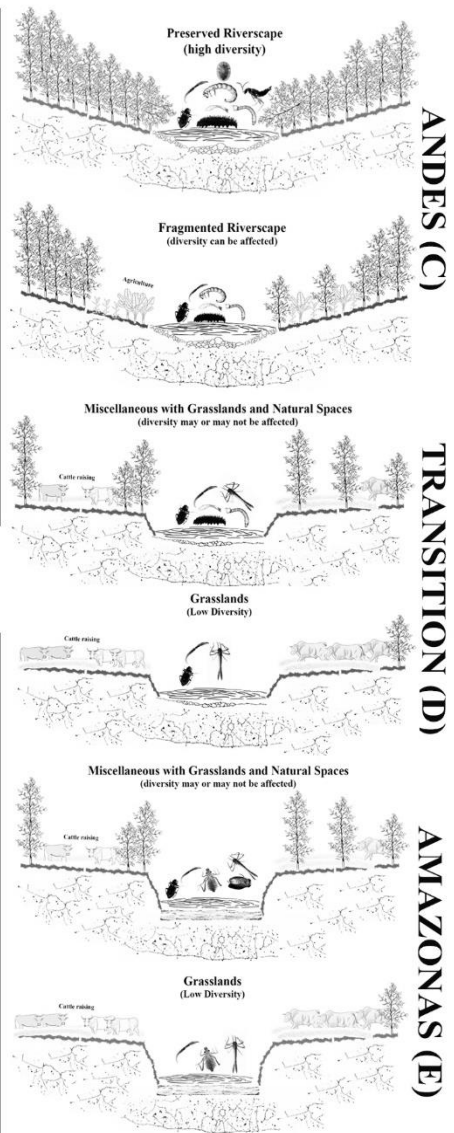
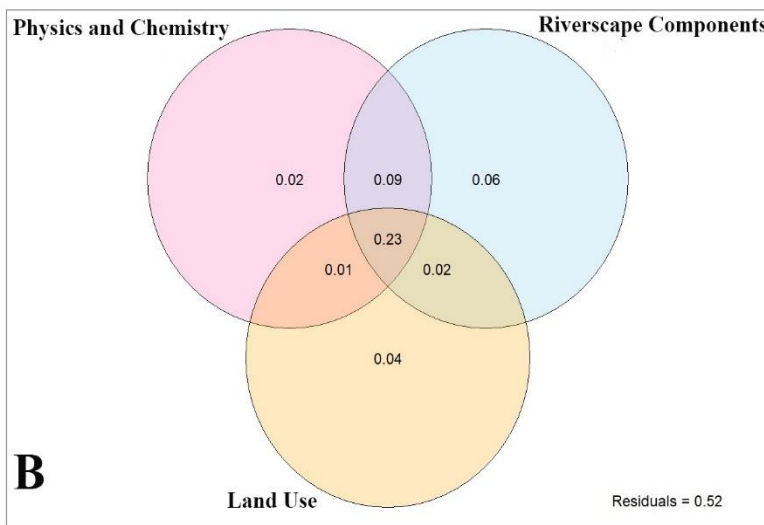
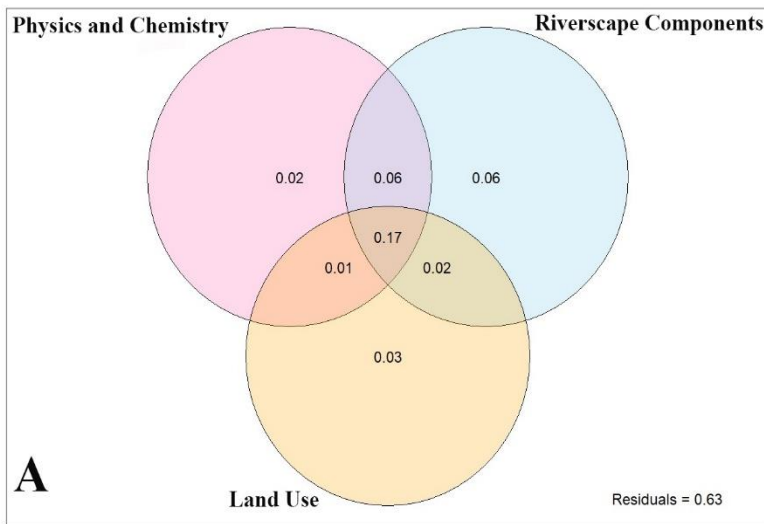


**Figure 4.** Estimates of functional diversity applied to the assembly of aquatic macroinvertebrates collected during field campaigns from October 2021 to April 2022 in the Andean-Amazonian region of the Caquetá Department.  $\alpha$  Functional diversity (A), Functional dispersion (B), Functional evenness (C), Total functional dissimilarity between Regions (D), Differences due to replacing functional space (E), and Differences in the amount of functional space (F).

### *Diversity of Aquatic Macroinvertebrates and Environmental Variables*

**Figure 5** details the results of the partitioned variance of environmental variables and their influence on the composition of aquatic macroinvertebrates (**Figure 5A**) and beta diversity (**Figure 5B**). The total interaction of these variables explains 0.17 of the composition and 0.23 of

beta diversity. For composition, according to **Table S8**, the most important physical and chemical predictor variables include dissolved oxygen (DO), conductivity (CO), and total dissolved solids (TDS), all with p value less than 0.05. Regarding the components of the river landscape, elements of plant origin (O.VEG), boulders (BLO), sand (ARE) and grasses (PA) also proved to be important. Regarding land use, grasslands (PAST.), forests (BOS), and recreational areas (REC) showed a notable influence. In relation to beta diversity taxonomic (Bray-Curtis), the same predictor variables are related, however the type of grassland vegetation (PA) is at the significant limit.



**Figure 5.** Venn diagrams relating the partitioned variance of environmental variables to the composition of aquatic macroinvertebrates (A) and their respective beta diversity (B), collected during the field campaigns from October 2021 to April 2022 in the Andean-Amazonian region of the Caquetá department. The illustration shows the conditions of the riverine riverscape across the three regions. In the Andes, a well-preserved riverine riverscape was observed, with some stations showing alteration in their components (C). In Transition (D) and Amazon (E), more significant deterioration of the riverine riverscape was evident, with miscellaneous stations without a riparian tree or shrub vegetation, where pastures and aquatic macrophytes dominated.

## Discussion

Our results, discussed below, support the research hypothesis that proposes a differentiation between the study regions in terms of composition, taxonomic diversity, and functional diversity of aquatic macroinvertebrates. Local environmental filters influence this differentiation between regions. However, we recognize that our findings serve as a starting point for future research incorporating processes of dispersion or phylogeny, as suggested by González-Trujillo et al. (2019; 2020) and Saito et al. (2015), for a more comprehensive understanding of the alpha and beta diversity patterns in the aquatic macroinvertebrate assemblage associated with the Andean-Amazonian region of the Caquetá department.

López-Erao et al. (2012), Cortés & Hernández (2016), Gutiérrez-Garaviz et al. (2016), Martínez-Dallos et al. (2016), and González et al. (2020, 2022), Bravo & Restrepo (2021), have previously documented numerous taxa of aquatic macroinvertebrates identified in this study in lotic ecosystems of the Andean-Amazonian region. However, our findings contribute to novel information for the study area, highlighting the presence of species and genera such as the Ephemeroptera *Andesiops peruvianus*, *Neotriplectides* spp. from the order Trichoptera, *Tholymis* spp. from the order Odonata, *Hydrochus* spp. from the order Coleoptera, and various taxa from the Chironomidae family, totaling 84 taxa (**Table S9**, supplementary material). Moreover, our data reveal a dissimilarity of 0.49 for the study area compared to previous studies.

These new contributions highlight the outstanding biodiversity that the Andean-Amazonian corridor of Caquetá can host. Indeed, this area has been considered a biodiversity hotspot in South America (Swenson et al., 2012; Asner et al., 2017; Pitman et al., 2021), and there is great concern over the loss of connectivity between regions due to the impact on the

riverscape from various anthropogenic activities, mainly deforestation (Anderson et al., 2018; Clerici et al., 2019; Linero et al., 2020; Murillo-Sandoval et al., 2022). These impacts are also evident in the department of Caquetá, focused on the Amazon, which has significantly increased after the peace process in 2016 between the government at that time and the Revolutionary Armed Forces of Colombia (FARC) (Clerici et al., 2019; Agudelo et al., 2023).

Our findings also reveal that the Amazon region hosts the highest richness of aquatic macroinvertebrates, with higher projected values based on estimates. Hoorn et al. (2010) explain this by demonstrating how the uplift of the Andes Mountains affected the climate, thus shaping the Amazon River landscape and defining biodiversity patterns that harbor high species richness. However, concerning Shannon-Wiener biodiversity (where wealth and equity are related) and the Simpson reciprocal, the Amazon shows a lower value than the Andes and Transition regions. The last two regions exhibit high environmental heterogeneity (climatic variation, altitudinal variation) that can support a wide range of ecological niches, favoring taxonomic distribution. Unlike the Amazon, which presents a relative homogeneity of environmental conditions, which can favor dominance in addition, there are other types of ecological processes, such as the flood pulse, which can be decisive in the composition of aquatic macroinvertebrates (Nessimian et al., 1998; Junk, 2005; McInerney et al., 2017; Araújo-Flores et al., 2021).

Regarding estimates of functional alpha diversity, we observed a different behavior compared to taxonomic diversity in the Andes and Transition regions, whereas in the Amazon, a notable level of diversity is maintained. Functional diversity assesses the richness of functional traits within a functional space in an ecosystem, while functional dispersion measures the variability of these functional traits within the functional space (Laliberté & Legendre, 2010; Cardoso et al., 2015; Shmera et al., 2017; Mammola & Cardoso, 2020). Furthermore, these estimates can reflect the role different species play in ecological processes such as primary productivity (Liu et al., 2021; Valina et al., 2017), metabolism (Gérino et al., 2003; Calapez et al., 2018), or energy flow (Merrit et al., 2014; Juvigny-Khenafou et al., 2021; Ao et al., 2023). Thus, the conditions of the riverine landscape for the Amazon and Transition can support a more remarkable coexistence of species with diverse functional strategies within a broader functional

space. In contrast, a narrower functional space in the Andes may exist whose adaptations are specific to mountain rivers, defining assemblages with more convergent ecological strategies.

The behavior of beta diversity among the study regions indicates a change in taxonomic composition and functional traits that respond to different environmental conditions, establishing niche distribution limits. The work of Shimabukuro et al. (2022) validates the hypothesis "mountain passes are higher in the Tropics" proposed by Janzen (1967), which suggests that geographical barriers such as mountain ranges (altitudinal variability) can be significant in the dispersion of species in tropical regions, unlike temperate areas where climatic oscillations are determinant in the establishment of an organism. Thus, the presence of the eastern Andes Mountain range in the Caquetá department defines a process of species rotation with the Amazon, thereby validating the indicated initial hypothesis. Furthermore, functional traits are defined by the local adaptations of taxa to the specific environmental conditions of each ecosystem (Heino et al., 2007; Tolomen et al., 2018; Ribeout et al., 2022), leading to functional specialization.

If we analyze the indicator species results for each region, in the Andes, genera associated with high mountain rivers stand out (belonging to the orders Diptera, Ephemeroptera, Trichoptera, and Coleoptera), with steep slopes, high current velocities, and rigid substrates (Domínguez et al., 2008; Roldán & Ramírez, 2022). If we evaluate an environmental variable such as hydraulic stress, which has an inverse relationship with the altitudinal gradient in the study area, we observe that genera such as *Leptohyphes* spp., *Nanomis* spp., or *Prebaetodes* spp. have optimized their bodies to resist water pressures; or genera like *Anchytarsus* spp., *Atopsyche* spp., or *Leptonema* spp. have developed strong claws to adhere to the substrate. Alternatively, the genus *Simúlium* spp. has developed a posterior sucker to adhere to the substrate and filaments on the head to filter food moving over the water current.

Regarding the Transition region, *Microcyllloepus* spp. stands out. It was collected on rocks with a significant flow of current, and its individuals, both adults and larvae, have developed tarsal claws that allow them to adhere to the substrate. The genus *Potabomobates* spp. was collected in calmer waters, which allows its mobility on the water surface.

About the Amazon, the riverscape conditions change, with a lesser influence of the altitudinal gradient and hydraulic stress (conditions more homogeneous than the Andes, as mentioned earlier), with characteristic genera of this region (Domínguez et al., 2008; Roldán & Ramírez, 2022). However, unlike the other two regions, these conditions provide greater diversity, leading to a functional species turnover reflected in a higher number of significant taxa. Comparing this region with the Andes and Transition regarding functional groups, collector or scraper organisms are prevalent for the latter two. In contrast, in the Amazon region, there is a wide variety of dipterans (genera of the families Chironomidae, Ceratopogonidae, or Culicidae), Coleoptera (genera of the families Dytiscidae, Noteridae, or Hydrophilidae), and Odonata (all the genera present) characterized by being predators within aquatic ecosystems. Our results align with the general concept of the river continuum proposed by Vannote et al. (1980), which indicates a prevalence of collectors and scrapers in the upper parts of rivers. As it decreases altitudinally towards the lowlands, collectors are maintained accompanied by predators.

Our findings underscore the significant role of local environmental filters in influencing the composition of the aquatic macroinvertebrate community within the Andean-Amazonian region, highlighting their impact on limiting species dispersion, as noted by Poff (1997), Coccia et al. (2021), and Sasahara et al. (2021). However, it would be of great interest to conduct future analyses on rare species, as their loss or substitution could have considerable consequences on specific ecosystem functions, as suggested by the study of Luiza-Andrade et al. (2023). For this, it is essential to understand the relationship that a component of the riverscape can have on the specific establishment of a taxon.

Wang et al. (2021) suggest that physical and chemical variables can influence the replacement or loss of functional traits. We further consider that they can also affect taxonomic replacement. Examining this consideration with the order Ephemeroptera and the availability of dissolved oxygen in the aquatic environment, in the Andes and Transition, tracheal gills with a more straightforward structure prevail (genera from the Baetidae family), which can easily capture the available gas. However, in the Amazon, this functional structure changes, with more complex tracheal gills observed, which directly relates to lower availability of dissolved oxygen

(for example, at station 30, which has a dissolved oxygen concentration of 0.52 mg L<sup>-1</sup>, the genus *Ulmeritoides* spp. with dense tracheal gills survives).

Olson & Hawkins (2017) have found that conductivity (CO) and total dissolved solids (TDS) can specifically affect the maintenance of ionic balance and the adaptive energy cost (mobility, growth, survival) of an aquatic insect, potentially limiting its distribution. However, our data do not measure that relationship, but they can be an essential reference for working with significant taxa in each of the study regions. Additionally, we can indicate that from a spatial scale perspective, there are differences between the study regions; for example, the average conductivity for Transition is the highest (30.13  $\mu\text{S cm}^{-1}$ ), compared with Andes (20.14  $\mu\text{S cm}^{-1}$ ) and Amazon (18.89  $\mu\text{S cm}^{-1}$ ), which may be related to an increase in lateral erosion attributed to the reduction of riparian vegetation cover. The opposite is true for TDS, which is more significant for Amazon (26,105) due to a greater accumulation of organic matter, recalling that this variable includes mineral and organic components (Chacón, 2016).

Revisiting Poff (1997) to discuss the river riverscape component, the substrate is a local-scale environmental filter crucial in establishing the aquatic macroinvertebrate community. Analyzing the regions, with a focus again on the order Ephemeroptera and specifically the Baetidae family, we observe the presence of *Baetodes* spp., *Camelobaetidius* spp., or *Andesiops peruvianus*, taxa associated with rocky substrates, which are replaced by *Callibaetis* spp. and *Apobaetis* spp. found in plant-based substrates.

Regarding the land use component, forest conservation stands out in the Andes region. However, in the Transition and Amazon regions, this conservation diminishes, with some stations showing a mix of natural spaces with pastures and stations with only pastures due to significant cattle farming activity that fragments riparian vegetation. Remember that riparian forests regulate river ecosystems and biodiversity; therefore, changes in vegetation cover can trigger taxonomic and functional changes within aquatic communities (Marques et al., 2021). Moreover, the functional and structural responses of the aquatic macroinvertebrate community can indicate the effectiveness of restoration processes (Espinoza-Toledo et al., 2021), something that would be

very interesting to evaluate in the future on stations like P26 and P27 in the Amazon region that have been completely modified.

In addition to the above, we consider that the presence of trees along the riparian margins in the Transition and Amazon regions allows for a high diversity of aquatic macroinvertebrates. For example, in stations P24 and P25, 54 and 70 different taxa were identified (the presence of trees and shrubs along the margins providing microhabitats in their roots for colonization). Thus, areas with natural spaces serve as a good reference for initiating ecological restoration work.

## **Conclusions**

Our research significantly contributes to the record of previously unknown taxa within the Andean-Amazonian region of the Caquetá department, presenting a dissimilarity value of 0.49 compared to reference studies. The findings of our study confirm the presence of marked differences in composition, taxonomic diversity, and functional diversity, notably highlighting the phenomenon of species turnover between the eastern Andes and the Amazonian plain. Importantly, our work also underscores the importance of physical and chemical variables and riverscape components, such as substrate and land use, which can act as environmental filters in determining the composition and diversity of aquatic macroinvertebrates.

Furthermore, it is essential to emphasize our concern about the loss of connectivity between the Andes and the Amazon. This issue is of utmost importance as it could have profound implications for the biodiversity of aquatic macroinvertebrates and ecosystem services in the region. Habitat fragmentation, driven by activities primarily associated with extensive livestock farming, may threaten the continuity of ecological functions and viability of native aquatic assemblages, underscoring the urgent need for conservation and restoration strategies to preserve ecological connectivity. Integrating our findings could provide a solid basis for developing sustainable management policies to mitigate adverse impacts on biodiversity and promote ecosystem resilience in the Andean-Amazon corridor.

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## Supplementary Material

**Table S1.** Geographic information related to the sampling stations where aquatic macroinvertebrates were collected in the Andean-Amazon region, during the 2021 and 2022 campaigns.

Estação	Altitude	XCoord	YCoord
P1	2205	-75,6796	1,8781
P2	2202	-75,6786	1,8773
P3	2070	-75,6700	1,8690
P4	1540	-75,6618	1,8404
P5	1000	-75,6470	1,7951
P6	957	-75,6394	1,7708
P7	2168	-75,7663	1,7627
P8	2041	-75,7496	1,7562
P9	1697	-75,7231	1,7359
P10	581	-75,6456	1,7294
P11	396	-75,6723	1,6236
P12	492	-75,9832	1,2977
P13	321	-75,9538	1,3350
P14	300	-75,9181	1,3622
P15	308	-75,8866	1,4083

P16	285	-75,2284	1,7665
P17	285	-75,2361	1,7539
P18	378	-75,3017	1,6362
P19	293	-75,3363	1,5427
P20	276	-75,3426	1,5205
P21	247	-75,3594	1,4283
P22	252	-75,3582	1,4253
P23	231	-75,3448	1,3754
P24	237	-75,3853	1,3572
P25	245	-75,4162	1,3358
E26	243	-75,4346	1,2999
E27	233	-75,4300	1,3052
E28	235	-75,4356	1,2954
E29	266	-75,4396	1,2821
E30	234	-75,4295	1,2973

**Table S2.** Physical and chemical variables related to the sampling stations where aquatic macroinvertebrates were collected in the Andean-Amazon region, during the 2021 and 2022 campaigns. Current velocity (VE), dissolved oxygen (OD), hydrogen ion potential (pH), conductivity (CO), total dissolved solids (TDS), water temperature (TE), sheet width (AL), Depth (PR).

STATION	VE	OD	PH	CO	TDS	TE	AL	PR
P1	0,22	7,5	7,363	28,85	14,4	14,4	2,6	0,05
P2	0,31	7,65	7,423	18,2	13,5	13,5	1,08	0,1
P3	0,29	7,775	7,393	14,875	13,875	13,875	8	0,2
P4	0,44	7,2	7,363	15,975	16,75	16,75	12	0,26
P5	0,41	7,075	7,273	20,475	19,7	19,7	15	0,26
P6	0,24	6,45	7,295	24,975	20,7	20,7	5,8	0,28
P7	0,36	7,08	8,998	12,475	13,225	13,225	2,5	0,07
P8	0,19	7,863	8,638	11,125	14,45	14,45	4	0,22
P9	0,23	7,693	7,325	23,975	17	17	1,2	0,04
P10	0,32	6,885	7,98	30,475	23,3	23,3	24	0,37
P11	0,21	6,425	7,72	36,5	24,1	24,1	7	0,28
P12	0,53	6,25	7,378	39,65	24,2	24,2	22	0,3
P13	0,39	6,3	7,26	28,7	24,375	24,375	16	0,37
P14	0,85	6,45	7,135	29,925	24,325	24,325	5	0,23
P15	0,71	6,6	7,505	32,775	23,55	23,55	13,5	0,35
P16	1,11	5,875	7,615	33,875	28,75	28,75	9	0,29
P17	0,44	5,175	7,26	30,625	29,368	29,368	18	0,14
P18	0,29	6,05	7,498	29,025	25,65	25,65	9	0,36
P19	0,45	5,825	7,223	19,025	25,675	25,675	9,5	0,15

P20	0,35	5,875	7,485	21,2	25,95	25,95	4,5	0,18
P21	0,72	5,925	7,07	18,9	25,775	25,775	22	0,36
P22	0,08	4,2	6,235	15,85	25,8	25,8	20	0,77
P23	0,08	3,95	6,115	18,75	24,975	24,975	5	0,84
P24	0,06	4,5	6,128	13,975	25,4	25,4	4	0,67
P25	0,06	3,25	6,108	13,6	25,2	25,2	3	0,58
P26	0,02	3,3	5,713	22,15	26,575	26,575	3,5	1,3
P27	0,02	1,875	6,06	25,15	27,575	27,575	25	0,22
P28	0,09	4,685	5,798	15,75	27,475	27,475	2	0,6
P29	0,06	3,45	6,098	13,675	26,5	26,5	10	0,91
P30	0,003	0,525	5,743	31,1	25,775	25,775	4	0,13

**Table S3.** Riverscape components related to the sampling stations where aquatic macroinvertebrates were collected in the Andean-Amazon region, during the 2021 and 2022 campaigns. Percentage (%) of vegetation cover (C.V), trees (ARB.), shrubs (ARBU.), herbaceous (HER.), grasses (PA.), blocks (BLO.), stones (CANT.), gravel (GRAV.), sand (ARE.), vegetable origin (O.VEG).

STATION	C.V	ARB.	ARBU.	PA.	HER.	BLO.	CANT.	GRAV.	ARE.	O.VEG
P1	0,95	0,7	0,3	0	0	0,7	0,1	0,05	0,1	0,05
P2	1	0,7	0,3	0	0	0,75	0,1	0,05	0,05	0,05
P3	0,9	0,2	0,4	0	0,4	0,65	0,1	0,1	0,1	0,05
P4	0,9	0,4	0,6	0,1	0,1	0,7	0,1	0,1	0,1	0
P5	0,6	0,4	0,2	0,2	0,2	0,6	0,2	0,1	0,1	0
P6	0,6	0,4	0,2	0,3	0,1	0,55	0,1	0,1	0,2	0,05
P7	0,9	0,6	0,3	0	0,1	0,2	0,35	0,2	0,2	0,05
P8	0,6	0,4	0,6	0	0	0,5	0,25	0,1	0,1	0,05
P9	0,7	0,2	0,6	0	0,2	0,1	0,55	0,2	0,1	0,05
P10	0,8	0,7	0,1	0,1	0,1	0,3	0,4	0,05	0,2	0,05
P11	0,5	0,3	0,1	0,4	0,2	0,4	0,2	0,1	0,3	0
P12	0,6	0,4	0,3	0,1	0,2	0,2	0,4	0,1	0,2	0,05
P13	0,6	0,4	0,2	0,2	0,2	0,2	0,4	0,2	0,2	0
P14	0,4	0,2	0,2	0,6	0	0,1	0,6	0,1	0,2	0
P15	0,5	0,3	0,2	0,5	0	0,2	0,4	0,3	0,1	0
P16	0,3	0,1	0	0,8	0,1	0,1	0,4	0,2	0,3	0
P17	0,8	0,6	0,2	0,1	0,1	0	0,5	0,2	0,3	0
P18	0,3	0,2	0	0,8	0	0,6	0,2	0,1	0,1	0
P19	0,3	0,2	0	0,8	0	0,1	0,4	0,2	0,3	0
P20	0,4	0,1	0	0,8	0,1	0,1	0,5	0,1	0,3	0
P21	0,4	0,4	0,2	0,4	0	0	0	0,2	0,6	0,2
P22	0,4	0,1	0,4	0,4	0,1	0	0	0	0,3	0,4
P23	0,4	0,2	0,4	0,4	0	0	0	0	0	0,3
P24	0,3	0,3	0,4	0,3	0	0	0	0	0	0,3

P25	0,5	0,1	0,4	0,4	0,1	0	0	0	0	0,3
P26	0	0	0	0,5	0,5	0	0	0	0	0,3
P27	0	0	0	0,5	0,5	0	0	0	0	0,3
P28	0,4	0,1	0,2	0,5	0,2	0	0	0	0	0,3
P29	0,5	0,5	0,1	0,3	0,1	0	0	0	0	0,3
P30	0,6	0,3	0,2	0	0,5	0	0	0	0	0,6

**Table S4.** Land uses identified in the study stations where aquatic macroinvertebrates were collected in the Andean-Amazon region, during the 2021 and 2022 campaigns. Forest (BOS.), agriculture (AGR.), recreation (RECR.), settlement (ASEN.), grazing (PAST.).

STATION	BOS.	AGR.	RECR.	ASEN.	PAST.
P1	1	0	0	0	0
P2	1	0	0	0	0
P3	0,8	0,2	0	0	0
P4	0,8	0,2	0	0	0
P5	0,3	0,5	0	0	0,2
P6	0,3	0,4	0	0	0,3
P7	1	0	0	0	0
P8	1	0	0	0	0
P9	0,2	0,6	0	0	0,2
P10	0,3	0,2	0,1	0,1	0,3
P11	0,1	0,1	0,1	0	0,7
P12	0,15	0,1	0,1	0,05	0,6
P13	0,2	0,2	0	0,05	0,55
P14	0,15	0,2	0	0,05	0,6
P15	0,15	0,2	0,1	0,05	0,5
P16	0	0	0,2	0	0,8
P17	0,4	0,2	0,1	0	0,3
P18	0	0	0,4	0	0,6
P19	0,1	0	0,3	0	0,6
P20	0	0,2	0,1	0	0,7
P21	0,1	0,2	0,1	0	0,6
P22	0,1	0,1	0	0	0,8
P23	0,1	0,1	0	0,05	0,75
P24	0,1	0,1	0	0,05	0,75
P25	0,35	0,1	0	0,05	0,5
P26	0	0	0	0	1
P27	0	0	0	0	1
P28	0	0	0	0	1
P29	0,1	0	0,1	0,05	0,75
P30	0,1	0	0	0	0,9

**Table S5.** Aquatic macroinvertebrates taxa collected and identified in the study region, during the 2021 and 2022 sampling campaigns.

PHYLUM	CLASE	ORDEN	FAMILIA	GENUS, SPECIE**, MORPHO***	CODE	
Annelida	Clitelata	Rhynchobdellida	Glossiphoniidae	<i>Helobdella</i> spp.	He	
	Oligochaeta	Haplotaxida	Naididae	Naididae_N.D	Na	
Arthropoda	Arachnida	Trombidiformes	Lumbriculidae	Lumbriculidae_N.D.	Lu	
			Limnesiidae	Limnesiidae_N.D.	Li	
			Hydryphantidae	Morfo 1***	Ac1	
			Limnocharidae	Morfo 2***	Ac2	
	Insecta	Coleoptera	Curculionidae	Sperchontidae	Morfo 3***	Ac3
				Dryopidae	<i>Dryops</i> spp.	Dri
				Dytiscidae	<i>Anodocheilus</i> spp.	An
					<i>Celina</i> spp.	Ce
					<i>Hydrovatus</i> spp.	Hy
					<i>Laccophilus</i> spp.	Lac
					<i>Laccodytes</i> spp.	Lacc
					<i>Liodessus</i> spp.	Li
					<i>Neoclypeodytes</i> spp.	Ne
					<i>Neobidesus</i> spp.	Neo
					<i>Pachydrus</i> spp.	Pa
	<i>Vatellus</i> spp.	Va				
Elmidae			<i>Austrelmis</i> spp.	Au		
			<i>Austrolimnius</i> spp.	Aus		
			<i>Cylloepus</i> spp.	Cy		
			<i>Disersus</i> spp.	Di		
			<i>Epodelmis</i> spp.	Ep		
			<i>Gyrelmis</i> spp.	Gy		
			<i>Heterelmis</i> spp.	Het		
			<i>Hexacylloepus</i> spp.	Hex		
			<i>Hexanchorus</i> spp.	Hexa		
			<i>Hintonelmis</i> spp.	Hi		
			<i>Huleechius</i> spp.	Hu		
			<i>Macrelmis</i> spp.	Ma		
			<i>Microcylloepus</i> spp.	Mi		
			<i>Neelmis</i> spp.	Neol		
			<i>Neolimnius</i> spp.	Neoli		
		<i>Notelmis</i> spp.	No			
		<i>Phanocerus</i> spp.	Pha			
		<i>Pharceonus</i> spp.	Phar			
		<i>Pilielmis</i> spp.	Pi			
		<i>Stegoelmis</i> spp.	Ste			

		<i>Pseudodisersus</i> spp.	Pse
		<i>Xenelmis</i> spp.	Xe
	Gyrinidae	<i>Gyretes</i> spp.	Gyr
		<i>Gyrinus</i> spp.	Gyri
		<i>Macrogyrus</i> spp.	Mac
	Hydrochidae	<i>Hydrochus</i> spp.	Hyd
	Hydrophilidae	<i>Anacaena</i> spp.	Ana
		<i>Hemiosus</i> spp.	Hem
		<i>Berosus</i> spp.	Be
		<i>Derallus</i> spp.	De
		<i>Enochrus</i> spp.	En
		<i>Laccobius</i> spp.	Lacco
	Lutrochidae	<i>Lutrochus</i> spp.	Lut
	Noteridae	<i>Hydrocanthus</i> spp.	Hydr
		<i>Notomicrus</i> spp.	Not
		<i>Suphisellus</i> spp.	Su
	Ptilodactylidae	<i>Anchytarsus</i> spp.	Anc
	Scirtidae	<i>Scirtes</i> spp.	Scir
		Scirtidae_N.D	Scirnd
	Psephenidae	<i>Pheneps</i> spp.	Phe
		<i>Psephenus</i> spp.	Psep
	Staphylinidae	Staphylinidae_N.D	Sta
Diptera	Blephariceridae	<i>Aposonalco</i> spp.	Ap
		<i>Limonicola</i> spp.	limon
	Ceratopogonidae	<i>Atrichopogon</i> spp.	Atri
		<i>Bezzia</i> spp.	Bez
		<i>Ceratopogon</i> spp.	Cera
		<i>Culicoides</i> spp.	Cul
		<i>Forcipomyia</i> spp.	For
		<i>Dasyhelea</i> spp.	Dasy
		<i>Paryphoconus</i> spp.	Pary
	Chironominae*	<i>Endotribelos</i> spp.	Endo
		<i>Tanytarsus</i> spp.	Tany
		<i>Rheotanytarsus</i> spp.	Rheot
		<i>Polypedilum</i> spp.	Polyp
		<i>Chironomus</i> sp1.	Chiro
	se cambia por maona	Chironomini***	Chirono
		<i>Xestochironomus</i> spp.	Xest
		<i>Riethia</i> spp.	Rieth
		Ge_x10***	Ge_10
		<i>Zavreliella</i> spp.	Zavre
		<i>Parachironomus</i> spp.	Pary
		<i>Caladomyia</i> spp.	Cala
		<i>Chironomus</i> sp2.	Chironom2

	<i>Oukuriella</i> spp.	Ou
	<i>Saetheria</i> spp.	Sa
	Ge_x1***	Ge
	Ge_x2***	Ge
Orthoclaadiinae	<i>Cricotopus</i> spp.	Cr
	<i>Parametrioctenemus</i> spp.	Pa
	<i>Thienemanniella</i> spp.	Thi
	<i>Antillocladius</i> spp.	Ant
	<i>Corynoneura</i> spp.	Co
	cf. <i>Cardiocladius</i>	Car
	<i>Onconeura</i> spp.	On
	<i>Orthocladus</i> spp.	Ort
	Ge_x8***	Ge9
	Ge_x9***	Ge10
Tanypodinae	<i>Larsia</i> spp.	La
	<i>Nilotanypus</i> spp.	Ni
	<i>Ablabesmyia</i> spp.	Ge
	<i>Pentaneura</i> spp.	Pe
	<i>Denopelopia</i> spp.	De
	cf. <i>Zavreliomyia</i>	Zav
	cf. <i>Macropelopia</i>	Ge
	<i>Labrundinia</i> spp.	La
	<i>Coelotanypus</i> spp.	Co
Culicidae	<i>Anopheles</i> spp.	An
	<i>Culex</i> spp.	Cu
	<i>Mansonia</i> spp.	Ma
	<i>Uranotaenia</i> spp.	Ur
Dixidae	<i>Dixella</i> spp.	Di
Dolichopodidae	<i>Rhaphium</i> spp.	Rh
Ephydriidae	Ephydriidae_N.D	Ep
Empididae	<i>Hemerodromia</i> spp.	He
	<i>Chelifera</i> spp.	Ch
Cf. Mycetophilidae	Mycetophilidae_N.D	My
Muscidae	<i>Limnophora</i> spp.	Li
Sciomyzidae	Sciomyzidae_N.D	Sc
Simuliidae	<i>Gigantodax</i> spp.	Gi
	<i>Simulium</i> spp.	Si
Psychodidae	<i>Maruina</i> spp.	Ma
	<i>Psychoda</i> spp.	Ps
	<i>Pericoma</i> spp.	Pe
Tipulidae	<i>Cheilotrichia</i> spp.	Ch
	<i>Hexatoma</i> spp.	He
	<i>Limonia</i> spp.	Li
	<i>Molophilus</i> spp.	Mo

		<i>Erioptera</i> spp.	Er
		<i>Tipula</i> spp.	Ti
Ephemeroptera	Baetidae	<i>Andesiops peruvianus</i> .	An
		<i>Americabaetis</i> spp.	Am
		<i>Apobaetis</i> spp.	Ap
		<i>Aturbina</i> spp.	At
		<i>Baetodes</i> spp.	Ba
		<i>Callibaetis</i> spp.	Ca
		<i>Camellobaetidius</i> spp.	Ca
		<i>Cloeodes</i> spp.	Cl
		<i>Guajirolus</i> spp.	Gu
		<i>Mayobaetis</i> spp.	Ma
		<i>Nanomis</i> spp.	Na
		<i>Paracloeodes</i> spp.	Pa
		<i>Prebaetodes</i> spp.	Pr
		<i>Varipes</i> spp.	Va
		<i>Waltzoyphius</i> spp.	Wa
	Caenidae	<i>Brasilocaenis</i> spp.	Br
		<i>Caenis</i> spp.	Ca
	Coryphoridae	<i>Coryphorus aquilus</i>	Co
	Euthyplociidae	<i>Campylocia</i> spp.	Ca
		<i>Euthyplocia</i> spp.	Eu
	Leptophlebiidae	<i>Ecuaphlebia</i> spp.	Ec
		<i>Farrodes</i> spp.	Fa
		<i>Fittkaulus amazonicus</i>	Fi
		<i>Hydrosmilodon</i> spp.	Hy
		<i>Miroculis</i> spp.	Mi
		<i>Terpides</i> spp.	Te
		<i>Thraulodes</i> spp.	Th
		<i>Ulmeritoides</i> spp.	Ul
	Leptohiphidae	<i>Amanahyphes</i> spp.	Am
		<i>Haplohyphes</i> spp.	Ha
		<i>Leptohiphes</i> spp.	Le
		<i>Traverhyphes</i> spp.	Tr
		<i>Tricorythodes</i> spp.	Tr
		<i>Tricorythopsis</i> spp.	Tr
		<i>Tricorythopsis rondonienses</i>	Tr
	Oligoneuridae	<i>Lachlania</i> spp.	La
	Polymitarciidae	<i>Campsurus</i> spp.	Ca
Lepidoptera	Crambidae	<i>Oxyelophila</i> spp.	Ox
		<i>Parapoynx</i> spp.	Pa
		<i>Petrophila</i> spp.	Pe
Megaloptera	Corydalidae	<i>Corydalus</i> spp.	Co
Plecoptera	Perlidae	<i>Anacroneuria</i> spp.	An

Trichoptera	Atriplectididae	<i>Neotriplectides</i> spp.	Ne	
	Calamoceratidae	<i>Phylloicus</i> spp.	Ph	
	Glossosomatidae	<i>Mortoniella</i> spp.	Mo	
	Hidrobiosidae	<i>Atopsyche</i> spp.	At	
	Helycopsychidae	<i>Helycopsyche</i> spp.	He	
	Hydropsychidae	<i>Leptonema</i> spp.	Le	
		<i>Macronema</i> spp.	Ma	
		<i>Smicridea</i> spp.	Sm	
		Hydroptilidae	cf. <i>Alisotrichia</i>	Aly
			<i>Metrichia</i> spp.	Me
			<i>Neotrichia</i> spp.	Ne
	<i>Oxyethira</i> spp.		Ox	
	Leptoceridae	<i>Grumichela</i> spp.	Gr	
		<i>Netopsyche</i> spp.	Ne	
		<i>Oecetis</i> spp.	Oe	
		<i>Triplectides</i> spp.	Tr	
		<i>Odontoceridae</i>	<i>Marilia</i> spp.	Ma
	Philopotamidae	<i>Chimarra</i> spp.	Ch	
	Polycentropodidae	<i>Cernotina</i> spp.	Ce	
		<i>Cyrnellus</i> spp.	Cy	
		<i>Polyplectropus</i> spp.	Po	
		<i>Xiphocentronidae</i>	<i>Xiphocentron</i> spp.	Xi
	Hemiptera	Belostomatidae	<i>Belostoma</i> spp.	Be
Corixidae		<i>Tenagobia</i> spp.	Te	
Guerridae		<i>Neogerris</i> spp.	Ne	
		<i>Trepobates</i> spp.	Tr	
		<i>Potamobates</i> spp.	Po	
		<i>Rheumatobates</i> spp.	Rh	
Naucoridae		<i>Ambrysus</i> spp.	Am	
		<i>Cryphocricos</i> spp.	Cr	
		<i>Limnocoris</i> spp.	Li	
		<i>Pelocoris</i> spp.	Pe	
		<i>Procryphocricos</i> spp.	Pr	
Nepidae		<i>Ranatra</i> spp.	Ra	
Notonectidae		<i>Buenoa</i> spp.	Bu	
		<i>Martarega</i> spp.	Ma	
		<i>Notonecta</i> spp.	No	
Mesoveliidae		<i>Mesovelia</i> spp.	Me	
Veliidae	<i>Microvelia</i> spp.	Mi		
	<i>Rhagovelia</i> spp.	Ra		
	<i>Stridulivelia</i> spp.	St		
Odonata	Aesnidae	cf. <i>Coryphaeschna</i>	Cor	
	Calopterygidae	<i>Hetaerina</i> spp.	He	
	Coenagrionidae	<i>Acanthagrion</i> spp.	Ac	

				<i>Argia</i> spp.	Ar
				<i>Enallagma</i> spp.	En
				<i>Epipleoneura</i> spp.	Ep
				<i>Protoneura</i> spp.	Pr
			Corduliidae	<i>Aeschnosoma</i> spp.	Ae
			Gomphidae	<i>Agriogomphus</i> spp.	Ag
				<i>Progomphus</i> spp.	Pr
				<i>Phyllocycla</i> spp.	Ph
			Megapodagrionidae	<i>Heteragrion</i> spp.	He
			Libellulidae	<i>Elga</i> spp.	El
				<i>Erythemis</i> spp.	Er
				<i>Erythrodiplax</i> spp.	Er
				<i>Diastatops</i> spp.	Di
				<i>Dythemis</i> spp.	Dy
				<i>Fylgia</i> spp.	Fy
				<i>Gynothemis</i> spp.	Gy
				<i>Miathyria</i> spp.	Mi
				<i>Micrathyria</i> spp.	Mi
				<i>Planiplax</i> spp.	Pl
				<i>Tholymis</i> spp.	Th
			Platystictidae	<i>Palaemnema</i> spp.	Pa
			Polythoridae	<i>Cora</i> spp.	Co
	Crustacea	Decapoda	Palaemonidae	<i>Macrobrachium</i> spp.	Ma
			<i>Trychodactilidae</i>	<i>Silviocarcinus</i> spp.	Si
	Copepoda	N.D	N.D	Copepoda N.D	Co
	Cladocera	N.D	N.D	Cladocera N.D	Cl
Mollusca	Gastropoda	Basommatophora	Planorbidae	<i>Acorbis</i> spp.	Acr
				<i>Biomphalaria</i> spp.	Bi
	Bivalvia	Veneriodes	Sphaeriidae	<i>Pisidium</i> spp.	Pi
Nematoda	N.D	N.D	N.D	Nematoda_N.D	Ne
Plathelminthes	Turbellaria	Tricladida	Planariidae	<i>Dugesia</i> spp.	Dug

**Table S6.** IndVal results of indicator taxa, related to the three study stations of the Andean-Amazon region, worked during the 2021 and 2022 sampling campaigns. Significance level (alpha): 0.05. Total number of species: 246. Selected number of species: 66. Number of species associated to 1 group: 45. Number of species associated to 2 groups: 21.

TAXA	ANDES	TRANSITION	AMAZONAS
<i>Mirocylis</i> spp.			0.001***
<i>Fittkaulus</i> spp.			0.001***
<i>Epipleoneura</i> spp.			0.001***
<i>Larsia</i> spp.			0.001***
<i>Callibaetis</i> spp.			0.001***

<i>Aturbina</i> spp.		0.001***
<i>Culex</i> spp.		0.001***
<i>Oecetis</i> spp.		0.001***
Copetoda_N.D		0.001***
<i>Denopelopia</i> spp.		0.001***
<i>Labrundinia</i> spp.		0.002**
<i>Erythrodiplax</i> spp.		0.005**
<i>Pachydrus</i> spp.		0.001***
<i>Brasilocaenis</i> spp.		0.001***
<i>Helobdella</i> spp.		0.004**
<i>Notomicrus</i> spp.		0.012*
<i>Culicoides</i> spp.		0.009**
<i>Microvelia</i> spp.		0.009**
<i>Tenagobia</i> spp.		0.008**
<i>Stegoelmis</i> spp.		0.012*
<i>Riethia</i> spp.		0.032*
<i>Waltzoyphius</i> spp.		0.022*
<i>Gyrelmis</i> spp.		0.019*
<i>Enochrus</i> spp.		0.019*
<i>Ceratopogon</i> spp.		0.021*
<i>Paryphoconus</i> spp.		0.023*
<i>Erioptera</i> spp.		0.023*
<i>Protoneura</i> spp.		0.030*
<i>Diastatops</i> spp.		0.020*
<i>Oukuriella</i> spp.		0.020*
<i>Ablabesmyia</i> spp.	0.001***	
<i>Atopsyche</i> spp.	0.001***	
<i>Leptohyphes</i> spp.	0.001***	
<i>Simulium</i> spp.	0.001***	
<i>Nanomis</i> spp.	0.001***	
<i>Anchytarsus</i> spp.	0.001***	
<i>Chelifera</i> spp.	0.002**	
<i>Pseudodisersus</i> spp.	0.001***	
<i>Leptonema</i> spp.	0.007**	
<i>Notelmis</i> spp.	0.010**	
<i>Phylloicus</i> spp.	0.010**	
<i>Prebaetodes</i> spp.	0.013*	
<i>Helycopsyche</i> spp.	0.010**	
<i>Microcylloepus</i> spp.		0.019*
<i>Potamobates</i> spp.		0.025*

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**Table S7.** Results of the analysis of variance (ANOVA) applied to the alpha functional diversity data of aquatic macroinvertebrates collected in the Andean-Amazon region during the 2021 and 2022 sampling campaigns.

<b>ESTIMATE</b>	Sum Sq	Mean Sq	F Value	Pr(>F)
Functional Diversity	0.001	0.0005	9.759	0.00064***
Functional Dispersion	0.002976	0.0014882	17.34	1.43e-05***
Functional Evenness	0.00202	0.001009	0.782	0.468
<b>TUKEY MULTIPLE COMPARISON OF MEAN</b>	Diff	lwr	upr	p adj
<b>Functional Diversity</b>				
ANDES-AMAZONAS	0.0125208041	0.020656797	0.004384811	0.0020132***
TRANSITION-AMAZONAS	0.0000671351	0.008068858	0.008203128	0.9997693
TRANSITION-ANDES	0.0125879392	0.004451946	0.020723932	0.0019095***
<b>Functional Dispersion</b>				
ANDES-AMAZONAS	-0.023629011	-0.03390189	0.013356136	0.0000135***
TRANSITION-AMAZONAS	-0.006549116	-0.01682199	0.003723759	0.2710539
TRANSITION-ANDES	0.017079895	0.00680702	0.027352770	0.0009055***
<b>Functional Evenness</b>				
ANDES-AMAZONAS	0.0125208041	0.020656797	0.004384811	0.0020132***
TRANSITION-AMAZONAS	0.0000671351	0.008068858	0.008203128	0.9997693
TRANSITION-ANDES	0.0125879392	0.004451946	0.020723932	0.0019095***

**Table S8.** Results of the RDA and dbRDA related to the influence of environmental variables on the composition and beta diversity of aquatic macroinvertebrates of the Andean-Amazon region, collected during the 2021 and 2022 sampling campaigns.

	<b>COMPONENT</b>	Variance	F	Pr(>F)	
<b>COMPOSITION</b>	<b>Physical and Chemical</b>				
		OD	0.13868	83.726	0.001***
		CO	0.03871	23.373	0.015*
		TDS	0.03230	19.503	0.030*
		PH	0.02752	16.613	0.057
		Residual	0.41408		
		<b>Riverscape component</b>			
		O.VEG	0.14340	91.539	0.001***
		BLO.	0.04781	30.519	0.004**
		ARE.	0.03118	19.905	0.028*
		PA.	0.02658	16.968	0.049*
		CANT.	0.02632	16.803	0.065
		Residual	0.37598		
		<b>Land use</b>			
		PAST.	0.11099	63.960	0.001***
		BOS.	0.04692	27.041	0.007**

	RECR.	0.03146	18.128	0.041*
	AGR.	0.02808	16.181	0.055
	Residual	0.43383		
<b>BETA DIVERSITY</b>	<b>Physical and Chemical</b>			
	OD	22798	126676	0.001***
	CO	0.5171	28732	0.011*
	TDS	0.4701	26122	0.019*
	PH	0.3384	18804	0.061
	Residual	44993		
	<b>Riverscape component</b>			
	O.VEG	23886	142401	0.001***
	BLO.	0.6783	40438	0.004**
	ARE.	0.4041	24091	0.015*
	PA.	0.3130	18662	0.051
	CANT.	0.2951	17594	0.077
	Residual	40257		
	<b>Land use</b>			
	PAST.	17500	89555	0.001***
	BOS.	0.6726	34420	0.005**
	RECR.	0.4364	22332	0.036*
	AGR.	0.3604	18442	0.063
	Residual	48854		

**Table S9.** Comparison of the results of the study with previous research, related to the composition of aquatic macroinvertebrates associated with the Andean-Amazon region of the department of Caquetá. Jaccard dissimilarity distance= 0.49.

ORDEN	FAMILIA	TAXON	REFERENCE	
			STUDY	STUDY
Haplotaxida	Lumbriculidae_N.D.	Lumbriculidae_N.D.	1	1
	Naididae	Naididae ND	1	1
Rhynchobdellida	Glossiphoniidae	<i>Helobdella</i> spp.	1	1
	cf. Lymnesiidae	Limnesiidae_N.D.	1	1
	Hydryphantidae	Morfo 1***	1	1
	Limnocharidae	Morfo 2***	1	1
	Sperchontidae	Morfo 3***	1	1
		Curculionidae_N.D	1	1
Coleoptera	Curculionidae	<i>Dryops</i> spp.	1	1
	Dryopidae	<i>Elmoparnus</i> spp.	1	0
		<i>Pelonomus</i> spp.	1	0
		<i>Celina</i> spp.	1	1
	Dysticidae	<i>Derovatellus</i> spp.	1	0
		<i>Hydrovatus</i> spp.	1	1
		<i>Anodocheilus</i> spp.	0	1
		<i>Laccophilus</i> spp.	1	1
		<i>Laccodytes</i> spp.	1	1
		<i>Pachydus</i> spp.	1	1
		<i>Liodessus</i> spp.	0	1

	<i>Neoclypeodytes</i> spp.	0	1
	<i>Vatellus</i> spp.	0	1
	<i>Rhantus</i> spp.	1	0
	<i>Uvarus</i> spp.	1	0
	ND	1	0
Chrysomelidae	<i>Austrelmis</i> spp.	1	1
Elmidae	<i>Austrolimnius</i> spp.	1	1
	<i>Cylloepus</i> spp.	1	1
	<i>Disersus</i> spp.	1	1
	<i>Epodelmis</i> spp.	0	1
	<i>Gyrelmis</i> spp.	1	1
	<i>Heterelmis</i> spp.	1	1
	<i>Hexacylloepus</i> spp.	1	1
	<i>Hexanchorus</i> spp.	1	1
	<i>Hintonelmis</i> spp.	0	1
	<i>Huleechius</i> spp.	1	1
	<i>Macrelmis</i> spp.	1	1
	<i>Microcylloepus</i> spp.	1	1
	<i>Neocylloepus</i> spp.	1	0
	<i>Neoelmis</i> spp.	1	1
	<i>Neolimnius</i> spp.	1	1
	<i>Notelmis</i> spp.	0	1
	<i>Phanocerus</i> spp.	1	1
	<i>Pharceonus</i> spp.	1	1
	<i>Pilielmis</i> spp.	0	1
	<i>Portelmis</i> spp.	1	0
	<i>Stegoelmis</i> spp.	1	1
	<i>Stenhelmoides</i> spp.	1	0
	<i>Pseudodisersus</i> spp.	1	1
	<i>Xenelmis</i> spp.	1	1
	<i>Georissus</i> spp.	1	0
Georissidae	<i>Gyretes</i> spp.	1	1
Gyrinidae	<i>Gyrinus</i> spp.	1	1
	<i>Macrogyrus</i> spp.	1	1
	<i>Hydrochus</i> spp.	0	1
Hydrochidae	<i>Anacaena</i> spp.	1	1
Hydrophilidae	<i>Crenitis</i> spp.	1	0
	<i>Chaetarthria</i> spp.	1	0
	<i>Hemiosus</i> spp.	1	1
	<i>Helobata</i> spp.	1	0
	<i>Hydrobius</i> spp.	1	0
	<i>Hydrophilus</i> spp.	1	0
	<i>Berosus</i> spp.	1	1
	<i>Derallus</i> spp.	1	1
	<i>Enochrus</i> spp.	1	1
	<i>Laccobius</i> spp.	1	1

		<i>Tropisternus</i> spp.	1	0
		Lampiridae N.D	1	0
	Lampiridae	<i>Lutrochus</i> spp.	1	1
	Lutrochidae	<i>Hydrocanthus</i> spp.	1	1
	Noteridae	<i>Mesonoterus</i> spp.	1	0
		<i>Notomicrus</i> spp.	1	1
		<i>Suphisellus</i> spp.	0	1
		<i>Anchytarsus</i> spp.	1	1
	Ptilodactylidae	<i>Scirtes</i> spp.	1	1
	Scirtidae	Scirtes_N.D	0	1
		<i>Eubrianax</i> spp.	1	0
	Psephenidae	<i>Ectopria</i> spp.	1	0
		<i>Psephenops</i> spp.	1	0
		<i>Pheneps</i> spp.	0	1
		<i>Psephenus</i> spp.	1	1
		<i>Stenus</i> spp.	1	0
	Staphylinidae	Athericidae N.D	1	0
Diptera	Athericidae	<i>Aposonalco</i> spp.	0	1
	Blephariceridae	<i>Limonicola</i> spp.	1	1
		<i>Atrichopogon</i> spp.	1	1
	Ceratopogonidae	<i>Bezzia</i> spp.	1	1
		<i>Ceratopogon</i> spp.	1	1
		<i>Culicoides</i> spp.	1	1
		<i>Forcipomyia</i> spp.	1	1
		<i>Dasyhelea</i> spp.	0	1
		<i>Paryphoconus</i> spp.	0	1
		<i>Endotribelos</i> spp.	0	1
	Chironomidae	<i>Tanytarsus</i> spp.	0	1
		<i>Rheotanytarsus</i> spp.	0	1
		<i>Polypedilum</i> spp.	0	1
		<i>Chironomus</i> sp1.	0	1
		Chironomini***	0	1
		<i>Xestochironomus</i> spp.	0	1
		<i>Riethia</i> spp.	0	1
		Ge_x10***	0	1
		<i>Zavreliella</i> spp.	0	1
		<i>Parachironomus</i> spp.	0	1
		<i>Caladomyia</i> spp.	0	1
		<i>Chironomus</i> sp2.	0	1
		<i>Oukuriella</i> spp.	0	1
		<i>Saetheria</i> spp.	0	1
		Ge_x1***	0	1
		Ge_x2***	0	1
		<i>Cricotopus</i> spp.	0	1
		<i>Parametricnemus</i> spp.	0	1

	<i>Thienemanniella</i>		
	spp.	0	1
	<i>Antillocladius</i> spp.	0	1
	<i>Corynoneura</i> spp.	0	1
	cf. <i>Cardiocladius</i>	0	1
	<i>Onconeura</i> spp.	0	1
	<i>Orthocladius</i> spp.	0	1
	Ge_x8***	0	1
	Ge_x9***	0	1
	<i>Larsia</i> spp.	0	1
	<i>Nilotanypus</i> spp.	0	1
	<i>Ablabesmyia</i> spp.	0	1
	<i>Pentaneura</i> spp.	0	1
	<i>Denopelopia</i> spp.	0	1
	cf. <i>Zavreliomyia</i>	0	1
	cf. <i>Macropelopia</i>	0	1
	<i>Labrundinia</i> spp.	0	1
	<i>Coelotanypus</i> spp.	0	1
	<i>Anopheles</i> spp.	0	1
Culicidae	<i>Culex</i> spp.	1	1
	<i>Mansonia</i> spp.	0	1
	<i>Uranotaenia</i> spp.	1	1
	<i>Dixella</i> spp.	1	1
Dixidae	<i>Rhaphium</i> spp.	0	1
Dolichopodidae	Ephy_N.D	1	1
Ephydriidae	<i>Hemerodromia</i> spp.	1	1
Empididae	<i>Chelifera</i> spp.	0	1
	Myce_N.D	0	1
Cf. Mycetophilidae	<i>Limnophora</i> spp.	1	1
Muscidae	Sarcophagidae N.D	1	0
Sarcophagidae	Sciom_N.D	1	1
Sciomyzidae	<i>Gigantodax</i> spp.	0	1
Simuliidae	<i>Simulium</i> spp.	1	1
	Phoridae N.D	1	1
Phoridae	<i>Maruina</i> spp.	1	1
Psychodidae	<i>Psychoda</i> spp.	0	1
	<i>Pericoma</i> spp.	0	1
	Stratioimyidae N.D	1	0
Stratioimyidae	N.D	1	0
Tabanidae	<i>Cheilotrichia</i> spp.	1	1
Tipulidae	<i>Hexatoma</i> spp.	1	1
	<i>Limonia</i> spp.	1	1
	<i>Molophilus</i> spp.	1	1
	<i>Erioptera</i> spp.	1	1
	<i>Tipula</i> spp.	1	1
	<i>Andesiops</i> spp.	0	1
Baetidae	<i>Americabaetis</i> spp.	1	1

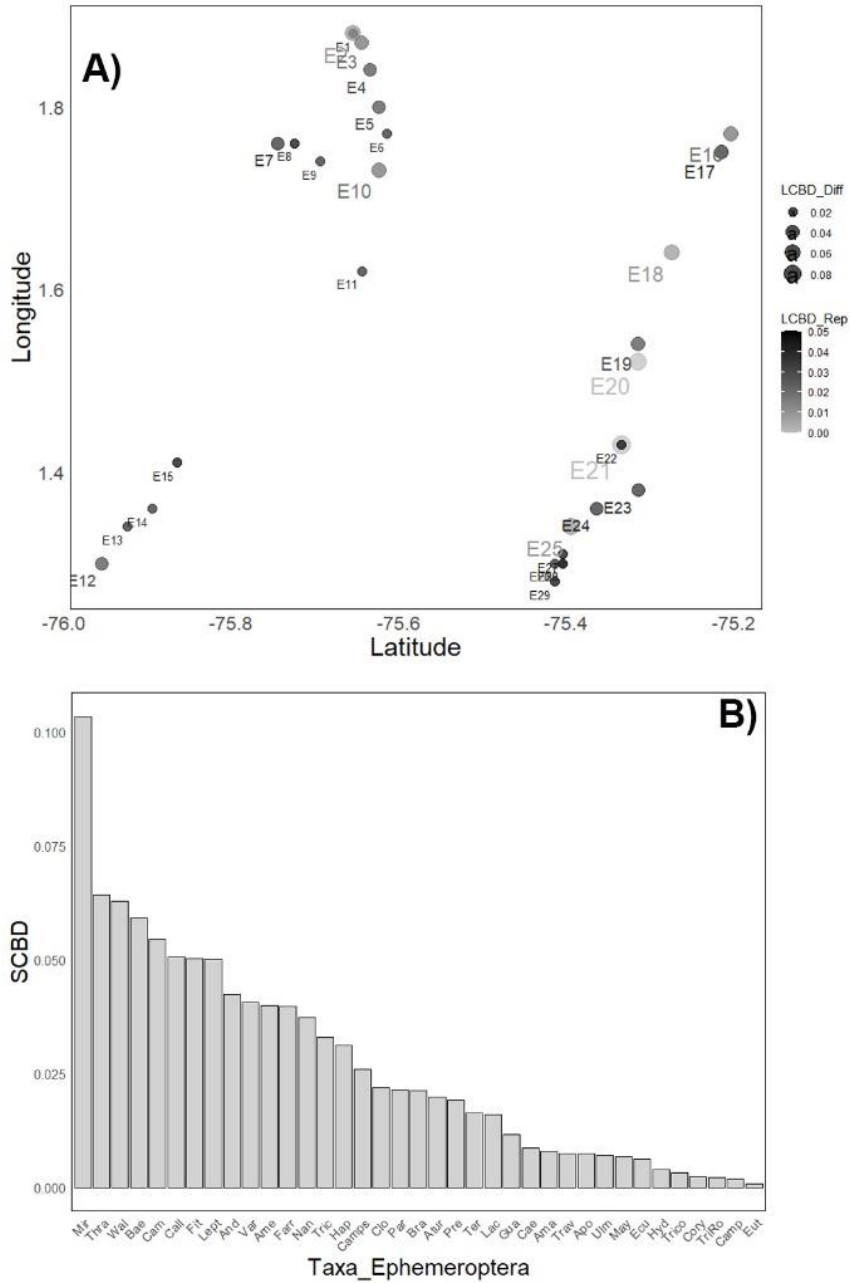
		<i>Apobaetis</i> spp.	1	1
		<i>Aturbina</i> spp.	1	1
		<i>Baetodes</i> spp.	1	1
		<i>Callibaetis</i> spp.	1	1
Ephemeroptera		<i>Camellobaetidius</i> spp.	1	1
		<i>Cloeodes</i> spp.	1	1
		<i>Guajirolus</i> spp.	1	1
		<i>Mayobaetis</i> spp.	1	1
		<i>Nanomis</i> spp.	1	1
		<i>Paracloeodes</i> spp.	1	1
		<i>Prebaetodes</i> spp.	0	1
		<i>Rivudiva</i> spp.	1	0
		<i>Varipes</i> spp.	1	1
		<i>Waltzophius</i> spp.	0	1
		<i>Zelus</i> spp.	1	0
		<i>Brasilocaenis</i> spp.	1	1
	Caenidae	<i>Caenis</i> spp.	1	1
		<i>Coryphorus</i> spp.	1	1
	Coryphoridae	<i>Campylocia</i> spp.	1	1
	Euthyplociidae	<i>Euthyplocia</i> spp.	1	1
		<i>Ecuaphlebia</i> spp.	0	1
		<i>Farrodes</i> spp.	1	1
	Leptophlebiidae	<i>Fittkaulus</i> spp.	0	1
		<i>Hydrosmilodon</i> spp.	1	1
		<i>Miroculis</i> spp.	1	1
		<i>Terpides</i> spp.	1	1
		<i>Thraulodes</i> spp.	1	1
	Leptohyphidae	<i>Traverella</i> spp.	1	0
		<i>Ulmeritoides</i> spp.	1	1
		<i>Amanahyphes</i> spp.	0	1
		<i>Haplohyphes</i> spp.	1	1
		<i>Leptohyphes</i> spp.	1	1
		<i>Traverhyphes</i> spp.	0	1
		<i>Tricorythodes</i> spp.	1	1
		<i>Tricorythopsis</i> spp.	0	1
		<i>Tricorythopsis rondonienses</i>	0	1
		<i>Lachlania</i> spp.	1	1
	Oligoneuridae	<i>Campsurus</i> spp.	0	1
	Polymitarcyidae	<i>Oxyelophila</i> spp.	0	1
Lepidoptera	Crambidae	<i>Paraponyx</i> spp.	1	1
		<i>Petrophila</i> spp.	1	1
		<i>Crambinae</i> spp.	1	0
		<i>Belostoma</i> spp.	1	1
Hemiptera	Belostomatidae	<i>Tenagobia</i> spp.	1	1
	Corixidae	<i>Gelastocoris</i> spp.	1	0

	Gelastocoridae	<i>Charmatometra</i> spp.	1	0	
	Guerridae	<i>Cylindrostethus</i> spp.	1	0	
		<i>Darwinivelia</i> spp.	1	0	
		<i>Halobatopsis</i> spp.	1	0	
		<i>Limmogonus</i> spp.	1	0	
		<i>Ovatametra</i> spp.	1	0	
		<i>Neogerris</i> spp.	1	1	
		<i>Trepobates</i> spp.	1	1	
		<i>Potamobates</i> spp.	1	1	
		<i>Rheumatobates</i> spp.	1	1	
		<i>Paratrephes</i> spp.	1	0	
	Helotrephidae	<i>Ambrysus</i> spp.	1	1	
	Naucoridae	<i>Cryphocricos</i> spp.	1	1	
		<i>Linnocoris</i> spp.	1	1	
		<i>Pelocoris</i> spp.	1	1	
		<i>Procryphocricos</i> spp.	1	1	
		<i>Ranatra</i> spp.	1	1	
		<i>Buenoa</i> spp.	1	1	
		<i>Martarega</i> spp.	1	1	
	Nepidae	<i>Notonecta</i> spp.	1	1	
	Notonectidae	<i>Mesoveloidea</i> spp.	1	0	
		<i>Mesovelia</i> spp.	1	1	
	Mesoveliidae	<i>Paraplea</i> spp.	1	0	
	Pleidae	Saldidae N.D	1	0	
	Saldidae	<i>Microvelia</i> spp.	1	1	
	Veliidae	<i>Rhagovelia</i> spp.	1	1	
		<i>Stridulivelia</i> spp.	1	1	
		<i>Corydalus</i> spp.	1	1	
Megaloptera	Corydalidae	<i>Chloronia</i> spp.	1	0	
		<i>Gynacantha</i> spp.	1	0	
		<i>Rhionaeschna</i> spp.	1	0	
Odonata	Aesnidae	<i>Coryphaeschna</i> spp.	1	1	
		<i>Hetaerina</i> spp.	1	1	
		<i>Acanthagrion</i> spp.	1	1	
	Calopterygidae	<i>Amphiagrion</i> spp.	1	0	
	Coenagrionidae	<i>Telebasis</i> spp.	1	0	
		<i>Argia</i> spp.	1	1	
		<i>Enallagma</i> spp.	1	1	
		<i>Epipleoneura</i> spp.	1	1	
		<i>Protoneura</i> spp.	0	1	
		<i>Aeschnosoma</i> spp.	0	1	
		Corduliidae	<i>Agriogomphus</i> spp.	0	1
		Gomphidae	<i>Aphylla</i> spp.	1	0
			<i>Epigomphus</i> spp.	1	0
			<i>Progomphus</i> spp.	1	1
	<i>Phyllogomphoides</i>		1	0	

		spp.		
		<i>Phyllocycla</i> spp.	1	1
		<i>Heteragrion</i> spp.	1	1
		<i>Megapodagrion</i> spp.	1	0
	Megapodagrionidae	<i>Oxystigma</i> spp.	1	0
	Libellulidae	<i>Argythemis</i> spp.	1	0
		<i>Brechmorhoga</i> spp.	1	0
		<i>Brachymesia</i> spp.	1	0
		<i>Macrothemis</i> spp.	1	0
		<i>Oligoclada</i> spp.	1	0
		<i>Perithemis</i> spp.	1	0
		<i>Tauriphila</i> spp.	1	0
		<i>Tramea</i> spp.	1	0
		<i>Sympetrum</i> spp.	1	0
		<i>Elga</i> spp.	1	1
		<i>Erythemis</i> spp.	1	1
		<i>Erythrodiplax</i> spp.	1	1
		<i>Diastatops</i> spp.	1	1
		<i>Dythemis</i> spp.	1	1
		<i>Fylgia</i> spp.	1	1
		<i>Gynothemis</i> spp.	0	1
		<i>Miathyria</i> spp.	1	1
		<i>Micrathyria</i> spp.	1	1
		<i>Planiplax</i> spp.	0	1
		<i>Tholymis</i> spp.	0	1
		<i>Palaemnema</i> spp.	1	1
	Platystictidae	<i>Cora</i> spp.	1	1
	Polythoridae	<i>Polythore</i> spp.	1	0
		ND	1	0
Orthoptera	Tetrigidae	<i>Anacroneuria</i> spp.	1	1
Plecoptera	Perlidae	<i>Neotriplectides</i> spp.	0	1
Trichoptera	Atriplectididae	<i>Phylloicus</i> spp.	1	1
	Calamoceratidae	<i>Banyallarga</i> spp.	1	0
	Ecnomidae	<i>Austrotinodes</i> spp.	1	0
		<i>Mortoniella</i> spp.	1	1
	Glossosomatidae	<i>Protoptila</i> spp.	1	0
		<i>Atopsyche</i> spp.	1	1
	Hidrobiosidae	<i>Helycopsyche</i> spp.	1	1
	Helycopsychidae	<i>Leptonema</i> spp.	1	1
	Hydropsychidae	<i>Macronema</i> spp.	1	1
		<i>Smicridea</i> spp.	1	1
	Hydroptilidae	cf. <i>Alisotrichia</i>	1	1
		<i>Anchitrichia</i> spp.	1	0
		<i>Cerasmatrixia</i> spp.	1	0
		<i>Hydoptila</i> spp.	1	0
		<i>Metrichia</i> spp.	1	1

		<i>Neotrichia</i> spp.	1	1
		<i>Ochrotrichia</i> spp.	1	0
		<i>Oxyethira</i> spp.	1	1
		<i>Rhyacopsuche</i> spp.	1	0
		<i>Zumatrichia</i> spp.	1	0
		<i>Atanatolica</i> spp.	1	0
	Leptoceridae	<i>Grumichela</i> spp.	1	1
		<i>Netopsyche</i> spp.	1	1
		<i>Oecetis</i> spp.	1	1
		<i>Tripletides</i> spp.	1	1
		<i>Marilia</i> spp.	1	1
	Odontoceridae	<i>Chimarra</i> spp.	1	1
	Philopotamidae	<i>Cernotina</i> spp.	1	1
	Polycentropodidae	<i>Cynellus</i> spp.	1	1
		<i>Polycentropus</i> spp.	1	0
		<i>Polyplectropus</i> spp.	1	1
	Xiphocentronidae	<i>Xiphocentron</i> spp.	1	1
Decapoda	Palaemonidae	<i>Macrobrachium</i> spp.	1	1
	<i>Trychodactilidae</i>	<i>Silviocarcinus</i> spp.	0	1
Copepoda	Copepoda	Copepoda N.D	1	1
Cladocera	Cladocera	Cladocera N.D	1	1
Basommatophora	Planorbidae	<i>c.f Acrorbis</i>	0	1
		<i>Biomphalaria</i> spp.	1	1
	Sphaeriidae	<i>Pisidium</i> spp.	1	1
	Ampullariidae	Ampullariidae N.D	1	0
	Ancylidae	Ancylidae N.D	1	0
	Hydrobiidae	Hydrobiidae N.D	1	0
	Physidae	Physidae N.D	1	0
	Thiaridae	Thiaridae N.D	1	0
Tricladida	Planariidae	<i>Dugesia</i> spp.	1	1
Nematoda_N.D	Nematoda_N.D	Mermithidae N.D	1	0
		Nematoda_N.D	1	1

## 8. Capítulo 3: Diversity and Distribution of Ephemeroptera along the Colombia Andes-Amazonian Transition



**Graphical abstract**

# **Diversity and Distribution of Ephemeroptera along the Colombia Andes-Amazonian Transition**

## **Abstract**

The Andean-Amazonian region of Caquetá is considered an area that hosts high biodiversity within various aquatic and terrestrial ecosystems. We investigated this region, seeking to understand the diversity and distribution of the Ephemeroptera order in different lotic ecosystems, from the Andes mountains to the Amazon plains. We defined three study regions: Andes, Transition, and Amazon. We established ten sampling stations for collecting biological material and recorded environmental parameters during four field expeditions. Based on the composition of Ephemeroptera, we evaluated the field sampling, estimated Hill numbers as alpha diversity, and calculated each taxon's importance value. For beta diversity, we calculated the beta diversity components, the local contribution to beta diversity (LCBD), and the species contribution to beta diversity (SCBD). Finally, we used RDA and dbRDA to identify predictive environmental variables on the assembly composition and its beta diversity (Bray-Curtis). We identified changes in the diversity of the order Ephemeroptera, distinguishing between the Andes and Transition from the Amazon, with species replacement as a beta diversity component. Substrate, land use for forest conservation, and vegetation cover dominated by grasses were the most significant variables in the Ephemeroptera assembly, acting as environmental filters on species distribution. The LCBD and SCBD values allowed us to identify stations and species of significant value for future conservation proposals. Our data contribute to studying the order Ephemeroptera in the Andean-Amazonian region, revealing new information about its distribution and diversity.

**Keywords:** turnover, ecoregional transition, neotropic, Caquetá.

## **Introduction**

The conservation of aquatic ecosystems and their biodiversity is a paramount challenge for humanity, which has sought a sustainable balance with nature but has also caused various

environmental disturbances (Flitcroft et al., 2019; Artaxo, 2020). Studying the biological components of these ecosystems, mainly aquatic macroinvertebrates, can provide fundamental insights that serve as a basis for developing river conservation and restoration strategies (Roldán-Pérez, 2016). These strategies, in turn, guide us toward a more responsible utilization and management of the diverse ecosystem services they provide (Sundar et al., 2020).

One of these groups of interest is the order Ephemeroptera, hemimetabolous organisms with simple metamorphosis (Zuñiga et al., 2004), considered primitive winged insects (Sam et al., 2004) due to their subimago stage situated between the nymph and the adult (Salles et al., 2004). These organisms, commonly known as mayflies, are of interest to aquatic ecology due to their diversity and extensive global distribution despite their small size (Waltz & Burian, 2008; Domínguez et al., 2009; Domínguez & Dos Santos, 2014; Jacobus et al., 2019).

Studying the order Ephemeroptera in the Andean-Amazonian region is essential for understanding its ecological role in the riverscape. Researchers recognize these organisms as indicators of water's biological quality due to their sensitivity to various anthropogenic impacts, an aspect extensively documented (Roldán & Ramírez, 2008, Domínguez et al., 2009). This bioindicator role, which it shares with other orders, including Trichoptera and Plecoptera, has even led to their categorization as the EPT group, for which a methodology for assessing biological quality exists (Aazami et al., 2020; Correa-Araneda et al., 2021). This may be relevant to the study area, considering the current significant concern over the loss of connectivity between the Andes Mountain range and the Amazon (Anderson et al., 2018; Clerici et al., 2018; Caldas et al., 2023). This concern is primarily associated with a historical accumulation of deforestation (Anderson et al., 2018; Clerici et al., 2019; Linero et al., 2020; Murillo-Sandoval et al., 2022), which has significantly increased after the peace process in Colombia in 2016 (Clerici et al., 2019; Agudelo et al., 2023). The absence of any documented taxon can raise concerns about the ecological consequences of fragmentation, especially along riparian zones.

Moreover, the Andean-Amazonian region presents an interesting peculiarity due to the presence of the eastern mountain range. According to Hoorn et al. (2010), forming the Andes mountains was crucial in shaping the current climatic conditions, leading to a significant

explosion of biodiversity, and establishing a new configuration of what we now know as the western Amazon. However, in the Caquetá department, we still need to deepen our understanding of this biodiversity, especially concerning aquatic assemblages such as the order Ephemeroptera. High forest fragmentation, mainly focused on the Amazon, may cause the loss of this biodiversity before it is fully known.

Previous research has linked the order Ephemeroptera (Cortes et al., 2016; Gutiérrez-Garaviz et al., 2016; Chaux et al., 2018; Chaux-Rojas et al., 2020; Bravo & Restrepo, 2021). However, these studies have primarily focused on characterization and have not delved into detailed analyses of alpha and beta diversity, which describe the richness and variation of taxa within and between habitats. Such analyses are vital for ecological assessments of aquatic ecosystems (Cox et al., 2017; Dodds, 2002).

Ephemeroptera studies addressing alpha diversity often estimate it using classic indices such as the Shannon-Wiener index, Margalef, Simpson, or Pielou (Rojas-Peña et al., 2021). ; Ballesteros et al., 2022 However, Jost (2006) criticizes using these indices, which often do not provide enough information to differentiate communities. Therefore, he proposes using adequate numbers or true diversity based on Hill's general equation (1973), something we consider essential for estimating this measure in the Andean-Amazonian region, facilitating the comparison between associated assemblies (Tolonen et al., 2017).

Researchers also expect to find studies on beta diversity based on values of similarity or dissimilarity between communities, estimated through Bray-Curtis, Euclidean distance, Jaccard, and Sorensen, among others (Moreno, 2001). However, these indices alone do not delve into whether this similarity or dissimilarity is due to species turnover or differentiation in species richness between communities, components that, according to Baselga (2010), would be part of the total beta diversity. We consider these components vital to understanding this diversity estimate, associating it with the process of taxon substitution of Ephemeroptera from the Andes to the Amazon. Moreover, it is essential to know the local contribution to beta diversity (LCBD) and the species contribution to beta diversity (SCBD), thereby obtaining a reference framework

that can be used for future conservation strategies (Legendre & Caceres, 2013; Valente-Neto et al., 2020).

Based on the previous context, our research aim was to determine the diversity and distribution of the order Ephemeroptera along an environmental gradient within the Andean-Amazonian corridor of the Caquetá department. We aimed to validate our hypothesis, positing that environmental filters influence a turnover of taxa between the Andes mountains and the Amazon plain. Furthermore, we aimed to establish the local contribution (LCBD) and species contribution (SCBD) within the beta diversity component as relevant ecological information that allows for identifying areas and taxa for future prioritization within river restoration programs.

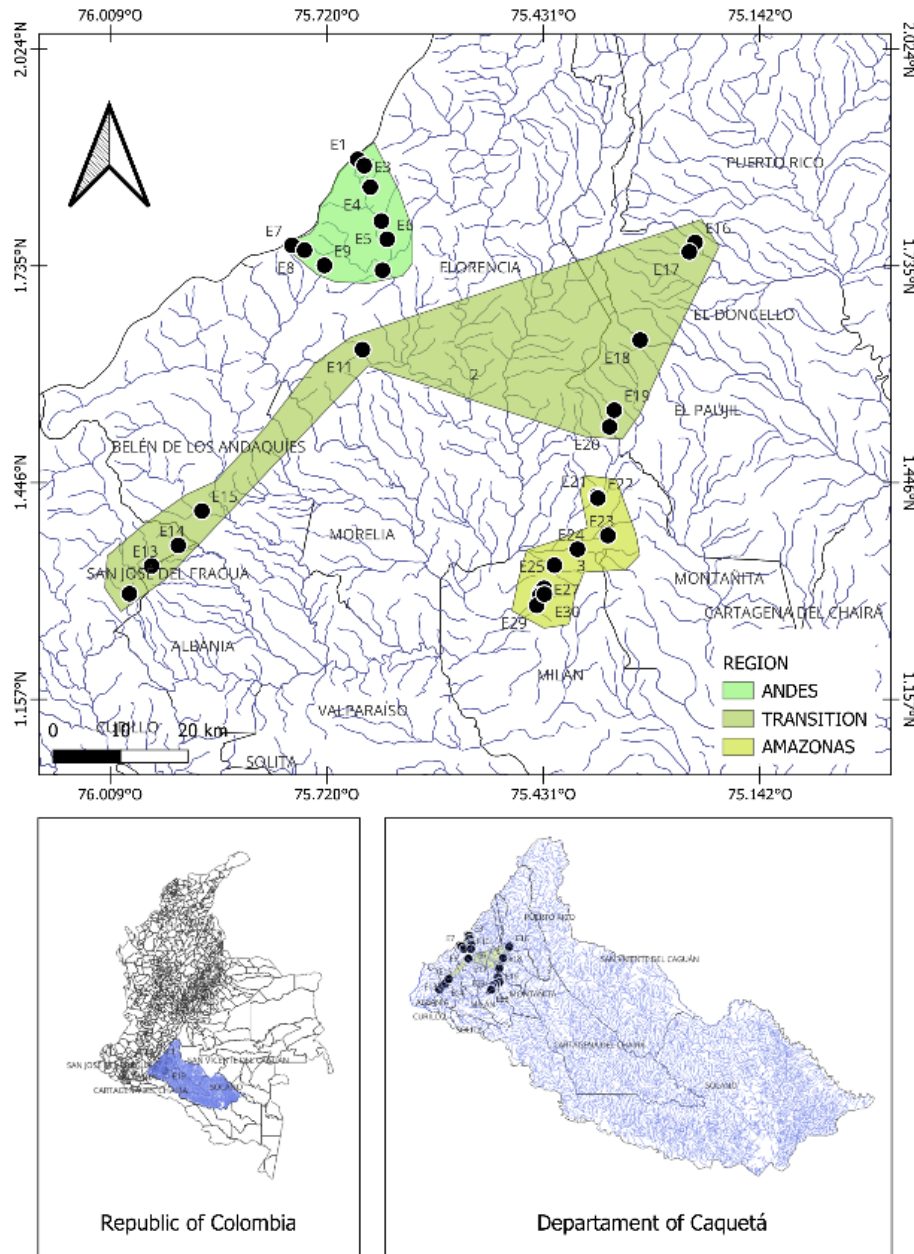
## **Material and Methods**

### ***Study area***

This study was carried out in the Andean-Amazonian region, in the Eastern Cordillera, Caquetá department, within the geographical coordinates of 2.024°N - 76.0090°W to 1.157°N - 75.1420°W, encompassing the municipalities of Belén de los Andaquíes, El Doncello, El Paujil, Florencia, Milán, Montañita, and San José del Fragua. Sampling sites capture an elevational gradient ranging from 2,205 to 231 meters above sea level (**Figure 1, Table S1** in the supplementary material). Following initial fieldwork, we designated three study regions: Andes, Transition, and Amazon, assigning ten sampling stations to each region. We demarcated each region based on observable changes in riverscape characteristics, including substrate composition, river hydrodynamics, and vegetation cover.

The Andes region exhibited, in most stations, a riparian vegetation cover dominated by forests, with a rocky riverbed substrate (boulders) and a higher presence of rapids in terms of river dynamics, which would allow for good oxygenation of the water column and additionally, observed small-scale agricultural activities. Regarding the Transition region, there was a higher fragmentation of riparian vegetation cover, with evidence of erosion on the slopes; the substrate remained rocky, with smaller sizes (pebbles, gravels, sands), rapids, and a higher percentage of

pools; moreover, observed agricultural activities on a larger scale. In the Amazon region, increased fragmentation of riparian vegetation was also evident; however, some stations presented mixtures of natural forests with grasslands. The substrate was soft and dominated by clays, and coarse particulate organic matter (CPOM) was present, which is closely related to river dynamics, where larger pools and a low current were identified. Regarding human activities, large-scale livestock farming was the major observed stressor.



**Figure 1.** Location and distribution of the 30 sampling stations for aquatic macroinvertebrates in the streams of the Andean-Amazonian Region in Caquetá, Colombia, from October 2021 to April 2022. Station abbreviation (E).

We carried out four bimonthly field campaigns (October and December 2021, February, and April 2022). For data collection, we implemented the composite-multihabitat sampling methodology for aquatic macroinvertebrates (Roldán & Ramírez, 2008; Rodríguez-Capítulo et al., 2009; Wantzen & Rueda-Delgado, 2009), using a Surber net with an opening of 0.3x0.3 meters and a mesh size of 250 microns. The sampling sections were 100 meters long, and ten sweeps were made against the current, totaling a collection area of 0.9 m<sup>2</sup> at each site (five sweeps over the dominant substrate, two over the second substrate, and three over the remaining available substrate). Subsequently, we homogenize the samples and packed all collected material in wide-mouthed plastic bottles of 500 milliliters, fixed with 75% alcohol. It is important to note that, based on the experience gained in the study area, for the Amazon region, most of the capture sweeps on substrates associated with roots, aquatic macrophytes, and plant material, which may harbor a more excellent composition of organisms, unlike the riverbed where clays dominate.

We used a Zeiss Stemi DV4 stereoscope and a Zeiss Primo Star microscope to classify and identify material related to the order Ephemeroptera based on the taxonomic keys from Domínguez et al. (2006, 2009), Salles et al. (2014), Ossa-López et al. (2017), Salles et al. (2018), and Salinas-Jiménez et al. (2018), and Miñano et al. (2019),

In addressing riverscape variables, we drew upon the methodologies and insights of Barbour et al. (1999), Elosegui & Sabater (2009), Hauer & Lamberti (2011), and Saito et al. (2020). We visually estimated the percentage of riparian vegetation cover (supported by geographic information), shade percentage, vegetation type (trees, shrubs, grasses, and herbaceous plants), and land use activity (forest conservation, agriculture, recreation, settlements, and grazing). Additionally, we determined the substrate composition, considering categories such as rounded rocks ("BLO." >25 cm in diameter), boulders ("CANT." 6 cm-25 cm), gravel ("GRAV." 2 cm-6 cm), sand ("SAND." 0.06 cm-2 cm), and vegetal origin ("O.VEG"). (**Table S2** in the supplementary material).

For physical and chemical variables, we measured dissolved oxygen (DO-mg L<sup>-1</sup>), hydrogen ion potential (pH), conductivity (CO-uS cm<sup>-1</sup>), total dissolved solids (TDS-ppm), and water temperature (WT-°C) using a professional AZ86031 multiparametric probe. Furthermore, we measured surface width, and flow speed and depth at each sampling station using a Global Water flow meter. (**Table S2** in the supplementary material).

### *Data analysis*

To assess the representativeness of our sampling in capturing regional diversity, we assessed sampling coverage for richness and then constructed a rarefaction curve that relates the number of individuals to richness (Rodriguez da Silva et al., 2022). Next, we calculated alpha diversity, following the recommendations of Jost (2006), who suggests that it is better to work with true diversity based on Hill numbers (Hill, 1973), where  $q=0$  relates to species richness,  $q=1$  is equivalent to the transformation of the Shannon-Wiener index and gives weight to species according to their frequency, and  $q=2$ , which constitutes the transformation of the Simpson dominance index, is associated with the most common species (Rodriguez da Silva et al., 2022). Additionally, we generated a plot to identify the importance value of each taxon, transforming abundance on a log<sub>10</sub> scale and comparing the three regions of interest on a positional range scale (Whittaker, 1972). For these analyses, we used the statistical packages iNEXT, ggplot2, and ggthemes.

We implemented a non-metric multidimensional scaling (NMDS) analysis with Bray-Curtis distance and the ‘elbow’ method for group selection using the vegan 2.6-4 library. The plot allowed us to visualize the turnover of taxa between the study regions in general, being a widely employed tool in ecology to discern similarities and dissimilarities among samples in a fitted spatial representation. Additionally, we conducted a Similarity Analysis (ANOSIM) using the Bray-Curtis distance. This analysis allowed us to validate if there are significant differences in taxon composition among the evaluated seasons, grouped into three regional categories that we established previously.

Regarding beta diversity, we followed the recommendations of Cáceres et al. (2013), Legendre (2014), and Schmera et al. (2020), calculating total beta diversity and the replacement and richness difference components. We supported the analysis using the Ružička dissimilarity measure of the Jaccard group of the Podani family (Podani & Schmera, 2011) as follows:

$$DR = (B+C) / (A+B+C)$$

Where DR is the Ružička dissimilarity and A, B, and C are the dissimilarity components for comparing communities.

Furthermore, we delved deeper into understanding the local contribution to beta diversity (LCBD) of each analyzed sampling station and the species contribution to beta diversity (SCBD) based on the identified taxa (Legendre & Cáceres, 2013; Valente-Neto et al., 2020), creating a map with geographical coordinates. In the beta diversity analyses, we used the `adespatia`, `ade4`, `ggplot2`, and `dplyr` libraries.

Finally, considering the environmental variables, we implemented two ordination analyses to identify their influence on Ephemeroptera's composition and beta diversity. This analysis organizes objects based on predictor variables (Rodríguez da Silva et al., 2022). For data preparation, abundances were transformed on a Hellinger scale (dependent variable), and environmental variables (independent variables) that were not highly correlated were selected using the “`forward.sel` function” and were subsequently standardized using the “`decostan` function”. In the first analysis, we used an RDA (Redundancy Analysis that accepts any independent variable and continuous dependent variable), analyzing the influence that selected environmental variables may have on ephemeropteran composition. In the second analysis, we used a dbRDA (Redundancy Analysis for beta diversity, which allows independent variables of any type and a distance matrix obtained from analyzed dependent variables), identifying the potential influence of independent variables on beta diversity supported by Bray-Curtis (it is recommended to review the book by Rodríguez da Silva et al., 2022).

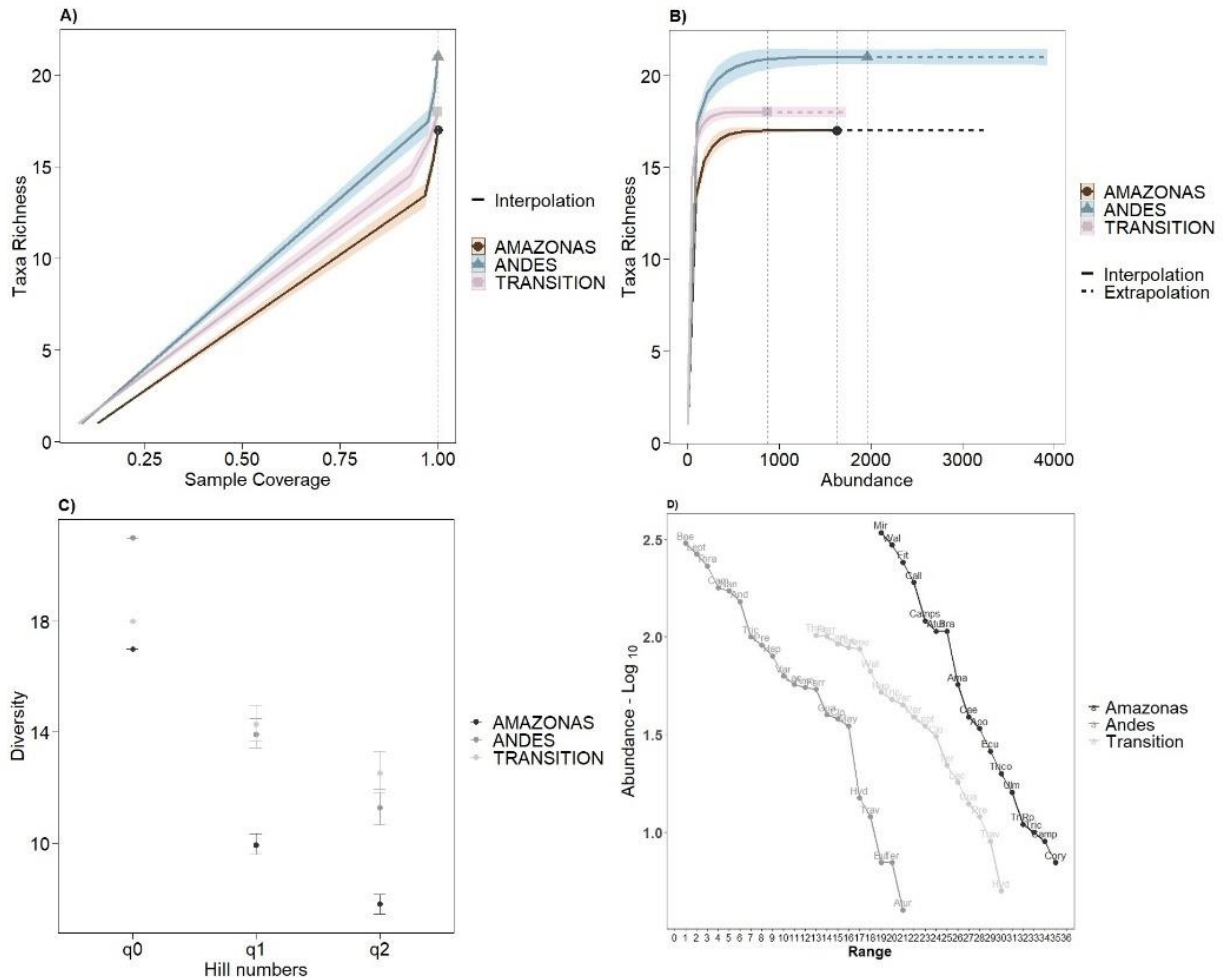
All analyses were conducted using the statistical software RStudio version 2023.09.1-494.

## Results

### *Spatial Distribution, Alpha Diversity, and Beta Diversity*

Eight families and 37 taxa were identified among 2.505 individuals associated with the 30 sampling stations (**Table S3** in the supplementary material). Reporting the first records for the Caquetá department are the genera *Prebaetodes* spp. and *Waltzoyphius* spp., as well as the species *Andesiops peruvianus* from the family Baetidae; *Coryphorus aquilus* from the family Coryphoridae; *Amanahyphes* spp., *Traveryphes* spp., *Trichorythopsis* spp., and the species *Tricorythopsis rondoniensis* from the family Leptohiphidae; *Ecuaphlebia* spp., *Ulmeritoides* spp., and the species *Fittkaulus amazonicus* from the family Leptophlebiidae; and *Campsurus* spp. from the family Polymitaecidae.

Sampling coverage had a value of 1 (**Figure 2-A**), indicating a solid representativeness of our samples. This is also reflected in the rarefaction curves, contrasting abundance with taxonomic richness (**Figure 2-B**). Regarding Hill numbers (alpha diversity), we observe that the Andes and Transition regions present similar values in terms of diversity ( $q=1$ ) and dominance ( $q=2$ ), differing in richness. On the other hand, the Amazon region separated from the two previous areas with lower values in the estimated indices (**Figure 2-C**). About the importance of each taxon (**Figure 2-D**), in the Andes region, the genera *Baetodes* spp. (Bae), *Leptohiphides* spp. (Lept), and *Thraulodes* spp. (Thra) stand out. In the Transition region, *Thraulodes* spp. and *Baetodes* spp. reappear, accompanied by *Farrodes* spp. (Farr), *Camelobaetidius* spp. (Cam), and *Americabaetis* spp. (Am). Finally, in the Amazon region, the genera *Miroculis* spp. (Mir), *Waltzoyphius* spp. (Wal), *Callibaetis* spp. (Call), and the species *Fittkaulus amazonicus* (Fit) predominate.

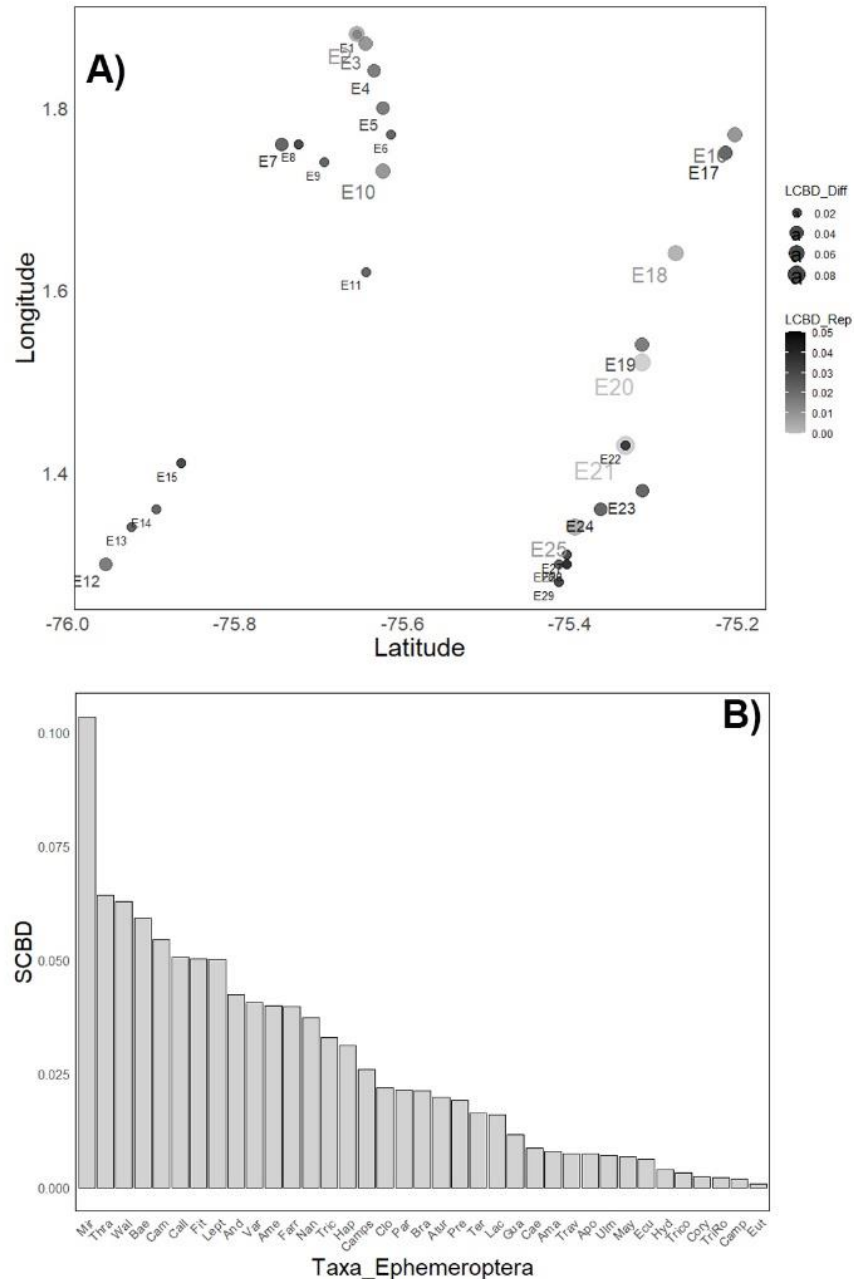


**Figure 2.** Sampling evaluation and alpha diversity taxa in streams of the Andean-Amazonian Region of the Caquetá Department, Colombia, sampled from October 2021 to April 2022. Sample Coverage (A), Rarefaction curve (B), True diversity (C), and importance value of each taxon (D).

Through the application of the elbow method, our results suggest the existence of two distinct groups ( $k=2$ ) for the multivariate NMDS analysis (**Figure 3**) from a spatial perspective. The resulting model exhibited a stress value of 0.081 and a non-parametric coefficient of determination ( $R^2$ ) of 0.993 for the two identified groups. In the first group, taxa from the Andes and Transition regions were associated, including *Americabaetis* spp., *Andesiops peruvianus*, *Aturbina* spp., *Baetodes* spp., *Camelobaetidius* spp., *Cloeodes* spp., *Euthyplocia* spp., *Farrodes* spp., *Guajirolus* spp., *Haplohyphes* spp., *Hydrosmilodon* spp., *Lachlania* spp., *Leptohyphes* spp., *Mayobaetis* spp., *Nanomis* spp., *Prebaetodes* spp., *Terpides* spp., *Thraulodes* spp.,



The beta diversity analysis yielded a Repl/BDtotal of 0.55, and RichDif/BDtotal of 0.44. Regarding local contribution to beta diversity (LCBD), stations E1, E8, E9, E15, E22, E26, E27, and E29 stand out for their contribution to replacement (LCBD-Rep). On the other hand, stations E2, E16, E18, E20, E21, and E25 significantly contribute to richness difference (LCBD-Diff) (**Figure 4-A**). Regarding species contribution to beta diversity (SCBD), the genus *Miroculis* spp. (Mir) emerges as the most ecologically significant (**Figure 4-B**).



**Figure 4.** Components of contribution to beta diversity in streams of the Andean-Amazonian Region of the Caquetá Department, Colombia, sampled from October 2021 to April 2022. Local

Contribution Beta diversity with turnover and richness of different (A), Species Contribution Beta Diversity (B).

### ***Environmental Variables and Diversity***

The results of Redundancy Analysis (RDA) and Distance-based Redundancy Analysis (dbRDA) are presented in **Table 1**. Out of the 20 environmental variables analyzed, the model selected 6: substrate types of vegetal origin (O.VEG), block (BLOQ.), stones (CANT.), current velocity (“VELO”), land use for forest conservation (BOS.), and type of vegetative cover of grasslands (PAST.). In RDA, the variables O.VEG, BLOQ., CANT., BOS., and PAST. were found to be significant, with a coefficient of determination ( $R^2$ ) of 0.584. In dbRDA, the same variables were significant, except grasslands.

**Table 1.** Multivariate analysis contrasting environmental variables with the composition and beta diversity of the Ephemeroptera assemblage present in streams of the Andean-Amazonian Region of the Caquetá Department, Colombia, sampled from October 2021 to April 2022.

Predictor Variable	Composition			Beta-Diversity		
	Variance	F	PR(>F)	Sum of Sqs	F	PR(>F)
O.VEG	0.228	17.7096	<b>0.001***</b>	39.856	279.176	<b>0.001***</b>
BLOQ.	0.051	4.0206	<b>0.002**</b>	0.6441	45.117	<b>0.006**</b>
CANT.	0.047	3.7130	<b>0.001***</b>	0.7377	51.670	<b>0.001***</b>
BOS.	0.037	2.9319	<b>0.013*</b>	0.4101	28.726	<b>0.040*</b>
PAST.	0.0294	2.2895	<b>0.038*</b>	0.3540	24.799	0.053

### **Discussion**

The new taxonomic records allow us to consider the Andean-Amazonian region as a crucial area for species dispersal, given its connectivity with other regions of the country, such as the Andes, the eastern plains, and the southernmost departments of the Amazon. For instance, *Coryphorus aquilus* (Peters, 1981; Molineri et al., 2001) and *Tricorythopsis rondoniensis* (Gomes Dias et al., 2016) have been reported for the eastern plains and areas further south of the Amazon. It is crucial to study the dispersal ability of species, considered a fundamental characteristic for environmental adaptation and spatial distribution (Moraes et al., 2014; Rádková et al., 2014; Horsák et al., 2015). However, our findings are a reference for future research on the

dispersal dynamics of many taxa and their relationship with the current loss of connectivity advancing between the Andes and the Amazon, intensifying after the peace agreement, as mentioned by Murillo-Sandoval et al. (2021).

Our study may provide valuable insights into the changes in diversity along the Andean-Amazonian transition. According to rarefaction curves, the three regions exhibit differences in richness and abundance, as 95% confidence intervals do not overlap. However, the Andes and Transition regions display similar values in terms of diversity ( $q=1$ ) and dominance ( $q=2$ ), distinguishing them from the Amazon region, which presents lower values in alpha diversity estimations. These results may be consistent on a broad scale with previous works by Murcia & Chavez (2009) and González et al. (2020) in the Caquetá region, who found differences in the aquatic macroinvertebrate assemblage within a similar altitudinal range to our study associated with the Andean-Amazonian corridor.

When analyzing groups separation in Non-Metric Multidimensional Scaling (NMDS), we also observe a notable differentiation in terms of assemblage composition. This differentiation can be associated with species turnover at spatial scales, as demonstrated by the beta diversity results, which is the outstanding component in the Andean-Amazonian region. In this context, taxa with wide and moderate altitudinal distribution, associated with the Andes and Transition regions, cluster separately from taxa with a more restricted distribution in the Amazon region (Romero et al., 2006; Gutiérrez & Reinoso-Flórez, 2010; Hoyos et al., 2014; Gutiérrez & Díaz, 2015; Rúa et al., 2015; Forero-Céspedes et al., 2016; Mosquera et al., 2022). This result is consistent with previous studies conducted by González et al. (2020) along the Andakí Trail in the Caquetá department, which demonstrated altitudinal influence as a regional variable in the turnover of aquatic macroinvertebrate species. Similarly, the work of Lujan et al. (2013) in the Andean-Amazonian region of Peru adds influential local elements in the establishment of aquatic macroinvertebrate assemblages, such as food availability (for functional groups), temperature, or anthropogenic impacts. Furthermore, the studies of Domínguez & Ballesteros (1992), Scheibler et al. (2014), and Múrria et al. (2014) also demonstrate the altitudinal relationship, which could be crucial for establishing a biological aquatic study group.

The preceding highlights the importance of considering spatial variability in biological diversity studies and suggests that altitude may be a determining regional factor in species composition across different ecosystems. However, we must note that the Altitude variable may be significant ( $p < 0.05$ ) in our results. However, it is highly correlated with other variables such as Block (Altitude-BLO = 0.71), temperature (Altitude-TEMP = 0.96), or forest cover (Altitude-BOS = 0.96), which excludes it from the model suggested by RDA and dbRDA as a predictor variable.

Focusing on the multivariate results, the variation in environmental variables can determine local factors in the establishment of the Ephemeroptera assemblage (do Amaral et al., 2019; Ghani et al., 2016). Looking at the results of RDA and dbRDA, the substrate block is significant, which dominates in the Andes region, with representative values in the Transition region. Conversely, for the Amazon, the primary collection substrate is of vegetal origin, allowing us to infer that this local variable may be crucial in the separation of the two groups in NMDS, as already demonstrated in the works of Principe et al. (2019), Schmitt et al. (2020). Furthermore, if we review the taxa associated with each region, the Andes and Transition, they are mainly associated with rocky substrates (Forero-Céspedes et al., 2016; Principe et al., 2019), unlike the Amazon, whose taxa are more characteristic of lowlands with soft substrates, aquatic macrophytes among others (Domínguez et al., 2002; Shimano et al., 2010).

Similarly, the conservation of forests plays a crucial role in preserving the Andean-Amazonian region, with the Andes serving as a reference point. Healthy riparian vegetation is critical for maintaining a greater diversity of insects in the order Ephemeroptera, contrasting with grassland areas primarily associated with the Amazon region, where more excellent livestock activity is evident. The negative impact of riparian forest fragmentation on aquatic insect diversity has already been demonstrated (Rawi et al., 2013; Meza-Salazar et al., 2020; Silva et al., 2022). However, in our study, the difference in richness between the Andes (21 taxa) and the Amazon (17 taxa) is less pronounced. For the latter, the presence of roots and aquatic macrophytes helps maintain significant values of the studied assemblage.

Additionally, evidence of a turnover of taxa related to changes in the riverscape components between the Andes and the Amazon serves as an essential reference for understanding the potential repercussions of connectivity loss due to deforestation in the study corridor. The work of Sierra et al. (2022) shows how accelerated deforestation in the Amazon can impact hydroclimatic connectivity with the Andes, leading to hydrological and energetic imbalances. This aspect could be modeled in the future, linking the dispersion or life cycle of the Ephemeroptera taxa with the river's hydrodynamics, which may be affected.

When analyzing the results of local contribution to beta diversity (LCBD) from the replacement component (LCBD-Rep), we observe that stations in the Amazon region may exhibit moderate (E22, E29, with the presence of miscellaneous vegetation consisting of natural vegetation and grasslands) or high fragmentation of riparian vegetation (E26, E27 dominated by aquatic macrophytes and grasslands), speaking in terms of forest presence. In these stations, the collection substrate we refer to as vegetal origin (O.VEG) may be crucial in species replacement. This idea is supported by the work of da Silva et al. (2023), who demonstrate that aquatic macrophytes are an essential predictor of EPT communities, driving changes in beta diversity. However, this dynamic differs in the Andes region, where there is high fragmentation of riparian forest in the mentioned stations. Though, there is also environmental heterogeneity that allows for taxon replacement.

Enríquez et al. (2020) found that the most excellent substitution of Ephemeroptera taxa is evident in preserved sites, limiting this component to mining activity as an anthropic stressor. This would lead us to think that even though stations in the Andes where more excellent replacement is evident have little or no vegetation cover, their loss is not anthropogenic but rather due to specific natural stochastic processes (avalanches), which could maintain the coexistence of taxa as proposed in the intermediate disturbance hypothesis (Battisti et al., 2016).

Regarding the local contribution to beta diversity by species turnover or nestedness (LCBD-Diff), the related stations significantly contribute to the variability in species richness of the Ephemeroptera assemblage among the sampling stations. Unlike turnover, where two communities exhibit non-shared species, in this case, the difference would establish subsets of

diversity among the communities, differing in the number of species (Baselga, 2010). Along a spatial gradient like ours, this may reflect the diversity of available niches (Legendre, 2014).

According to Legendre (2013), high values of LCBD can indicate sites with unusual species combinations and a high degree of conservation or degraded sites with low species richness. In our case, stations E1, E18, E20, E21, E26, and E27 exhibit the lowest values of taxonomic richness across the entire study region (see **Table S3** in the supplementary material). This leads us to consider prioritizing river restoration mechanisms at these stations, which could enhance the diversity of the Ephemeroptera order. This assertion is based on the works of Ruhí et al. (2017), Valenten-Neto et al. (2020), and Beaujour et al. (2024), who emphasize the importance of analyzing beta diversity through local contribution (LCBD) in regional dynamics to identify priority areas for the conservation of biological diversity.

About species contribution to beta diversity (SCBD), this metric is related to the degree of variation of individual taxa in the study area. In our case, the prominent taxon is *Miroculis* spp., which is collected in all Amazon region stations and is highly abundant within the Ephemeroptera assemblage. According to Rodríguez-Lozano et al. (2023), taxa with a high contribution to beta diversity are prioritized less for conservation, unlike taxa with lower contributions, which may be more vulnerable to extinction. We could mention species like *Coryphorus aquilus* or *Tricorythopsis rondoniensis*, which are present in one or two stations with low abundances. We are proposing these two species as critical indicators for the Amazon region.

## **Conclusions**

Our results highlighted differences in the diversity and distribution of the Ephemeroptera order along the environmental gradient studied in the Andean-Amazonian region of the Caquetá department. The Andes showed the highest taxa richness, with diversity and Simpson's reciprocal similar to the Transition region. In contrast, the Amazon had lower values in estimates of taxonomic alpha diversity. Regarding taxonomic beta diversity, species turnover between the study regions was prominent, induced by successional changes in landscape components such as substrate composition or land use, which can have an indirect influence.

Furthermore, our results also demonstrated the importance of understanding beta diversity and how each station's local contribution to beta diversity (LCBD) can identify prioritization points. Similarly, the species contribution to beta diversity (SCBD) can be a crucial indicator of riparian vegetation fragmentation, especially in the Amazon region, where the most significant deforestation has historically been evidenced. Today's activity has repercussions in terms of losing connectivity between the Andes Mountain range and the Colombian Amazon.

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## Supplementary material

**Table S1.** Geographic information related to the sampling stations where Ephemeroptera were collected in the Andean-Amazon region, during the 2021 and 2022 campaigns.

<b>Estação</b>	<b>Altitude</b>	<b>XCoord</b>	<b>YCoord</b>
E1	2205	-75,6796	1,8781
E2	2202	-75,6786	1,8773
E3	2070	-75,6700	1,8690
E4	1540	-75,6618	1,8404
E5	1000	-75,6470	1,7951
E6	957	-75,6394	1,7708
E7	2168	-75,7663	1,7627
E8	2041	-75,7496	1,7562
E9	1697	-75,7231	1,7359
E10	581	-75,6456	1,7294
E11	396	-75,6723	1,6236
E12	492	-75,9832	1,2977
E13	321	-75,9538	1,3350
E14	300	-75,9181	1,3622
E15	308	-75,8866	1,4083
E16	285	-75,2284	1,7665
E17	285	-75,2361	1,7539
E18	378	-75,3017	1,6362
E19	293	-75,3363	1,5427
E20	276	-75,3426	1,5205
E21	247	-75,3594	1,4283
E22	252	-75,3582	1,4253
E23	231	-75,3448	1,3754
E24	237	-75,3853	1,3572
E25	245	-75,4162	1,3358
E26	243	-75,4346	1,2999
E27	233	-75,4300	1,3052
E28	235	-75,4356	1,2954
E29	266	-75,4396	1,2821
E30	234	-75,4295	1,2973

**Table S2.** record of the different components of the riverscape for each of the sampling stations, where ephemeropterans were collected in the 2021 and 2022 campaigns. Current velocity (VE), dissolved oxygen (OD), hydrogen ion potential (pH), water temperature (TE), Depth (PR). Percentage of vegetation cover (C.V), trees (ARB.), shrubs (ARBU.), herbaceous (HER.), grasses (PA.), blocks (BLO.), stones (CANT.), gravel (GRAV.), sand (ARE.), vegetable origin (O.VEG). Forest (BOS.), agriculture (AGR.), recreation (RECR.), settlement (ASEN.), grazing (PAST.).

STATION	ALTITUDE	C.V	BLO.	CANT.	GRAV.	ARE.	O.VEG	PRO.	VEL.	OD.	PH.	TEM.	ARB.	ARBU.	PAST.	HERB.	BOS.	AGR.	RECR.	PASTOR.
E1	2205	0,8	0,7	0,1	0,05	0,1	0	0,05	0,75	7,5	7,363	14,4	0,4	0,6	0	0	1	0	0	0
E2	2202	0,8	0,75	0,1	0,05	0,05	0	0,1	1,04	7,65	7,423	13,5	0,4	0,6	0	0	1	0	0	0
E3	2070	0,8	0,65	0,1	0,1	0,1	0	0,2	0,96	7,775	7,393	13,875	0,1	0,5	0	0,4	0,7	0,3	0	0
E4	1540	0,9	0,7	0,1	0,1	0,1	0	0,26	1,45	7,2	7,363	16,75	0,3	0,4	0,2	0,1	0,7	0,2	0	0,1
E5	1000	0,6	0,6	0,2	0,1	0,1	0	0,26	1,35	7,075	7,273	19,7	0,4	0,2	0,2	0,2	0,3	0,5	0	0,2
E6	957	0,6	0,55	0,1	0,1	0,2	0	0,28	0,82	6,45	7,295	20,7	0,3	0,3	0,2	0,2	0,3	0,4	0	0,3
E7	2168	0,8	0,2	0,35	0,2	0,2	0	0,07	1,2	7,08	8,998	13,225	0,6	0,4	0	0,1	1	0	0	0
E8	2041	0,4	0,5	0,25	0,1	0,1	0	0,22	0,63	7,863	8,638	14,45	0,6	0,4	0	0	1	0	0	0
E9	1697	0,6	0,1	0,55	0,2	0,1	0	0,04	0,77	7,693	7,325	17	0,2	0,6	0	0,2	0,2	0,6	0	0,2
E10	581	0,8	0,3	0,4	0,05	0,2	0	0,37	1,08	6,885	7,98	23,3	0,7	0,1	0,1	0,1	0,1	0,2	0,1	0,6
E11	396	0,4	0,4	0,2	0,1	0,3	0	0,28	0,72	6,425	7,72	24,1	0,2	0,3	0,3	0,2	0,1	0,3	0,1	0,5
E12	492	0,5	0,2	0,4	0,1	0,2	0	0,3	1,77	6,25	7,378	24,2	0,55	0,2	0,05	0,2	0,2	0,2	0,1	0,5
E13	321	0,4	0,2	0,4	0,2	0,2	0	0,37	1,31	6,3	7,26	24,375	0,4	0,2	0,2	0,2	0,15	0,2	0	0,65
E14	300	0,4	0,1	0,6	0,1	0,2	0	0,23	2,82	6,45	7,135	24,325	0,2	0,4	0,4	0	0,2	0,2	0	0,6
E15	308	0,5	0,2	0,4	0,3	0,1	0	0,35	2,33	6,6	7,505	23,55	0,5	0,2	0,3	0	0,1	0,2	0,2	0,5
E16	285	0,2	0,1	0,4	0,2	0,3	0	0,29	3,67	5,875	7,615	28,75	0,1	0,1	0,6	0,2	0,1	0	0,1	0,8
E17	285	0,7	0	0,5	0,2	0,3	0	0,14	1,47	5,175	7,26	29,368	0,6	0,2	0,1	0,1	0,1	0,1	0,1	0,7
E18	378	0,3	0,6	0,2	0,1	0,1	0	0,36	0,96	6,05	7,498	25,65	0,2	0,2	0,5	0,1	0	0	0,2	0,8
E19	293	0,3	0,1	0,4	0,2	0,3	0	0,15	1,5	5,825	7,223	25,675	0,3	0,1	0,6	0	0,1	0	0,3	0,6
E20	276	0,3	0,1	0,5	0,1	0,3	0	0,18	1,18	5,875	7,485	25,95	0,1	0	0,8	0,1	0	0,2	0,1	0,7
E21	247	0,7	0	0	0,2	0,6	0	0,36	2,38	5,925	7,07	25,775	0,4	0,4	0,2	0	0,1	0,2	0,1	0,6
E22	252	0,4	0	0	0	0,3	0,7	0,77	0,29	4,2	6,235	25,8	0,2	0,3	0,4	0,1	0,1	0,1	0	0,8
E23	231	0,6	0	0	0	0	0,6	0,84	0,29	3,95	6,115	24,975	0,2	0,3	0,3	0,2	0,1	0	0	0,9
E24	237	0,5	0	0	0	0	0,6	0,67	0,2	4,5	6,128	25,4	0,2	0,3	0,4	0,1	0,1	0,1	0	0,8

E25	245	0,5	0	0	0	0	0,6	0,58	0,2	3,25	6,108	25,2	0,1	0,3	0,4	0,2	0,1	0,1	0	0,8
E26	243	0	0	0	0	0	0,6	1,3	0,07	3,3	5,713	26,575	0	0	0,6	0,4	0	0	0	1
E27	233	0	0	0	0	0	0,6	0,22	0,09	1,875	6,06	27,575	0,1	0,4	0,4	0,1	0	0	0	1
E28	235	0,4	0	0	0	0	0,65	0,6	0,32	4,685	5,798	27,475	0,1	0,3	0,3	0,3	0	0	0	1
E29	266	0,5	0	0	0	0	0,65	0,91	0,2	3,45	6,098	26,5	0,7	0,1	0,1	0,1	0,1	0,1	0	0,8
E30	234	0,6	0	0	0	0	0,4	0,13	0,01	0,525	5,743	25,775	0,4	0,2	0	0,4	0,1	0	0	0,9

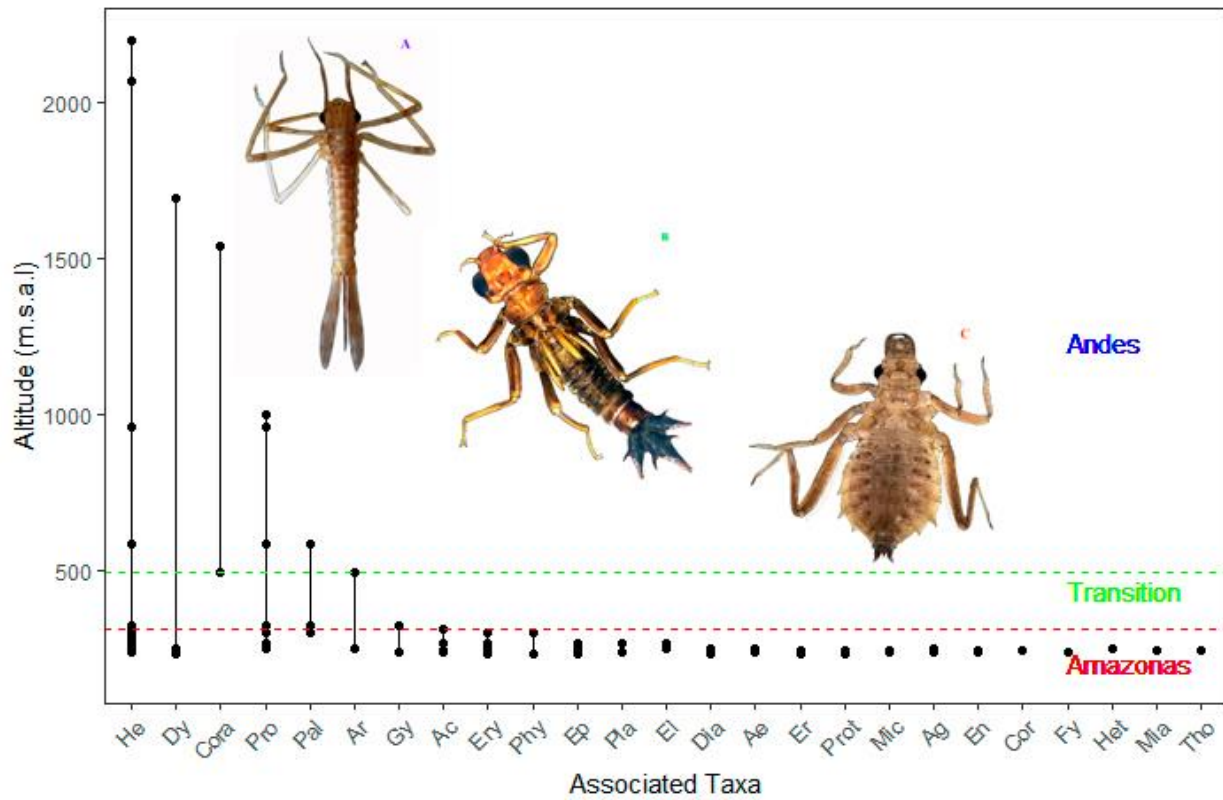
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## 9. Capítulo 4: Odonata (Insecta) in the Andean-Amazonian Corridor, Caquetá-Colombia: Composition, Richness, and Regional Distribution



Graphical abstract

## Odonata (Insecta) in the Andean-Amazonian Corridor, Caquetá-Colombia: Composition, Richness, and Regional Distribution

### Abstract

Dragonflies are a group of insects that play significant ecological roles, such as energy transfer and terrestrial pollination. Additionally, these organisms are valuable as bioindicators of water quality and riparian vegetation fragmentation. **Objective:** A study was conducted to determine the composition, specific richness, and regional-scale distribution of the Odonata order in the Andean-Amazonian corridor, specifically in the Caquetá Department. **Methodology:** Four bi-monthly field trips were conducted between October 2021 and April 2022, in 25 sampling stations. Biological material was collected using a Surber net, covering different available microhabitats. For data analysis, a line graph of altitudinal ranges was created for each identified taxon, and boxplots and barplots were used to relate species richness and abundance to vegetation cover. Additionally, a generalized linear model (GLM) was implemented, contrasting altitude, latitude, and longitude with Odonata specific richness and abundance. **Results:** Nine families and 25 genera of Odonata were identified, with Libellulidae and Coenagrionidae standing out. Additionally, the genera *Aeschnosoma* spp., *Gynothemis* spp., *Planiplax* spp., *Tholymis* spp., and *Agriogomphus* spp. are reported for the first time in the studied region of Caquetá. Concerning altitude, *Hetaerina* spp., *Dythemis* spp., and *Progomphus* spp. exhibited the widest distribution range. Regarding the GLM statistical models, specific richness and abundance presented an inverse relationship with Altitude, Latitude and Longitude. Concerning vegetation cover, grasslands hosted the highest Odonata abundance and species richness, particularly in the Amazonas ecoregion. **Conclusion:** Genera were identified that are new reports for the department of Caquetá, in addition to the relationship that regional variables may have with the order Odonata, finding the highest values of specific richness and abundance in the Amazon ecoregion, especially in stations with plant fragmentation.

**Keywords:** fragmentation, bioindication, ecoregion, regional variable, altitudinal range.

## Introduction

Aquatic ecosystems offer a range of essential environmental services to humanity, including potable water supply, climate regulation, food production, and recreational opportunities (Garcia, 2015; Milcu et al., 2013). Nevertheless, inadequate regulation of anthropogenic activities within riverine landscapes has led to the disruption of initial conditions in water systems, ultimately resulting in the loss of biodiversity (Borgwardt et al., 2019; Infan & Alatatawi, 2019). In this context, the analysis of different biological assemblages yields valuable information that can support river restoration and conservation proposals.

One of the assemblages capable of providing valuable information is Odonata, popularly known as 'dragonflies or damselflies (Pessacq et al., 2018). They are distinguished by their hemimetabolic development (Neiss & Amada, 2014) and are classified into two major suborders, Zygoptera and Anisoptera (Ramírez, 2010a; Roldán & Ramírez, 2022). The larval and nymphal stages inhabit both lotic ecosystems (Mendes et al., 2017; Tobias-Loaiza and Tamaris-Turizo, 2019) and lentic ecosystems (Altamiranda-S et al., 2010; Letsch et al., 2016; Vilenica et al., 2020).

These organisms serve as bioindicators of environmental conditions in an ecosystem (Garzón-Salamanca et al., 2020), being associated with various microhabitats such as aquatic macrophytes (Silva et al., 2020; Vilenica et al., 2022), riparian grasslands (Bried et al., 2018; Pires et al., 2022), hard substrates (Pires et al., 2020 a, b), and soft substrates (Luke et al., 2017; Worthen & Horacek, 2015).

Globally, the order is estimated to comprise around 6,300 known species (Bybee et al., 2021). According to Puget Sound Portal (2023), their listing as of December 23, 2020, breaks down the species into 3,092 anisopterans and 3,217 zygopterans. Furthermore, this order can be found in the Oriental, Australasian, Afrotropical, Palearctic, Nearctic, Pacific, and Neotropical biogeographic regions (Suhling et al., 2015). For the Neotropical region, Neiss & Hamada (2014) report 1,768 species, and according to Olaya (2019), the countries with the highest number of registered species include Brazil, Peru, Venezuela, Colombia, and Ecuador.

In Colombia, this order has been of growing interest due to its ecological role in ecosystems and the species richness it harbors within the country (Bota-Sierra et al., 2021; Palacino-Rodríguez, 2016), with approximately 410 species (Palacino-Rodríguez et al., 2022). Furthermore, it is worth noting that Colombia is globally recognized as one of the most megadiverse countries (Bota-Sierra et al., 2018), hosting a wide variety of ecosystems, including the Amazon rainforest and the Andean paramos.

In the Caquetá Department, where this research is being conducted, Bota-Sierra et al. (2015) recorded 26 species of Odonata, while Stand-Pérez et al. (2021) found 40 species in the Chiribiquete National Natural Park. Although these data are valuable, most of the records are somewhat distant from study area and focus on adults, highlighting an initial need to address the group at the immature stage to enrich local information. Additionally, it is important to mention that some studies on aquatic macroinvertebrates (Bravo and Restrepo, 2021; González et al., 2022; Gutiérrez-Garaviz et al., 2016) and biological databases such as GBIF (2023) allow for an approximate reference of 62 genera. Therefore, this study can validate that information and provide new data to this region of interest.

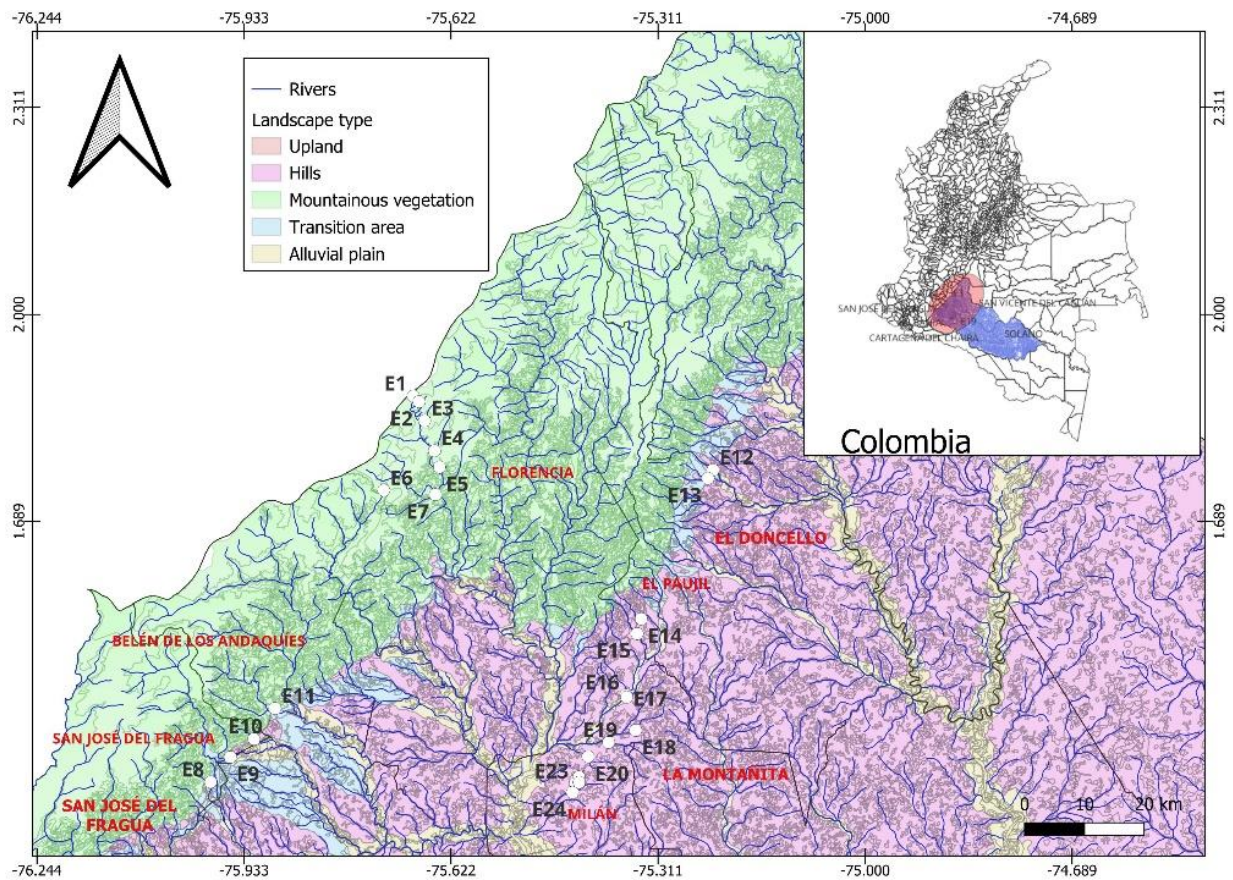
Another crucial aspect, distinct from understanding the composition or richness of the Odonata order, is comprehending how the altitudinal gradient (Palacino-Rodríguez et al., 2020; Pešić et al., 2017) and geographic variations in latitude (Sniegula et al., 2016) and longitude (Vorster et al., 2020) influence the distribution of its organisms. It's essential to consider that regional variables influence the local characteristics of the river habitat (Dodds et al., 2019), including the quality of riparian vegetation concerning the altitudinal gradient (Latsiou et al., 2021). Therefore, there is a second compelling reason to investigate these relationships in the Andean-Amazonian region of the Caquetá Department, especially since it has been demonstrated that many taxa of the Odonata Order serve as indicators of riparian vegetation fragmentation (Seidu et al., 2017), a phenomenon notably evident in the Caquetá Amazon.

This research determines the composition, specific richness, and regional distribution of the Odonata order in selected lotic ecosystems of the Andean-Amazonian corridor of Caquetá. Furthermore, it establishes a connection between the interest group and vegetation cover.

## Material and Methods

### Study area

The study stations included seven municipalities in the Caquetá Department, covering an altitudinal range between 231 meters above sea level (m.a.s.l.) and 2202 m.a.s.l. (**Figure 1**, **Table S1** in supplementary material with geography information). Stations one (E1) to seven (E7) encompassed the greatest altitudinal variation, primarily associated with the Andean Ecoregion featuring mountainous vegetation landscape type. Stations eight (E8) to twelve (E12) were associated with a transition area. And, from station fourteen (E14) to twenty-five (E25), they were associated with vegetation cover in alluvial plain, hills and upland, characteristics of the Amazonas Ecoregion, with little altitudinal variation.



**Figure 1.** Distribution of the 25 sampling points in the Andes-Amazon region, Caquetá-Colombia, sampled from October 2021 to April 2022. Modified from the Land Cover of the

Colombian Amazon shapefile for the year 2022, Scale: 1:100,000 (Open data - SINCHI Institute, 2023).

### ***Collection and identification***

Four bimonthly sampling campaigns were conducted between October 2021 and April 2022, during which aquatic macroinvertebrates were collected using a composite multi-habitat sampling approach. A Surber net 0.3x0.3 meter with a mesh size of 250 micra was employed, following the guidelines proposed by Merritt et al. (2008), Ramírez (2010b), and Roldán (1988). For the reference stretch, 100 meters of length were selected, and 10 trawls samples were taken against the current. The collected material was preserved in 95% ethanol for subsequent separation and counting in the laboratory, utilizing a Zeiss Stemi DV4 stereoscope.

Regarding the identification of different morphotypes of the Odonata order, taxonomic guides, and keys of Anjos-Santos et al. (2018), Costa et al. (2004), Ellenrieder & Garrison (2008), Lago and Pessacq (2018), Lozano et al. (2018), Neiss & Hamada (2014), Neiss et al., (2018), Novelo-Gutiérrez et al. (2018), and Pessacq et al. (2018), Ramírez (2010a) were used.

Additionally, altitude and geographical information for each study region were recorded using a GARMIN 64 SC GPS device.

### ***Data analysis***

The ggplot2 library (Wickham, 2016) was utilized to construct a graph representing the altitudinal distribution based on Odonata abundance data. Additionally, employing the same library along with GGally (Schloerke et al., 2018), a bubble plot was generated to illustrate the relationship between species richness and abundance with geographic distribution.

To assess the potential influence of regional environmental variables (Altitude, Latitude, and Longitude) on species richness and abundance, a Generalized Linear Model (GLM) was employed. This model allows for the analysis of data when the assumptions of traditional linear regression are not met (López-González & Ruiz-Soler, 2011). A Poisson distribution was chosen

for this analysis, which is suitable for discrete data and obviates the need for data transformation (O'Hara & Kotze, 2010).

The Akaike's Information Criterion (AIC) was employed to identify the model with the best fit and greatest parsimony (Satha et al., 2017). Likewise, the same Generalized Linear Model (GLM) was utilized to construct a data matrix that discriminated between suborders (Anisoptera, Zygoptera) of Odonata. This involved considering species richness and abundance values as independent variables about altitude as the dependent variable. The results of these analyses were elaborated upon in the discussion section of this study.

All these analyses were done using the statistical programming software RStudio version 2023.03.1.

QGIS software v. 3.30.2 (QGIS Development Team, 2023) was used to relate vegetation cover with the richness and abundance of odonates. Through the "Union" geoprocessing tool, the shape layer obtained from the SINCHI Institute (Open Data-SINCHI Institute, 2023) was overlaid with the stations, identifying five vegetation covers: dense forest (B.D), fragmented forest with secondary vegetation (B.F.V.S), secondary vegetation (V.S), grasslands (P.S), and miscellaneous with pastures and natural spaces (M.P.E.N). Subsequently, the information was visualized using the graphical functions boxplot (Abundance) and barplot (Specific Richness) in RStudio.

## Results

### *Taxonomic Composition of the Odonata*

394 individuals were collected, identifying nine families (four anisopterans with 186 individuals, five zygopterans with 208 individuals) and 25 genera (**Table 1**), associated with the 25 sampling stations. New reports for the Caquetá department are highlighted, including the genera *Aeschnosoma* spp., *Gynothemis* spp., *Planiplax* spp., *Tholymis* spp., and *Agriogomphus* spp. Two of the found families constitute over 50% of the odonate composition. The family

Libellulidae has eleven genera, contributing 34.68% to the order's composition. Coenagrionidae ranks second, with five genera representing 26.07% of the total.

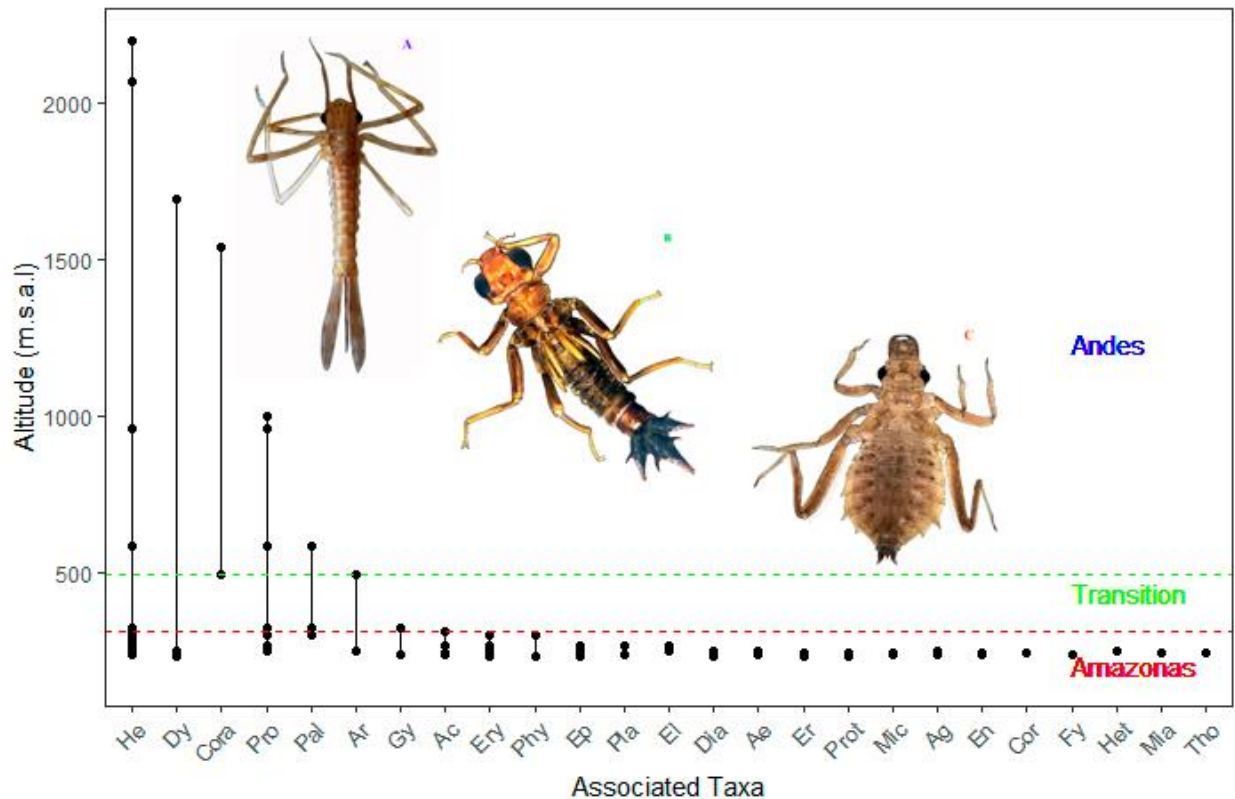
**Table 1.** Composition of the Odonata order in the Andean-Amazonian region, Caquetá, Colombia, collected from October 2021 to April 2022. Within parentheses are the abbreviations of each taxon to consider in Figure 2 and Figure 5.

Taxa	E 1	E 2	E 3	E 4	E 5	E 6	E 7	E 8	E 9	E1 0	E1 1	E1 2	E1 3	E1 4	E1 5	E1 6	E1 7	E1 8	E1 9	E2 0	E2 1	E2 2	E2 3	E2 4	E2 5	
<b>Anisoptera</b>																										
<b>Aeschnidae</b>																										
cf.																										
Coryphaeschna <sup>(Co)</sup> Williamson, 1903	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2	-	-	-	-	-
<b>Corduliidae</b>																										
Aeschnosoma spp. <sup>(Ae)</sup> Selys, 1870	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	8	3	-	-	-	-	3
<b>Libellulidae</b>																										
Elga spp. <sup>(El)</sup> Ris, 1909	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	4	-	-	-	3	-
Erythemis spp. <sup>(Er)</sup>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	9	4	-	-	-	-
Erythrodiplax spp. <sup>(Ery)</sup> Brauer, 1868	-	-	-	-	-	-	-	-	-	2	-	-	-	-	-	-	2	3	-	-	10	7	1	1	1	-
Diastatops spp. <sup>(Dia)</sup>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	4	31	15	2	-	-
Dythemis spp. <sup>(Dy)</sup> Hagen, 1861	-	-	-	-	3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3	-	2	1	-	-	
Fylgia spp. <sup>(Fy)</sup> Kirby, 1889	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	
Gynothemis spp. <sup>(Gy)</sup> Calvert, 1909	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	3	-	-	1	-	-	
Miathyria spp. <sup>(Mia)</sup> Kirby, 1889	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	7	-	-	-	-	
Micrathyria spp. <sup>(Mic)</sup> Kirby, 1889	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3	-	-	-	2	
Planiplax spp. <sup>(Pla)</sup> Muttkowski, 1910	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	5	3	
Tholymis spp. <sup>(Tho)</sup> Hagen, 1867	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3	-	-	-	-	
<b>Gomphidae</b>																										
Agriogomphus spp. <sup>(Ag)</sup> Selys, 1869	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	6	2	-	-	-	-	
Phyllocycla spp. <sup>(Phy)</sup> Calvert, 1948	-	-	-	-	-	-	-	-	-	2	-	-	-	-	-	-	-	-	1	-	-	-	-	-	-	
Progomphus spp. <sup>(Pro)</sup> Selys, 1854	-	-	-	4	6	-	4	-	3	2	-	-	-	-	-	-	-	-	-	1	-	-	-	2	-	
<b>Zigoptera</b>																										
<b>Calopterygidae</b>																										

<i>Hetaerina</i> spp. <sup>(He)</sup> Hagen, 1853	2	4	-	-	2	-	1	-	3	1	9	1	3	3	3	4	5	-	-	4	-	-	4	3	-
<b>Coenagrionidae</b>																									
<i>Acanthagrion</i> spp. <sup>(Ac)</sup> Selys, 1876	-	-	-	-	-	-	-	-	-	-	3	-	-	-	-	-	-	-	-	-	5	-	-	2	2
<i>Argia</i> spp. <sup>(Ar)</sup> Rambur, 1842	-	-	-	-	-	-	6	-	-	-	-	-	-	-	-	-	-	-	-	2	-	-	-	-	-
<i>Enallagma</i> spp. <sup>(En)</sup> Selys, 1876	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3	-	6	-	-	-	-
<i>Epipleoneura</i> spp. <sup>(Ep)</sup> Williamson, 1915	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	5	2	5	7	5	4	9	5	3
<i>Protoneura</i> spp. <sup>(Prot)</sup> Selys, 1857	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	-	12	12	4	-	-
<b>Megapodagrionidae</b>																									
<i>Heteragrion</i> spp. <sup>(Het)</sup> Selys, 1862	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	6	-	-	-	-	-
<b>Platystictidae</b>																									
<i>Palaemnema</i> spp. <sup>(Pal)</sup> Selys, 1860	-	-	-	-	-	-	9	-	2	5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
<b>Polythoridae</b>																									
<i>Cora</i> spp. <sup>(Cora)</sup> Selys, 1853	-	-	4	-	-	-	6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

### *Altitudinal distribution of Odonata*

According to the altitudinal distribution of Odonata genera (**Figure 2**), *Hetaerina* spp., *Dythemis* spp., and *Progomphus* spp. exhibited the widest distribution, spanning from the Andes to the Amazonas ecoregion. The genus *Cora* spp. was collected above 492 m.a.s.l., and 76% of the recorded taxa were below 310 m.a.s.l. within the Amazonas ecoregion.



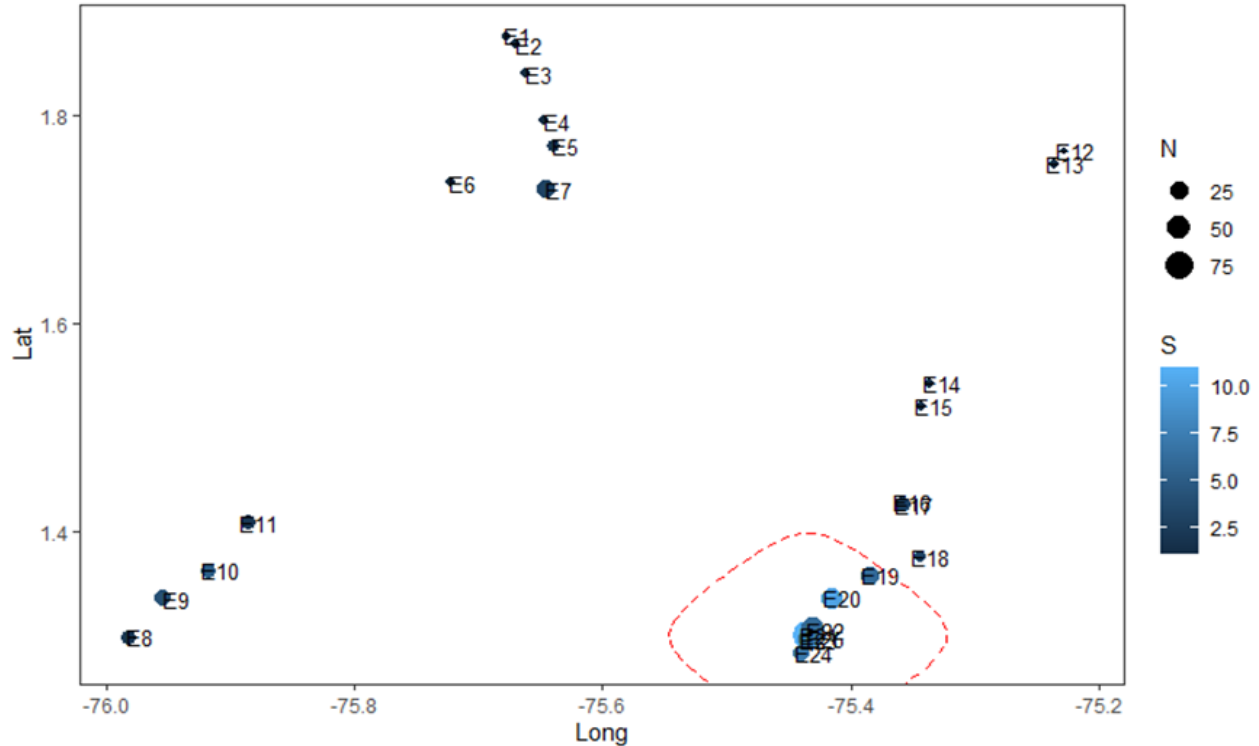
**Figure 2.** Altitudinal distribution of Odonata genera in the Andean-Amazonian region, Caquetá, Colombia, collected between October 2021 and April 2022. Abbreviations can be identified in Table 1. *Hetaerina* spp. (A), *Cora* spp. (B), *Agriogomphus* spp. (C).

***Abundance and Specific Richness of Odonata, Geographical Distribution and Vegetation Cover***

Figure 3 clearly depicts a notable concentration of Odonata richness and abundance in stations 19 to 25, located at points near a longitude of -75.4W and below a latitude of 1.4N, area associated with the Amazonas ecoregion. When examining the potential influence of regional environmental variables (Altitude, Latitude, Longitude) on the two specified variables (species richness and abundance) through generalized linear models (GLM), significant results were obtained (**Table 2**).

The analyses revealed that Altitude (p-value = 0.0008), Latitude (p-value = 3.75E<sup>-07</sup>), and the interaction between Latitude\*Longitude (p-value = 0.048) were significant for species

richness. However, according to the Akaike Information Criterion (AIC), the interaction model proved to be the best fit for the data (AIC = 93.891). Regarding abundance, Altitude (p-value =  $2.41E^{-13}$ ), Latitude (p-value =  $2.00E^{-16}$ ), Longitude (p-value = 0.0315), as well as the interactions Altitude\*Latitude (p-value = 0.001), Latitude\*Longitude (p-value =  $1.28E^{-08}$ ), and AltitudeLatitude\*Longitude (p-value = 0.026) were significant. The latter model achieved the best fit (AIC = 281.14).



**Figure 3.** Bubble chart depicting the relationship between calculated values of richness and abundance, based on data collected during a sampling period between October 2021 and April 2022, in the Andean-Amazonian region, Caquetá, Colombia. Stations (E) with higher richness and abundance are highlighted in red.

**Table 2.** GLM Models Associating Odonata Specific Richness and Abundance with Regional Variables. Data obtained during a sampling period between October 2021 and April 2022, in the Andean-Amazonian region, Caquetá, Colombia.

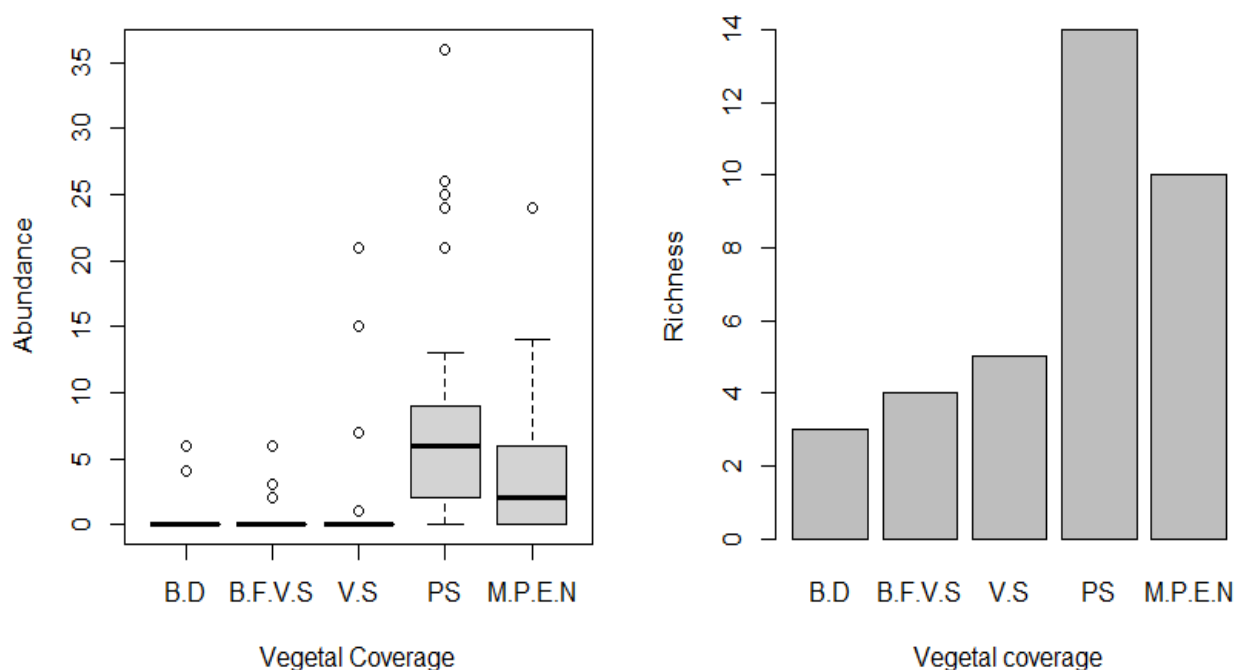
Models	Estimate	SE	Z	P	AIC
<b>(a) Richness</b>					
Altitude	-1.11E-03	0.0001	-3.329	<b>0.000872</b>	114.044
Latitude	-3.669	0.722	-5.081	<b>3.75E-07</b>	<b>96.071</b>

Longitude	0.606	0.504	1.203	0.229	130.540
Altitude*Latitude	0.005	0.003	1.835	0.066	<b>96.082</b>
Altitude*Longitude	-0.002	0.006	-0.434	0.665	117.683
Latitude*Longitude	-9.748	4.939	-1.974	<b>0.048</b>	<b>93.891</b>
Altitude*Latitude*Longitude	8.69E-04	3.98E-02	0.022	0.983	100.011

**(b) Abundance**

Altitude	-0.001	0.0001	-7.324	<b>2.41E-13</b>	426.9
Latitude	-3.958	0.355	-11.13	<b>2.00E-16</b>	341.5
Longitude	0.508	0.236	2.151	<b>0.0315</b>	516
Altitude*Latitude	0.004	0.001	3.313	<b>0.0009</b>	333.22
Altitude*Longitude	-0.002	0.003	-0,788	0,431	427.79
Latitude*Longitude	-16.729	2.941	-5.688	<b>1.28E-08</b>	<b>295.56</b>
Altitude*Latitude*Longitude	4.37E-02	1.97E-02	2.222	<b>0.026</b>	<b>281.14</b>

As can be observed in Figure 4, the highest values of taxa richness and abundance of the genus of Odonata were associated with the vegetation coverage of grasslands (PS), followed by the vegetation coverage of miscellaneous with pastures and natural spaces (M.P.E.N).



**Figure 4.** Comparison for taxa richness and abundance of the Odonata, considering vegetation coverages, elaborated from data obtained during a sampling period between October 2021 and April 2022 in the Andean-Amazonian region, Caquetá, Colombia. dense forest (B.D), fragmented forest with secondary vegetation (B.F.V.S), secondary vegetation (V.S), grasslands (P.S), and miscellaneous with pastures and natural spaces (M.P.E.N).

## Discussion

### *Taxonomic Composition of the Odonata*

According to the taxonomic composition found, considering the referenced works for the Caquetá department regarding genera (Bota-Sierra et al., 2015; Bravo & Restrepo, 2021; GBIF, 2023; González et al., 2022; Stand-Pérez et al., 2021), the collected and identified specimens encompass 32.25% (20 genera), with a reference of 62 genera. Furthermore, new taxa would be added to the reference data, resulting in a total of 67 genera present in the Caquetá department.

Focusing on the new records, it is known that they are present at other localities in Colombia, near the study area; therefore, the data from this study allows for an expansion of their geographical distribution:

*Aeschnosoma* spp. is a genus that was first recorded in the year 2015 (Rodríguez, 2015), located in the Meta department. Subsequently, it was published for the Chocó department (Mosquera-Murillo and Mosquera-Mosquera, 2021), and its closest occurrence to the Caquetá department is in Caño Cristales, Sierra de la Macarena, Meta department (Bohorques et al., 2018). Although González et al. (2022) has listed the family Corduliidae for Caquetá, it is possible that the collected individual belongs to the same genus occurring in the present study.

The genus *Gynothemis* spp. is recorded in the departments of Amazonas (González et al., 2022), Meta (Amaya-Perilla & Palacino-Rodríguez, 2012), and Putumayo (Bota-Sierra et al., 2015). *Planiplax* spp. is reported for the Meta department (Álvarez-Álvarez et al., 2023) and Putumayo (González et al., 2022). *Tholymis* spp. is associated with the departments of Amazonas (Palacino-Rodríguez, 2009), Guaviare (González et al., 2022), Meta and Nariño (iNaturalist,

2023). Lastly, the genus *Agriogomphus* spp. is documented in the Meta department (Martínez, 2019) and Vichada (Trujillo, 2018).

Regarding the dominant families Libellulidae (Anisoptera) and Coenagrionidae (Zigoptera), Pérez-Gutiérrez and Palacino (2011) mention that these are the two most common taxonomic groups in Colombia. Similarly, the studies by Mosquera-Murillo and Mosquera-Mosquera (2021), Palacino-Rodríguez et al. (2020a), and Tobias-Loaiza and Tamaris-Turizo (2019) showed a similar dominance as the one observed in this research.

The family Libellulidae comprises 143 genera and 1000 species, distributed worldwide. In the Neotropics, 390 species are listed across 48 genera (Neiss et al., 2018), which, according to this reference of genera, the study present would encompass 22.91%. Concerning Colombia, the study it would account for 33% of 33 genera list (Hincapie-Montolla et al., 2022).

Regarding Coenagrionidae, it is the most diverse family within the Order Zigoptera, encompassing 1267 species across 114 genera (Lozano et al., 2018). In the Neotropics, 650 species in 70 genera are listed (Lozano et al., 2018), with the study region accounting for 7.14% of data referent. Concerning Colombia, understands 18.51% of 27 genera list (Hincapie-Montolla et al., 2022).

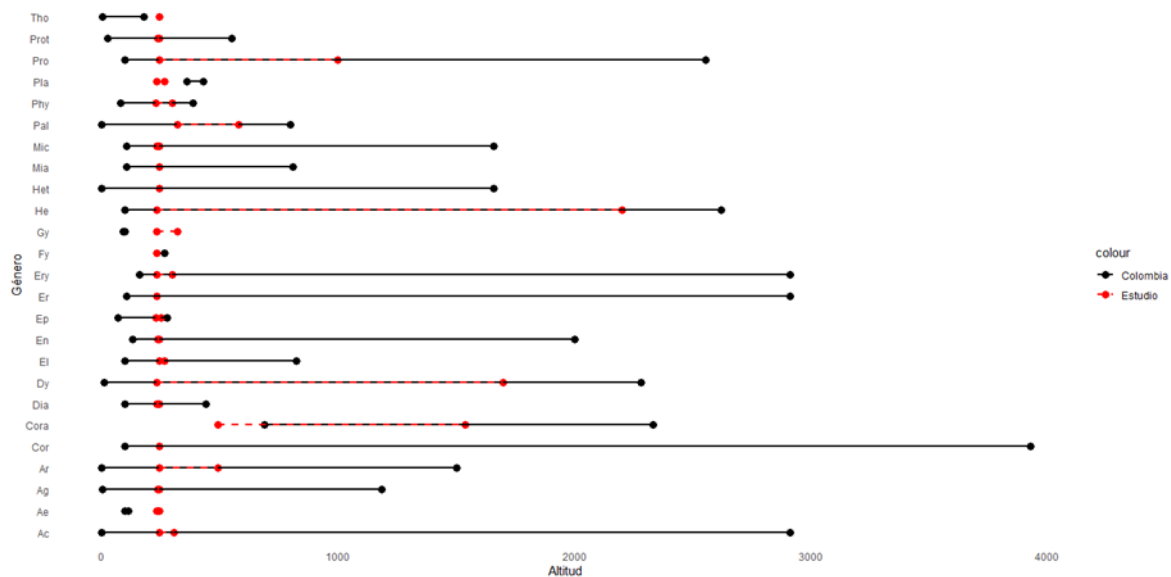
### ***Altitudinal distribution of Odonata***

The change in altitudinal gradient can influence the community of aquatic macroinvertebrates (Jacobsen, 2003; Jacobsen, 2004), as evidenced in the present study at the level of Odonata. This has been the subject of interest in numerous scientific studies, aiming to understand distribution patterns and the environmental factors that influence their composition and diversity (Cancellario et al., 2022; Gómez-Anaya et al., 2011; Harabiš & Dolný, 2010; Maoduš et al., 2022).

Reviewing the databases of SIB-Colombia (2023), GBIF (2023), and the works of Cuéllar-Cardozo et al. (2019), Mosquera-Murillo & Mosquera-Mosquera (2021), Tobias-Loaiza

and Tamaris-Turizo (2019), it can be observed that most genera found in the study have a broader altitudinal range (**Figure 5**), even surpassing 3000 m.a.s.l, as is the case of *Coryphaeschna* spp. reported for the Tolima department (Lozano, 2021). However, the present study also provides new information for the Caquetá region regarding the altitudinal distribution of the genera *Gynothemis* spp., *Palaemnema* spp., *Tholymis* spp., and *Aeschnosoma* spp.

It appears therefore, that is necessary to collect in many other locations within the department in the future to expand the information, especially in sites further north, for example, near the Los Picachos National Natural Park where higher altitudes occur. This need can be supported by the works of Bota-Sierra & Sandoval-H (2017), Torres Pachón & Realpe-Rebolledo (2015), Palacino-Rodríguez et al. (2020b), who have collected odonates in other departments at higher altitudes compared to the current study.



**Figure 5.** Altitudinal distribution ranges of Odonata genera found in the study (Red line) vs. reviewed information for Colombia (Black line). Source: elaborated by Gutiérrez-Garaviz (2023).

*Abundance and Specific Richness of Odonata, Geographical Distribution and Vegetation Cover*

Analyzing how biodiversity is distributed in response to geographical factors is a topic of great interest within the field of Ecology (Jourdan et al., 2019). For instance, Jacobsen (2004) found a decrease in the richness of the Odonata order with increasing altitude, a pattern similar to that recorded in the study where higher richness values, combined with abundance, were associated with stations at lower altitudes, within the Amazonas Ecoregion. Furthermore, when differentiating between the two suborders, Palacino-Rodríguez et al. (2020c) discovered that the Anisoptera group can be affected by altitude, unlike Zygoptera. This observation is also supported by the present study, as a GLM analysis was conducted to differentiate between Odonata suborders. The results indicate that altitude significantly impacts both the specific richness (p-value= 0.009) and abundance (p-value=  $1.14e^{-07}$ ) of Anisoptera, while in the case of Zygoptera, specific richness is not significant (p-value= 0.055), but abundance can indeed be affected (p-value=  $1.92e^{-06}$ ).

Other studies have also demonstrated how altitude and latitude influence the establishment of an organism when relating to habitat conditions (Birrell et al., 2020; Ceia-Hasse et al., 2023; Cerini et al., 2020; Heidrich et al., 2018; Pires et al., 2018; Villamarín et al., 2020). Furthermore, according to the results, it is possible that geographical length plays a decisive role in the richness of Odonata, considering that this measure decreases when approaching the eastern mountain range of the Andes, and increases towards the Amazon plain. However, it is important to carry out a future study focused on evaluating this regional variable on a larger geographic scale, evaluating changes associated with local environmental variables such as substrate, vegetation cover, temperature, or life zone, among others. For instance, the work of Kalkman et al. (2022) identifies a longitudinal decrease in Odonata diversity from European countries towards Russia and desert country (Africa, Asian), where temperature and type of ecosystem are determining factors.

When analyzing the relationship between the richness and abundance of Odonata and the vegetation cover, some lower altitude stations exhibit a high fragmentation of the riparian forests. This is the case of stations 20 and 21, where the highest specific richness is evident (10 and 11 taxa). Additionally, stations 21 and 22 are dominated by aquatic macrophytes and grasses in near-land habitats. Some studies mention that odonates benefit from degraded areas, forest

fragmentation, and the presence of aquatic macrophytes Carvalho et al. (2013), Cuéllar-Cardozo et al. (2020), Mendes et al. (2019), Suárez-Tovar et al. (2022); serving as groups indicator of the environmental conditions of the rivers (Da Silva et al., 2013; Ribeiro & Brasil, 2022).

The above explains why some taxa consulted in the bibliography have more altitudinal distribution, considering that there may be more significant vegetal fragmentation in those places compared with the research where there are more conserved areas, such as the Andes region.

## **Conclusions**

The Odonata assemblage in the Andean-Amazonian region of the Caquetá department, has high richness and abundance of genera of Odonata, with dominance of the Libellulidae and Coenagrionidae families. Moreover, the genera *Aeschnosoma* spp., *Gynothemis* spp., *Planiplax* spp., *Tholymis* spp., and *Agriogomphus* spp. represent new records in this region, expanding their geographic distribution within the country.

Regarding altitudinal distribution, contrasting the results with the literature, most of the recorded taxa have a broader range, especially the genera from the Amazon Ecoregion collected below 310 m.a.s.l. It is noteworthy that new information is provided on the altitudinal distribution of the genera *Gynothemis* spp., *Palaemnema* spp., *Tholymis* spp. and *Aeschnosoma* spp.

Generalized linear models (GLM) demonstrated that regional variables can be related to specific richness and abundance of Odonata, identifying an inverse relationship with Altitude, Latitude and Longitude, concentrating their highest values in the Amazonas ecoregion.

Regarding vegetation coverage, grasslands exhibited the highest abundance and richness of Odonata genera, particularly associated with the Amazonas Ecoregion. In this way, it can be considered that in the Amazonas Ecoregion, it is necessary to initiate fluvial restoration processes, especially in rivers where grasses dominate as vegetation cover. If a clear policy regarding ecosystem services and the use of natural resources is not implemented in the Andes

Ecoregion, which has better vegetation cover conditions (dense forests), the potential increase in Odonata abundance and specific richness could serve as an alarm regarding biodiversity loss.

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## Supplementary materials

**Table S1.** Geographic information related to the sampling stations in the Andean-Amazonian region, Caquetá, Colombia

STATION	ALTITUDE	XCOORD	YCOORD
E1	2202	-75,6786	1,8773
E2	2070	-75,6700	1,8690
E3	1540	-75,6618	1,8404
E4	1000	-75,6470	1,7951
E5	957	-75,6394	1,7708
E6	1697	-75,7231	1,7359
E7	581	-75,6456	1,7294
E8	492	-75,9832	1,2977
E9	321	-75,9538	1,3350
E10	300	-75,9181	1,3622
E11	308	-75,8866	1,4083
E12	285	-75,2284	1,7665
E13	285	-75,2361	1,7539
E14	293	-75,3363	1,5427

E15	276	-75,3426	1,5205
E16	247	-75,3594	1,4283
E17	252	-75,3582	1,4253
E18	231	-75,3448	1,3754
E19	237	-75,3853	1,3572
E20	245	-75,4162	1,3358
E21	243	-75,4346	1,2999
E22	233	-75,4300	1,3052
E23	235	-75,4356	1,2954
E24	266	-75,4396	1,2821
E25	234	-75,4295	1,2973

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## 10. Conclusões gerais

Nossa investigação ressalta a dinâmica da paisagem fluvial dos ecossistemas lóticos, por meio da análise de processos sucessionais relacionados uma série de variáveis ambientais locais nas três áreas de estudo situadas no corredor Andino-Amazônico de Caquetá. Identificamos alterações significativas em diversos aspectos, tais como o substrato, a velocidade do fluxo da água, a profundidade, o oxigênio dissolvido, o pH e a cobertura vegetal ripária, entre outros. Adicionalmente, observamos que o uso do solo, particularmente voltado para o desenvolvimento da pecuária extensiva, exerce um impacto marcante sobre os componentes da paisagem fluvial, evidenciando um processo de degradação ambiental que se estende da região dos Andes em direção à Amazônia. Além de isso, tendo em conta a cobertura vegetal, propomos duas rotas de gestão baseadas na cobertura vegetal. Para os Andes que apresentam melhor conservação da paisagem fluvial, a rota de recuperação pode ser implementada em algumas das estações que apresentam alteração. Para Transição e Amazonas, o melhor caminho é a transformação, que teria a finalidade de criar espaços naturais e pastagens diversas, baseadas em sistemas agroecológicos ou silvipastoris.

Os processos sucessionais dos diferentes componentes da paisagem influenciam a composição e diversidade dos macroinvertebrados aquáticos. Nossos resultados mostram como a assembleia se altera, em termos taxonômicos e funcionais, evidenciando uma substituição de espécies, influenciada por diferentes filtros ambientais como o tipo de substrato ou atividades de uso do solo como pastoreio que podem influenciar indiretamente a composição em termos de perda cobertura vegetal ribeirinha. Além disso, a Amazônia contempla maior riqueza, porém, os Andes e a Transição apresentam melhor diversidade. Estes dados são muito importantes, tendo em conta que a área de estudo se enquadra numa vulnerabilidade em termos da perda de ligação entre a cordilheira dos Andes e a Amazônia colombiana.

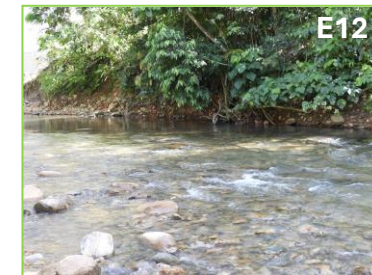
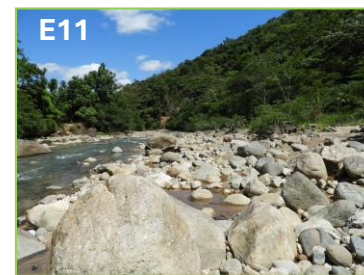
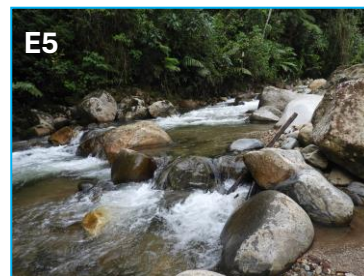
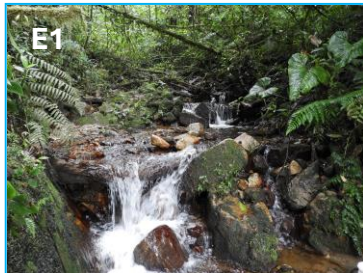
Focando apenas na ordem Ephemeroptera, organismos que são reconhecidos pela sua capacidade bioindicadora, também conseguimos identificar a influência que diferentes filtros ambientais podem ter na sua composição e diversidade, gerando também uma substituição de

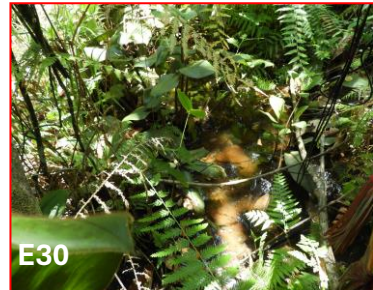
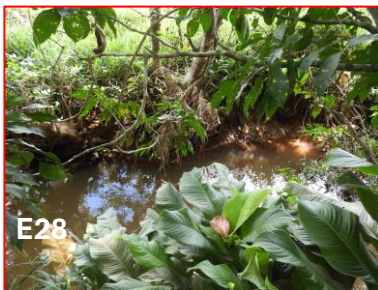
espécies entre as áreas de estudo. Além disso, este grupo nos indicou a importância de analisar as contribuições locais e de espécies para a diversidade beta, e como ela pode estar relacionada com estratégias de priorização de rios para restauração e conservação. Desta forma, consideramos que a região Andina-Amazônica é referência de estudo para a ordem Ephemeroptera.

Da mesma forma, a ordem Odonata mostra-nos a influência que a altitude, a latitude e a longitude podem ter, embora neste caso a análise seja feita a partir de uma escala regional. Além disso, esses organismos possuem grande relevância bioindicadora para fragmentação florestal, permitindo-nos associar maior abundância e riqueza a estações com alta fragmentação de mata ciliar relacionada à cobertura arbórea.

## 11. Anexos da tese

### Anexo 1. Estações de amostragem.





Anexo 2. Alguns táxons de macroinvertebrados aquáticos



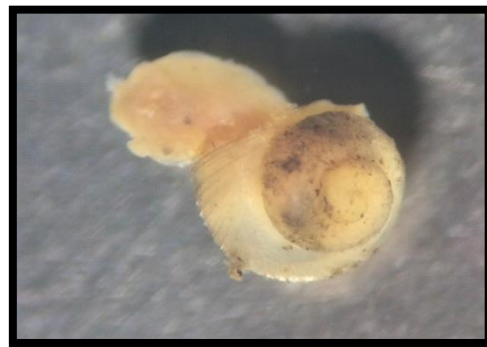
**Familia Glossiphoniidae**



**Familia Lumbriculidae**



**Familia Limnesiidae**



***Acrorbis* spp.**



***Biomphalaria* spp.**



***Cylloepus* spp.**



***Sylviocarcinus* spp.**



***Aposonalco* spp.**



*Coryphorus aquilus*



*Cryphocricos spp.*



*Petrophila spp.*



*Corydalis spp.*



*Aeschnosoma spp.*



*Anacroneuria spp.*



*Neotriplectides spp.*



Copepoda