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Diversity analysis of Chironomidae from the Andean-Amazonian transition (Caquetá, Colombia): a regional perspective

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ABSTRACT

Introduction: The family Chironomidae is one of the most diverse and abundant groups of aquatic macroinvertebrates in aquatic ecosystems.

Objective: To analyze Chironomidae's composition and diversity in the Caquetá department's Andean-Amazonian corridor, Colombia.

Methods: Three study zones (Andean, Transition, and Amazonian), each with ten sampling stations, were selected. We conducted four field campaigns to collect biological material and record riverscape characteristics. We assessed alpha diversity using Hill numbers, a set of diversity measures providing a unified framework for diversity analysis. We also analyzed beta diversity by considering species richness differences and turnover. Using multivariate RDA analysis, physical and chemical variables of the riverscape contrast with taxonomic composition and beta diversity.

Results: 36 chironomid taxa were identified, with the subfamily Chironominae and the genera *Cricotopus*, *Chironomus*, *Larsia*, and *Polypedilum* standing out. Hill numbers showed differentiation in Shannon-Wiener diversity (q_1) and inverse Simpson diversity (q_2), but richness (q_0) showed less variation across study zones. The most significant component of beta diversity was species richness differences. Current velocity, total dissolved solids (TDS), vegetation cover, and substrates of sandy and vegetative origin (roots, aquatic macrophytes), as well as other components of the riverscape, significantly influenced species composition and beta diversity.

Conclusions: Significant genus level information is provided for the Chironomidae family in the Andean-Amazonian region of the department of Caquetá, revealing dissimilarity among the study areas driven by differences in richness, as a measure of taxonomic beta diversity. Moreover, these findings indicate that the transition region functions as an ecotone for Chironomidae, serving as an intermediate zone between the Andes and the Amazon.

Key words: beta diversity; riverscape; non-biting midges; Hill numbers.



RESUMEN

Análisis de la diversidad de quironómidos de la transición andino-amazónica (Caquetá, Colombia): una perspectiva regional

Introducción: La familia Chironomidae es uno de los grupos de macroinvertebrados acuáticos más diversos y abundantes en los ecosistemas acuáticos.

Objetivo: Analizar la composición y diversidad de Chironomidae en el corredor Andino-Amazónico del departamento del Caquetá, Colombia.

Métodos: Se seleccionaron tres zonas de estudio (Andina, Transición y Amazónica), cada una con diez estaciones de muestreo. Se realizaron cuatro campañas de campo para recolectar material biológico y registrar características del paisaje fluvial. La diversidad alfa se evaluó utilizando los números de Hill, un conjunto de medidas que proporciona un marco unificado para el análisis de diversidad. Además, se analizó la diversidad beta considerando las diferencias en riqueza y reemplazo de especies. Mediante un análisis multivariado de RDA, se contrastaron las variables físicas y químicas del paisaje fluvial con la composición taxonómica y la diversidad beta.

Resultados: Se identificaron 36 taxones de quironómidos, destacando la subfamilia Chironominae y los géneros *Cricotopus*, *Chironomus*, *Larsia* y *Polypedilum*. Los números de Hill mostraron una diferenciación en la diversidad de Shannon-Wiener (q_1) y el inverso de Simpson (q_2), aunque la riqueza (q_0) mostró menos variación entre las zonas de estudio. El componente más significativo de la diversidad beta fue la diferencia en riqueza de especies. La velocidad de la corriente, los sólidos totales disueltos (TDS), los sustratos de origen arenoso y vegetal (raíces, macrófitas acuáticas), al igual que otros componentes del paisaje fluvial, influyeron significativamente en la composición de especies y la beta diversidad.

Conclusiones: Se proporciona información significativa a nivel de género para la familia Chironomidae en la región Andino-Amazónica del departamento del Caquetá, encontrando disimilitud entre las áreas de estudio a partir de la diferencia en riqueza, como estimación beta taxonómica. Además, estos hallazgos también indican que la región de transición funciona como un ecotono para Chironomidae, sirviendo como una zona intermedia entre los Andes y la Amazonía.

Palabras clave: números de Hill; diversidad beta; paisaje fluvial; mosquitos no picadores.

INTRODUCTION

The Chironomidae family, belonging to the order Diptera, is one of the most diverse groups of aquatic macroinvertebrates in terms of richness and individual abundance, present in nearly any aquatic ecosystem (Karima, 2021; Pero et al., 2023). Approximately 6 200 chironomid species have been reported globally, with an estimated 900 species in the Neotropical region (da Silva et al., 2018). A review of the list of aquatic macroinvertebrates of Colombia documented 65 genera distributed throughout the national territory (Hincapié-Montoya et al., 2022). In addition, the research of Bejarano-Narváez et al. (2023), Hoyos-Jaramillo & Dias (2020), Ospina-Torres et al. (2018), and Rojas-Céspedes et al. (2024) addresses the study of the Chironomidae family in the country. However, data on the Chironomidae family remains limited in biodiverse and transitional areas such as

the Andean-Amazonian corridor in the Caquetá Department, a region of particular interest due to its unique ecological characteristics and high biodiversity. This information gap underscores the need to deepen taxonomic research to understand their distribution and diversity in this ecologically significant area better.

Chironomids play a fundamental role in aquatic ecosystems as organisms that transform organic matter (Ocon et al., 2023; Prat & Castro-López, 2023) and serve as an essential food source within energy flows (Dong et al., 2021). Like other aquatic biological communities, various environmental conditions can influence their establishment and distribution. For instance, studies by Cerba et al. (2022), Garay et al. (2020), and Gething et al. (2020) have demonstrated a significant relationship with substrate composition. Chironomid distribution is also associated with factors such as current velocity (Rossaro, Marziali, & Boggero,

2022; Rossaro & Marziali, 2024), water temperature (Engels et al., 2020; Rossaro, Marziali, Montagna, et al., 2022), dissolved oxygen levels (Nunes et al., 2020), vegetation cover (Leszczyńska et al., 2019; Roque et al., 2010), and conductivity (Rossaro, Marziali, Montagna, et al., 2022). These variables are part of local-scale environmental filters that can determine the presence or absence of any aquatic macroinvertebrate (Liu et al., 2023; Rideout et al., 2022). Understanding these interactions is essential for interpreting how changes in riverscapes can influence biological diversity, especially in the Andean-Amazonian corridor. No detailed study has evaluated the relationship between the Chironomidae family and local physical and chemical variables in this region.

Analyzing the taxonomic diversity of the Chironomidae family in the Andean-Amazonian corridor provides a comprehensive view of its structure and dynamics in this transitional region. Alpha diversity, estimated through Hill numbers, enables the assessment of species richness and distribution within a specific area, thereby elucidating local diversity patterns within ecosystems (Hill, 1973). Regarding beta diversity, focusing on components of richness difference (nestedness) and species turnover (Legendre, 2014) offers insights into community variation across aquatic localities between the Andes and the Amazon. Richness differences between localities suggest that one community or locality is a subset of a larger community or locality, while species turnover highlights change in species composition among the studied communities or localities (Baselga, 2010; Legendre, 2014). These beta diversity components are essential for understanding how geographical barriers, such as the uplift of the Andes, which shapes the richness of the Western Amazon (Hoorn et al., 2010), generate variation in the structure of the Chironomidae family related to both habitat availability and environmental conditions.

The objective of this research was to examine the taxonomic composition and diversity of the Chironomidae family in different lotic ecosystems associated with the Andean-Amazonian

region of the Caquetá department while also evaluating the influence of environmental variables of riverscape in the family. This study provides important information to fill knowledge gaps through a taxonomic and diversity analysis approach.

MATERIALS AND METHODS

Study area: The area extends between coordinates 2.0240° N & 76.0090° W and 1.1570° N & 75.1420° W, on the Eastern flank of the Eastern Cordillera in Southern Colombia, Caquetá Department, with an altitudinal range varying between 2 205 m above sea level (m.a.s.l.) and 231 m.a.s.l. (Fig. 1, SMT 1). Three study regions were defined (10 stations per region) based on field observations of riverscape components and accessibility to sampling stations: 1. The Andean region has lotic ecosystems with high water flow dynamics, rocky substrates, and good vegetation cover, and dominance of preserved forest (altitudinal range = 581-2 205 m.a.s.l.). 2. The Transition region is characterized by high to moderate water flow, transitioning from rocky to soft substrates, and fragmented riparian vegetation with some stations presenting mixtures of natural forests with pasture (altitudinal range = 276-492 m.a.s.l.). 3. The Amazonian region features slower water flow, soft substrates, and fragmented riparian vegetation, also with some stations presenting mixtures of natural forests with pasture (altitudinal range = 231-266 m.a.s.l.).

Field phase: Four field campaigns were conducted between October and December 2021 and February and April 2022, aiming to ensure a representative sample composition. We collected biological material using a multi-habitat sampling method, following the recommendations of Rodríguez-Capítulo et al. (2009) and Roldán-Pérez & Ramírez-Ramírez (2022). Using a Surber net (0.3 × 0.3 m, 250 µm mesh size), we performed ten sweeps over a 100 m study segment for each station (five sweeps over the dominant substrate, two over the secondary substrate, and three over the remaining

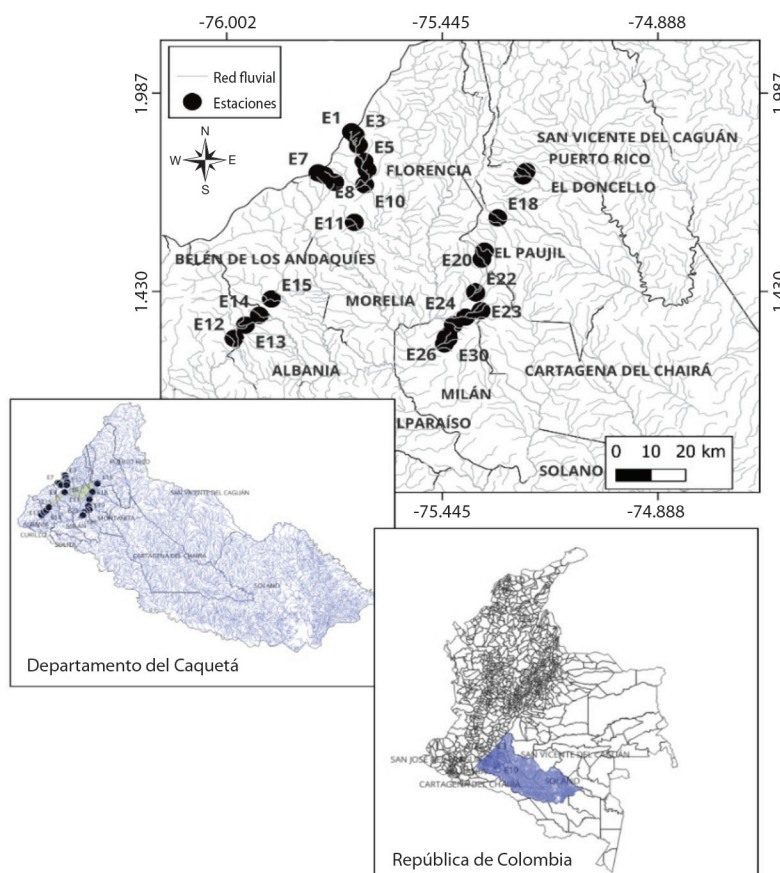


Fig. 1. Location and distribution of the 30 sampling stations associated with the Andean-Amazonian region of the Caquetá department. E1:E10 corresponds to the Andean region, E11:E20 to the Transition region, and E21:E30 to the Amazonian region.

available substrate). We homogenized the sample in a bucket, separated the collected material using a 250 μm sieve, and labeled and preserved it in 75 % ethanol.

For riverscape components (environmental variables), dissolved oxygen (DO, mgL^{-1}), pH, conductivity (Co., $\mu\text{S cm}^{-1}$), water temperature (Te., $^{\circ}\text{C}$), channel width (Wi., m) and total dissolved solids (TDS, ppm) were recorded using a professional multiparametric probe (AZ86031). We used a Global Water flow meter to measure current velocity (Ve., ms^{-1}) and depth (dep., m). We visually identified vegetation cover types and classified them into trees (Tre.), shrubs (Shr.), pasture (Pas.), and herbaceous plants

(Her.). We estimated the percentage of vegetation cover (C.V) with the assistance of GIS data (Open Data – SINCHI Institute, 2023). For substrate composition (adapted from Hynes (1972), we defined the categories as follows: boulders (> 25 cm in diameter), cobbles (6 cm-25 cm), gravel (2 cm-6 cm), sand (0.06 mm-2 mm), and organic material of vegetal origin (O.Veg.) (SMT 2).

Laboratory phase: The contents of each collected sample were washed, sieved, and placed in Petri dishes for the separation, identification, and counting of taxa associated with the Chironomidae family, with a level of

taxonomic resolution up to genus. Following the recommendations of Trivinho-Strixino (2011), the individuals were dissected by separating the head from the body, mounting the parts on a slide, fixing the preparation with Hoyer's medium at room temperature for 5 days, and subsequently sealing the mounts with Euparal. We used the taxonomic guides and keys of da Silva et al. (2018), Paggi (2009), Trivinho-Strixino (2011), and Wiederholm (1983) to identify the collected Chironomidae taxa.

Data analyses: It is into all analyses using statistical software R version 4.4.1 (R Core Team, 2024). We analyzed the three study regions based on their composition and abundance at each associated sampling station. Following the framework proposed by Chao et al. (2014), we evaluated the field data using extrapolation and interpolation components applied to sample coverage and rarefaction curves. Subsequently, based on the total abundances of each taxon for each region, we calculated taxonomic alpha diversity values by estimating Hill numbers, as Jost (2006) recommended. We expressed these as observed richness (q_0), Shannon-Wiener diversity (q_1), and inverse Simpson diversity (q_2), using the "iNEXT" library version 3.0.1 (Chao et al., 2016), along with the "ggthemes" (Arnold, 2025) and "ggplot2" (Wickham, 2016) libraries.

We followed approach Legendre (2014) for taxonomic beta diversity, employing the Ružička dissimilarity measure from the Jaccard group, Podani family (Podani & Schmera, 2011), which considers abundances. This analysis calculated the components of total beta diversity, richness difference, and species turnover using the "beta.div.comp" function from the "adespatial" (Dray et al., 2026) library. To complement the taxonomic beta diversity data, we applied Principal Coordinate Analysis (PCoA) with Bray-Curtis distance, which allowed us to visually identify clusters between regions based on composition (transformed the biological abundance data to the Hellinger scale), implementing the R statistical packages "vegan" (Oksanen et al., 2026) and "ggplot2"

(Wickman, 2016). In addition, we also performed Permutational Multivariate Analysis of Variance (PERMANOVA), seeking to validate significant differences between the clusters.

Finally, we worked with biological data transformed to the Hellinger scale, and average data environmental variables were standardized with a mean of 0 and a standard deviation of 1. We used a subset of non-correlated variables, whose selection we used the "forward.sel" function of the "vegan" package (Oksanen et al., 2026). Next, using Redundancy Analysis (RDA), we identified the environmental predictor variables that influence chironomid composition. We also performed a distance-based redundancy analysis (dbRDA) to assess the relationship between environmental variables and beta diversity, using the "vegdis" function to generate the distance matrix. We also used the RDA graph to contrast riverscape components and the abundances of the different collected taxa of the Chironomidae family, focusing the analysis on the dominant genera. The libraries implemented in this final analysis were "vegan" (Oksanen et al., 2026) and "adespatial" (Dray et al., 2026).

RESULTS

Chironomidae composition in the Andean-Amazonian region: According to the sampling coverage, the analysis required the entire collected material, indicating a high efficiency of the implemented methodology in capturing (Fig. 2A). The rarefaction curves stabilized for the Amazon and Transition regions, with higher richness estimated in the Andes region (Fig. 2B).

We identified 36 taxa from the Chironomidae family among 3 766 individuals collected in the Andean-Amazonian region of the Caquetá department (SMT 3, SMF 1). Of these taxa, 17 belonged to the subfamily Chironominae, 10 to Orthocladiinae, and 9 to Tanypodinae. The most abundant genera were *Cricotopus*, *Chironomus*, *Larsia*, and *Polypedilum*. Fig. 3 shows the behavior of the genres mentioned above.

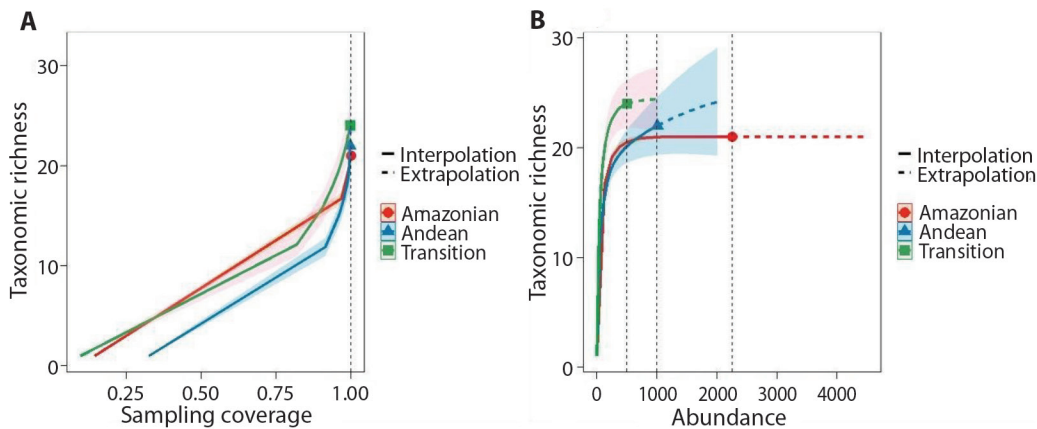


Fig. 2. A. Sampling coverage. B. Rarefaction curves estimated for the Chironomidae family collected in different lotic ecosystems of the Andean-Amazonian region in the Caquetá department during the 2021 and 2022 sampling campaigns.

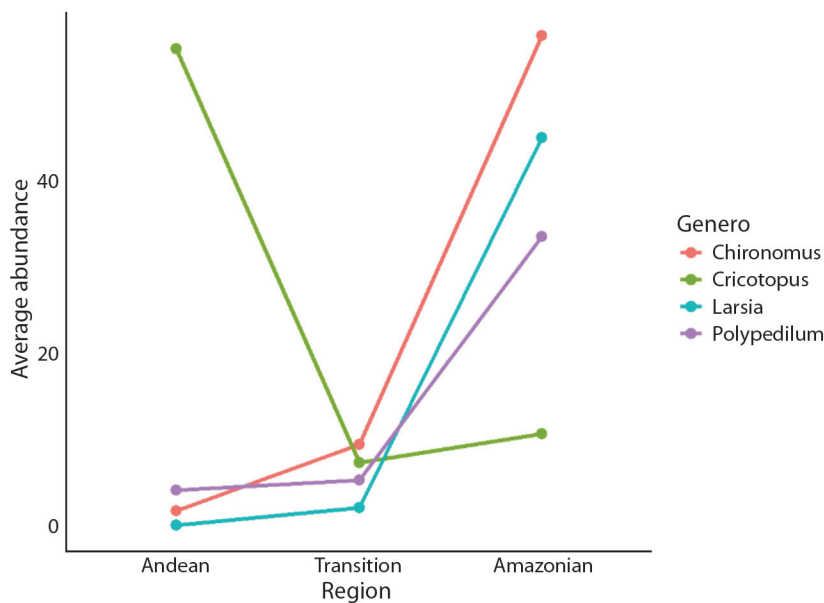


Fig. 3. The average abundance behavior of the most outstanding genera of the Chironomidae family in the Andean-Amazonian region of the Caquetá department, based on samples collected during the 2021 and 2022 sampling campaigns.

Chironomidae Diversity in the Andean-Amazonian Region: Based on the results of taxonomic alpha diversity (Fig. 4A), we observe that the bars in the three regions overlap, however, the greatest richness is observed in the Andes region, followed by Transition and

Amazonas. Regarding Shannon-Wiener diversity (q_1) and Simpson's inverse diversity (q_2), regional differentiation was observed from a visual analysis. Regarding taxonomic beta diversity, the most prominent component was richness difference ($RichDif/Btotal$), with a

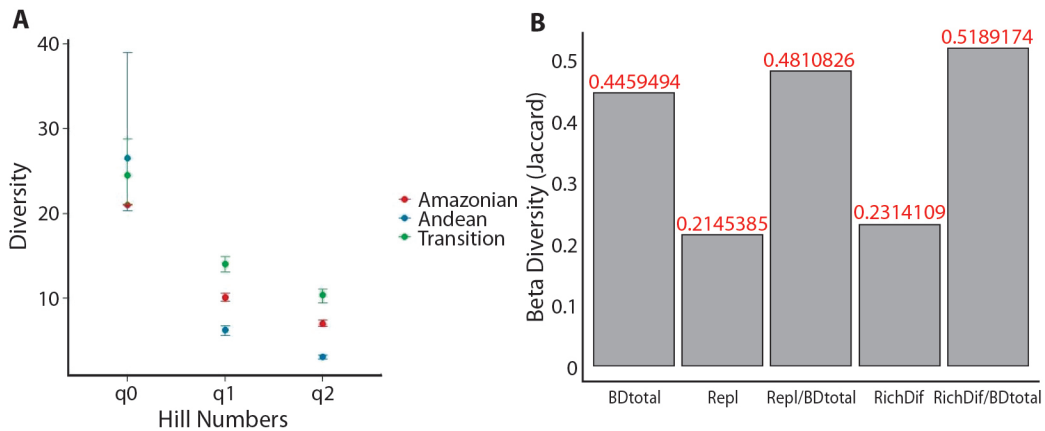


Fig. 4. A. True taxonomic alpha diversity. B. Components of taxonomic beta diversity estimated for the Chironomidae family collected in different lotic ecosystems of the Andean-Amazonian region in the Caquetá department during the 2021 and 2022 sampling campaigns. Taxonomic richness (q0), Shannon-Wiener diversity (q1), and inverse Simpson diversity (q2), Beta diversity Total (BDtotal), Replace (Repl), richness difference (RichDif).

value of 0.5189 about the total beta diversity (Fig. 4B).

The PCoA with 45.31 % of explained variance (Fig. 5), shows a progressive environmental gradient in the composition of chironomids, highlighting a marked separation between the

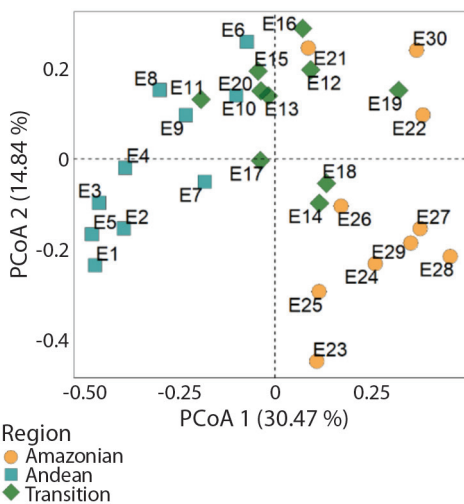


Fig. 5. Principal coordinate analysis (PCoA) was applied to the Chironomidae family collected in different lotic ecosystems of the Andean-Amazonian region in the Caquetá department during the 2021 and 2022 sampling campaigns. Station (E).

Andean stations (E1:E10) and the Amazonian stations (E2: E30). In contrast, the stations in the transition region (E11:E20) position themselves immediately, reflecting the combination of community attributes typical of the extreme zones. The PERMANOVA test ($F = 4.040$, $p = 0.001$) statistically corroborates this pattern and confirms significant differences in the structure of the communities among the three regions.

Chironomidae and riverscape components: The results of the RDA identified TDS, current Ve., sand, and vegetal origin (O.Veg.) as predictor variables for the composition of Chironomidae, with a coefficient of determination (R^2) of 0.38 (Table 1). These variables were significant for beta diversity, with an $R^2 = 0.42$. SMT 3 in the supplementary material provides detailed information on the various characterized components of the riverscape.

The redundancy analysis graph (Fig. 6) results indicate that the first two axes explain 47 % of the total variance. On the first axis, altitude, hard substrates, dissolved oxygen, and tree-dominated vegetation cover positively influence the Andean region, where *Cricotopus* thrives with greater abundance. In contrast, at low-altitude sites (Amazon),

Table 1
Results of the Redundancy Analysis (RDA) contrast the taxonomic composition and beta diversity of the Chironomidae family with riverscape variables.

Analise	Variable	Component	Variance	F	p-value
RDA	Composition	TDS	0.102	6.4432	0.001***
		Ve.	0.040	2.5079	0.007**
		Sand	0.034	2.1561	0.017*
		O.Veg.	0.033	2.0945	0.015*
		C.V	0.026	1.6215	0,089
		Residual	0.380		
	Beta Diversity	TDS	Sum of Sqs 1.524	8.0081	0.001***
		Ve.	0.573	3.0074	0.002**
		Sand	0.410	2.1512	0.022*
		O.Veg.	0.437	2.2936	0.014*
		C.V	0.339	1.7779	0.053
		Residual	4.569		

Total dissolved solids (TDS), current velocity (Ve.), sand, vegetal origin (O.Veg.), and vegetation cover (C.V). *p < 0.05, **p < 0.001, ***p < 0.0001.

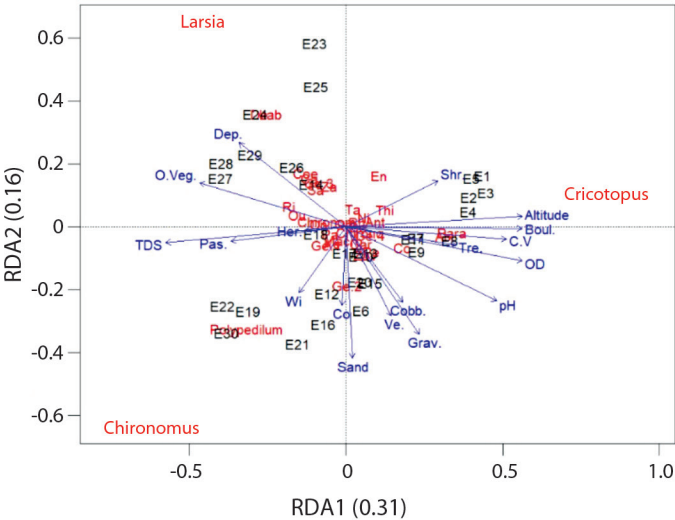


Fig. 6. Redundance Analise Graph (RDA) was applied to the Chironomidae family collected in different lotic ecosystems of the Andean-Amazonian region in the Caquetá department during the 2021 and 2022 sampling campaigns. Station (E), red (taxa), blue (riverscape component). The meaning of each abbreviation is found in the supplementary material. SMT 2 indicates the riverscape component, |SMT 3 indicates the biological component.

environmental conditions change significantly, with the presence of soft substrates, such as sand and plant material, greater depth, a cover dominated by grass and herbaceous plants, and an increase in the concentration of TDS. Current velocity remains high in the Andean and transition regions but decreases markedly in the Amazon. *Larsia*, *Chironomus*, and

Polypedilum show a greater association in these lowland environments.

DISCUSSION

The increase in taxonomic richness and estimated abundance in the Andes region suggests that new taxa continue to emerge despite completing four sampling campaigns, reflecting the area's complexity and biological richness. The rarefaction curve's lack of stabilization indicates that we must intensify the sampling effort to estimate the true diversity associated with the Chironomidae family accurately. Previous studies, such as the one conducted by Acosta and Pratt (2010) in the Andean rivers of Peru, have documented this phenomenon and highlighted that the true diversity of Chironomidae genera in the region remains unknown. Similarly, in the work of Rojas-Céspedes et al. (2024) on high Andean streams in the Tolima department of Colombia, a representativeness between 78 and 100 % was reported, emphasizing the possibility of recording new genera. In contrast, the sampling is more representative of the Transition region, and especially the Amazon, indicating a low probability of encountering new taxa within the study area.

The 36 taxa identified in this study represent new geographic records for the Andean-Amazonian region of Caquetá. Unlike previous research in the department, we focused these results on the genus level, moving beyond the family-level analyses of earlier studies (Bravo-Chaves & Restrepo-Franco, 2021; Chaux et al., 2018; Cortés-Cadena et al., 2016; Gutiérrez-Garaviz et al., 2016) and contributing to a higher taxonomic resolution of the Diptera group. Furthermore, the greater generic richness associated with the subfamily Chironominae observed in this study aligns with the findings of Bejarano-Narváez et al. (2023) and Oviedo-Machado & Reinoso-Flórez (2018) in Colombia.

Various studies in the Neotropical region frequently report the dominant genera found in this study. Additionally, the results of the RDA graph illustrate how environmental variables can be key determinants of the abundance and

distribution of chironomids. *Cricotopus* exhibits a broad altitudinal distribution, ranging from lowlands to mountainous areas (Acosta & Prat, 2010). In the study area, *Cricotopus* dominates particularly in the Andean region, where dense tree cover, predominantly rocky substrates, high water flow velocity, low temperatures, and good oxygenation create favorable conditions. However, its abundance decreases as it moves toward the lowlands. These findings are consistent in part with Rojas-Céspedes et al. (2024) and Oviedo-Machado & Reinoso-Flórez (2018).

In relation with *Chironomus* inhabits a wide altitudinal range (Matthews-Bird et al., 2016) and stands out as a bioindicator of pollution due to its high tolerance to various levels of contamination (Molineri et al., 2020; Prat & Castro-López, 2023). Similarly, *Polypedilum* is frequently found in high abundance across altitudinal gradients (Bejarano-Narváez et al., 2023; Shimabukuro & Trivino-Strixino, 2018; Villamarín et al., 2021), while *Larsia* has been reported in springs, small streams, and stagnant waters (Wiederholm, 1983), with individuals collected at altitudes below 321 m.a.s.l. in the study area.

According to this study's results, *Chironomus*, *Polypedilum*, and *Larsia* increase in abundance as altitude decreases towards the Amazon, suggesting that these genera find more favorable conditions at lower elevations. In these areas, slow-moving waters with greater depth, higher concentrations of TDS, and substrates of plant origin prevail, along with vegetation cover dominated by grasses and herbaceous plants, which indicate fragmentation of riparian vegetation.

Regarding the diversity of the Chironomidae family, the absence of clear differentiation in taxonomic richness (q_0) among the study regions supports the moderate dissimilarity component of beta diversity, reflecting a pattern of ecological nestedness (richness difference). This pattern suggests that the species present at one site are a subset of those found at sites with greater species richness (Baselga, 2010; Legendre, 2014). Furthermore, a detailed analysis of



the PCoA reveals that the stations from different regions show a degree of compositional mixing, particularly with those in the transition region. This pattern suggests a limited contribution of species replacement, which would otherwise result in more distinctly separated groups. These findings indicate that the transition region is an ecotone for the Chironomidae family with taxonomic superposition, serving as an intermediate zone between the Andes and the Amazon.

In reviewing other studies conducted in the Neotropics, González-Trujillo et al. (2019) reported low values of both replacement and nestedness in Chironomidae communities, emphasizing that many of these taxa exhibit high tolerance to environmental disturbances, with resilience to pollution stress as noted by Williams-Subiza et al. (2022). On the other hand, the observed values of Shannon diversity (q1) and inverse Simpson diversity (q2) allowed differentiation among the three study areas, indicating a better spatial distribution of the various Chironomidae taxa in the Transition and Amazon regions. These results partially align with the findings of Villamarín et al. (2021), where Hill numbers separated rivers associated with high altitudes, which showed higher values, from rivers at medium and low altitudes. However, in the present study, rivers at medium altitudes (Transition) and low altitudes (Amazonian) displayed the highest values of Shannon diversity (q1) and inverse Simpson diversity (q2).

Finally, in the Andean-Amazonian region, substrate type and water flow velocity are key environmental factors shaping the composition and richness of aquatic communities, acting as critical predictors of aquatic biodiversity. Several studies, such as Glowacki et al. (2023), support this finding by reporting significant correlations between these environmental factors and the local species richness of Chironomidae. Similarly, Garay et al. (2020) identified that variables such as water temperature, conductivity, hardness, substrate type, and flow velocity significantly influence the establishment of Chironomidae communities,

as observed in our study. They also highlighted that, in high-mountain rivers, low temperatures and gravel favored the colonization of specimens from the subfamily Orthocladiinae, such as the genus *Cricotopus*. In contrast, taxa from the subfamily Chironominae predominated in warm waters with low conductivity, as exemplified by *Chironomus*. Additionally, Barboza et al. (2015) suggested that an increase in the heterogeneity of environmental variables could lead to greater beta diversity within the Chironomidae family.

Ethical statement: The authors declare that they all agree with this publication and made significant contributions; that there is no conflict of interest of any kind; and that we followed all pertinent ethical and legal procedures and requirements. All financial sources are fully and clearly stated in the acknowledgments section. A signed document has been filed in the journal archives.

See supplementary material
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