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Ecoregions, climate, topography, physicochemical, or a combination of all: which criteria are the best to define river types based on abiotic variables and macroinvertebrates in neotropical rivers?

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Abstract

The baseline conditions for a particular river or stream type are essential to classify aquatic ecosystems based on physical and biological characteristics. In this study, we proposed a river typology for different ecoregions, climate and topography of northwestern Argentina using parameters, and combined key variables to establish reference conditions. A set of geographical, hydro-morphological, hydrological, geological (pedology and sedimentology) and physicochemical variables were measured from different rivers and analyzed with clustering and ordination techniques to develop a typology. We analyzed the correspondence of the physical river conditions and benthic macroinvertebrate assemblages using non-metric multidimensional scaling analysis, dissimilarity among assemblages, ANOSIM approach and *envfit* analysis in order to make an ecological validation of the classification. Our results allowed us to classify the neotropical rivers studied, according to typological systems adapted from the European Water Framework Directive. The combination of ecoregions and topography along with other variables associated (system B), was better corresponded with biological arrangements. Hence, ecoregions and topography combined turned out to be more precise as a criterion to define river types and their local abiotic and biotic reference conditions. Macroinvertebrate distribution corresponded with the classifications proposed and was related with abiotic features of the rivers. The physical variables as altitude, grain size, water temperature and turbidity were key parameters to develop a schematic model to define river types that could be implemented and tested in other countries of the region. Five river types have been identified, characterized, and included in three large groups: Mountains, Foothills,

and Lowlands (Plains). Our results showed that topography and climate are two aspects that strongly influence South American freshwater biota. We propose the schematic model developed in our study as a baseline to define freshwater biomes based on altitude (topography), ecoregions (climate) and biological functional traits at a broad spatial scale (continental or global).

Key words: Freshwater ecosystems, classification, landscape, South America,

1. Introduction

The use of ecological classification of freshwater ecosystems helps us to assessing whether human activity has altered ecosystems, because biological assemblages can show natural variability (Hawkins et al., 2010). The most widely used classification schemes (regionalization and typologies) are based on the fact that fluvial ecosystems have a hierarchical structure in which ecoregion, catchment, reach and site level are present along rivers (Frissell et al., 1986, Munne and Prat, 2004). Regional scale factors such as geology, climate, and basin size influence the characteristics of rivers at local scale (Sandin and Verdonschot, 2006). We expect these classification schemes would allow us to infer the environmental conditions and biota expected at specific individual water bodies based on their type. Accurate classifications reduce the probability of inferring impairment when it does not exist or even not detecting it when is absent (Gibson et al., 1996). Following Ward (1998), when the scale of analysis is the riverine landscape, the rivers must be approached from a holistic perspective observing the large-scale patterns (e.g. geomorphology of floodplains, channel belts and terraces) and processes associated with the fluvial systems (drainage and channel patterns). In addition, identifying the most relevant abiotic (physical) variables that allow defining different river types is essential to establish the reference conditions and assess the ecological quality of these water bodies (Pardo et al. 2012). The ecological classification of freshwater ecosystems into water body types has been extensively tested in North America and Europe, but this issue has only recently been studied in other continents, such as Asia (Kim et al., 2017; Cai et al*.,* 2019)

and South America (Martins et al., 2017; Agra et al., 2018; Pero et al., 2019; Gonzalez-Trujillo et al., 2019). In South America there is a lack of a common regional, legal or management framework to classify and assess the water bodies of the continent, which is fundamental for bio-monitoring processes that are urgently needed considering the increasing pressure over our fluvial ecosystems (IPBES, 2018; Albert et al. 2020).

After the publication of the Water Framework Directive (WFD) (WFD2000/ 60/EC; European Commission, 2000) many European typological classifications have been used to test the concordance between landscape attributes and the structural and functional aspects of biological communities (Brunke, 2004; Lorenz et al., 2004; Rawer-Jost et al., 2004; Verdonschot and Nijboer, 2004; Dodkins et al., 2005; Ferréol et al., 2005; Verdonschot, 2006; Sanchez-Montoya et al., 2007; Skoulikidis et al., 2009; Pardo et al., 2010). Currently, the WFD approach is still used to define river types such as the typology of large rivers from Europe developed by Borgwardt et al. (2019). Calibrations of ecological classifications are also been conducted to achieved common management objectives for aquatic ecosystems (Birk et al., 2013; Lyche Solheim et al., 2019). In addition, some future climate and socio-economic scenarios suggested that some eco-hydrological river types could change their types becoming another type or even new types, with the potential to create novel river ecosystems (Laizé et al. 2017). The approach proposed by the WFD recognizes two systems for river classification, based on the ecoregions proposed by Illies and Botosaneanu (1963) and Illies (1978). The typological systems proposed by the WFD define 1) system A, as a fixed range of

altitude, geology and drainage area within of a broad ecoregional framework, and 2) system B, as a more flexible combination of the same factors (altitude, geology, size) plus other physical and chemical characteristics. The WFD also proposes the establishment of hydromorphological, physicochemical, and fundamentally biological reference conditions for each type of water body. Regionalizations based on ecoregions have also been broadly tested to predict freshwater fauna distribution and reference conditions (Hawkins et al., 2010). Furthermore, a recent conceptual framework posits that biomes (ecoregions) provide a significant way of understanding how lotic ecosystem structure and function varies across macrospatial scales (Dodds et al., 2015).

In this study and due to their ecological importance we develop effective classification systems for macroinvertebrate assemblages. Aquatic macroinvertebrates are widely used to understand distributional patterns across spatial scales (Johnson et al., 2007), and are also used extensively as indicators of the biological quality of freshwater ecosystems (Resh et al., 1995, Moya et al. 2011, Dos Santos et al., 2011, Birk et al. 2012). Macroinvertebrates play an important role in freshwater ecosystem functioning by cycling nutrients, processing organic matter, and providing food to higher trophic levels. Furthermore, many groups of aquatic insects that are well represented in river environments are among the most threatened insects around the world (Sánchez-Bayo and Wyckhuys, 2019). In general, studies showed that segregation of macroinvertebrate assemblages among regions is more strongly related to topography and associated physiochemical variables (Hawkins et al., 2000; 2010;

Sandin and Verdonschot, 2006; Lyche Solheim et al., 2019). Many previous studies have found this hierarchical pattern; for example, biotic variation among stream sites is higher when ecoregions have marked differences in topography (Hawkins et al., 2000). Sandin and Verdonschot (2006) analyzed macroinvertebrate datasets in relation to environmental and biogeographical variables from Europe and found three major stream types that corresponded with three major landscape types: Mountains, Lowlands, and Mediterranean. Similarly, in a recently developed broad typology, common river types have been defined within regions of Europe and mainly corresponded to very large, lowland, mid-altitude, highland and glacial and Mediterranean rivers (Lyche Solheim et al., 2019). The extensive and varied antecedents of North America and Europe, and also experiences of other regions (e.g., Australia, Marchant et al. 2000), support the idea of having abiotic characterizations to classify the different types of rivers and validate them ecologically by comparing them with distributional patterns of biological communities such as those of benthic macroinvertebrates to develop better systems for environmental assessment and monitoring. Although important knowledge has been accumulated about aquatic communities in South America, such as those based on fishes (Oberdorff et al., 2019), the relationships between landscape units, their abiotic features and the structure and composition of benthic macroinvertebrates are still poorly studied. Therefore, it is necessary to increase and improve our knowledge about the influence that environmental factors at large spatial scale may have on the distribution of South American aquatic macroinvertebrates.

In South America there are recent experiences of regionalization or river typologies in countries such as Bolivia (Moya et al. 2003), Brazil (Vasconcelos et al., 2013; Martins et al., 2017; Agra et al., 2018), Chile (Fuster et al., 2015) and Argentina (Pero et al., 2019). Recently, an ecoregional classification from northwestern Argentina had been proved to be useful as a base to classify fluvial ecosystems (Pero et al. 2019). In addition, Agra et al. (2018) highlighted the importance of defining an *a priori* ecoregional classification system and *a posteriori* nested system of river typology to better explain the spatial variability of macroinvertebrate assemblages. In general terms, in the set of South American studies it was observed that variables associated to topography (altitude, substrate size, river size and hydrology) together with physicochemical variables (temperature and conductivity) were the best descriptors to discriminate river types. However, it is not clear yet what variables among those abiotic components are more important to define South American river types with different macroinvertebrates composition.

The main objective of our study is to develop a river typology for northwestern Argentina following a top-down approach according to the WFD and then to validate the resulting typology with a biological community such as macroinvertebrate assemblages (bottom-up approach). We decided to use a strong and practical approach, such as WFD, as a base to develop a South American classification scheme and included additional analyses to generate a schematic model to define river types that could be implemented and tested in other countries of the region. To design such a model, we identify and propose key variables that allow synthesizing the relationship between the abiotic features and

river types according to the biotic assemblage's structure. Consequently, we pose the following questions: 1) is it possible to identify different river types based on the variations of abiotic features along the landscape? 2) Is there a correspondence between macroinvertebrate assemblage structures and abiotic classifications? and 3) what are the main abiotic variables related to assemblage's distribution and river types?

2. Material and methods

2.1 Study Area

The study area is located between S 26° - 28° and W 66° - 64°, and covers approximately 20 thousand km^2 including most part of Tucumán province and its limits with Santiago del Estero province in Northwestern Argentina (Fig. 1). The area is wide with heterogeneous landscapes containing diverse ecosystems such as mountain cloud forests, dry forests and grasslands (Brown and Pacheco, 2006). In this study we sampled reaches of fluvial channels located in two different ecoregions: Yungas subtropical cloud forest and Western Chaco dry forest.

The Yungas subtropical cloud forest or Yungas forest is a narrow belt of mountain rainforest, ranging from 400 to over 3000 m.a.s.l. (Brown, 2000). The climate is warm and humid, with annual average temperatures ranging from 14°C to 26°C and rainfall from 1000 to 2500 mm (Brown et al., 2001). The Yungas forest is stratified into 3 vegetation floors or bands. In general, Yungas altitudinal floors are not considered sub-ecoregion units, but in this study we evaluated them as differentiated units within the Yungas forest because each altitudinal floor presents particular climatic features and floristic composition (Brown and Pacheco, 2006). The high montane forest (1500-3000 m.a.s.l.) contains monospecific tree stands that are usually either *Alnus acuminata* or *Podocarpus parlatorei*. Rainfall reaches 1000 mm. The low montane forest (700-1500 m.a.s.l.) has the most diverse vegetation, with many evergreen species, and is dominated by *Cinnamomum porphyrium* and *Blepharocalyx salicifolius*. The low montane forest also has the highest precipitation (2000 mm annual) and least seasonal hydrological regime. The foothill forest (400-700 m.a.s.l.) contains deciduous trees and is dominated by

Tipuana tipu and *Enterolobium contortisiliquum*. The annual rainfall varies between 1000-1500 mm during the wet season, and the 6-month dry season (≤ 50 mm rainfall) extends from June to November (Brown et al., 2001).

The Western Chaco ecoregion is a vast sedimentary fluvial plain formed by the streams and rivers that run northwest to southeast and includes parts of northwestern Argentina, southeastern Bolivia, northwestern Paraguay, and southwestern Brazil (Great South American Chaco). The headwaters are located in the mountains, outside the region to the west, and they transport great quantities of sediments into the region. Mean annual temperatures range between 19° and 24° C. Annual rainfall varies between 400 and 900 mm, with most precipitation falling in the summer and little falling in the winter (Minneti, 1999). The vegetation is composed of dry forests and segregated grasslands. This ecoregion is classified into three sub-ecoregions: Arid Chaco, Semiarid Chaco, and Chaco Serrano (Brown and Pacheco, 2006). Only the latter two are represented in the study area. The Chaco Serrano is part of the western border of the ecoregion and is characterized by low mountain topography. It is bordered in some places by the Yungas forest. The Semiarid Chaco occupies the greater portion of the ecoregion and is a continuous xerophytic and semi-deciduous forest. A wide transition zone occurs between the Western Chaco and the Yungas forest, which includes species common in both ecoregions (Cabrera, 1976), although it is currently highly modified by agricultural use (Gasparri, 2016).

2.2 Survey design and methods

We studied 24 sites (Fig. 1, Supplemental material). Sites were distributed across ecoregions and sub-ecoregions as follows: eighteen in the Yungas subtropical cloud forest (four in high montane [HM], ten in low montane [LM], and four in foothill forests [FH]), and six in the Western Chaco (two in Chaco Serrano [CS] and four in Semiarid Chaco [SC]). Each site consisted of a fluvial stream reach ~100 m long. We chose sites that were minimally disturbed, without upstream industrial or others human activities, and with well-preserved native riparian vegetation at least 100 m wide.

Data from 14 of the 24 sites (HM3, HM4, LM3, LM5, LM9, LM10, FH1, FH2, CS1, CS2, SC1, SC2, SC3 and SC4) were collected between 2014 and 2018 by the authors. Data for the ten other sites (HM1, HM2, LM1, LM2, LM4, LM6, LM7, LM8, FH3, FH4) were obtained from the IBN (Neotropical Biodiversity Institute, National Council of Technological and Scientific Research – Universidad Nacional de Tucumán) database. The IBN sites were sampled between 2005 and 2007 following the same collection methods. Climate conditions were similar during these two periods according to local climate databases, and both periods corresponded to the ENSO phase of El Niño according to the Oceanic Niño Index (ONI) (https://origin.cpc.ncep.noaa.gov/products/analysis_monitoring/ensostuff/ONI_v5.php). In addition, previous studies in the region observed that macroinvertebrate assemblages composition and structure changes seasonally rather than annually (Mesa et al., 2009; Mesa, 2012). All sites were sampled once at the end of the low water season (October-December) and once at the end of the high water season (March-June) during two years, totalizing four visits per site.

2.2.1 Environmental variables

For the delimitation of river types a dataset was generated, comprising geographical, hydro-morphological, hydrological, geological (pedology and sedimentology) and physicochemical variables measured at the 24 sites (Table 1).

Geographical variables: We recorded altitude (m.a.s.l.) with a Garmin eTrex 20™ global positioning system. To define the size of the basin of each sampling site, the area in hectares was calculated using a layer of basins through the Geographical Information System (QuantumGIS, 2014). We used the ecoregional classification proposed by Brown and Pacheco (2006) to define an ecoregion correspondence for each site.

Hydro-morphological (geomorphological) variables: For the hydromorphology of the rivers, channel width (m) was measured in the field, and satellite images and digital elevation models were used to determine slope, sinuosity and braiding parameters (Miall, 1977). The Sinuosity (P) of a current is defined as the relationship between the length of the channel axis or thalweg and the straight length of the valley (Mueller, 1968; Schumm, 1977), that is: P = Long. Thalweg/Long. Valley. The braiding parameter is the splitting of channels around bars or islands and the degree of braiding (braiding index) is best measured as the mean number of active channels or braid bars per transect across the channel belt (Bridge and Demicco, 2008).

Hydrological variables: discharge (m^3/s), we estimated discharge by measuring cross-sectional area, taking depth measurements every 25 cm (for streams ≤11 m wide) or 1 m (for rivers ≥11 m wide) along 1 cross-sectional transect across the channel, and measuring velocity with a velocity meter at 2/3 the depth at each point (Global Water Flow Probe FP111). Stream power (Watts/m) was estimated from the formula given by Gordon *et al*. (2004): W = *pgQS,* where W is power in Watts, *Q* is discharge (m^3/s), *S* is the stream slope (m/m) obtained from a digital elevation map (ASTER DEM 30x30 m resolution), *p* is the density of water (kg/m^3), and *g* is the acceleration due to gravity (m/s^2).

Geological variables: For this study, "System A" was modified with respect to the variable "geology" of the WFD because the lithological detail used in European typologies is not available in our study area. For this reason, “geology” was replaced by "Soils", as an approach to the lithological variable assuming that it is a parameter that influences the rivers on a wide scale of the landscape and is described in detail in our studied area. The more recent classification of soils for Tucumán corresponds to Puchulu and Fernández (2014). Following the proposal of Soil Survey Staff (2010); soil mapping and description performed by Puchulu and Fernández (2014) consider the different physiographic units and their geoforms. Also, the grain size, shape and lithology of the sediments (granulometry) of the river were recorded by transverse transects to the channel. We measured in the field pebbles and cobbles, and sampled sediments from the top of channel bars (Bridge and Demicco, 2008). To estimate sediment grain size at each site, we measured 20 to 130 clasts that were >2 mm in diameter in a cross-section of the

fluvial bar close to the channel where we took the invertebrate samples. The sediment grain size deposited at a mid-central fluvial bar is related to the slope and discharge and hence stream power (Bridge and Demicco, 2008). In addition, sand samples were collected at each site to be analyzed in the Sedimentology laboratory of the Facultad de Ciencias Naturales and Miguel Lillo Institute (IML) of the Universidad Nacional de Tucumán (UNT). The grain size of sandy sediments (Wentworth, 1922) was obtained in the sedimentological laboratory using mechanical sieving separation equipment. The data were ordered in diameter class intervals, locating them according to Udden-Wentworth grain size scale (in millimeters) (Wentworth, 1922) in the following classes: Boulder (> 256); Coarse cobble (256 to 64); Fine cobble (64 to 16); Pebble (16 to 8); Granule (8 to 4) and Sand (<4).

Physicochemical variables: water temperature (°C), pH, electrical conductivity (µS/cm), turbidity (NTU), total dissolved solids (mg/L), and dissolved oxygen (DO mg/L) were measured at every visit with a Horiba™ multi-probe water quality checker U-50 series. Measurements were taken in every site at similar time of the day and within the same week to minimize daily changes that naturally occur. In addition, in each site, two water samples were taken (one in a low water season and another in a high water season) to be analyzed in the laboratory. Quantities (mg/L) of the following major ions were measured according to APHA (2005) from each sample: Total Alkalinity, Sodium, Potassium, Calcium, Magnesium, Chlorine and Sulfate (using a Metrohm ion chromatograph, model 881 Compact IC pro). In the samples of the low-water season the nutrients Nitrate, Nitrite (using a Metrohm

ion chromatograph) and Phosphate (using the ascorbic acid method) were also measured (mg/L), this season was only considered because the concentrations during high waters are usually very low to be detected by the analyzes performed.

2.2.2 Benthic macroinvertebrates

At each site and visit we collected quantitative and qualitative samples. Three quantitative samples were collected with a Surber sampler (net area 0.09 m^2 with a 300-µm mesh), and were subsequently pooled into a single, composite sample. We took these samples in fast-water habitat units (riffles or runs, sensu Hawkins et al., 1993) that were separated by 50 m along a longitudinal transect. The qualitative samples consisted of samples collected with a D-frame net (300-µm mesh), a kick-net (500-µm mesh), and by manual sampling. Manual sampling included directly picking specimens from boulders, cobbles, leaves, and algae. The qualitative sampling took approximately 30 minutes to cover all habitats. Riffles, pools, and marginal vegetation habitats were most common.

Quantitative data were used to analyze abundance patterns, and the combined quantitative and qualitative data were used to analyze presence-absence data. We brought all samples to the lab after collection, where we processed and identified each entire sample. Macroinvertebrates from all samples were identified by the same group of taxonomists. We conducted the analyses at two targeted taxonomic levels of resolution: genus and family (see Appendix B for a list of all taxa). We identified individuals based on the regional keys of Domínguez and Fernández (2009). When possible, individuals of Ephemeroptera, Odonata, Plecoptera, Trichoptera, Megaloptera, Lepidoptera, Coleoptera (Elmidae), Diptera (except for

Chironomidae), Hydracarina and Mollusca were identified to genus level. Individuals of Crustacea and the rest of Coleoptera were identified at family level. The latter groups were only at family-level analyses. Representative individuals of Nematoda, Platyhelminthes, and Annelida were not included in the analyses because they could not be identified to family. A list of identified taxa is included in Appendix.

2.3 Data analysis

All statistical analyzes and graphs were produced via the R platform (version 3.6.1, 2019, R Foundation for Statistical Computing, Vienna).

2.3.1 River typology

To define system A, we used the main geographical criteria to define river types: ecoregion, altitude, drainage area and soil type. To define system B, we analyzed the geographical variables, plus the rest of the abiotic variables surveyed. The statistical analyses utilized to develop the typology were done in two steps following the methods used by Ferréol et al. (2005) and Borgwardt et al. (2019). In a first step, a classification procedure was employed in order to create the typology. Prior to the analysis, variables were scaled using the *scale* function. A matrix was composed with Euclidean distances of the sites. Then the distance values were classified in a tree structure using clustering techniques from the *hclust* function. The number of groups was graphically determined upon the relative lengths of the tree branches using the *k-means* function. The criterion for delimiting the maximum number of clusters was that no cluster should be made up

of a single site. In a second step, abiotic data were analyzed by computing an ordination. A principal components analysis (PCA) was used to describe how abiotic factors varied within and across sampling sites. The function *dudi.pca* in the *ade4* R package (version 1.7-8; Dray et al., 2017) was used for these analyses. The PCA was based on the mean values of each variable across all sampling points at each site. The superposition of the classification results upon a PCA´s plan for rows enabled to detect wrongly represented objects which can be close in the ordination but far in the space (Ferréol et al., 2005).

2.3.2 Dissimilarity

We used the Sørensen and the Positive Matching indices (PMI, Dos Santos and Deutsch, 2010) to analyze the presence-absence data. We used the Bray-Curtis and Dissim indices (Nieto et al., 2017) to estimate compositional dissimilarity between assemblages based on our abundance data (number of individuals per sample). The PMI can vary between 0 and 1 and represents the mean proportion of “positive matches” relative to the complete list of taxa that could occur at a site. The PMI covers the range of richness encompassed by the two lists – i.e., the smaller and longer ones (Dos Santos and Deutsch, 2010). Hence, if 2 lists of different lengths are compared, for example of 10 and 100 specimens, and the PMI is 0.3, that result indicates that the 2 lists share 30% of taxa, on average, given the list sizes range from the smaller to the longer one (Dos Santos and Deutsch, 2010). In contrast, Euclidean and Bray–Curtis distances are 2 dissimilarity indexes that are frequently used in ecological analyses (Nollet and De Gelder, 2014).

However, both of these indices are strongly influenced by dominant species and are only weakly affected by rare species (Valentin, 2012) and are therefore not as useful when there are gradual changes in composition along a gradient. The Dissim index can be used when the observed taxa are assumed to have been sampled from a common regional pool of species. The Dissim Index assesses whether assemblages are similar based on both the taxa present and their abundance. Thus, two sites would be considered more similar if they grouped consistently near each other after successive orderings of sites by increasing values of consecutive taxa abundance (Nieto et al., 2017).

We used ANOSIM (Legendre and Legendre, 1998) to determine if site taxonomic composition differed statistically among ecoregional, sub-ecoregional and typological classifications based on presence-absence and abundance data. We also used multivariate analyses to determine if differences in assemblage composition among sites were associated with regional classifications. We used Nonmetric Multidimensional Scaling (NMDS) based on dissimilarity values obtained from presence-absence and abundance data to visualize if the positions of sites in taxa space were concordant with ecoregional, sub-ecoregional and typological classifications. We interpreted how discrete the ecoregions, sub-ecoregions and river types were by drawing a convex polygon around each group of river types on the NMDS plot. These polygons were based on whichever classification and index had the highest ANOSIM value. We considered NMDS and ANOSIM to be complementary analyses.

It is well known that benthic macroinvertebrate assemblages can vary markedly with season (Minshall, 1988; Poff and Ward, 1989). We therefore separated the data by low and high water periods to verify that the differences among ecoregions, sub-ecoregions and river types were greater than the seasonal differences within each site.

2.3.3 Selection of variables for the schematic model for river types

The variables that correlated more strongly with PCA axes were selected to exclude those that were redundant or highly inter-correlated (Munne and Prat, 2004). A correlation coefficient over |0.7| ($p < 0.05$) was used as the criterion to reduce the number of variables of each axis (Munne and Prat, 2004). To assess the set of environmental variables that best correlate with biological ordinations, vectors (selected environmental variables) were fitted to the existing NMDS plots of sample dissimilarities using the function "*envfit*" (from R package vegan). The *envfit* scales these vectors based on their correlation coefficient, and the resulting plot allows to quickly identify the most important variable gradients represented by the NMDS plot (Clarke and Ainsworth, 1993). For the development of the schematic model, those highly correlated variables were retained, thereby synthesizing environmental information.

3. Results

3.1 Classification based on system A.

From the classification analysis carried out on a matrix of Euclidean distances based on the descriptive variables of system A, a classification dendrogram was obtained (Fig. 2A) in which six river types have been identified (Table 2). Those river types can in turn be grouped into larger groups. In a first division there are two groups: a) Mountains and b) Foothills and lowlands rivers. In second place, the Mountains rivers can be subdivided into two groups: High montane rivers (2000-1200 m.a.s.l.) and low montane rivers (1200-700 m.a.s.l.). In turn, the second group was divided into Foothill forest rivers (700-400 m.a.s.l.) and lowlands rivers, this last group can be distinguished based on the sub-ecoregion in which they are found: Chaco Serrano (1000-400 m.a.s.l.) or Semiarid Chaco (<400 m.a.s.l.). The values or categories of each variable of system A per site can be seen in Appendix A.

3.2 Classification based on system B.

From the classification analysis carried out on a matrix of Euclidean distances based on the descriptive variables of system B, a classification dendrogram was obtained (Fig. 2B) in which five river types have been identified (Table 3). The five river types can be grouped into larger groups. In a first division two groups are distinguished, that respond to Altitude: Mountain rivers (over 400 m.a.s.l.) and lowland rivers (below 400 m.a.s.l.). In second place the mountain rivers can be

subdivided into two groups: high and middle mountain rivers (between 2000 and 700 m.a.s.l.) and foothills rivers, which includes the Yungas foothill forest (between 700 and 400 m.a.s.l.) and the Chaco Serrano (located in semi-arid valleys between 1000 and 400 m.a.s.l.). On the other hand, the lowland rivers, which in turn correspond to the Semiarid Chaco sub-ecoregion, are subdivided according to their size and textural class of dominant sediment: medium-sized rivers with predominance of pebbles and large rivers with predominance of sand.

The PCA made with the total of variables showed an ordering of the sites in accordance with the river types established from the classification analysis (Fig. 3). The axis 1 of the PCA (explaining 41.4% of the total inertia) allowed to separate the types I and II and from the III, IV, and V. The variables that correlated most strongly with axis 1 were:% of Coarse Cobble, % Boulder, Altitude (-); and electrical conductivity (EC), total dissolved solids (TDS), concentration of all ions (in high and low water season), water temperature (in low water season), turbidity (in high water season), and sinuosity (+). On the axis 2 (19% of the total inertia) types III, IV and V were separated from each other. The sites corresponding to the river type IV were located towards the negative side of axis 2, with which braiding, % fine cobble, % pebble and water temperature (in high water season) were strongly correlated. The sites corresponding to river type V were located towards the positive side of axis 2, with which the channel width and discharge (in high and low water seasons), % of sand and concentration of nitrite were positively correlated. The sites corresponding to river type IIII were located near the intersection of the axes in an intermediate position to the rest of the river types.

Major ions composition was different at the broader classification scale, the Mountain Rivers had a sodium bicarbonate or calcium bicarbonate waters and Lowlands had sulphate-chloride waters. In addition, those differences in water chemistry were observed as gradual compositional changes along the altitudinal gradient in concordance with conductivity changes. The values or categories of each variable of system B per site can be seen in Appendix A.

3.3 Validation of typologies with benthic macroinvertebrates

Dissimilarity and ordering: The general structure of the macroinvertebrate assemblages corresponded with the river typologies, both with the classification system A and B. The assessment through the ANOSIM approach yielded a positive and significant R value (p = 0.001) for both typologies at all levels of analysis (Table 4). However, the classification scheme with the higher ANOSIM score was that of the typology based on system B. Although, there was a correspondence between assemblages and classifications in all taxonomic levels analyzed and with both types of dataset (presence-absence and abundance), the correspondences were generally greater when using the taxonomic level of genus and abundance dataset.

3.4 Correlations among abiotic and biotic ordinations

In the correspondence of the assemblages and system B (Fig. 4), the ordering pattern was common at all the levels analyzed. The axis 1 of the plane allowed separating, on its positive side the group of assemblages from lowland rivers, and

on its negative side that of mountain rivers. On the other hand, axis 2 internally separates each group. Among the mountain rivers, the foothill forest and Chaco Serrano assemblages segregated on the positive side, and on the negative side the assemblages from high and low montane forests; while the assemblages of lowland rivers separated in pebble rivers towards the positive side of the axis and sandy rivers towards the negative side.

According to the *envfit* analyses the axes of the NMDS plots were significantly correlated with some abiotic features of the rivers (Table 5). In a first analysis the variables altitude and total dissolved solids highly co-vary with coarse cobble and conductivity respectively, thus the two first mentioned variables were excluded from the final analysis. Finally, the grouping of the mountain assemblages (river type I) was more related to larger sediment size (% of coarse cobble), while lowland assemblages were strongly related to a greater proportion of sand in river bed and high values of water temperature, conductivity and turbidity (Fig.4). Foothills assemblages (river types II and III) had an intermediate position in the observed biotic-abiotic gradient. Finally, typology based on system B was preferred to establish the final river typology (Fig. 5). Following these results, we performed a schematic model to visualize the spatial location of river types according to their altitude and the relations among abiotic variables (Fig. 6).

4. Discussion

Our results allowed us to classify the neotropical rivers studied, according to both typological systems. The ecoregional scheme (system A) was consistent to classify the rivers in a broad spatial scale, in coincidence with previous studies (Pero et al. 2019; Gonzalez-Trujillo et al. 2019). The combination of ecoregions and topography, along with other variables associated (system B), was better corresponded with biological arrangements. Hence, ecoregions and topography combined turned out to be a more precise criterion to define river types and their local abiotic and biotic reference conditions. Macroinvertebrates distribution corresponded with the classifications proposed and was related to the variations of environmental features along the landscape. Within abiotic parameters, some features strongly influenced by altitude, such as sediment size, water temperature and turbidity were key variables to develop a schematic model of river types. These results agree with those obtained by several authors that identified river types using typological or regional classifications (Hawkins et al., 2000; Verdonshot, 2006; Borgwardt et al., 2019), but also showed gradual variations along the landscape that could follow the premise of observed/expected models like RIVPACS and similar modeling approaches (Moss et al. 1987, Wright 1995).

An abiotic variable more related to topography, such as sediment size, appears to be more important than physiochemical variables to define river types and predict invertebrate composition. Spatial variations in bed material character (size, shape, and sorting) have been seen to produce macroinvertebrate responses in different

ways. The grain characteristics determine the inertial, hiding, and, to some extent, structural properties (packing, pivot angles, arrangement) that control particle entrainment and define substrate stability (Buffington and Montgomery, 1997; Downes et al., 1997). Although the altitude turned out to be one of the most explanatory variables of our model, we believe that in itself it should not influence the size of the bed sediments or the hydrology of the reach. The hydrological, hydro-morphological (geomorphology) and grain size of sediments features of a river are variables that would be expected to be mainly related to the slope of the section (González del Tanago and García del Jalón, 2006; Bridge and Demicco, 2008). Probably, due to the observed co-variation between altitude and slope in our study, the importance of the slope may have been masked by altitude (Griffith et al., 2001). It would be important to also analyze cases of ecoregions with low slope relief and located at high altitudes, such as the arid fluvial valleys of the Monte (Pero et al., 2019) or the highlands of the Puna (Nieto et al., 2017), to test whether the slope can be an important variable to define river types regardless of the altitude at which they are located. In addition, the number of sites was low to have many replicates for each river type because it was difficult to found minimally impacted sites, mainly in lowland regions. Because of that, the wider classification of rivers in three main types (mountains, foothills, and lowlands) was more statistically rigorous.

Seasonality influenced the hydrological and physicochemical characteristics of the rivers across ecoregions. Some physicochemical variables also appear to be important factors to characterize river types, such as water temperature and

turbidity. Nonetheless, some of these physiochemical variables showed different results depending on the hydro period surveyed. Temperature is a factor related to latitude, altitude and seasonality and limits macroinvertebrates distribution and affects the community structure (Hynes, 1960; Biggs et al., 1990; Hussain and Pandit, 2012; Dos Santos et al., 2018). Additionally, we found an important contribution of EC, TDS, and the concentration of all ions to classify the neotropical rivers studied. These results are in agreement with those obtained by Ometo et al. (2000), Miserendino and Pizzolon (2003), and Epele et al. (2019) who compared the variability of chemical composition and macroinvertebrates. They also found that macroinvertebrate community structure changes with local physical and chemical variables.

It would be important to test whether seasonality might affect classifications that include different climatic ecoregions. Climate appears to be a factor that drives differences between river types that have similar altitudinal and topographical characteristics. For example, rivers that had topographical similarities but correspond to two different climatic conditions (humid or semiarid), had similar assemblages but enough differentiated to define different river types, as was also observed in previous studies (Pero et al., 2019). This fact could be related to a mix of assemblage's functional adaptations to the diversity of environmental features (Gallardo et al., 2013). It will be expected that those assemblages (e.g. from humid and semiarid foothills) share a set of similar adaptations to topography but have different ones to climate (seasonality). The use of assemblages functional traits to develop freshwater ecosystems classifications at large spatial scales is scarce

(Heino et al., 2013), and it would be interesting to be used in further studies in South America.

The combination of methods used resulted to be useful to define neotropical river types. We hope that the employed methodologies can be applied to other regions to fill in the spaces in the schematic model proposed and to test the generality of the model. Comparisons among assemblages of river types from different regions or continents are difficult because of taxonomic and historical biogeography differences. However, we could expect that between river types corresponding to the same biome, similar functional traits will be found in macroinvertebrate or other biotic assemblages (Statzner et al., 2004; Ernst et al., 2012; Doods et al., 2015).

5. Conclusions

Our results suggest that topography and climate could be two aspects that strongly influence South American freshwater biota structure. Thus, they could constitute useful variables to classify fluvial ecosystems at a broad spatial scale (continental or global), as was observed for Europe (Verdonshot, 2006; Borgwardt et al., 2019) and North America (Hawkins et al., 2010). We propose the schematic model established in our study as a baseline to develop and test a similar scheme to define freshwater biomes based on altitude (topography), climate and biological functional traits. We expect that the development of a classification of freshwater biomes based on a few key abiotic variables and functional aspects of biotic communities will be a powerful tool for the study, conservation and management of freshwater ecosystems at a continental and global scale.

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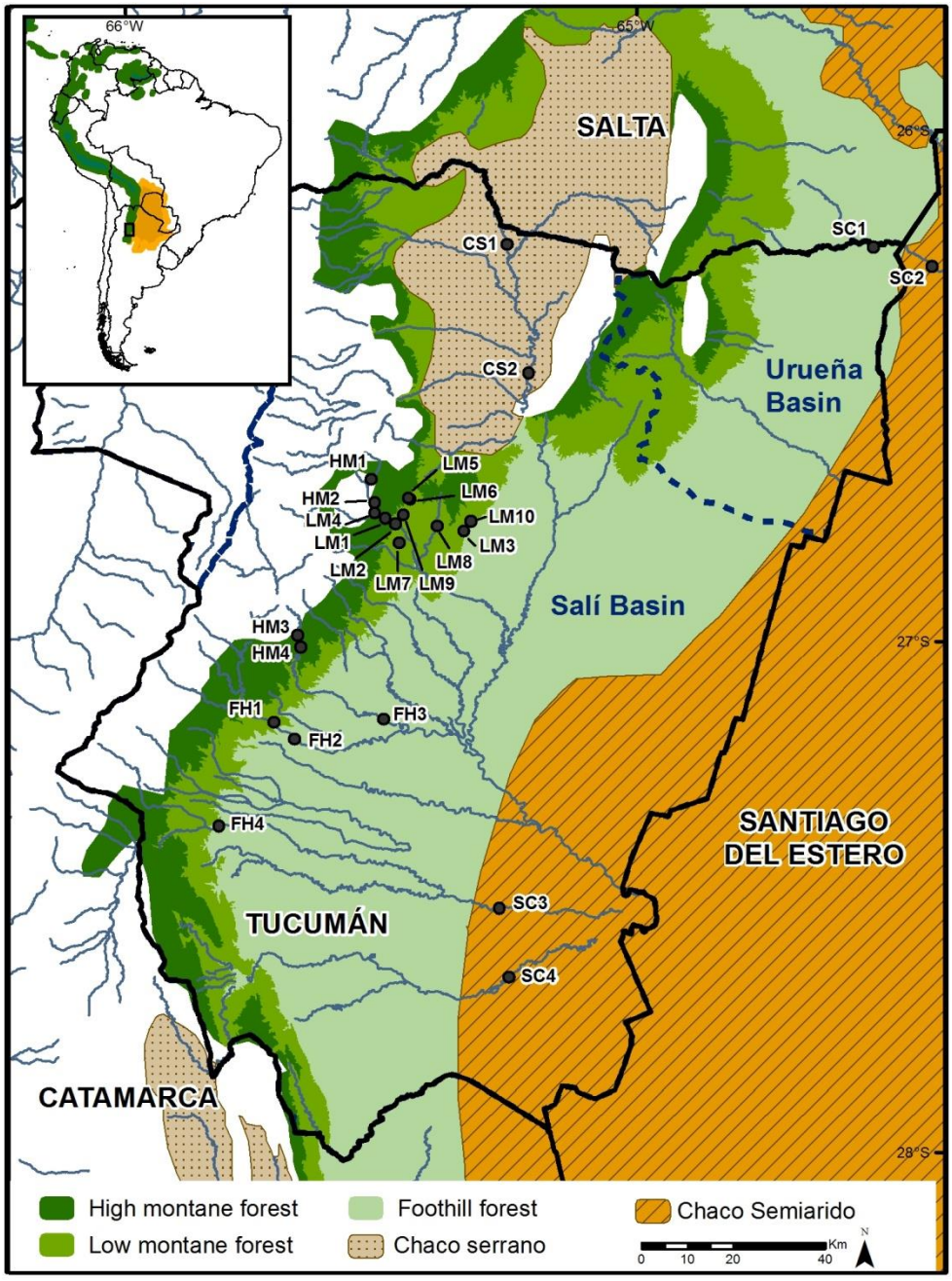

Fig.1. Study area and sampling site location.

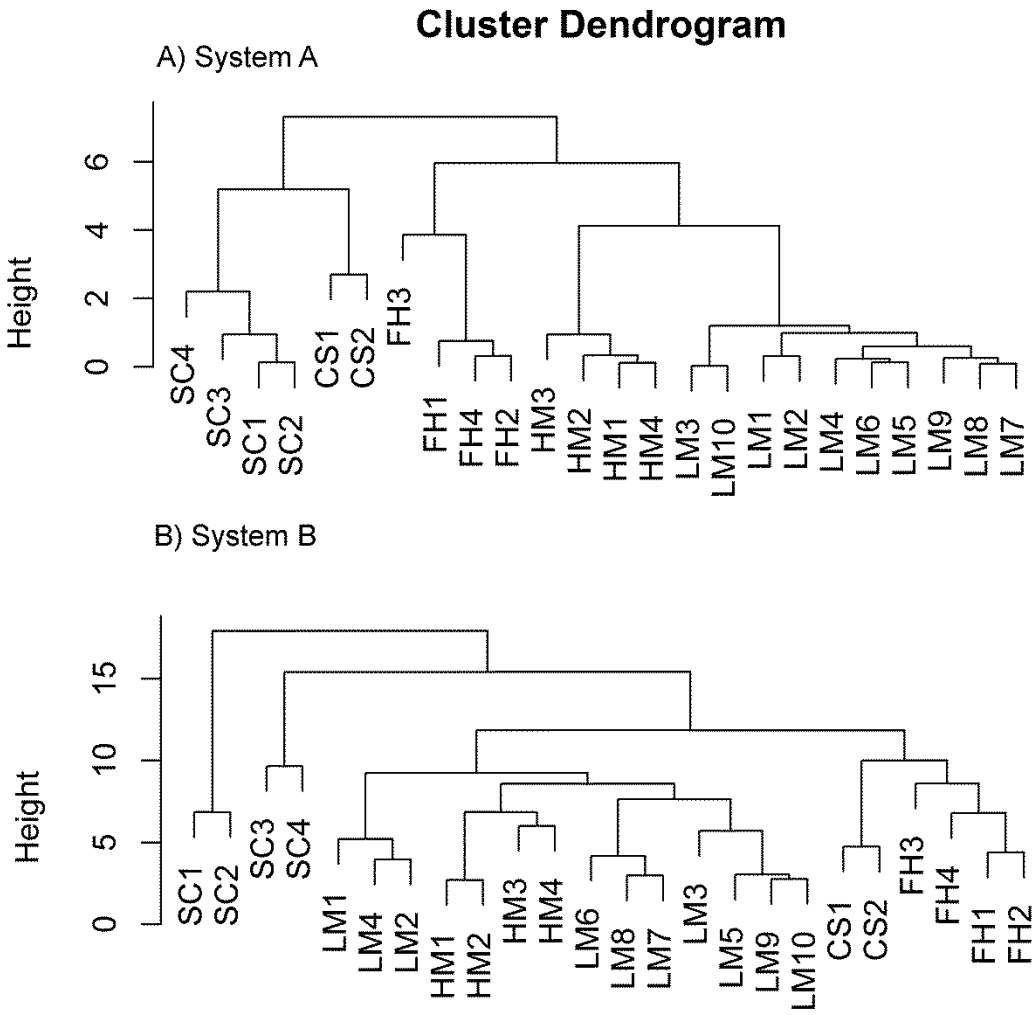

Fig.2. Cluster dendrogram with euclidean distances between sites according to systems A (A) and B (B). HM: High montane, LM: Low montane, FH: Foothill forest, CS: Chaco Serrano, SC: Semiarid Chaco.

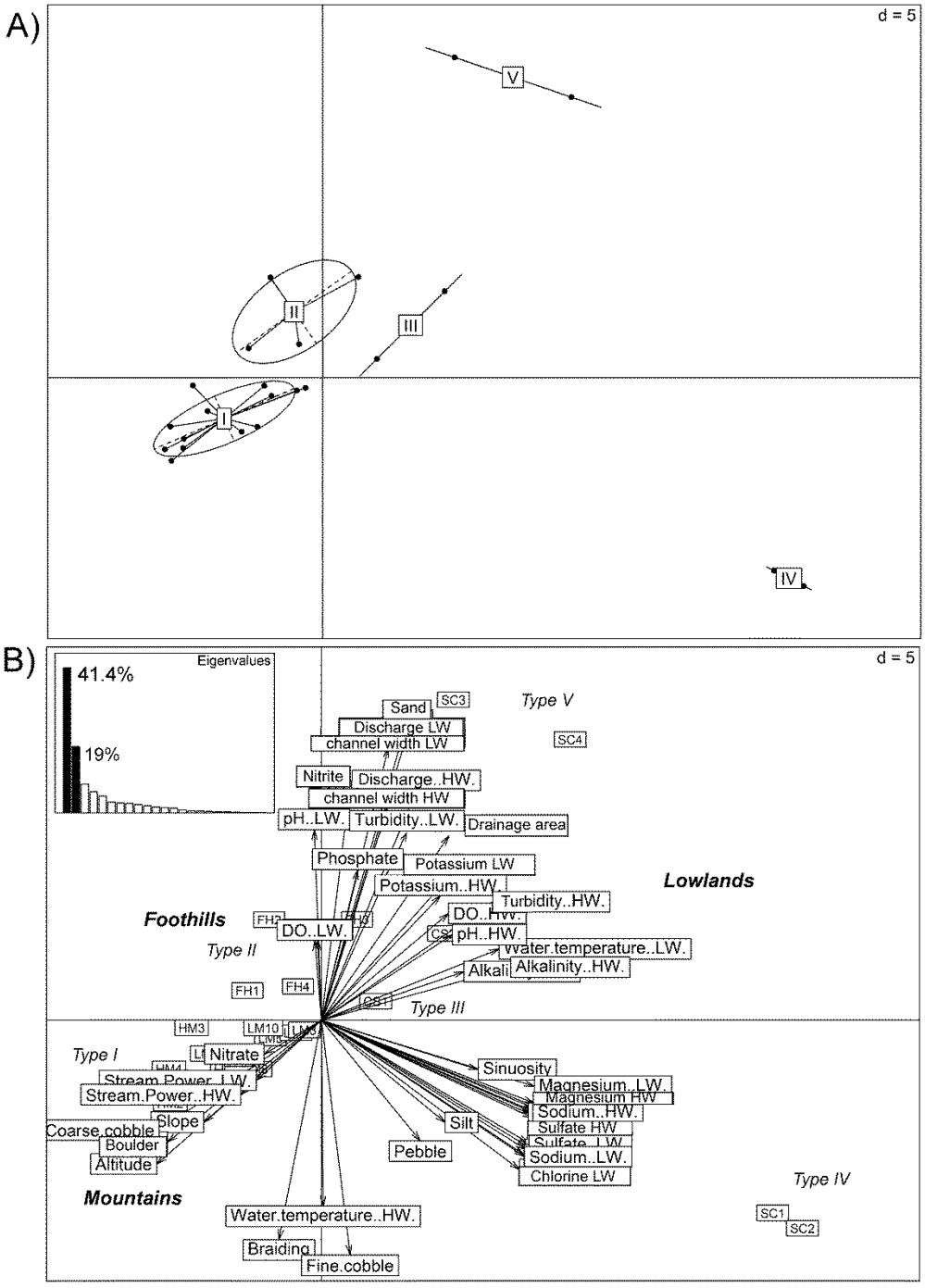

Fig. 3. Principal Components Analysis (PCA) ordination of environmental variables measured at the 24 sampling sites. A. The 95% confidence ellipses for river types. B. PCA biplot of environmental variables and sampled sites, with the inset showing the barplot of eigenvalues. LW: Low waters, HW: High waters.

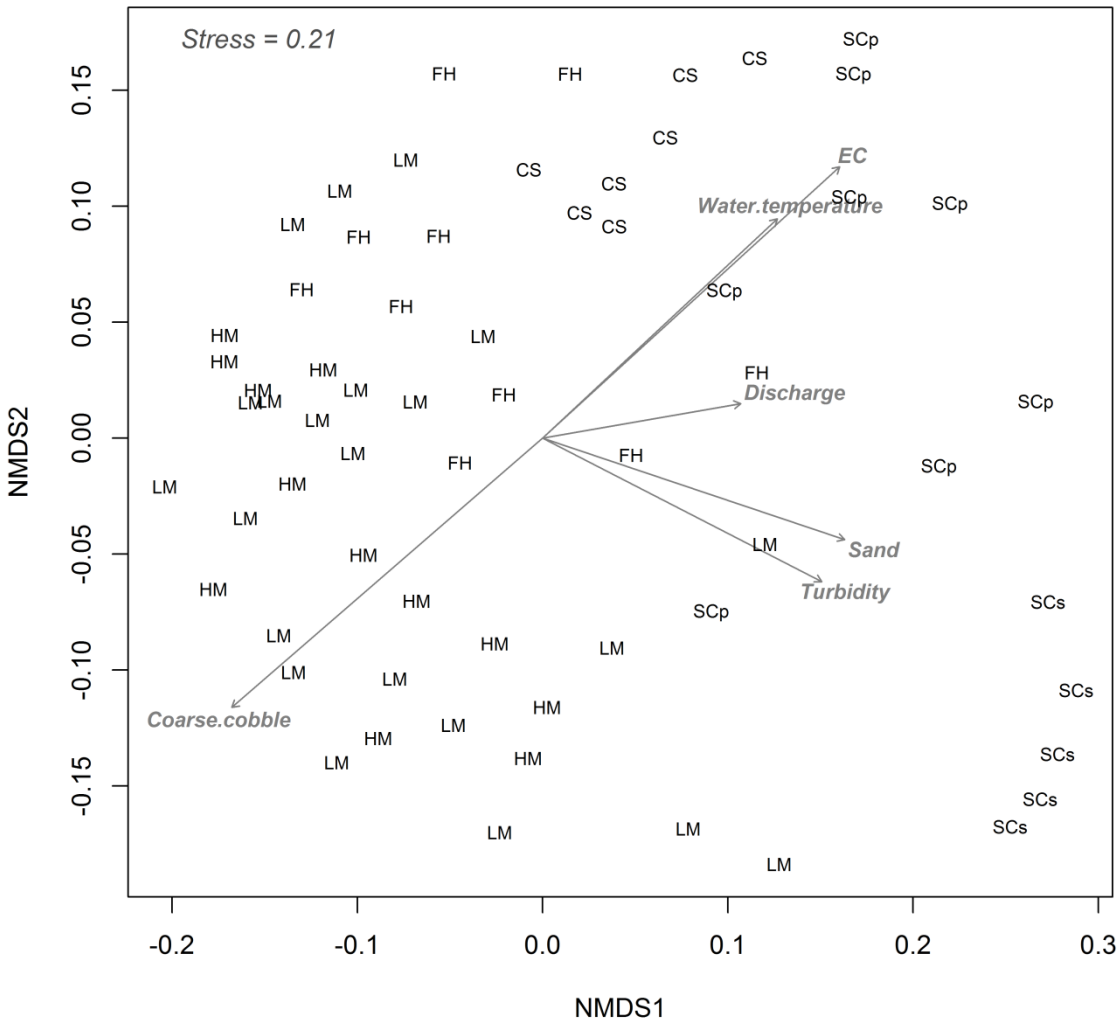

Fig. 4. Non-metric multidimensional scaling (NMDS) plot of macroinvertebrates samples dissimilarity at the genus level using abundance data (Dissim index) with best correlated environmental variables from Envfit analysis. HM=high montane; LM=low montane; FH=foothill forest; CS=Chaco Serrano; SCp= Semiarid Chaco pebble; SCs=Semiarid Chaco sand.

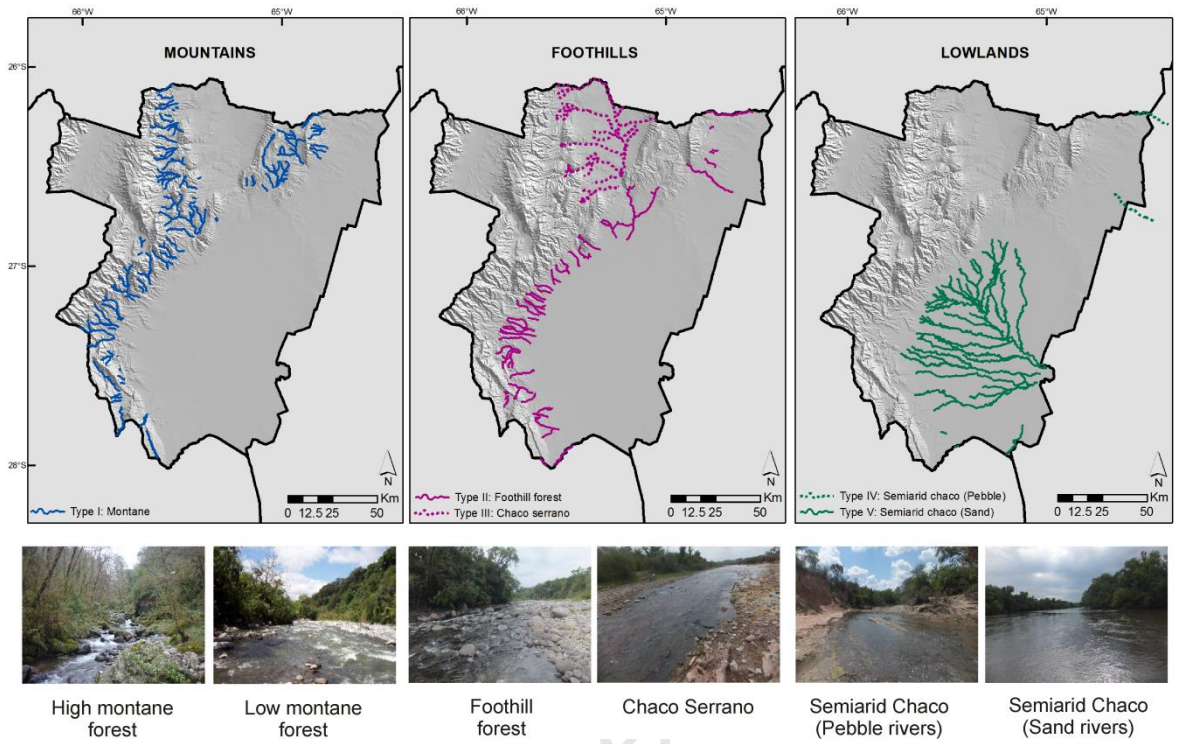

Fig. 5. Maps of the three main groups of river types (Mountains, Foothills and Lowlands) with river types delimitated. Photos of river types examples: high montane forest and low montane forest [type I], foothill forest [type II], Chaco Serrano [type III], Semiarid Chaco pebble [type IV], Semiarid Chaco sand [type V].

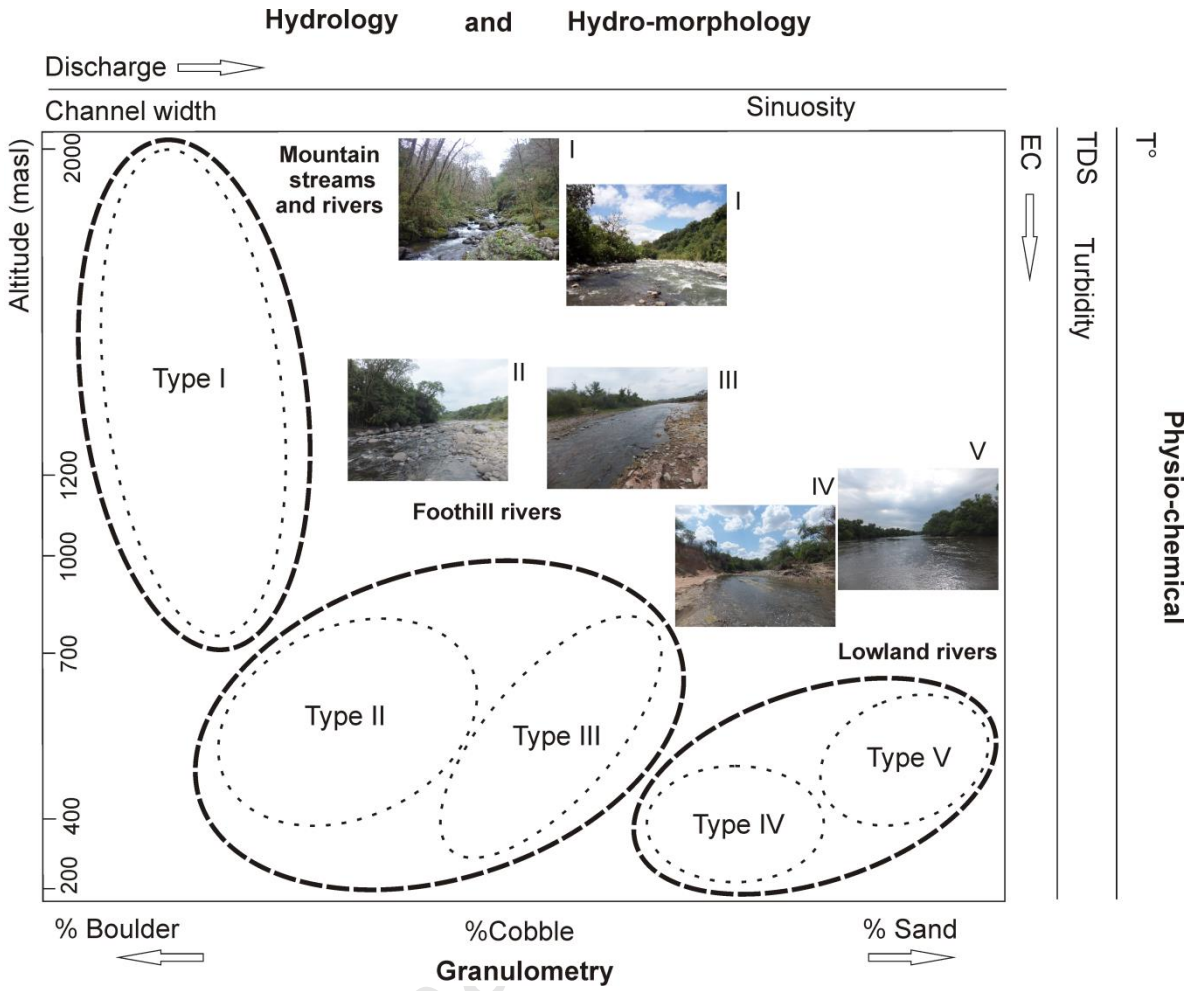

Fig. 6. Schematic model to visualize river types, their location according to altitude (m.a.s.l., meters above the sea level), and the relations among abiotic variables. EC: electrical conductivity T°: Celsius temperature; TDS: Total Dissolved Solids.

Tables and appendix

Table 1. Environmental variables used in each classification system (A and B).

Variables A		Variables B	
Ecoregion	Yungas		
	Western Chaco		
Sub-ecoregion	High montane forest	Hydro-morphology	Sinuosity
	Low montane forest		Braiding
	Foothill forest		Slope
	Chaco Serrano		Channel width
	Semi-arid Chaco		
Altitude		Hydrology	Discharge
Drainage area			Stream Power
		Granulometry	% Boulder
			% Coarse cobble
			% Fine cobble
			% Pebble
			% Granule
			% Sand
			% Silt
Soil type (Order)	Inceptisols	Physicochemistry	Water temperature
	Molisols		Electrical Conductivity
	Entisols		pH
	Alfisols		Total dissolved solids
			Dissolved Oxygen
			Turbidity
			Main ions Composition
			Nitrate
			Nitrite
			Phosphate

Table 2. River typology according to system A.

Mountains	Mountains zone	I.	High montane forest (2000-1200 m.a.s.l.)
		II.	Low montane forest (1200-700 m.a.s.l.)
Foothills and lowlands	Foothill forest zone	III.	Foothill forest (700-400 m.a.s.l.)
	Western Chaco zone	IV.	Chaco Serrano (1000-400 m.a.s.l.)
		V.	Semiarid Chaco (pebble rivers) (<400 m.a.s.l.)
		VI.	Semiarid Chaco (sand rivers) (<400 m.a.s.l.)

Table 3. River Typology according to system B.

Mountain rivers (>400 m.a.s.l.)	High and middle mountain	I. Montane forest rivers (2000-700 m.a.s.l.)
	Foothills	II. Foothill forest (700-400 m.a.s.l.)
		III. Chaco Serrano (1000-400 m.a.s.l.)
Lowland rivers (<400 m.a.s.l.)	Semiarid Chaco	IV. Pebble rivers (<400 m.a.s.l.)
		V. Sandy rivers (<400 m.a.s.l.)

Table 4. ANOSIM analysis statistics for the correspondence between benthic macroinvertebrates and systems A and B, according to the use of the Sørensen, PMI, Bray-Curtis and Dissim indices.

Index	Ecoregion				Typology A			
	Genus		Family		Genus		Family	
	R	*p*	R	*p*	R	*P*	R	*p*
Sørensen	0.49	0.001	0.49	0.001	0.51	0.001	0.51	0.001
PMI	0.49	0.001	0.47	0.001	0.41	0.001	0.42	0.001
Bray Curtis	0.31	0.001	0.20	0.001	0.36	0.001	0.25	0.001
Dissim	0.47	0.001	0.50	0.001	0.46	0.001	0.55	0.001
Index	Topography				Typology B			
	Genus		Family		Genus		Family	
	R	*P*	R	*p*	R	*P*	R	*P*
Sørensen	0.57	0.001	0.57	0.001	0.66	0.001	0.65	0.001
PMI	0.53	0.001	0.51	0.001	0.48	0.001	0.47	0.001
Bray Curtis	0.39	0.001	0.31	0.001	0.48	0.001	0.32	0.001
Dissim	0.56	0.001	0.57	0.001	0.54	0.001	0.49	0.001

Table 5. Environmental vectors overlaid with the non-metric multidimensional scaling (NMDS) plot of macroinvertebrate samples dissimilarity at the genus level using abundance data (Dissim index) (dimensions 1 and 2) using the *envfit* function (R package vegan).

Vectors	Dimension 1	Dimension 2	R^2	*p*
% Coarse cobble	-0.82	-0.57	0.54	0.001
% Sand	0.96	-0.26	0.37	0.001
Water temperature	0.80	0.60	0.32	0.001
Electronical Conductivity (EC)	0.81	0.59	0.51	0.001
Turbidity	0.92	-0.38	0.34	0.001
Discharge	0.99	0.14	0.15	0.009

Appendix A.

Table A.1 Geographical features of each sampling site. masl: meters above the sea level.

Sites	Geographical variables				
	Ecoregion	Sub-ecoregion	Soil type	Altitude (masl)	Drainage area (ha)
HM1	Yungas forest	High montane	Inceptisols	1360	53.20
HM2	Yungas forest	High montane	Inceptisols	1278	159.6
HM3	Yungas forest	High montane	Inceptisols	1622	90.73
HM4	Yungas forest	High montane	Inceptisols	1394	95.2
LM1	Yungas forest	Low montane	Inceptisols	1069	515.8
LM2	Yungas forest	Low montane	Inceptisols	960	580.5
LM3	Yungas forest	Low montane	Inceptisols	710	73.69
LM4	Yungas forest	Low montane	Inceptisols	1105	164.66
LM5	Yungas forest	Low montane	Inceptisols	1126	22.1
LM6	Yungas forest	Low montane	Inceptisols	1080	26.5
LM7	Yungas forest	Low montane	Inceptisols	942	27
LM8	Yungas forest	Low montane	Inceptisols	908	25
LM9	Yungas forest	Low montane	Inceptisols	1002	27.5
LM10	Yungas forest	Low montane	Inceptisols	713	88
FH1	Yungas forest	Foothill	Molisols	711	105.8
FH2	Yungas forest	Foothill	Molisols	543	486.3
FH3	Yungas forest	Foothill	Molisols	350	576.2
FH4	Yungas forest	Foothill	Molisols	660	497.56
CS1	Western Chaco	Chaco Serrano	Entisols	761	708.4
CS2	Western Chaco	Chaco Serrano	Entisols	649	2415
SC1	Western Chaco	Semiarid Chaco	Entisols	429	706.4
SC2	Western Chaco	Semiarid Chaco	Entisols	398	765.0
SC3	Western Chaco	Semiarid Chaco	Entisols	307	1271
SC4	Western Chaco	Semiarid Chaco	Entisols	291	2088

Table A.2 Hydro-morphological features of each sampling site. LW: low waters, HW: high waters. Mean values for channel width.

Sites	Hydro-morphological variables				
	Slope (°)	Sinuosity	Braiding	Channel width (m) (LW)	Channel width (m) (HW)
HM1	17.84	1.117	1.2	2.10	2.60
HM2	32.3	1.048	1.5	6.00	9.25
HM3	10.26	1.180	0.5	2.05	5.20
HM4	9.9	1.076	1.0	1.85	1.60
LM1	5.85	1.049	1.0	9.85	10.8
LM2	7.58	1.053	0.5	11.1	12.7
LM3	17.36	1.018	1.0	0.23	1.97
LM4	34.8	1.036	1.0	4.57	7.40
LM5	5.78	1.072	1.0	1.15	1.45
LM6	4.53	1.032	1.0	2.75	4.55
LM7	5.20	0.977	0.5	7.05	9.50
LM8	5.71	1.095	0.5	4.10	3.60
LM9	4.64	1.062	1.0	1.75	1.75
LM10	5.09	0.974	1.0	1.45	2.95
FH1	7.6	1.105	0.5	5.35	9.00
FH2	2.14	1.138	0.5	21.7	26.0
FH3	0.4	2.028	0.6	55.0	100
FH4	13.83	1.076	0.6	11.0	20.0
CS1	0.75	1.213	0.5	10.5	32.0
CS2	0.79	1.024	0.5	21.4	31.7
SC1	0.82	1.534	1.0	6.06	13.8
SC2	0.86	2.207	1.0	4.45	12.8
SC3	0.61	1.123	0.5	79.0	80.0
SC4	0.32	1.331	0.0	44.0	29.9

Table A.3 Hydrological features of each sampling site (mean values).LW: low waters, HW: high waters.

Sites	Hydrological variables			
	Discharge (m^3/s) (LW)	Discharge (m^3/s) (HW)	Stream Power (Watts/m) (LW)	Stream Power (Watts/m) (HW)
HM1	0.010	0.28	4.94	164.5
HM2	0.440	1.57	116	523.3
HM3	0.320	0.40	3.28	4.100
HM4	0.037	0.15	0.37	1.544
LM1	1.170	2.64	701	1578
LM2	0.920	4.27	168	1047
LM3	0.008	0.05	0.14	0.870
LM4	0.430	1.40	231	754.1
LM5	0.026	0.05	0.15	0.329
LM6	0.025	0.16	12.0	109.8
LM7	0.040	0.24	8.17	47.05
LM8	0.070	0.17	10.9	26.25
LM9	0.052	0.14	0.24	0.649
LM10	0.031	0.13	0.16	0.692
FH1	0.160	1.71	1.22	12.90
FH2	1.640	8.88	3.50	19.00
FH3	2.560	6.28	1.02	2.512
FH4	1.680	3.01	23.2	41.65
CS1	1.260	5.94	0.94	4.450
CS2	3.280	9.72	2.60	7.670
SC1	0.630	2.55	0.52	2.100
SC2	0.230	2.52	0.20	2.200
SC3	5.540	6.00	3.40	3.660
SC4	4.130	16.1	1.32	5.150

Table A.4 Granulometrical features of each sampling site.

Sites	Granulometrical variables						
	% Boulder	% Coarse Cobble	% Fine Cobble	% Pebble	% Granule	% Sand	% Silt
HM1	52.0	31.0	17.0	0.00	0.00	0.00	0.00
HM2	50.0	32.0	18.0	0.00	0.00	0.00	0.00
HM3	57.1	28.6	14.3	0.00	0.00	0.00	0.00
HM4	44.4	33.3	22.2	0.00	0.00	0.00	0.00
LM1	38.0	36.0	22.0	4.00	0.00	0.00	0.00
LM2	35.0	31.0	21.0	8.00	5.00	0.00	0.00
LM3	15.0	40.0	15.0	10.0	10.0	10.0	0.00
LM4	41.0	38.0	19.0	2.00	0.00	0.00	0.00
LM5	12.5	16.6	20.8	37.5	12.5	0.00	0.00
LM6	8.00	15.5	19.5	42.8	14.2	0.00	0.00
LM7	35.0	31.0	29.0	4.00	1.00	0.00	0.00
LM8	31.0	35.0	26.0	6.00	2.00	0.00	0.00
LM9	6.20	0.00	18.7	56.2	18.7	0.00	0.00
LM10	10.7	7.1	21.4	35.7	7.1	17.8	0.00
FH1	9.80	13.1	8.20	4.90	34.4	13.1	0.00
FH2	7.20	23.2	4.30	1.40	43.4	20.2	0.00
FH3	0.00	0.00	22.8	42.1	19.2	10.5	5.2
FH4	11.5	29.2	14.1	14.1	13.3	13.3	4.4
CS1	0.00	11.4	34.1	3.80	30.3	19.7	0.00
CS2	0.00	0.00	12.0	2.00	30.0	56.0	0.00
SC1	0.00	0.00	15.6	53.1	0.00	0.00	31.2
SC2	0.00	0.00	48.0	44.0	4.00	4.00	0.00
SC3	0.00	0.00	0.00	0.00	0.00	100	0.00
SC4	0.00	0.00	0.00	0.00	0.00	100	0.00

Table A.5 Physicochemical features of sampling sites including water temperature, pH and dissolved oxygen (mean values). LW: low waters, HW: high waters.

Sites	Physicochemical variables					
	Water temperature (C°) (LW)	Water temperature(C°) (HW)	pH (LW)	pH (HW)	Dissolved oxygen (mg/L) (LW)	Dissolved oxygen (mg/L) (HW)
HM1	19.5	17.5	7.65	6.00	9.00	9.40
HM2	17.0	18.5	6.90	6.00	8.70	9.40
HM3	10.5	10.8	8.00	8.14	12.9	10.6
HM4	14.4	11.1	7.46	7.58	9.60	9.92
LM1	15.5	18.0	6.95	6.00	8.60	9.00
LM2	15.5	21.0	6.95	6.00	9.00	8.80
LM3	16.5	14.7	8.02	8.16	8.36	12.2
LM4	18.0	20.0	7.70	6.00	9.00	9.00
LM5	15.7	18.1	8.20	8.79	11.4	11.1
LM6	18.5	17.0	7.10	6.00	9.00	9.00
LM7	18.0	21.0	8.05	6.00	9.00	9.00
LM8	17.5	19.5	6.90	7.00	9.00	9.00
LM9	18.5	11.6	8.46	8.27	11.0	10.4
LM10	18.8	13.2	8.28	7.74	10.5	10.9
FH1	23.1	15.0	7.56	6.86	7.62	12.5
FH2	23.5	12.2	8.80	7.31	8.38	10.1
FH3	24.0	18.0	8.00	7.43	9.00	10.8
FH4	17.5	14.5	8.00	7.62	11.0	11.5
CS1	25.2	14.6	7.59	7.50	10.0	9.60
CS2	22.3	16.6	7.00	8.26	10.3	9.65
SC1	25.2	19.2	7.07	8.32	9.68	11.5
SC2	32.5	18.3	7.25	8.26	7.97	11.8
SC3	28.5	10.6	8.45	8.15	9.82	11.2
SC4	23.8	12.4	8.36	8.04	10.5	13.1

Table A.6 Physicochemical features of sampling sites including electronical conductivity (EC), turbidity and total dissolved solids (TDS) (mean values). LW: low waters, HW: high waters.

Sites	Phisicochemical variables					
	EC (µS/cm) (LW)	EC (µS/cm) (HW)	Turbidity (LW)	Turbidity (HW)	TDS (g/L) (LW)	TDS (g/L) (HW)
HM1	164.6	88.60	0.00	1.00	0.06	0.05
HM2	114.7	91.10	1.00	2.00	0.06	0.05
HM3	84.75	72.35	0.60	0.00	0.06	0.05
HM4	82.60	55.00	0.00	0.00	0.05	0.03
LM1	122.2	104.3	1.00	1.50	0.08	0.07
LM2	131.8	108.4	0.00	1.50	0.08	0.07
LM3	359.5	296.5	0.00	0.00	0.27	0.26
LM4	90.00	78.60	0.00	1.00	0.05	0.07
LM5	272.0	181.0	0.00	0.00	0.17	0.11
LM6	220.5	113.8	1.00	2.00	0.18	0.20
LM7	612.0	173.5	1.00	2.00	0.08	0.10
LM8	321.5	252.5	1.00	2.00	0.15	0.20
LM9	357.5	318.0	0.00	0.00	0.23	0.20
LM10	246.5	210.0	0.00	0.00	0.18	0.13
FH1	70.50	34.50	0.43	0.65	0.04	0.02
FH2	112.0	67.50	1.71	3.50	0.07	0.04
FH3	150.0	90.00	1.70	10.0	0.10	0.20
FH4	200.0	150.0	1.00	15.2	0.10	0.30
CS1	728.0	384.0	1.33	3.50	0.47	0.24
CS2	989.0	612.0	1.01	13.4	0.32	0.39
SC1	2645	2095	12.2	47.5	1.62	1.34
SC2	3175	1970	1.35	83.7	2.22	1.29
SC3	504.0	260.0	21.8	52.5	0.32	0.16
SC4	676.5	1125	137	144	0.43	0.71

Table A.7 Physicochemical features of sampling sites including major ions: Total Alkalinity, Chlorine and Sulfate (mean values). LW: low waters, HW: high waters.

Sites	Major ions (mg/L)					
	Total Alkalinity (LW)	Total Alkalinity (HW)	Chlorine (LW)	Chlorine (HW)	Sulfate (LW)	Sulfate (HW)
HM1	54.28	30.00	2.98	1.80	2.50	1.50
HM2	39.85	30.00	2.23	1.80	2.50	1.70
HM3	27.90	32.10	0.50	0.42	2.52	1.96
HM4	00.00	00.00	0.51	0.36	1.92	1.10
LM1	49.80	35.05	2.98	2.06	3.00	3.40
LM2	52.85	44.04	3.72	3.21	3.00	3.70
LM3	206.9	148.1	1.86	1.67	6.38	4.82
LM4	00.00	00.00	2.23	1.70	3.20	1.80
LM5	111.6	93.85	1.20	1.13	4.47	3.29
LM6	92.81	75.20	3.72	3.20	8.20	6.60
LM7	75.84	61.50	5.21	4.75	8.50	6.80
LM8	119.5	88.00	3.72	3.33	10.0	7.80
LM9	00.00	00.00	2.98	2.13	69.6	52.0
LM10	00.00	00.00	0.90	0.69	3.81	2.80
FH1	29.10	24.70	0.40	0.42	6.80	4.45
FH2	56.00	44.40	0.80	0.62	6.30	5.51
FH3	142.7	37.83	8.90	5.70	8.64	7.68
FH4	181.2	49.42	5.70	17.8	15.3	10.5
CS1	142.7	37.83	40.0	11.1	106	61.0
CS2	210.0	163.0	104	59.0	154	97.0
SC1	187.0	181.7	509	354	482	396
SC2	203.0	207.8	480	379	519	418
SC3	172.0	202.8	21.2	14.2	38.4	4.80
SC4	200.1	140.3	49.6	88.7	121	228

Table A.8 Physicochemical features of sampling sites including major ions: Calcium and Magnesium (mean values). LW: low waters, HW: high waters.

Sites	Major ions (mg/L)			
	Calcium (LW)	Calcium (HW)	Magnesium (LW)	Magnesium (HW)
HM1	9.40	6.00	1.90	1.01
HM2	3.76	3.20	0.89	0.66
HM3	11.1	10.5	3.03	2.44
HM4	9.55	8.07	2.22	1.56
LM1	5.85	5.05	1.26	1.05
LM2	6.89	5.88	1.14	0.96
LM3	51.1	35.8	17.2	11.7
LM4	4.80	3.90	1.01	0.86
LM5	33.7	25.7	6.18	5.39
LM6	13.4	12.6	2.38	1.90
LM7	11.1	10.5	3.40	2.70
LM8	17.8	15.2	8.15	6.02
LM9	52.9	43.4	10.0	8.84
LM10	36.0	23.1	12.0	8.68
FH1	6.50	5.02	1.64	1.33
FH2	12.0	10.7	4.20	3.78
FH3	27.6	8.00	5.64	1.32
FH4	36.6	16.2	8.80	4.55
CS1	62.0	46.4	9.80	5.80
CS2	66.0	56.0	11.1	8.70
SC1	153	133	42.0	35.2
SC2	143	134	44.0	36.5
SC3	44.0	26.0	17.8	7.80
SC4	33.4	49.0	16.1	18.0

Table A.9 Physicochemical features of sampling sites including major ions: Sodium and Potassium (mean values). LW: low waters, HW: high waters.

Sites	Major ions (mg/L)			
	Sodium (LW)	Sodium (HW)	Potassium (LW)	Potassium (HW)
HM1	9.06	5.10	2.72	2.05
HM2	9.96	7.02	3.28	2.87
HM3	3.68	2.99	2.65	2.59
HM4	3.26	2.08	1.74	1.38
LM1	11.3	10.5	3.50	3.05
LM2	11.7	10.6	3.44	3.15
LM3	13.0	9.52	3.26	3.06
LM4	10.2	8.70	3.14	2.78
LM5	9.36	7.03	2.91	2.09
LM6	17.8	16.9	2.44	2.12
LM7	16.5	15.8	3.34	3.05
LM8	17.1	16.1	4.49	4.11
LM9	15.6	11.4	3.29	1.97
LM10	8.98	5.84	1.08	0.95
FH1	3.60	3.05	2.70	2.50
FH2	4.70	3.79	2.80	2.42
FH3	19.5	8.00	2.00	3.90
FH4	17.2	11.5	1.60	5.90
CS1	77.0	52.0	3.70	2.30
CS2	134	97.0	4.10	3.10
SC1	393	291	5.00	4.14
SC2	429	313	5.20	4.24
SC3	39.5	17.2	10.1	3.90
SC4	60.0	163	5.08	7.80

Table A.10 Physicochemical features of sampling sites including nutrients: nitrate, nitrite and phosphate (mean values).

Sites	Nutrients (mg/L)		
	Nitrate	Nitrite	Phosphate
HM1	1.20	0.005	0.12
HM2	1.20	0.005	0.12
HM3	1.30	0.006	0.12
HM4	11.1	0.030	0.10
LM1	1.20	0.005	0.12
LM2	1.10	0.005	0.12
LM3	3.30	0.006	0.47
LM4	1.20	0.005	0.10
LM5	2.50	0.030	0.21
LM6	1.20	0.005	0.20
LM7	1.20	0.005	0.10
LM8	1.50	0.005	0.12
LM9	1.41	0.030	0.20
LM10	2.03	0.030	0.25
FH1	0.17	0.000	0.16
FH2	3.28	0.000	0.10
FH3	0.17	0.000	0.25
FH4	0.37	0.000	0.00
CS1	1.05	0.000	0.10
CS2	2.10	0.000	0.12
SC1	1.23	0.000	0.12
SC2	0.27	0.000	0.17
SC3	0.09	0.090	0.62
SC4	1.18	0.050	0.15

Appendix B. Macroinvertebrates taxa lists.

Table B.1 Odonata and Plecoptera taxa list. Indet. = Indeterminate genus.

Order	Family	Genus	Species
Odonata	Gomphidae	*Progomphus*	*P. complicatus*
		Phyllocycla	*P. argentina*
	Libellulidae	*Brechmorhoga*	*B. nubecula*
		Elasmothemis	*E. cannacrioides*
		Perithemis	*P. moma*
	Calopterygidae	Indet.	
	Coenagrionidae	*Argia*	*A. joergenseni*
		Neoneura	*N. confundens*
Plecoptera	Gripopterygidae	*Claudioperla*	*C.tigrina*
	Perlidae	*Anacroneuria*	

Table B.2 Ephemeroptera taxa list.

Order	Family	Genus	Species
Ephemeroptera	Baetidae	*Americabaetis*	*A. alphus*
		Andesiops	*A. peruvianus*
		Apobaetis	
		Baetodes	*B. huaico*
		Callibaetis	
		Camelobaetidius	*C. penai*
		Cloeodes	
		Guajirolus	*G. queremba*
		Nanomis	*N. galera*
		Paracloeodes	
		Varipes	
	Caenidae	*Caenis*	*C. ludrica*
		Alloretochus	*A. peruvianus*
	Leptohyphidae	*Haplohyphes*	*H. baritu*
		Leptohyphes	*L. eximius*
		Lumahyphes	*L. huacra*
		Tricorythodes	*T. popayanicus*
			T. quizeri
	Leptophlebiidae	*Farrodes*	
		Meridialaris	
		Thraulodes	*T. cochunaensis*
			T. bolivianus
		Traverella	
	Oligoneuriidae	*Homoeoneuria*	
		Lachlania	
	Polymitarcidae	*Tortopsis*	*T. sarae*

Table B.3 Hemiptera, Megaloptera, Trichoptera and Lepidoptera taxa list.

Order	Family	Genus	Species
Hemiptera	Corixidae	*Trichocorixa*	
	Gelastocoridae	*Gelastocoris*	
		Nerthra	
	Naucoridae	*Ambrysus*	
		Limnocoris	
Megaloptera	Corydalidae	*Corydalus*	
Trichoptera	Hydropsychidae	*Smicridea*	
		Leptonema	
	Philopotamidae	*Chimarra*	
	Polycentropodidae	*Polycentropus*	
	Hydrobiosidae	*Atopsyche*	*A. callosa*
			A. maxi
		Cailloma	
	Glossosomatidae	*Mortoniella*	
		Protoptila	
	Hydroptilidae	*Hydroptila*	
		Ithytrichia	
		Metrichia	
		Oxyethira	
	Leptoceridae	*Nectopsyche*	
		Oecetis	
	Calamoceratidae	*Banyallarga*	
	Helicopsychidae	*Helicopsyche*	
	Odontoceridae	*Marilia*	*M. cinerea*
			M. flexuosa
Lepidoptera	Crambidae	*Petrophila*	

Table B.4 Diptera taxa list (except family Chironomidae). “?” = dubious identification. Indet. = indeterminate genus.

Order	Family	Genus
Diptera	Tipulidae	*Hexatoma*
		Molophilus
		Prionocera
		Tipula?
	Blephariceridae	Indet.
	Psychodidae	*Maruinia*
		Pericoma
	Dixidae	Indet.
	Simulidae	*Simulium*
	Ceratopogonidae	*Alluaudomya*
		Bezzia
		Atricopogon
		Dasyhelea
	Stratiomydae	*Nemotelus*
		Odontomya
	Empididae	*Chelifera*
		Hemerodromia

Table B.5 Coleoptera taxa list.). Indet. = indeterminate genus. “?” = dubious identification.

Order	Family	Genus	Species
Coleoptera	Dytiscidae	Indet.	
	Dryopidae	Indet.	
		Helichus	
	Elmidae	*Austrelmis*	
		Cylloepus?	
		Heterelmis	
		Macrelmis	*M.isis*
			Indet.
		Microcylloepus	
		Neoelmis	
		Phanocerus	
	Hydraenidae	*Gymnochthebius*	
	Hydrophillidae	Indet.	
		Enochrus	
	Lutrochidae	*Lutrochus*	
	Psephenidae	*Psephenus*	
	Staphillinidae	Indet.	

Table B.6 Crustacea taxa list. Indet. = indeterminate genus. “?” = dubious identification.

Order (Subphyllum)	Family	Genus
Decapoda (Crustacea)	Aeglidae	*Aegla*
Copepoda (Crustacea)	Indet.	Indet.
Amphipoda (Crustacea)	Bogidiellidae	Indet.
	Hyalellidae	*Hyalella*
Ostracoda (Crustacea)	Indet.	

Table B.7 Acari taxa list.

Subclass	Family	Genus	Species
Acari	Hygrobatidae	*Atractides*	
		Atractidella	*A. porophora*
		Corticacarus	
		Dodecabates	
		Hygrobatella	*H.multiacetabulata*
		Hygrobates	
		Tetrahygrobatella	
	Rhynchohydracaridae	*Clathosperchon*	
	Torrenticolidae	*Torrenticola*	*T.columbiana*
	Limnesiidae	*Neomamersa*	
		Protolimnesia	
	Aturidae	*Axonopsella*?	
	Hydryphantidae	*Neocalonyx*	
	Limnocharidae	*Rhyncholimnochares*	

Table B.8 Platelmyntha, Annelida and Mollusca. Indet. = indeterminate genus.

Class (Phyllum)	Family	Genus	Species
Turbellaria (Platelmyntha)	Indet.	Indet.	
Oligochaeta (Annelida)	Indet.	Indet.	
Hyrudinea (Annelida)	Indet.	Indet.	
Bivalvia (Mollusca)	Sphaeriidae	*Pisidium*	
	Corbiculidae	*Corbicula*	
Gasteropoda (Mollusca)	Succineidae	*Omalonyx*	
	Planorbidae	*Biomphallaria*	*B.tenagofila*
	Physidae	*Physa*	*P.acuta*
	Cochliopidae	*Heleobia*	

Credit authors' statement

Edgardo J. I. Pero: Conceptualization, Methodology, Software, Formal analysis, Investigation, Resources, Data Curation, Writing - Original Draft, Visualization, Project administration. **Sergio M. Georgieff**: Conceptualization, Methodology, Investigation, Resources, Writing - Review & Editing, Supervision, Funding acquisition. **M. de Lourdes Gultemirian**: Conceptualization, Software, Validation, Writing - Review & Editing. **Fátima Romero**: Validation, Data Curation. **Guillermo E. Hankel**: Validation, Data Curation. **Eduardo Domínguez**: Conceptualization, Investigation, Resources, Writing - Review & Editing, Supervision, Project administration, Funding acquisition.

Declaration of interests

- ✓ ☐ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

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Highlights

- Ecoregions and topography combined were more precise to define river types.
- River types were included in three large groups: Mountains, Foothills, and Lowlands.
- Macroinvertebrate distribution was related to abiotic features along the landscape.
- Sediment grain size, temperature and turbidity were key factors to define river types.
- Topography and climate could strongly influence South American freshwater biota.