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Diversity of Freshwater Macroinvertebrate Communities in Los Tuxtlas, Veracruz, Mexico

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Abstract: The objective of this work is to contribute to the knowledge of the freshwater macroinvertebrate communities of Los Tuxtlas, Veracruz, Mexico. For this region, there is only limited knowledge of its aquatic crustaceans and mollusks. A total of 13,399 freshwater macroinvertebrates were collected from four river sections in each of the three sub-basins of the region using the Surber network in four seasons of an annual cycle (2021–2022) and were preserved in 70° alcohol. Organisms belonging to seven phyla, nine (sub)classes, 21 (sub)orders and 65 families were identified. The most abundant orders were Ephemeroptera (42.03%), with greatest abundance of the family Baetidae, and the orders Trichoptera (19.11%), Diptera (15.43%), and Coleoptera (3.98%). Four families exceeded 10% relative abundance, and together they total 61.02%: Baetidae (23.84%), Hydroptilidae (13.58%), Leptohyphidae (13.03%), Chironomidae (10.57%), and Elmidae (3.23%). The order Plecoptera was recorded for the first time in Los Tuxtlas, with three families. The orders Hydrachnidae and Ostracoda, as well as six families of the order Ephemeroptera, with only one previously recorded family, and six more families of the order Diptera, were also documented. Two species of invasive aquatic mollusks were found in several rivers and basins. In this work, a high diversity of freshwater macroinvertebrates occurred compared to other sites studied in Veracruz and Mexico, and new records of these taxa are provided for the region of Los Tuxtlas.

Keywords: rivers; water quality; environmental monitoring; Los Tuxtlas; macroinvertebrates

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1. Introduction

Mexico is a megadiverse country [1], and Veracruz is one of the states with the most biodiversity in the country [2]. Los Tuxtlas region is one of the best-studied regions in the Neotropics due to the presence of Los Tuxtlas Tropical Biology Station of the National Autonomous University of Mexico (Estación Los Tuxtlas (unam.mx)). Since the 1960s, this station has facilitated extensive research on the biology and ecology of this region by several academic and research institutions. Many taxonomic groups of its flora and fauna have been studied, including numerous groups of invertebrates. However, aquatic macroinvertebrates (AqMI) have been poorly studied in Los Tuxtlas, where freshwater ecosystems are among the most vulnerable due to the loss of connectivity. AqMI are small organisms (<200–<500 µm, depending on the authors) without a backbone that inhabit aquatic ecosystems such as rivers, streams, lakes, ponds, and wetlands. They are widely distributed and abundant, and are thus commonly used as bioindicators to assess water quality and environmental health. Their presence, abundance, and diversity can provide valuable insights into the ecological condition of aquatic environments. Despite the importance and abundance of water resources in the region, and the inclusion of a Biosphere Reserve, little has been published on its AqMI. Existing publications focus on specific groups such as mollusks and crustaceans, mainly in the faunistic-checklist [3–5], except for the work of Álvarez et al. (2009) [6], which presents the development of a biotic integrity index based on the aquatic crustaceans and mollusks in two rivers in the region. There are no specific publications in the region for other groups such as aquatic insects. García-Aldrete (2017) [7] presents a list of insects for Los Tuxtlas region; however, Leptophlebiidae is included as the only recorded family of Ephemeroptera, and there are no records for Plecoptera. For Diptera, six families with aquatic larvae are recorded.

Macroinvertebrates are used as bioindicators worldwide, but the high diversity of macroinvertebrate species and endemism in Latin America requires greater knowledge of this group to increase the effectiveness of biomonitoring [8]. Various indices have been developed to utilize AqMI for this purpose, with taxonomic determination often focused at the family level [9,10]. Taxonomic richness, which reflects species diversity within ecosystems, plays a crucial role in comprehensive environmental evaluations. AqMI are valuable indicators, offering insights into aquatic ecosystems' health, composition, and responses to environmental stressors [11]. However, the Neotropical region, characterized by its rich biodiversity, presents unique challenges for AqMI studies. Despite successful AqMI bioindicator applications in many Neotropical countries, knowledge gaps persist due to limited expertise, study discontinuity, and exceptional biodiversity [12]. This gap in taxonomic knowledge about AqMI hinders their potential use as bioindicators in the Los Tuxtlas region and comprehensive knowledge of the region's river ecosystems. AqMI studies have not only improved our understanding of environmental quality but also sparked increased interest in these organisms. Research on AqMI has deepened our knowledge of their taxonomic richness, community composition and structure, ecological functions, and responses to environmental stressors [13]. This work presents the basic information on the regional richness and diversity of AqMI and serves as a basis to take advantage of the potential of AqMI as environmental bioindicators in the region. The study of AqMI in both the Neotropics and Mexico has focused, above all, on their use as bioindicators of water and the environmental quality of aquatic ecosystems. From this point of view, AqMI have been studied in several countries, such as Brazil [14], Argentina [15,16], and Costa Rica [17], and in the Andean region [18–20]. For Mexico, there are works from the central highlands [21–24]. Specifically, there are several published studies for the state of Veracruz on AqMI, highlighting those of the Pánuco River in the north of the state [25], the Totoapan and Paso Real rivers of the Actopan River basin [26], the Blanco River [27] and the Atoyac River [28,29] in the center of the state, and the La Antigua River [30,31], as the closest to the region of Los Tuxtlas. Most of the works are at the family taxonomic level due to the difficulty in assigning the genus or species; some records for America report between three to five phyla and 6 to 10 classes. In the Atoyac River in Veracruz, four phyla, 10 classes, 20 orders, and 67 families have been recorded [29]. Other studies in this basin report fewer groups [28]. In the Antigua basin, Armas (2015) [30] recorded six classes, 15 orders, 59 families and 101 genera; Astudillo (2014) [31] recorded 31 families. In the Actopan River basin, Castro et al. (2015) [26] recorded 12 families. In response to the fragmentary available information and as the first part of a study for the development and application of a biotic integrity index based on the AqMI in the Los Tuxtlas region, this paper records and analyses the richness and diversity of the AqMI of Los Tuxtlas, Veracruz.

2. Materials and Methods

2.1. Study Site Description

Freshwater macroinvertebrates were collected in 12 stretches of distinct rivers from Los Tuxtlas, in the southern center of the state of Veracruz. This is considered a priority hydrological region in the Gulf of Mexico, extending along ≈3200 km^2 of volcanic mountain range (0–1700 m.a.s.l.) and isolated on the coastal plain of the Gulf of Mexico, with a predominantly humid, tropical climate, high precipitation (2000–4500 mm) [32], and 6771.6 km of surface currents (elaborated from INEGI, 2010) [33]. Rivers and bodies of water that drain inland are part of the Papaloapan River or Coatzacoalcos River basin. Short rivers on the coastal slope flow directly into the Gulf of Mexico. Los Tuxtlas forests constitute the northernmost relicts of tropical humid forest on the American continent.

The region belongs to the floristic province of the Gulf of Mexico and shares around 70% of its species with the Mesoamerican flora and the rest with South America. In the region, nine vegetation types are defined [34]. The predominant natural vegetation in the area and in the surroundings of the rivers studied—up to 900 m.a.s.l.—is the high and medium evergreen forest, generally disturbed, or secondary vegetation of these. In general, the sites are in a landscape with a different degree of fragmentation where corridors of jungle vegetation are preserved on the riverbanks, and outside them, along the lines of living tree fences and some fragments of jungle or disturbed vegetation in the ravines and steeper terrain, where extensive livestock-induced pastures with African grasses predominate and, in some cases, corn, tobacco, and sugar cane crops. The study sites are located between the coordinates 2,030,684 to 2,065,758 north latitude and between 257,381 to 289,872 west longitude (WGS 84 UTM Zone 15N). To sample a representative example of the area, and considering the accessibility of the localities, 4 river stretches corresponding to four micro-basins of each of the three main sub-basins of the region were included: Tecolapilla or coastal (As), Catemaco (Ar), and San Andrés (Aq) [33] (Figure 1). In each section, AqMi and water sampling were carried out with a quarterly frequency in each of the four climate seasons that occur in the region: the tropical cyclones season with intense rains (September–November, 2021), the northern winds season (December, 2021–February, 2022), the dry season (March–May, 2022), and the summer rainy period (June-August, 2022) (based on Soto 2004) [32]. Water sampling included physicochemical parameters with Hanna HI multiparametric probes HI-9829 and HI-98194: pH, DO % and mg/L, electrical conductivity, total dissolved solids (TDS, ppm), and temperature (°C). A characterization of the reaches including length, width, and depth [35], and upstream micro-basin area using digital elevation models [36] with the software Quantum GIS 3.22, their location in the basin system, and the condition of the type of river order following Strahler (1957) [37] was carried out (Table 1).

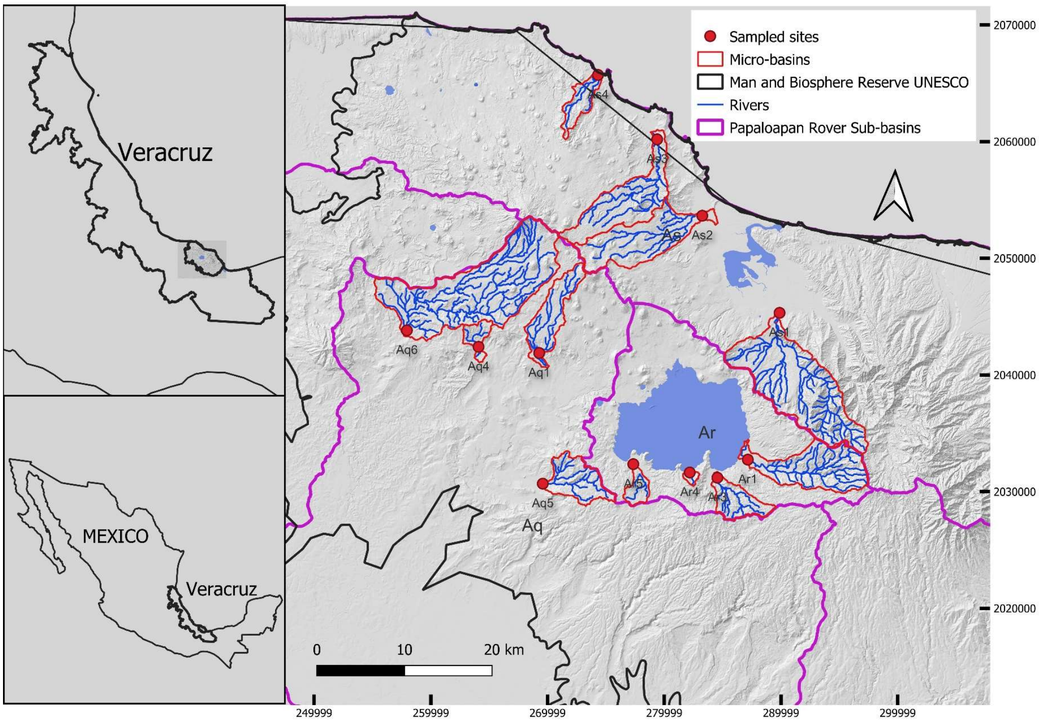

Figure 1. Location of the 12 sampling sites or micro-basins, with four rivers in each of the three sub-basins studied belonging to the administrative basin of the Papaloapan River (basin RH28, Aq, Ar, As sub-basins) in Los Tuxtlas region, Veracruz, Mexico (elaborated from INEGI, 2010) [33,36].

Table 1. Location and hydro morphological characterization of the studied streams of the micro-basins in Los Tuxtlas basin system, state of Veracruz.

Code	Micro-Basin	Latitude (UTM)	Longitude (UTM)	Mainstream Length (km)	Area (ha)	Perimeter (m)	Order Strahler	Depth (cm)	Width (m)
Aq1	Seco	2,041,891	269,283	9.823	1701.98	32,784	2	19.11	8.7
Aq4	Tepancan	2,042,351	264,115	3.375	470.26	15,393	2	12	3.5
Aq5	Chuniapan	2,030,684	269,585	8.097	1509.31	26,147	3	5.57	8.5
Aq6	Tepango	2,043,393	257,381	23.702	7529.94	63,328	4	130	1.8
Ar1	Cuetzalapan	2,032,919	287,110	14.330	2971.20	40,014	3	6.88	18
Ar3	Margarita	2,031,385	284,469	7.205	1086.97	21,600	3	4.03	11.5
Ar4	Porvenir	2,031,599	282,226	1.268	116.15	5805	1	6.5	1
Ar5	Victoria	2,032,088	277,414	4.854	463.25	12,906	2	55	1.6
As1	Coxcoapan	2,045,323	289,872	17.369	5811.29	55,052	4	7.88	18
As2	Palma	2,053,606	282,713	14.056	3189.62	45,370	3	6.03	11
As3	Máquina	2,059,799	279,474	14.8865	3061.46	44,992	3	8.49	21
As4	San Martín	2,065,758	274,331	6.241	775.61	20,975	2	29.6	8.3

2.2. Macroinvertebrate Sampling, Identification, and Analysis

At each described season and sampling site, the AqMI were collected using a Surber-type net (30 × 30 cm opening, and mesh size of 0.3–0.5 mm), and the bottom substrate was removed and rubbed with the hands just upstream of the entrance to the net to catch organisms in it. We sought to sample three microhabitats at each site for about 30 min in total, with 12 collections distributed proportionally to the availability and extent of each microhabitat in 50 m stretches of river. The captured AqMI were separated from the rest of the sediments and organic material and were counted and identified directly or with the use of a magnifying glass or digital microscope. The samples of the specimens were preserved in 70° alcohol. Identification in general was carried out using identification guides and keys [38–41] and other specific keys and primary literature for some orders or families, as well as with online identification resources [42]. The data were entered into a dataset including a faunal list following the taxonomic criteria of Pineda et al. (2014) [43], recording the presence and abundance of each taxon for each of the 12 sites (Appendix A). For each site, species richness (S), abundance (N), Shannon-Wiener Diversity Index (H'), and Berger-Parker Dominance Index (DB-P) were calculated. Species richness and uniformity were analyzed using the Whittaker range abundance curve. The similarity between the sampling sites was also analyzed using the unweighted pair-group average (UPGMA) where clusters are joined based on the average distance between all members in the two groups. For this analysis, we used Ward's method, based on taxa composition and abundance, and the Jaccard Similarity Index [44], based on taxa composition. Non-metric Multidimensional Scaling (NMDS) and Analysis of Similarities (ANOSIM), applying Jaccard's and Bray-Curtis indexes, were used for assessing the similarity of AqMI communities at the 12 study sites distributed across three different sub-basins. All the above calculations were made using Past V. 4.12 [45].

3. Results

3.1. Environmental and Stream Characteristics

In general, the 12 sampling sites are shallow rivers (<50 cm), and some of them occasionally have very narrow sections and higher current speeds in sections of their upper parts (Table 1). Among the 12 sections considered, a fast current (>0.3 m/s) predominates in five (Aq1, Aq6, As1, As2, and As3) even in the dry season; in two, an intermediate current predominates (Ar1 and As4); and in five, a slow current predominates (Aq4, Aq5, Ar3, Ar4, and Ar5). The environmental factors varied: 17.16–27.78 °C, pH 7.35–8.82, O_2 (mg/L) 5.28–8.8 with a saturation % of 62.7–102.3%, electrical conductivity of 73–533 μS/cm, and 37–267 ppm total dissolved solids (Table 2).

Table 2. Physicochemical characterization of the sections of the studied streams of the micro-basins in Los Tuxtlas basin system, state of Veracruz.

Code	River Stretch	pH	Temp (°C)	O_2 (mg/L)	O_2 %	EC (μS/cm)	TDS (ppm)
Aq1	Seco	8.05–8.82	20.56–27.51	5.28–7.8	70.1–93	305–352	154–177
Aq4	Tepancan	8.41–8.68	22.23–26.7	5.45–8.12	68.20–93.38	290–533	154–267
Aq5	Chuniapan	8.5–8.72	22.7–27	6.9–8.32	86.24–97.34	210–387	105–193
Aq6	Tepango	8.3–8.79	19.6–26.2	8–8.8	94.3–98.96	260–304	130–152
Ar1	Cuetzalapan	7.77–8.6	20.68–26.08	6.5–8.65	82.5–100	84–129	42–62
Ar3	Margarita	7.41–8.68	20.03–27.78	6.98–8.14	91.7–98.2	73–102	37–51
Ar4	Porvenir	7.49–8.71	20.87–26.2	6.23–7.65	76.56–89.046	114–363	57–166
Ar5	Victoria	7.35–8.1	19.33–27.09	5.4–6.23	62.70–73.26	108–189	54–94
As1	Coxcoapan	8.2–8.66	24.33–26.62	6.78–8.56	84.5–102.3	195–311	97–156
As2	Palma	7.82–8.22	17.16–25.1	7.53–8.3	83–98.3	136–196	69–98
As3	Maquina	7.92–8.59	22.53–25.3	6.93–8.61	82.7–99.5	122–157	61–74
As4	San Martin	7.85–8.3	23.8–25.27	6.65–7.47	80.5–91.7	146–165	65–83

3.2. Distribution, Taxonomic Composition, and Abundance of Freshwater Macroinvertebrates in the Study Sites

In total, 13,399 individuals of AqMI from seven subphyla or phyla (Annelida, Chelicerata, Crustacea, Hexapoda, Arthropoda, Mollusca, and Nematoda), nine (sub)classes, 21 (sub)orders, and 65 families were recorded (Appendix A). To these, the phylum Nematoda and the order Ostracoda were added to the analysis, for which it was not possible to reach a lower level in taxonomic determination. The coastal micro-basin of the La Palma River (As2) presented the highest number of families or equivalent taxa (38), followed by the Maquina River (36). The micro-basins with the lowest values were the Tepancan (20 taxa), the Seco River (24), and the Victoria (26). In general, from 24 to 38 taxa were recorded at the family level or lower. The richness of families per micro-basin was on average 32.17 ± 4.34 species (Table 3). The Insecta class (phylum Hexapoda) was the most abundant. The order with the most frequency of organisms was Ephemeroptera (5632 ind., 42.03%, at the 12 sites), followed by Trichoptera (2560 ind., 19.11%, 12 sites) and Diptera (1205 ind., 15.43%, 12 sites). The next five orders were in the 2–5% range, and another three 1–2%. The remaining nine orders had relative abundances <1%, seven orders <50 ind., and five orders <10 ind. Only four families exceeded 10% relative abundance, and together they total 61.02%. These families were Baetidae (n = 3194, 23.84%), Hydroptilidae (n = 1820, 13.58%), Leptohyphidae (n = 1746, 13.03%), and Chironomidae (n = 1416, 10.57%). Five families exceeded 3%: Simuliidae (n = 633, 4.72%), Hydrospsychidae (n = 525, 3.92%), Leptophlebiidae (523, 3.9%), Thiaridae (n = 456, 3.4%), and Elmidae (n = 433, 3.23%). Seven families presented 1–2%, and the remaining 51 presented values <1%, totaling 9.14%.

There were 12 to 16 AqMI families per site, out of a total of 65, with a percentage greater than 1% and representing an average of 89.96% of the total number of individuals (82.52–95.2%) (Figure 2)). In the coastal sub-basin, there was an average of 15.5 families (As1: 15, As4: 15, As2:16, and As3:16), and in the San Andrés basin, there was an average of 14.25 (Aq1:13, Aq6: 14, Aq4, and Aq5: 15). In the Lake Catemaco basin, the average was 13 families. One site presented all 16 families; one site presented 15; and two sites presented only 12 families.

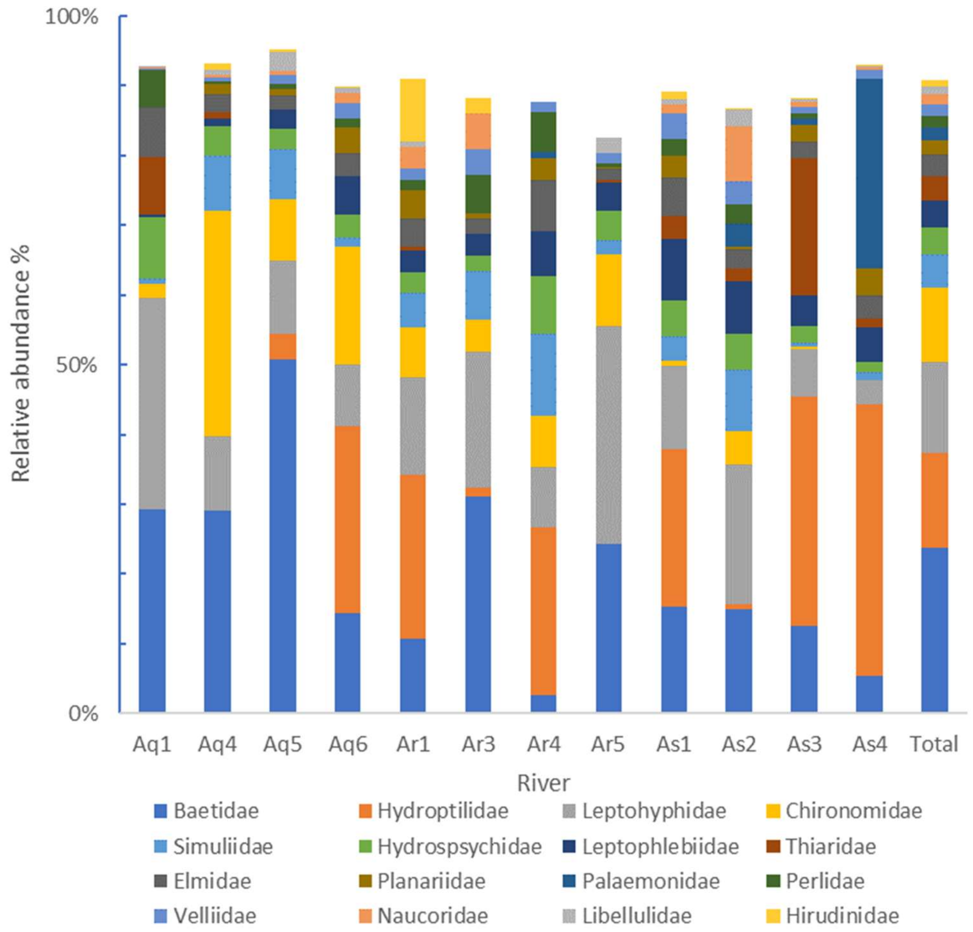

Figure 2. Relative abundances of AqMI families (only those with >1% relative abundance are shown) in the four rivers studied in each of the 3 sub-basins in Los Tuxtlas region, Veracruz: Aq: San Andrés sub-basin; Ar: Lake Catemaco sub-basin; As: coastal and estuarine sub-basin (Tecolapilla).

Table 3. Richness and diversity of aquatic macroinvertebrates in 12 micro-basins belonging to 3 sub-basins (Aq, Ar, As) of the hydrologic region (RH28 Basin, Papaloapan river) in Los Tuxtlas region, Veracruz.

Index	Aq1	Aq4	Aq5	Aq6	Ar1	Ar3	Ar4	Ar5	As1	As2	As3	As4	Total
S	24	35	34	35	33	34	28	26	34	38	36	29	67
N	879	2193	2273	1500	864	578	442	618	906	839	1524	783	13399
H′	1.96	2.05	1.95	2.49	2.64	2.49	2.64	2.25	2.68	2.80	2.23	2.00	2.70
J′	0.62	0.58	0.55	0.70	0.76	0.71	0.79	0.69	0.76	0.77	0.62	0.59	0.64
DB-P	0.30	0.32	0.51	0.27	0.23	0.31	0.24	0.31	0.23	0.20	0.33	0.39	0.24
Dominant taxon	Leptohyphidae	Chironomidae Baetidae	Baetidae	Hydroptilidae	Hydroptilidae	Baetidae	Hydroptilidae	Leptohyphidae	Hydroptilidae	Leptohyphidae	Hydroptilidae	Hydroptilidae	Baetidae

S—species richness; N—abundance of individuals; H′—Shannon–Wiener diversity index; J′—Pielou equity index; DB-P—Berger–Parker dominance index.

3.3. Diversity Indices of Freshwater Macroinvertebrates in the Study Sites

The diversity of the total sites was H′ = 2.70 and that of Pielou was J′ = 0.64; all were similar to values from other sites studied, both in Mexico and the Neotropics [20,46,47]. For the sites in this study, the lowest value was obtained in the Chuniapan River (Aq5), with H′ = 1.667, and the highest H′ = 2.094 in the La Palma River (As2) (Table 3). Overall, the average values were 2.35 ± 0.31, like those of other studies in Mexico. For the sites in this study, the dominant taxa were at the level of the order Ephemeroptera in 5 of 12 sites (nearly 6), with the family Leptohyphidae dominant in 3 of 12 and Baetidae in 2(3) of 12. Order Trichoptera follows with the Hydroptilidae family as dominant in six sites, reaching high densities on suitable substrates. The DBP index ranged from 0.20 to 0.51, with an average of 0.30 ± 0.08. This dominance index is similar to that obtained in other works. The Whittaker diagram (Figure 3) summarizes and compares the rank-abundance curves and the uniformity and richness of taxa found for each site.

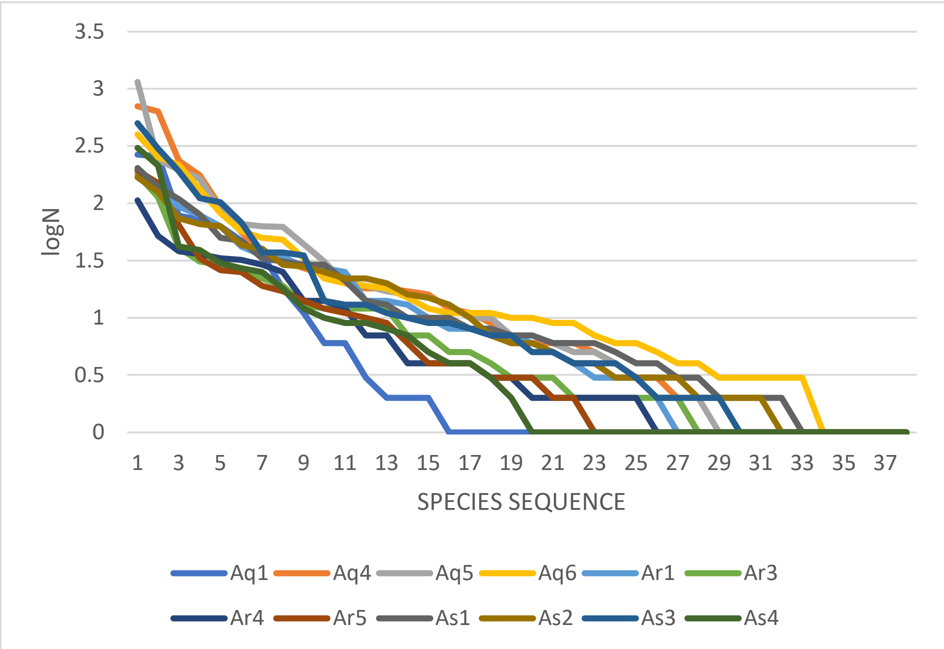

Figure 3. Whittaker diagram or rank-abundance curve (logN) from 12 reaches in rivers corresponding to 12 micro-basins (3 sub-basins: Aq, Ar, As) in Los Tuxtlas, Veracruz, Mexico.

The overall average species richness was 24.00 to 38 (32.2 ± 4.3). The mean abundance of individuals per site varied from 442 to 2273 (1116.58 ± 614.64). The interval for the Shannon H index was from 1.95 to 2.80 (2.35 ± 0.31). The DB-P index varied from 0.2 to 0.51 (0.3 ± 0.08). These indices provide similar and even high values in terms of richness and diversity compared to other regional studies in Mexico and tropical America.

The UPGMA for cluster analysis, using the Jaccard similarity index (Figure 4), showed little grouping, with only some subgroups with similarities of 67.5–70%: Aq5 and Ar3 and somewhat less Aq6 presented close to 70%; in contrast, As2 and As3, as coastal basins, showed a similarity of 67.5%. At the other end of the scale, Aq1 was shown to be the most different, more than the total. The sites Ar4, As4, Ar5, and Ar1 showed increasing similarity towards the branches of the aforementioned groups. There are no clear groups by sub-basins in terms of composition. In the UPGMA-Jaccard Cluster, a few rivers from the same sub-basins cluster together. There are also similar similarities with rivers in other sub-basins. A quite different grouping and similarity for Jaccard and proximity for Ward's method resulted from the two grouping calculation methods: by Jaccard similarity index and by Ward's method (Figure 5).

In the Analysis of Similarities (ANOSIM) with the use of Jaccard's similarity index that considers only composition and presence of taxa, R = 0.11 was obtained; between pairs of sub-basins, the greatest difference was between Ar and As (R = 0.2396), and between Aq and Ar, a similarity was observed (R = -01198); between Aq and As, there was a minor difference (R = 0.224). The ANOSIM was also performed using the Bray–Curtis index, based on composition and abundance, with which an R = 0.3403, p(same) = 0.0108 was obtained, which indicates a somewhat greater difference considering abundance, not only the presence used in the Jaccard Index. Unlike the ANOSIM with Jaccard, with the Bray–Curtis index, the greatest difference was between the Aq and Ar micro-basins (R = 0.5417), which indicates that basins with similar composition can present significant differences in the abundance of taxa. Taking into account abundance, in addition to the composition, between the sub-basins Aq and Ar, the difference was very low (R = 0.032), in correspondence with the ANOSIM with Jaccard, where a small similarity in composition was obtained (R = −0.1198).

The non-metric multidimensional scaling (NMDS) analysis was similar to the Bray–Curtis ANOSIM in showing a greater similarity between the continental Aq and Ar sub-basins, and a somewhat more distant group of coastal micro-basins As. Consistent with the UPGMA analysis, the NMDS showed Aq1 as the most distant micro-basin with less similarity with respect to any other (Figure 5). As the ellipses overlap significantly in the center, this suggests that there is a large overlap and similarity in species composition and abundance between the three groups of basins. That is, most species are present in all basins, regardless of the sub-basin to which they belong.

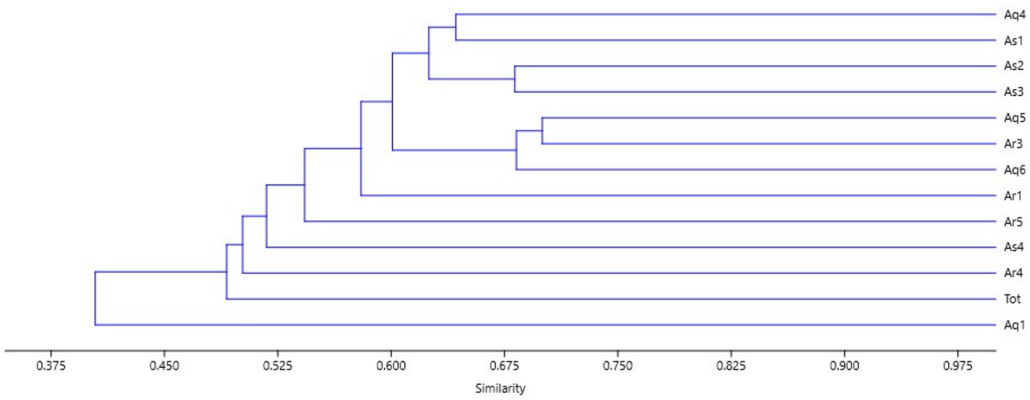

Figure 4. Cluster with Jaccard's similarity index of the 12 rivers and respective micro-basins in Los Tuxtlas, Veracruz, Mexico.

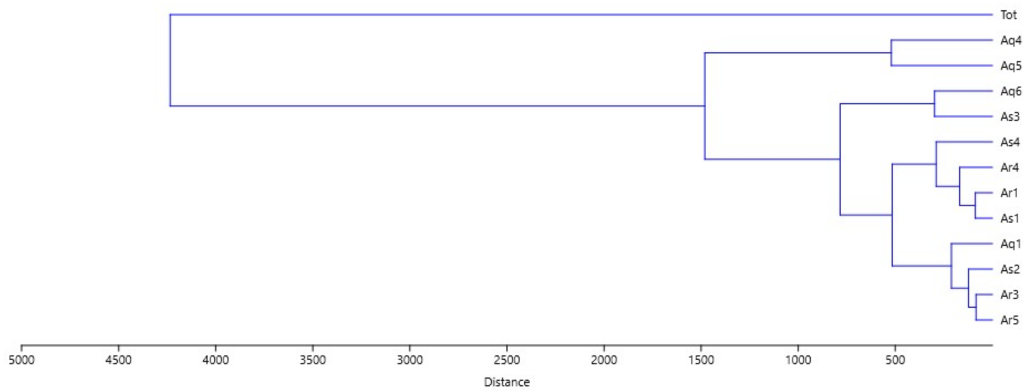

Figure 5. Cluster with Ward's method distance index of the 12 rivers and respective micro-basins in Los Tuxtlas, Veracruz, Mexico.

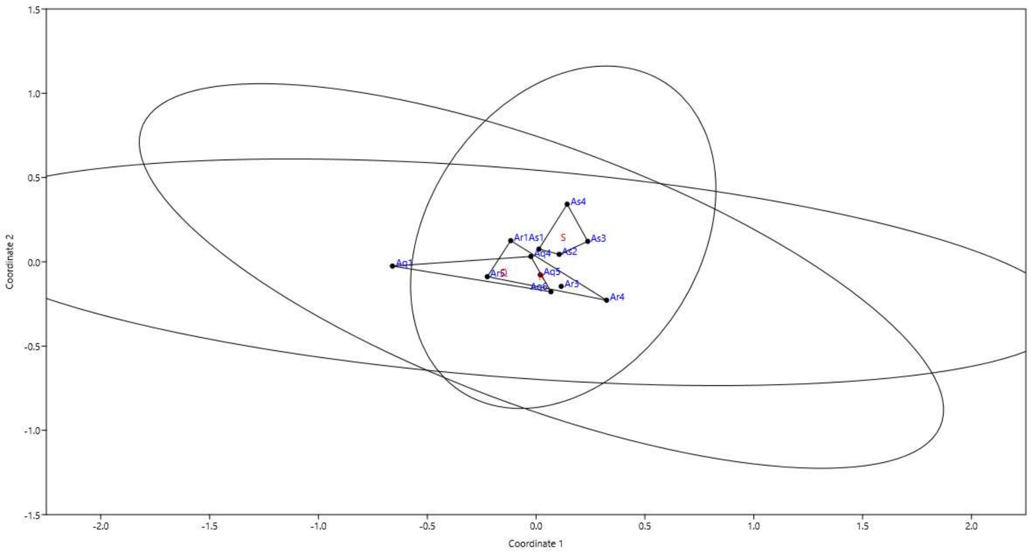

Figure 6. Scatter plot for non-metric multidimensional scaling (NMDS) for composition and abundance of freshwater macroinvertebrates taxa in the 12 micro-basins in three sub-basins (Aq, Ar, As) in Los Tuxtlas, Veracruz, Mexico.

The values of the physicochemical parameters (Table 2) are in good-to-acceptable ranges for aquatic life. The pH of all sites remained within normal ranges for river systems and suitable for aquatic life, with values between 7.35 and 8.82. Higher pH can be related to carbonated waters and photosynthetic activity in the case of the Rio Seco (Aq1), which originates from a volcanic lake (Laguna Encantada). Dissolved oxygen varied seasonally with temperature. The lowest value corresponds to a slow-flowing river in an agricultural and livestock environment (Ar5), with a predominance of pools and a slow flow that is rich in fine sediments. The temperature varied between 17.16–27.78 °C in relation to the station temperatures and the ambient temperature in the previous hours or days, given the thermal inertia of the water. The conductivity was found to be between 73 and 533 μS/cm. The total dissolved solids (TDS) presented values between 37 and 267 mg/L, which corresponds to excellent quality, even for drinking water, according to the World Health Organization and Mexican Standard "Norma Mexicana NOM-127-SSA1-1994".

4. Discussion

It was found that the composition and taxonomic abundance of AqMI at the phylum (seven phylum) and class levels in the Los Tuxtlas micro-basins is similar to that reported in other works on the Neotropical river system. In Los Tuxtlas, a high richness of taxa (S

= 67) of AqMI was obtained at the family level (or at the lowest taxon level that could be determined), similar to or higher than that found in several national basins [24] and the state of Veracruz by several authors in the Atoyac River [28,29], in the La Antigua River [30], and in the Actopan River [23,26]. In this work, the Insecta class dominates and, at the order level, Ephemeroptera (42.03%), Trichoptera (19.11%), Diptera (15.43%), and Coleoptera (3.98%) dominate. By families, only four families exceeded 10% relative abundance, and together they totaled 61.02%. These families were Baetidae (*n* = 3194, 23.84%), Hydroptilidae (*n* = 1820, 13.58%), Leptohyphidae (*n* = 1746, 13.03%), Chironomidae (*n* = 1416, 10.57%), and Elmidae (3.23%). At the family level, in Chinchiná, Colombia, Diptera (Chironomidae, Simuliidae), Ephemeroptera (Baetidae), Trichoptera (Hidrobiosidae), and Coleoptera (Elmidae, Scirtidae) dominated [48]. In the upper basin of the La Antigua River, Veracruz, the order Diptera (Simuliidae) dominated. Other dominant families, with differences depending on the sampling sites were Ephemeroptera, Trichoptera, Hemiptera, and Coleoptera [30]. In this study, Diptera was also among the dominant ones with two families: Chironomidae, which was abundant in somewhat disturbed sites, and Simuliidae, which was quite abundant in clean-to-regular-quality streams. In the order Coleoptera, Elmidae stood out in larval and adult forms in current areas with good water quality. In the order Ephemeroptera, at the family level, the Baetidae family especially dominated in some microhabitats of water of good to fair quality, as reported in the Balsas River basin [47]. Invasive species of Gastropoda, of the Thiaridae family (*Melanoides, Tarebia*), and Bivalva (*Corbicula fluminea*) occurred in some sites in large quantities, as dominant in some seasons, stretches, and micro-basins, and represent a threat to diversity and conservation. At least two rivers are invaded by *Corbicula*, one in the Aq, San Andrés sub-basin (Aq1, Seco River) and the other in coastal sub-basin As, with extreme abundance in some sections in As3. At least 9 of the 12 rivers—the 4 rivers of the coastal sub-basin (As), the 3 rivers in the San Andrés (Aq), and 2 in the Catemaco (Ar)—are invaded by invasive snails of the Thiaridae family, especially critical in some reaches in the Máquina River (As3) and the Seco River (Aq1). Residents report that they are associated with the disappearance of native snails, "tornillo" or "jutes", that were consumed (*Pachychilus*). The order of the Strahler [37] stream section did not correlate with diversity, but it did limit the number of organisms and, therefore, sample size. The number of total AqMI collected in the only Order 1 river was N = 442 (Ar4, El Porvenir River), but it no longer appears to be a significant factor in higher orders. This river had the lowest flow and width and the lowest N, but had the fourth highest diversity index H', because although it also occupied the tenth place in the number of species, it had a relatively low dominance of species. There were no significant differences in the general relative abundance in the four seasons of the yearly cycle, but variations in absolute quantities were observed by basins and which require more sampling in different years and more detail in the analysis. Individually, the basin with the least richness was Río Seco Aq1, with 24 species, followed by the La Victoria River (Ar5), with 26 species, a rather disturbed and slow riverbed, and with an abundance of pools much of the year, but with seasonal increases and the presence of small sections of current (Ar5). The two transects with the highest richness corresponded to coastal rivers (As2), with 38 species and its mouth in the estuary of the Sontecomapan Lagoon, and As3, with 36 species and its mouth directly into the sea. Two rivers follow with 35 families each from the internal sub-basin, Aq4 (the Xoteapan River) and Aq6 (the Tepango River) of San Andrés, and rivers from the Aq basins continue to alternate with those from the Ar and As basins. A wealth of families greater than 30 was observed in relatively undisturbed rivers that have not passed through and received discharges from urban populations. There is a certain influence of enrichment in species and influence on dominance and diversity indicators due to the presence of highly abundant coastal species and invasive species; this may be reflected in the results of the ANOSIM and NMDS analysis in which a lower similarity of the As basin group and a greater similarity between Aq and Ar were found (Figure 6).

The rich hydrography in Los Tuxtlas shows a high richness and diversity of AqMI, until now little known, except for crustaceans and mollusks, and is comparable or superior to that recorded for other basins in Mexico [22–31,46,47,49,50], Central and South America [14–20,51–53], and the Great Antilles of the Caribbean [54]. With 21 orders and 67 families, this work surpasses those obtained in works reviewed in other basins of Veracruz and in other states of Mexico. This highlights regional diversity and the importance of inventorying and monitoring AqMI in their river systems to conserve them as bioindicators of environmental quality. In terms of abundance, at the phylum level are insects, highlighting the order Ephemeroptera, followed by Diptera, Trichoptera, and Coleoptera. At the phylum, class, and order levels, AqMI recorded in this study are already recorded in many regions or basins of Mexico, the Gulf-Caribbean Region, and Central and South America. Some taxa found had not been recorded in previous reviews or compilations of aquatic macroinvertebrates for Mexico [43], such as Pseudothelphusidae and Atyidae. New taxa are reported for the region in the river systems Plecoptera, Hydrachnida, and Ostracoda, at the order level. At the family level, new taxa are reported for the order Plecoptera, with three families, Hydrachnidae and Ostracoda at the order level, and several families in the order Ephemeroptera, for which only the family Leptophlebiidae was recorded in Los Tuxtlas [7]. With this work, six more families are added: Baetidae, Caenidae, Ephemerellidae, Heptageniidae, Leptohyphidae, and Oligoneuriidae. In relation to the same work [7], three families are recorded for the first time for the order Diptera region—Ceratopogonidae, Empididae, and Pedicidae—in the micro-basins of this work, and another three families in other impacted sections: Ephydridae, Psychodidae, and Stratiomidae. It is important to continue and expand the inventory and monitoring of rivers and bodies of water, as some sections and basins continue to be impacted by pollution from residual discharges from growing urban areas and by agrochemicals and the use of different poisons and agrochemicals for illegal fishing of "acocil" and "acamaya" shrimps in coastal rivers, with an effect on other aquatic macroinvertebrates. In relation to beta diversity, at the regional level, there are three sub-basins, two of which are sub-basins of the Papaloapan River and present similar diversity. The rivers of the coastal basin are similar to the previous ones, but they add coastal groups or species. The two studied coastal rivers that flow indirectly through the Sontecomapan estuarine lagoon lack some groups of littoral species, but mainly maintain crustaceans from low coastal areas not present in the continental interior basins. The Lake Catemaco basin is a tributary of the San Andrés basin. The Eyipantla Waterfall has been a natural factor of isolation and endemism for species of fish and some aquatic macroinvertebrates in these two basins, such as crustaceans and mollusks. Lake Catemaco connects or separates, but does not appreciably differentiate, the rivers of the two basins, the tributaries of the lake from those of the San Andrés basin. The differences are likely due to the characteristics of each channel. The replacement of species is not appreciated at the family level, but it does occur at the genus or species level in the cases of some Pseudothelphusid crabs: *Tehuana*, with different species distributed in the three basins [5,55], as well as *Hyalella*. Basins with coastal currents are especially enriched in coastal crustaceans and mollusks: Decapoda (*Potimirim, Macrobrachium*, and Atyidae (*Atys scabra*)) and mollusks such as Neritina, are present only in the rivers of the coastal sub-basin (As). The invasive Asian clam *Corbicula* occurs in high abundance in the Máquina River (As3). It has also been detected as an invasive species in some interior micro-basins (the Laguna Encantada basin). *Macrobrachium* and *Procambarus* present the replacement of some species, with different species on the continental slope from those present in the coastal micro-basins, but at the family level, they can be present in nearly all basins. A more detailed study of sampling and identification is required, as well as a literature review to determine the presence and regional distribution and more detailed taxonomic determinations. In the inland basins, there is also a high diversity of AqMI, with the presence of some endemism of mollusks and crustaceans (*Pomacea catemacensis*, *Macrobrachium tuxtlensis*, and several species of Pseudothelphusid crabs). There are rivers with great abundance in some sections or microhabitats of invasive species of mollusks: an Asian clam *Corbicula fluminea*, and trumpet snails of the F. Thiaridae (*Melanoides, Tarebia*), which especially affect some coastal rivers, specifically the Maquina River.

The inhabitants remember its impact on the almost disappearance of native mollusks in the Coxcoapan River (*Pachychilus*). The rivers of the Lake Catemaco basin appear less affected presently by these invasive species. Knowledge of current abundance and distribution of AqMI is essential for a baseline to develop management proposals, and for the restoration and monitoring of river systems in the region, being an excellent group of bioindicators. The registration of new families or taxa for this AqMI community of Los Tuxtlas is highly probable. These results represent a baseline and a significant contribution to fill a gap in information on river ecosystems and associated aquatic fauna in the region, so studied in other aspects and groups, and provide elements for their conservation, management, and restoration.

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Appendix A

Table A1. Macroinvertebrate taxa in 12 sampling sites in the Los Tuxtlas region, Veracruz, Mexico. Based on Pineda-López et al. (2014) [43].

TAXA*	Aq1	Aq4	Aq5	Aq6	Ar1	Ar3	Ar4	Ar5	As1	As2	As3	As4
Annelida		✓	✓	✓	✓	✓			✓	✓	✓	✓
Clitellata		✓	✓	✓	✓	✓			✓	✓	✓	✓
Arhynchobdellida		✓	✓	✓	✓	✓			✓	✓	✓	✓
Hirudinidae		✓	✓	✓	✓	✓			✓	✓	✓	✓
Chelicerata				✓						✓	✓	✓
Arachnida				✓						✓	✓	✓
Parasitengona				✓						✓	✓	✓
Hydrachnidia				✓						✓	✓	✓
Crustacea	✓	✓	✓	✓		✓	✓		✓	✓	✓	✓
Malacostraca	✓	✓	✓	✓		✓	✓		✓	✓	✓	✓
Amphipoda	✓											
Hyallelidae	✓											
Decapoda	✓	✓	✓	✓		✓	✓		✓	✓	✓	✓
Armasidae												✓
Atyidae									✓	✓	✓	✓
Cambaridae			✓			✓				✓		✓
Palaemonidae							✓			✓	✓	✓
Pseudothelphusidae	✓	✓	✓	✓		✓	✓			✓	✓	
Sesarmidae									✓			
Oligostraca	✓						✓					
Ostracoda	✓						✓					
Ostracoda	✓						✓					

Hexapoda	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Insecta	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Anisoptera	✓	✓	✓	✓	✓	✓		✓	✓	✓	✓	✓
Aeshnidae	✓	✓	✓	✓	✓			✓	✓	✓		
Coenagrionidae		✓										
Gomphidae		✓	✓	✓	✓	✓		✓	✓	✓		
Libellulidae	✓	✓	✓	✓	✓			✓	✓	✓	✓	✓
Coleoptera	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Chrysomelidae									✓			
Curculionidae									✓			
Elmidae	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Haliplidae	✓											
Hydrophilidae					✓	✓	✓			✓	✓	
Lutrochidae									✓			
Noteridae	✓				✓							
Psephenidae		✓	✓	✓		✓	✓	✓	✓			✓
Ptilodactylidae				✓	✓		✓	✓				
Scirtidae								✓	✓	✓	✓	✓
Diptera	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Ceratopogonidae		✓										
Chironomidae	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Empididae	✓	✓					✓				✓	
Muscidae		✓	✓									
Pediciidae					✓							
Simuliidae	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Tipulidae			✓	✓		✓	✓		✓			
Ephemeroptera	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Baetidae	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Caenidae				✓		✓		✓				
Ephemerellidae	✓	✓			✓	✓		✓		✓		✓
Heptageniidae		✓			✓	✓	✓		✓	✓	✓	
Leptohyphidae	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Leptophlebiidae	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Oligoneuriidae		✓	✓		✓	✓		✓	✓	✓	✓	
Hemiptera	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Belostomatidae		✓	✓	✓		✓	✓	✓	✓	✓	✓	
Gerridae			✓	✓		✓						
Naucoridae	✓	✓	✓	✓	✓	✓			✓	✓	✓	✓
Pleidae			✓		✓							
Velliidae	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Lepidoptera		✓			✓							
Crambidae		✓										
Pyralidae					✓							
Megaloptera	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	
Corydalidae	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	
Plecoptera	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	
Leuctridae	✓											
Perlidae	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	
Perlodidae						✓						
Trichoptera	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Calamoceratidae		✓		✓				✓		✓		✓
Glossosomatidae				✓		✓	✓			✓		
Helicopsychidae			✓									
Hydrobiosidae		✓	✓	✓	✓	✓	✓		✓	✓	✓	✓
Hydroptilidae		✓	✓	✓	✓	✓	✓		✓	✓	✓	✓
Hydropsychidae	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Leptoceridae		✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓

Philopotamidae		✓	✓	✓	✓	✓	✓		✓	✓	✓	✓
Polycentropodidae			✓	✓		✓	✓			✓	✓	✓
Zygoptera	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Calopterygidae		✓	✓	✓	✓	✓		✓	✓	✓	✓	✓
Coenagrionidae	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Mollusca	✓	✓	✓	✓	✓			✓	✓	✓	✓	✓
Bivalvia				✓							✓	
Venerida				✓							✓	
Corbiculidae				✓							✓	
Gastropoda	✓	✓	✓		✓			✓	✓	✓	✓	✓
Caenogastropoda	✓	✓	✓		✓			✓	✓	✓	✓	✓
Pachychilidae					✓						✓	
Thiaridae	✓	✓	✓		✓			✓	✓	✓	✓	✓
Cycloneritida											✓	✓
Neritidae											✓	✓
Hygrophila		✓									✓	
Physidae		✓									✓	
Planorbidae											✓	
Nematoda				✓						✓	✓	
Nematoda				✓						✓	✓	
Nematoda				✓						✓	✓	
Nematoda				✓						✓	✓	
Plathyhelmintha	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Rhabditophora	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Tricladida	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Planariidae	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓

*In bold: taxa of (sub) Phylum, (sub) Class, (Sub) order. In plain text: Taxa at Family level or higher for which it was not possible to reach a lower level of their taxonomic determination.

Appendix B

Photo gallery of macroinvertebrates from Los Tuxtlas.

(**a**) (**b**) (**c**)

Figure A1. Class Insecta, order Ephemeroptera: (**a**) Leptophlebiidae; (**b**) Leptohyphidae; (**c**) Baetidae.

(**a**) (**b**) (**c**)

Figure A2. Order Trichoptera: (**a**) Hydropsychidae; (**b**) Hydrobiosidae; (**c**) Hydroptilidae.

(**a**) (**b**) (**c**)

Figure A3. (**a**) Order Trichoptera: Calamoceratidae; (**b**) order Plecoptera: Perlidae; (**c**) order Coleoptera: Ptilodactylidae**.**

(**a**) (**b**) (**c**)

Figure A4. (**a**) Order Coleoptera, Psephenidae; (**b**) Elmidae: larvae and adults; (**c**) Scirtidae**.**

(**a**) (**b**) (**c**)

Figure A5. Order Diptera: (**a**) Simuliidae; (**b**) Tipulidae (Limoniidae); (**c**) Chironomidae.

(**a**) (**b**)

Figure A6. Order Diptera: (**a**) Stratiomidae; (**b**) Pediciidae.

(**a**) (**b**) (**c**)

Figure A7. (**a**) Order Zygoptera: Coenagrionidae; (**b**) order Anisoptera: Libellulidae; (**c**) Gomphidae.

(**a**) (**b**) (**c**)

Figure A8. Order Hemiptera: (**a**) Naucoridae; (**b**) Gerridae; (**c**) Velliidae.

(**a**) (**b**) (**c**)

Figure A9. (**a**) Order Hemiptera: Belostomidae; (**b**) order Megaloptera: Corydalidae; (**c**) Mollusca: Physidae.

(**a**) (**b**) (**c**)

Figure A10. (a) Class Crustacea: Pseudothelphusidae G° Tehuana; (b) order Decapoda: Palaemonidae; (c) order Decapoda: Cambaridae.

(**a**) (**b**)

Figure A11. (**a**) Sublcass Hirudinea; (**b**) phylum Mollusca: Neritidae.

Appendix C

Images of study sites.

(**a**) (**b**)

Figure A12. (**a**) Cuetzalapan River (Ar1); (**b**) El Porvenir River (Ar4).

(**a**) (**b**)

Figure A13. (**a**,**b**) La Victoria River (Ar5).

(**a**)

(**b**)

Figure A14. (**a**) River Coxcoapan (As1), (**b**) Tepango Rriver (Aq6).

(**a**)

(**b**)

Figure A15. (**a**,**b**) Maquina River (As3).

(**a**)

(**b**)

Figure A16. (**a**,**b**) La Palma River (As2).

(**a**)

(**b**)

Figure A17. (**a**,**b**) San Martin River (As4).

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