UNIVERSIDAD SAN FRANCISCO DE QUITO USFQ

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A preliminary view of the biogeography, phylogeography and ecology of freshwater shrimps (Atyidae) from Isla San Cristóbal, Galápagos Archipelago

Proyecto de Investigación

.

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RESUMEN

Las islas Galápagos representan a nivel mundial un excelente escenario para el estudio de los procesos evolutivos y ecológicos debido a su aislamiento geográfico. La fauna de los ecosistemas de agua dulce en el archipiélago ha sido poco estudiada pese a sus singulares procesos de colonización y evolución influenciada por las características geológicas de las islas. Bajo este contexto, se desconoce sobre los camarones de agua dulce pertenecientes a la familia Atyidae, quienes tienen funciones ecológicas claves en los ecosistemas lóticos. Esta investigación busca proporcionar una perspectiva evolutiva, biogeográfica, taxonómica y ecológica de las especies de camarón existentes en la Islas San Cristóbal. Se plantea la hipótesis de que las islas y las zonas continentales cercanas al archipiélago podrían ser los lugares que proporcionan las especies ancestrales que colonizaron el área. Para lograr estos objetivos, se muestreó 6 arroyos en la isla San Cristóbal, y se identificó los taxa según la morfología y ADN. Los resultados morfológicos sostienen la existencia de tres géneros (*Australatya*, *Atya* and *Archeatya*) mientras que la genética resulta en solo dos clados presentes en la isla. Los riachuelos de la Isla San Cristóbal tienen una química característica debido a su origen volcánico y estas condiciones distintivas podrían haber influenciado en la evolución de nuevos taxones. Los arroyos de San Cristóbal se caracterizaron por un bajo caudal y altos valores de turbidez. Todos los arroyos mostraron una gran riqueza química, así como grandes variaciones entre sus componentes, causadas principalmente por las características volcánicas de la isla. Las variables ambientales más importantes para la abundancia de Atyidae fueron caudal, turbidez, rocas de sustrato de tamaño mediano y materia orgánica particulada fina, ya que estas pueden afectar algunas funciones biológicas de los camarones. Las condiciones adecuadas para los camarones Atyidae también incluyen la presencia de calcio, magnesio, potasio y sodio. En el aspecto biogeográfico, la información morfológica y genética es aún escasa, y se necesitan estudios más profundos para comprender los procesos de colonización y evolución en la isla, sin embargo está claro que hubo dos colonizaciones independientes de Atyidae. La química distintiva de los arroyos de San Cristóbal pudo resultar en la evolución de nuevos taxones.

Palabras clave: camarones, arroyos, islas, Atyidae, filogeografía, colonización, evolución, *Australatya, Atya, Archeatya*.

ABSTRACT

The Galápagos Islands are worldwide considered as a model system for the study of evolutionary and ecological processes because of their geographical isolation and recent Island formation processes. The fauna of freshwater ecosystems in the archipelago has been little studied despite its unique processes of colonization and evolution influenced by the geological characteristics of the islands. In this context, the information we currently have about freshwater shrimps of Atyidae family is even more limited. This research seeks to provide an evolutionary, biogeographic, taxonomic, and ecological perspective of existing shrimp species at San Cristóbal Island. It was hypothesized that the islands and the continental zones near the archipelago could be the places that provide the ancestral species that colonized the area. To achieve these objectives, 6 streams in San Cristóbal Island were sampled; shrimps were identified according to morphology and DNA. Morphological results support the existence of tree genera (*Australatya*, *Atya* and *Archeatya)*, while genetics reports only two taxonomic clades present on the island. San Cristóbal´s streams were characterized by low dynamic flow and high turbidity values. All the streams showed a large chemical richness, as well as great variations among their components, mainly caused by the volcanic feature of the island. The most important environmental variables for Atyidae abundance were flow, turbidity, cobble and fine particulate organic matter percentages as they can affect some biological functions of shrimps. Suitable conditions for Atyidae shrimps also include calcium, magnesium, potassium and sodium presence. In the biogeographical aspect, morphological and genetic information is still scarce, and deeper studies are needed to understand the processes of colonization and evolution on the island; however it is clear that there have been two independent colonizations of Atyidae. The distinctive chemistry of San Cristóbal´s streams could result on the evolution of new taxa.

Key words: shrimps, streams, islands, Atyidae, phylogeography, colonization, evolution, Australatya, Atya, Archeatya.

TABLE OF CONTENT

LIST OF TABLES AND APPENDICES

LIST OF FIGURES

INTRODUCTION

The Galápagos Archipelago is a group of volcanic islands that are considered a natural biological laboratory to study life history evolution. The archipelago is a chain of volcanic hotspot islands (Geist et al., 2014) located in the Pacific Ocean, at 1000 km from the continent and belong to Ecuador. Nine large islands, six small islands and 40 islets formed the Galápagos. The islands are also located on the Nazca Plate, moving towards the continent at an annual speed of 7 cm (Hey et al., 1977). As the islands originate due to a submarine hot spot of continuous eruptions, the emerged islands have an age of up to 6 million years, while the submerged islands can exceed 11 millions of years (Lanteri, 2001). Given the movement of the Nasca plate, and the position of the submarine hot spot, the oldest islands are the ones closer to the continent and the youngest are the farthest (Fig. 1); hence, many Islands on the farthest sites, like Fernandina and Isabela, have high and very recent volcanic activity (d'Ozouville1 et al.,)

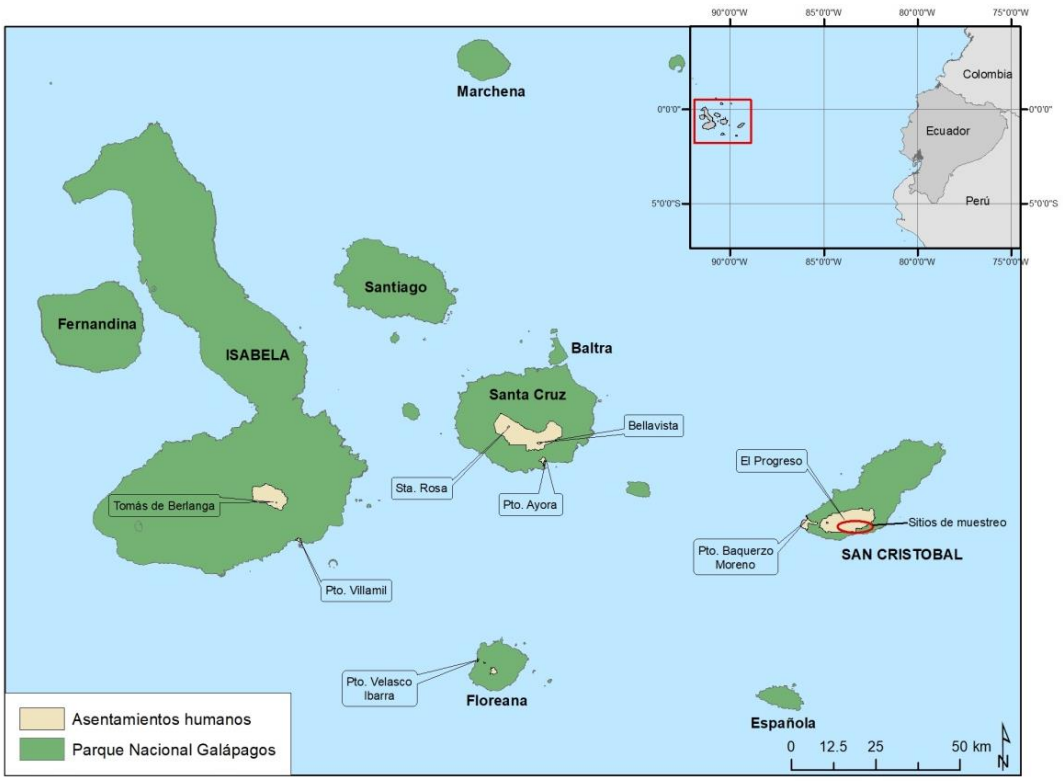

Figure 1. Map of Galápagos Archipelago, with its main nine large islands, areas of human settlements (cream) and National Park (green).

The remarkable endemism of Galápagos is caused by its geographic history and separation from the continent; as a consequence, colonization and dispersion of organisms result in high levels of speciation and adaptive radiation based on genetic drift (Grant & Grant, 2002; Petren et al., 2005). Due to these characteristics, many Galápagos endemic species have evolved following the allopatric model of speciation: First, an ancestral species colonizes an island, then this new established population evolves by natural selection, adapting to different current conditions. As a result of this new population being geographically isolated from the ancestral taxa, it would become a different species (Grant & Grant, 2002). In this process, the inherent and intrinsic dispersal capacity of each taxon is

also an essential factor, thus groups with limited dispersion or reduced migration will be the most prone to evidence speciation (Incagnone et al., 2015).

As freshwater bodies are sparse in the Galápagos, in underground and superficial forms, they stand as potential habitats for colonization and evolution. The freshwater accumulates in: coastal lagoons, freshwater projections to the sea, aquifer groundwater, pools, lakes (perennial or temporary), streams, and groundwater of the upper part (last two restricted only to San Cristóbal). The streams have exceptionally weak flows and the rivers run sporadically as they depend on climate and underground storage (d'Ozouville, 2007b).

The Archipelago, similar to other islands on formation (Craig, 2003), started with no water, but they have accumulated water on ground depressions, forming small ponds and lakes. Moreover, river and streams started to form in the slopes and natural creeks in mountains, through soil erosion triggered by precipitation events. Hence, only the oldest islands that have mountains have formed streams while the newest ones have not formed any yet (Craig, 2003). The water that enters the hydrological cycle varies from year to year, as these incomes include rain and ¨*garúa*¨, their abundance is affected in exceptional years of very heavy rains (El Niño) and years of drought (La Niña) (d'Ozouville, 2007b). Due to the low altitude of Galápagos Islands, they receive much less rainfall than their tropical counterparts as Hawaii, also land evaporation is very high (d' Ozouville, et al., 2008). The outputs of the hydrological cycle are: (i) evaporation, quite high in winter and low in the ¨*garúa*¨ season on the islands; (ii) infiltration, which is very rapid due to the high permeability of the soil; (iii) runoff, both non-perennial, sustained by very heavy rains, and permanent ones, supplied by underground water sources (d'Ozouville, 2007b). The quantity and type of rainfall play a major role in generating runoff and interlinking the

individual small freshwater ecosystems: feeding swamps and ponds, semi-perennial streams, and recharging the basal aquifer (d' Ozouville, et al., 2008).

Under this geographical and evolutionary context, freshwater ecosystems and their diversity have not been studied as much as their terrestrial and marine counterparts. Within the fauna of freshwater ecosystems, shrimps have key ecological roles, either as filters or as predators, depending on the taxonomical family (Crowl et al., 2001; Mantel & Dudgeon, 2004). The families with the most freshwater shrimp species are: Atyidae (filters) and Palaemonidae (predators), although the last one can also include amphidrome and marine species (Christodoulou, et al., 2016) with nauplius (larvae) that could feed on detritus. According to previous studies, four reported species of freshwater shrimps in the Galápagos belonging to the two families already mentioned: *Macrobrachium americanum* in Santa Cruz and *Macrobrachium hancocki* collected at San Cristóbal (Palaemonidae) (Wicksten, 1991). Two species are reported for Atyidae in the Galápagos: *Archeatya chacei* found in the Academy Bay at Santa Cruz Island (Wicksten, 1991) and *Typhlatya galapagensis* at Santa Cruz and Isabela islands (Botello, et al., 2013).

Atyidae is the most diverse freshwater shrimp family, with 42 extant genera. Its main morphological characteristic is a pair of chelipeds with setae and used for passively filtration and scrape detritus (Fryer, 1977; De Grave et al., 2008). They mostly inhabit tropical fast flowing streams and rivers (Han & Klotz, 2015). Regarding ecological interactions and trophic relationships, atyids play an important role as components of stream food webs in tropical freshwater habitats. For instance, since all atyids are detritivores and shredders, extreme variations in their population affect the overall level of litter biomass and inorganic sedimentation with the substrate (Page et al., 2008). Although

this family is primarily adapted to freshwater, some species such as those of the genus *Atya* and similar, have marine or estuarine larvae, making it possible for them to inhabit the Pacific islands. Marine long distances dispersions have been suggested for some atyid taxa (Page et al., 2008), allowing the colonization of the eastern Pacific islands by the Sub-family Atyinae of America (*Archeatya* in Galápagos and Cocos Islands) and non-Atyinae (*Typhlatya* in Galápagos) (Page et al., 2008).

SPECIFIC OBJECTIVES

As specific objectives, it is proposed to:

1) Report the existing shrimps´ species in freshwater ecosystems of San Cristóbal Island, and compare this information with previous studies and discover the possible biogeographic origins of the taxon. It is hypothesized that the islands and continental zones near the archipelago could be the places that provide the ancestral species that colonized the area.
2) Characterize the main environmental attributes of stream habitats in San Cristóbal Island
3) Assess potential relationships between environmental characteristics and composition and structure of shrimp communities.

METHODOLOGY

STUDY AREA

The research was carried out on the island of San Cristóbal, Galápagos, Ecuador (Fig. 1, Table 1). This is a basaltic island located on the Nazca´ oceanic plate, at the eastern end of the Galápagos Archipelago. Its total surface reaches to 558 km2 (Pyret et al., 2012), and it is one of the oldest Island of the Archipelago, as it first became emergent ~ 2.35 ±0.03 m.y. ago (Gei47st et al., 2014).

Figure 2. Map of study sites at San Cristóbal Island, including the seven sampling sites in. The striped area shows the agricultural zone, the smooth section corresponds to National Park areas, and orange lines represents roads.

The island has a total of 17 permanent streams, where the amount of water is always variable depending on rainfall (Salazar Espinoza, 2017). The streams were originated by the prolonged erosion of the lands caused by the rains, located at the highlands without ocean influence.

The study was focused at the following sampling sites: La Toma Cerro Gato, Milton Aguas, Pachai, Jatun Sacha, Gutiérrez, and Encañada Crecida (Fig. 2, Table 1). All sites are small freshwater streams with widths that average from 0,80 m to 1,90 m, and the maximum average deep reach 0,75 m. Streams´ substrate was mainly composed by rocks (with a variable size between < 1cm and ~ 15 cm) and abundant leaf litter coming from the riparian vegetation. The composition of the riparian vegetation includes 60% of introduced plants and 40% of native plants, with introduced species such as bamboo, blackberry and avocado trees, and few native species of fern, manzanillo, *Miconia robinsoniana* (cacaotillo), and *Psychotria rufipes* (cafetillo).

Samples were collected during two field trips; on February 1st to 9th and on November 9th to 17th in 2018.

Table 1. Geographic information of the study sites

Obs.	***Site***	***Latitude***	***Longitude***	***Elevation (m.a.s.l.)***
1	*La Toma Cerro Gato1*	0°55'22.69"S	89°28'31.66"O	138
2	*La Toma Cerro Gato2*	0°55'22.97"S	89°28'31.59"O	180
3	*Milton Aguas*	0°55'36.95"S	89°29'12.75"O	190
4	*Pachai*	0°54'41.31"S	89°27'34.81"O	202
5	*Jatun Sacha*	0°55'29.85"S	89°29'57.63"O	175
6	*Gutiérrez*	0°55'42.57"S	89°30'41.08" O	179
7	*Encañada Crecida*	0°55'25.48"S	89°28'13.75"O	172

Specimen sampling

This study is part of the project ¨Diversidad y Hábitats de los Ecosistemas de agua dulce de las Galápagos¨ corresponding to the contrato marco MAE-DNB-CM-2016-0041-M-0003 and No. PC- 45 – 18. The collections and physical chemical information was obtained by other researchers: Andrea Encalada, Andrea Tapia and Patricia Cárdenas.

At each habitat, shrimps were collected using a Surber sampler, or D-net as necessary and an equal sampled effort was applied in each stream (Fig. 2). Samples were obtained during day time. The specimens were clean and separated at the Galápagos Science Center and then preserved in alcohol 99%. For further identification species were transported to the Laboratorio de Ecología Acuática, at Universidad San Francisco de Quito (LEA – USFQ) and will be returned to Galápagos when all the analyses are performed.

Figure 3. Sampling picture at Milton Aguas stream, February 9th 2018.

Morphological identification of samples

Shrimp samples were identified to the lowest taxonomic level (genus, species or morphospecies), using the taxonomic keys by Holthuis (1993). The main traits for identification were the pterygostomian margin of carapace, third maxiliped, and chelae.

Genetic analysis

In order to assess the phylogenetic and biogeographic relationships of the shrimp species from the Galápagos Islands, DNA was obtained from the mitochondrial gene Cytochrome oxidase subunit 1 (COI). DNA extraction was done following the Guanidine Thiocynate protocol (see Appendix 8). To achieve standardized DNA concentration of all samples, DNA quantification of each sample was done using Thermo Scientific™ NanoDrop™ 2000, then dilutions of the sample with TE were made, until reaching a concentration of 5 ng / µL. PCR concentrations for all cases samples were Buffer 10 x, MgCl2 50mM, dNTP's 10mM, Primer forward (JgLCO1490) 10pM, Primer reverse (JgHCO 2198) 10 pM, Taq polymerase 5 u / µL and 0.5µL of DNA, with a final volume of 25 µL. The amplification conditions followed were proposed by Folmer et al. (1994): 1 cycle from 94 ° C to 3 minutes; 35 seconds at 42 ° C, 1 minute at 72 ° C, 30 seconds at 93 ° C, 35 seconds at 42 ° C, all of them for 35 cycles, and finally 1 cycle for 1 minute at 72 ° C. Single PCR products were visualized in 2% agarose gel, and unincorporated primers and dNTPs were removed from PCR products with illustra ExoStar enzymes (GE Healthcare Life Sciences). Laboratory phase was done in the Laboratorio de Biología Evolutiva at Universidad San Francisco de Quito (LBE – USFQ). Sequencing was conducted at

Macrogen Inc. (South Korea). Chromatographs were aligned and manually edited using Geneious 11.1.5 (created by Biomatters and available from http://www.geneious.com).

The resulting sequences were aligned using MAFTT v. 7 (Katoh et al., 2002), with the strategy Q-INS-i. Possible errors of base calls and gaps were examined with Mesquite 3.51 (Maddison & Maddison, 2018) and verified with chromatograms in Geneious 11.1.5. In order to obtain the best fit evolutionary model for the aligned sequences, I used IQ-TREE v1.6.8 (Lam Tung Nguyen et al., 2015). Maximum likelihood method in the IQ-TREE program v1.6.8 was used to infer a phylogenetic tree, with 10000 ultrafast bootstrap approximations (Bui Quang Minh et al., 2013).

The resulting COI sequences from Galápagos shrimps´ samples were included in the phylogenetic analysis, as well as its closest resulting taxa of ¨BLAST Sequence Analysis Tool¨ (Madden, 2002) and 23 more sequences corresponding to Atyidae genera available (Page et al., 2008) (GenBank accession numbers are detailed in Table 2). Finally, genetic distances between clades were calculated using the IQ-TREE evolutionary model.

Characterization of Freshwater Habitat

In order to characterize each sampling location, the physical and chemical variables of each stream were measured. For physical factors: width was quantified with a measuring tape, for flow and depth, we used the velocity meter OTT MFpro. Temperature was measured with the YSI ProDSS probe. Substrate size and composition for benthos was determined by direct observation, sediments in suspension were also measured as flowing: Fine particulate organic matter (FPOM), detritus particles of less than 1mm were collected as Appendix 9. Upon reaching the laboratory, the filters were placed in a freezer at <10 ° C,

then left for 48 hours in an oven at 60 ° C with the aluminum envelope opened, thus obtaining the dry weight. The filters were then placed in pre-weighed crucibles and burned for 5 hours in a muffle at 550 ° C, obtaining the weight of the inorganic material. Finally, this was subtracted from dry weight, obtaining the ash free dry mass (AFDM). Coarse particulate organic matter (CPOM) (more than 1mm), was collected with surber stream bottom sampler across the streams. The collected samples were stored in Ziploc© bags, labeled, and transferred to the laboratory where each was placed in a labeled aluminum tray and dried at environmental temperature for two days, then left for 48 hours in an oven at 60 ° C, to obtain the dry weight. Next, a subsample was extracted and placed in previously weighted crucibles and burned for 5 hours in a muffle at 550 ° C, to get inorganic material´s weight, which then was used as a conversion factor to obtain the ash free dry mass (AFDM). Also index of riparian quality (QBR) and fluvial habitat index (IHF) were calculated based on visual observations.

The chemical characteristics: pH, percentage of oxygen, turbidity, and dissolved oxygen (mg / L), were measured *in situ* using the YSI ProDSS probe. The analysis of trace metals and major elements for chemical information was performed on samples of filtered and acidified water, using an atomic emission spectrometer with inductive plasma coupling (ICP-OES) brand Thermo Scientific, model iCAP 7400, in the Laboratorio de Ingeniería Ambiental at Universidad San Francisco de Quito (LIA - USFQ). Measurements of the following chemical elements were taken: chloride, sulfates, Al, Ba, Cu, Mn, V, Zn, Ca, Si, Mg, K, Na and Fe. Due to logistics complications no physicochemical data were taken from Encañada Crecida.

Statistical Analyses

To understand if there were differences between environmental characteristics of the streams, physicochemical data were statistically analyzed with an ANOVA and a Tukey´s with JMP® data analysis software. A principal component analysis (PCA) was performed in Primer 6 BETA to analyze the abiotic characteristics.

A Cluster and an nMDS were realized to compare the diversity of the biological structure between the sites. To explore the relationship between environmental characteristics and shrimp diversity correlations between all the physicochemical variables and the abundance of the found genera were performed, as well as a BEST analysis using Primer 6 BETA.

RESULTS

Morphological identification of samples

Using morphological characteristics, tree genera of the Atyidae family were identified: *Australatya*, *Atya* and *Archeatya*, each genus with one morphospecie. *Australatya* was the most abundant genera and was present at all streams. *Archeatya* was found in low quantities at four of the seven study sites. The largest number of Atya individuals was found in La Toma Cerro Gato1 (Fig. 4). Only *Archeatya* genus has been previously reported for the Archipelago, with *A. chacei* species in the Academy Bay at Santa Cruz Island (Wicksten, 1991).

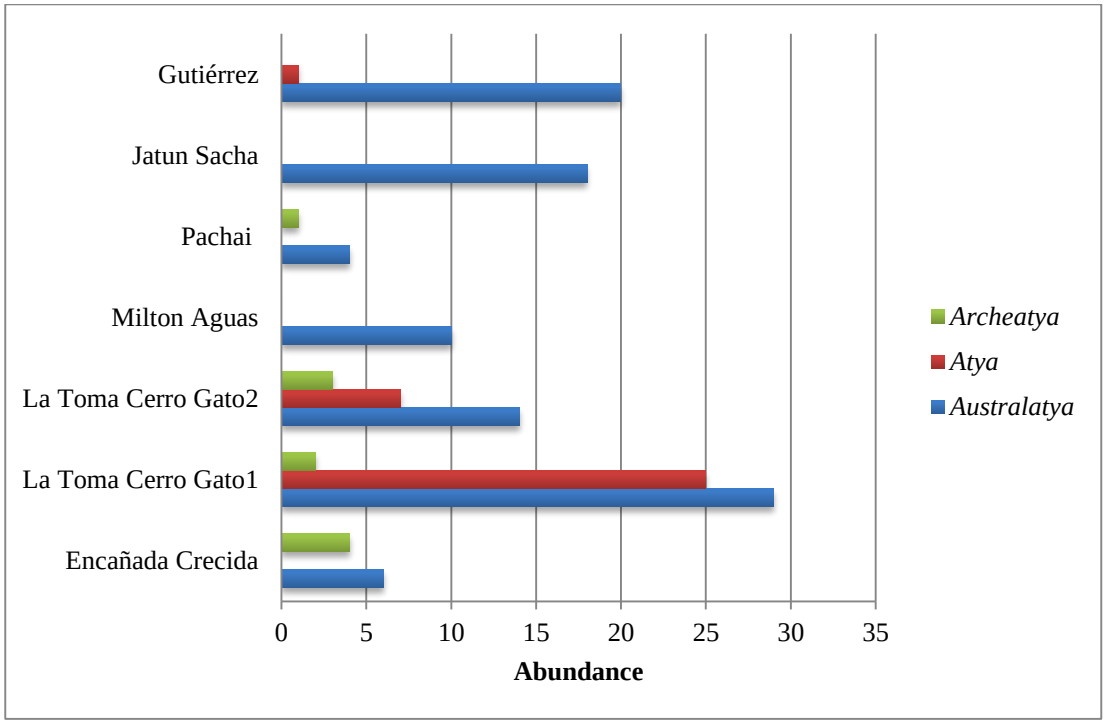

Figure 4. Abundances of the tree genera identified by morphological method founded at seven study sites: *Australatya*, *Atya* and *Archeatya*.

Genetic analysis

The genetic results differ from the morphological classification. With the genetic approach only two clades were inferred. The samples morphologically assigned to *Australatya* and *Atya* fall together in one clade (the 2 genera are not reciprocally monophyletic), while *Archeatya* individuals formed another group (Fig. 5). According to the maximum likelihood tree, the closest taxa from *Australatya –Atya* clade are *Potimirim brasiliana* (15% of genetic distance) and *Micratya cooki* (12% of genetic distance). *Atyopsis spinipes* is the nearest species to *Archeatya* genera (18% of genetic distance) (Appendix 1). These results strongly support the existence of two different genera in San Cristóbal Island different from those included in the phylogenetic tree. However, the evolutionary relationships among taxa are not completely clear since taxon sampling in Atyidae is scarce and several nodes in the phylogenetic tree are not well supported. As there are no available sequences of *Archeatya* genus yet, it is not possible to determine if the San Cristóbal´s specimens belong to a different taxa, the same happens with *Australatya*; since the available sequences of the species *A. striolata* could not be included in the analysis due to alignment problems, caused by very variable regions. Nevertheless, due to the large genetic distance between the clade *Australatya - Atya* and *Atya intermedia* (~ 20%) it is clear that they are two distinct genera.

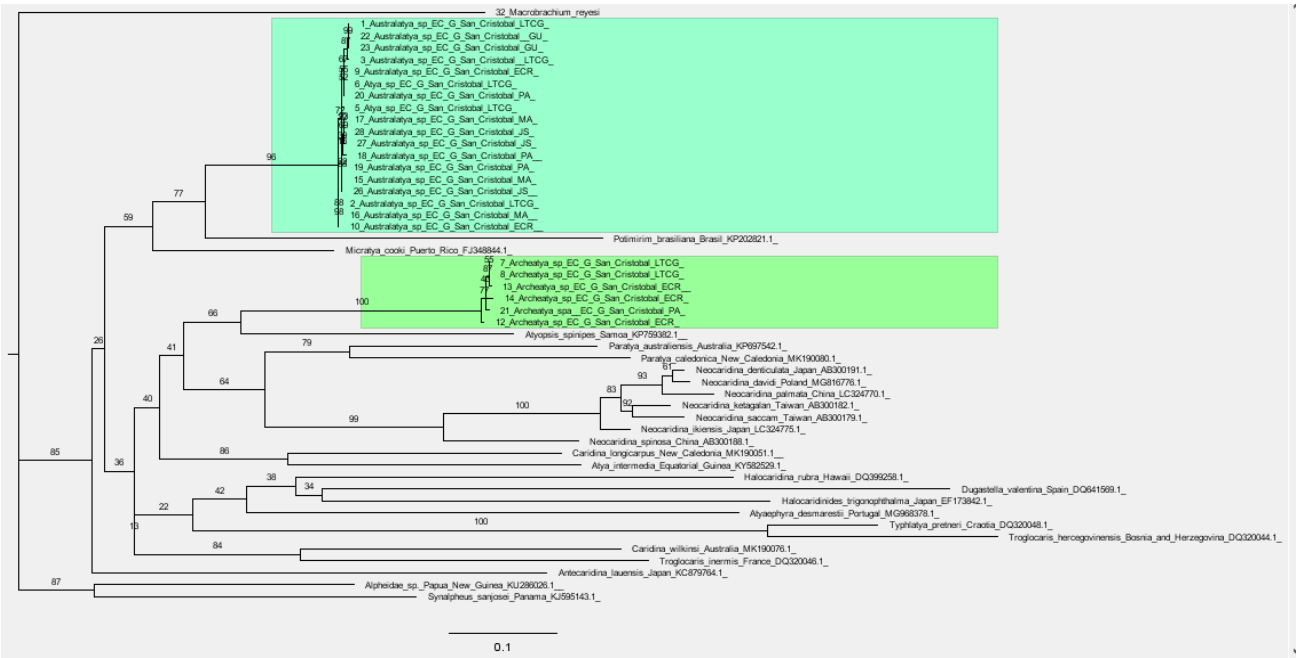

Figure 5. Maximum likelihood tree of Atyidae inferred from the mtDNA COI gene. Numbers on branches represent nodal support values, using ultrafast bootstrap approximation (Bui Quang Minh et al., 2013). EC: Ecuador, G: Galápagos, LTCG: La Toma Cerro Gato, GU: Gutiérrez, ECR: Encañada Crecida, PA: Pachai, MA: Milton Aguas, JS: Jatun Sacha.

Characterization of Freshwater Habitat

Streams at San Cristóbal Island look like a typical tropical montane stream with dynamic flows (fluctuating from < 0,004 m^3/s to a maximum average of 0, 0150 m^3/s), steep gradients and a sequence of riffle/pool habitats. The results of the IHF index in general show low alterations in the original form of the streams channels (> 80) (Pardo, et al., 2002). Since they are surrounded by dense riparian vegetation (tropical montane trees), the amount of organic matter, as litter input, that they receive is relatively large, varying from ~ 80,00 (g/m2) to 290, 00 (g/m2) (Appendix 4). The ratings of the QBR index (< 75) show strong alterations in the riparian vegetation making it of poor quality (Munné, Solà & Prat, 1998).

Although similar in appearance, most physical-chemical characteristics differed between each other streams (Figure 6, Appendix 2,4, 5 & 6), with clear differences in some parameters, like conductivity and turbidity (Appendix 2), fluctuating from 49 to ~ 100 µS/cm and from 4 to 1~ 18 UNT respectively. Streams benthos was different also between sites (Appendix 7), with substrate composition comprised on average by boulder (>20%), pebble (<40) and cobble´s (~60%) (Appendix 5). FPOM was very similar between streams, with very little variation between sites (~ 6 x 10^{-6} (g/ml)).

Due to their volcanic origin, the chemical elements where different between each stream (Appendix 7). La Toma Cerro Gato presented the highest values of chloride, aluminum, barium, calcium, sodium, ODO% and conductivity. Milton Aguas had high conductivity and was the only site with zinc presence and Pachai was the only stream with absence of aluminum. Finally, Jatun Sacha and Gutiérrez had an absence of barium and low values of chloride and sodium. The results of ANOVA and Tukey´s test support clear abiotic differences ($p - values \leq 0.05$) between sites for almost all variables (Appendix 7). CPOM, sulfates and iron concentration were not significantly different among streams.

PCA results also stays this outcome, as no assembly grouping more than 1 stream was obtained (with a 75% of similarity), getting 5 statistically different groups interpreted as distinct environmental plots. According to the results, PC 1 explains 46, 4 % of variation, PC 2 explains 21, 9% and PC 3 explains 14, 2 %, summing up a total of 85,5 % of cumulative variations. The principal components that characterize the stream were: chloride, barium, calcium, sodium, pH, conductivity, salinity and specific conductivity (coefficients in the linear combinations of variables > 0,240), as they were the most important variables contributing to the separation.

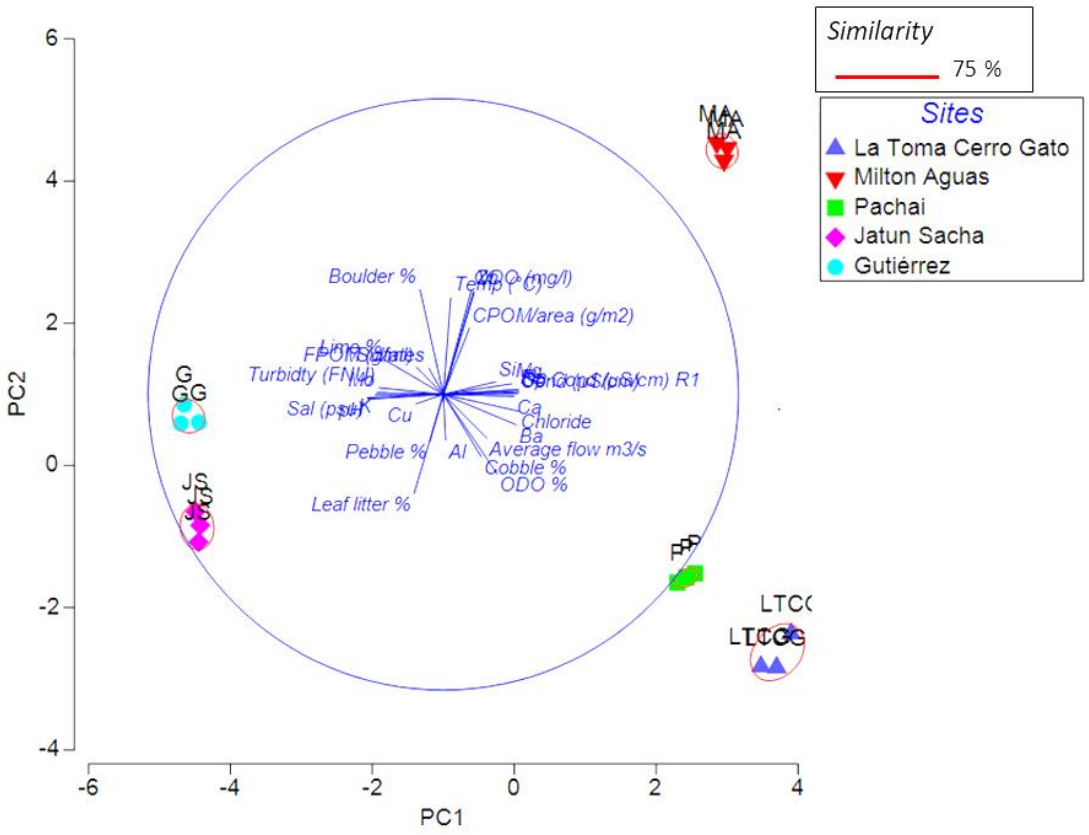

Figure 6. PCA between physicochemical variables and streams from San Cristóbal Island, realized in Primer 6 Beta. G = Gutiérrez, JS = Jatun Sacha, LTC1 = La Toma Cerro Gato1, LTCG2 = La Toma Cerro Gato2, MA = Milton Aguas, P = Pachai PC 1 explains 49,4% of the variation percentage Principal components: chloride, barium, calcium, sodium, pH, conductivity, salinity and specific conductivity and conductivity *(*coefficients in the linear combinations of variables > 0,240)

Ecology

To assess potential relationships between environmental characteristics and composition and structure of shrimp communities, a biological characterization was also realized. The Cluster and nMDS results reveal a strong division of study sites (Fig. 7). Two different groups were found, based on the structure of the shrimp community, grouping first Gutiérrez, Jatun Sacha and Milton Aguas (80% similarity), and as a second group

composed by La Toma Cerro Gato 1 and 2, Encañada Crecida and Pachai, with 70% similarity (Fig. 6 and Appendix 10).

Figure 7. nMDS, realized in Primer 6 Beta with the tree genera abundances at each study site. A 75% similarity percentage shows two main groups o streams.

For analyze if there exist some environmental variables that explains the biological aspects, correlation´s results suggest that the shrimp abundances of the tree genera are positively influenced mainly by the presences of cobble, and average flow (R > 0,5) (Appendix 11), in contrast lime and boulder substrates seem to generate a negative effect on *Archeatya* abundances (R >0,5). The abundances of specimens morphologically identified as *Atya*, are positively influenced by higher concentrations of chlorides and barium. *Australatya* was not related to any specific variable, possibly showing better adaptation and more generalistic characteristics. The remaining variables did not show statistical significant relations with the abundances of the shrimp genera.

BEST analysis finds the best match between the samples patterns and environmental variables. For this study, it reflects the degree to which the physicochemical data explains the genera abundances in streams, and also identifies the variables that have the highest correlation with this biotic pattern. An R = 0,77 was obtained in BEST result, showing a strong association between biotic and abiotic factors. The main environmental variables that influences *Australatya*, *Atya* and *Archeatya* abundances were FPOM, cobble, flow and turbidity (Figure 8).

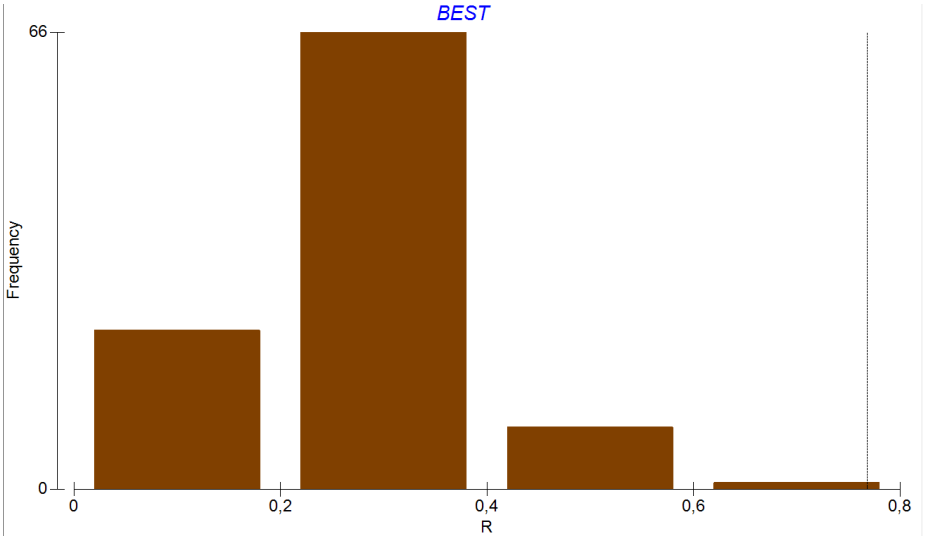

Figure 8. BEST analysis performed in Primer 6, with 499 permutations. The dotted line represents an R = 0, 77, that shows a strong association between environmental and shrimp genera abundances, explained by FPOM, cobble, flow and turbidity.

DISCUSSION

Morphology *vs.* genetics

The results clearly show discrepancies between the morphological and genetic approaches. The main dissimilarity is the genetic clade formed by the two genera morphologically identified as *Australatya* and *Atya*. These genera were split up based on the terminal spine or setae in the third maxilliped; however, the genetic distances between them are minimal (<1%) and there is nor reciprocal monophyly, suggesting that it is a single species (Costa et al., 2007). The genus *Australatya* was described in 1983, conformed by a single species. The terminal spine in the third maxilliped was one of the main diagnostic features, used in several morphological keys to separate the genus from others (Chace Jr, 1983). Although, a revised diagnosis of the genus was presented in 2015 with the description of a new species, establishing that third maxilliped is dimorphic between sexes, with a terminal spiniform seta (nail) in males and tip rounded, without a nail in females (Han & Klotz, 2015). It is worth mentioning, that within Galápagos samples, all collected shrimps that lack a terminal spine were ovigerous females. Consequently, a morphological misidentification could be the explanation of these morphological and genetic discrepancies, due to ontogenetic change or sexual dimorphism in the *Australatya* - *Atya* species complex.

The maximum likelihood tree suggests that both *Australatya–Atya* and *Archeatya* clade could represent new genera distinct from those included in the analysis. Also, the closest genera resulting from BLAST are very divergent (p distances > 13%), which could be an artifact of poor sampling of Atyidae species in other geographic areas. On the other hand, as the revised diagnosis of the genus *Australatya*, dispute the morphology used for its

taxonomic separation, that does not contemplate sexual dimorphism (Han & Klotz, 2015), it is suggested to carry out more extensive research including all the ideal genetic sequences to resolve evolutionary aspects, as well as a deeper morphological revision with the collaboration of experts.

The morphological identification of *Archeatya* genus matches the previous reports of *Archeatya chacei* in Santa Cruz Island (Wicksten, 1991), but not for San Cristóbal Island. Unfortunately, the genetic information of this species (and for other *Archeatya* species) is not available, so is not possible to assure that both of them are the same species. In consequence, it is necessary to analyze the morphology and genetics of *Archeatya chacei* to provide more accurate information.

Most of the taxonomy of Atyidae family remains unclear (Chace Jr, 1983; von Rintelen et al., 2012), there have been many changes in the genera that comprise it, and in the same way many of them are composed of a single species and inhabit rivers of tropical islands, showing high levels of speciation by geographical isolation. As a result, both San Cristóbal´s Atyidae taxes could be new or endemic to the area, but it is necessary to make a descriptive morphological and genetic study to confirm this postulate.

Colonization and evolution

Due to the isolation of the Galápagos Archipelago, two colonization scenarios are possible. Usually, freshwater shrimps exhibit little dispersal capacity, but some Atyidae genera can have an amphidromous live cycle (Cook, et al., 2006), a characteristic that gives them the capacity to colonize islands by oceanic dispersal (Page et al., 2005, 2008), as it is documented for Caribbean atyids (Page et al., 2008). Amphidromy is considered as an ancestral feature. Under this context, an ancestral amphidromous species could have reached San Cristóbal Island transported by oceanic currents, and then adapted to the existing freshwater conditions.

As another possible colonization scenario, passive dispersal of shrimp´s larvae by waterbirds needs to be considered. Darwin's postulates (1859) the theory of long-distance dispersal (LDD), which applies for many aquatic invertebrates' taxa, referring to the ability of waterfowl to contribute to the dispersal of organisms to very distant places, either by external or internal carrying (Green & Figuerola, 2005). Nowadays there are numerous studies that support this theory also for shrimps (Sanchez, et al., 2012; Coughlan, et al., 2017; Chen, Tsai & Tzeng, 2009). In 2005, Green et al. reported the dispersion of *Artemia* shrimp by redshank and godwits (shorebirds) through thousands of kilometers in the European continent. Around 25 species of birds in the Galápagos are register as migrants moving between North, Central and South America (Peck, 1994), which could act as a possible conveyor for shrimps' larvae.

According to the reports in Costa Rica, Isla de Cocos, Gulf of Panama and Perlas Archipelago, the genus *Archeatya* can inhabit brackish waters (Chace, 1983). The

geographic closeness of these areas with the Galápagos Islands points out a high probability that these could be the source countries for the San Cristóbal Island, agreeing with the established biogeographic hypothesis of this study.

Australatya has only been reported in Taiwan and Australia (Han & Klotz, 2015), but, unfortunately, the lack of homologous sequences in our genetic matrix forbid a biogeographic interpretation. However, the enormous distance between the Galápagos Islands and Taiwan and Australia, makes this colonization scenario unlikely for organisms with little dispersal capacity. No report for shrimps' dispersal between these countries exist yet, but some active flying insects that inhabit the Galápagos have come from Asia and Australia, by air currents, plants or accidental transported by humans (Roque Albelo & Causton, 1999). Also plants genera as *Nicotiana* and *Sicyos* shows birds transport as plausible mechanism for trans-Pacific dispersal events (Sebastian, et al., 2012).

In any case, biogeographic patterns require good taxon and geographic sampling, something that is currently lacking in Atyidae. Nevertheless, the results of two distinct existing genera strongly assure that Atyidae colonization to Galápagos has happened twice independently.

Characterization of Freshwater Habitat

The evolutionary histories and diversity of the organisms found in island streams highly depend on the geologic and physical characteristics of the islands (Smith, Covich & Brasher, 2003). On volcanic islands, lotic ecosystems dynamics starts from its formation, which is closely related to the age of the island itself. At first, the volcanic material is initially too porous to support running surface water, so rainwater percolates down through

the substrate (Menard 1986), until vegetation becomes well established and soil develops, with consequent water retention and infiltration. Later, groundwater starts to leak out joining with the surface water to create a true channel. The erosive force of water leads to eventual formation of diverse habitats and creates opportunities for organisms to adapt to a complex topography over evolutionary time (Craig, 2003). In the case of San Cristóbal Island, these processes have led to the formation of streams principally characterized by a lower dynamic flow and higher turbidity than Ecuadorian continental streams (average flow ~ 1.5 m^3 / s, turbidity ~ 0.06 NTU) (Encalada, A.C. et al., 2014; Encalada A.C. et al., 2016); differences that are probably caused by the land relief characteristic of the island, and the large amount of chemical elements present in the streams.

San Cristóbal streams have elevated levels of conductivity (~ 100 μS) unlike other tropical islands, as Hawaii Archipelago or the Polynesian islands (~ 30 μS). Inputs of San Cristóbal´s aquifer groundwater could be the cause of this high conductivity, as is also thought to occur in the Tahiti Islands (~ 250 μS) (Craig 2003).

Streams study sites were also characterized by significant quantity of chlorides, calcium, silica, magnesium, potassium, sodium and conductivity, varying between each other site due to volcanic features. One of the main attributes of volcanic rocks is it´s silica content (40 - 70%), and also minerals like the ones mentioned above. The natural waste of the stones and water erosion causes the mineral dissolution, giving special characteristics to these waters, including also barium, zinc and copper presence (Toulkeridis, 2011). Despite the physical measurements did not vary so much, the chemistry separates each one of the streams as unique. Although most of the chemical elements were common between streams, what make each other different are the great variations in the components

concentrations. This indicates that the volcanic chemistry is so varied and strong that causes a special chemical print for each stream defining it. As the flow was significantly different between sites (probably caused by the land relief) this could also affect the rate of natural waste of the volcanic rocks from each stream.

Ecology

High IHF index ratings indicate that San Cristóbal´s streams have experienced little alterations in its initial form, showing scarce human intervention. (Pardo, et al., 2002) and good ecological quality for macroinvertebrates fauna. The biological division of the study sites in two clear groups, also matches with the geographical distribution of the streams, being divided in eastern area: La Toma Cerro 1 and 2, Encañada Crecida and Pachai; and west zone: Milton Aguas, Jatun Sacha and Gutiérrez. *Archeatya* and *Atya* are found mostly in the eastern zone, as these sites have the appropriate requirements for the genera, according to what correlations results stay (poor presence of lime and boulder substrates, and high values or chloride and barium). The large presence of *Australatya* in all study sites could show more general environmental requirements, and/or higher tolerance levels to environmental conditions. According to the literature, the only requirements for the genus are a permanent water flow, rocky areas and moderate turbidity (Chace Jr, 1983).

Streams show not significant difference in CPOM, but low punctuation of QBR index, probably caused by the fact that all streams were located near or inside agricultural zone mainly dominated by introduced plants. Changes in the riparian forest can also affect fluvial ecosystem´s fauna (Pozo et al., 2009). As shrimps feed on fine particles, the low quality of the shore vegetation does not seem to have direct effects on them.

BEST analysis shows that FPOM, cobble, flow and turbidity are variables that can dictate genera abundance (*Australatya, Archeatya* and *Atya*), due to they affect some biological functions. For example, cobble substrate is suitable for Atyidae shrimps as they feed by rapidly removing small sediments and detritus from this rock substratum, making shrimps key processors of both FPOM and inorganic deposits (Pringle et al., 1999). Shrimps prefer moderate water flow as it´s increases also produce increments in the turbidity of the streams, caused by a greater drag of solid material in suspension by the river (Göransson, Larson, & Bendz, 2013). As turbidity is caused by fluctuation of suspended particles deposits, when it reaches high levels affects shrimp respiration, because suspended particles can clog gills and hinder gas exchange (FAO, n. D.).

The stream that presented the highest total abundance of shrimp was La Toma Cerro Gato, characterized by high values of magnesium (~ 2700 ppm), potassium (~ 3100 ppm) and sodium (5200 ppm), as these are essential macro elements for the growth of the crustacean, this site offers a perfect combination of suitable conditions, including cobble´s presence (60%) and appropriate average flow (0.0150 m3 / s). Probably the most important macroelement is calcium, as it has a significant relevance in the formation and hardening of the cuticular structure (Alday-Sanz, 2010), incorporated from the environment to shrimp body through branchial epithelia (Davis and Gatlin, 1996)

As a consequence, it is presumed that in streams with poor chemical setting and unsuitable substrates, Atyidae shrimps would not be present. The ecological importance of these crustaceans is not only limited to stream ecosystem-level processes such as storage, transport and export of FPOM. Atyidae shrimps keep streams clean as they process organic material, sweeping off the sediment and then using it into their own bodies, which will

become a food source for other slaves in the food chain, keeping linkages between the whole biotic community. This result in enhancement of resilience of streams to abiotic disturbances, as huge organic discharges (Pringle et al., 1999).

All the environmental features mentioned throughout this study, determining of San Cristóbal´s streams, could finally have led to the evolution of new taxa, as has occurred in Hawaii Archipelago which presents similar geological characters to Galápagos, where a high adaptation and diversification of the Atyidae family (Eldredge & Miller, 1997) at the genera level rather than species has occurred presumably based on the allopatric model of speciation and environmental adaptations.

RECOMMENDATIONS

Due to the huge lack of taxonomic information of this family of freshwater shrimps I recommend:

1. Make the formal descriptions of the two founded genera *Australatya* and *Archeatya*
2. Expand the genetic analysis including other genes to try to resolve the phylogenetic relationships, as well as molecular markers that allow to understand the colonization times
3. Include genetic analyzes with samples from *Australatya* and *Archeatya* already identified from other geographical locations
4. Expand research to the remaining San Cristóbal streams and increase sampling times, to capture the complete life cycle of shrimp and the possible movement patterns of the species
5. Include geological aspects to understand better the differences of streams´ chemical dynamic

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APPENDICES

Appendix 1. Individual sequence information data.of shrimp specimens and sequences included genetic analysis of cytochrome oxidase I (COI). Specimen sequences from other sites different to San Cristóbal Island were obtained from GenBank.

Species	***Sample site***	***Latitude***	***Longitude***	***Elevation (m.a.s.l)***	***GenBank accessions***	***Voucher***
Atya intermedia	Equatorial Guinea	N/A	N/A	N/A	KY582529.1	N/A
Potimirim brasiliana	Brasil: Ilheus, Bahia	N/A	N/A	N/A	KP202821.1	N/A
Typhlatya pretneri	Croatia	N/A	N/A	N/A	DQ320048.1	N/A
Micratya cooki *	Puerto rico streams	N/A	N/A	N/A	FJ348844.1	N/A
Atyopsis spinipes *	Samoa: Samoa Islands	N/A	N/A	N/A	KP759382.1	MNHN-IU-2012-1066
Caridina longicarpus *	New Caledonia	N/A	N/A	N/A	MK190051.1	N/A
Caridina wilkinsi	Australia	N/A	N/A	N/A	MK190076.1	N/A
Atyaephyra desmarestii	Portugal: Raia River	N/A	N/A	N/A	MG968378.1	Ades_PORT01_2015_01
Dugastella valentina	Baldovi, Valencia, ES	N/A	N/A	N/A	DQ641569.1	N/A
Troglocaris hercegovinensis	Bosnia and Herzegovina	N/A	N/A	N/A	DQ320044.1	N/A
Troglocaris inermis	France	N/A	N/A	N/A	DQ320046.1	N/A
Synalpheus sanjosei *	Panamá	N/A	N/A	N/A	KJ595143.1	681_sanjo
Alpheidae sp. *	Papúa New Guinea: Ili Ili Bua Bua	N/A	N/A	N/A	KU286026.1	N/A
Neocaridina denticulata	Lake Biwa, Shiga Prefecture, Honshu, Japan	N/A	N/A	N/A	AB300191.1	N/A
Neocaridina davidi	Poland	N/A	N/A	N/A	MG816776.1	Ndent_AQUA_2017_05
Neocaridina ketagalan	Taiwan:Beifeng R., Sijhih, Taipei County	N/A	N/A	N/A	AB300182.1	N/A
Neocaridina palmata	China:Leye, Guangxi	N/A	N/A	N/A	LC324770.1	N/A
Neocaridina saccam	Taiwan:Houjha, Tainan City	N/A	N/A	N/A	AB300179.1	N/A
Neocaridina ikiensis	Japan:Nagasaki, Iki Island	N/A	N/A	N/A	LC324775.1	N/A

Neocaridina spinosa	China:Tongan, Fujian Province	N/A	N/A	N/A	AB300188.1	N/A
Halocaridina trigonophthalma	Japan: Irabu Island, Okinawa	N/A	N/A	N/A	EF173842.1	N/A
Halocaridina rubra	Oahu, Hawaii	N/A	N/A	N/A	DQ399258.1	N/A
Paratya caledonica	New Caledonia	N/A	N/A	N/A	MK190080.1	N/A
Paratya australiensis	Brisbane River, Queensland, Australia	N/A	N/A	N/A	KP697542.1	MDFRC_DE0001
Antecaridina lauensis	Japan: Tarama-jima	N/A	N/A	N/A	KC879764.1	N/A
Australatya sp.	La Toma Cerro Gato, San Cristóbal Island, Galápagos	0°55'22.69"S	89°28'31.66" O	138	N/A	N/A
Australatya sp.	La Toma Cerro Gato, San Cristóbal Island, Galápagos	0°55'22.69"S	89°28'31.66" O	138	N/A	N/A
Australatya sp.	La Toma Cerro Gato, San Cristóbal Island, Galápagos	0°55'22.69"S	89°28'31.66" O	138	N/A	N/A
Atya sp	La Toma Cerro Gato, San Cristóbal Island, Galápagos	0°55'22.69"S	89°28'31.66" O	138	N/A	N/A
Atya sp	La Toma Cerro Gato, San Cristóbal Island, Galápagos	0°55'22.69"S	89°28'31.66" O	138	N/A	N/A
Archeatya sp	La Toma Cerro Gato, San Cristóbal Island, Galápagos	0°55'22.69"S	89°28'31.66" O	138	N/A	N/A
Archeatya sp	La Toma Cerro Gato, San Cristóbal Island, Galápagos	0°55'22.69"S	89°28'31.66" O	138	N/A	N/A
Australatya sp.	Encañada Crecida, San Cristóbal Island, Galápagos	0°55'25.48"S	89°28'13.75" O	172	N/A	N/A
Australatya sp.	Encañada Crecida, San Cristóbal Island, Galápagos	0°55'25.48"S	89°28'13.75" O	172	N/A	N/A
Archeatya sp	Encañada Crecida, San Cristóbal Island, Galápagos	0°55'25.48"S	89°28'13.75" O	172	N/A	N/A
Archeatya sp	Encañada Crecida, San Cristóbal Island, Galápagos	0°55'25.48"S	89°28'13.75" O	172	N/A	N/A
Archeatya sp	Encañada Crecida, San Cristóbal Island, Galápagos	0°55'25.48"S	89°28'13.75" O	172	N/A	N/A
Australatya sp.	Milton Aguas, San Cristóbal Island, Galápagos	0°55'36.95"S	89°29'12.75" O	190	N/A	N/A
Australatya sp.	Milton Aguas, San Cristóbal Island, Galápagos	0°55'36.95"S	89°29'12.75" O	190	N/A	N/A
Australatya sp.	Milton Aguas, San Cristóbal Island, Galápagos	0°55'36.95"S	89°29'12.75" O	190	N/A	N/A
Australatya sp.	Pachai, San Cristóbal Island, Galápagos	0°54'41.31"S	89°27'34.81" O	202	N/A	N/A
Australatya sp.	Pachai, San Cristóbal Island, Galápagos	0°54'41.31"S	89°27'34.81" O	202	N/A	N/A
Australatya sp.	Pachai, San Cristóbal Island, Galápagos	0°54'41.31"S	89°27'34.81" O	202	N/A	N/A

Archeatya sp	Pachai, San Cristóbal Island, Galápagos	0°54'41.31"S	89°27'34.81" O	202	N/A	N/A
Australatya sp.	Gutiérrez, San Cristóbal Island, Galápagos	0°55'42.57"S	89°30'41.08" O	179	N/A	N/A
Australatya sp.	Gutiérrez, San Cristóbal Island, Galápagos	0°55'42.57"S	89°30'41.08" O	179	N/A	N/A
Australatya sp.	Jatun Sacha, San Cristóbal Island, Galápagos	0°55'29.85"S	89°29'57.63" O	175	N/A	N/A
Australatya sp.	Jatun Sacha, San Cristóbal Island, Galápagos	0°55'29.85"S	89°29'57.63" O	175	N/A	N/A
Australatya sp.	Jatun Sacha, San Cristóbal Island, Galápagos	0°55'29.85"S	89°29'57.63" O	175	N/A	N/A

*****Closest resulting species of BLAST (NCBI)

Appendix 2. Mean and standard deviation of environmental variables of streams from San Cristóbal Island, taken 2018.

Site	***pH***	***ODO %***	***ODO (mg/l)***	***Turbidity (NTU)***	***Conductivity (µS/cm)***	***Std. Conductivity (µS/cm) R1***	***Salinity (psu)***	***Temp (°C)***
La Toma Cerro Gato1	7,62 ± 0,01	102,37 ± 0,06	8,71 ± 0,01	17,87 ± 0,97	101,7 ± 0,0	104,83 ± 0,06	0,05 ± 0,0	23,39± 0,00
La Toma Cerro Gato2	7,62 ± 0,01	102,37 ± 0,06	8,71± 0,01	17,87 ± 0,97	101,70 ± 0,00	104,83 ± 0,06	0,05 ± 0,00	23,29 ± 0,00
Milton Aguas	7,50 ± 0,01	99,63 ± 0,06	8,05 ± 0,01	14,38 ± 0,24	104,90 ± 0,00	105,23 ± 0,06	0,05 ± 0,00	24,83 ± 0,00
Pachai	7,05 ± 0,01	89,47 ± 015	7,71 ± 0,01	5,74 ± 0,09	117,50 ± 0,00	122,80 ± 0,00	0,06 ± 0,00	22,72 ± 0,00
Jatun Sacha	6,63 ± 0,00	95,03 ± 0,06	8,05 ± 0,01	4,07 ± 0,04	50,50 ± 0,00	51,80 ± 0,00	0,02 ± 0,00	23,67 ± 00
Gutiérrez	6,32 ± 0,00	68,97 ± 0,06	5,87 ± 0,00	15,94 ± 0,03	49,60 ± 0,00	51,20 ± 0,00	0,02 ± 0,00	23,39 ± 0,00

* ODO stands for concentration of Dissolved Oxygen

Appendix 3. QBR and IHF punctuation of study streams (QBR: index of riparian quality; IHF: index of fluvial habitat)

Site	***QBR***	***IHF***
La Toma Cerro Gato	50	94
Milton Aguas	40	87
Pachai	75	84
Jatun Sacha	70	82
Gutiérrez	25	79

Appendix 4. Mean of organic matter and flow measurements of streams from San Cristóbal island

Sites	***FPOM (g/ml)***	***CPOM/area (g/m^2)***	***Average flow (m3/s)***
La Toma Cerro Gato 1	5,90E-06	219,70	0,0150
La Toma Cerro Gato 2	5,90E-06	219,70	0,0150
Milton Aguas	5,70E-06	699,05	0,0040
Pachai	6,10E-06	185,28	0,0015
Jatun Sacha	6,10E-06	79,20	0,0020
Gutiérrez	6,20E-06	291,37	0,0010

Appendix 5. Substrate composition of study sites

Site	***Boulder %***	***Cobble %***	***Pebble %***	***Sand %***	***Lime %***	***Leaf litter%***
La Toma Cerro Gato1	20	60	0	0	20	28
La Toma Cerro Gato2	20	60	0	0	20	28
Milton Aguas	67	0	0	0	33	0
Pachai	28	0	36	0	36	37
Jatun Sacha	31	0	23	0	46	23
Gutiérrez	60	0	0	0	40	40

Appendix 6. Mean and standard deviation of chemical variables of streams from San Cristóbal Island.

Sites	***Chlorides (ppm)***	***Sulfates (ppm)***	***Al (ppm)***	***Ba (ppm)***	***Cu (ppm)***	***Mn (ppm)***	***V (ppm)***	***Zn (ppm)***	***Ca (ppm)***	***Si (ppm)***	***Mg (ppm)***	***K (ppm)***	***Na (ppm)***	***Fe (ppm)***
La Toma Cerro Gato1	449,433 ± 4,25	3,33 ± 1,53	31,73 ± 4,51	8,01 ± 0,02	0	0	0,01	0	3245,72 ± 17,54	5313,99 ± 23,56	2768,13 ± 22,43	314,93 ± 23,59	5241,67 ± 468,93	89,88 ± 24,20
La Toma Cerro Gato2	449,43 ± 4,25	3,33 ± 1,53	31,73 ± 4,51	8,01 ± 0,02	0	0	0,01 ± 0,01	0	3245,72 ± 17,54	5314,00 ± 23,56	2768,13 ± 22,43	314,93 ± 23, 59	5241, 67 ± 468,93	89,88 ± 24,20
Milton Aguas	114,13 ± 3,25	4,33 ± 0,58	3,59 ± 6,23	2,45 ± 0,03	0	0	0,41 ± 0,05	4,51 ± 0,10	2954,55 ± 31,82	5714,30 ± 37,67	2698,52 ± 18,40	268,14 ± 7,64	4477,25 ± 19,02	97,57 ± 17,93
Pachai	148,17 ± 18,15	4,67 ± 0,58	0	3,71 ± 0,04	364 ± 0,11	0	0	0	2177,99 ± 24,19	3773,51 ± 22,52	2154,20 ± 24,36	190,13 ± 4,13	3283,61 ± 47,36	115,02 ± 69,32
Jatun Sacha	85,60 ± 11,60	3,67 ± 0,58	0,44 ± 0,77	4,53 ± 0,04	0	0	0	0	564,32 ± 488,78	1212,52 ± 1,70	552, 82 ± 478,90	22,55 ± 19,89	1942,77 ± 1299,94	101,73 ± 63,20
Gutiérrez	86,60 ± 6,93	6,00 ± 2,65	39,23 ± 3,80	3,51 ± 0,01	0	7,63 ± 0,11	0	0	683,72 ± 13,45	567,55 ± 7,07	641,26 ± 13,82	90,85 ± 8,06	1806,51 ± 26,74	114,69 ± 62,25

Appendix 7. Summary results of one-way ANOVA for physicochemical data, where the response variable was each environmental characteristic and the factors were each stream site. (P and F values, degrees of freedom of error term and the model).

Parameter	***P value***	***F value***	***Degrees of freedom***
Log e FPOM (g/ml)	0.0002274	12234	F5, 12
*Log e CPOM/area (g/m2) **	0.1031	7775	F5, 12
Log e Boulder %	2.2e-16	2,3644	F5, 12
Log e Cobble %	2.2e-16	4,4484E+35	F5, 12
Log e Pebble %	2.2e-16	6,9784E+35	F5, 12
Log e Lime %	2.2e-16	2,6743E+34	F5, 12
Log e Leaf litter %	2.2e-16	1,4263E+35	F5, 12
Log e Average flow m3/s	2.2e-16	9,1729E+33	F5, 12
Chlorides	2,523E-12	1014.7	F5, 12
*Sulfates **	0.2627	63,621	F5, 12
Al	0,00003227	17575	F5, 12
Ba	2.2e-16	31423	F5, 12
Log e Cu	2.2e-16	319225	F5, 12
Log e Mn	2.2e-16	319225	F5, 12
Log e V	0.0184	4,265	F5, 12
Log e Zn	2.2e-16	61752	F5, 12
Ca	0,000001038	115,08	F5, 12
Si	2.2e-16	29431	F5, 12
Mg	5.25e-09	87,144	F5, 12
K	1,354E-07	162,78	F5, 12
Na	0,01651	20,586	F5, 12
*Fe **	0.9714	0,1632	F5, 12
Log e Ph	2.2e-16	46403	F5, 12
ODO %	2.2e-16	645716	F5, 12

Log e ODO (mg/L)	2.2e-16	5860021	F5, 12
Turbidty (TNU)	1,439E-09	350,45	F5, 12
Log e Cond	2.2e-16	2,959E+32	F5, 12
Sp Cond (µS/cm) R1	2.2e-16	1696517	F5, 12
Sal (psu)	2.2e-16	2,0285E+34	F5, 12
Log e Temp	6,6801E+31	6,6801E+31	F5, 12

* Points out the variables not significantly different

Appendix 8. Guanidine Thiocynate protocol for DNA Extraction

Day 1

For tissue samples - ExtADN02

a. Cell lysis and inactivation of nucleases

1. Heat the Lysis Buffer to dissolve the SDS again (less than 1 minute in the microwave or at 55 ° C the stove)

2. In each tube pour 300 µL of Lysis Buffer (100 mM NaCl, 10 mM Tris-HCl (0.1 M) pH 8.0, 25 mM EDTA pH 8.0, 0.5% SDS). The NaCl and Tris-HCl serve to regulate the pH and osmolality of the lysate recreating the internal conditions of the cell. EDTA inhibits the action of nucleases by sequestering Ca and Mg ions, necessary for the action of these enzymes. SDS is a detergent used to break down cell membranes and to break down non-covalent bonds of proteins and solubilize them.

3. Place the tissue on a slide with tweezers and with a stylet, pierce it as thin as possible (make sure that the materials are sterile (autoclaved), while the tweezers and the stylet (knife / razor) must be immersed in alcohol and flamed in the lighter)

4. Introduce the tissue in the corresponding tube (containing the lysis buffer) and with a sterile pistil (autoclaved) further marinate the tissue

5. Add 3 µL of Proteinase K solution (20 mg / mL). Proteinase K is responsible for denaturating the nucleases and other proteins embedded in the cell membrane.

6. Mix with vortex and make sure that all the tissue is in the solution (you can make a spin down to force pieces of tissue that are on the walls to fall)

7. Incubate the samples at 55 ° C with 600 or 900 rpm shaking until the next day

8. Before leaving and leaving the samples overnight, add 3 µL more Proteinase K

Day 2

a. Protein precipitation

1. Incubate the tubes at 95 ° C for 10 minutes. To inactivate Proteinase K

2. Add 4 µL of RNAse A Solution and mix with vortex for 20 seconds

3. Incubate at 37 ° C for 30 minutes

4. Add 100 µL of the Protein Precipitation Solution (4 M Guanidine Thiocyanate, 0.1 M Tris-HCl pH 7.5) and mix with vortex for 20 seconds.

5. Centrifuge for 10 minutes at 13,000 rpm

6. Decant 400 µL of supernatant in second phase tubes (1.5 mL transparent eppendor) and discard the rest

b. Precipitation of DNA:

1. Add 300 µL of cold Isopropanol and mix carefully by inverting the tubes several times

2. Centrifuge at 13,000 rpm for 10 minutes and discard the supernatant

3. Add 300 µL of 70% Ethanol and mix carefully by inverting the tubes several times. To wash the pellet

4. Centrifuge at 13,000 rpm for 10 minutes and discard the supernatant

5. Allow the pellet to dry in the tube leaving it open at room temperature

6. Add 100 µL of 10 mM Tris-HCl pH 8.0, resuspend the pellet and store at 4 ° C until the next day

Day 3

a. Precipitation and washing of DNA:

1. Add 10 µL of 3 M NaOAc and mix by inverting the tubes. Sodium Acetate helps to precipitate DNA.

2. Add 100 µL of cold isopropanol and mix carefully by inverting the tubes several times.

3. Centrifuge at 13,000 rpm for 10 minutes and discard the supernatant

4. Add 300 µL of 70% Ethanol and mix carefully by inverting the tubes several times. To wash the pellet

5. Centrifuge at 13,000 rpm for 10 minutes and discard the supernatant

6. Allow the pellet to dry in the tube leaving it open at room temperature

7. Resuspend the pellet in 50 µL of TE 0.1 pH 8.0

8. Incubate the tubes at 37 ° C with shaking at 500 rpm for one hour. It helps the solubilization of DNA

9. Incubate the tubes at 4 ° C until the next day. Allows the total solubilization of the AND

10. Store at -20 ° C

Appendix 9. FPOM Processing Protocols

1. For the >250m fractions (steps 1-5), wash contents of the sample container with tap water into a large pail and resuspend in water.

2. Pour the resuspended material through nested 500-m and 250-um mesh sieves. Allow time to drain samples thoroughly and transfer material to separate, labeled paper bags.

3. Oven-dry material and bags at 60°C to constant weight (24 hr to several days, depending on sample size). Place bags in a desiccator for 24 hr.

4. Remove material from bags and weigh on a top-loading balance to determine dry mass.

5. Ash material at 500°C (small, heavy-gauged, aluminum baking pans work well for this purpose), and reweigh to obtain AFDM for the 250 to 500um and 500 um to 1.0 mm size fractions.

6. For the <250 um fraction (steps 6-11), set up a microfiltration unit as described in step 1 of "Standard Processing Protocols".

7. Individually pour each of the three replicate subsamples into separate 1-L graduated cylinders and record the subsample volumes.

8. Pour the first subsample into the funnel of the filtration unit. Wash any material clinging to the subsample bag or graduated cylinder into the funnel with distilled/deionized, prefiltered water. Draw material down onto a GFF, washing sides of funnel with distilled/deionized prefiltered water. Remove filter with blunt forceps and return to aluminum square.

9. Repeat steps 7 and 8 for remaining replicates.

10. Dry, weigh, ash, and reweigh FPOM samples and GFFS following steps 7 and 8 of "Standard Processing Protocols"

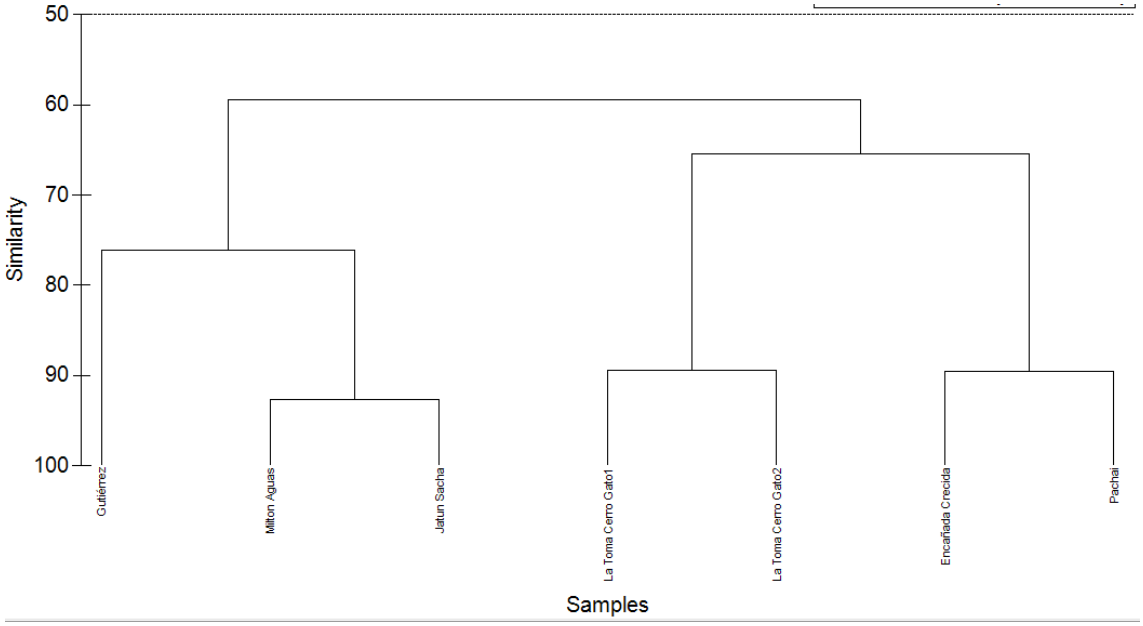
11. AFDM of the 0.45 um to 250um size fraction is estimated as the mean of the following quantity calculated for each of the three subsamples:

12. FBOM quantity is normally expressed as g AFDM/m^2 of stream bottom. This requires you to know the area of your sampling device (in cm^2). Use the following equation for FPOM standing stocks estimated for each size fraction to express your results:

g AFDM/m^2 = (mg AFDM ÷ 1000) X (10, 000 ÷ cm^2 of area sampled)

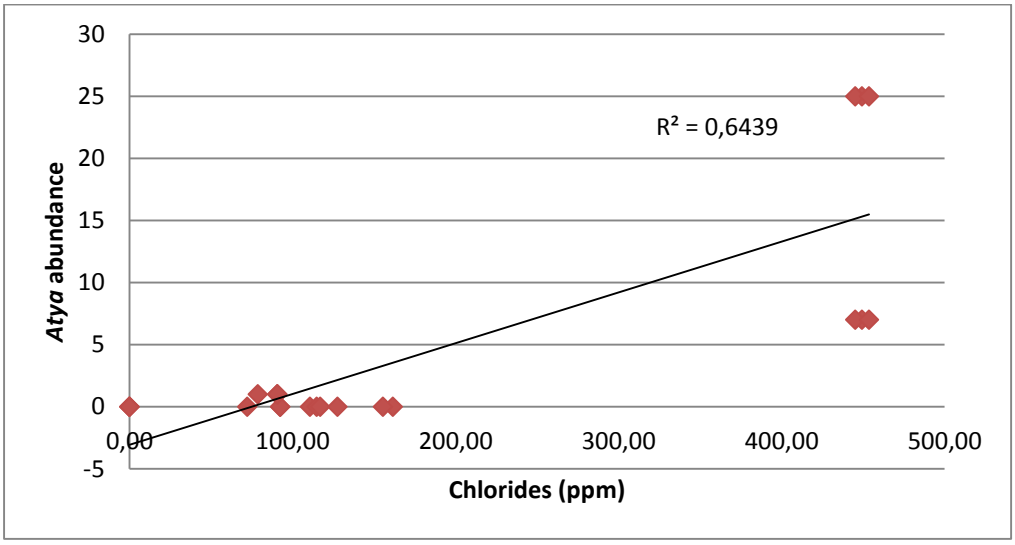
The g AFDM/m^2 for each size fraction are summed to obtain total FPOM standing crop (in AFDM) in your sample.

Appendix 10. Cluster with biological information and streams realized in Primer 6 Beta with the tree genera abundances at each study site. Resemblance was performed with Bray Curtis similarity and samples were previously transformed with fourth root.

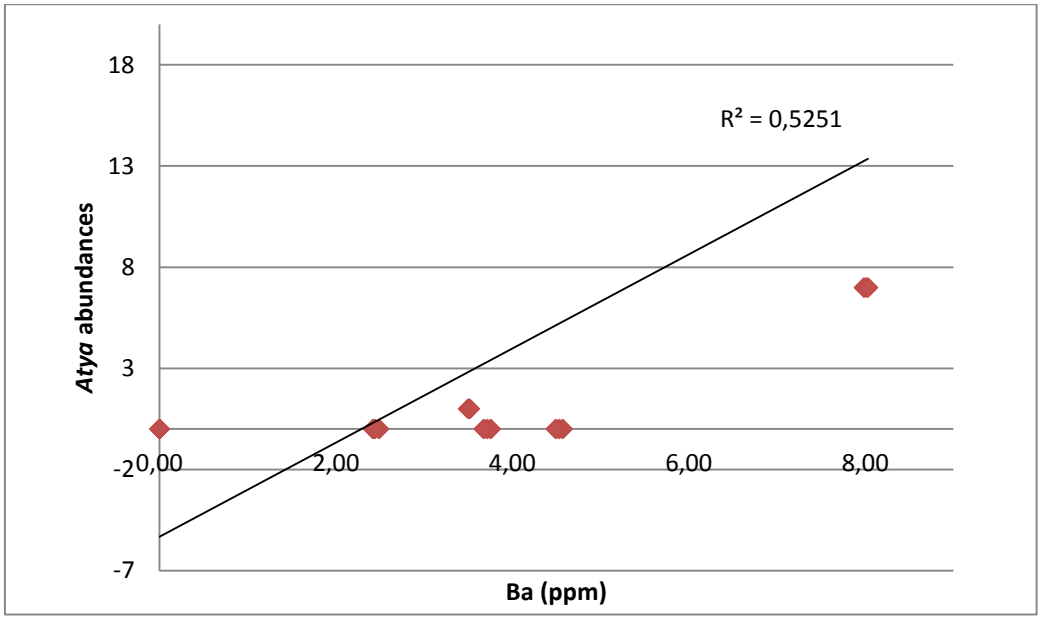


Appendix 11. Multiple correlations between Shrimps (of different taxa) and environmental factors.

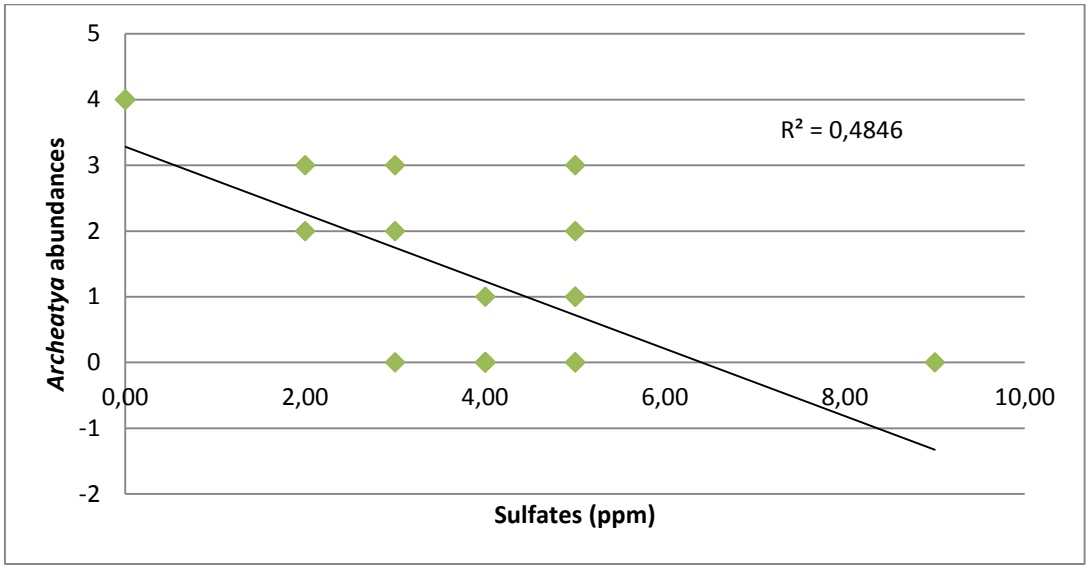
11.1 Correlation between *Atya* abundance and Chlorides



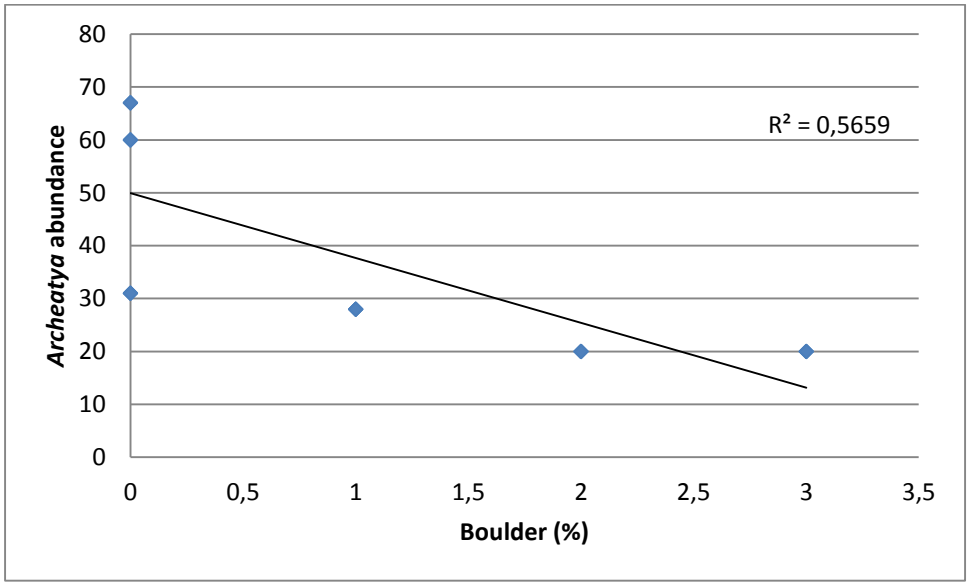
11.2 Correlation between *Atya* abundances and barium



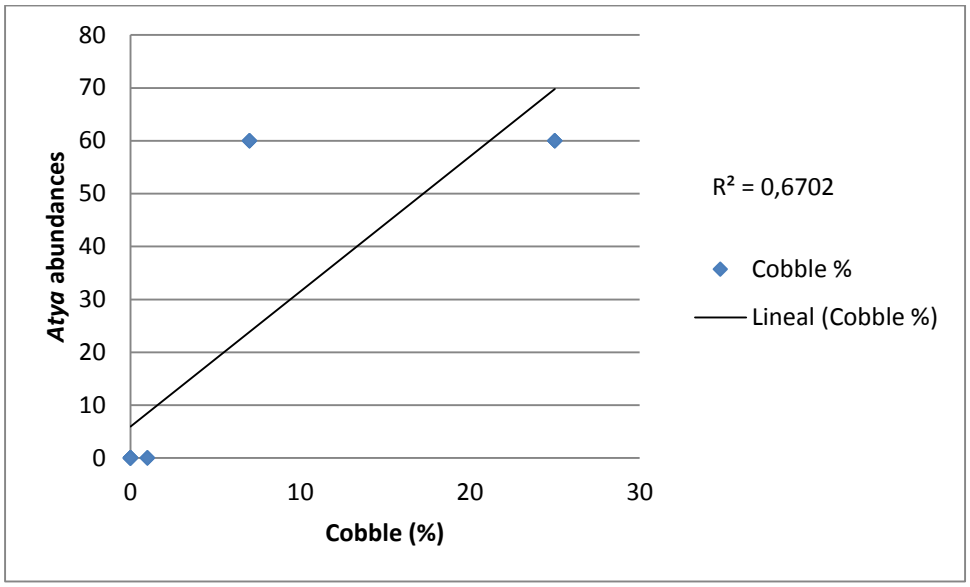
11.3 Correlation between *Archeatya* abundances and sulfates



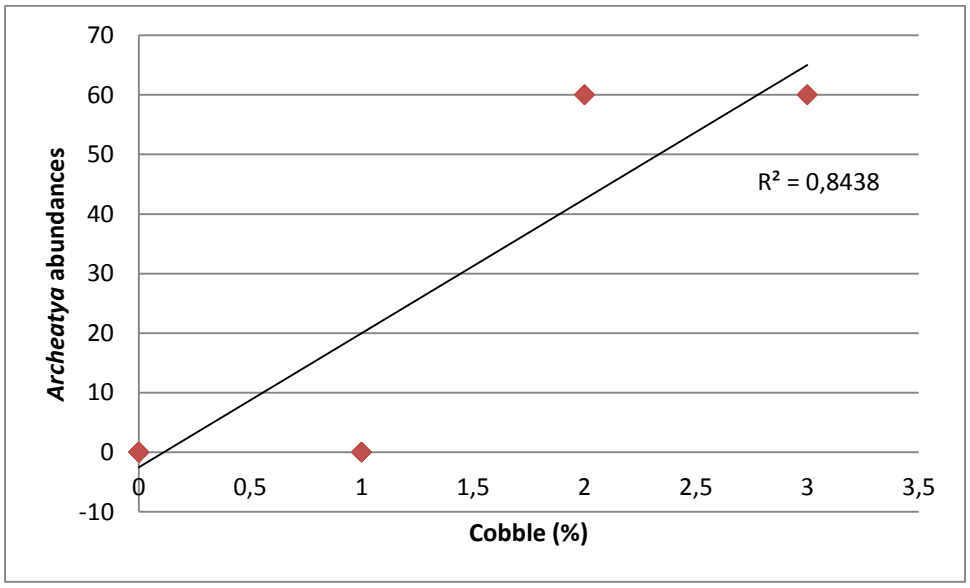
11.4 Correlation between *Archeatya* abundances and boulder



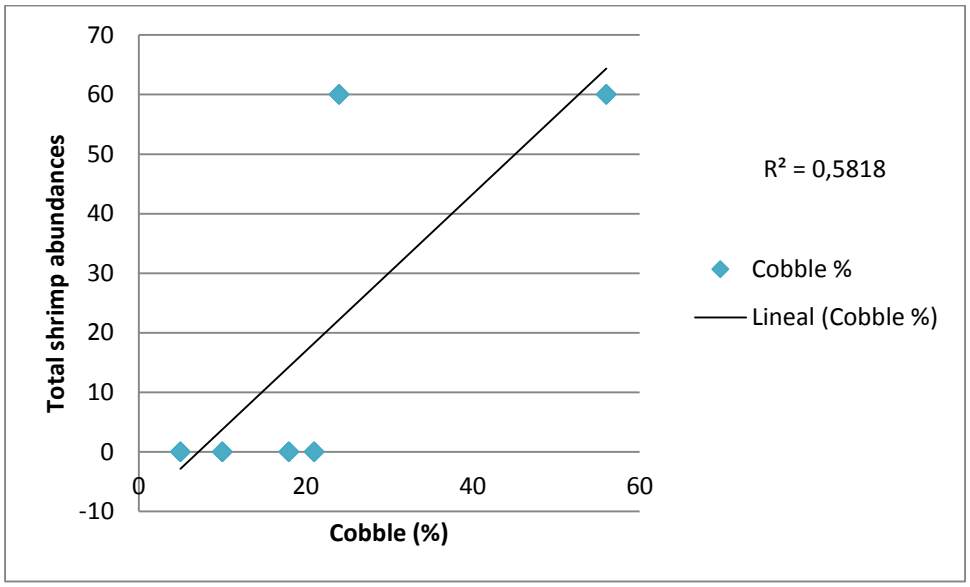
11.5 Correlation between *Atya* abundances and cobble



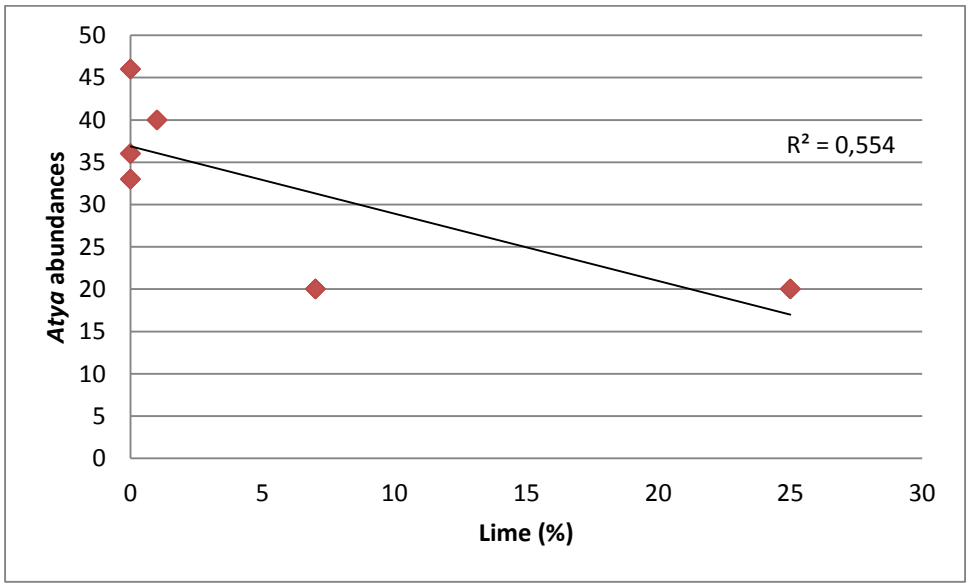
11.6 Correlation between *Archeatya* abundances and cobble



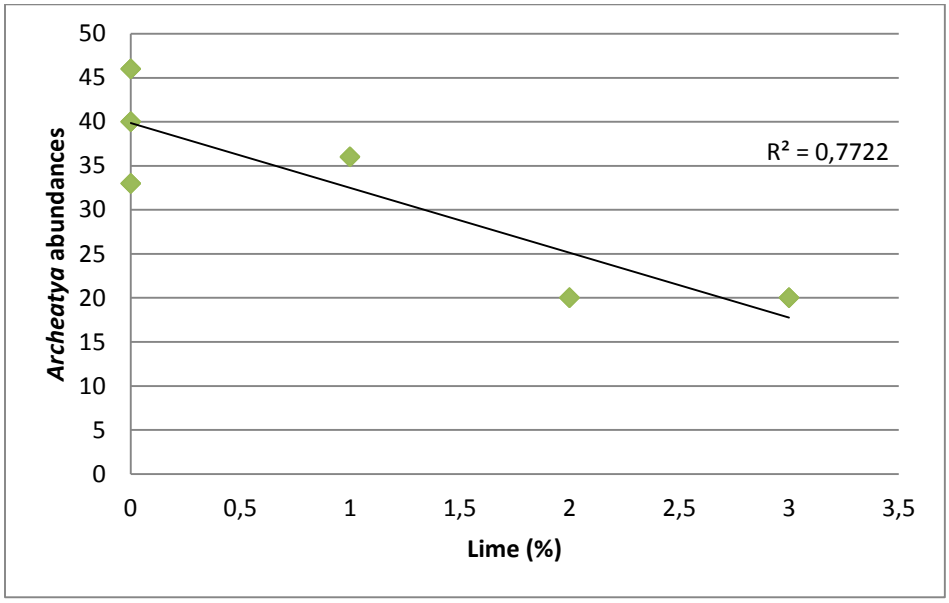
11.7 Correlation between total shrimp abundances and cobble



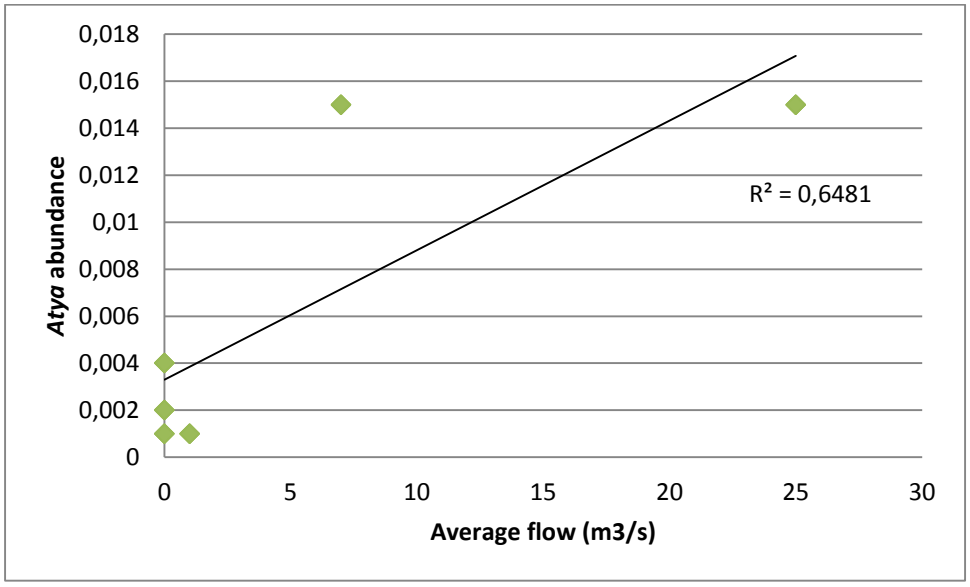
11.8. Correlation between *Atya* abundances and lime



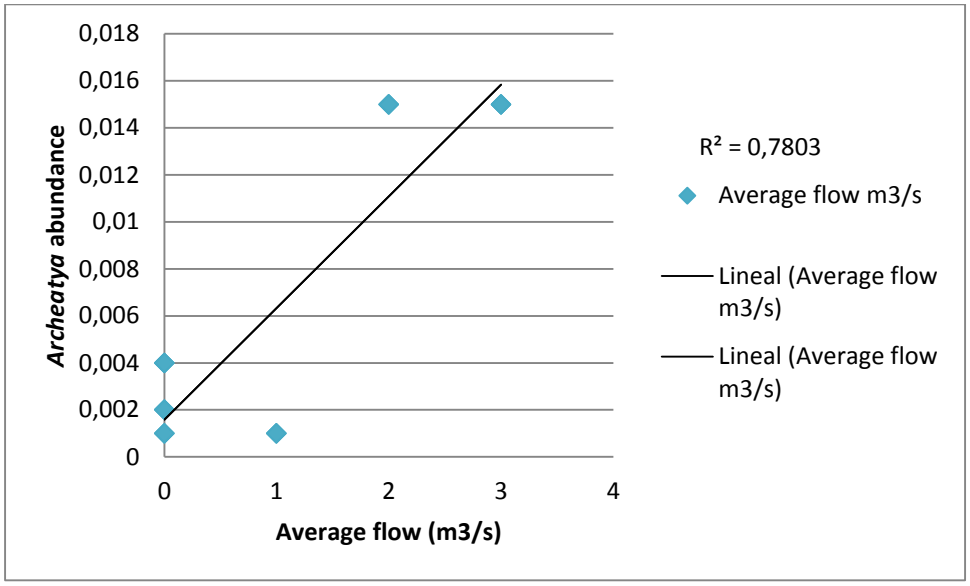
11.9 Correlation between *Archeatya* abundances and lime



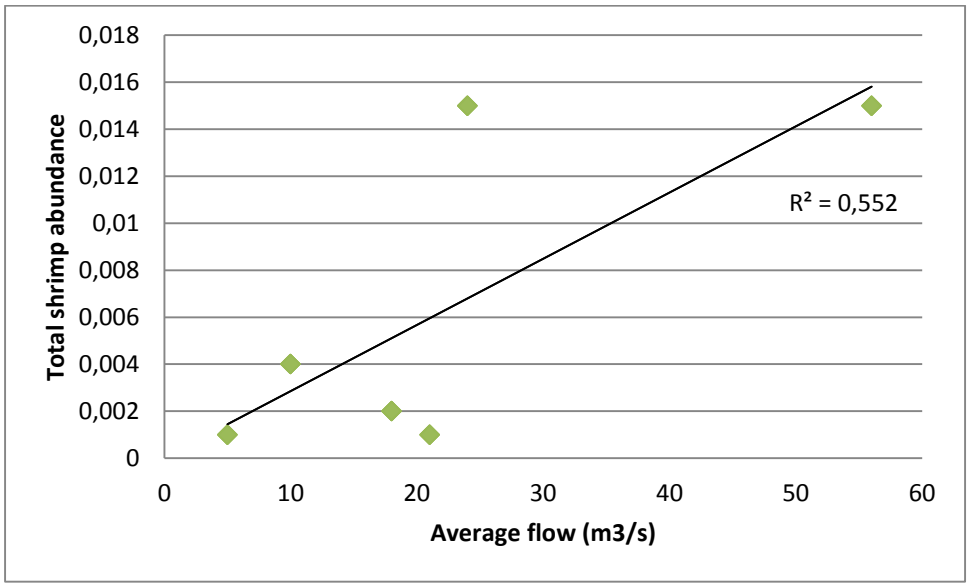
11.10. Correlation between *Atya* abundances and average flow

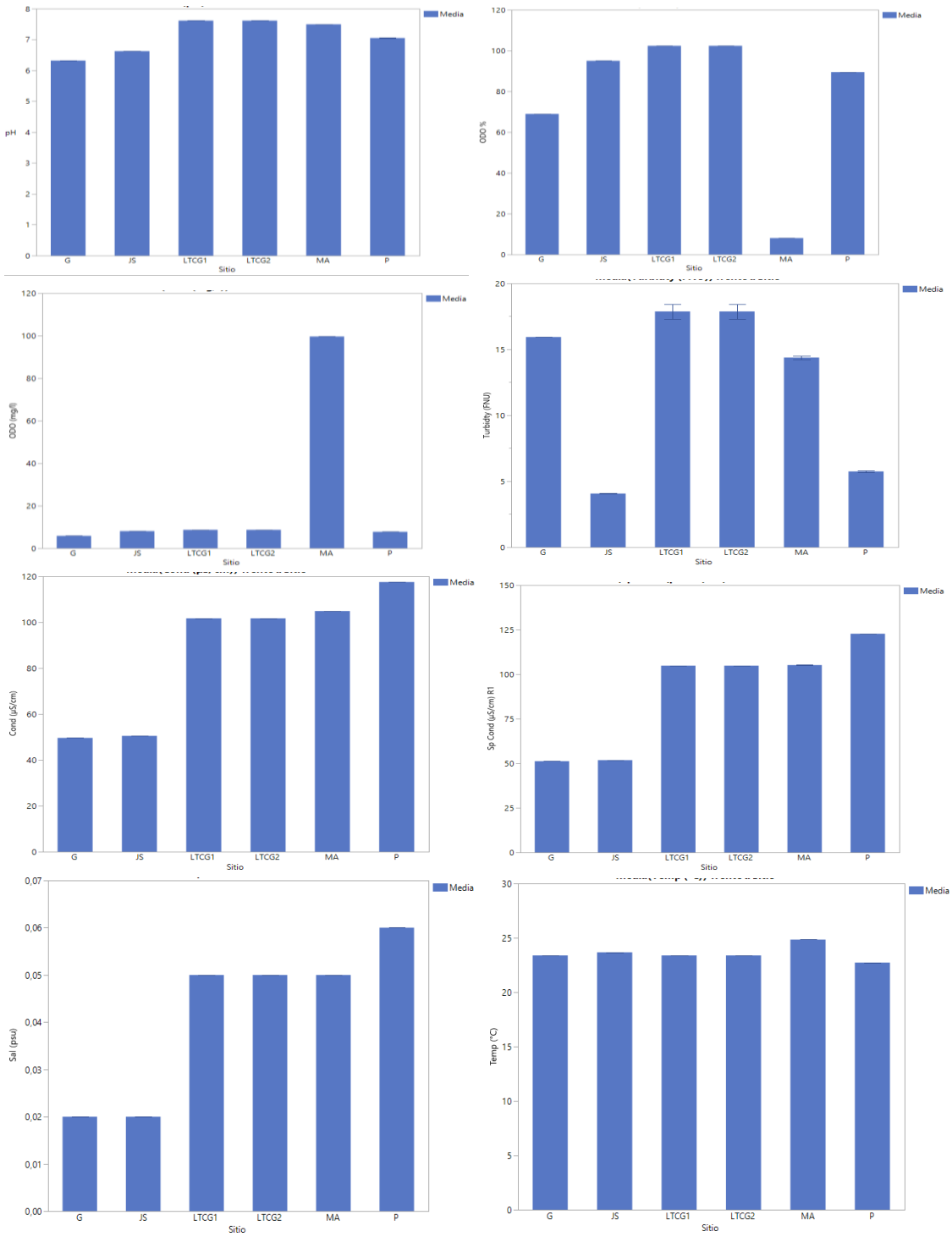


11.11. Correlation between *Archeatya* abundances and average flow

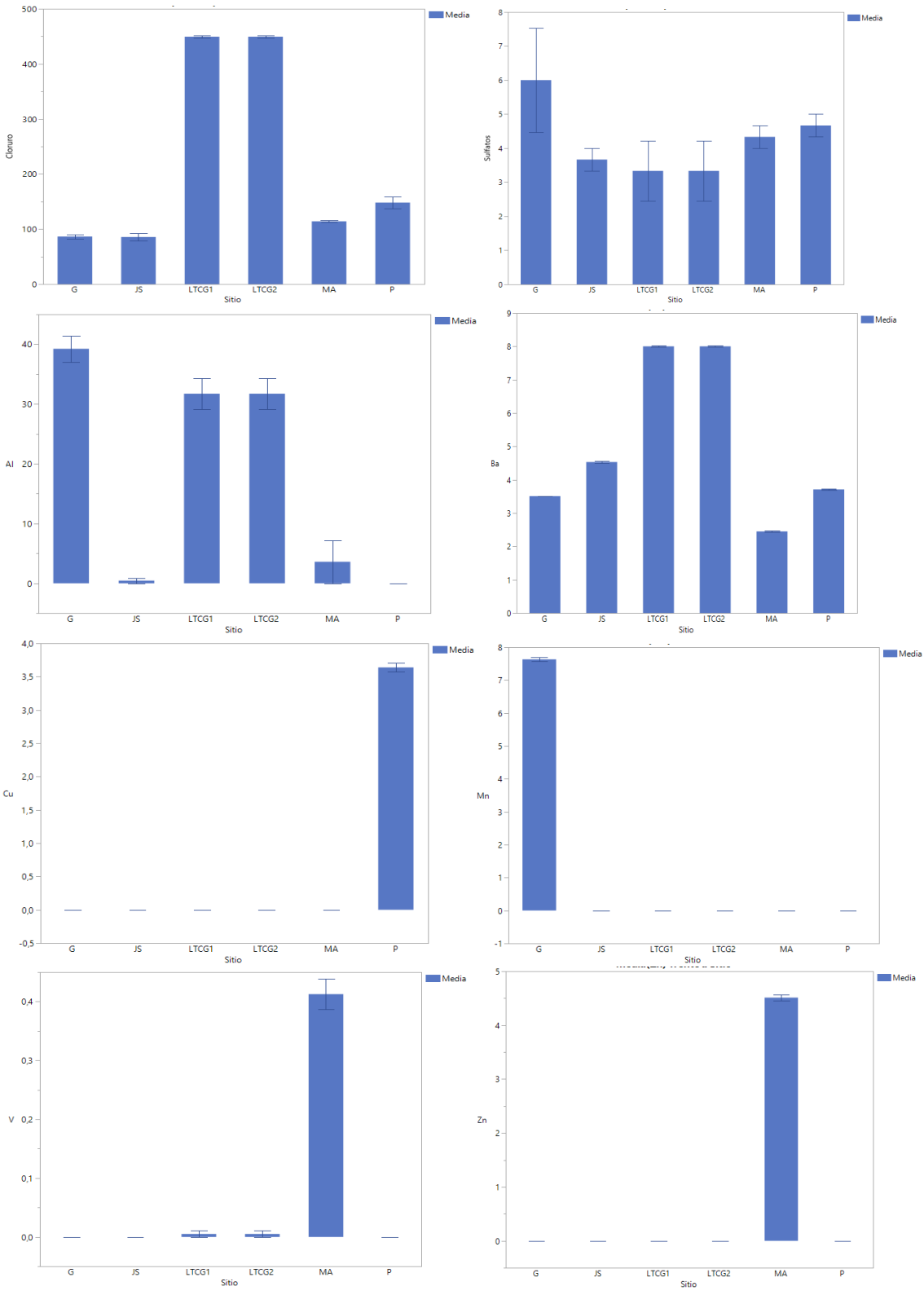


11.12. Correlation between total shrimp abundances and average flow

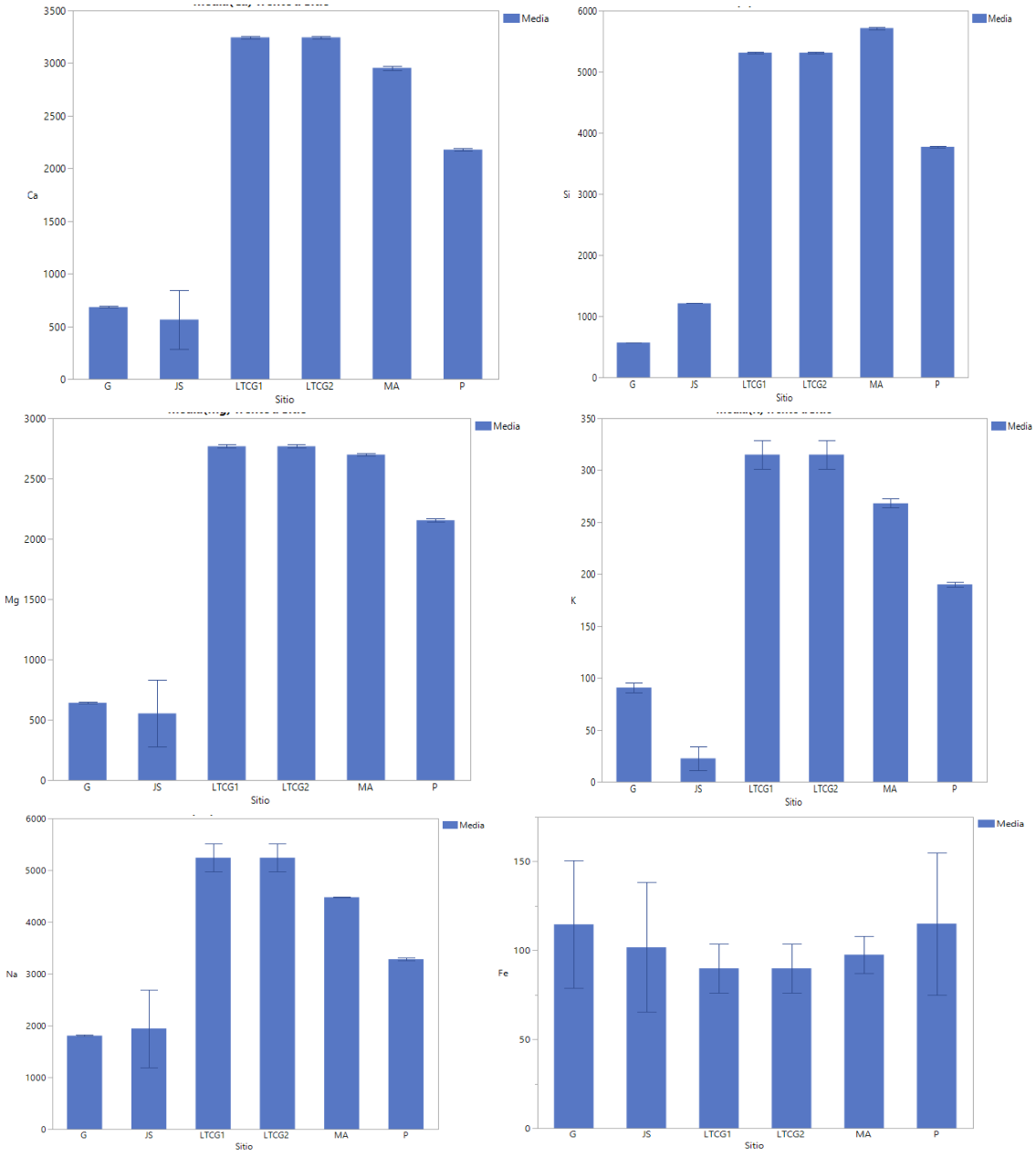




Appendix 12. Mean concentration of environmental variables (pH, ODO %, ODO (mg/L), Tutbidity, Cond, Sp Cond, Sal, Temp). G = Gutiérrez, JS = Jatun Sacha, LTC1 = La Toma Cerro Gato1, LTCG2 = La Toma Cerro Gato2, MA = Milton Aguas, P = Pachai.



Appendix 13. Concentrations of chemical elements (Chlorides, sulfates, Al, Ba, Cu, Mn, V and Zn). G = Gutiérrez, JS = Jatun Sacha, LTC1 = La Toma Cerro Gato1, LTCG2 = La Toma Cerro Gato2, MA = Milton Aguas, P = Pachai.



Appendix 14 Continuation of chemical concentrations (Ca, Si, Mg, K, Na and Fe). G = Gutiérrez, JS = Jatun Sacha, LTC1 = La Toma Cerro Gato1, LTCG2 = La Toma Cerro Gato2, MA = Milton Aguas, P = Pachai.