



Evaluation of the diversity of the tardigradus (bear water) on the tourist route of the Puyo River of Pastaza province as an input On Its Importance for further research on the benefits it offers.

Veronica L. Cordova-Juepa*, Greys Elizabeth Vásquez Cabrera, María José Méndez Solórzano, Michelle Alexandra Dávila Sánchez, Edgar R. Chicaiza-Reisancho.

^{to} Department of Life Sciences, Amazon State University, Puyo, Pastaza, Ecuador. E-mail: pblg2016003@uea.edu.ec.

^bAmazon State University, Puyo, Pastaza, Ecuador. E-mail: echicaiza@uea.edu.ec.

Graphical Abstract

Paseo turístico Río



Abstract

The objective of this article is the literature review of the components of the study of *Tardigradus* or (bear water), followed by the analysis of the information to describe the main peculiarities of this organism, with the objective of determining the importance of the water bear to knowledge of new technological applications (such as a protein from tardigrades protects human DNA from sunlight) and many other applications offered by this organism, which due to lack of information limits understanding as a fundamental source for future applicable research in the study of organism. With a description of their cryptobiosis state. The research will also contain a sample of the *Tardigradus* species in the tourist hike of the Puyo River (Pastaza province), increasing the knowledge of its distribution, in which the abundance existing in the area will be measured. A sample of the tardigrade will be taken in three different parts of the place, with strata of tree bark and stone to continue observing them in a digital microscope, followed by the identification of the class with its respective taxonomic key.

Keywords: tardigradus, technology, organism, cryptobiosis.

Introduction

The tardigrade is an invertebrate that lives in all kinds of environments on earth. Tardigrade comes from the Latin *tardigradus* which means slow movements, they are also known as a water bear because of its particular plump shape, which resemble those of a bear and also because they need a layer of water around their body to perform their necessary activities to lifetime. It appeared, this species 600 million years ago has been described in at least 1,000 different species (Zenteno de León, 2014). They were discovered in 1773 by Johann August Ephraim Goeze. This species has achieved an interest on the part of scientists in the last four decades for the recent discoveries about the many contributions they can offer in the field of medicine, space exploration in genetic engineering and many other benefits that this species can offer throughout the investigations. Thanks to its incredible capacity to adapt to extreme climates, its diverse states of cryptobiosis, tardigrades can be used as a model for the investigation of strategies to achieve technological progress (Pérez-Plancarte, Trejo-Moreno, & Xocopa-Castro, 2016). According to this, the objective of the work is to gather more information about the importance of adaptations of the tardigrade to know the benefits offered by this organism in various fields.

The Phylum Tardigrada comprises two classes, the Eutardigrades with characteristics that lack dorsal plates, cirrus (hairs), have two pairs of claws on each leg (more often), are fresh and terrestrial water (more often) and lay eggs ornamented and the Heterotardigrada class has the dorsal plates and the cirrus with four or more solitary claws for each leg (more frequently), are terrestrial / marine (more frequently) and eggs without ornaments or flat (Guayasamín, 2018). In Pastaza province, they have not conducted considerable research on tardigrades, so they are one of the least studied groups. This research becomes the first one carried out on the Río Puyo tourist promenade, which contributes to the knowledge of the diversity of the country, which includes 14 individuals from the class of eukaryotes and Heterotardigrada distributed in the place, contributing to the study of a new investigation.

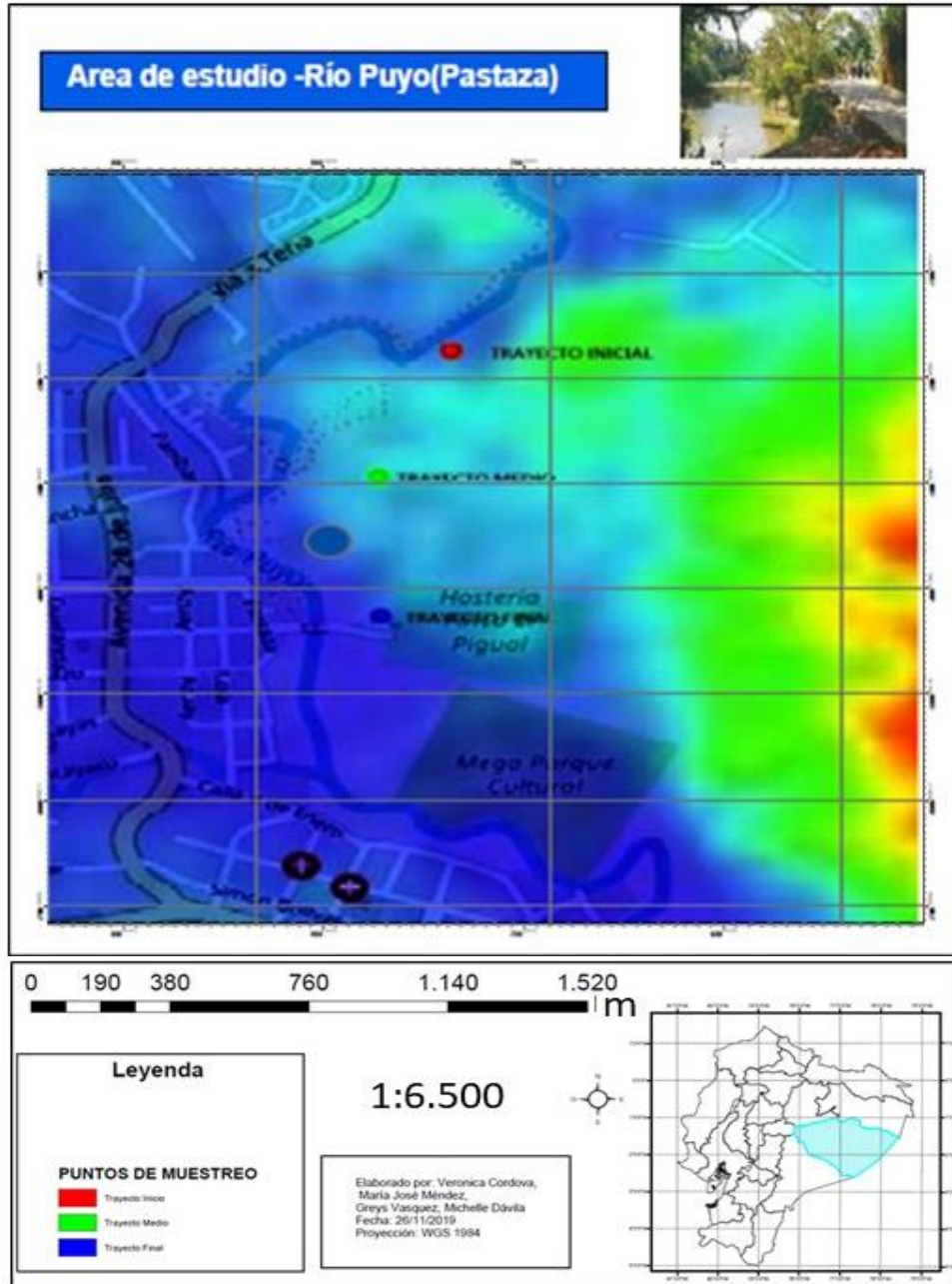
Materials and Methods

The tools used in this research are experimental and bibliographic, covering a collection of interesting studies of tardigrades, backed by a sampling area on the Tourist route of the Puyo River.

Sampling area: the samples were collected in moss, bark and stone substrates, in three different sites, in the initial part of the Puyo river tourist route to the coordinates **S 01 ° 28.651 'W 077 ° .59.914'**, middle part **S01 ° 28. 460 'W077 ° 59.890'** and final part of the Paseo **S01 ° 28. 182 'W077 ° 59.697'**. The place where the sampling is performed is located in the Barrio Obrero, an ecological path known as Paseo Resort, this was the first tourist complex that was created in the province of Pastaza with ecological and natural vision built by the city municipality, the sector is publicly owned, is located in the Park del Barrio Obrero. It is a kilometer and a half road that runs along the river bank, is about 20 meters wide. It has a humid climate that makes it essential for the collection of tardigrade. The average temperature is from 22 to 23 ° C. (Independiente, 2015).

Collection and identification: 6 samples, 2 were collected in each of the Carrige, samples were run level tree moss on substrates and stone. The samples were stored on paper and then insert them in plastic sleeves to be transported to the workplace. Then he proceeded to remove samples of plastic sleeves to introduce water in Petri dishes for them to soak overnight. A next followed to drain the samples and water-soaked in another Petri dish for further analysis in which a digital microscope of 1000X was used, one observed after the specimen was photographed to continue identification. instruments like open books and publications, slide and cover glass, Petri dish and GPS coordinates to take place was also used.

Figure 1. Map of the study area. Collection zones are shown on the Tourist route of the Puyo River, in three respective areas of the site.



(Cordova Davila Vasquez Mendez, 2019)

Bibliographic results

Internal and External morphology of the *Tardigradus*

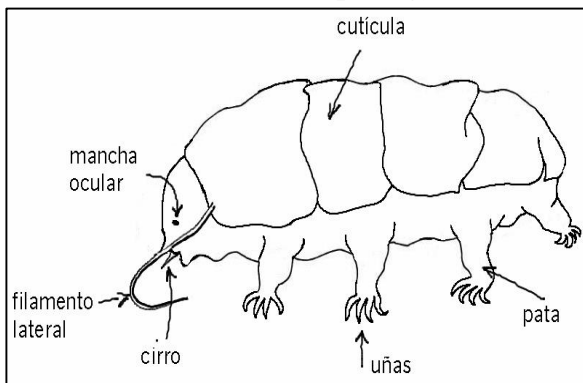


Figure 2. External parts of the tardigrade (Guayasamin, 2018)

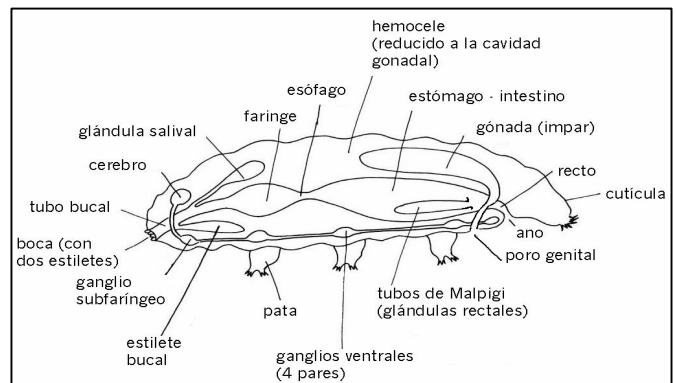


Figure 3. Internal parts of the tardigrade (Guayasamin, 2018)

Covered with cuticle (endo, exo and meso), with legs unsegmented ending in claws, mouthparts are a tube with two stylets to suck juices prey. They live in marine habitats, fresh water and land, have bilateral symmetry, triploblastic-protostomes body, breathing through the surface of the body (gills, trachea, lungs book). In each sex tardigrades presents a unique gonad; male genital pore is located in front of the anus and female genital pore is located in front of the anus or along with it. Females reproduce by parthenogenesis often (is the development of an individual from a female sex cell that has not been fertilized) (Pliego Robles , 2014). External or internal fertilization (within molting) are oviparous, have atypical holoblastic segmentation that produces a stereogastrula (a formation of the enterocoelomic coelom) where five pairs of pockets are formed, the first four disintegrate and the last pair binds and causes the gonad. There are five types of cryptobiosis, one that is Anhydrobiosis that occurs in extreme desiccation Anoxibiosis happens in situations absence of oxygen, Quimiobiosis that occurs in response to high concentrations of toxins, Cryobiosis occurring against low temperatures and Osmobiosis which is a response to an external environment extremely concentrated (Guayasamín, 2018).

Studies to tardigrades in technological applications

Cryptobiosis: A Survival Phenomenon

Tolerance radical changes is one of the salient features in tardigrades. Because of this ability, they have managed to survive environmental conditions in which most living things die, as temperatures ranging from absolute zero to very high temperatures. In space as evidenced in 2011 from two species of tardigrades: *Richtersius coronifer* and *Milnesium tardigradum*. In the process of cryptobiosis there are certain compounds that play an important role as the disaccharide. Among the most important are trehalose and sucrose functioning as bio protective agents. There hypothesis suggesting that the protective capacity of these disaccharides is due to changes in the tetrahedral arrangement of water molecules in the hydrating layer carbohydrate. This was observed in the trehalose dissolved because many forms hydrogen bonds with the solvent. As a result of this property, trehalose can generate a protective barrier dry at high temperatures and rehydrated time does not harm cellular function is maintained. Accordingly, it has been considered as a means to retain proteins and cells at room temperature without performing the freeze-drying process (drying process wherein the solvent (water) is frozen and subsequently removed by sublimation under vacuum atmosphere) This was observed in the trehalose dissolved because many forms hydrogen bonds with the solvent. As a result of this property, trehalose can generate a protective barrier dry at high temperatures and rehydrated time does not harm cellular function is maintained. Accordingly, it has been considered as a means to retain proteins and cells at room temperature without performing the freeze-drying process (drying process wherein the solvent (water) is frozen and subsequently removed by sublimation under vacuum atmosphere) This was observed in the trehalose dissolved because many forms hydrogen bonds with the solvent. As a result of this property, trehalose can generate a protective barrier dry at high temperatures and rehydrated time does not harm cellular function is maintained. Accordingly, it has been considered as a means to retain proteins and cells at room temperature without performing the freeze-drying process (drying process wherein the solvent (water) is frozen and subsequently removed by sublimation under vacuum atmosphere) (Fernández Arévalo, 2010)It is noteworthy that cryptobiosis is not the same as diapause, because although both are idle states in the cryptobiosis there are factors specific triggers which comes in a state of genetically predetermined development, the process occurs regeneration when conditions become favorable. Diapausing, however, metabolic activity remains low even when environmental changes become favorable. (Estrada García , Ascencio Rosado, & Hernández Dávila, 2017)

¿How it happens and how the tardigrades survived in these states cryptobiosis?

A crucial element is the protective layer called "tun" which is the result of the invagination of its unarticulated legs, the body is contracted and wound longitudinally wraps and cuticle of the body inward. For the formation of the same, relative humidity is required, between 70 and 95% in their metabolism, in addition to trehalose synthesis (is a carbohydrate with little sweetness, very soluble in water and used as an energy source and for forming of the exoskeleton of chitin in many insects. It is part of cell membranes of various insects and microorganisms who synthesize) (Parada Puig, 2011).This allows the Tardigrade enter cryptobiotic state in this state trehalose and

sucrose (and other molecules) become amorphous crystals that are responsible for protecting the cell and to stop their metabolic processes. (Pérez-Plancarte, Trejo-Moreno, & Xocopa-Castro, 2016).

¿How a protein of the tardigrade could protect human DNA from sunlight?

Natural publication of Communications has identified which gives the tardigrade their super powers: a protective protein that provides resistance to harmful rays X. Researchers have succeeded in transferring this resistance to human cells. "It is believed that tolerance is a secondary X-rays characteristic to the adaptability of animals to intense extreme dehydration," says Takekazu Kunieda, molecular biologist at the University of Tokyo and author of the study. According to Kunieda, extreme dehydration cause havoc intense molecules of living beings. You can even destroy the DNA, and the like X-rays The researchers wanted to find out how tardigrades were protected against such harsh conditions. In this way, Kunieda and his colleagues began by sequencing the genome of *Ramazzottius varieornatus*, a species particularly stress tolerant. Is easier to study processes within cells of the Tardigrade when the genome of the animal is inserted into mammalian cells, said Kunieda. So the researchers decided to manipulate human cell cultures to produce the components of the internal machinery of the tardigrade, in order to determine which parts confer resistance to animals.

Ultimately, Kunieda and colleagues found that a protein known as Dsup ('protein damage suppression') prevented radiation and desiccation destroy the DNA of the animal. And also they found that human cells with certain cellular components of the Tardigrade managed remove 40% damage induced by X-rays "Protection and repair of DNA are a fundamental feature of all cells and central to many diseases human, including cancer and aging," says Ingemar Jönsson, evolutionary ecologist who studies the tardigrades in Kristiansand University in Sweden. Hence the findings of the new article prove of great interest for medicine, says Jönsson. With them opens the possibility of improving stress resistance of human cells, which could one day benefit people who must undergo radiation treatments. Kunieda adds that these discoveries in the future could protect radiation workers in nuclear facilities or possibly help us develop crops in extreme environments, such as exist on Mars. Bob Goldstein, a biologist at the University of North Carolina at Chapel Hill, who helped sequence the genome of another species of tardigrade highlights the interest of the investigation. Agrees with the authors predict that their discovery represents only the first of many more. "The tardigrade is resistant to a wide variety of extreme situations," says Goldstein. Which means that animals should have many different ways to protect themselves. "We have only just begun to explore the genetic treasure that represents the genome of the tardigrade" says Jönsson (Bittel, 2016).

In the field of genetic engineering allow tardigrades What?

With the use of seed banks generate cryptobiosis could to keep different species of plants through genetic engineering techniques, allowing maintenance conditions of these sites are less strict and more lasting benefits. This would decrease the rate of extinction and loss of diversity to provide the tools to colonize habitats in need. (Estrada García & Ascencio Rosado, 2017)

The tardigrade, the only living thing to survive an astronomical cataclysm

The tardigrade is the last survivor on Earth. No matter if an asteroid collides against the surface of the planet or if a supernova explodes in a nearby galaxy, in any case, be a way of life that survive on our planet. Only the death of the Sun could end the tardigrade. A new study published in the journal "Scientific Reports" says the tardigrade (*Hypsibius dujardini*) could survive a possible extinction caused by any possible astrophysical catastrophe. According to the authors, it will remain on the face of the Earth much longer than humans. Lamp is turned off only if the Sun, scientists estimate what will happen in 10,000 million years, would end this species. To reach this conclusion, scientists have considered three potential astronomical events that could end life on the planet. These are the impact of a meteorite, stellar explosion as a supernova and a burst of gamma rays. None of them would be a risk for tardigrades, according to the study (Autonomo, 2017).

Field Results

15 individuals of Eutardigrade and Heterotardigrada class were found, with 10 individuals of the first class and the second class 4. medication diversity index which will be used in index Margalef based on the numerical distribution of individuals of different species (in this case is the 2 kinds of tardigrades) depending on the number of individuals existing in is also included the sample. In this method uses values <2 is the low range and values > 5 which is highly diverse. (Moreno C. , 2001)

Table 1. Catalog Class Tardigrada and Eutardigrade collected, in 3 different sampling areas (A, B, C) of the Paseo Resort Rio Puyo. The samples belong to the microhabitat is the Moss (M), which were collected on substrates: 1 (bark) and 2 (stone) 0 (no substrate) Class: EU (Eutardigrade) HETERO (Heterotardigrada). CI (number of individuals)

<i>Area's</i>	<i>Clases</i>	<i>Microhábitats</i>	<i>Substrate</i>	<i>CI</i>
A	EU	M	1, 2	5
	HETERO		1	1
B	EU	M	2	3
	HETERO		1	3
C	EU	M	1,2	2
	HETERO		2	0
Total				14 I.

(Córdova, 2019)

table 2. Diversity indices tardigrades, and Heterotardigrada eutardigrades class. S = total number of species, N = total number of individuals.

<i>Class</i>	<i>No. of individuals</i>
<i>Eutardigrade</i>	10
<i>Heterotardigrada</i>	4
Total	14

(Mendez 2019)

$$Dmg = \frac{S-1}{\ln N} = \frac{2-1}{\ln(14)} = \frac{1}{3} = 0,3 < 2 \text{ baja diversidad}$$

Taxonomic key used for species identification is: Eutardigrade with features lacking dorsal plates, cirrus (hairs), have two pairs of claws on each leg, are terrestrial and freshwater and lay eggs ornamented and Heterotardigrada class has dorsal plates and cirrus with four or more claws lonely by each leg are / seascapes and eggs without ornament or planes. (Guayasamín, 2018). In this case no eggs were found in these kinds of tardigrades. In the diversity index tardigrades in Carrige, we note that the results showed a low diversity of tardigrades of Eutardigrade and Heterotardigrada class.



Figure 4. Photograph taken from a digital microscope of the Eutardigrada Class. (Vásquez, 2019)



Figure 4. Photograph taken from a digital microscope of the Heterotardigrada Class. (Dávila, 2019)

Discussion

According to our analysis, as part of our final result of the investigation, we can make a comparison in our sampling area, which was held in moss two kinds of tardigrades Eutardigrada and Heterotardigrada in two different strata. What are they; tree bark and stone according to this we can say that was accessible sample it on moss because in this non-vascular plant there is much more likely to find this microorganism. Unlike what was spoken of tardigrades found in this study. two types of lichen and a community of bryophytes: three different microhabitats were selected. In the sampling area cohabiting systematically *Xanthoria parietina* species and species *Physcia adscendens* (as the most common of its kind). As for bryophytes, spp *Orthotrichum* samples were collected on substrates cortex, whereas in the petrous substrates samples were communities comprising the genera *hypnum* L, *Bryum* Hedw and *Ditrichum* Hampe. In these communities in which genres alternate with clustered growth is impracticable to address each of them separately because they grow so cushion as if it were a single individual. To refer to both these groups as gender *Orthotrichum* we will generically as "moss". In these communities in which genres alternate with clustered growth is impracticable to address each of them separately because they grow so cushion as if it were a single individual. To refer to both these groups as gender *Orthotrichum* we will generically as "moss". In these communities in which genres alternate with clustered growth is impracticable to address each of them separately because they grow so cushion as if it were a single individual. To refer to both these groups as gender *Orthotrichum* we will generically as "moss" (Alíer Giménez, 2016). Relatively this work is done with greater complexity, as sampled species, while analyzed in our work is more accessible and accurate sampling at tardigrades class level.

Conclusions

In conclusion, research on the tardigrade have been very important, emphasizing the cryptobiosis has been one of the main features for new knowledge of technological applications that make it an ideal model for many studies also are a good strategy for the study of medicine, especially because these organisms have the ability to repair DNA damage, they represent a breakthrough in the study of genetics and finally knowledge can be obtained from its study is very broad and can be taken in many areas, hence the importance and the need to expand research.

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