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MICROFOSSILS BIOSTRATIGRAPHY AND PALEOENVIRONMENTAL RECONSTRUCTION OF THE LATE PLEISTOCENE LACUSTRINE SAN MIGUEL FORMATION, GUAYLLABAMBA BASIN, ECUADOR.

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DEDICATION

This work is mainly dedicated to my beloved mother and father, Narcisa Villon Mora and Ernesto Zamora Vera respectively, who have supported me in this chapter of my life.

Of course, a special dedication to my current beloved girlfriend Karla Miño who will be my future wife and has been next to me almost since the beginning of this college period.

I also dedicate this work to a reference person in my life, the constitutional former president of Ecuador, Ec. Rafael Correa Delgado, who is affectionately known as 'Mashi'. He has inspired me to fight for my dreams in a correct way, with science, and with conscience, in order to reach the well-being of my country. This achievement is principally due to him, whose love for a better Ecuador, decided to start the creation of this important higher-education public-project to change the future of this country based on the formation of pure and competent scientists focused on the researching of pure sciences. Currently, graduated professionals that were formed at the public Yachay Tech University, are representing the name of Ecuador in different places of the world, by developing important research projects, master's programs, PhD programs, and many other important social and professional activities. I am sure the most important results will be visible in some years in the world and in Ecuador.

To some of my former professors and teachers of my high school period: Leonardo Carrion that taught me the science of mathematics, Nelson Lindao that taught me the science of physics, Holger Choez that taught me the science of chemistry, Oswaldo Granados that taught me scientific research subject, and Miguel Vera that taught me economy subject. Of course, also to all my professors of the college, that have taught me all the necessary knowledge I need for my professional career. I consider that all my previous professors were relatively important, but honestly, I really think that the professors I dedicate this work, were the most important during my personal formation, which is reflected in the person I am right now.

To special friendships of the high school: Richard Garcia who is now a Petroleum Eng., Richard Bauz who is now studying laws, and Julissa Saula who is now studying medicine. They have been my most close friends since I finished the high school period in my hometown.

Finally, a crazy dedication to my lovely deceased dogs, 'peluchin', 'princesa' and 'vaco', and to my current dogs 'oso' and 'melcocho'. All of them have showed me the real unconditional love and loyalty that any person in this world needs in their life.

Joaquin Zamora Villon

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Joaquin Zamora

Resumen:

La cuenca de Guayllabamba se encuentra en la depresión interandina central (DIA) del Ecuador. También conocida como depresión de Guayllabamba, es clasificada como una semicuenca separable que comenzó a formarse en el Pleistoceno tardío. La mayor parte de la investigación previa realizada en Guayllabamba se ha enfatizado más en una perspectiva general de enfoques geodinámicos, estructurales, sedimentológicos y litológicos, y carece de análisis detallados en el campo paleoambiental. Dentro de la depresión de Guayllabamba, específicamente en la Formación San Miguel, se pueden encontrar depósitos fluvialeslacustres. En este trabajo, se utilizaron técnicas micropaleontológicas basadas en análisis de microfósiles para comprender las implicaciones paleoambientales en la Formación San Miguel. Así, el propósito principal de este trabajo es desarrollar y proponer una reconstrucción paleoambiental del paleolago, basada en análisis sedimentológicos, bioestratigráficos y paleoecológicos con microfósiles de diatomeas de agua dulce extraídos de sedimentos lacustres varvados y masivos de dos secciones estratigráficas de la Formación San Miguel, Tanda y Puéllaro. Finalmente, realizamos dos registros estratigráficos en cada sección, y mediante la técnica de deslizamiento de frotis, identificamos un total de 6 morfotipos, 13 géneros y 22 especies de diatomeas utilizando un microscopio óptico. Mediante correlación de las condiciones paleoecológicas que requirieron las diatomeas, se pudo comprender las implicaciones paleoecológicas en la evolución del lago, y así, se propuso una reconstrucción paleoecologica de 3 fases y una reconstrucción paleoambiental del lago.

Palabras claves:

Diatomeas, Cuenca de Guayllabamba, formación San Miguel, paleoecología, paleoambiente.

Abstract:

Guayllabamba Basin is located at the Central inter-Andean Depression (IAD) of Ecuador. Also known as Guayllabamba depression, is classified as a pull-apart semi-basin that started to form in the Late Pleistocene. Most of the previous research done in Guayllabamba have been more emphasized to a general perspective of geodynamical, structural, sedimentological and lithological approaches, and lack of detailed analyses in the paleoenvironmental field. Inside the Guayllabamba depression, specifically at the San Miguel Formation can be found fluvial-lacustrine deposits. In this work, micro-paleontological techniques based on microfossils analyses, were applied in order to understand the paleoenvironmental implications in the San Miguel Fm. Hence, the main purpose of this work is to develop and propose a paleoenvironmental reconstruction of the paleolake, based on sedimentological, biostratigraphical, and paleoecological analyses with freshwater-diatoms microfossils extracted from the lacustrine varved and massive sediments of two stratigraphic sections of the San Miguel Formation, Tanda and Puéllaro sections. We finally performed two stratigraphic logs in each section, and by smear slide technique we identified a total of 6 morphotypes, 13 genera and 22 diatoms species using a light microscope. By correlating the paleoecological conditions that diatoms required, it was understood the paleoecological implications during the paleolake evolution, and therefore, are proposed a three-phases paleoecological reconstruction and a one paleoenvironmental reconstruction of the lake.

Keywords:

Diatoms, Guayllabamba basin, San Miguel formation, paleoecology, paleoenvironment.

Table of Content

List of Tables	ii
List of Figures	iii
1. Introduction	1
1.1 Problem statement	
1.2 General and specific objectives	4
2. Review of freshwater diatoms and freshwater components as paleoecologic paleoenvironmental indicators	al and:4
2.1 Freshwater diatoms	4
2.2 Freshwater sponges' spicules	6
2.1 Freshwater phytoliths	7
3. Review of varves in lake sediments	8
4. Theoretical Framework	10
4.1 Regional geologic framework of Ecuador	10
4.2 Geology of the inter-Andean depression (IAD)	10
4.3 Geology and stratigraphy of the Guayllabamba basin	11
4.3.1 General geologic setting of Guayllabamba basin	11
4.3.2 Stratigraphy of Guayllabamba	12
4.3.2.1 Pisque Formation	12
4.3.2.2 San Miguel Formation	13
4.3.2.3 Guayllabamba Formation	14
4.3.2.4 Chiche Formation	14
4.3.2.5 Mojanda Formation	15
5. Methodology	15
5.1 Description of stratigraphic logs	16
5.2 Paleoecological analyses	16
5.2.1 Diatom microfossils preparation by smear slide	17
5.2.2 Counting of abundances of the components	18
5.2.3 Definition of morphotypes and identification of some species of diatoms	18
5.2.4 Biostratigraphic analyses	20
5.3 Paleoenvironmental analyses	20
6. Results	21
6.1 Description of the stratigraphic successions	22
6.1.1 General description of the Tanda (GT) section	22
6.1.1.1 Description of the stratigraphic succession of the Tanda (GT) section	22
6.1.2 General description of the Puéllaro (GP) section	25
6.1.2.1 Description of the stratigraphic succession of the Puéllaro (GP) section	25
6.2 Description of the components and counting of their abundances	28
6.2.1 Description of the components	28

6.2.2 Counting of abundance of components
6.2.2.1 Results of the counting of abundance of components in Tanda (GT) section 30
6.2.2.2 Results of the counting of abundance of components in Puéllaro (GP) section 33
6.3 Definition of morphotypes and identification of some species of freshwater diatom 35
6.3.1 Definition of morphotypes
6.3.2 Identification of some diatom species
6.3.2.1 Planktonic (Centrales) diatoms 41
6.3.2.2 Benthic (Pennales) diatoms
6.4 Biostratigraphy of freshwater diatoms in the Tanda and Puéllaro sections
6.4.1 General distribution of freshwater diatoms in the Tanda and Puéllaro sections 52
6.4.2 Biostratigraphy of freshwater diatom in the Tanda and Puéllaro sections
6.4.2.1 Biostratigraphy of freshwater diatom in the Tanda (GT) sections
6.4.2.2 Biostratigraphy of freshwater diatom in the Puéllaro (GP) sections
6.4.3 The attempt to constructing a lithostratigraphic correlation between the Tanda (GT) and Puéllaro (GP) sections
6.4.4 The attempt to constructing a biostratigraphic correlation between the Tanda (GT) and Puéllaro (GP) sections
. Discussion
7.1 About the results of sponges' spicules and phytoliths in Tanda and Puéllaro sections . 59
7.1.1 Abundance values, scarce and dissolution of sponges' spicules and phytoliths in dependence of the paleoecological conditions of the paleolake
7.1.1.1 Sponges' spicules implications60
7.1.1.2 Phytoliths implications
7.2 About the results of freshwater diatoms in the Tanda and Puéllaro sections
7.2.1 Interpretation of the counting results: the changes in the abundance values of freshwater diatoms found in the Tanda (GT) and Puéllaro (GP) sections
7.2.1.1 Dominance, scarce and dissolution of diatoms in some specific stratigraphic levels of Tanda (GT) and Puéllaro (GP) sections
7.2.2 Interpretation of the diatom biostratigraphy logs, and the changes in the associations of freshwater diatom species identified in the Tanda (GT) and Puéllaro (GP) sections 64
7.2.2.1 Dominance of planktonic and benthic diatoms species in the Tanda (GT) and Puéllaro (GP) sections respectively
7.2.3 About the paleoecological conditions of the identified diatoms species
7.2.3.1 Planktonic (Centric) freshwater diatoms
7.2.3.2 Benthic (Pennate) freshwater diatoms
7.3 Paleoecological and paleoenvironmental reconstruction of this paleolake
7.3.1 Paleoecological reconstruction of this paleolake71
7.3.2 Paleoenvironmental reconstruction of this paleolake
3. Conclusions
9. References

List of Tables

Table 1. Suprageneric classification of the diatoms	6
Table 2. Geographic coordinates of the two outcrops studied in this research project	21
Table 3. Results of the counting of abundances of components in the Tanda (GT) section	31
Table 4. Results of the counting of the components in the Puéllaro (GP) section	33
Table 5. Summary of the morphotypes identified in the Tanda (GT) and Puéllaro (GP) section	ons 40
Table 6. Results of the identified morphotypes and species of freshwater diatoms microfoss in the 40 plates	sils 52

List of Figures

Figure 1. Centric diatom(s) with their respective parts	5
Figure 2. Pennate diatom(s) with their respective parts	5
Figure 3. Drulia uruguayensis freshwater sponge	7
Figure 4. Images of typical phytolith morphotypes	8
Figure 5. Geotectonic and geomorphological map of Ecuador and surrounding basins	. 10
Figure 6. Stratigraphy of Guayllabamba basin	. 13
Figure 7. Classification of diatom morphotypes	. 19
Figure 8. Study area of this project	. 21
Figure 9. Photos of the Tanda (GT) section	. 23
Figure 10. Stratigraphic log of the Tanda (GT) section with its sedimentary structures and for content.	ossil 24
Figure 11. Photos of the Puéllaro (GP) section	. 26
Figure 12. Stratigraphic log of the Puéllaro (GP) section with its sedimentary structures fossil content	and 27
Figure 13. Complete and fragmented needle-shaped sponges' spicules in the light microsc	ope 28
Figure 14. Complete and fragmented phytoliths in the light microscope	. 29
Figure 15. Fragmented detritic grains in the light microscope	. 29
Figure 16. Complete and fragmented types of diatom in the light microscope	. 30
Figure 17. Stratigraphic log and results of the counting of abundances of components in Tanda (GT) section	the 32
Figure 18. Stratigraphic log and results of the counting of abundances of components in Puéllaro (GP) section	the 34
Figure19. Monoraphid diatoms from Tanda (GT) and Puéllaro (GP) sections	. 36
Figure 20. Three types of centric morphotypes diatoms from Tanda (GT) and Puéllaro (sections	GP) 37
Figure 21. Epithemioid diatoms from Tanda (GT) and Puéllaro (GP) sections	. 38
Figure 22. Asymmetric biraphid diatoms from Tanda (GT) and Puéllaro (GP) sections	. 39
Figure 23. Symmetric biraphid diatoms from Tanda (GT) and Puéllaro (GP) sections	. 39
Figure 24. Araphid diatom just from Puéllaro (GP) section	. 40
Figure 25. Non-identified morphotypes of diatoms from Tanda and Puéllaro sections	. 40
Figure 26. Aulacoseira pusilla species	. 42
Figure 27. Aulacoseira granulata species	. 43
Figure 28. Aulacoseira herzogii species	. 43
Figure 29. Aulacoseira temperei species	. 43
Figure 30. Cyclotella meneghiniana species	. 44
Figure 31. Discostella pseudostelligera species	. 44
Figure 32. Discostella stelligera species	. 45

Figure 33. Cocconeis placentula var. Euglypta species
Figure 34. Cocconeis pseudothumensis species
Figure 35. Rhopalodia gibba species
Figure 36. Rhopalodia gibberula species
Figure 37. Rhoicosphenia curvata species
Figure 38. Cymbella tumida species
Figure 39. Gomphosphenia lingulatiformis species
Figure 40. Gomphosphenia grovei species
Figure 41. Gomphoneis pseudo-okunoi species
Figure 42. Sellaphora alastos species
Figure 43. Navicula lundii species
Figure 44. Navicula radiosa species 50
Figure 45. Karayevia laterostrata species
Figure 46. Karayevia laterostrata species
Figure 47. Synedra goulardii species
Figure 48. Biostratigraphic log of the Tanda (GT) section
Figure 49. Biostratigraphic Log of Puéllaro (GP) section
Figure 50. Proposed paleoecological reconstruction of the paleolake formed in San Miguel Fm., Guayllabamba, Ecuador during the Late Pleistocene
Figure 51. Proposed paleoenvironmental reconstruction of the paleolake formed in San Miguel Fm., Guayllabamba, Ecuador during the Late Pleistocene

1. INTRODUCTION

Micropaleontological techniques are commonly used for developing paleoenvironmental and paleoproductivity reconstructions of a determined area. Since the XIX century is already known that diatoms microfossils, that have been deposited in lacustrine environments, are considered useful paleoecological tools for interpreting the history of paleolakes (Hecky & Kilham, 1973). Currently, the use of microfossils in applied geological investigations as in paleoenvironmental and biologic assemblages, has become crucial (Saraswati & Srinivasan, 2015). Thanks to their size and large abundance, a relatively small quantity of dry sediment with microfossils content can usually yield enough data for the application of more rigorous quantitative methods of analysis and at the same time, those microfossils can, therefore provide many important and relevant clues for biostratigraphic and paleoecological interpretations (Berggren, 1998). Due to both, past organisms and their environments cannot be observed directly, the use of paleoecological proxies are used for interpreting past occurrences, distributions, and abundances of organisms. Therefore, the data from that geological and biological evidence can be used to reconstruct past populations, communities, landscapes, environments, and ecosystems (Birks, 2008).

Continental Ecuador is crossed by the N-S trending Andean ranges. In the core of these ranges appears a tectonic depression, named in this research as inter-Andean depression (IAD). This depression hosts several intramontane basins, called from north to south as: Chota, Guayllabamba and Ambato-Latacunga. The sedimentary filling of these intermontane basins is an excellent record of the uplift, erosion and climate history of the nearby ranges (Nocentini *et al.*, 2017; Sobel *et al.*, 2003; Streit *et al.*, 2017). The Guayllabamba basin is interpreted as a pull-apart semi-basin that started to form in the Late Pleistocene (Villagómez, 2003). This basin is filled by volcanic, fluvial, alluvial and lacustrine sediments. The Guayllabamba basin contains a thick lacustrine stratigraphic unit, called San Miguel Formation, which is made up of massive and varved diatomites interbedded with volcanic, fluvial, deltaic, turbiditic and gravity flow deposits. Those diatomaceous lacustrine successions have important and relevant microfossils content that serve as tracking tools for understanding the changes in the behavior of a paleolake and their relationship with the paleoenvironment and paleoclimate of the area.

Freshwater diatoms, that are siliceous microfossils, can be analyzed for understanding the past environment of the San Miguel Fm. in the Guayllabamba Basin. Those lacustrine sediments are characterized by millimeter rhythmic interbedded of light and dark laminas named varves, where the couple light-dark lamination represents one year. These alternations are related to seasonal climatic oscillations. In this case the study area is located at the tropical area of Ecuador, where dry and wet seasons seems to be the most adequate for the analyses. Alhonen (1986) stated that lacustrine microfossils are useful for a good reconstruction of typological water level changes in lakes and climatic fluctuations. Hence, lacustrine microfossils are also useful for inferring paleo-productivity and lake regimes. So much so that during last years, many micropaleontologists have been constantly using several microorganisms in order to solve contemporary issues of environment and climate change (Jones, 2006).

Quaternary diatoms are useful indicators of local habitat changes from terrestrial to deep marine environments and provide insight into relative lake-level and sea-level changes and water chemistry (Armstrong & Brasier, 2005). The diatoms assemblages have been classified -the Halobian system (Hustedt, 1957)- according to their salinity preferences. Therefore, these siliceous microfossils are paleoecologically important, and a careful identification, sound taxonomy, and unambiguous nomenclature are essential (Birks, 2008) for a good understanding of the area. The classification of diatoms has been traditionally based on two parameters: (i) frustule form, that is the cellular wall made up of opaline silicon, and (ii) sculpture. Two orders are widely recognized, namely the Centrales (Fig. 1) and Pennales (Fig. 2). If frustules have a radial symmetry are considered centric diatoms, and when frustules have an elongated bilateral symmetry are considered pennate diatoms (Round & Crawford, 1990). From 10% to 30% of the valve surface area is covered by tiny pores that are called punctae, the arrangement of which is also significant for classification, perforate surface. Moreover, the region of overlap between the epivalve and hypovalve is called the girdle, and a study of the valve and the girdle view aids the identification (Armstrong & Brasier, 2005).

Moreover, in this work we also have to take into account that freshwater diatoms are not the only microorganisms that can appear in the sediments of the two studied stratigraphic sections. Sponges' spicules (in fossil state) and phytoliths (biominerals of vegetal origin) are the other siliceous components necessary to analyze in order to correctly understand the paleoecology of the paleolake we are studying.

Paleoecology is mainly concerned with reconstructing past biota, population communities, landscapes environments, ecosystems, or with the description of the features of an organism from the available geological and biological (fossil) evidence (Birks, 2008). In few words, paleoecology is the ecology of the past that deals with the understanding of the relationships between organism and their environment in the past. Advantageously, paleoecology can generally be studied in any period of the Earth's history in which life was present (Birks, 2008) as long as there is geological and paleontological (fossils) evidence.

Biostratigraphy deals with the documentation, analysis, and interpretation of the ordered succession of fossils, their relationships to evolving earth and life history, and the application to the elucidation thereof (Emery & Myers, 1996; Jones, 1996; Doyle & Bennett,

1998; Briggs & Crowther, 2008; Coe *et al.*, 2003; Harries, 2003; Gradstein *et al.*, 2012). Biostratigraphy can also provide a high-resolution basis for the division of strata, and therefore, we can correlate among different successions.

Paleoenvironments are highly dependent on many aspects and conditions throughout time. Environmental information (e.g.: climate, lake level, and water temperature) can sometimes be obtained from the sediments in which fossils are preserved by analyzing those sediments. For example, changes in lake-water pH can be inferred from changes in the composition of fossil diatom assemblages, under the assumption that the ecological preferences of the modern taxa are the same as they were in the past (Birks, 2008). Paleoenvironmental interpretation based on various microfossil groups -in this case for diatoms- is essentially based on: analogy with their living counterparts, functional morphology, associated fossils, and sedimentary facies (Jones, 2006). In fact, a wide range of environmental interpretations can be made with diatoms on the basis of: their life histories, habitats, and ecological preferences (Bradbury & Krebs, 1995). Since diatoms have been considered useful paleoecological tools (Hecky & Kilham, 1973), they can also be successfully used to describe the paleoenvironmental history of the paleolake studied in this research project. The intention in this section is focused on interpreting past limnological conditions from diatoms and subsequently extrapolating the paleoenvironmental implications of the San Miguel Formation, that is determining past lacustrine conditions of the Guayllabamba Basin.

Finally, in order to reach the main purpose of this research project, that is proposing a paleoenvironmental reconstruction of San Miguel Formation, is necessary to correlate the entire data obtained mainly from the paleobiological (fossil), sedimentological and stratigraphical evidence in the two stratigraphic sections studied here.

1.1. Problem statement

Most of the previous research done in the Guayllabamba basin have been more emphasized on tectonic and stratigraphic approaches, and lack of detailed micropaleontological analyses. In this work, micro-paleontological tools, based on microfossils analyzes, were used in the lacustrine San Miguel Fm. in order to determine: the vertical evolution of diatom-productivity and to reconstruct the paleoecology of this lake.

This study represents an important input for the knowledge of the central IAD of Ecuador during the Pleistocene. The study of the sedimentary infilling of the San Miguel Fm. has a great importance due to the need to know more about the climate during the Pleistocene. In fact, it is already know that Pleistocene is characterized by many changes in the climate (Park & Maasch, 1993; Nie, 2018), and in the continental Ecuador, is then important to register

those paleoenvironmental changes related to the fast uplifting of the Andean ranges (Nocentini *et al.*, 2017; Sobel *et al.*, 2003; Streit *et al.*, 2017; Delfaud *et al.*, 1999; Spikings *et al.*, 2010).

1.2. General and specific objectives

The main purpose of this research project is to develop and propose a paleoecological and paleoenvironmental evolution of the lacustrine San Miguel Formation, within the Guayllabamba basin. We carried out the identification and counting of diatoms together with other siliceous microfossils and components, as well as the interpretation of their abundances. Two stratigraphic sections of the San Miguel Fm. have been sampled to carry out this research, one in distal reaches of the paleolake and the other one in proximal realms. The specific objectives of this work were the following:

- To describe the two stratigraphic successions studied.
- To calculate the abundances values of each one of the components such as diatoms, sponges' spicules, phytoliths and detritic grains in each sample of both stratigraphic sections.
- To define morphotypes and to identify some species of freshwater diatom.
- To reconstruct the paleoecology of the lake based on the differences in the associations of the diatom species between the two stratigraphic sections.

2. REVIEW OF FRESHWATER DIATOMS AND FRESHWATER COMPONENTS AS PALEOECOLOGICAL AND PALEOENVIRONMENTAL INDICATORS.

2.1 Freshwater diatoms

Diatoms, or bacillariophytes, are an extant Jurassic-Recent group of unicellular algae that occurs as solitary cells or in colonies that are characterized by golden-brown or yellowish photosynthetic pigments that differ from other chrysophytes in lacking flagella. Their main feature is their cell wall or shell is composed by opaline silica, this is, silicified, and is known as frustule, which also comprises two valves, one overlapping the other like the lid of a box (Round *et al.*, 1990; Blome *et al.*, 1996; Stoermer & Smol, 1999; Armstrong & Brasier, 2005; Hasle *et al.*, 1996). The larger of the two valves (dorsal) is called the epivalve or epitheca, while the smaller one (ventral) is called the hypovalve or hypotheca (Fig. 1, 2). In the fossil state, most frustules are separated, so this distinction is not especially important (Burckle, 1998).

It is well known that diatoms can live in almost all kind of aquatic and semi-aquatic environments that are exposed to light, and their remains may accumulate in enormous numbers forming diatomites (Armstrong & Brasier, 2005). Therefore, diatoms are autotrophic, that is, they manufacture their own food in the photic zone through the process of photosynthesis. In fresh-water and marine environments, diatoms can be found occupying a great number of niches. On land, diatoms can be found in soils and occasionally on wetted rocks and plants, while in streams, lakes, and ponds, diatoms can be found attached to rocks, plants, and in bottom muds (Burckle, 1998).



Fig. 1. Centric diatom(s) with their respective parts. (A) SEM image (taken from Taylor *et al.*, 2007), and (B) drawing images of valve and girdle views of: (a) *Melosira*, valve view (left) and girdle view of colony (right, about \times 342). (b) *Coscinodiscus*, valve view, about \times 535. (c) *Actinoptychus*, valve view (left, about \times 277) and girdle view (right, about \times 340). (d) *Thalassiosira*, valve view (above) and girdle view of colony (below, both \times 670). Scale bar = 10 µm (taken from Armstrong & Brasier, 2005. Sources: After van der Werff & Huls, 1957–1963).

Historically, for classifying diatoms have been rigorously taken into account the frustule's shape, based on the distribution of their pores and ornamentation (Round & Crawford, 1990). According to Armstrong & Brasier (2005), frustules are usually either circular (centric) or elliptical (pennate) in valve view, these kinds also comprising the two orders of diatoms: Centrales and Pennales (Table 1). In few words, diatoms are divided into two groups that are distinguished by the shape of the frustule: the centric diatoms (Fig. 1) and the pennate diatoms (Fig. 2). In diatoms, the nutrient content of the water is extremely important for its growth and reproduction based on the fact that three nutrients such as phosphorous, nitrate and silica are considered essential for almost all diatoms species.



Fig. 2. Pennate diatom(s) with their respective parts. (A) SEM image (taken from Taylor *et al.*, 2007), and (B) drawing images of valve and girdle views of: (a) *Pinnularia*, oblique view with raphe ×320. (b)

Fragilaria, valve view with pseudoraphe (left) and girdle view of colony (right, about ×545). (c) *Achnanthes*, hypovalve with raphe (left), epivalve view with pseudoraphe (centre) and girdle view (right, all about ×545). (d) Detail of diatom punctae. Scale bar = 10 μ m (taken from Armstrong & Brasier, 2005. Sources: (a) After Scagel *et al.*, 1965; (b) and (c) after van der Werff & Huls 1957–1963; (d) after Chapman & Chapman, 1973).

Freshwater diatoms microfossils have constantly been used to study the history of lakes since the last glaciation, revealing the effects of changing pH and climate (Battarbee, 1984; Battarbee & Charles, 1987; Mackay *et al.*, 1998; Leng *et al.*, 2001; Marshall *et al.*, 2002; Ampel *et al.*, 2010) and the effects of human pollution (Jones *et al.*, 1989; Stewart *et al.*, 1999; Joux-Arab *et al.*, 2000; Ek & Renberg, 2001).

Table 1. Suprageneric classification of the diatoms (taken from Armstrong & Brasier, 2005. Sources:Redrawn after Baron in: Lipps1993, after Simonsen 1979).

Order	Suborder	Family
Centrales: Central point formed by a point, auxospore formation by oogamy.	Coscinodiscineae: Valves with a ring of marginal pores, symmetry primarily without development of polarities, e.g. <i>Coscinodiscus</i> .	Thalassiosiraceae Melosiraceae Coscinodiscaceae Hemidiscaceae Asterolampraceae Heliopeltaceae
	Rhizosoleniineae: Valves primarily unipolar, strongly elongated in the direction perpendicular to the plane at which the two valves are joined in the frustule, e.g. <i>Pyxilla</i> .	Pyxillaceae Rhizosoleniaceae
	Biddulphineae: Valves primarily bipolar, secondarily tri- to multipolar to circular, e.g. <i>Triceratium</i> .	Biddullphiaceae Chaetoceraceae Lithodemiaceae Eupodiscaceae
Pennales:	Araphidineae: Valves without a raphe, e.g. <i>Thalassiothrix</i> .	Diatomaceae Protoraphidaceae
Structural centre normally formed by a line, auxospore formation not by oogamy.	Raphidineae: Valves with a raphe, e.g. <i>Nitzchia.</i>	Eunotiaceae Achanthaceae Naviculaceae Auriculaceae Epithemiaceae Nitzsciaceae Surirellaceae

2.2 Freshwater Sponges' spicules

Freshwater sponges (Fig. 3a) are dispersed in both lentic and lotic habitats, in continental and insular waters, with perennial or temporary regimes, from coast lines to high plain and from high mountains to subterranean environments (Manconi & Pronzato, 2008). Sponges are usually common and can be abundant inhabitants of a wide variety of freshwater habitats. In some situations, sponges are related to benthic fauna and may play important roles in ecosystem processes in freshwater (Frost *et al.*, 2001).



Fig. 3. *Drulia uruguayensis* freshwater sponge. (A): a hand sample, and (B) the tree-like morphology, and some of its isolated spicules in light microscope (taken from Magalhães *et al.*, 2011).

Freshwater sponges occur worldwide except so far in the Antarctica region, is stated that their geographical distribution is related to both geological and climatic conditions of the continents, and to the long-term dynamics of hydrogeographic basins (Manconi & Pronzato, 2008). Freshwater sponges are considered real extreme survivors under chemico-physical conditions ranging from permafrost, thermal waters, long-lasting dry-up, fluctuating water levels, stagnant to high speed waters, anoxic, oligotrophy to eutrophy, high levels of chemicals, and natural man-made pollution by hydrocarbons and heavy metals (Harrison, 1974; Pronzato & Manconi, 2002).

Species of freshwater sponges display a huge variable body shape and dimensions, consistency and colour. The main key feature for identifying freshwater sponges are the skeletal architecture, range of spicule geometry, size and shape of spicules and gemmular traits (Manconi & Pronzato, 2008). All freshwater sponges exhibit skeletal systems comprised of siliceous spicules (Fig. 3b) and collagen (Frost *et al.*, 2001) In fact, Penney & Racek (1968) stated that the classification of freshwater sponges is based fundamentally on the structure of gemmule spicules. However, in this work the description, classification or identification of the freshwater sponges' species is not addressed for the development of the main purpose.

2.3 Freshwater Phytoliths

A phytolith is a microscopic mineral of vegetal-origin (Fig. 4). They formed by the metabolism of the plant related with set of processes known as biomineralization. In other words, phytoliths are formed by the accumulation of silica in the vegetal tissues (Hart, 2015) during thousands of years. The silica can be absorbed from the water of the soil. Phytoliths are commonly found in: the plants, in the leaf epidermis, outermost covering of seeds and fruits, the epidermis of bracts which surround and protect grass seeds, and in the subepidermal tissue of orchid and palm leaves (Shakoor *et al.*, 2014).



Fig. 4. Images of typical phytolith morphotypes. (a, b) Long saddle, (c) Rondal, (d, e) Bilobate, (f) Globular echinate, (g) Bamboo bulliform, (h) Bulliform, (i) Square, (j) Rectangle, (k) Point, (l) Wavy-trapezoid, (m) Wavy-narrow-trapezoid, (n-q) Broad-leaf-type. Scale: black bar $\frac{1}{4}$ 10 mm; white bar $\frac{1}{4}$ 20 mm. (taken from Zuo *et al.*, 2016).

Phytoliths allow us to know the past of the vegetation and as corollary of the bioclimatology, paleoenvironments, archaeology, antiagriculture, etc. Therefore, these phytoliths represents a great record of the past of the soils' conditions, and their usefulness is relevant in several research. As occur with the freshwater sponges' spicules, in this work we are not focused on the description, classification or identification of the phytoliths for addressing the main purpose.

3. REVIEW OF VARVES IN LAKE SEDIMENTS

Initially, the term 'varved sediments' was used synonymously for annually laminated proglacial lake deposits. It often was called as 'varved clays' that refers to the interpretation of alternating pale and dark laminated non-glacial sediments in Swiss outcrops as a potential source of chronological information (Zolitschka *et al.*, 2015). Then, the term was used to define one complete annual depositional cycle consisting of a coarse-grained pale summer layer and a fine-grained dark winter layer in varved clay sequences. Varves quickly gained international recognition as a powerful concept with its rapid adaptation in glacial (Reeds, 1926) and non-glacial lake sediments (Whittaker, 1922; Perfiliev, 1929). Finally, the varve term, was finally

extended to all annually laminated sediment records not only on continents (O'Sullivan, 1983; Saarnisto, 1986) but also in marine settings (Kemp, 1996).

Lacustrine sediments have offered important depositional studies whose sedimentation rate term 'varved' and 'annually laminated' can be affected by past temperatures, precipitation, volcanism, solar activity, biomass burning, pollution and even other factors (Zolitschka *et al.*, 2015). Lacustrine sediments consist of material from two sources: (1) allochthonous, derived from outside the lake or transported into the lake from catchment area or beyond, and (2) autochthonous, derived from the lake itself or produced within the lake from lacustrine productivity or via precipitation. Specifically, autochthonous material includes fossils of limnic organisms such as: diatoms, chrysophytes, other algae and their pigments, bacteria, aquatic macrophytes, cladocerans, ostracods, chironomids or fishes. Birks (2008) remarks that the input of allochthonous material to a lake has three main sources: groundwater, catchment or watershed, and atmospheric. However, Anderson and Dean (1988), stated that the vast majority of lacustrine sediments are mixtures of these components or mixed-domain varves, but under certain conditions, these lacustrine sediments can be accumulated and preserved as a succession of laminae representing a seasonal cycle of sedimentation that is often driven by annual climate variability (Zolitschka *et al.*, 2015).

It is already known that the limnic sedimentary-varves are mainly formed by the variable seasonal periods (or climatic processes), at which several geological processes (or also called catchment geology) took place (Zolitschka *et al*, 2015). In fact, climate and geology jointly control the water chemistry and plankton communities (Wetzel, 2001). Zolitschka *et al.*, (2015) also stated that visually all varve types contain at least two or more seasonal laminae with distinctly contrasting colour, composition, texture, structure and/or thickness, and that these successions of seasonally deposited layers reflect a repetitive annual cycle. The appearance of varves along a depth transect can be used to reconstruct the history of anoxia within a lake basin (Jenny *et al.*, 2013). Hence, laminated sediments are more likely to be found in relatively deep lakes with small surface areas than in shallow lakes with large surface areas (Saarnisto, 1986; Larsen *et al.*, 1998; Tylmann *et al.*, 2013). Therefore, varved sediments are currently considered as invaluable sources of data for quantitative paleoclimate reconstructions (Larocque-Tobler *et al.*, 2015). The best conditions of these annually laminated sediments are found in eutrophic lakes with a low surface area/depth ratio and at least seasonal anoxic conditions in the hypolimnion (Zolitschka *et al.*, 2015).

The nature of paleoclimate reconstruction based on annually laminated sediments strongly depends on the type of varves. In fact, organic and some other types of varves store a variety of biological proxy information that enables qualitative and quantitative paleoclimatic reconstructions based on fossil remains like pollen, plant macrofossils, charcoal, non-pollen palynomorphs, diatoms, and chrysophytes cysts, as well as chironomids, coleoptera, ostracods and cladocera (Smol *et al.*, 2001a, b). Annually laminated sediment records provide accurate time control for a multitude of paleoenvironmentally relevant sedimentary parameters and document the frequency and rates of annual to millennial-scale climatic oscillations.

4. THEORICAL FRAMEWORK

4.1 Regional Geologic Framework of Ecuador

The principal morpho-structural features of the continental Ecuador (Fig. 5) are consequence of the active regional dynamic controlled by the interaction of the oceanic Nazca Plate that subducts under the continental South-American plate with a rate of relative convergence of 58 mm/y in the N and 78 mm/y in the S (Trenkamp, 2002).

This tectonic interaction provoked the rising up of the Andes Cordillera about 70 Mya (Lonsdale & Klitgrod, 1978), and formed six geomorphological regions that can be recognized with different tectonic and stratigraphy. They are, from W to E: Coastal basin (CB), Western Cordillera (WC), Inter-Andean Depression (IAD), Real Cordillera (RC), sub-Andean Zone (SAZ) and Oriente Basin (OB). The study area of this research project is developed in the center of the Inter-Andean depression (IAD) which started to form since the Late-Pliocene Miocene (Winkler *et al.*, 2002).



Fig. 5. Geotectonic and geomorphological map of Ecuador and surrounding basins. Blue square shows the location of Guayllabamba basin area (taken and modified from Ruiz, 2002 Alvarado *et al.*, 2014).

4.2 Geology of the inter-Andean Depression (IAD)

The inter-Andean depression is a topographic depression with a trending from N-S toward NNE-SSW (Winkler *et al.*, 2005) in which there are 19 volcanoes. This depression geographically extends from ~2°30'S to the Colombian border (Winkler *et al.*, 2005) and is

mainly understood as a tectonic-geomorphological depression below 3000 m located between and simultaneously flanked by the Occidental Cordillera (CO) and the Real Cordillera (CR). According to Litherland *et al.*, (1994), Spikings *et al.*, (2001) and Hughes & Pilatasig (2002), the main structural limits of the regional depression are credited to the reactivation of crustal scale faults that can be traced along the Andean chain in Colombia (Toussaint & Restrepo, 1994), which formed during successive Cretaceous and early Tertiary accretionary events along the Ecuadorian continental margin. The inception and tectonic development of the IAD have been temporally and geographically coeval by the concentration of volcanic activity in the Ecuadorian Andes since ~5 Ma (Pliocene-Quaternary; Winkler *et al.*, 2005).

The inter-Andean depression is composed by three main intermontane basins. They are: Chota basin in the N, Guayllabamba basin in the Centre, and the Latacunga-Riobamba basin in the S. The sedimentary sequences of the inter-Andean depression overlie either exposures of basement rocks of the cordilleras (Pallatanga and Guamote units) (Litherland *et al.*, 1994; Hughes & Pilatasig, 2002; Villagómez, 2003) or Oligocene-late Miocene volcanic successions, which are also exposed to the south of the present IAD in the Sierra of southern Ecuador. These sequences are made up by thick stratigraphic successions of volcanic, fluvial, alluvial and lacustrine deposits. This research project is developed in the Guayllabamba intermontane basin.

4.3 Geology and Stratigraphy of the Guayllabamba basin

Guayllabamba basin is specifically located at the Central inter-Andean depression where the sedimentary rocks of this basin unconformably overlie either basaltic rocks of the Cretaceous Pallatanga unit (CO) or Pliocene volcanic rocks of the basal Pisque Formation (Villagómez *et al.*, 2002). Villagómez in (2003) also provided the necessary tectonic and morphological evidence for classifying the Guayllabamba depression as a pull-apart semi-basin that started to form in the Late Pleistocene.

4.3.1 General Geologic Setting of Guayllabamba basin

In the Guayllabamba depression can be found fluvial-lacustrine deposits specifically in the lacustrine San Miguel Formation, that is the study area of this work and is where are located the two stratigraphic sections studied here. What has been well studied throughout time in Guayllabamba basin is the lithostratigraphy and, in less proportion, the chronostratigraphy.

This research project takes place in northern area of the Guayllabamba Basin, that is Late Pleistocene in age, currently surrounded by an active system of faults. This basin is better known by being an intermontane basin; where were identified a lacustrine environment composed of diatomites interbedded with primary volcanic, gravity flows and fluvial deposits (Villagómez, 2003). Two stratigraphic sections have been strategically selected for taking the samples of diatom microfossils, which belonged to an ancient lake that existed during the deposition of the lacustrine San Miguel Formation in the Guayllabamba basin. The location of those two sections are useful to study and understand how the diatoms microfossils, that lived in the paleolake are associated with the paleoenvironment of the lacustrine San Miguel Fm.

4.3.2 Stratigraphy of Guayllabamba basin

In this work, the stratigraphic succession of Guayllabamba basin marks important information of the basin. This succession has been previously described by few authors such as Lavenu *et al.*, (1995, 1996), Villagómez (2003) and Winkler *et al.*, (2005). The units of this succession are shown in Fig. 6 where San Miguel Formation is the focus of this research project. The sedimentology that appears in San Miguel Fm. are valuable for understanding the history of the paleolake that formed. The stratigraphic series that appear in the Guayllabamba basin are described based on the available data of the lithostratigraphy and chronostratigraphy. The description was mostly taken from Villagómez (2003), Winkler *et al.*, (2005) and other authors.

4.3.2.1 Pisque Formation

The Pisque Formation is mainly composed by conglomerates, breccias and sandstone with basaltic clast of intermediate to basaltic composition (Martin, 2020 *in prep.*). This Formation is subdivided in the basal, lower and upper Pisque Formation:

A. The basal Pisque Fm. constitutes the strictly volcanic basement of Guayllabamba basin. It includes the Basal Lava Member with ~200 m thick, that consists of andesitic and basaltic lavas and scorias toward the base and unconformably overlain by breccias, tuffs and lahars toward the top of the lower Pisque Formation.

B. The lower Pisque Fm. is composed by Puente Viejo (NW) and Golden Tuffs (NE) Members. The Puente Viejo Member with ~160 m height, consists of stratified thick lahars (with andesitic blocks, basalts, pumice and clasts of green rocks) interbedded with light colored tuffs (Villagómez, 2003). The Golden Tuffs Member (probably lower Pleistocene) with ~100m thick, consist of mud flows at the base, then appear white and gold tuffs and the sequence becomes richer in metric strata of tuffs (Villagómez, 2003).

C. The upper Pisque Fm. is composed by the Fluvial (NE) and alluvial fan (NW) Members. The Alluvial Fan Member (probably lower Pleistocene in age) with ~80m thick, consists of prograde alluvial-fan sequences. The Fluvial Member with ~50 m thick, consists of metric bodies of fluvial sandstones, alluvial-fan facies (sheet flows and debris flows) with interbeds of lacustrine sediments.



Fig. 6. Stratigraphy of Guayllabamba basin (taken and modified from Martin, 2020 *in prep.* with permission). The lacustrine San Miguel Fm. was the interval studied in this work with eight to nine identified units.

4.3.2.2 San Miguel Formation

The San Miguel Formation is the stratigraphic unit at where is located the study area of this work. DGGM (1982), Samaniego *et al.*, (1994) and Ego & Sebrier (1996) defined that this formation was deposited un a lacustrine environment with volcanic contributions. Villagómez (2003) interpreted the San Miguel Fm. as a volcanic, tuff-rich sequence of eastward prograding deltaic and lacustrine deposits, where the latter units were strongly deformed during synsedimentary, gravity-driven displacements in response to the loading exerted by younger lahars deposited during the deposition of the Guayllabamba Formation.

San Miguel Formation can be subdivided into two members:

A. The San Miguel Member, probably lower Pleistocene in age, with ~80 m thick, consists of sandstones layers interbedded with tuff-siltstone and with primary and reworked tuffs. The

facies analyses indicate that correspond to fluvial, deltaic and lacustrine deposits and that much of the deposition was in a subaqueous medium (Villagómez, 2003).

B. The Lacustrine San Miguel member, probably lower Pleistocene, ~ 200 m thick, is limited toward the current Guayllabamba depression and also toward the N and E limits of the depression suggesting that there would had had a tectonic control in the installation of the paleolake.

4.3.2.3 Guayllabamba Formation

The Guayllabamba Formation implies an important geologic period of intense volcanism and tectonic activity in all the Central IAD, producing some mud flows (Villagómez, 2003; Winkler *et al.*, 2005). To the W and S can be found primary volcanic deposition reflected in lavas, pyroclastic flows and avalanches (Winkler *et al.*, 2005). When the Guayllabamba Formation was deposited, the lake had disappeared, and as consequence, the San Miguel Formation was strongly deformed during the deposition of Guayllabamba Formation.

Guayllabamba Fm. is composed by four members: **A.** The Lahar Member, ~80 m thick, consists of mudflows bodies that contain clasts of siltstones and deformed sandstones of the underlying units, next to volcanic pumices clasts in a muddy matrix. **B.** The Domos Member corresponds to two small dome-shaped volcanic necks: Pacpo and Catequilla with different chemical composition. **C.** The Volcanic Member, middle Pleistocene in age, ~200 m thick, is located to the W, and consists of several primary volcanic products such as: lava flows, pyroclastic flows of the type block & ash, and avalanche deposits. **D.** The Alluvial Member, probably middle Pleistocene in age, ~100 m thick, consists of a stratified alluvial sequence. The blocks correspond to andesitic lavas. The deposit is grain-supported with sandy matrix.

4.3.2.4 Chiche Formation

The Chiche Formation consists of conglomerates and coarse sandstones interstratified with tuffs. Winkler *et al.*, (2005) established this unit was deposited in calm, low energy, lacustrine, and fluvial environments in the Quito and Guayllabamba basins.

Chiche Fm. is composed by four members: **A.** The Fluvio-Lacustre I Member, upper Pleistocene in age, with ~60 m thick, consists of cream siltstones, white tuffs interbedded with grey sandstones. **B.** The Lahar Member (upper Pleistocene in age) with ~40 m thick, corresponds to mudflows and hyperconcentrated flows. **C.** The Fluvio-Lacustre II Member, upper Pleistocene in age, ~80 m thick, consist of white tuff-siltstones, diatomites and even grey sandstones. **D.** The Chiche s.s. Member, upper Pleistocene in age, ~100 m thick, consists of conglomerates, coarse sandstones interbedded with tuffs.

4.3.2.5 Mojanda Formation

The Mojanda Formation, upper Pleistocene on age, with ~100 m thick, consists in volcanic deposits and volcanic-sedimentary products from the Mojanda volcanic complex. Lahars are rich in dark andesitic lithics and even in scoria. Debris flows contain andesitic and dacitic blocks. To the N was derived from the middle-late Pleistocene Mojanda volcanic complex (0.6 Ma, K/Ar whole-rock andesite, Barberi *et al.*, 1988).

5. METHODOLOGY

Lakes and their sediments provide the most diverse records of past biota and environment because lacustrine sediments integrate a range of regional and local biological and environmental signals (Birks, 2008). This work is focused on the reconstruction and on the understanding the paleoenvironments of the San Miguel Formation, part of the Guayllabamba basin, based on paleoecological and biostratigraphical analyses of diatoms microfossils that have been sampled in two lacustrine varved and massive successions of the San Miguel Formation. These diatoms are considered paleoenvironmental indicators or climate tracers. Diatoms valves, and their capacity for preservation, represent an important tool in order to reconstruct their paleoecology and paleoenvironmental conditions. The most remarkable advantage in working with microfossils is that a single sample will serve for analyzing and interpreting all the data required to reach the goals proposed in this research project.

In this work, two stratigraphic sections of the San Miguel Formation were sampled. Those two locations are strategic areas of entrances of the paleolake that have important geological features as well as the biological evidence (microfossils) for understanding the geodynamic of the paleolake and the paleoenvironments of the area. In each location were performed its respective stratigraphic log in order to understand and describe the stratigraphic successions, as well as to interpret the lacustrine facies. The stratigraphic section of Tanda (GT), 96-m height, corresponds to distal areas of the paleolake; while the stratigraphic section of Puéllaro (GP), 113-m height, represents the fluvial discharges of the paleolake in proximal areas of the paleolake. Once the stratigraphic successions were analyzed, the process of sampling was carried out. In both lacustrine sections, several claystone sediments were sampled, marking a red circle in the selected point at which each sample was taken. It was also registered the height and each sample was saved in a plastic bag for avoiding contamination.

The laboratory analyses of diatoms (siliceous microfossils) samples were treated and analyzed with the available laboratory tools (heating plate, weight balance, etc.), chemical reactives (H₂O₂, Xylene + Canadian balsam solution, and alcohol), and equipment of the micro-paleontology laboratory (light microscopes) of Yachay Tech University. The treated samples

do not need to be in big proportions because for microfossils analyses is only needed very few weight (just ~10 mg) of the entire sample for obtain good results. Once the samples of claysediments were scraped, they were treated with the 'Smear' technique for elaborating the plates of each sample. All these plates were then analyzed on an Olympus BX53 light microscope. It was used the microscope's micrometer, and were taking several micro-photos that were useful for defining the diatoms' morphotypes, sizes, and some diatom species for the counting process. Then was applied biostratigraphical interpretations between the two stratigraphic successions by analyzing the different associations of diatoms species for understanding the paleoecological conditions. Finally, by correlating all the obtained data, a paleoenvironmental reconstruction of the study area composed of paleoecological-phases, can be proposed in a sketch.

In this chapter, each technique that was used to obtain the results, is schematically explained with its respective process(es) depending on the objective(s) we wanted to reach. This is, there are some techniques or methodologies for the biostratigraphic goal, for the paleoecological goal, and for the paleoenvironmental goal.

5.1 Description of stratigraphic logs

Field-work was required for the register and the performing of the two stratigraphic logs with a good detail, and for simultaneously collecting all the clay-sediment samples at different heights in the two outcrops. The two stratigraphic logs were elaborated in Strater software and edited in Affinity Design software. Strater is a professional software provided by Golden Software company, useful for elaborating stratigraphic logs. And Affinity Design software is a vectoral design software useful for editing not only the stratigraphic logs of this work but also for the rest of maps and images of this work. A detailed analysis of those stratigraphic successions was useful for complementing the ongoing work that is in progress by other authors in the Guayllabamba basin. Then, the height, lithology, grain size variations, sedimentary structures, and fossils content were described.

Once the two stratigraphic logs with their sedimentary structures and fossil content, were elaborated and described in digital versions, we could decide at which stratigraphic levels the clay-sediments samples can be extracted and analyzed in each stratigraphic succession. In spite of there are many claystone layers for sampling the sediments, it was decided to take each sample in one point separated at least by 1.5-m or more from each other.

5.2 Paleoecological analyses

In this research project, we used paleontological techniques for obtaining data about the paleoecology of diatoms species and for developing a paleoenvironmental reconstruction of the San Miguel Fm. All the samples of claystone sediments with diatoms microfossils content,

were extracted from the two studied stratigraphic sections, which belong to entrances areas of the paleolake located at into the Guayllabamba basin. Moreover, we expect coherent distributions in the two varved and massive lacustrine deposits of the paleolake, due to those distributions of the diatoms (paleoecology) were driven by environmental conditions such as: lake paleocurrents, depth, salinity, insolation and nutrient supply.

5.2.1 Diatom microfossils preparation by Smear Slide

Frotis (a.k.a smear slide) technique is a relatively quickly technique for treating with diatoms microfossils. A total of 40 plates (slides) were prepared using the Smear technique, which involves several laboratory tools and chemical reactives. The procedure was the following:

1. In a cleaning work area, organize all the laboratory equipment (heating plate), tools (scrapper), materials (pieces of sheets of papers, ice cream flat wood sticks, slides, coverslips), chemical reactives (alcohol, distilled water, xylene + Canadian balsam), sediment samples saved in a plastic bags, as well the personal protection equipment (facial mask).

2. Scrap the fragmented sediment sample with a scrapper for obtaining the required amount of clay-sediment rich in diatoms and saving in a small plastic bag. We must be sure whether the fragmented sample of claystone is massive or is varved. In massive samples is required just one scrap. In varved samples is required one scrap for light laminae and one scrap for dark laminae.

3. In an analytical weight, weight ~ 10 mg of the scrapped sediments and save it in a separate rigid plastic recipient (centrifuge recipients).

4. Label each plastic bag, each centrifuge plastic recipient, and each slide with a small sticker. Mandatorily each slide must be sterilized with alcohol.

5. Turn on the heating plate at ~80 °C, and put some free slides and coverslips to heating on plate.
6. Put the slides over a clean piece of sheet of paper and drop the ~10 mg of scraped sediments on the slide and put one drop of distilled water over the sediment.

7. With a flat ice cream stick, apply the Smear technique on the wet sediment and homogeneously distribute it across the ³/₄ of the slide's area. Then throw out the used ice cream stick.

8. Once a homogeneous distribution of sample is reached, put the slide on the heating plate at 80 °C for drying all the slide's area and simultaneously over the coverslip drop one drop of xylene + Canadian balsam solution (50:50), and wait 1 to 2 minutes until the slide dries completely and until the drop in the coverslip is well spread. Finally, once the spread sediment in the slide is totally dry, join the coverslip with the slide in the center of the slide and with a standard flat ice cream stick, apply few pression over the coverslip in order to spread the solution for covering all the coverslip's area. For getting uniform distribution, leave the slides in the heating plate at ~30 °C for 24 hours.
9. After the 24 hours, turn off the heating plate and wait for other 2 to 3 hours (or more if is

necessary) until all the plates be completely dry. At this moment, both the slide and cover slide do

not have to move, this is, well adhered one from other. Then, once the slide is ready, is used a fine piece of paper for cleaning out the unnecessary sediment on the coverslip. Finally, you can put on a cold surface for leave them more time and reach a best final product. You can save each slide in a paper cover. Then you can observe each plate in the light microscope.

5.2.2 Counting of abundances of the components

This technique consists in having a control in the micro-area on the slide for counting the following components, in complete parts or fragments: diatoms, sponges' spicules, phytoliths, and detritus. A light microscope was mandatorily required for this goal. The light microscope available in the Micro-paleontology Laboratory at Yachay Tech University was an Olympus BX53. It was also used the associated software charged in that microscope, the Olympus Stream Essential, which was used to take photos in live during the examination of the 40 plates, and for adjusting the focus depending on the sample we were observing.

We also need a reference limit-number for stopping the counting of components in a sample. That reference number has to be statistically representative for the entire sample. Then, we are mainly concerned in determining at which number, all the relative abundance (%) values of each component keep constant in a sample. However, as have been usually done in similar works, and following the recommendations of Schrader & Gersonde (1978), we counted 300 individual fragments on each slide. Then diatom valves on the slides were observed, enumerated and identified under oil immersion (1000x magnification). There were slides where diatoms were totally abundant and others scarce. Each central area of a pennate diatom, or more than half a valve, was counted as a whole single individual. In some samples, frustules were so poorly preserved that they could only be identified to at generic level.

5.2.3 Definition of morphotypes and identification of some species of diatoms

Here, the morphology of diatom -that is based on their valves' shape, ornamentation, and even the shape of pores-, were carefully observed in the light microscope in order to define the morphotypes and to identify some species of diatoms that appears at different intervals in each stratigraphic succession studied. It is expected some changes in the associations of diatoms species depending on the depth of the paleolake.

For defining the morphotypes of diatoms, was used the classical classification of diatom based on their morphological features from https://diatoms.org. This classification is composed by nine artificial (not strictly evolutionary) categories (Fig. 7) for visual classification purposes, based on the diatoms' morphology. These categories are:



Fig 7. Classification of diatom morphotypes. (A) Centric, (B) Araphid, (C) Eunotioid, (D) Symmetric biraphid, (E) Monoraphid, (F) Asymmetric biraphid, (G) Epithemioid, (H) Nitzschioid, and (I) Surirelloid morphology (images taken from https://diatoms.org/morphology). Scale bar = $10 \,\mu m$.

1. Category A: Centric morphology - Valves with radial symmetry (symmetric about a point). Cells lack a raphe system and lack significant motility. Cells may possess fultoportulae (strutted processes) and rimoportulae (labiate processes). Sexual reproduction is oogamous.

2. Category B: Araphid morphology - Valves with bilateral symmetry (symmetric about a line). Cells lack a raphe system and lack significant motility. Rimoportulae (labiate process) may be present.

3. Category C: Eunotioid morphology - Valves with bilateral symmetry (symmetric about a line). Valves often asymmetrical to the apical axis. Raphe system is short and provides weak motility. Raphe located on valve mantle and face. Cells may possess 2 or more rimoportulae (labiate processes).

4. Category D: Symmetric biraphid morphology - Valves with bilateral symmetry (symmetric about a line). Valves symmetric to both apical and transapical axis. Raphe system

well developed and cells may be highly motile. This group has the greatest diversity among the freshwater diatoms.

5. Category E: Monoraphid morphology - Valves with bilateral symmetry (symmetric about a line). Raphe system present on one valve (raphe valve). Raphe system absent on one valve (rapheless valve). Heterovalvar ornamentation.

6. Category F: Asymmetric biraphid morphology - Valves asymmetric to apical axis or asymmetric to the transapical axis, or both. Raphe system well developed. Some genera possess apical porefields that secrete mucilaginous stalks. Other genera secrete mucilagous tubes.

7. Category G: Epithemioid morphology - Valves with bilateral symmetry (symmetric about a line). Valves asymmetrical to apical axis. Raphe system well developed and enclosed within a canal. Raphe system positioned near the valve margin.

8. Category H: Nitzschioid morphology - Valves with bilateral symmetry (symmetric about a line). Valves usually symmetrical to both apical and transapical axes. Raphid system well developed, and positioned near the valve margin. Raphe is enclosed within a canal and may be raised onto a keel.

9. Category I: Surirelloid morphology - Valves with bilateral symmetry (symmetric about a line). Raphe system extremely well developed and enclosed within a canal. Raphe positioned around the entire valve margin and raised onto a keel.

And for identifying the genera and when is possible the species of diatom, we used: (1) many previous bibliographic studies and, (2) digital databases focused on the identification of diatoms species based on the description of their frustules' shapes and their general morphology using a light microscope (LM). A good source used here was a the same previous digital-database for diatom learning (https://diatoms.org), which is really useful for identifying not only the main morphotypes of the diatoms but also for the genera and species of diatoms. Moreover, several bibliographic studies based on study of diatoms species were used as a guide.

5.2.4 Biostratigraphic analyses

The intention is trying to develop biostratigraphical analyses between the two stratigraphic successions of San Miguel Fm., that were sampled in order to understand how much and why the associations of freshwater-diatoms species have changed in each vertical succession. Those changes would allow to infer the paleoecology of the paleolake and therefore to develop the paleoenvironmental reconstruction of the San Miguel Fm.

5.3 Paleoenvironmental proxies

Once we have obtained the data from the counting process and biostratigraphy of the two stratigraphic sections studied in this work, we can propose a simplified paleoenvironmental

reconstruction sketch of the paleolake formed in the San Miguel Fm., within the Guayllabamba basin, by correlating all the results. It means, the simplified reconstruction has to keep a direct relationship with the paleoecological conditions of the freshwater-diatoms identified in this work, and a relative dependence on the depth of the lake in function of the paleogeographic location of the two stratigraphic sections (Tanda & Puéllaro) where all the samples were taken.

6. RESULTS

Inside Guayllabamba basin was possible to identify two principal entrances of terrigenous (detrital) sediments toward the paleolake, whose sedimentary deposits currently belong to the San Miguel Fm., within the Guayllabamba basin. One entrance is located at the NE of the basin in the Guayllabamba-Tabacundo road, close to the entrance to the Tanda ranch, and represents the pelagic (deep-waters) areas of the paleolake. We labeled this section as 'GT'. Meanwhile the other entrance is located at the NW of the basin in the Río Pisque-Puéllaro road and corresponds to the littoral (shallow-waters) areas of the paleolake. We labeled this section as "GP'. Hence, the study area of this research project has two locations (Fig. 8, Table 2). We chose these two sections due their good exposure -stratigraphically talking- from the base toward the ceiling of the San Miguel Fm. Through a detailed study of the stratigraphy in each section, this research project would mark an import input for understanding the processes associated to the development of the Guayllabamba basin.

				J		
	Latitude (x)	Longitude (y)	Height (z) [m]	Name	Label	Thickness [m]
Section 1	0° 00' 18.1" S	78° 18' 59.9" W	2010	Tanda	GT	~ 96
Section 2	0° 00' 20.7" S	78° 23' 39.9" W	2104	Puéllaro	GP	~ 103

Table 2. Geographic coordinates of the two outcrops studied in this research project.



Fig. 8. Study area of this project. Tanda (GT) and Puéllaro (GP) sections are marked in the map.

Several techniques, digital tools, analyses of the data and considerations were used for showing the main results of this research project. For this chapter, each secondary objective has its own section of results in order to keep a coherent order and for better understanding.

6.1 Description of the stratigraphic successions

We provide a general description of the two sections studied in this research project. Several photos were taken in each section at different stratigraphic levels for visualizing the potential that both sections have for being considered in this work. These features were put in their respective stratigraphic successions and are most related to sedimentary processes that are the clearest evidence of the paleolake evolution that took place in this area. Advantageously, both GT and GP section are high quality outcrops -geologically speaking- that allow the development of a detailed 2D stratigraphic profiles for each section. In each profile, we could identify key surfaces, some structures and the geometry of sedimentary bodies.

6.1.1 General Description of the Tanda (GT) section:

Tanda (GT) section (Fig. 9) evidences a remarkable paleotectonic activity, whose lithologies as well as sedimentary structures are associated to the past limnological activities inside the paleolake. The tectonic events that occurred here are evidenced as active normal-faults with huge massive blocks displacement, and the sedimentary processes observed here, allow us to infer that this station belongs to the pelagic (deep-waters) zone of the paleolake.

6.1.1.1 Description of the stratigraphic succession of Tanda (GT) section (Fig. 10):

We found facies associated to distal (deep-waters) areas of the paleolake, where the varves of the lower part, and the varved-facies of the upper part are well developed. Therefore, this succession is dominated by deltaic deposits where the identified lacustrine are heterolytic facies (Martin, 2020 *in prep.*). These facies are defined as a mix-up of alternation of millimetric claystone laminae and very-fine grained sandstone laminae. We divided this stratigraphic succession in eight lithological units:

Unit A: Deltaic deposits, composed by alternation of coarse sandstones and coarse conglomerates.

Unit B: Medium to very-coarse sandy deposits.

Unit C: Varved-lacustrine deposits of clays and silts intercalated with pumices and scorias.

Unit D: Silty deposits intercalated with deformed sandstone bodies, scorias and pumices.

Unit E: Massive coarse sandy-pumices deposits.

Unit F: Massive silty deposits with a FeO_x layer.

Unit G: Sandy body intercalated with a granule conglomerate body.


Fig 9. Photos of the Tanda (GT) section. (A) Lower conglomeratic unit of deltaic areas, (B) Middle deformed, slumped and faulted lacustrine varves, (C) Upper package of lacustrine varves, (D-N) several massive and varved sediments where samples of this section were taken. The red points indicate where were extracted the claystone-sediment samples. Scales: hammer = 35 cm, chisel = 30 cm, backpack = 1 m.



Fig. 10. Stratigraphic log of the Tanda (GT) section with its sedimentary structures and fossil content.

6.1.2 General Description of the Puéllaro GP) section:

Puéllaro (GP) section (Fig. 11) shows more chaotic sedimentary processes clearly from a palustrine environment that coincide with the inferred location in the proximal (shallower) areas of the paleolake. Here were found several fossil leaves, trunks, gastropods molds and even bioturbation. Moreover, there exist active tectonism with several remarkable active faults that are affecting the road.

6.1.2.1 Description of the stratigraphic succession of Puéllaro (GP) section (Fig.

12): We found facies associated to proximal areas of the paleolake with a high rate of entrance of detritic sediments. Those processes facilitated the formation of massive conglomeratic lithologies in major proportions than varved-sequences in this section. Moreover, we found fossil plant leaves and some fossil trunks which are evidence of a palustrine environment at the lower unit of this succession. These features are related to the shallower areas of this paleolake. We divided this stratigraphic succession in nine lithologic units:

Unit A: Massive granule to boulder conglomerate bodies intercalated with coarse sandstone stratas.

Unit B: Sandstone with a pumice stratum.

Unit C: Conglomerates intercalated with coarse sandstone bodies, claystone and silty stratas.

Unit D: Varved-lacustrine deposits of clays and silts, intercalated with sandstone stratas.

Unit E: Pebble conglomerates intercalated with sandstone, a reference FeO_x layer, and pumices stratas.

Unit F: Varved-lacustrine deposits of silts and clays, intercalated with sandstones and scorias stratas.

Unit G: Granule conglomerates intercalated with coarse sandstones stratas.

Unit H: Coarse sandy deposits intercalated with granule and pebble conglomerate bodies.

Unit I: Varved-lacustrine deposits of clays and silts intercalated with sandstone stratas (not studied).



Fig 11. Photos of the Puéllaro (GP) section. (A) An active fault, (B) Lacustrine varves, (C) Massive sandstones in the upper part, (D) Fossils of wood and plant-leaves at the lower unit, (E) cross-bedded fine sandstones, (F) Deformed and slumped sandstones, (G) Well-developed varves, (H) Massive cross-bedded sandstones, (I) Sigmoidal fine sandy layers, (J-L) Lacustrine varves packages. The red points indicate where were extracted the claystone-sediment samples. Scales: hammer = 35 cm, chisel = 30 cm, backpack = 1 m.



Fig. 12. Stratigraphic log of the Puéllaro (GP) section with its sedimentary structures and fossil content.

6.2 Description of the components and counting of their abundances:

The counting of components for determining their relative abundance (%), was done in 40 samples. The components considered for the counting process were diatoms (and fragments), sponges' spicules (and fragments), phytoliths (and fragments), and detritic sediments (Fig. 13-16).

6.2.1 Description of the components:

• Sponges' spicules (and fragments) observed in Tanda (GT) and Puéllaro (GP).

The sponges' spicules were relatively easy to identify in the samples. As was previously said, we have to deal with skeletal architecture, range of spicule geometry, size and shape of spicules and gemmular traits (Manconi & Pronzato, 2008). The spicules found in our samples were monaxons (needle shape) with a central channel and no ramifications (Fig. 13). Their length is ranged from 100 μ m to 150 μ m, and their width is ~15 μ m. Almost all spicules we observed belong to the same morphotype and no ornamentation was observed.



Fig. 13. Complete and fragmented needle-shaped sponges' spicules in the light microscope. Scale bar=10 µm, magnification=1000x. (A-C) Spicules from Tanda section, and (D-F) spicules from Puéllaro section.

• Phytoliths (and fragments) observed in Tanda (GT) and Puéllaro (GP).

Phytoliths are silica bodies (Fig. 14) produced by plants when soluble silica in the ground water is absorbed by the roots and carried up to the plant via the vascular system (Yost & Taylor, 2008). The phytoliths we found were subjected to mechanical breakage, deterioration with irregular shapes. These phytoliths were not deeply studied nor identified or classified.



Fig. 14. Complete and fragmented phytoliths in the light microscope. Scale bar=10 μ m, magnification=1000x. (A-C) Phytoliths from Tanda section, and (D-F) phytoliths from Puéllaro section.

• Detritic grains observed in Tanda (GT) and Puéllaro (GP).

Detritic sediments are small fragmented lithics or small angular mineral grains (Fig. 15). Some showed pleichroism propertied and probably could belong to amphiboles minerals.



Fig. 15. Fragmented detritic grains in the light microscope. Scale bar=10 μm, magnification=1000x. (A-C) detritic from Tanda section, and (D-F) detritic from Puéllaro section.

• Diatoms (and fragments) observed in Tanda (GT) and Puéllaro (GP).

Diatoms are siliceous microorganisms with several shapes and sizes (Fig. 16). We observed variations in these parameters depending on the morphotype and the species. Diatom species were found in solitary or in colonies conditions, and were deeply studied in this work.



Fig. 16. Complete and fragmented types of diatom in the light microscope. Scale bar=10 μm, magnification= 1000x. (A-E) Centric diatoms from Tanda section, and (F-I) benthic and central diatoms from Puéllaro section.

The results presented are totally dependent on the general and specific paleoecological conditions of each component (diatoms, sponges' spicules and phytoliths). However, is necessary to consider that both sections, GT and GP, are paleoecologically and paleoenvironmentally related, but they are paleogeographically different. This clear difference is better appreciated in the sedimentological evolution of each stratigraphic section (Fig. 10, 12). In this work we did not carried out a deeper counting with the diatom component. It means, planktonic and benthic diatoms were not counted separately, even the specific diatom species of each group that were identified in the next sections, were not also counted separately. Then the relative abundances of each component (diatom, sponges' spicules, phytoliths and detritus) in each stratigraphic section studied here, are shown in the next subsections:

6.2.2 Counting of abundance of components

6.2.2.1 Results of counting of abundances of components in the Tanda (GT) section

Due to Tanda section belongs to pelagic (deep-waters) zones of the paleolake, is expected a very low rate of entrance of detritic sediments in this area. So, Tanda section would relatively guarantee most appropriate conditions for a bloom event of planktonic diatoms, but not the best conditions for the development of benthonic diatoms (Table 3 & Fig. 17).

# OF	# OF	(LABEL)		HEIGHT	ABUNDANCE (%) OF COMPONENTS					
PLATE	POINTS			[m]	DIATOMS	SPICULES	PHYTOLITHS	DETRITIC	30101	
1	1	GT	М	9.2	67	0.33	9.67	23	100	
2	2	GT	М	12.6	46	6	19.33	28.67	100	
3	3	GT	М	21.3	0.33	35.33	30.33	34	100	
4	4	GT	М	25.95	83.33	5.33	5.33	6	100	
5	5	GT	М	28.4	86.67	2.33	5	6	100	
6	6	GT	V-L	32.82	5.33	3.33	49.33	42	100	
7	0	GT	V-D	32.82	22.67	0.67	41.67	35	100	
8	7	GT	М	35.45	73.67	13.33	1.67	11.33	100	
9	8	GT	М	40.55	45	1	31.67	22.33	100	
10	9	GT	М	44.45	88	0.67	6.67	4.67	100	
11	10	GT	V-L	72.65	87	3.33	5.67	4	100	
12	10	GT	V-D	72.65	71.67	10	10	8.33	100	
13	11	GT	V-L	77.55	38.67	45.33	9	7	100	
14	11	GT	V-D	77.55	84	2.67	6.67	6.67	100	
15	12	GT	V-L	79.6	70.33	15.33	9	5.33	100	
16	12	GT	V-D	79.6	85.33	3.33	6	5.33	100	
17	13	GT	V-L	80.8	82.67	5.67	4.33	7.33	100	
18	15	GT	V-D	80.8	79	8.33	6.67	6	100	
19	14	GT	V-L	85.7	83	3.33	5.33	8.33	100	
20	14	GT	V-D	85.7	83	5	6.67	5.33	100	
21	15	GT	М	86.5	88.33	3.33	5	3.33	100	
22	22 16		V-L	87.2	85.67	2	7.33	5	100	
23	10	GT	V-D	87.2	87.67	0	8.33	4	100	
24	17	GT	V-L	89.85	89.67	0	6.33	4	100	
25	1/	GT	V-D	89.85	84	1.67	8.33	6	100	

Table 3. Results of the counting of abundances of components in the Tanda (GT) section.

In Fig. 17 are shown the results of the counting process for the Tanda (GT) section. It is necessary to remark that in Tanda (GT) there were more varved successions and better chances to collect more samples than in Puéllaro (GP) section. Here, we also remark that the abundance ranges in the horizontal-axis vary depending on the component. Only diatoms have its abundance range from 0% to 100%, while spicules, phytoliths and detritic, have their abundance ranges from 0% to 50%.

In Fig. 17 can be observed the distribution of the counting results for each component in Tanda (GT) section. In order to facilitate the analyses, based on the sedimentary processes and the paleoecological conditions of this section of the paleolake, we divided this distribution in three subzones. Then, the three zones of the counting results of Tanda (GT) were:



Fig. 17. Stratigraphic log and results of the counting of abundances of components in the Tanda (GT) section. Red points between the lithology log and grain size log indicate the stratigraphic level at which each sample (17 in total) was taken. Those red points horizontally coincide with the horizontal bars that indicates the abundance (%) values of each component in this section.

- *Zone A* (09m - 13m): Deltaic areas composed by massive sands and conglomerates. Here, there is a medium-high rate of entrance of detritic sediments between 20% - 30%, while diatoms present abundances between 40% - 70%.

- *Zone B* (21m - 45m): Composed by silts and clays intercalated with coarse-sands, pumices and scorias. The rate of entrance of detritic sediments is in low and medium rates, but there are two pikes of very high rate in the sample GT-21.30 M and in both samples of GP-32.82 V in the light and dark laminae. These two stratigraphic levels have the highest rate of entrance of detritic sediments in this section. Moreover, in those points with the highest rate of detritic abundances, diatoms have almost disappeared in the point GT-21.30 M and have a very low abundance in the both samples of the point GP-32.82 V in the light and dark laminae, which is interesting sur to in this section, diatoms present abundances between 45% - 90%.

- *Zone C* (72m - 90m): Composed by varves intercalated with medium to coarse-sandstones. Here, there is not much to deduce, all the trends are coherent except in the abundance value of the sample GT-77.55 V with a light and a dark lamina. This sample has an interesting result because it has the two opposite abundance values for diatoms vs spicules for each laminae sample. The sample of the light lamina, GT-77.50 V-L, has low abundance of diatom and a high abundance of spicules, while the sample of the dark lamina, GT-77.55 V-D, has a high abundance values of diatom and a low abundance of spicules. In fact, this point is the only one that have this particularity in this section. In this section, diatoms present abundances >85%.

6.2.2.2 Results of counting of abundances of components in the Puéllaro (GP) section

Due to Puéllaro section belongs to littoral (shallow-waters) zones of the paleolake, is expected a major rate of entrance of detritic than Tanda. So, conditions of Puéllaro are the most appropriate for a bloom event of benthic diatoms. However, due to this section is close to the superficial land, Puéllaro section can simultaneously have good conditions for a bloom event (in less proportion) of planktonic diatoms (Table 4 & Fig. 18). Hence, the shallow-waters of Puéllaro (GP) section would be adequate for both kind of diatoms, but it has better conditions for benthic diatoms, which is reflected in the high biodiversity grade of this group.

# OF	# OF			HEIGHT	ABUNDANCE (%) OF COMPONENTS					
PLATE	PLATE POINTS		BEL)	[m]	DIATOMS	DIATOMS SPICULES PHYTOLITHS D		DETRITIC	SUIVI	
1	1	GP	М	12.3	49.33	0.33	28.33	22	100	
2	2	GP	V-L	53.85	88.67	0	3	8.33	100	
3	Z	GP	V-D	53.85	87	0	5.67	7.33	100	
4	3	GP	М	56.67	75.67	0	16	8.33	100	
5	Δ	GP	V-L	58.82	94.67	0	0	5.33	100	
6	4	GP	V-D	58.82	89.33	1.33	1.67	7.67	100	
7	F	GP	V-L	62.5	91.67	0	0	8.33	100	
8	5	GP	V-D	62.5	76.67	0.33	10.33	12.67	100	
9	6	GP	М	65.17	28.33	0.33	43	28.33	100	
10	7	GP	М	77.3	19	0.33	45.33	35.33	100	
11	8	GP	М	82.45	79	0.67	11.67	8.67	100	
12	0	GP	V-L	84.43	62.33	0.33	19	18.33	100	
13	9	GP	V-D	84.43	72.33	0	15.33	15.33	103	
14	10	GP	М	85.67	77	1.67	12.67	8.67	100	
15	11	GP	М	89.38	63	0.33	20.67	16	100	

Table 4. Results of the counting of the components in the Puéllaro (GP) section.

In Fig. 18 are shown the results of the counting process for Puéllaro (GP) section. It is necessary to remark that in Puéllaro (GP) section there were less varved successions and less chances to take the appropriate samples for the analyses. Similar to the previous section of Tanda, here is also important to remark the variation of the abundance ranges in the horizontal

axis depending on the component. Diatoms have the abundance range from 0% to 100%, spicules have the abundance range from 0% to 2%, and phytoliths and detritic have their abundance ranges from 0% to 50%.



Fig. 18. Stratigraphic log and results of the counting of abundances of components in the Puéllaro (GP) section. Red points between the lithology log and grain size log indicate the stratigraphic level at which each sample (11 in total) was taken. Those red points horizontally coincide with the horizontal bars that indicates the abundance (%) values of each component in this section.

In Fig. 18 can be observed the distribution of the counting results for each component in the Puéllaro (GP) section. In order to facilitate the analyses, based on the sedimentary processes and the paleoecological conditions of this section of the paleolake, we divided this distribution in three subzones. The three zones of the counting results of Puéllaro (GP) were:

- *Zone A* (12m - 13m): Composed by huge-massive conglomerates. Here was taken just one sample with medium rate of entrance of detritic. Diatoms has an abundance of 50%.

- *Zone B* (53m - 66m): Composed by lacustrine varves of clays and silts intercalated with conglomerates. The rate of entrance of detritic sediments is in low rates, but there is pike of very high rate in the sample GP-65.17 M, which is also the highest rate of entrance of detritic

in this interval. Diatoms present abundances between 75% - 95%, except in the sample GP-65.17 M with an abundance value close to 30%.

- Zone C (77m - 90m): Composed by lacustrine varves of clays and silts intercalated with conglomerates. Here, the rate of entrance of detritic sediments is in low rates, but there is pike of very high rate in the sample GP-77.30 M, and is the highest rate of entrance of detritic in the Puéllaro section. Diatoms present abundances between 65% - 80%, except in the sample GP-77.30 M with an abundance value close to 20%.

Once we have obtained the plots of the counting results in each section and their respective analyses, we can state that in both, Tanda (GT) and Puéllaro (GP) sections, there were three general patterns among the four components. These are:

(i) The abundances values of detritic are slightly similar to the phytoliths abundances values. This is, both increase or decrease at the same stratigraphic level.

(ii) The abundances values of diatoms are opposite to the sponges' spicules abundances. This is, if one increase, the other decrease, and the opposite.

(iii) The abundance values of detritic sediments are opposite to the diatoms' abundance values. This is, if one increase, the other decrease, while whether one decrease, the other increase.

6.3. Definition of morphotypes and identification of some species of freshwater diatom:

For this set of results, we used: (a) the bibliographic data of the reliable public digitaldatabase for diatom learning <u>https://diatoms.org</u>, (b) some academic books, and (c) scientificresearch articles for defining the main morphotypes and for identifying some species of diatoms in this work. Previous bibliographic studies focused on the identification of diatoms species based on the description of their frustules' shapes and their general morphology. So, they were also used as a guide to identify the morphotypes and the species of diatoms that appeared in the two stratigraphic successions of San Miguel Fm. studied here. Some of the sources we used for this section were: Zachariasse *et al.*, 1978; Garcia *et al.*, 1993; Gladenkov & Barron, 1992; Mitlehner, 1996; Bradbury, 2000; Denys, 2003; Kingston, 2003; Kociolek & Spaulding, 2003a, b; Lowe, 2003; Stoermer & Julius, 2003; Tuji & Williams, 2007; Dias *et al.*, 2009; McLaughlin, 2012; Ognjanova-Rumenova, 2012; Solak & Kulikovskiy, 2013; Buendía-Flores *et al.*, 2015; Bicudo, 2016; Borges *et al.*, 2016; Tofilovska *et al.*, 2016; Ognjanova-Rumenova & Yaneva, 2018; Plata *et al.*, 2018; Solak *et al.*, 2018; and Sabater, 2010.

However, it is important to remark again that here was not used a SEM microscope for reaching this objective with a total reliability. But we also remark that the process we carried out for defining morphotypes and for identifying diatom species, is well supported by reliable bibliographic studies previously cited. In those previous studies, authors have only used the light microscope for the identification of many diatom species. Therefore, we just compared all those visual features for selecting each morphotype and we strictly observed particular features of the species for identifying them correctly. Therefore, for illustrative purposes, we randomly selected several photos of diatoms and we grouped in the six morphotype categories identified in this work, which are shown in the next section. The same was applied for each of the 22 species of diatom identified in this work. All pictures were taken in the light microscope at the same conditions, using the immersion oil, at 1000x of magnification.

6.3.1 Definition of the categories of diatom morphotypes.

In this work, in order to define correctly each morphotype, we observed carefully all the morphological features such as: frustule's shape, orientation of pores, length, wide, particular body marks, raphe features, etc. Therefore, we must be sure that those visual features reported in the bibliography previously cited, coincide with the characteristics of each category of morphotype we propose in this work, or at least those features have to be very close.

Then in the 40 analyzed samples from the two stratigraphic sections studied here, we identified a total of six categories of diatom morphotypes. These six categories of diatom morphotypes were: monoraphid (Fig. 19), centric (Fig. 20), epithemioid (Fig. 21), asymmetric biraphid (Fig. 22), symmetric biraphid (Fig. 23), and araphid (Fig. 24).

• Morphotype of diatom #1: Monoraphid.

These kind of diatoms have an 'oval-shape' with different internal features (Fig. 19a-i). In Tanda section, these diatoms were very rarely to find one. Their valve are bilaterally symmetric about the line.



Fig. 19. Monoraphid diatoms from Tanda (GT) and Puéllaro (GP) sections. Scale bar=10 μ m. The vertical orange bar divides the type of morphotype in complete parts (A-F) in the left side, and in fragments (G, H) in the right side.

• Morphotype of diatom #2: Centric

Diatoms of this cathegory were the most common in Tanda and also the dominants. This is thanks to the paleogeography of Tanda that belongs to deeper areas of the paleolake where planktonik (centric) diatoms can grow with the conditions they need. Moreover this morphotype

cathegory was subdivided in three subtypes based on visual remarkable features of the diatoms depending on the view they were observed. These three types are:

- *Type A*: Diatoms with 'staggered shape' (girdle view). These diatoms vary in the number of steps sections from one to seven or more steps sections (Fig. 20a-e).

- Type B: Diatom with 'porous rectangular shape' (girdle view) with elongated peak (Fig. 20f-i).

- *Type C:* Diatoms with 'circular shape' (in valve view) with several features such as: one or more internal rings (Fig. 20k-w), with peaks around the circular edges (Fig. 20x-af), and with no hole but with internal lines (Fig. 20ag-ah).



Fig. 20. Three types of centric morphotypes diatoms from Tanda (GT) and Puéllaro (GP) sections. Scale bar=10 μ m. The vertical orange bar divides the type of morphotype in complete parts in the left side and in fragments in the right side. (A-E) Centric morphotypes of type A: staggered shape, (F-I) centric morphotypes of type B: porous rectangular shape, (K-AH) centric morphotype of type C: circular shape.

• Morphotype of diatom #3: Epithemioid

This category of diatoms was very common in some levels of Puéllaro section. In this case, this category of Puéllaro that have their valves with a bilateral symmetry about a line and a well-developed raphe system, can be subdivided in three subtypes:

- *Type A*: Diatoms with elongated-rounded shape with thick internal perpendicular lines and rounded ends (Fig. 21a-c).

- Type B: Diatoms with thick semi-elongated shape and a thick raphe system (Fig. 21d-g).

- *Type C:* Diatoms with semi elongated-rounded shape with internal perpendicular thin lines and elongated-oblique pike(s) in the ends (Fig. 21h-o).



Fig. 21. Epithemioid diatoms from Tanda (GT) and Puéllaro (GP) sections. Scale bar=10 μ m. (A-C) Epithemioid morphotypes of type A: elongated-rounded shape with thick internal perpendicular lines and rounded ends, (D-G) Epithemioid morphotypes of type B: thick semi-elongated shape and a thick raphe system, (H-O) Epithemioid morphotype of type C: semi elongated-rounded shape with internal perpendicular lines and elongated-oblique pike in the ends.

• Morphotype of diatom #4: Asymmetric biraphid

These diatoms were common in Puéllaro (GP) section. These diatoms (Fig. 22a-g) have a well-developed raphe system. Their ends can also have several shapes.



Fig. 22. Asymmetric biraphid diatoms from Tanda (GT) and Puéllaro (GP) sections. Scale bar=10 μ m. The vertical orange bar divides the type of morphotype in complete parts in the left side and in fragments in the right side.

• Morphotype of diatom #5: Symmetric biraphid

This category of diatom morphotype has the particularity of being the gratest diversity among the freshwater diatoms. In Puellaro due this section belongs to shallower areas of the paleolake, bethic diatoms have best conditions to grow. Therefore, benthic diatoms are the dominants in Puellaro section. In this case, this category of Puéllaro that have a bilateral symmetry about a line and a well-developed raphe system, can be subdivided in two subtypes: *Type A*: Diatoms with elongated shape and punctuated ends of different forms. In the photos of Fig. 23a-j, this subtype can have the presence, absence or several combinations of these shapes and the end of the punctuated pikes.

- *Type B*: Diatoms with elongated shape and semicircular ends (Fig. 23k-n).



Fig. 23. Symmetric biraphid diatoms from Tanda (GT) and Puéllaro (GP) sections. Scale bar=10 µm. The vertical orange bar divides the type of morphotype in complete parts in the left side and in fragments in the right side. (A-I) Symmetric biraphid morphotypes of type A: elongated shape and punctuated ends of different forms, (J-N) symmetric biraphid morphotypes of type B: elongated shape and semicircular ends.

• Morphotype of diatom #6: Araphid

This is diatom almost did not appear in Puellaro. The one that appeared (Fig. 29) is one of the most representative of this category. Its valve does not have raphe system and central dilation usually appears.



Fig. 24. Araphid diatom just from Puéllaro (GP) section. Scale bar=10 µm.

• Not identified fragments of diatom: n/d

These fragments (Fig. 25a-r) could not be related with any morphotype category.



Fig. 25. Non-identified morphotypes of diatoms from Tanda and Puéllaro sections. Scale bar=10 μm.

In summary, from the 9 types of morphotypes categories of diatoms that exist, in this work, we identified a total of 6 morphotypes in the 40 plates observed under the light microscope. The following table indicates the morphotypes that appear in each station:

DIATOM MORPHOTYPE	LABEL	GT TANDA	GP PUELLARO				
Centric	С	Х	Х				
Araphid	А	-	Х				
Eunotioid	EU	-	-				
Symmetric Biraphid	SB	Х	Х				
Monoraphid	М	Х	Х				
Asymmetric Biraphid	AB	Х	Х				
Ep ithemioid	EP	Х	Х				
Nitzschioid	N	-	-				
Surirelloid	S	-	-				

Table 5. Summary of the morphotypes identified in the Tanda (GT) and Puellaro (GP) sections.

6.3.2 Identification of some species of diatoms

In this point, was superficially identified a total of 22 diatoms species. A high-detailed visual description and a deep descriptive analysis for each one of the identified species of diatoms, is not addressed in this research work because we did not have the availability of a SEM equipment in the laboratory. However, we grouped the diatoms-candidates with similar morphological features. Then we compared all those morphological features with: (1) the species of the digital database, and (2) with all the preliminary bibliographic studies done about the identification of diatom species. Some species were easy to identify, others required more time with more careful observations, for figuring out the particular features of a specie; and other species were very complex to determine its taxonomy.

In this work, we followed the suggestion of Stoermer & Julius (2003), who stated that the taxonomical identification of planktonic (centric) diatoms can be based on the observation of the valve morphology. In theory, the structures of these centric diatoms are stable and consistent, which provide a reliable means of consistent identification. We also followed the same suggestion for the identification of the benthic (pennate) diatoms.

The identified diatom species vary according to their paleogeographical and paleoecological conditions in the two studied stratigraphic sections. It was found both planktonic and benthic diatoms species. It is important to remark that the results of the proposed identification for each diatom specie, is highly close to the true identification, and that approximation is really useful for the general purposes of this work. The diatom species identified, based upon their visual features were the following:

6.3.2.1 Planktonic (Centrales) diatoms

Due to centric diatoms have a high variability in frustule structure and forms of species (see Fig. 7), is highly suggested that researchers must be able to properly interpret all those types of variations before a formal systematic analysis proposal, which eventually provide a clear classification system for centric diatoms. Even those morphological differences are attributed to environmental, geographic or other factors (Stoermer & Julius, 2003). Centric diatoms have centric frustules rarely of bilaterally symmetrical construction. The frustules' structures are: concentric, radial or irregular. They have no raphe or pseudoraphe. The valves surface is: circular, polygonal or elliptical, rarely irregular boat-shaped (McLaughlin, 2012).

• Genus 01: Aulacoseira (Centric morphology - Planktonic)

Aulacoseira was previously classified as *Melosira*, a genus now restricted primarily to marine species (Round *et al.*, 1990). *Aulacoseira* genus contains a large number of species and several different forms as a result of either growth processes (Muller, 1903, 1906; Cleve-Euler,

1911a, b; Bethge, 1925) or silica limitation (Stoermer *et al.*, 1985). *Aulacoseira* appears best adapted to life in: lakes, ponds, and lager rivers. *Aulacoseira* is considered one of the best distributed species in time and space, of all freshwater centric diatoms. This genus is found in modern lakes from all parts of the world, and ancient tectonic lakes each appear to have their swarms of indigenous species (Edlund *et al.*, 1996).

• Species #1: Aulacoseira pusilla (Fig. 26). It was found in GT and GP.

They have cylindrical cells and form short chains. Valves are $4.5 - 7.5 \mu m$ in diameter, with a mantle height (width range) of $2.2 - 4.5 \mu m$. The ratio of the mantle height to valve diameter is slightly less than 1. They present some convex sides in the mantle, and the valve face can be flat or very slightly convex. The collum is ~ 1.4 - 1/5 of the mantle height. They have robust spines originated from two or three pervalvar costae. They could occur in lake sediments (Potapova, 2010a).



Fig. 26. *Aulacoseira pusilla* species in valve view (A-F) and in girdle view (G-K), that appear in the Tanda and Puéllaro sections. Scale bar=10 μm.

• Species #2: Aulacoseira granulata (Fig. 27). It was found in GT and GP.

They have cylindrical frustules with a direct face-to-face jointing forming filamentous colonies. Valves are 4-17 μ m in diameter with a mantle eight of 4-20 μ m. The ratio of mantle height to valve diameter is 0.8 – 5. The mantle has straight sides and a flat valve face. Their valve face has small scattered areolae. The linking spines are short, triangular and are located at the end of each pervalvar costa. They appear in lake sediments (Potapova & English, 2010a).



Fig. 27. *Aulacoseira granulata* species in girdle view (A-E) and in semi oblique valve view (G-M) that appear in the Tanda and Puéllaro sections. Scale bar=10 µm.

• Species #3: Aulacoseira herzogii (Fig. 28). It was found in GP.

Cylindrical frustules form short colonies. Their valves are 3-7 μ m in diameter and have a mantle height between 7-14 μ m. Ratio of mantle height to valve diameter is >= than 1. The mantle is straight. The valve face is flat and have small areolae with circular shape in light microscope and with irregular shape in the SEM microscope (Potapova & English, 2010b).



Fig. 28. Aulacoseira herzogii species in girdle view the Puéllaro section. Scale bar=10 µm.

• Species #4: Aulacoseira temperei (Fig. 29). It was found in GP.

Their frustules are cylindrical composed by a thick cell wall united in straight. Valve diameter: 2-22.5 μ m, mantle length: 8-22 μ m, mantle areolae arranged in 6-9 μ m straight rows in 10 μ m. The valve face is circular and flat, covered by peripheral areolae; the areolae are circular to elongate, occluded by rotate (Ognjanova-Rumenova, 2012).



Fig. 29. Aulacoseira temperei species in girdle view (A-F) that appear in the Puéllaro section. Scale bar=10 µm.

• Genus 02: Cyclotella (Centric morphology - Planktonic)

• Species #5: Cyclotella meneghiniana (Fig. 30). It was found in GT and GP.

It presents disc-shaped valves, with a narrow mantle. The valve can be transversely undulate or flat. The central area covers $\frac{1}{2}$ to $\frac{1}{3}$ of the valve face. Can appear marginal spines positioned in line with each costa. It is wide spread and common in shallow nutrient-rich waters, and in lake sediments (Lowe & Kheiri, 2015).



Fig. 30. *Cyclotella meneghiniana* species in valve view (A-E) that appear in the Tanda and Puéllaro sections. Scale bar=10 μm.

• Genus 03: *Discostella* (Centric morphology - Planktonic)

• Species #6: *Discostella pseudostelligera* (Fig. 31). It was found in GT.

Their valves may be flat, or can be a convex or concave center. The central area of the valve has a floral pattern, that is also surrounded by a marginal ring of radiate striae that can be bifurcated by ribs. It is wide spread in nutrient-rich plankton streams (Lowe, 2015a).



Fig. 31. *Discostella pseudostelligera* species in valve view (A-E) that appear in the Tanda section. Scale bar=10 µm.

• Species #7: Discostella stelligera var. tenuis (Fig. 32). It was found in GT.

The valves have a well-defined marginal ring and a distinct central area. The center can be either concave or convex. The central area presents a stellate pattern of alveoli. Depending on the specimen, the central pattern may be absent or composed by a ghost alveolus. They occur in lakes and large rivers with increased nutrients (Lowe, 2015b).



Fig. 32. *Discostella stelligera* species in valve view (A-F) that appear in the Tanda section. Scale bar=10 µm.

6.3.2.2 Benthic (Pennate) diatoms

Benthic diatoms are microalgae used worldwide for several research purposes. They are of particular interest in the context of bioassessment because of their taxonomic diversity, different sensitivity, and resistance to pollution. In general, their frustules are bilaterally symmetrical, they present raphe or pseudoraphe, and the most part of the valve surface is rod-or boat-shaped (McLaughlin, 2012).

• Genus 04: Cocconeis (Monoraphid morphology - Benthic)

• Species #8: Cocconeis placentula var. Euglypta (Fig. 33). It was found in GT and GP.

Valves are elliptic and relatively flat. The raphe is straight and filiform. Their striae are radiate and interrupted by a hyaline ring positioned close to the valve margin. The areolae can be well distinguished in light microscope. Rapheless valve has a linear to linear-lanceolate axial area and radiate striae. It occurs in lake sediments (Potapova & Spaulding, 2013).



Fig. 33. *Cocconeis placentula* var. *Euglypta* species in valve view (A-D) that appear in the Tanda and Puéllaro sections. Scale bar=10 µm.

• Species #9: Cocconeis pseudothumensis (Fig. 34). It was found in GT and GP.

Their values are broadly elliptical. Raphe has a narrow axial area. The striae are fine and radiate. The rapheless value has a broad, lanceolate axial area that covers 1/3 to $\frac{1}{2}$ of the value. It is common in lotic (lakes) ecosystems (Lowe, 2015).



Fig. 34. *Cocconeis pseudothumensis* species in valve view (A-B) that appear in the Tanda and Puéllaro sections. Scale bar=10 μm.

• Genus 05: *Rhopalodia* (Epithemioid morphology – Benthic)

• Species #10: *Rhopalodia gibba* (Fig. 35). It was found in GP.

Valves are best appreciated in valve view, being linear with apices bent towards the ventral margin. The central valve is inflated. Their raphe is positioned in a canal along the dorsal margin. Costae are prominent, and extend across the valve face. It occurs in lake sediments (Kociolek, 2011a).



Fig. 35. *Rhopalodia gibba* species in girdle view (A, B) and in valve view (C-E) that appear in the Puéllaro section. Scale bar=10 µm.

• Species #11: *Rhopalodia gibberula* (Fig. 36). It was found in GT and GP.

Valves are lunate, with strongly convex dorsal margins. The ventral margin is slightly concave to straight. In girdle view, frustules are widely lanceolate or elliptical. Raphe is positioned on the dorsal margin. The apices can be slightly bent ventrally or may be protracted and rounded. It tends to grow in saline lakes, and in nitrogen-poor habitats (Jordan, 2015).



Fig. 36. *Rhopalodia gibberula* species in girdle view (A-D) that appear in the Tanda and Puéllaro sections. Scale bar=10 μm.

• Genus 06: *Rhoicosphenia* (Asymmetric Biraphid morphology – Benthic)

• Species #12: Rhoicosphenia curvata (Fig. 37). It was found in GT and GP.

This species is currently regarded as a synonym of *Rhoicosphenia abbreviata* (Agardh, 1831). Valves are clavate with rounded apices. Frustules are heteropolar, appearing bent in girdle view. Ventral and dorsal valves are concave and convex, respectively. Central area is oval to elliptic. Areolae are apically elongated. Cells occur on stalks, forming colonies. They are found in freshwater lakes -high in ionic content- and in brackish water (Potapova, 2009).



Fig. 37. *Rhoicosphenia curvata* species in girdle view (A-C) that appear in the Tanda and Puéllaro sections. Scale bar=10 μm.

• Genus 07: Cymbella (Asymmetric Biraphid morphology – Benthic)

• Species #13: Cymbella tumida (Fig. 38). It was found in GP.

This species is currently regarded as a synonym of *Cymbella cymbiformis* (Agardh, 1830). The valves are lanceolate and dorsiventral with bluntly rounded apices. The dorsal margin is moderately arched. Central area is small and elliptic. The raphe is distinctly lateral. Striae are widely spaced and nearly parallel. Areolae are distinct in light microscope and widely spread (Bahls, 2016).



Fig. 38. *Cymbella tumida* species in girdle view (A-C) that appear in the Puéllaro section. Scale bar=10 µm.

- Genus 08: Gomphosphenia (Asymmetric Biraphid morphology Benthic)
 - Species #14: Gomphosphenia lingulatiformis (Fig. 39). It was found in GP.

Valves are at the higher end of the size range are linear-clavate with undulate margins, while smaller valves are clavate with smooth margins. Raphe is straight. The striae are radiate and are formed by a single areola. It has been found in rivers (Kociolek & Bishop, 2017).



Fig. 39. *Gomphosphenia lingulatiformis* species in girdle view (A, B) that appear in the Puéllaro section. Scale bar=10 µm.

• Species #15: Gomphosphenia grovei (Fig. 40). It was found in GP.

Valves are linear-clavate with a broadly rounded headpole. The footpole is narrow and subcapitate to capitate. Raphe is filiform and straight. Striae are usually short and near the margin, composed of two or more isolated areolae. It occurs in lake sediments (Kociolek, 2011b).



Fig. 40. *Gomphosphenia grovei* species in girdle view (A, B) that appear in the Puéllaro section. Scale bar=10 µm.

• Genus 09: *Gomphoneis* (Asymmetric Biraphid morphology - Benthic)

• Species #16: Gomphoneis pseudo-okunoi (Fig. 41). It was found in GP.

Valves are clavate, with a broadly rounded headpole and narrow footpole. Central area has four isolated stigmoids. Raphe is straight and filiform. Striae appear costate, without resolvable puncta. The axial area is very narrow, straight and broadening at the center. It can be common in circum-neutral to alkaline environments (Kociolek, 2011c).



Fig. 41. *Gomphoneis pseudo-okunoi* species in girdle view that appears in the Puéllaro section. Scale bar=10 μm.

• Genus 10: Sellaphora (Symmetric Biraphid morphology – Benthic)

• Species #17: Sellaphora alastos (Fig. 42). It was found in GP.

Valves are broadly linear, with parallel or weakly concave margins. Axial area is narrow and undulate. The conopea are continuous through the central area that is wide and transversely rectangular. Raphe is filiform and follows the undulate margins of adjacent conopea. Striae are parallel, straight, shorter and more widely spaced. This species is found in small lakes and rivers (Bahls, 2014).



Fig. 42. Sellaphora alastos species in girdle view (A-D) that appear in the Puéllaro section. Scale bar=10 µm.

• Genus 11: Navicula (Symmetric Biraphid morphology - Benthic)

• Species #18: Navicula lundii (Fig. 43). It was found in GT and GP.

Valves are lanceolate with rounded weakly apices. Axial area is narrow. Central area is transapically expanded and asymmetric. Raphe is filiform and straight. Striae are curved and radiate. Areolae in the striae are fine and difficult to resolve in light microscope. It is widely distributed in wet habitats (e.g.: moist soils), and in streams and lake shores (Bahls, 2012).



Fig. 43. *Navicula lundii* species in girdle view (A, B) that appear in the Tanda and Puéllaro sections. Scale bar=10 μm.

• Species #19: Navicula radiosa (Fig. 44). It was found in GP.

Their valves are narrow and lanceolate, with acutely rounded ends. The axial area is narrow and linear. The central area is rhombic. Raphe is straight. The transapical striae are strongly radiate. The apices are acutely rounded. Some of this species are distributed in lake sediments (Potapova, 2011).



Fig. 44. *Navicula radiosa* species in girdle view (A-C) that appear in the Puéllaro section. Scale bar=10 µm.

• Genus 12: Karayevia (Monoraphid morphology - Benthic)

• Species #20: Karayevia laterostrata (Fig. 45). It was found in GP.

Valves are elliptical with narrowly capitate to subcapitate apices. The raphe valve has a lanceolate axial area and no distinct central area. Raphe is straight, without terminal fissures. Striae on raphe are radiate, in the valve center are short, and on the rapheless valve are punctate. It appears in lake sediments (Potapova, 2010b).



Fig. 45. *Karayevia laterostrata* species in girdle view that appears in the Puéllaro section. Scale bar=10 μm.

• Species #21: Karayevia laterostrata (Fig. 46). It was found in GP.

Valves are elliptical. Raphe is straight, without terminal fissures. Raphe is straight (11-16 μ m in length). Striae on the raphe valve are radiate. The rapheless valve has linear axial area and no central area. This specie usually occurs in lake sediments (Potapova, 2010c).



Fig. 46. *Karayevia laterostrata* species in girdle view that appears in the Puéllaro section. Scale bar=10 μm.

- Genus 13: Synedra (Araphid morphology Benthic)
 - Species #22: Synedra goulardii (Fig. 47). It was found in GP.

Valves are linear-lanceolate with a distinctly constricted mid-valve. Axial area is linear and very narrow. Central area is large, circular, and asymmetric. Striae are parallel, becoming slightly radiate at the apices. They are common in benthic habitats (Bishop, 2015).



Fig. 47. Synedra goulardii species in girdle view that appears in the Puéllaro section. Scale bar=10 µm.

Table 6 summarizes the results of the identification of the diatom species. A total of 6 morphotypes, 13 genera and 22 species of diatoms between planktonic and benthic, were identified in this work. For planktonic diatoms we have 1 morphotype, 3 genera and 7 species; while for benthic diatoms we have 5 morphotypes, 10 genera and 15 species.

In Table 6 was also indicated the section at which, each diatom specie identified in this work appears, however, due to in the same table cannot be expressed the relative abundances of each specie, we cannot correctly establish how dominant or scarce each specie is in each section. Then, for now we can say that planktonic diatom species are more abundant in the deeper waters of Tanda (GT) section, while benthic diatom species are more abundant in the shallower waters of Puéllaro (GP) section.

Table 6. Results of the identified morphotypes and species of freshwater diatoms microfossils in the 40 plates. It is also indicated location as well as the theoretical paleoenvironments that each identified species of freshwater diatoms, are associated to (Source of paleoenvironments: <u>https://diatoms.org</u>). It was also assigned a reference color for each morphotype and a derived color with less intensity for each identified genus and species that belongs to that morphotype. The paleoenvironment labels are: (1) Lotic ecosystem, (2) small lakes, (3) lake sediments, (4) wetlands, (5) lakes shore, (6) streams, (7) river shallow waters, and (8) brackish waters.

Type	Mornhotypo	Conus	Species		Location		Paleoenvironment						
туре	wrorphotype	Genus			GP	1	2	3	4 5	56	7	8	
		Aulacoseira	Aulacoseira pusilla	Х	Х			Х					
			Aulacoseira granulata	Х	Х			Х					
Planktonia	Centric		Aulacoseira herzogii	-	Х								
Planktonic (Centreles)			Aulacoseira temperei	-	Х								
(Centrales)		Cyclotella	Cyclotella meneghiniana	Х	Х			Х			Х		
		Discostella	Discostella pseudostelligera	Х	-					X	Х		
			Discostella stelligera var. tenuis	Х	-			Х		X	X		
	Monoraphid	Cocconeis	Cocconeis placentula var. Euglypt	Χ	Χ			Χ					
			Cocconeis pseudothumensis	Χ	Χ	Х		Χ					
		Karayevia	Karayevia laterostrata	Χ	-			Χ					
			Kolbesia ploenensis	-	Χ			Χ					
	Enithemioid	Rhopalodia	Rhopalodia gibberula	Х	Х								
	Ершенною		Rhopalodia gibba	-	Х			Х					
Benthic (Pennates)		Rhoicosphenia	Rhoicosphenia curvata	Х	Х			Х		X		Х	
	Asymmetric	Cymbella	Cymbella tumida	-	Χ								
		Gomphosphenia	Gomphosphenia lingulatiformis	-	Х					X			
	Birapilia		Gomphosphenia grovei	-	Х			Х					
		Gomphoneis	Gomphoneis pseudo-okunoi	-	Χ								
	Symmetric	Sellaphora	Sellaphora alastos	-	Х		Х			X			
	Dironhid	Navicula	Navicula lundii	-	Х				XУ	Χ			
	ытарию		Navicula radiosa	-	Х		Х						
	Araphid	Synedra	Synedra goulardii	-	Χ								

6.4 Biostratigraphy of freshwater diatoms in the Tanda (GT) and Puéllaro (GP) sections.

6.4.1 General distribution of freshwater diatoms in the Tanda and Puéllaro sections.

Once the sedimentological evidence (see Fig. 10, 12) for inferring the paleogeographic location of each section studied here, and supported by the counting results of the abundance of the components (see Table 3, 4 and Fig. 17, 18) in GT and GP sections, the distribution of siliceous microfossils (freshwater diatoms) that was obtained, was the one we expect in each section. We agree that those distributions were driven by several ecological and environmental conditions of the paleolake. Advantageously, in both sections (GT & GP), we collected enough sedimentological and paleontological evidence for understanding and interpreting a good interval of the evolution of this paleolake, and therefore, to do a good paleoecological study based on the diatom species previously identified.

Tanda section (GT) belonged to deeper zone of the paleolake, whose paleogeographical conditions facilitated the formation of several varved successions during the evolution of the paleolake. While, the Puéllaro (GP) section belonged to shallower zone of the paleolake where

its paleogeographical conditions, limited the normal development of the varved-succession in this section. In both sections, GT and GP, we found massive units of grained coarse-sandstone and boulder-conglomeratic deposits that were not sampled due to these lithologies do not have the required conditions that guarantee the normal development of microbiological life and activity. Then, these units are totally covered by coarse-grains and detritic-sediments, and have no or very limited siliceous microfossils content. In the same way, in both sections, GT and GP, there are fine-grain massive units and silty-clayed varved sediments that were sampled due to these lithologies had much better conditions for a good development of the microbiological life and activity, especially for siliceous microorganisms. Therefore, those lithologies have a relatively high siliceous-microfossil content, especially in diatoms, sponges' spicules and even the non-fossil siliceous phytoliths of vegetal origin.

6.4.2 Biostratigraphy of freshwater diatom in the Tanda and Puéllaro sections.

After the definition of morphotypes, and the identification of several genera and species of diatoms, paleoecological analyses are required. Then, we used biostratigraphical analyses for understanding how the distribution of the species of diatoms have varied in the two stratigraphic successions (paleoecology of the Tanda and Puéllaro).

In both biostratigraphic logs were used different filling colors in the circles' body in order to differentiate the morphotype category at which each diatom species belongs to. Those filling colors were assigned as: in yellow are centric, in red are monoraphid, in green are epithemioid, in dark-orange are asymmetric biraphid, in grey are symmetric biraphid, and in blue are araphid morphology. Moreover, in the same biostratigraphic results of GT and GP, for indicating which diatom species is dominant in each stratigraphic level studied, we added an outline color for this purpose. Those outline colors in the border of the circles indicate that: (i) a black-outline is the diatom species of total dominance (>40% or >50%) in that stratigraphic level, (ii) a blue-outline is the 2nd dominant diatom in that stratigraphic level, and (iii) a grey-outline is the 3rd dominant diatom species were very diverse and there was not a real diatom with total dominance over the rest. For these cases, where was not possible to assign a black outline color in the outline of a circle, we used the blue outline to indicate a diatom specie as the dominant (>30 %), and then the grey outline as the 2^{nd} dominant diatom in that level.

Moreover, we considered that the best way to do the biostratigraphic analyses is dividing the biostratigraphic logs in subzones, exactly as was done in the previous plots of the results of the counting of components. Therefore, these divisions are useful for understanding how the associations of diatom species vary per intervals in the two studied stratigraphic successions. The results of the biostratigraphy of Tanda (GT) and Puéllaro (GP) sections, are then shown in Fig. 48 and Fig. 49 respectively.

6.4.2.1 Biostratigraphy of freshwater diatoms in the Tanda (GT) section

We divided the biostratigraphic log of Tanda section (Fig. 48) in three zones. These three zones are the same zones of the plots of counting of components. These are:

Zone A (09m – 13m): Two stratigraphic levels were sampled in this zone, where *Aulacoseira* genus is more abundant here. *Aulacoseira pusilla* is the absolute dominant in the first level and the apparition of a benthic specie (*Rhoicosphenia curvata*) is reported in the second level. In this lower zone, the paleoecological conditions are not good for both planktonic or benthic diatom species. Besides the specie *Aulacoseira* genus is the most abundant, just two of its species are registered in this level, which is relatively interesting in terms of the paleoecological conditions in that level for this species in particular.

Zone B (21m - 45m): Seven stratigraphic levels were sampled in this zone, and centric diatoms of the genus *Aulacoseira*, *Cyclotella* and *Discotella* are the most abundant and dominants here. *Aulacoseira pusilla* appears in all levels of this zone, and is the main dominant specie in two levels. A particular event occurred in the level GT-21.30 M where diatoms did not appear in those samples, while spicules had a great abundance (see Fig. 17), which makes this level interesting in terms of the paleoecological conditions that provoke that event. Moreover, 2 species of benthic diatoms (*Cocconeis placentula* var. *Euglypta* and *Rhoicosphenia curvata*) appear in the two upper levels of this zone, which seems to keep a paleoecological and environmental relationship with the first levels of the next zone C.

Zone C (72m - 90m): Eight stratigraphic levels were sampled in this zone. Centric diatoms are the remarkable dominants here with the species of the genera *Aulacoseira, Cyclotella and Discotella*. The species *Aulacoseira pusilla* and *Aulacoseira granulata* are the most dominant and in the majority of the levels of this zone. However, some benthic species of the genera *Cocconeis, Rhopalodia, Gomphosphenia* and *Navicula*, had some few representants in the lower levels of this zone, from 72 m to 81 m specifically, which keeps a probable relationship with the last two levels of the previous zone B. And the last levels of this succession do not have any benthic diatom specie registered in our samples.

Finally, biostratigraphically talking, is clear that diatoms species of the planktonic group, are the main dominants in Tanda (GT) section. These biostratigraphic results of Tanda were what we expected, and has the distribution that the deeper zones of Tanda has to have. However, some interesting events occurred and those have to be discussed later.



Fig. 48. Biostratigraphic log of the Tanda (GT) section with relative abundances for specific diatoms species that are dominants in a specific stratigraphic level. Circles filled in: yellow are centric; red are monoraphid, green are epithemioid, brown are asymmetric biraphid, in grey are symmetric biraphid, and in blue are araphid morphology. Circles' outline colored with: black is a total dominant (>40%) diatom, blue is the 2nd dominant, and grey is the 3rd dominant. In case of no total diatom dominant was used the blue border as dominant.

6.4.2.2 Biostratigraphy of freshwater diatoms in the Puéllaro (GP) section

We divided the biostratigraphic log of Puéllaro section (Fig. 49) in three zones. These three zones are the same zones of the plots of counting of components. These are:

Zone A (12m - 13m): In this zone, just one sample was taken at the level GP-12.30 M. Here was mostly observed fragments of diatoms that were not possible to identify. However, just two benthic species (*Navicula radiosa* and *Karayevia laterostrata*) rarely appeared in this level. The high rate of fragmented parts of diatoms, has to be related to, that this thin claystone layer was deposited among several massive and fragmented conglomerates at the base of this succession, whose weight could affect by pressure the conservation of those diatoms.

Zone B (53m – 66m): Five levels were sampled in this zone where benthic diatoms species show more diversity distributed than planktonic diatoms. The interesting results were:

- Planktonic diatoms of the genera *Aulacoseira* and *Cyclotella* appears with less diversity, but is interesting that: (i) the specie *Aulacoseira granulata* is the total dominant in the levels GP-58.82 V and GP-62.50 M, and in these two levels, benthic diatoms have no representants, and (ii) the specie *Aulacoseira pusilla* is the dominant in the level GP-53.85 V, where benthic diatoms have more representants.

- The level GP-56.67 M is the only one where planktonic diatom did not appear. Just were found benthic diatoms where the species *Cocconeis placentula* is the dominant in this level.

- In the levels GP-53.85 V and GP-56.67 M benthic diatoms have the highest grade of biodiversity not only in this zone but also in the entire section of Puéllaro.

- In the interval from 58 m to 66 m, benthic diatoms are very scarce and just the benthic specie *Cocconeis placentula* seems to be constant specie in this zone B.

Zone C (77m - 90m): Five levels were sampled in this zone where again benthic diatoms, in less proportion, are the dominants. In spite of planktonic diatoms tended to decrease in this zone, they again have their dominants species in some levels. The interesting results were:

- In the level GP-77.30 M did not appear planktonic species, just benthic species without a clear dominant(s) due to the high rate of entrance of detritus in this level (see Fig. 18).

In the levels GP-82.45 M and GP-84.43 V just appear the specie *Cocconeis placentula* var. *Euglypta* in for the benthic group, while in the planktonic group, just appear *Aulacoseira* genus. *Aulacoseira pusilla* is the dominant specie in the level GP-85.67 M and GP-89.38 M where benthic diatoms have more diversity, and *Aulacoseira granulata* is the absolute dominant in the level GP-84.43 V where the benthic specie *Cocconeis placentula* var. *Euglypta* appeared.

In the biostratigraphy of Puéllaro (GP) section, is clear that diatoms species of the benthic group, are the main dominants. The distribution of benthic diatom species in this section was also as we expected in an area with shallow-waters. The benthic diatom specie *Cocconeis placentula* was the most common, there were levels where planktonic diatoms were dominants.



Fig. 49. Biostratigraphic Log of Puéllaro (GP) section with relative abundances for specific diatoms species that are dominants in a specific stratigraphic level. Circles filled in: yellow are centric; red are monoraphid, green are epithemioid, brown are asymmetric biraphid, in grey are symmetric biraphid, and in blue are araphid morphology. Circles' outline colored with: black is a total dominant (>40%) diatom, blue is the 2nd dominant, and grey is the 3rd dominant. In case of no total diatom dominant was used the blue border as dominant.

6.4.3 The attempt of constructing a lithostratigraphic correlation between the Tanda (GT) and Puéllaro (GP) sections.

This approach cannot be done due to the two selected stratigraphic successions are very complex and is not easy to identify all the units that compose the study area if the San Miguel Fm. Besides the attempt to correlate Tanda with Puéllaro seems very complex and difficult, we figured out that in both stratigraphic successions, the lack of required markers that would serve as reference guides, are not exposed, which does not facilitate us a correct analyses and interpretation for developing a correct lithostratigraphical correlation. Moreover, the Guayllabamba basin is a relatively small basin and in the two stratigraphic successions studied here, do not appear the remarkable events necessary to develop a stratigraphic correlation based on sequential stratigraphy by using transgressive or regressive surfaces, or we also consider that progradational and retrogradational sequences, as suggested by Shanley & McCabe (1994).

Therefore, the intention of developing a lithostratigraphic correlation is difficult and not correctly supported with enough data. Moreover, basing on the ongoing work of Martin (2020, *in prep.*), where have been studied eight stratigraphic successions, by correlation, this author has identified at least four lithostratigraphic units in his study area, which is bigger than this work's study area. Conversely, in this research project we identified at least eight lithostratigraphic units between the two stratigraphic successions studied in the San Miguel Fm. To better appreciate the limitation and lack of clear field-evidences that prevent us in developing a lithostratigraphic correlation, in the work of Martin (2020, *in prep.*), has been identified a volcanic input unit of deformed lava, which does not appear in either of the stratigraphic successions (GT and GP) studied in this work. Therefore, the best input of the present work is provided in analyses of the two studied stratigraphic successions (see Fig. 10, 12), based on the particular geologic processes related with the paleolake's evolution.

6.4.4 The attempt of constructing a biostratigraphic correlation between the Tanda (GT) and Puéllaro (GP) sections.

Similarly, as a consequence of a not provided lithostratigraphical correlation analysis, the intention of providing a complete biostratigraphic correlation between Tanda and Puéllaro sections, is equally affected by the same limitation and lack of data for constructing an adequate identification of clear biostratigraphic markers that could serve as reference for developing the biostratigraphic correlation between them. Hence, the best input of the present work is provided in the two biostratigraphic logs (see Fig. 48, 49) previously analyzed, and can be appreciated remarkable differences in the associations of freshwater diatoms between them, which is the data we needed.
In summary, in the Tanda (GT) section domain the planktonic diatoms and in Puéllaro (GP) section domain the benthic diatoms. These biostratigraphic results depend on several micro-, macro- and external natural-conditions of the Guayllabamba paleolake that, in this research project are not deeply studied. However, the paleogeographic location in the paleolake of the two stratigraphic sections studied here, are the key conditions for the results in the diatoms' dominances. Then, the biostratigraphic results showed us a reliable and coherent distribution of the associations of freshwater diatoms species in the two sections studied here based on the paleoecological conditions that acted on the paleolake.

7. DISCUSSION

In the subchapter 6.2 was carried out the counting process of the four components (diatoms, sponges' spicules, phytoliths and detritic sediments) in each stratigraphic section studied here, whose results were shown in the Tables 3, 4 and plotted in the Figs. 17, 18. At the end of the counting results, three general trends among the four components were identified: (i) The abundances values of detritic sediments are slightly similar to the phytoliths abundances values. This is, both increase or decrease at the same stratigraphic level, (ii) The abundances values of diatoms are opposite to the sponges' spicules abundances. This is, if one increase, the other decrease, and the opposite, and (iii) The abundance values of detritic sediments are highly dependent on specific conditions that each component -at least the siliceous such as the diatoms, spicules and phytoliths- needs in lacustrine areas. Therefore, we analyzed the results of the counting process and biostratigraphic analyses of each component based on the biological conditions they needed in order to understand the paleoenvironmental implications of the paleolake formed in San Miguel Fm.

7.1 About the results of the sponges' spicules and phytoliths in Tanda and Puéllaro sections. 7.1.1 Abundance values, scarce and dissolution of sponge spicules and phytoliths in dependence of the paleoecological conditions of the paleolake.

In the subchapter 6.2 of results of the counting process, was addressed the counting for the sponges' spicules and phytoliths components. They also reflect important paleoecological and paleoenvironmental conditions for us, therefore, the understanding of the changes of the abundance values of those two siliceous components are useful for the purpose of this work. The several paleoecological conditions that spicules and phytoliths have in dependence of the paleolake are not deeply studied here, just superficially. Previously, were stated that the counting results of Tanda and Puéllaro sections showed three common patterns that correlate all components. In this section, we focus on spicules and phytoliths behaviors and their paleoecological and environmental implications that acted on the paleolake evolution.

We mandatorily consider the paleogeographic location of Tanda and Puéllaro in the paleolake. Tanda section belongs to the pelagic (deep-waters) zones of the paleolake, which means that the entrance of detritic sediments would be caused by several natural factors such as wind transportation, superficial water-current transportation, internal water currents, etc. While Puéllaro section belongs to the littoral (shallow-waters) zone of the paleolake where the entrance of detritic sediments tend to be more chaotic and appears palustrine environment in that area. Our assertation is in agreement with a source area of coarse sediment located to the west of Puéllaro section, and the deeper zone of the paleolake near to the Guayllabamba county, as evidenced in this work (see Fig. 17, 18 & Table 6), and as stated by Villagómez (2003).

7.1.1.1 Sponges' spicules implications:

The grow of sponges is regulated by the availability of nutrients in a suitable habitat. The development of populations of freshwater-sponges also depend on the availability of hard substrates, and the majority of sponges seems to also depend on the presence of some solid surface from boulders conglomerates or exposed bedrock to the branches of fallen trees to the leaves and stems of aquatic macrophytes (Frost, 2001). We consider this fact really important for the analyses because it makes sense and keeps a coherent distribution with the results of abundances values of sponges' spicules in the Tanda and Puéllaro sections. This is, the abundances values of spicules in Tanda (Fig. 17) have two highest pikes in the levels GT-21.30 M and GT-77.55 V (just in the light laminae), while the abundances values of spicules in Puéllaro (Fig. 18) have the highest pikes in the levels GP-58.82 (just in the dark laminae) and GP-85.67 M. If we look carefully the counting results of the spicules in both sections, we would note that those highest pikes previously cited, are located over coarse-grain conglomeratic lithologies from pebble to boulder bodies and even over a very coarse sandy body. This makes sense with the Frost's (2001) interpretation which we taking it as reference for establishing that those stratigraphic level points are important reference points that served as soil solid surface for the development of more populations of the freshwater-sponges.

It is also known that sponges are among the few animals that require large amounts of silica for their spicules' development. The spicule is bound together by collagens to form an inorganic skeleton. It is well known that sponges are restricted largely to water of moderate silica content (>0.5 mg liter⁻¹) (Jewell, 1935). The distribution of the species of spicules are directly correlated with the calcium content of the water but declines in very hard waters (high in dissolved minerals, largely calcium and magnesium) (Jewell, 1939; Strekal & McDiffett, 1974); Hutchinson, 1993). However, the causal mechanism for this relationship still remain not

clear, due to spicules are probably related to food abundance rather than to a direct demand for calcium (Wetzel, 2001). We also consider that sponges are distributed by biogeographic factors such as light, substrata, and wave action (Frost, 2001).

We also noted that spicules are more abundant in Tanda section (from 2% to 45%) than in Puéllaro section (from 0% to just 2%). We attribute this to the fact that freshwater sponges, which usually occur only in relatively clear, unproductive waters, rarely become a major component of benthic communities, this is, their significance on benthic productivity is minor in most situation (Wetzel, 2001). The previous biological condition, coincide with the obtained results of the counting of abundances of sponges' spicules, which forced us to modify the abundance range in the counting plots of the sponges' spicules component. What we did was that in Tanda section the range of abundance was constrained between 0% to 50 %, while in Puéllaro section was constrained between from 0% to just 2%. However, the most reliable biological interpretation that is coherent with our sponges' spicules results is that in lakes, sponges can be limited in shallow waters -Puéllaro section in this case- by wave action or ice scour (Bader, 1984), while in deeper water -Tanda section in this case- they can be limited by low oxygen or by colder temperatures (Frost, 2001).

7.1.1.2 Phytoliths implications:

Several kinds of phytoliths are decay-resistant plant remains and can be deposited in the soil as the plant or plant parts die and break down, but they are subjected to mechanical breakage, erosion and deterioration in high pH soils. The mechanisms by which phytoliths can be transported can by through animal consumption, gathering of plants by humans, or by erosion or transportation of the soil by wind, water or ice (Yost, 2008).

In spite of phytoliths are also used by paleoclimatic reconstruction in conjunction with pollen and diatom data from cores samples, in those studies, the distribution of phytoliths are compared with variations of the genera of pollen. Even the most appropriate way to work with phytoliths is determining whether their provenance is from a C_3 or C_4 plant and then developing a comparison with the diatom data in order to determine lake-level changes or salinity (Rosen, 2008). Disadvantageously, in this work we are not addressing a deep analysis for identifying the species of the phytoliths components found in the two sections studied here, we just counted them and we used them as reference for the analyses and for finding correlations with the rest of the components counted in both sections.

Finally, what we infer here is that the nature of both sponges' spicules and phytoliths tend to be part of plants or other biological organisms that could easily be transported by the wind, superficial currents, internal waves or even at the bottom of the lake. This support that in several samples were found phytoliths and spicules close to each other, and in some cases accompanied by detritic sediments. Similarly, the previous statement coincides with the previous patterns between phytoliths and detritic sediments components. Those components keep similar abundance values and we infer that the detritic sediments that entered toward the paleolake were transporting phytoliths in similar proportions by natural attachment/factors.

7.2 About the results of freshwater diatoms in the Tanda and Puéllaro sections.

7.2.1 Interpretation of the counting results: the changes in the abundance values of freshwater diatoms found in the Tanda (GT) and Puéllaro (GP) sections.

We analyzed several specific conditions that diatoms need in separate subsections.

7.2.1.1 Dominance, scarce and dissolution of diatoms in some specific stratigraphic levels of Tanda (GT) and Puéllaro (GP) sections:

Diatoms are the dominant siliceous-components in both stratigraphic sections. For the analyses, we decided to divide the highest abundance values of diatoms component, in two ranges: (i) a dominance between 45% - 70% we call these diatoms as the 'dominant' component in that stratigraphic level, and with (ii) a dominance of >70% we call these diatoms as the "total dominant" component in that stratigraphic level.

Specifically, the stratigraphic levels of Tanda (GT) section where diatoms are considered the 'total dominant' are in GT-25.95 M, GT-28.40 M, GT-35.45 V, GT-44.45 M, GT-72.65 V (in both light and dark laminae), GT-80.80 M (in both light and dark laminae), GT-79.60 V (in both light and dark laminae), GT-80.80 M (in both light and dark laminae), GT-87.70 V (in both light and dark laminae), GT-86.50 M, GT-87.20 V (in both light and dark laminae), and in GT-89.67 V (in both light and dark laminae). While in Puéllaro (GP) section where diatoms are considered the 'total dominant' are in GP-53.85 V (in both light and dark laminae), GP-56.67 M, GP-58.82 V (in both light and dark laminae), GP-62.50 V (in both light and dark laminae), GP-82.45 M, GP-84.43 V(just in dark laminae), and in GP-84.67 M. Therefore, we are interested in understanding why in those specific stratigraphic-levels of Tanda (GT) and Puéllaro (GP) sections, diatoms have very high abundance values compared with the rest of component that show abundance values that are opposite, the same, or in some levels are very scarce or almost do not appear in that level.

Since we observed this trend of diatom dominance in the majority of the analyzed stratigraphic levels in the two sections, we infer that during the evolution of the paleolake, when the lake-level was in those stratigraphic level-points, a bloom of diatoms could occur due to, there were simultaneously the right conditions for having a dependent biologic event that facilitates such bloom of diatom. It means, that a high diatom abundance is driven by high

nutrient lake productivity, a high siliceous content that facilitates the diatom growing, reproduction, preservation and even good conditions for a good diversity of diatom species.

However, the dominance of diatoms in many stratigraphic levels were not in the best terms, this is, that they were affected by biological and chemical conditions that fragmented them and were not able to have an appropriate preservation of their valves. In those levels where was common to find fragmented (dissolution) valves of diatoms, we consider that diatoms were absent from some lacustrine sediments where the conditions were unfavorable to a biogenic silica preservation (Westover, 2019). Frequent resuspension cycles and grazing also can cause breakage of diatom frustules and diffusion of dissolved silica, making them more liable to dissolution (Jewson et al., 1981; Beyens & Denys, 1982; Rippey, 1983). We also observed dissolution (fragmentation) of planktonic assemblages, which suggests that the lake remained relatively deep even as lake levels fell and alkalinity increased due to evaporite concentration (Westover, 2019). Moreover, we consider that in these levels where diatoms suffered high rates of fragmentation, was due to they were very abundant, and therefore, in those levels existed high diatoms concentrations. As consequence of high diatom concentrations, several processes could take place, such as (i) an increasing of diatom production; (ii) preservation of valves, which may be lost by dissolution and/or fragmentation; or (iii) a dilution by detrital components of the sediment (Westover, 2019). We then attribute that the levels associated to high abundances of diatoms must have suffered a dissolution by detrital components of the sediments, which produce unfavorable conditions to a biogenic silica preservation. This seems to be the most appropriate theory for justifying the levels with a high rate of fragmented diatom valves, and this indicates that preservation of diatoms is dependent on several biological and chemical conditions that were occurred in the paleolake.

According to Lowe (1996), in shallow waters, this is, in the littoral zone of a lake, some of the favorable direct factors that act on the benthic diatom populations are typically, light, nutrients, grazing, and physical disturbance. By definition, the best conditions for a substantial population of benthic diatoms are provided by clear-waters in the littoral zone (Liboriussen & Jeppesen, 2003) where light is plenty supply. Burkholder (1996), stated that the abundance of benthic diatoms in shallow-waters may be closely tied to the available substrata, and the seasonal occurrence of host plants. Therefore, we infer that several of the previous biological and external natural conditions must have also existed during the paleolake evolution for a good availability of benthic diatoms, especially in Puéllaro section that belongs to the littoral zone.

Conversely, there were also few stratigraphic levels with the lowest abundance values of diatoms where there were high scarce and almost a total disappearance of diatoms. Specifically, the stratigraphic levels of Tanda (GT) section where diatoms are considered 'very scarce' (<25%) is just in GT-32.82 V (in both light and dark laminae). While in Puéllaro (GP) section

where diatoms are considered 'very scarce' are in GP-65.17 M and in GP-77.30 M. We attribute that in those stratigraphic levels are usually characterized by features indicating poor preservation, including fragmentation, dissolution, and apparent post-depositional alteration (Westover, 2019) in really high rates. Similarly, in those samples with very low abundance values of diatom (insufficient for counts), we can also attribute that those stratigraphic levels can be part of: (i) transitional zones adjacent to and likely as extensions of intervals of higher abundance, and (ii) separate intervals (i.e., multiple adjacent samples) of poor preservation; or (iii) solitary samples (Westover, 2019). Hence, we infer that those levels can be considered intervals of poor preservation or levels with a solitary-weird diatom sample.

7.2.2 interpretation of the diatom biostratigraphy logs, and the changes in the associations of freshwater diatom species identified in the Tanda (GT) and Puéllaro (GP) sections:

We studied the specific conditions that the most important or index diatom species need in both sections, due to besides lithofacies analyses, a microfossil biostratigraphy may be decisive for the interpretation of the paleolimnological conditions of the study area (Bao *et al.*, 1999). We also are interested the conditions that each section implies for diatoms growing.

7.2.2.1 Dominance of planktonic and benthic diatoms species in the Tanda (GT) and Puéllaro (GP) sections respectively:

This fact is mostly related to the paleogeographic location of each section studied in this paleolake. Tanda (GT) section is part of the pelagic (deep-waters) zone of the paleolake while Puéllaro (GP) section is part of the littoral (shallow-waters) zone of the paleolake. As was previously stated, in the diatom biostratigraphic logs of Tanda and Puéllaro sections, planktonic (centric) diatoms are dominant in Tanda (GT) section while benthic (pennate) diatoms are dominant in Puéllaro (GP) section. This fact is due to shallow waters of Puéllaro can have a greater potential for benthic algal growth than in deep waters of Tanda (Wetzel, 1983; 2001), because of in the littoral (shallow-waters) zones of the paleolake, can be supported a range of habitats that promote a diverse and productive benthic diatom assemblage which, in turn, fuel food-web pathways (Lindeman, 1942). However, according to Vadeboncoeur et al., (2003), the contribution % of benthic algae to overall lake production varies as a function of lake morphometry and trophic status. And with an increasing event of nutrient loading in the lake, is well known that pelagic (deep-waters) production tends to increase, while benthic production contracts as light starts to limit the production of bottom-dwelling algae (benthic) and submerged macrophytes (Scheffer & van Nes, 2007). The previous statement supports why benthic diatoms are dominant in the shallow-waters of Puéllaro (GP) section while planktonic diatoms are dominants in the deep-waters of Tanda (GT) section.

We also infer that: (i) due to Tanda section represents the pelagic (deep-waters) zone of the paleolake, is expected a moderate to low rate of entrance of detritic sediments in this area, where the most appropriate conditions for a bloom event would be more suitable for planktonic diatoms, but at the same time, those conditions are not the best for the development of benthic diatoms, which is what the biostratigraphic results of Tanda section (see Fig. 48) show us.; and (ii) due to Puéllaro section represents the littoral (shallow-waters) zone of the paleolake, is expected a high rate of entrance of detritic sediments in this area where the conditions are better for a bloom event of benthic diatoms, and simultaneously, the conditions are good (in less proportion) for planktonic diatoms, however, this section has better conditions for benthic diatoms which is reflected in the high biodiversity according to the biostratigraphic results in Puéllaro section (see Fig. 49). Our interpretation agrees with the W-E extensional setting interpreted for the lacustrine basin during the deposition of the lacustrine San Miguel Member, of the San Miguel Formation, and the provenance directions, from N to S and from SW to NE, of the terrigenous sandy and conglomeratic sediments, as stated by Villagómez (2003).

According to Smetacek (1985), sinking is considered a direct factor that act on the diatom populations in the deep-waters (pelagic zone) of a lake (in this case Tanda section). It means, if diatoms are not able to maintain buoyancy, then the mechanism to reduce grazing or out-complete other individuals for nutrient are of diminished value (Bennion *et al.*, 2001). We coincide with this idea because, this support the fact that in the three lowest levels of Tanda section and Puéllaro sections, we do not have a good diversity of diatom species, just two to three in each section in those stratigraphic levels. Sinking can also occur in shallow-waters.

In shallow-waters (in this case Puéllaro section), Bennion *et al.*, (2001) stated that in littoral zones of the lake is often subjected to intermittent mixing events that can promote the growth of considerable planktonic diatom through resuspension, which can occur over the lake area (Hellstrom, 1991). These mixing events are usually initiated by wind and local weather patterns (Padisák *et al.*, 1988; Carrick *et al.*, 1993) or disturbance imposed by internal forces such as fish movement (Roozen *et al.*, 2007). Even according to Bennion *et al.*, (2001), the bioturbation by fish, benthic invertebrates, and wind-induced resuspension events are common in shallow-waters and can destroy much of the integrity of the stratigraphic record. We then agree with this idea because it supports two facts: (i) the appearance of planktonic diatoms in Puéllaro section, and (ii) the chaotic sedimentology we found in Puéllaro section.

According to Saros & Anderson (2014), a regime of water-column mixing can also support the abundance of centric diatoms in sedimentary records. By themselves, planktonic diatoms are generally not very sensitive indicators of water depth, because they require only sufficient water depth to remain suspended in the water column. Variability in the composition of planktonic diatom assemblages is best understood as a response to changing nutrient concentrations, mixing/stratification, and light availability (Kilham *et al.*, 1986; Owen & Crossley, 1992). These factors are, of course, dependent on/and influenced by climate, basin morphometry, and catchment characteristics.

Planktonic diatoms usually indicate wet conditions. While pennate diatoms are cosmopolitan, occurring in many sediments indicating at least some soil moisture (Yost, 2008). Littoral diatom communities can act as valuable biological indicators in shallow standing waters (Bennion *et al.*, 2001). The scarcity of littoral (shallow-waters) diatoms may indicate rapid expansion and contraction of the lake, and/or a rapid change in lake conditions (e.g., pH) (Westover, 2019). We can consider the previous fact as part of the paleolake evolution in the Puéllaro section where there a decreasing in the highest levels.

7.2.3 About the paleoecological conditions of the identified diatoms species in both sections:

Diatoms preserved in the sediments of lake systems constitute powerful biological paleoenvironmental indicators due to their rapid response to hydrochemical changes, their diversity and their wide cosmopolitan distribution (e.g. Gasse *et al.*, 1989, 1997; Bradbury, 1988; Fritz *et al.*, 1993). In this research project were identified a total of 22 species of diatoms among 13 genera. However as was mentioned, those identifications are a reliable approximation to the real one. Therefore, we analyzed the particular ecological conditions that the identified planktonic and benthic diatoms need. Some of these features are related with the pH content, nutrient content, the siliceous concentration, etc. Advantageously, silica (SiO₂) is moderately abundant in fresh waters, which is really significance to diatomaceous algae and sponges. Even diatoms assimilate large quantities of silica and markedly modify the flux rates of silica in lakes and streams (Wetzel, 2001). Hence, some of the general and specific conditions that the identified planktonic and benthic diatom species needed, are shown below:

7.2.3.1 Planktonic (Centric) freshwater diatoms:

Are a major component of many freshwater systems and can be obtained strong inferences regarding the ecological requirements of some species, based on their distribution and occurrence relative to measurable ecological parameters (Stoermer & Julius, 2003). In freshwater diatoms, life cycle adaptations play an important part in ecological success. The most important genera of the planktonic diatoms group identified in this work were:

Aulacoseira genus: Is a very large, complex, and widely distributed genus, that is best adapted to life in lakes, ponds, and larger rivers. This genus has mechanisms that allow some species to thrive in deep lakes (see Table 6) during winter circulation or during summer in shallow water bodies, enough to be mixed to the bottom by wind stress. Diatoms of this genus are dominant

when Si and/or P content is high. Periods of dominance by *Aulacoseira* are produced by deeper mixing, and they are generally considered good competitors for light (Westover, 2019). *Aulacoseira granulata sp.:* It is associated with abundant Si and P (Kilham *et al.*, 1986).

Cyclotella genus: Is environmentally important. Taxa within the group are recognized as important environmental indicators in a broad range of environments. Diatoms of this group are commonly found in low to very low nutrient environments, in particular for lakes and shallow nutrient-rich waters. *Cyclotella meneghiniana sp:* is often observe in shallow, nutrient-rich waters (Lowe & Kheiri, 2015), and has been observed as a dominant taxon in alkaline lakes (Hecky & Kilham, 1973).

Discotella genus: Diatoms of this genus are oligotrophic and are important indicators of a continental stressed paleoenvironments as: lakes, stream rivers and shallow nutrient-rich waters. *Discotella stelligera* can represent high lake-level conditions without elevated salt concentrations (Tapia *et al.*, 2003). These species also well preserved in low-energy environments (e.g., Rühland *et al.*, 2008), and thanks to their small-size, these species also have enhanced their buoyancy which facilitates them the nutrient uptake under conditions of lower nutrients (Bao *et al.*, 2015). Diatoms of the *Discostella stelligera* group are currently more abundant during austral summer, when stratification is favored (Dorador *et al.*, 2003).

7.2.3.2 Benthic (Pennate) freshwater diatoms:

There are few studies that relates the diatom assemblage composition to water chemistry, geography and catchment area characteristics. The genera of the benthic diatoms group that appeared in this research work were:

Cocconeis genus: Diatoms of this genus are a periphytic taxa of high-nutrient guild. They live adnate to the substratum being haptobenthic. *Cocconeis* has shown the ability to sequester excess nutrients in polyphosphate bodies (Stevenson & Stoermer, 1982).

Karayevia genus: Diatoms of this genus prefer alkaline water (Kingston, 2003). They grow and live adnate on sand grains.

Rhopalodia genus: Diatoms of this genus are usually heavily silicified and robust and although they may be highly motile, they often adnate on aquatic vegetation (epiphytic) (Marks & Lowe, 1993; Lowe, 1996). Species of this genus are more common in alkaline water, while occupying microhabitats that are relatively poor in quantities of fixed nitrogen (NO3, NH4) (Fairchild *et al.*, 1985). This genus is found in hard-water nitrogen-poor benthic habitats.

Rhoicosphenia genus: Diatoms of this genus are found in fresh waters. *Rhoicosphenia* is common in lotic ecosystems or along the shores of lakes. It is found across a wide range of nutrient and other chemical regimes (Kociolek & Spaulding, 2003a).

Cymbella genus: Diatoms of this genus grow commonly in benthic habitats often surrounded by mucilage (gluey substance produced by nearly all plants and some microorganisms) or producing mucilaginous stalks. This genus is often characterized as occurring in circumneutral freshwater (pH~7) across a wide spectrum of nutrient concentrations and water chemistries (Kociolek & Spaulding, 2003a). They can be also found in lentic or lotic habitats (Cholnoky, 1965; Patrick & Reimer, 1975).

Gomphosphenia genus: Two species of this genus, *Gomphosphenia. grovei* and *Gomphosphenia lingulataeforme*, have been collected from widely distributed benthic habitats of fresh waters (Kociolek & Spaulding, 2003a).

Gomphoneis genus: Diatoms of this genus produce long, mucilaginous stalks, with the cells growing upward from the point of attachment of the stalk. The colonies form thick, mucilaginous masses attached to surfaces along the shores of lakes, rivers, and streams (Kociolek & Spaulding, 2003b).

Sellaphora genus: Diatoms of this genus are widespread in North America in alkaline fresh to brackish waters. It occurs in habitats of circumneutral pH (Kociolek & Spaulding, 2003b).

Navicula genus: Diatoms of this genus are broadly distributed in nearly every freshwater habitat (Kociolek & Spaulding, 2003a).

Synedra genus: Diatoms of this genus can be planktonic or benthic. Planktonic *Synedra* taxa can be very abundant in oligotrophic, mesotrophic, and eutrophic lakes during different seasons (Stoermer & Yang, 1970; Agbeti & Smol, 1995; Agbeti *et al.*, 1997). Benthic *Synedra* populations can be major components of river communities (Main, 1988).

In general, according to Reynolds (1984), the seasonal dominance by diatoms in most deep lakes is restricted to relatively discrete, seasonal blooms that gradually transition into dominance by other phytoplanktonic groups. Finally, the previous set of identified diatom species represent valuable information which correlated to the rest of sedimentological and counting results we are useful for understanding how variable was the paleoecology of the paleolake study here.

7.3 Paleoecological and paleoenvironmental reconstruction of this paleolake

Working with the consideration that individual taxa requires specific conditions for growing in a lake, the presence of diatoms in paleoenvironmental context provides important data about the nature of the study area. Those data coupled with input about the local geology, sedimentology, paleoecology and others provide evidence of the paleoenvironmental setting (Yost, 2008) we need for this study. The morphometry of the lake, however, dictates the influence of lake-level variation on nutrient cycling, thereby modulating the magnitude of the climate imprint in the sedimentary record (Bao *et al.*, 2015).

The key element that controls the primary production on mountain lakes throughout time, is the coupling of lake dynamics with the biogeochemistry of the catchment (Catalan *et al.*, 2006). Nutrient availability depends not only on the external inputs but also on internal recycling due to the existing water column structure at a given time. Its change prompts shift in phytoplankton communities, which, in turn affect primary productivity as well as higher trophic production (Margalef, 1978; Winder & Hunter, 2008). Conversely, changes in the lake level alter the effects of mixing (Imboden & Wuest, 1995), causing a change in productivity levels. The changes in the diatom record allow a tentative reconstruction of the palaeohydrological evolution. But this interpretation can be complicated by the fact that diatom assemblages of a sampled level are usually composed of a mixture of diatoms depicting different environmental conditions (Bao *et al.*, 1999).

In the littoral zone (shallow-waters), we expect a mixing (polymictic) event with a consequent lack of stratification of temperature or oxygen with increased potential for nutrient recycling and physical water circulation patterns (Carrick *et al.*, 1994). Many lowland, shallow water are relatively alkaline and warm for much of the year, and is well established that diatom preservation declines with increasing pH and temperature as the opaline silica becomes susceptible to dissolution under such conditions (Flower, 1993; Barker *et al.*, 1994). Conversely, in the pelagic (deep-waters) zone can occur several variations in the water-column by a mixing regime that is strongly dependent on changes in the water-level. These mixing events act as a key driver in long-term productivity conditions that can be compensated for losses produced at times of decreased nutrient availability related to runoff (Bao *et al.*, 2015).

However, in general we consider that climate changes constitute a primary driver in long-term productivity conditions but the magnitude of change can be strongly amplified or reduced by factors intrinsic to the lake that vary during its ontogeny. Therefore, we need to consider the previous facts during the interpretation of lacustrine paleoproductivity record as evidence of late Quaternary climatic change (Bao *et al.*, 2015).

Hence, a paleoecological reconstruction of the paleolake that existed in the San Miguel Formation is proposed in this section. This proposal was based on the analyses of diatoms microfossils sampled from the two stratigraphic successions of lacustrine massive and varved sequences that belonged to a paleolake can be proposed now with a good approximation.

We do not know how exactly was the climate during the evolution of the paleolake studied here, but we can infer it, with the available data obtained from the ecological conditions that the paleolake had during the diatom bloom events. Considering that the amount of nutrients is reflected in the amount of productivity in a lake, we can use the ecological conditions of diatoms for inferring the different phases that the lake had during its evolution. The proposed paleoecological reconstruction also indicates the relative paleogeographic locations of the two studied sections in this work, Tanda (GT) and Puéllaro (GP) sections, in the Late Pleistocene paleolake. Moreover, the morphotypes of diatoms, that appeared in each section, are shown in order to have a general perspective of the biological and paleoecological condition that the paleolake had for being suitable for the identified diatom species. Then, the correlation among the sedimentological, paleontological and paleoecological results were used for this proposed reconstruction.

The paleolake productivity is better in Tanda section than in Puéllaro section, due to the paleoecological implications that acted based on their paleogeographic locations. In Tanda section, there were better conditions, for a good life-conditions mostly for planktonic diatom. Conversely, in Puéllaro section was found a palustrine environment at the initial meters of the succession with remains of fossil plant-leaves and other past biological activity tracers such as trunks, but with an important input of coarse- and sand-grained sediments sourced from the N, and SW. Therefore, Puéllaro section is more chaotic with several thick massive lithologies.

In the biostratigraphic results of the two sections studied here (see Figs. 48, 49), we appreciated the distribution of diatom species in both sites. By definition we agree that the amount of diatom productivity is of course related with the productivity of the lake. This is, if the productivity of the lake is low (oligotrophic with clear waters and low nutrients), diatom productivity is also low, while if the productivity of the lake is high (eutrophic with colored waters and high nutrients), the diatom productivity is high. Moreover, climatic variations associated with a decreasing in the productivity in a lake, reflects low oxic (anoxic) situation, a stratified condition, and arid phases (Bao *et al.*, 2015). When we have an increasing pattern of diatom frustule dissolution, suggests an increased alkalinity (Westover, 2019).

Previously we decided to divided the biostratigraphic logs of Tanda and Puéllaro sections (see Figs. 48, 49) in three zones. Those zones are limited intervals where samples were taken and the identified diatoms species were grouped in genera. In both biostratigraphic logs is highly remarkable that in their lowest unit, appears the lowest diatom productivity, which is reflected in the scarce of diatoms that appear in the samples of those stratigraphic levels. Then in the middle zones of both biostratigraphic logs are shown two behaviors: (i) the highest diatom productivity in Puéllaro section, which is reflected in the huge diatom diversity that appear in the samples of those levels, and (ii) a relative moderate to high diatom productivity in Tanda section. And finally, in the upper zones of both biostratigraphic logs are also shown two behaviors: (i) the highest diatom productivity in Puéllaro section productivity in Puéllaro section but less than its middle zone's productivity, and (ii) a moderate productivity in Tanda section.

Regarding the periods of dominance of several diatom species, we decided to analyze just the main genera that were identified as 'dominant' (45% - 70%) and as 'total dominant'

(>70 %) in a stratigraphic level in both Tanda and Puéllaro sections. Those genera were: (i) *Aulacoseira* and *Cocconeis* genus in Tanda section, and (ii) *Aulacoseira*, *Cyclotella*, *Discotella* and *Cocconeis* genus in Puéllaro section.

Periods of dominance by Aulacoseira reflects high Si flux and enhanced mixing of the water column while the absolute abundance of Aulacoseira in the sediments is likely due to better preservation as lake levels rose and enhanced diatom production in response to higher ambient nutrient levels. (Westover, 2019). Enhanced Si flux from the catchment via streamflow would be expected under a more humid climate, whereas increased aridity would be expected to reduce the flux of Si from the landscape (Stager et al., 2003). A high relative abundance and a high concentration of Aulacoseira suggest that the lake was frequently well mixed, and conversely, a diminution of abundance of Aulacoseira suggest a complete mixing of the lake during the diatom-producing freshwater phases (Westover, 2019). Cyclotella diatoms are commonly found in low to very low nutrient environments., and Cyclotella meneghiniana is often observe in shallow, nutrient-rich waters (Lowe & Kheiri, 2015). Cyclotella meneghiniana has been observed as a dominant taxon in alkaline lakes in East Africa (Hecky & Kilham, 1973). Discotella diatoms are oligotrophic. Discotella stelligera can represent high lake-level conditions without elevated salt concentrations (Tapia et al., 2003) These diatoms are currently more abundant during austral summer, when stratification is favored (Dorador et al., 2003). Cocconeis diatoms are a periphytic taxa of high-nutrient guild. They live adnate to the substratum being haptobenthic. Cocconeis has shown the ability to sequester excess nutrients in polyphosphate bodies (Stevenson & Stoermer, 1982).

7.3.1 Paleoecological reconstruction of this paleolake

Finally, by correlating all the paleoecological and sedimentological results obtained from the identified diatom species in the two sections studied here, we propose a paleoecological reconstruction composed by three stages (Fig. 50), that correlate the paleoproductivity with the paleoecological conditions that identified planktonic and benthic diatoms species imply during the evolution of paleolake studied in this work. Therefore, the paleoenvironmental reconstruction model proposed is:







~ 8 Km Puéllaro Tanda NE - ~ 2200 masl NW (GP) (GT) 0. 0111/2 O 01% C 10 С ~ 2100 masl ~ 2000 masl Δ Asymmetric Biraphid Morphology: Symmetric Biraphid Morphology: **Centric Morphology:**



Fig. 50. Proposed paleoecological reconstruction of the paleolake formed in San Miguel Fm., Guayllabamba, Ecuador during the Late Pleistocene. It is included the location of two section studied here: Tanda in the pelagic (deep-waters) zone and Puéllaro in the littoral (shallow-water) zone. Some symbols as reference of diatoms morphotypes and some genera are included in function of their paleoecological conditions they need. A paleobatimetry scale is not included here due to we do not have precise data about the real depth of the paleolake studied here.

In Fig. 50 we propose a paleoecological reconstruction composed by these three simplified phases based on the results obtained from diatom species:

Stage 1: Oligotrophic phase with low diatom productivity.

Stage 2: Eutrophic phase with high diatom productivity in Puéllaro section, and a Mesotrophic to eutrophic phase with moderate to high diatom productivity in Tanda section.

Stage 3: Mesotrophic to eutrophic phase with moderate to high diatom productivity in Puéllaro section, and Eutrophic phase with high diatom productivity in Tanda section.

In summary, we finally inferred that this Late Pleistocene paleolake formed in the San Miguel Formation, evolved in an initial phase with a low to moderate productivity, and after several changes, it continued toward a high productivity. We also stated that diatoms species of this paleolake could form in relatively good mesotrophic to eutrophic conditions.

7.3.2 Paleoenvironmental reconstruction of this paleolake

As was stated at the beginning of this work, the main purpose of this work is to provide a and propose a paleoenvironmental reconstruction of this Late Pleistocene paleolake. Because diatom species are specific to the habitats in which they grow, they are valuable as environmental indicators (Smol & Stoermer, 2010). Therefore, in a more simplified model we propose the following sketch:



Fig. 51. Proposed paleoenvironmental reconstruction of the paleolake formed in San Miguel Fm., Guayllabamba, Ecuador during the Late Pleistocene. It is included the location of two section studied here: Tanda in the pelagic (deep-waters) zone and Puéllaro in the littoral (shallow-water) zone. Some symbols as reference of diatoms morphotypes and some genera are included in function of their paleoecological conditions they need. A paleobatimetry scale is not included here due to we do not have precise data about the real depth of the paleolake studied here.

We correlated the majority of the data obtained from the results of the sedimentological and the freshwater-diatom analyses addressed in the stratigraphy, biostratigraphy, counting of components, and paleoecology in the two sections studied in this research project. We also considered dynamic factors during the deposition of this model in function of the paleogeographic locations of the Tanda (GT) and Puéllaro (GP) sections. We finally interpret palustrine environments close to the Puéllaro (GP) section, where were found fossils of plantleaves and trunks with a remarkable coarse-grained clastic sediments input, and in the deepwaters of the Tanda (GT) section, mixing events allowed best conditions for planktonic diatoms to growing resuspended with a fine-grained clastic sediments input in this section.

8. CONCLUSIONS

What was relevant for this work is the consideration that the formation of lacustrine sediments is mostly controlled by climatic processes and by the catchment geology whose analyses represent a relevant indicator of the variability of past environmental conditions. Moreover, in lacustrine basins the variations recorded in lakes and their sediments are predominantly related to climate variability (Zolitschka, 2015).

In this research project we provide the first input of a biostratigraphical analysis of freshwater diatoms in order to propose a paleoenvironmental reconstruction of the lacustrine Late Pleistocene San Miguel Formation, that is part of Guayllabamba basin. Four objectives were attained in order to reach the main purpose of this work. The most important findings in this research were:

Tanda (GT) and Puéllaro (GP) stratigraphic successions have remarkable sedimentological evidences for understanding what were their paleogeographic locations in the paleolake. In this work, the sedimentological and micropaleontological evidences, allow us to established that Tanda (GT) section belongs to the pelagic/distal (deep-waters) zones of the paleolake, and Puéllaro (GP) section belongs to the littoral/proximal (shallow-waters) zones of the paleolake.

In the two stratigraphic successions studied here, the changes in the distribution of abundance values of the freshwater diatoms, freshwater sponges' spicules, freshwater phytoliths and detritic sediments, showed a direct relationship with the paleolake sedimentological-evolution. We agree that the abundance values of each component are dependent on several natural and external biological conditions that acted on this paleolake. Hence the rate of entrance of sediments toward the paleolake was considered as one of the direct factors that directly could had affected the dominance or scarce of the freshwater diatoms species in several stratigraphic levels. These events could be related to variable energy conditions where the stress and turbulence in the environment could also constrain the diatom abundance.

Sponges require important amounts of silica, nutrients and a suitable habitat for their population growth and maintenance. Sponges represent a natural resource for their functional role involved in natural processes of water purification in freshwater ecosystems. Losses are influenced by physical environmental conditions and, potentially, by interactions with other organisms. As was previously discussed, sponges are distributed across habitats that exhibit a broad range of silica conditions, and conversely sponges in habitats with low silica, may be limited to growing closely attached to substrates or in waters with little wave action which becomes them in vulnerable to consumption. However, both diatoms and sponge spicules can be transported with those sediment.

The paleoecological study of the morphotypes (6 in total), genera (13 in total), and the species (22 in total) of freshwater diatoms of this work, was used for reconstructing the paleoecological phases of the paleolake formed in the San Miguel Fm. These paleoecological interpretations, keep a close relationship with the sedimentological evidence and reconfirm that the Tanda section belongs to deeper waters of the paleolake and the Puéllaro section belongs to shallower waters of the paleolake.

All the identified freshwater diatom species have a coherent vertical-distribution in both stratigraphic successions. We attribute this behavior to all the specific paleoecological conditions that each diatom specie required, which, in turn, coincide with the proposed paleoecological reconstruction during the evolution of the paleolake in this area.

Analyzing the paleoenvironments detailed in the Table 6, we can evidence that (i) in the Guayllabamba basin (close to the Tanda section) occurred several facies and paleoenvironments (fine-grained lake sediments with few stream rivers and shallow nutrientrich waters) indicating this section surely belongs to the central part and receive in minor proportion the input of shallow waters, and (ii) in the Guayllabamba paleolake borders (close to the Puéllaro section), occurred shallow lake deposits (sandy lake sediments, lake shoreline, the input of stream river sediments, and wetland facies too). These facies and paleoenvironments point out at the different facies' distribution, depending on the paleodepth, this is, more fine-grained (varves et al.) sediments in the central lake (Tanda area), and more coarse-grained (stream rivers and shore) sediments in the lake borders (Puéllaro area).

Biostratigraphic analyses in both stratigraphic successions were as we expected, and reconfirm the paleoecological conditions that the identified diatoms of this area, required for surviving during the paleolake's evolution. The results indicated that in the Tanda section dominated planktonic freshwater-diatom species (rich in centric morphotypes), while in the Puéllaro section dominated benthonic freshwater-diatom species (rich in monoraphid, epithemioid, biraphid -symmetric and asymmetric-, and araphid morphotypes). We finally attribute these results to: (i) the fact that energetic conditions during deposition, could

control/affect diatom species reproduction: centric in calm waters (with varves), and the other five morphotypes (with coarse- and sandy-grained sediments) in stressed waters; and **(ii)** the pelagic (deep-waters) zone has high-productivity natural-conditions for being more suitable for planktonic diatoms thanks to mixing events that allow them to live resuspended, while in the littoral (shallow-waters) zone thank to the light and other biological factors is more suitable for benthic diatoms.

In both, Tanda and Puéllaro sections we attribute that the changes in the dominance/scarcity, and in the increasing/decreasing of abundance values among the identified index diatoms (e.g.: *Aulacoseira*, *Cyclotella*, *Discotella* and *Cocconeis* species), are driven by the paleoecological variations in response to the paleoclimatic conditions of the area. Those changes are displayed in the proposed three-stages paleoecological reconstruction of this paleolake. We also infer that some biological consequences of those paleoecological variations, are best reflected in the mixing-events, changes in the Si/P amount, dissolution of diatom valves, etc., during the paleolake evolution studied here.

In general, the evolution of the paleolake marks a good trend regarding its productivity. We concluded that the paleolake started with a low to moderate productivity, and after several changes, it continued toward a high productivity. We also stated that diatoms species of this paleolake could form in relatively good mesotrophic to eutrophic conditions, where the amount of silica and phosphorous were enough to sustain moderate to high diatom production in combination to low allochthonous inputs (detritic sediments). Therefore, the paleontological evidences obtained from the freshwater diatoms, indicates that this paleolake was mainly mesotrophic to eutrophic with low rate of entrance of detritic sediments.

In this work, we remark that climatic conditions could affect the long-term lacustrine paleoproductivity record of a lake as evidence of Quaternary climatic change. Therefore: (i) In spite of we were able to provide a reliable identification of several freshwater-diatom species that are close to the real one, by using the light microscope, we highly suggest the use of a SEM microscope for correctly identify more freshwater-diatom species in future research at the same study area, with similar purposes of this work; and (ii) we also suggest the application of other multidisciplinary proxies, such as isotopic analyses, geochemical studies, paleoclimatic studies, and others, in order to get a complete set of data of the study area, and hence, develop a enhanced understanding of this area during the Pleistocene.

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