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rpl-11.1 Knock-Down Disturbs Translation Machinery and Proteostasis in *Caenorhabditis elegans*

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Summary- An array of human chronic diseases, such as Alzheimer's disease, Huntington's disease, and Parkinson's disease, are related to defects in cellular proteostasis and the formation of protein aggregates. Using Caenorhabditis elegans PP563, a model organism developed for studying proteostasis stress, we can elucidate the biological role of specific genes and proteins involved in translation and proteostasis, advancing our understanding of relevant pathologies and therapeutics. Here, we report the knockdown of rpl-11.1 in C. elegans leads to disturbance in protein translation and proteostasis pathways, including the Ubiquitin-Proteosome System (UPS) and selective autophagy. We confirmed the importance of rpl-11.1 in ensuring correct ribosome biogenesis and translation accuracy.

Keywords: rpl-11.1, caenorhabditis elegans, RNAi, ubiquitin-proteosome system, selective autophagy, germline apoptosis.

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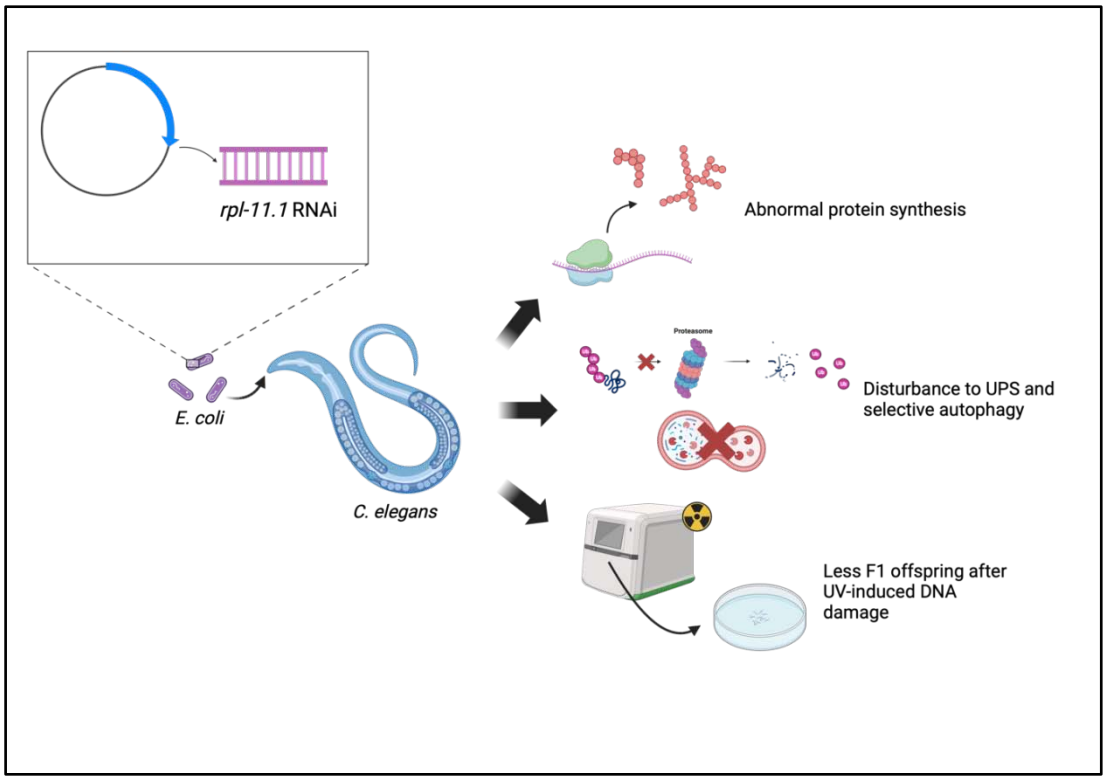
Keywords: *rpl-11.1, caenorhabditis elegans, RNAi, ubiquitin-proteosome system, selective autophagy, germline apoptosis.*

Graphical Abstract



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I. Introduction

Protein degradation is a major cellular process that maintains proteostasis and cellular physiology (Papaevgeniou & Chondrogianni, 2014). It primarily consists of degrading normal proteins in excessive amounts or eliminating damaged proteins, which might be a result of disturbances to normal

protein synthesis in the ribosomes. A major proteostasis pathway in charge of this role is the Ubiquitin-Proteosome System (UPS), which tags damaged or misfolded proteins with ubiquitin and digests them with the 26S proteosome complex (Kipreos, 2005).

Since the genes involved in UPS are generally evolutionarily conserved, their biological roles can be investigated in simple model organisms such as *Caenorhabditis elegans*, a well-studied nematode for understanding fundamental biological mechanisms. A specific strain of *C. elegans*, PP563, has been developed to specifically investigate the UPS pathway of proteostasis. Having a GFP linked to the ubiquitin protein, *C. elegans* PP563 provides a direct approach to monitoring UPS activity in nematodes through fluorescence signals (McCue et al., 2015). In a genetic screen that uses RNAi to silence 35 genes at the post-transcriptional level in *C. elegans* PP563, we discovered a particular gene, *rpl-11.1*, whose knockdown causes a marked increase in fluorescence signal in both intensity and localization. Such phenotype led us to deduce that *rpl-11.1* knockdown causes excessive UPS activity by increasing the number of active ubiquitin tags, which in turn disrupts proteostasis at the organismal level.

The biological role of *rpl-11.1*, a gene primarily expressed in the germline cells and pharyngeal muscle cells of *C. elegans* (Bgee, 2024), is still not completely elucidated. It was predicted to encode the 60S ribonucleoprotein L11-1 (RPL-11.1) in *C. elegans*, a homolog of ribosomal protein L11 (RPL-11) in humans (WormBase, 2022). RPL-11.1 constitutes a part of the large ribosomal subunit, which contains the peptidyl transferase center that catalyzes the formation of peptide bonds during mRNA translation (UniProt, 2024). Thereby, *rpl-11.1* knockdown might lead to the absence of RPL-11.1 in the ribonucleoprotein complex, affecting the biogenesis of ribosomes and forming a defect in translation machinery. The incorrectly assembled ribosomes will produce misfolded proteins with altered thermodynamic stability that may be functionally damaged or prone to aggregation after exposing hydrophobic regions. An excess of misfolded proteins and protein aggregates can be the source of proteostasis disturbance observed previously: a plethora of proteins are tagged with active ubiquitin and hence emit a substantial amount of fluorescence signal.

We thereby hypothesize that knocking down *rpl-11.1* via RNAi in *C. elegans* results in structurally incomplete ribosomes, which might produce misfolded proteins, recruit excessive ubiquitin for tagging and clearing through the UPS pathway, and ultimately overwhelm the proteostasis. Answering this hypothesis would reveal the biological role of *rpl-11.1* more clearly and possibly shed light on its relationship with translation efficiency and proteostasis regulation. To verify the hypothesis, two aims have been formulated: first, to determine if *rpl-11.1* has a role in ensuring correct protein synthesis. This will be achieved by conducting protein assays such as SDS-PAGE to compare the pattern of protein expression in *rpl-11.1* knockdown and that in *C. elegans* PP563 on an empty vector (L4440). We will further examine whether the other pathway of proteostasis that also makes use of ubiquitin tagging, selective autophagy, is involved in the clearance of *rpl-11.1* knockdown-induced protein aggregates and misfolded proteins as well. This would require knocking down *rpl-11.1* in *C. elegans* MAH215, which is built for studying autophagy (Chang et al., 2017), and conducting a double-gene RNAi knockdown in *C. elegans* PP563 for comparison with *rpl-11.1* single knockdown. Another minor aim of this study would be to determine the role of *rpl-11.1* in protecting germline cells. The human orthologue of *C. elegans* RPL-11.1 is involved in p53 pathway regulation through RPL11-MDM2 antagonism and acts as a tumor suppressor(Chène, 2003). Similarly, *C. elegans* also has a p53-like pathway (*cep-1*) that regulates DNA damage-induced apoptosis in germline cells(Derry, 2001). We hypothesize that knocking down *rpl-11.1* will inhibit cep-1 stabilization and hence germline apoptosis after UV-induced DNA damage, allowing increased germline cell proliferation and more egg deposition. This will be investigated through a F1 progeny count following parent generation exposure to UV.

As mentioned, the functionality and mechanism of *rpl-11.1* in *C. elegans* is not fully clarified in literature. Some of the of the earliest genome-wide screens indicate that *rpl-11.1* knockdown resulted in phenotypes such as life-span extension(Hsin & Kenyon, 1999) and gigantism(Patel et al., 2002). More recent studies have focused on the germline proliferation aspect of the gene as well as its evolutionary history in the *C. elegans* genome(Maciejowski et al., 2005). A more interesting study, without any experimental evidence, suggested the potential involvement of the gene in mitochondrial activity.(Sun et al., 2019). These various studies seemingly covered the role of *rpl-11.1* in *C. elegans* from a broad perspective, but none of them point out the specific mechanism by which this gene works in terms of its fundamental role: encoding a ribosomal protein. Additionally, none of the prior research exclusively focused on studying *rpl-11.1*. Here, we solely investigated *rpl-11.1* knockdown from the perspective of translation and proteostasis disturbance and germline proliferation. Elucidating the biological role of *rpl-11.1* in nematodes is of great importance as it can give insight into similar mechanisms in the translation machinery and UPS pathway of humans. A substantial homogeneity exists between humans and *C. elegans(Lai, 2000)*, so confirming the gene's role inthe nematode can potentially pave the way to developing therapeutic strategies for diseases related to protein aggregation and irregular proteostasis, such as Alzheimer's disease,

Huntington's disease, Parkinson's disease, and prion disorders(Papaevgeniou & Chondrogianni, 2014).

II. Methods

Culture of C. elegans. C. elegans PP563 and *C. elegans* MAH215 were used in this study and obtained from the *Caenorhabditis* Genetics Center (University of Minnesota, St. Pau, MN, USA). They were maintained at 25˚C under standard conditions on nematode growth media (NGM; 2% (w/v) agar, 0.3% (w/v) NaCl, 0.25% (w/v) peptone, 1 mM $CaCl_2$, 5 µg ml^{-1} cholesterol, 25 mM KH_2PO_4, 1 mM $MgSO_4$) agar plates(Sun et al., 2019). Nematodes were fed on *E. coli* OP50.

PP563 RNAi screen. The following screen protocol was followed:

Day 1. 100 uL of cultures of the Htt115 strain of *E. coli* containing the L4440 plasmid alone or containing an RNAi sequence targeting a specific gene were seeded onto NGM + carbenicillin p6 plates and allowed to dry overnight. Each plate has one targeting RNAi. (Refer to these plates as RNAi p6s.)

Day 2. All RNAi p6s had 50 uL of IPTG added to enhance expression of the RNAi. This was allowed to dry (~1 hour). Gravid worms were bleached to obtain a synchronized population of eggs. Approximately 50 eggs were plated onto the RNAi p6s and were allowed to develop to adulthood (~3 days) at 20˚C.

Day 5. About 10 worms were randomly selected from each RNAi p6, immobilized with sodium azide, and aligned for imaging. Micrographs were imaged at 150x magnification and 20ms exposure with LED at 16 and white-balanced. For the GFP signal, *C. elegans* PP563 was imaged at 800 ms exposure, and *C. elegans* MAH215 was imaged at 500 ms exposure. For the mCherry signal, *C. elegans* MAH215 was imaged at 500 ms exposure.

We conducted a preliminary screen for *C. elegans* PP563 with 36 different RNAi knockdowns. Of all the knockdowns that showed some degree of change in fluorescence signal in comparison to the L4440 genetic control, *the rpl-11.1* knockdown displayed the most drastic increase in both signal intensity and localization. We therefore decided to follow up on our investigation on *the RPL-11.1* knockdown exclusively.

The knockdown efficiency of *rpl-11.1* RNAi was confirmed using RT qPCR, where the mRNA level of the housekeeping gene *ama* was used as the internal reference. Primers for the target genes were designed by primer-BLAST (NCBI, 2019) and synthesized by a commercial company. The RT qPCR result showed that *rpl-11.1* RNAi achieved a near-complete (0.0025 fold change) knockdown of *rpl-11.1* in *C. elegans* PP563. It should be noted that the same gene knockdown in *C. elegans* MAH215 was not verified by RT qPCR.

Nematode population maintenance: Nematodes were synchronized by hypochlorite bleaching (2% sodium hypochlorite and 0.5 mol/L NaOH) according to standard protocols (Stiernagle, 2006). Approximately 1,000 extracted worm eggs were cultured on one NGM plate for protein isolation and RNA isolation (for RT qPCR cDNA synthesis).

Protein isolation: Proteins from *C. elegans* PP563 *rpl-11.1* knockdown and L4440 are isolated by washing the worms off the plate following standard protocols (Stiernagle, 2006), lysing through RIPA (50mM Tris-HCl, 150 mM NaCl, 1 mM EDTA, 1% or 5% SDS, tablet ofprotease inhibitor), centrifugation and incubation on ice. Proteins treated with 1% SDS are generally small-sized and soluble, while the 5% SDS aims to separate out proteins that are large-sized or aggregated.

SDS-PAGE: RIPA-treated proteins (including 1% SDS-treated and 5% SDS-treated) were loaded onto protein gel to run SDS-PAGE at 170V for the first 10 minutes and then at 200V for 1 hour. The gel was destained and visualized in imaging system.

Rpl-11.1 RNAi knockdown in C. elegans MAH215: C. elegans MAH215 were fed with *rpl-11.1* RNAi-expressing bacteria during development. Procedure similar to PP563 RNAi screen was followed. Micrographs were imaged at 31.6 ms exposure and 2x gain. GFP and mCherry fluorescence signal are later overlayed to create merged fluorescence micrographs.

Double-gene RNAi knockdown: Two genes (*rpn-6* and *lgg-1*, *rpl-11.1* and *lgg-1*, *rpn-6* and *rpl-11.1*) were knocked down via RNAi at once in *C. elegans* PP563 to evaluate the involvement of UPS and autophagy pathways of proteostasis in translation-defect cells' protein degradation. Bacteria expressing both types of RNAi are used as food source for worms and the procedure is similar to PP563 RNAi screen. Micrographs were imaged at 31.6 ms exposure and 2x gain.

UV radiation exposure: Two plates of *rpl-11.1* knockdown *C. elegans* PP563 and two plates of L4440 *C. elegans* PP563 were placed under a UV source (imaging system), with one plate exposed to UV for 30 sec and the other for 120 sec. Then, 5 adult worms from each plate is transferred to new plates seeded with *E. coli* OP50 and cultured for 3 days at 25˚C till the F1 progeny reaches adulthood. The number of worms on each plate was then counted manually. After worm (population) count, 10 worms from each group were transferred to new plate, immobilized and imaged under fluorescent microscope.

III. Results

a) 5% SDS-PAGE Reveals Potential Protein Aggregation

In *Figure 1*, it is observed that under 1% SDS treatment, which separates out smaller proteins, there is

no clear difference between the bands of *rpl-11.1* knockdown and the control (L4440). Nonetheless, under 5% SDS treatment that targets larger proteins, *rpl-11.1* knockdown exhibits markedly more bands and higher intensity in comparison with the control. These extra bands might be protein aggregates accumulated in the cells.

b) Rpl-11.1 Knockdown in MAH215 Induces More Merged Fluorescence Signal

In *Figure 2,* merged micrographs generally reveal more stand-alone m Cherry fluorescence in the control (L4440) and more merged fluorescence (mCherry and GFP) in *rpl-11.1* knockdown. One exception is that there are two nematodes in *rpl-11.1* knockdown that exhibit GFP fluorescence unaccompanied by any mCherry fluorescence. These two nematodes were believed to be dead by the time of imaging and were displaying auto-fluorescence because the GFP signal must be accompanied by the mCherry signal in *C. elegans* MAH215. Other than the two abnormalities, *rpl-11.1* knockdown displays mostly merged fluorescence.

It should be noted that in an attempt to verify if the autophagy pathway of proteostasis is involved in misfolded protein clearance, a double-gene knockdown experiment was also performed (refer to *Methods*). However, no valid results were obtained because there is minimal difference between the control and experiment groups (*Figure 3*).

c) Rpl-11.1 Knockdown Produces Less F1 Progeny after UV Exposure

Figure 4A reveals that there is no obvious difference between UV-treated *rpl-11.1* knockdown and the control (L4440) in terms of F1 progeny phenotype (fluorescence, size, mobility, etc.). However, when a worm count was conducted, it turned out that in both the 30s and 120s UV exposure groups, there are more nematodes surviving in the control group, with a 6.4-fold increase in the 30s exposure and a 2.5-fold increase in the 120s exposure. The knockdown of *rpl-11.1* inhibits nematode proliferation after UV exposure.

IV. Discussion

a) Protein Aggregation as a Result of Error in Translation Machinery

Our SDS-PAGE experiment substantiated that there are protein aggregates formed in *C. elegans* PP563 cells after *rpl-11.1* knockdown, as indicated by extra bands of proteins that are not seen in the control (*Figure 1*). Moreover, although the expression of smaller proteins appears to be similar in both the control and the *rpl-11.1* knockdown, we observed an overexpression of larger proteins (5% SDS-treated) in the *rpl-11.1* knockdown, suggesting a difference in protein expression pattern. This aligns with our hypothesis that abnormal protein synthesis will arise due to the absence of RPL-11.1 ribonucleoprotein in ribosome biogenesis. The abnormality can take the form of producing misfolded polypeptides that have altered thermodynamic stability, which are prone to exposing their hydrophobic regions and hence become aggregated (Berrill et al., 2011). Alternatively, the abnormal protein synthesis might also simply result in an overexpression of a group of large, insoluble proteins. Both outcomes will cause severe disruption to the UPS because excessive amounts of ubiquitin will be tagged to abnormal proteins, overwhelming the proteostasis pathway.

It is confirmed that knocking down *rpl-11.1* makes a difference in cellular protein synthesis in *C. elegans*. Next, we will attempt to identify which specific proteins are affected (overexpressed, misfolded, or become prone to aggregation) by such errors in translation machinery. Our preliminary RNAi screen indicated that most proteostasis defects occur in C. elegans intestinal cells after knocking down *rpl-11.1.* We will select a set of proteins whose expressions may be disturbed by the knockdown and use immunoblotting to confirm their identity.In addition, we will evaluate the degree of impact on ribosome biogenesis and translation efficiency caused by knocking down *rpl-11.1* through RT qPCR on rRNAs and polysome profiling, respectively.

b) Autophagy Pathway is Disturbed by Rpl-11.1 Knockdown Alongside UPS

Alongside UPS, the selective autophagy pathway of proteostasis also makes use of ubiquitin tagging to achieve protein degradation (Kocaturk & Gozuacik, 2018). We indeed observed a disturbance to the autophagy pathway in the *C. elegans* MAH215 *rpl-11.1* knockdown (*Figure 2*). The abundant mCherry signal in the control suggests that most GFP tagged to the autophagosomes has been quenched after fusing with the lysosome in the autophagy pathway. Conversely, in *rpl-11.1* knockdown, an increase in merged fluorescence signal indicates that autophagosomes are not efficiently undergoing lysosomal fusion, suggesting a delay in protein degradation and a buildup of protein waste. This is likely attributable to the protein aggregates and other large misfolded proteins generated by the translation error. Therefore, the hypothesis that selective autophagy is also involved in this knockdown-induced proteostasis disturbance is supported. Interestingly, autophagy differs from the UPS in that it primarily degrades long-lived proteins, insoluble protein aggregates, and organelles, whereas the UPS pathway targets short-lived proteins and soluble misfolded proteins (Kocaturk & Gozuacik, 2018). This aligns with our findings from the protein assay, which show that knocking down *rpl-11.1* produces protein aggregates and other insoluble large proteins,

necessitating the involvement of selective autophagy in maintaining proteostasis.

Our hypothesis could be better supported with results from the double-gene knockdown experiment. If simultaneously knocking down *rpl-11.1* and *lgg-1*, a key gene involved in autophagy (Romane Leboutet et al., 2023), produces a result that is no different from knocking down *rpl-11.1* alone, we could conclude that the autophagy pathway of proteostasis is already disturbed by knocking down *rpl-11.1* in addition to the UPS. However, unfortunately, the experiment did not yield valid data to draw any conclusions. We suspect that the RNAi knockdown efficiency might be problematic, resulting in an incomplete silencing of the targeted gene. To ensure validity in re-performing the double-gene knockdown experiment, we will include a confirmation of RNAi knockdown efficiency using RT qPCR.

c) Rpl-11.1 Might Be Integral to Germline Proliferation

We previously predicted that knocking down *rpl-11.1* will lead to more F1 offspring as germline apoptosis is inhibited by the deactivation of cep-1, which is under RPL-11.1 regulation(Schumacher et al., 2001). However, the experiment result indicates that *rpl-11.1* knockdown produces much less F1 progeny in comparison with the control after UV exposure for either 30s or 120s. The original hypothesis is hence rejected. We need to reconsider the role of *rpl-11.1* in maintaining germline proliferation.

According to Chang et al. (2017), cep-1, while activating DNA damage-induced germline apoptosis, is also required for meiotic chromosome segregation in the germline. Hence, it is reasonable that knocking down *rpl-11.1*, the stabilizer of *cep-1*, leads to less progeny after UV-induced DNA damage in the parent generation. Also, the mechanism by which cep-1 is stabilized by RPL-11.1 in *C. elegans* might be different from how p53 (the human homolog of cep-1) is stabilized by RPL-11 (the human homolog of RPL-11.1) in humans, requiring us to figure out the specific mechanism of cep-1 activation before making predictions. It can be concluded that *rpl-11.1* might be integral to germline proliferation in *C. elegans* in the face of radiation stress, based on the UV exposure experiment result.

In further investigation, we attempt to monitor the process of meiotic chromosome segregation in the germline of *rpl-11.1* knockdown *C. elegans* using live microscopy imaging. We will observe the gonads of the nematodes under fluorescent microscopes after their chromosomes are stained with fluorescent markers. This will allow us to evaluate our new hypothesis.

In conclusion, our original hypothesis was partially supported. The role of *rpl-11.1* in ensuring correct protein synthesis in *C. elegans* is confirmed. We believe the selective autophagy pathway of proteostasis is involved in the clearance of misfolded proteins alongside UPS, but evidence from the double-gene RNAi knockdown experiment is lacking. On the other hand, the role of *rpl-11.1* in protecting germline cells requires re-consideration and further investigation. It appears that the gene may be a necessity for meiotic chromosome segregation, as it plays an important role in nematode reproduction after radiation-induced DNA damage.

Due to the substantial homogeneity between humans and *C. elegans* (Lai, 2000), it is of utter importance to continue elucidating the biological role of *rpl-11.1* and its protein product in translation and cellular proteostasis, which relates closely to an array of human diseases involving proteomic defects. We would like to further evaluate the disturbance caused by RPL-11.1 dysfunction to translation efficiency by conducting polysome profiling and rRNA qPCR. The immunoblotting of specific proteins involved in the disturbance is equally crucial for developing therapeutic strategies toward relevant proteomic diseases.

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Abbreviations

UPS	Ubiquitin-Proteosome System
RNAi	Ribonucleic acid interference
mRNA	Messenger ribonucleic acid
DNA	Deoxyribonucleic acid
GFP	Green fluorescent protein
UV	Ultraviolet
SDS-PAGE	Sodium dodecyl-sulfate polyacrylamide gel electrophoresis
RT qPCR	Quantitative reverse transcription polymerase chain reaction
NGM	Nematode growth medium

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Spark of First Life and Consciousness

By Chandra Prakash Trivedi

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It has been observed in the ultra-resolution image that one purine and one pyrimidine base differing only in Nitrogen are complimentary to each other shed with cosmology. The elementary particles adhered to space, the sound of vibration touched, press the mark, and rebound. The colliding protons, decaying into hadron jets, the electrons converted them into electric vibrations to join the purine and pyrimidine base in series with mass.

The electrostatic interaction between the charged ions of the water with dehydration separated the hydrogen bond. It has formed a covalent Hydrogen bond between the purine and pyrimidine complementary base. The complementary wavelength of hydrogen bond activated the nucleotide pair with transfer of electron.

Keywords: *spark of life, phonon, slime soup.*

GJSFR-C Classification: *DDC Code: 843.914*

Strictly as per the compliance and regulations of:

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The electrostatic interaction between the charged ions of the water with dehydration separated the hydrogen bond. It has formed a covalent Hydrogen bond between the purine and pyrimidine complementary base. The complementary wavelength of hydrogen bond activated the nucleotide pair with transfer of electron. The hydrogen triple bond converts into the double bond, and reunited on the opposite side with change in the electron with oxidation and reduction in chain with the first genetic code and amino acid in series. The synthesized chromosomes divided into four with the first prokaryotic cell. Life appears with the streaming of the protoplasm and disappears with aging of the cell. The complementary wavelength of hydrogen triple bond of the nucleotide pair led the development from generation to generation with new life.

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I. Introduction

Origin of life consciousness is a great puzzle, life appears with the streaming of the protoplasm and disappears with aging of the cell body. I have traced the roots of life consciousness in pre-cosmic condition. The phonon wave appeared first and activated the dark matter with blast and light. The phonon and photon run parallel with equal and opposite wavelength. The purine and pyrimidine base differing only in Nitrogen shed like bullet with incandescent gaseous clouds with phonon photon interaction with the vibrations. The electrostatic force governs the motion of the elementary particles, which caused them to aggregate or collide in various ways with oxidation and reduction with the transfer of electrons in the primordial soup. It has activated the purine and the pyrimidine complementary base pair with resonance.

The electrostatic interaction between the charged ions of the water with dehydration separated the hydrogen bond. It has formed a covalent Hydrogen bond between the purine and pyrimidine complementary base. The complementary wavelength of hydrogen bond activated the nucleotide pair with transfer of electron. The hydrogen triple bond converts into the double bond, and reunited on the opposite side with change in the electron with oxidation and reduction in chain with the first genetic code and amino acid in series. The synthesized chromosomes divided into four with the first prokaryotic cell. Life appears with the streaming of the protoplasm and disappears with aging of the cell. The complementary wavelength of hydrogen triple bond of the nucleotide pair led the development from generation to generation with new life.

II. Early Work

The Russian Chemist A.I. Oparin 1922 and English Geneticist J.B.S. Haldane 1928 first conceived of the theory of the pre-biotic origin of life. DNA Watson and Crick 1953, Darwin Origin of Species 1859, Life evolved from the single DNA with Genetic recombination and cell division. How did the first Life begin? NASA researchers noticed polycyclic aromatic hydrocarbons (PAHs) in meteorites. Extra hydrogen or oxygen called Quinone has the potential for the origin of life.

Higgs field 1914, phonon scattered the photon in a crystal Lie et al 2014, Einstein 1923 there must be two equal and opposite forces. The photon is the smallest unit of light, and immortal phonon is smallest unit of the sound wave vibration are connected at the molecular level with equal and opposite wavelength.

The DNA with photon-phonon interaction is universally present. Hence its complimentary resonant wave blackouts radio communications on the earth and the protons damage human beings in space if not protected properly. Because the entry of radiation rays with protons checked by the magnetosphere and ozone layer and complimentary resonance finds its counterpart protons astronaut human in space.

a) *Life on the Earth*

The incandescent gaseous cloud cooled down with time and the movement of the molten mass generated the geomagnetic field and magnetosphere around the earth has given the place for the ionization of the solar flares trapped by the magnetosphere and interacts with the sun's magnetic field. The ions flow down and filled the earth with the water.

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b) Ozone Layer

Stratospheric ozone is formed naturally through the interaction of solar ultraviolet (UV) radiation with molecular oxygen (O2). Ozone absorbs the toxic UV rays with the entry of visible light, it has given the way for the origin of life on the earth.

III. Theory

I have traced its root in pre-cosmic cosmology and the sun.

The earth is a part of our solar system, which is one unit of the cosmos. The human body is a microcosm inside a macrocosm. All can be searched just like a drop of water in the sea can reveal the character of the ocean I have studied the sun with the naked eyes with my yogic practice otherwise it is impossible to face the sun even for a second with confirmation from Egypt Rosetta granite stone, pyramids of the Egypt, Gold plate Grand Canyon North. America, NASA pictures & Veda,

I have observed the nuclear reactions on the sun's surface with blast and light. The photon and phonon run in a straight way in concentric circles. It has been confirmed from the Sun disc gold plate Grand Canyon and Veda.

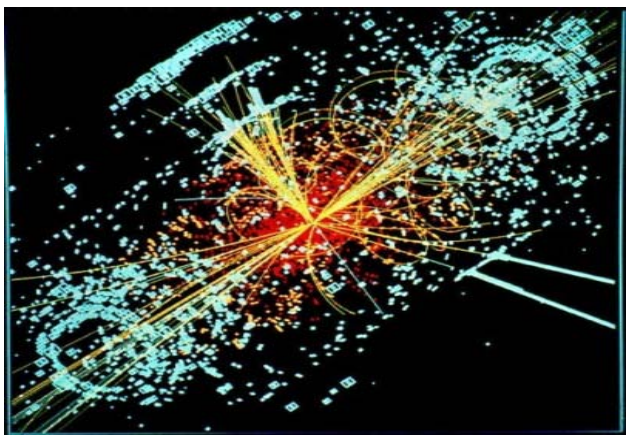
The digitally stacked sequence reveals that the photon and phonon running in concentric circles from the sun Grand Canyon Star Trails NASA - March 3, 2013. The Scientists are searching the Dark matter, is not a matter. The dark atmosphere is hidden in the interior of the sun, black caters and sunspots, which explode with blast and light.

The shock waves are antimagnetic, white, and travel with supersonic speed, and dark matter is an inactive condensed zone of magnetism without movement, just like a waste. The shock waves are 'anti-matter of dark matter, immortal with opposite character. It appears first in the pre-cosmic darkness like the shock waves appear before the earthquake, and activated the dark matter with resonance with blast and light. The photon and photon are complementary to each other.

The activation of dark matter is activation of inherent magnetism in cosmos, with formation of charged elementary particles. The electrostatic force is the force which governs the motion of the elementary particles, which caused them to aggregate or collide in various ways with oxidation and reduction with the transfer of electron.

The photon and phonon have broad complementary spectrum from gamma rays to radio waves with equal and opposite wavelength. The immortal phonon stimulate the event with electron configuration and half-spin change in the opposite wavelength and photon undergoes the synthesis and degradation with time Einstein's Equation$E=Mc^2$.

The flow of the photon and photon has been halted with the Higgs field underlying space imparted mass to the elementary particles.



Higgs Field and Mass to the Elementary Particles

All elementary particles are vibrating with the resonance of vibration and their respective charge. They are complementary to each other from gamma rays to radio waves. They find their resonant with resonance. The resonant vibrations of electromagnetic rays, touch, press-mark, and rebound. The colliding protons, decaying into hadron jets and electrons, converted them into electric vibrations to join them in series with phonic compression electromagnetic force. It has maintained its continuity in the molecules and the matter with Higgs field 2013 with asteroids and planets.

IV. Discussion

All elementary particles are vibrating with the resonance of vibration and their respective charge. They are complementary to each other from gamma rays to radio waves. They find their resonant with resonance. The phonon touch press mark and rebound with electron configuration and the half spin change in the opposite wavelength, and the photon undergoes the synthesis and degradation with time Einstein's

equation $E=Mc^2$

The first life arose in the primordial soup with the streaming movement of the charged ions in the colloidal solution. The respective complementary wavelength of the charged ions caused them to vibrate with streaming

The vibratory movement of the ions with streaming developed a dynamo in the center with actions and interactions in series with electron transfer and photon undergoes synthesis and degradation with time.

The electron transfer is associated with the oxidation loss of an electron and reduction gain of electron in anaerobic condition. The electrostatic interaction between the charged ions developed dynamo in the center with the electromagnetic field. The vibration waves activated the equal and opposite wavelengths of purine and pyrimidine base differing only in Nitrogen The elementary particles adhered to space, the sound of vibration, touched, press the mark, and rebound. The colliding protons, decaying into hadron jets and electrons, converted them into electric vibrations to join them in series with electron configuration and half spin change in the opposite wavelength.

The electrostatic interaction between the charged ions of the water with dehydration separated the hydrogen bond. It has formed a covalent Hydrogen bond between the purine and the pyrimidine base.

Ultra resolution image of DNA with Electron transfer

The phonon wave strike and rebound with a press mark with the electron configuration in the opposite direction of hydrogen triple bond, it triggered off the chain of oxidation and reduction reaction, and the hydrogen triple bond converted into double bond and Nitrogen reunite it on the other side simultaneously.

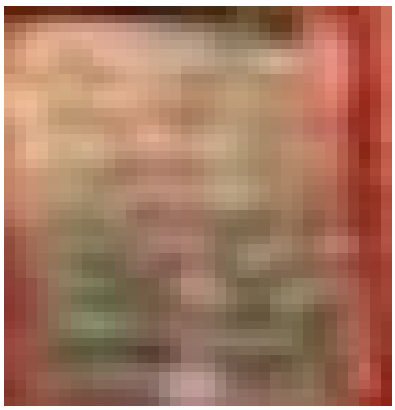
The equal and opposite wavelength of hydrogen triple bond led the development with electron transfer. With the first genetic code and amino acid in series synthesized the chromosomes. The chromosomes divided into four with first prokaryotic cell. Life appears with the streaming of protoplasm with the food metabolism as source of life and disappears with aging and death of the cell body. The complementary wavelength of hydrogen triple bond of the nucleotide pair led the development from generation to generation with new life.

T Nucleotides Divide in air Like Image in the Mirror with Electron Transfer

The equal and the opposite wavelength of hydrogen triple bond led the development with electron transfer in series and the hydrogen triple converted into double bond and reunited in the opposite direction simultaneously with Nitrogen in series as identity of the individual cell with equal and opposite wavelength. Hence, even the time twins have different genetic identity and fate in life.

The prokaryotes evolved into the eukaryotic autotrophic cell with the entry of the red wavelength of light made apparent the three places of nucleotide pair with the photosynthesis and generation of immortal chemical energy. The immortal phonon wave follows the immortal DNA from generation to generation with new life and cell division.

At the Point of Two Different DNA the Complementary Phonon Wave Strike and Rebound with Generation of Triplet code in air with Electron Configuration and Half Spin Change in the Opposite Wavelength



The Complementary Wavelength Led the Development Vigorously

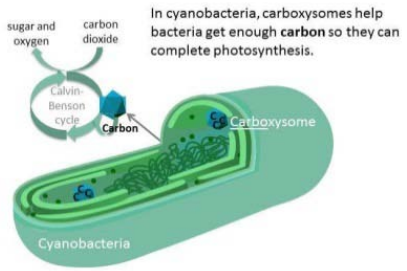
The Triplet Genetic Code of DNA Divides in Chain with Never Breaking Nitrogen

The complementary phonon wavelength acts as antennae and speaker to execute the functions of life, It led the development with the synthesis of amino acids and proteins in chain to synthesize the chromosome pair with the first prokaryote.

The Hydrogen triple bond Nitrogen triple bond with oxidation and reduction separate and unite simultaneously on other side, with oxidation the hydrogen bond break and Nitrogen reunite it on other side, due to this the double helix chain never break.

V. Result and Conclusion

The entry of the Red wavelength of light through the plasma membrane activated the place of the chlorophyll pigment on the DNA.



The first prokaryotic cell with an incipient nucleus maintained its continuity with cell division, and immortal phonon follow it from generation to generation with new life.

The entry of the Red wavelength of light through the plasma membrane activated the place of the chlorophyll pigment on the DNA as source of life with food metabolism.

It has given double horsepower to the developing cells and the prokaryotic autotrophic cell evolved into the eukaryotic cell and moved on the path of evolution with genetic recombination and cell division with the hereditary characters and the complementary phonon wave follow it from the generation to generation with new life as hereditary life principle.

Life appears with the streaming of the protoplasmic vibrations with food metabolism and disappears with the aging of the cell body.

It is like this that all the rotating astronomical bodies rotate at their axis with the generation of the dynamo in the centre with the magnetic field and the magnetosphere around them. In the same fashion, the streaming of the protoplasm with the nucleus in the centre generates dynamo in the centre with a magnetic field and magnetosphere but is hard to detect, which disappears with death, aging of the cell body.

The purine and the pyrimidine base pair of DNA differing only in Nitrogen have shed from the Nebula with the cosmological event. It divides in the air just like the image in the mirror. The Purine and pyrimidine base pair of the DNA has an inbuilt mechanism for the transcription and translation with time, with three immortal and three stages of life. The three immortals are, 1- the Higgs field ensign of the existence, 2- the immortal chemical energy of photosynthesis, with food metabolism is the source of life. 3. The immortal DNA with resonant vibrations light of life for all.

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Middle Jurassic Lithostratigraphy of the Tecocoyunca Group in the Numí Riber Area (Close to Tlaxiaco), Oaxaca

By Raúl Sabino Carrasco-Ramírez

Abstract- The Jurassic lithostratigraphy of the Mixtec Region is relatively well known, however the system on which is based includes formational descriptions somewhat deficient (e.g. vagueness, characterization by fossil content, insufficient cartographic discrimination). In order to contribute to correct such deficiency, we undertook a detailed study of the Río Ñumí Area, vicinity of Tlaxiaco, where the Middle Jurassic units that make up the Tecocoyunca Group display their attributes, thus allowing to supplement the formational descriptions. It was found that locally, the Tecocoyunca Group includes in the lower part the associated Formations Zorrillo/Taberna (Early to Late Bajocian), consisting of ~287m of carbonaceous siltstone, mudstone and subarkosic very fine-grained sandstone and siltstone; this composite unit bears pelecypods and continental plants, as well as two carbon zones; it is interpreted that they were part of a delta complex. These associated formations conformably underlie the Simón Formation (Middle-Late Bathonian), it consists of ~270m of subarkoses and siltstone set in thin to thick strata; it is interpreted as a transitional deposit.

Keywords: mexico, oaxaca, middle jurassic, tecocoyunca group, lithostratigraphy, paleontology.

GJSFR-C Classification: FOR Code: 850301

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Keywords: mexico, oaxaca, middle jurassic, tecocoyunca group, lithostratigraphy, paleontology.

Resumen- La litoestratigrafía jurásica de la Región Mixteca es relativamente bien conocida, sin embargo, el esquema en que se basa incluye descripciones formacionales un tanto deficientes (e.g. vaguedad, caracterización por contenido fósil, delimitación cartográfica insuficiente). Con el propósito de contribuir a subsanar esta deficiencia, realizamos un estudio detallado del Área Ñumí cercanías de Tlaxiaco, donde las unidades mesojurásicas integrantes del Grupo Tecocoyunca despliegan sus atributos, permitiendo así suplementar las descripciones formacionales. Se encontró que El Grupo Tecocoyunca localmente incluye en la parte inferior a las Formaciones Asociadas Zorrillo/Taberna (Bajociano Temprano-Tardío inicial), constituidas por ~287m de limolitas carbonosas, lodolitas y subarcosas, porta pelecípodos y plantas fósiles, así como dos zonas de carbón; se les interpreta como parte de un complejo deltaico. Estas unidades subyacen en concordancia a la Formación Simón (Batoniano Medio-Tardío), integrada por ~270m de subarcosas y limolitas dispuestas en estratos delgados y gruesos; se le considera un depósito transicional. Esta unidad subyace en concordancia a la Formación Otatera (Batoniano Tardío), consiste de ~170m de coquinas de pelecípodos con intercalaciones de estratos calcáreos de intraespatita; se le interpreta como un depósito nerítico somero, con un componente subordinado de playa. Esta unidad subyace en concordancia a la Formación Yucuñuti (Calloviano Medio), constituida por ~118m de areniscas finas, coquinas, limolitas y biomicritas que portan pelecípodos y gasterópodos; se le interpreta como un depósito transicional a nerítico somero. A esta unidad le sobreyace en discordancia la Caliza con "*Cidaris"* del Oxfordiano, que no forma parte del Grupo Tecocoyunca. El Grupo Tecocoyunca incluye paleofauna y paleoflora mesojurásica,; comunes en la Región Mixteca. Finalmente, se considera que la descripción detallada de las formaciones que constituyen al Grupo Tecocoyunca, es *de facto* un avance en la redefinición de las unidades mesojurásicas de la Región Mixteca.

Palabras Clave: méxico, oaxaca, jurásico medio, grupo tecocoyunca, litostratigrafía, paleontología.

I. Introduction

The Mixteca Region (noreast of Guerrero, norwest of Oaxaca and south of Puebla states) was studied by many geologist (e.g. Wieland 1909; 1914-1916; 1926; Burckhardt, 1927; Guzman, 1950; Cortés-Obregón et al., 1957; Alencaster, 1963; Ochoterena-Fuentes, 1960; Pérez-Ibarguengoitia et al., 1965; Ojeda-Rivera, 1975; Ortega-Gutierrez, 1978; Westermann, 1983, 1984; López-Ticha, 1985; Morán-Zenteno et al. , 1994; Meneses- Rocha et al., 1994; Ortíz-Martínez et al., 2013). One of the most important because of its paleontology and stratigraphy is Burckhardt (1927) which is the first and initial full- monography with descriptions and plates of fauna fossil, this work is basis of the Middle Jurassic paleontology of Mexico; Erben (1956) did the first stratigraphy of the Jurassic for the region, which includes the following lithostratigraphic units for the Tecocoyunca Group: Zorrillo, Taberna, Simon, Otatera and Yucuñuti Formations. However, the

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system on which it is based includes somewhat deficient formational descriptions (e.g. vagueness, characterization by fossil content and insufficient cartographic discrimination).

Moreover, the Erben (1956) stratigraphic units had been used, since then, for geologic studies; in this sense the Tecocoyunca Group (Middle Jurasssic) had been identified commonly in the Mixteca. That is why, was considered convenient the study of this group of rocks at the Numí Riber area, close Tlaxiaco, which is next to Diquiyu area, Tezoatlán, Oaxaca (Fig. 1). Outcrops of this group show clearly stratigraphic and paleontologic characteristics, this information permit us to make full descriptions of the Jurassic lithostratigraphic units, and contribute for best knowledge of the regional geology. Also in this area there are Jurassic coal beams (Ramírez, 1882; Birkinbine, 1911; Cortés- Obregón at al., 1957), which give to the area economic interest.

Study area. The study area is at both sides of the Numí Riber, in the Mixteca Region, northwest of Oaxaca state, which is 10 km long and 4 km width, distributed NW-SW, almost 40 km^2 inside the followings coordinates as: 17° 16' - 17° 22' N and 97° 41' – 97° 46' W (Fig. 1) and 2000-2200m over sea level; next locality is Santiago Nundichi, which is arrived by a No.125 second class highway, also it is 5 km NE of Tlaxiaco.

II. Material and Methods

Cartographic material used includes: Topographic map E14D34 Tlaxiaco, scale 1: 50 000 (INEGI, 2000); Geologic and Mines map E14D34 Tlaxiaco scale 1: 50 000 (SGM, 2000), and Geologic Map Oaxaca E14-9 scale 1: 250 000 (INEGI, 1994). Field geology methodology was applied using maps (Finkl, 1988). It was searched prior geologic information in order to know available reports and elaborate a preliminary map to be used as initial field geologic map. The geologic cartography was mapped following geologic contacts by foot and the structural stratigraphic sections also were mapped. The lithostratigraphy described have been done following the North American Stratigraphic Code (2005), spanish version (Barragan et al. 2010).

Petrographic and lithologic descriptions were done by geologic observations and field pictures, this is basically supported by 80 lithic samples and 60 thin sections; petrographic and lithologic terminology came from Folk (1974) and Boggs (2009). Fossils were collected during field geology.

III. Discussion and Results

a) Lithostratigraphy

i. *Tecocoyunca Group*

The Tecocoyunca Group name, Erben (1956) is corresponding to a sequence of sandstones, siltstone, carbonaceous shales and limestones that are outcropping in the Mixteca Region, its Type Locality is a homonymous creek, located between Cualac and Huamuxtitlan, Guerrero state (~150 km west of Numí area), since then was designed as Middle Jurassic age, also at the same time was indicated that consisted of five Formations as follow: Zorrillo, Taberna, Simón, Otatera and Yucuñuti. This author also recognized the same units at Diquiyú area, Tezoatlán region, which is ~ 70 km west of the study area. He pointed out that both Jurassic areas are very similar, which complement Numí River lithologic descriptions.

In the studied area, the Tecocoyunca Group it is outcropping both sides the Numí River (Fig. 3) and is ~800 m thick; the geologic structure observed is the flank of a syncline NNE-SSW direction and ~35-80° dip, through ESE direction; also is affected by faults at 90 degrees respect to the synclinal flank (Fig. 3). Lithostratigraphy units is as follow.

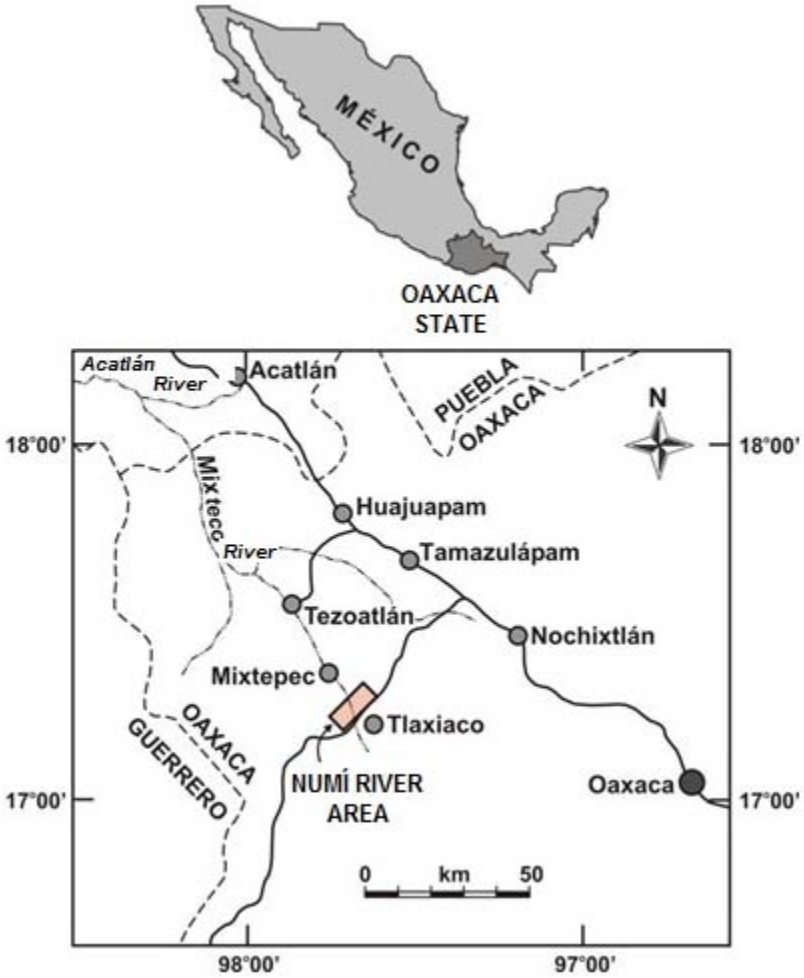

Figure 1: Localization Map of the Numí River area, close to Tlaxiaco, Oaxaca

ii. *Zorrillo/Taberna "Unit"*

Erben (1956) gave the name to the Zorrillo Formation, he toke the name from the Zorrillo hill west of San Juan Diquiyú, Tezoatlán region, Oaxaca state, and also assigned Type Locality and lower Bajocian age. The Taberna Formation also was described by Erben (1956), reported the Tierra Amarilla Hill as Type Locality which is located at side south of the Taberna stream, northeast of San Juan Diquiyú, giving Middle Bajocian to Lower Bathonian age.

Lithologic descriptions of both formations are very similar and they are related transitional, this circumstance make to be difficult recognize them out the type area. This is the reason why were not possible to be recognized at Numí area. As a consequence, was decided to be considered together in one stratigraphic association called Zorrillo/Taberna "Unit", and as a result those formations were not considered individual (Figs. 2 and 3).

Outcrops of this "Unit" are very close to northwest side of Numí Riber, and are recognized with very similar lithologic and stratigraphic sequences outside in the study area.

Lower contact is transitional with Cualac Conglomerate, upper contact also is transitional with the base of Simón Formation. Total thick of this unit show variations, however an average overall from the geologic work done during this study is of 277 m. The Reference Section was measure at the Yuticuani Stream (Fig. 4A).

Lithology: Main rocks of the Zorrillo/Taberna "Unit" are carbonaceous siltstone (Fgi. 5A, B, C y D) and invertebrate and plants fossils. Is clear grey and dark grey color, predominantly first. The stratifications beds are middle to thick (30 to 40 cm width); upper beds are alternatively sandstone and argillaceous siltstone.

Coal zones indicate marsh environment conditions, where tectonic and geographic conditions giving rise to peat accumulation. Two coal beams indicate cyclic of tectonic and sedimentary conditions. Were identified three follow lithology varieties:

1) Phyllite and carbonaceous siltstone (Fig. 6A). It is main variety of the Zorrillo/Taberna "Unit". Consists 60-70% of clastic grains of the sediment, its size is

from fine silt to very fine sand, predominantly middle silt; grains are sharp to sub-sharp, are well classified to middle well classified, some grains show bimodal distribution. Mostly of grains are of quartz (75%); symmetrical and elongate shape; 10% show wave extinction which indicate metamorphic origin. The left grains show parallel or little wave extinction, it contains inclusion as bubbles. Probably alkaline feldspar is present in 5 to 10% of grains mostly argillaceous altered.

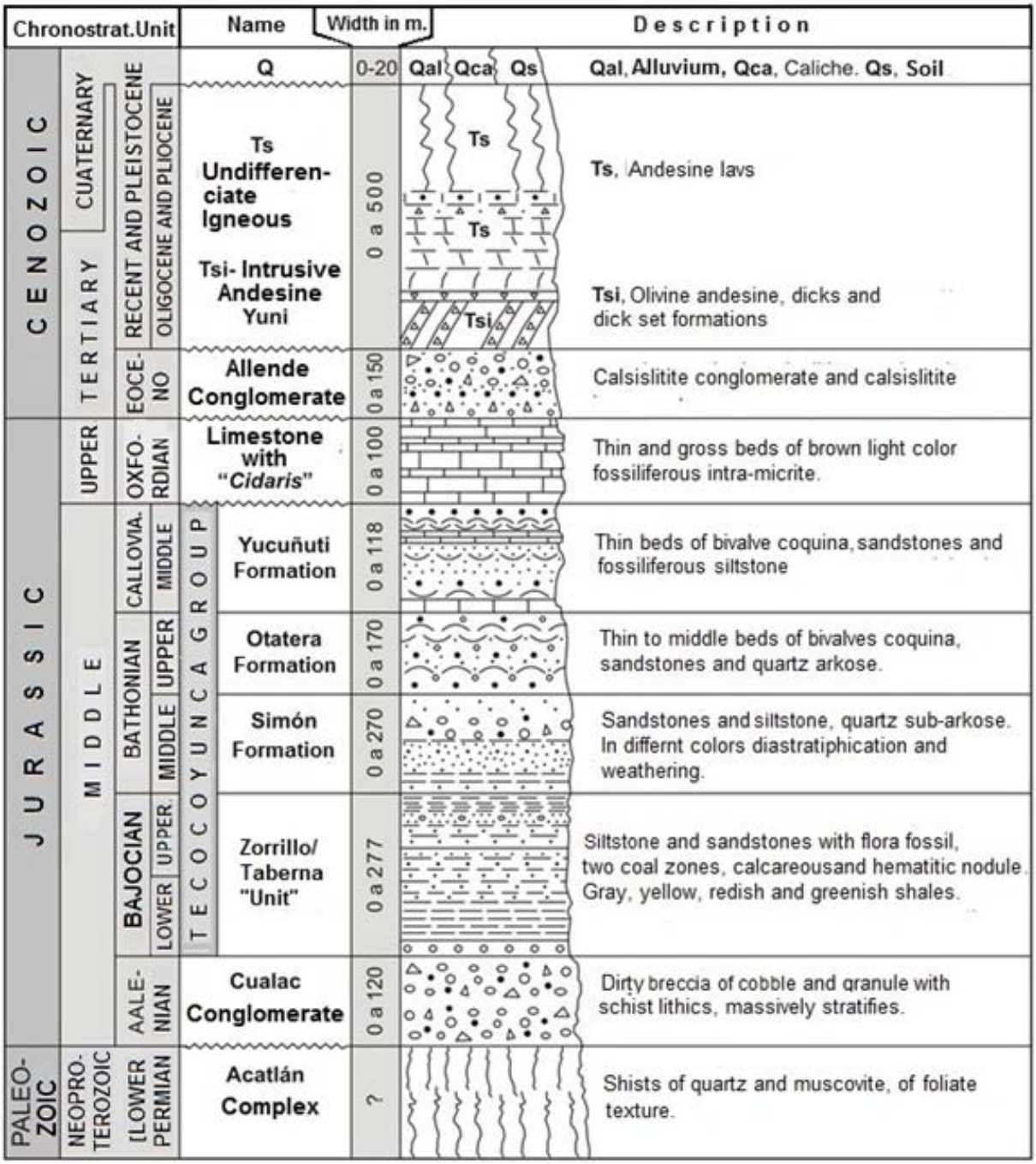

Figure 2: General stratigraphic column of the Numí River Area

Grains of mica are in 10 to 15%, mostly of which are reddish or bluish color biotite with 40 to 60 mμ size. There is calcite (~1%) of secondary origin. Sparing carbonaceous material consists of polymorphs and kerogeno. 30-40% is matrix material, it consists of kaolin, chlorite,?illite and not identified ferromagnesian (?hiperstene). With X ray diffraction equipment was identified chlorite and kaolin (done free by Mexican Geological Survey).

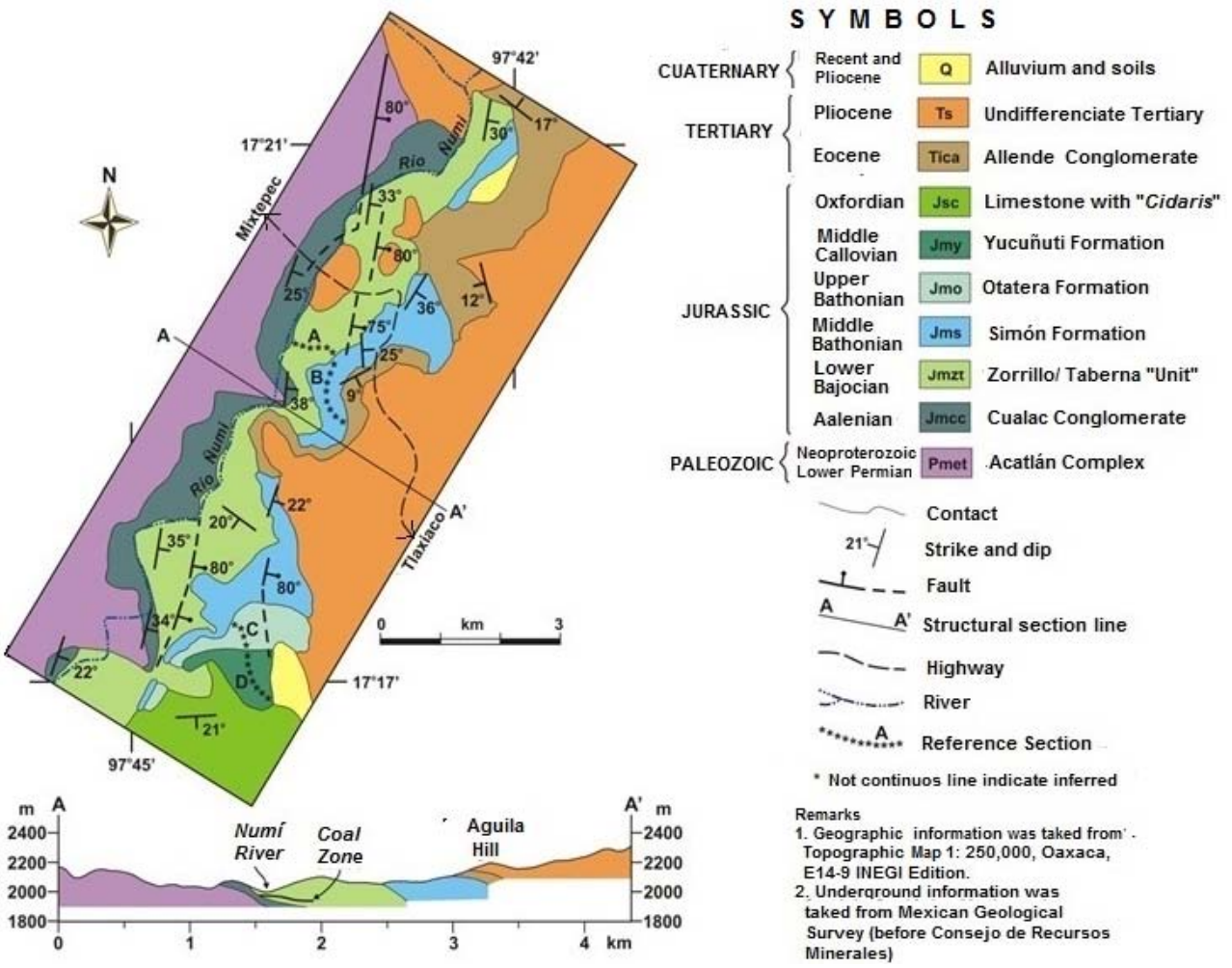

Figure 3: Geologic map and general structural section of the Numí River area, close to Tlaxiaco

2) Quarzite - phyllite mudstone (Fig. 6B). Consist of clastic material included in an abundant argillaceous matrix (30-40%). Grains are 60-70 %) of the sediment, its size is from middle silt to very small sand with predominance of gross silt. Mostly of gains are sharp and unclassified. This variety mineralogy is mainly of quartz (80-90%), grains are mostly elongate and with parallel extinction, bubbles rare. Mica grains (10 to 20%) are almost completely altered, they do not have regular shape, brown reddish and brown bluish color. Matrix consists of unidentified clay and ferruginous material.
3) Quarzite - phyllite subarkose sandstone (Fig. 6C). Grains of quartz are 80 to 85% of rock classified as middle sand. Mostly of grains are sub-spherical, almost well classified. Shape of grains is almost equigranular to elongate; third part of grains of wave extinction, left grains show parallel extinction. Bubbles are rare. Grains of feldspar (15 to 20%) show not-regular shape and alteration to clay.

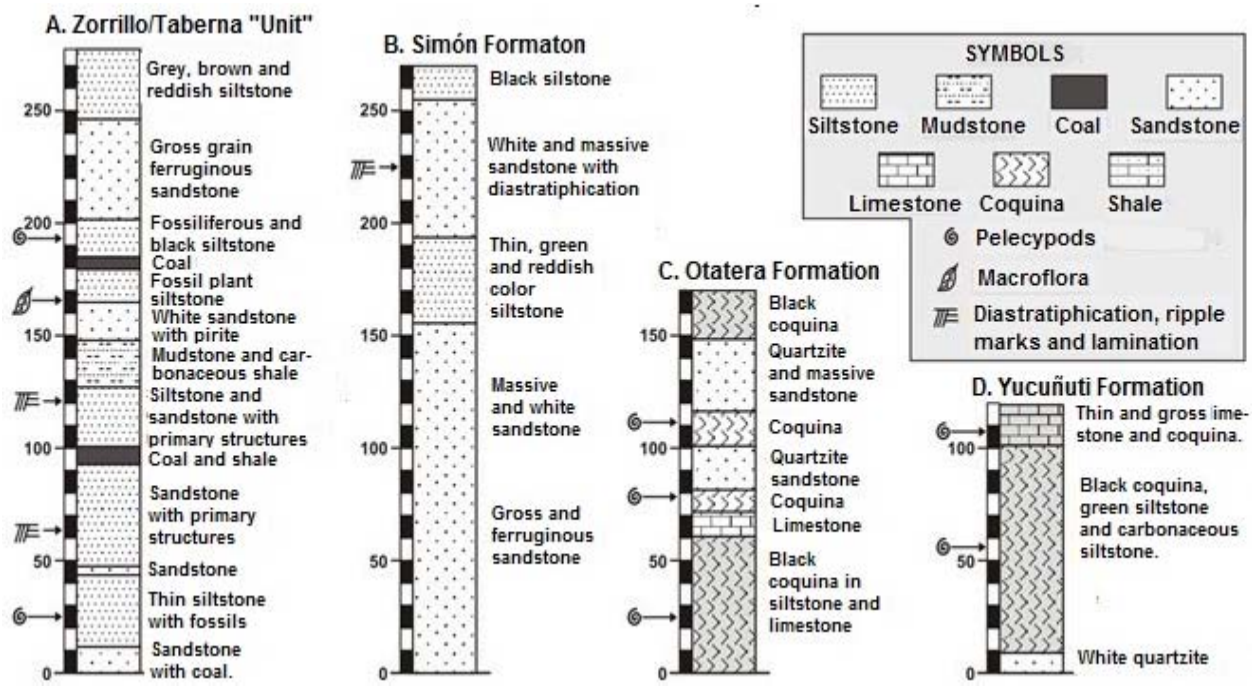

Figure 4: Main Reference Sections of the Tecocoyunca Group formations

Deposit environment. Sedimentological characteristics observed in the Zorrillo/Taberna "Unit" (e.g. spherical fragments, well to almost well classified – i.e., *shoe string*) suggest that this formations was deposited during fluvial channels belonged to a delta system. The environment interpretation became because of the sedimentological texture, showed by the carbonaceous siltstone which belonged to a transitional deposit with fluvial and marine influence. The abundant organic material indicate marsh conditions. The argillaceous siltstone was deposited in similar environment, also green and rose color of beds indicate some aerial expositions of the deposit. The relatively thickness unit indicate constant slow downfall of the basin, also quite tectonic conditions.

Additionally, the mineral conditions as: quartz of wave extinction, clay and mica less abundant, point out to a metamorphic source which probably was the Acatlán Complex Formation which holds granite.

At the same time findings of argillaceous feldspar fragments suggests strong wheathering of sediments before were deposited; this was happening easily with humid and hot weather climate of the source area. From middle to long distance of transport are suggested by taking account texture and silty argillaceous sediments.

As a summary the observed features give the conclusion that the Zorrillo/Taberna "Unit" represent two deposit environments: Main one was coastal marsh and the other less sized fluvial; at the same time both were part of a deltaic system (see Reineck and Singh, 1980; Howard and Reineck, 1981; Reading, 1996).

Moreover, the two coal seams that were founded in this "Unit" indicate that happening following conditions (Stach, 1975; Tatsch, 1980): a) Constant and slowly slowdown, b) Marsh protected by beach and sand dikes, also natural dykes against marine inundations and river inundations, c) An slow energy environment of the continent and slowly sediment deposit, otherwise were not be possible peat deposits.

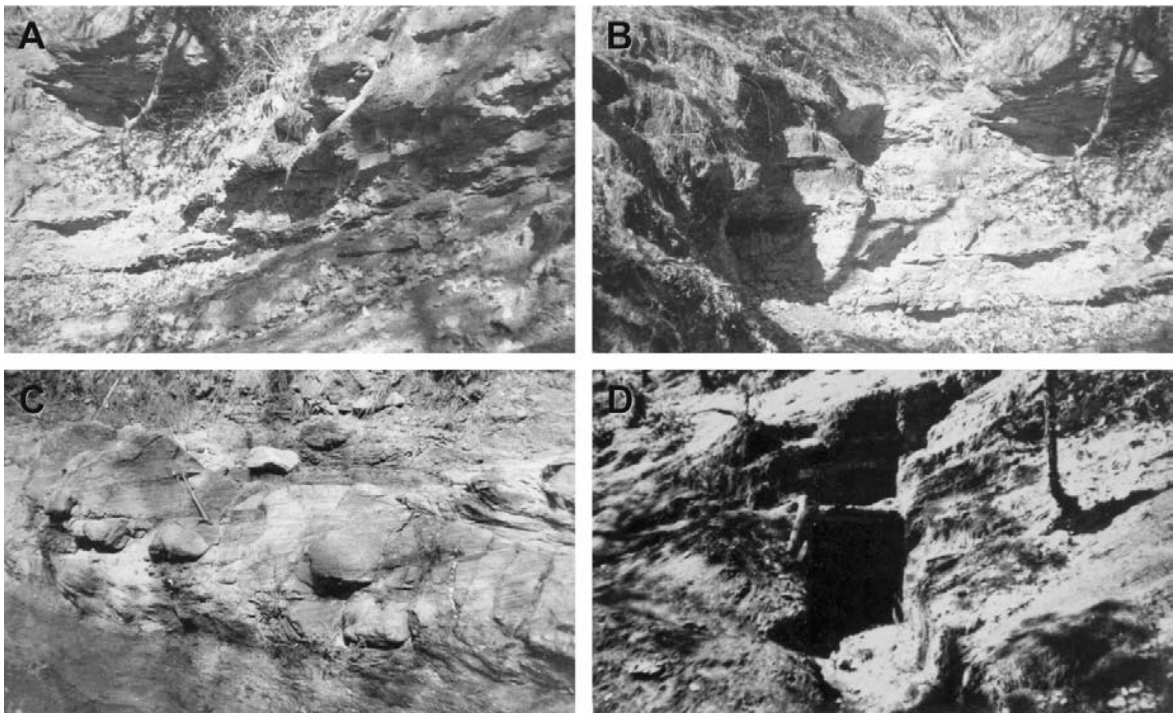

Figure 5: Outcrops of the Zorrillo/Taberna "Unit": A) La Carbonera Stream, show fine grained siltstone and sandstone, road to San Juan Mixtec, Km 6. B) Chicavandicuche Stream, show fine grain siltstone and sandstone, *Idem* km 6 C) Bridge on the Mixtepec road, show siltstone, *Idem*, km 8. D) Small Tunnel, show carbonaceous siltstone and lamination of coal, *Idem*, km 8.5

Fossil collected. Mollusks fauna collected was pelecypods (see Fig. 13E-H) such as: *Lucina cf. L.bellona, Astarte sp, Vaugonia (Vaugonia) v. costata var. Mexicana, Trigonia (Indotrigonia) impressa* (Alencaster, 1963; Alencaster and Buitrón, 1965) which were of cosmopolitan distribution from marine or brackish environment. The continental macro flora collected are taxa such as: *Zamites oaxacensis, Zamites lucerensis, Williamsonia netzahualcoyotlii, Ptillophyllum acuiforme* (see Fig.13A-D) well known from the Mixteca Region, they belonged to humid and hot weather environment of continental flora (Wieland, 1914-1916; Silva – Pineda,1970;Silva-Pineda et al, 1986a, b; Pearson, 1976; Ortíz-Martínez et al., 2013).

Age. The fossils taxa stratigraphic lapse collected in the Río Numí area is as follow: The bivalve are from Bajocian to Callovian age. Stratigraphic lapse of flora fossil founded is longest (Pearson, 1976; Sandoval and Westermann, 1986; Carrasco-Ramírez, 1999).

However taking in consideration that Mixtepec and Río Numí areas are next, also because the Taberna Formation hold ammonites of Early to Late Bajocian age, is deigned this age to the Zorrillo/Taberna "Unit".

iii. *Simón Formation*

Erben (1956) described this unit, and assigned to the Middle Bathonian age, selected as Type Locality the Stream of Simón at the Carrizo clift, noreast of San Juan Diquiyú, in the Tezoatlán region, Oaxaca.

At the study area, Simón Formation is outcropping mainly in the Allende Stream where was measured the Reference Section (Fig. 4B). The lower contact is transitional with Zorrillo/Taberna "Unit", upper contact is not transitional but concordant with the Otatera Formation. The Simón Formation thickness is ~270 m.

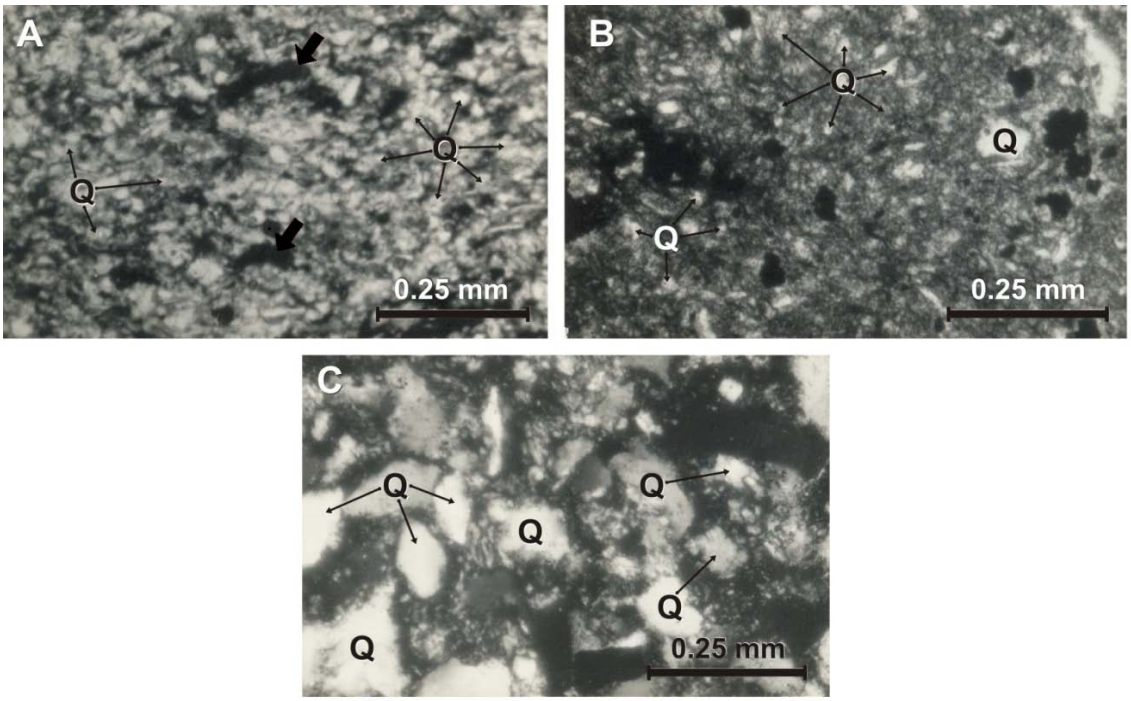

Figure 6: Micro-photographs of the Zorrillo/Taberna "Unit": A) Carbonaceous- phyletic siltstone, at natural light conditions. Clay cement, grains are mainly of quartz (Q) and carbonaceous material is in small forms, pointed out by arrows. B) Quarzite-phylitic mudstone observed by crossed nicol. It is observed mainly clay and clastic grains of quartz and mica (M). C) Subarkosic quartzite phyletic mid-grain sandstone observed by cross nicol. Quartz grains are plentiful

Litology: Main part of the Simón Formation is a middle to gross grain subarkose sandstone, grey to white color, with beds of 0.40 to 1 m and to 1.5 m when grain size increase, this beds belonged to a group of beds mainly of reddish or bluish siltstone, which thickness is from 10 to 50m. There are some red ferruginous nodules. There is mainly *diastratiphication* as primary structure (Figs 7A-7B). Description of the common lithology identified is as follow:

1) Middle grain quartzite-subarkose sandstone. This lithologic variety is main part of this formation (Fig. 8A). 95% are made of grains which ~80% are of quartz, from this 25% show wave and composed extinction, which suggest metamorphic provenience, left of grains show parallel extinction. Irregular grains of? Alcaline feldspar (15 to 20%) show fractures well done. Weathering not permit good identification. Were found trace of mica mainly biotite and of heavy minerals as garnet and turmaline; last are suggesting schists and pegmatite rocks provenience (see Folk, 1974). Matrix is 5% of rock which is of middle silt made of argillaceous mica and clay; secondary minerals are hematite and calcite, this last is cement and is filling porous places.
2) Phyletic siltstone. Clay or fine silt texture, it is made of grains of quartz with wave extinction, argillaceous mica and ferromagnesian alteration; its matrix is argillaceous (Fig. 8B).

Figure 7: Outcrops of the Simón Formation, are observed conglomeratic sandstone with diastratiphication structures: A) Allende stream, Allende road, km 11 B) Yuni stream, San Juan Mixtepec road, km 6

Deposit environment. Wave extinction of quartz, suggest that provenience area was metamorphic rocks, probably the Acatlán Complex. Grains of quartz of parallel extinction indicate that provenience area was formed by igneous rocks (?granite). Besides, size of grains (fine silt) indicate middle length to too long transport; also *diastratiphication* indicate fluvial environment (inundations plains and front of delta facies); at the same time reddish colorations indicate this deposits were exposed to aired oxidation (see Reineck and Sing, 1980; Reading, 1996). As a summary we can said that Simón Formation had a sedimentation development similar to Zorrillo/Taberna "Unit"; Simón Formation sedimentation was fluvial but mainly beach environment.

Age. There is not paleontologic or radiometric information that support age of this unit, at this circumstance the stratigraphic relationship is useful. The Simón Formation transitionally overlay the Zorrillo/Taberna "Unit" which is of Bajocian age and its upper contact is concordant with Otatera Formation of Late Bathonian age (Fig. 9). So, Simón Formation is probably Late to Middle Bathonian age.

iv. *Otatera Formation*

Erben (1956) gave the name to this sedimentary rocks which have their Type Locality at central and southcentral of the Otatera creek, Rosario River in the Tezoatlán region, Oaxaca, giving Late Bathonian age. Its distribution is small area in the Doña Chona Stream, south of the study area (Fig. 3), it consist of ~170 m width of sandstones and coquina (Figs 2 and 9) and is interlayered with basal beds of the Simón Formation, upper part is over layered by the Yucuñuti Formation. Reference Section was measure at Doña Chona Stream (Fig. 4C).

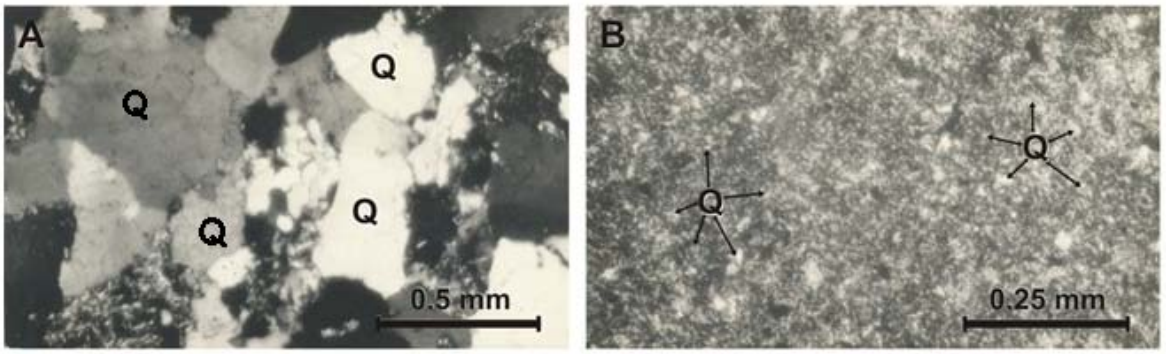

Figure 8: Micro photographs of Simón Formation: A) Quartzite subarkose middle grain sandstone observed with crossed nicole. There are igneous quartz composed (grey) and igneous included in silt. B) Phyletic siltstone parallel light observed. Microcrystals of clay and fine silty size are plentiful with poor quartz

Lithology: Mostly of Otatera Formation is constituted by beds of black coquina of following genus of bivalves: *Eocallista, Pleuronya, Crenotrapezium* and ostracoda (*Gryphaea*), with sparite cement (Fig. 9). Coquina beds are mainly interlayered with calcareous beds of brown grey color, which consists of small fragments of calcspar and black color shells. Whole group of coquina are interlayered by thin beds of limestones from 4 to 5 cm width. Upper part is constituted of thin beds (1 to 20 cm width) of fine and gross grains of sandstones.

The Otatera Formation include few lithic variation, most common is sparite (Fig. 10). This is sub mature calcarenite, it contains few lithic fragments (gross sands size), constituted by tuffs (~3%), subarkose (3%), quartz (2%) and probably alkaline

feldspar (2%). Grains are cemented by sparite calcspar (~90%), which crystals are 5 to 10 mμ.

Deposit environment. Sedimentary features of this unit indicate a shallow marine environment (neritic) with short terrigenous deposit, where water movement was able to erode unconsolidated microcrystalline calcite and re-deposit it as sparite. Relative close terrigenous material, indicate topographic high. It is important to know that upper middle of this unit are thin beds that consists of sandstones (fine to gross grains) become thick as increase grain size. This deposit characteristics suggest probably beach environment.

Moreover, coquina beds indicate continuous buried, which not permit groups of bivalve subaerial destruction; only relatively fast sinking of the basin could be the reason.

Fossil collected. Were collected mainly bivalves of the genus *Eocallista, Pleuromya, Crenotrapezium* (Fig. 14 I-L) (Alencaster, 1963; Alencaster and Buitrón, 1965) which were living in tide zone environment (Reineck and Singh, 1980); consolidated remains of this mollusks made coquina.

Age. Stratigraphic extension of taxa collected is large and is not possible to be assigned Jurassic age to this formation. However, in Tezoatlán area, the Otatera Formation hold ammonites among which is *Epistrenoceras paracontrarium* from Late Bathonian age (Erben, 1956), this make possible to be assigned same age to this unit.

v. *Yucuñuti Formation*

Erben (1956) designed as Yucuñuti Formation a sedimentary sequence outcropping at the (homonymous) Yucuñuti Stream (chose as Type Locality), located east of Santa Maria Yucuñuti, Tezoatlán region, Oaxaca, and assigned Callovian age.

In the Numí River area this formation is located at south position, particularly where the Doña Chona Stream is. Its covering is short (Fig. 3). Lower beds interlayered with Otatera Formation and upper beds are discordantly overlay by Limestone with "*Cidaris*"; it is ~118 m width (Fig. 2). Reference Section was measured in the mentioned stream (Fig. 4D).

Lithology. The Yucuñi Formation is a sequence that starting with fine grains of whitish and rose colors sandstones (Fig. 11); middle part consists of black color ostracods coquina interlayered by light grey siltstone with several *burrows*. Upper parts consists of middle thick beds (20 to 30 cm) of fossiliferous limestones interlayered by thin beds of *Lucina* coquina. Restricted beds of coquina alternate with ostracoda and pelecypod species. Main identified lithology are described as follow:

1) Quartzite siltstone. Is gross siltstone, middle classified, with sub mature texture (Fig. 12A). Grains are ~70 % of rock volume, its constitution is of quartz with parallel (~80%) or wavy (20%) extinction; grains size are ~30 μm. Besides there is argillaceous matrix (30% of rock); sparite is cement and fill micro structures and hollows.

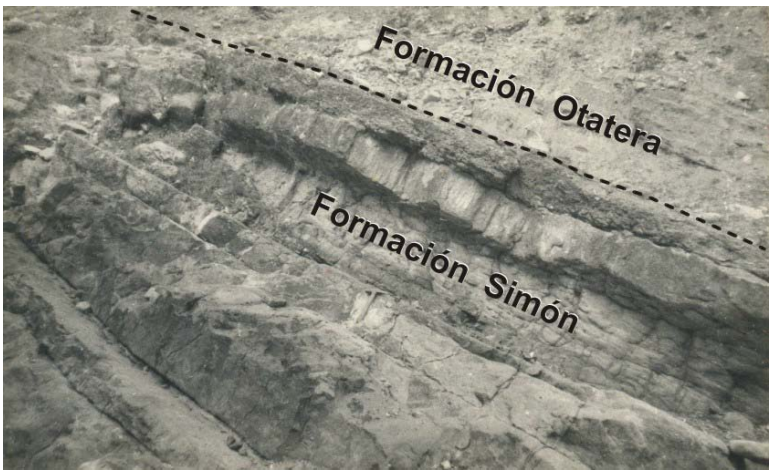

Figure 9: Outcrop show Simón Formation with Otatera Formation contact. It is observed black coquina resting on sandstone

2) Quartzite - subarkose siltstone. Rock constitution is 70-80% of grains with argillaceous matrix short abundant (20-30% of rock), which give a sub mature texture (Fig. 12B). Grains are constitute by quartz (70-80%), sharp grains with parallel extinction (wavy extinction are rare), size typically is bimodal (10 μm and 40 μm). Left grains (20 to 30%) are constitute by feldspar (?alcaline), crystals are fractured and change, its average size is 40 μm. Matrix consists of clay, mica and calcite micro-crystals. Plutonic origin suggested by quartz type.
3) Biomicrite with silt quartzite. This characteristic variety, consists of bio clasts (30%) and micrite (40%) (Fig. 12C). Bio clasts typically are fragments of bivalves. Terrigenous clasts are ~20 μm size, they include parallel and wavy extinction quartz as well as lithic clasts (?ignimbrite).

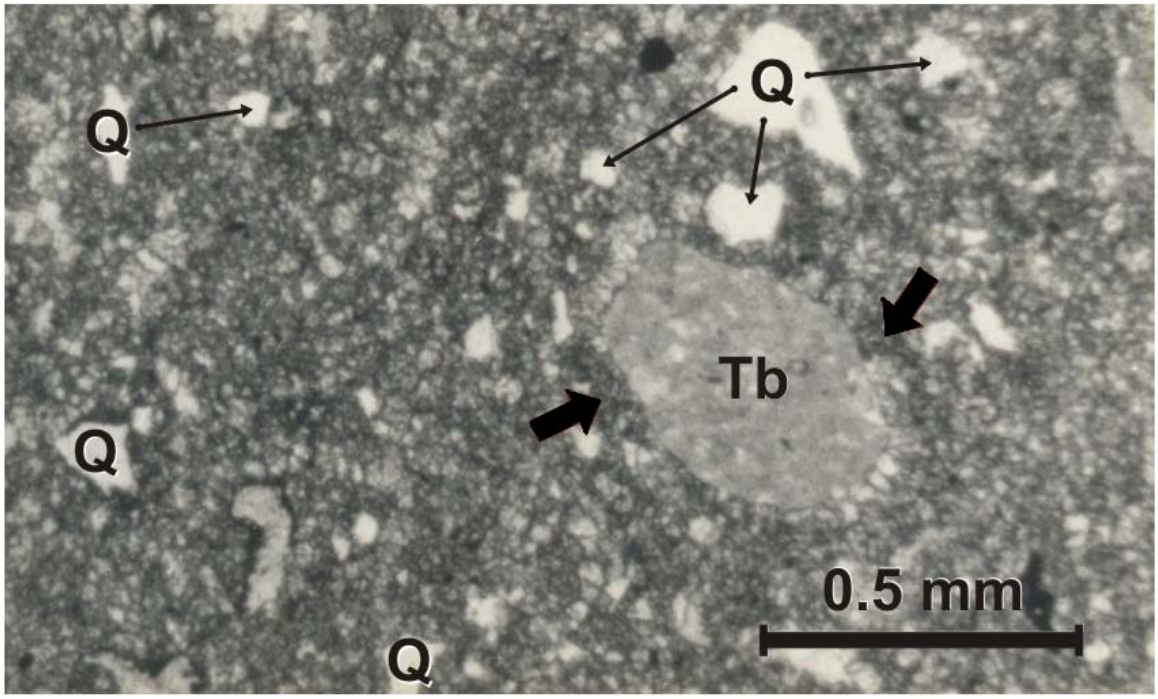

Figure 10: Otatera Formation: Micro photograph using parallel light. There is big tuff grain (Tb) surrounded by spathite (arrows point out)

Deposit environment. Lithic variety suggest a shallow marine/transitionally deposit environment, 1) and 3) varieties are of neritic environment, meanwhile variety 2) indicate terrigenous supply at plain stagnation water basin. Mineral composition and texture of described lithic varieties suggest that supply area, could consists mainly of igneous (?granite) and metamorphic bodies, which were located relatively far from the sedimentary basin.

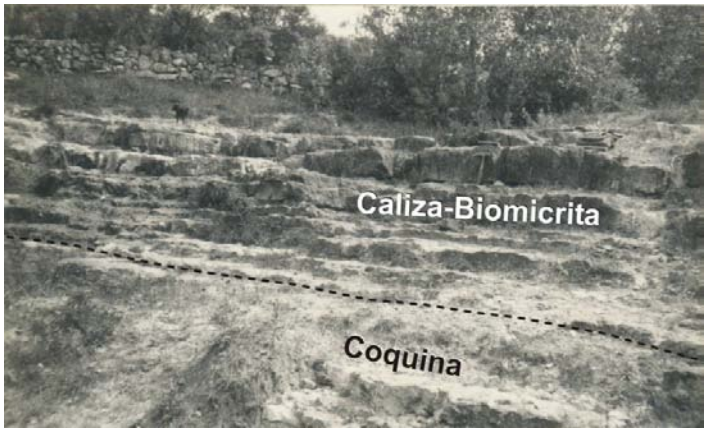

Figure 11: Outcrop of Yucuñuti Formation. Thin beds of light grey biomicrita and coquina

Fossils collected. In the Yucuñuti Formation were collected cosmopolite mollusks such as genus: *Lucina, Astarte, Vaugonia, Gryphaea, Eocallista, Crenotrapezium, Pleuromya*, and *Lima*, (Fig. 14 M – Ñ) (Alencaster, 1963; Alencaster and Buitrón, 1965) which belonged to a marine or saline environment.

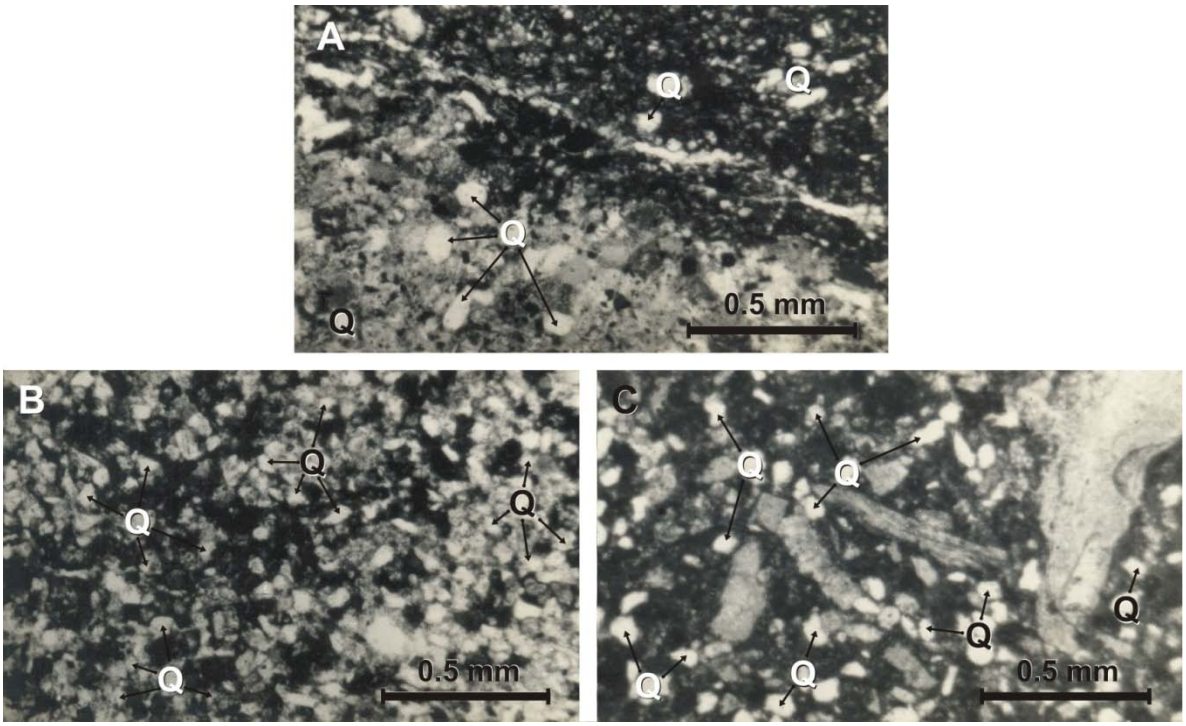

Figure 12: Micro photograph of the Yucuñuti Formation. A) Gross grains of quartz sand siltstone using parallel light. Have a look that grains of quartz are abundant. C) Biomicrite and quartzite

Age. The stratigraphic length of mollusks do not give age to this formation. However in the Mixtepec area Carrasco-Ramírez (2003) collect Middle Callovian ammonites; moreover in Mixtepec and Numí River areas outcrop same stratigraphic sequences, this circumstance give age to the Yucuñuti Formation.

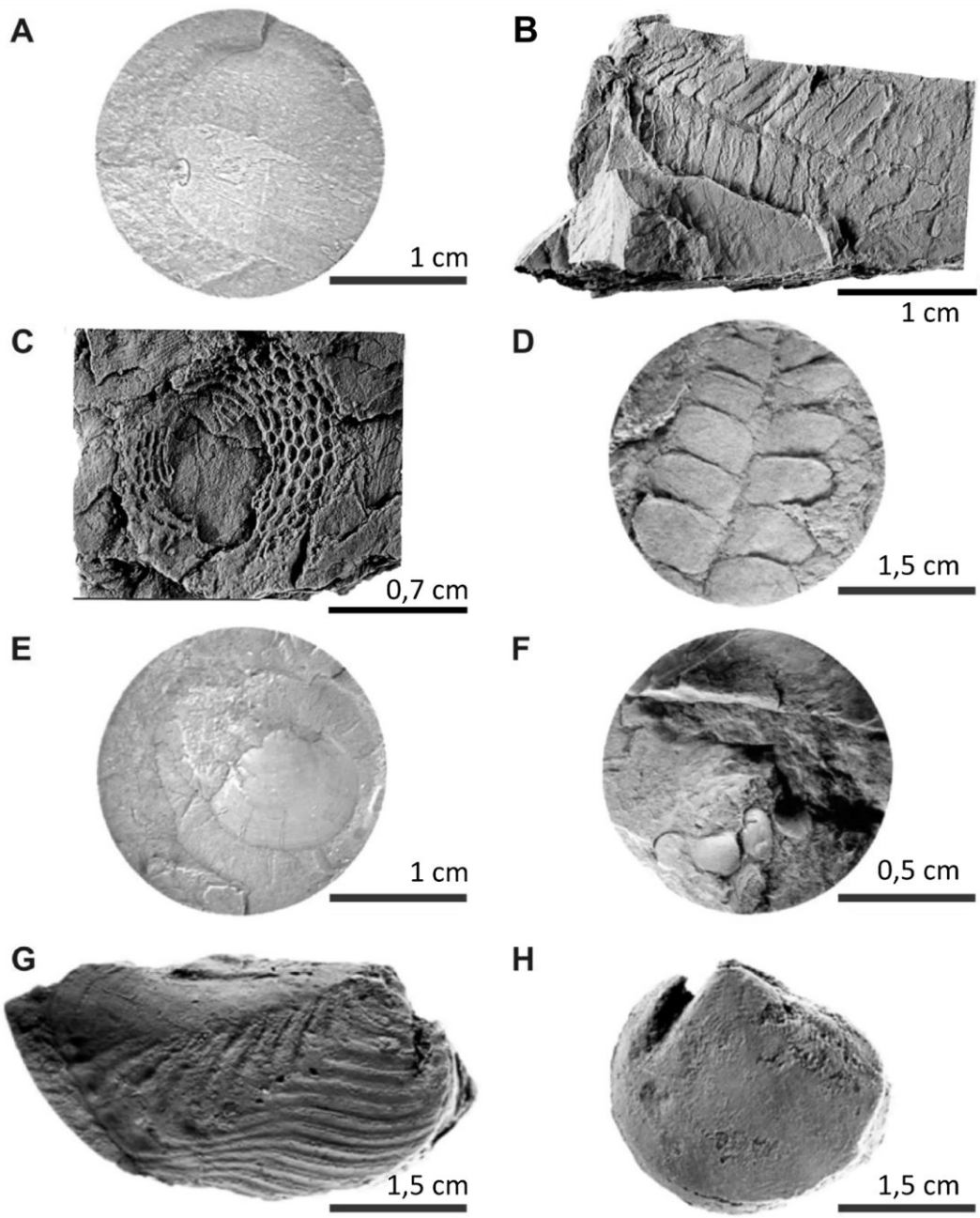

Figure 13: Specimens collected from the Zorrillo/Taberna "Unit". A) *Zamites oaxacencis* B) *Zamites lucerensis,* C) *Williamsonia netzahualcoyotlii*, D) *Ptilophyllum acutifolium*, E) *Lucina* cf. L. *bellona*, F) Astarte sp., G) *Vaugonia* (*Vaugonia*) *v-costata* var. *mexicana*, H) *Trigonia* (*Indotrigonia*) *impressa*

IV. Conclusions

Tecocoyunca Group in the Numí River area, Tlaxiaco region, Oaxaca, consists at its lower part of the Zorrillo/Taberna "Unit" of Lower (late part) Bajocian and Late Bajocian (begin part) age, transitional is overlaying Cualac Conglomerate of Aalenian age, they are ~287 m thick constituted by carbonaceous siltstone, mudstone, subarkose, very fine grained sandstone and siltstone, hold bivalves and fossil flora, as well as two coal beams, one at the lower part and the other at middle of upper part. The inferred deposit environment of this unit is a delta complex, composed by coastal marsh and fluvial contributions. This units are overlaying by Simón Formation, of Middle-Late Bathonian age, which is constituted by ~270m of thin and gross beds of siltstone and subarkose set; its deposit environment inferred is transitional (beach zone). The Simón Formation is transitionally overlay by Otatera Formation of Late Bathonian age, which consists of pelecypods coquina with interlayers of spathite limestone strata of ~170 m thick; the inferred deposit environment of this unit is shallow marine, and some contribution of beach. This unit is concordantly overlay by Yucuñuti Formation

of Middle Callovian age, which is constituted by fine grain sandstone, coquina and biomicrita ~118 m thick, holding bivalves; the inferred deposit environment is transitional (flood plain, coastal marsh and shallow neritic zone); this unit is discordant overlay by Limestone with "*Cidais*" of Oxfordian age.

Taking in consideration detailed descriptions of the Tecocoyunca Group formations, is *de facto* re-definition advance work for the Middle Jurassic units of the Mixteca Region (see NACSN, 2005, Art. 18, Remark b).

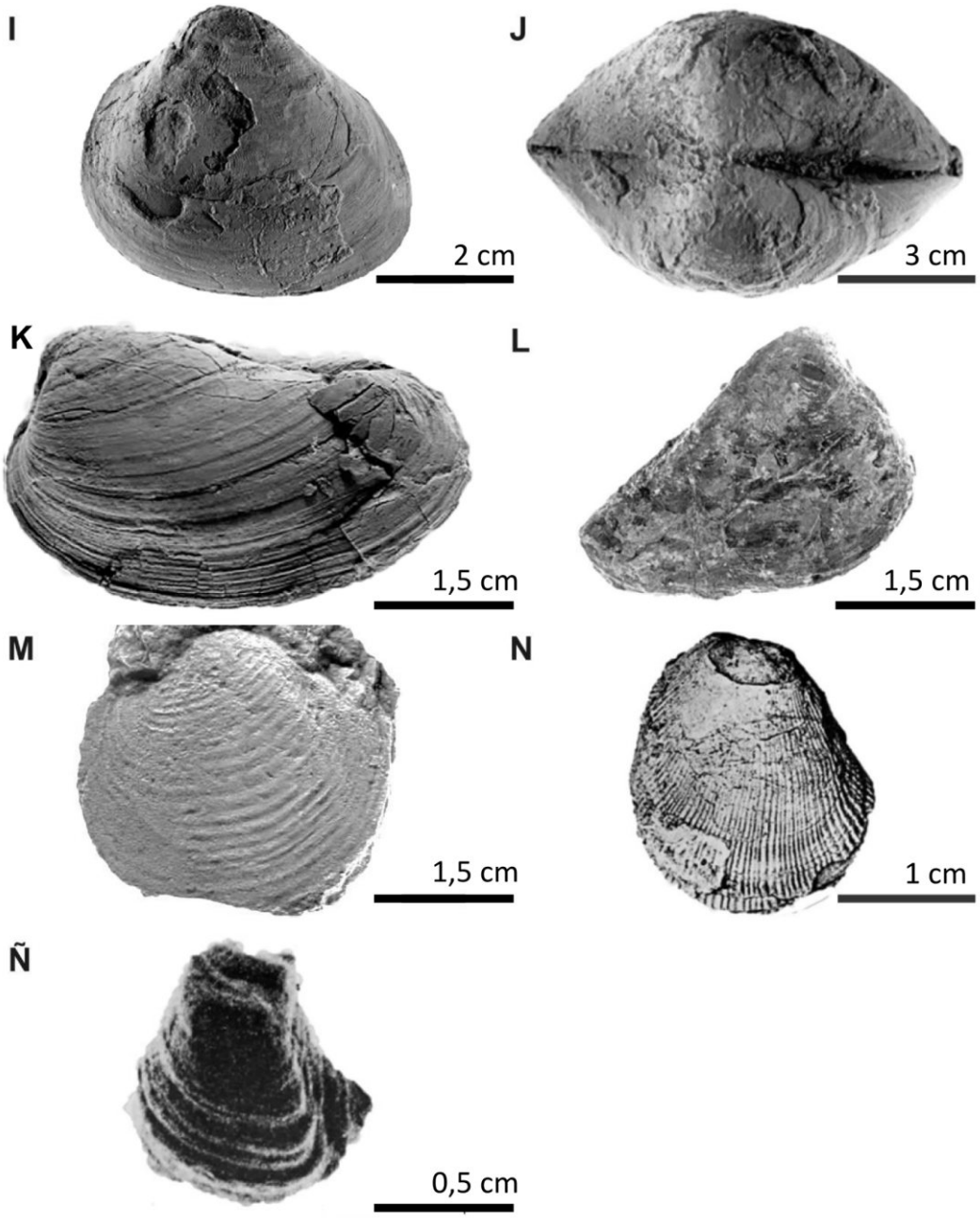

Figure 14: Specimens collected from the Oatera Formation. I) y J) *Eocallista imlayi*, K) *Pleuromya* sp., L) *Crenotrapezium hayam.* Specimens collected from the Yucuñut Formationi. M) *Lucina* sp., N) *Lima* (*Plagiostoma*) sp., Ñ) *Gryphaea mexicana*

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First Detection and Molecular Characterisation of *Eimeria* Spp on Mugilidae Fish *Mugil cephalus* Linnaeus, 1758 in Algerian Coast

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Abstract- The objective of this work is to develop and optimise the ESSP841/CRP999 real-time PCR targeting the 18S ribosomal rRNA of Eimeridae (*Eimeria* spp) found in *Mugil cephalus* from the east coast of Algeria and to determine their pathogenicity and the risk of contamination of consumers. Mugilidae from the east coast of Algeria were captured in search of parasites that could infect them. In the laboratory 816 *Mugil cephalus* were weighed, dissected and eviscerated. In our study we found 378 samples positive for *Eiemeria* sp (46.3%) out of 816 fish samples, i.e. a prevalence of 46.3%, the average intensity was 1.6. The results of the direct examinations are confirmed by real-time PCR using the primer pair ESSP841 CRP999 which was positive for all samples tested. We obtained curves with variable TCs.

Keywords: *mugilidae, mugil cephalus, eimeria spp, RTPCR.*

GJSFR-C Classification: *LCC: QL391.E53*

Strictly as per the compliance and regulations of:

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Racha Boubekeur [α], Khaled Abdelouahed [σ], Salim Bekhouche [ρ], Haeit Adjmi Hammoudi [ω] & Zouhir Ramdane [¥]

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I. Introduction

The genus *Eimeria* comprises obligate intracellular protozoan parasites belonging to the phylum Apicomplexa. Members of this genus cause enteric disease in a wide range of vertebrate hosts, including fish, reptiles, birds and mammals. These parasites complete their development in a single host species and their sporocysts can be recognised by the presence of a Stieda body, an organelle through which the sporozoites exyst. Duszynski and Wilber 1997 several species cause high levels of morbidity and/or mortality in certain hosts, resulting in economic losses in various animal production industries (Daugschies and Najdrowski 2005; Aarthi et al. 2010; Sharma et al. 2018).

A total of 157 species of fish-parasitic *Eimeria* have been described on the basis of sporulating oocyst morphology, host specificity, pathology and geographical distribution (Belova and Krylov 2000). Although these characteristics have traditionally been used to identify *Eimeria* species (Duszynski and Wilber 1997), they are often insufficient for reliable differentiation between species due to overlapping morphometric and biological characteristics (Long et al. 1984; Zhao and Duszynski 2001). A combination of morphological and molecular analyses is therefore necessary to delimit species and determine phylogenetic relationships between them.

The development of molecular tools has allowed not only diagnosis but also the study of genetic variability of pathogens from small quantities of oocysts using molecular markers (Schnitzler et al, 1998; Costa et al, 2001). Fernandez et al (2003) identified species-specific markers for *Eimeria* spp from a cluster of SCAR (Sequence-Characterized Amplified Region) markers. This allowed the use of the polymerase chain reaction (PCR) technique as an efficient and integrated diagnostic method, capable of detecting *Eimeria* species individually or simultaneously in a single reaction (Fernandez et al, 2003; Lien et al, 2007).

Of the forty-two species of Coccidia described in marine fishes (Dykova and Lom 1983), sixteen exist in Mediterranean fishes and are divided into four genera: *Crystallospora* Labbé 1896, *Eimeria* Schneider 1875, *Epieimeria* Dykova and Lom, 1981, and *Goussia* Labbé 1896. Little research has been carried out on these parasites since the end of the last century; the main works are those of Thélohan (1892), Labbé (1896), Léger and Hollande (1922) and finally Lom and Dykova (1981, 1982).

Molecular information on the diversity of *Eimeria* species infecting fish is scarce. Thus, only a few species of *Eimeria* isolated from different marine, estuarine and freshwater fish have been genetically characterised: *Eimeria* percae from perch (*Perca fluviatilis*); *Eimeria anguillae* from the European eel (*Anguilla anguilla*); *Eimeria variabilis*, from the long-billed bullhead (*Taurulus bubalis*); *Eimeria daviesae* on gudgeon (*Gobius fluviatilis*); *Eimeria rutili* on roach (*Rutilus rutilus*); and *Eimeria nemethi* on bleak (*Alburnus alburnus*). (Molnár et al. 2012).

This work constitutes the first study of Coccidia Eimeriidae in the Mugilidae of the Algerian coast. The objective of the present study was to molecularly characterise, at the small subunit ribosomal RNA (rRNA-

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US) locus using the primer pair ESSP841 CRP999, the *Eimeria* isolates obtained from the mullet (*Mugil cephalus*), and to develop a quantitative PCR for rapid detection.

II. Materials and Methods

a) Collection and Processing of Samples

From February 2017 to March 2018 a total of 816 *Mugil cephalus* were caught by fishermen in the east coast of Algeria.

The first step of the present study is to identify positive samples from whole fish samples and examine them under the microscope for oocysts, either by direct methods where samples are examined either freshly, using a 10% concentrated natural formalin buffer, or by staining the samples with iodine or Giemsa to make the internal components clearer.

– The gastrointestinal tract was differentiated into the pyloric cecum and the intestine. The pyloric caeca were homogenised using an Ultra-Turrax® T10 homogeniser (Ika®-Werke GmbH and Co., KG, Staufen, Germany). The intestinal contents were removed by scraping with a scalpel blade and then ground in a mortar with 0.04 M phosphate buffered saline (PBS) pH 7.2. The resulting homogenates were filtered through a set of two sieves (mesh size, 150 and 45 μm) before being subjected to a two-phase concentration of 0.04 M PBS pH 7.2/ diethyl ether (2:1) by centrifugation at 1250xg, 4°C, for 15 min. The supernatants were carefully discarded and the concentration step was repeated until lipid-free sediments were obtained. Finally, the pellets were resuspended in 500-1000 μL of PBS and stored at -20°C.

Aliquots of 10 L of sediment were examined under brightfield microscopy to detect *Eimeria* oocysts (×400 magnification), which were confirmed by appearance, presence of four sporocysts and thin wall. A total of 50 oocysts from several fish specimens were observed under differential interface contrast (DIC) microscopy (×1000 magnification) and measured under a light microscope (AX70 Olympus Optical Co., Ltd., Tokyo, Japan) using a micrometer eyepiece and DP Controller 2.1.1.183 software (©2001-2004 Olympus Optical Co., Ltd.).

b) DNA Extraction

Genomic DNA was extracted from the samples using the Qiamp Stools Quiagen DNA extraction kit.

c) DNA Profile

For the detection of DNA that is extracted from stool samples by using a Nanodrop spectrophotometer (THERMO. USA) for the detection and measurement of the concentration of nuclear acids (DNA and RNA), where the concentration of DNA is detected (ng/μl) and the measurement of the purity of the DNA by reading the absorbance at a wavelength between (280-260 nm) Figure 1.

Wavelength 260 nm: represents the area of maximum absorbance of nucleic acids.

The 280 nm wavelength: is used to establish the ratio and to control the purity of the extraction.

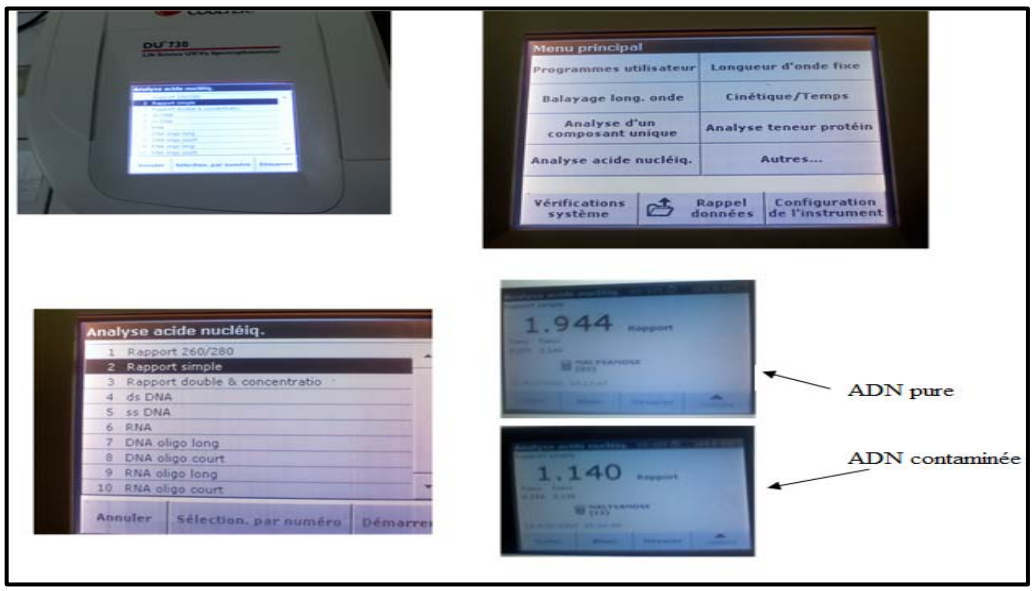


Figure 1: Detection of DNA concentration (ng/μl) and measurement of DNA purity with a Nanodrop spectrophotometer (THERMO. USA)

d) Real-Time PCR Protocols

Real-time PCR performed for the detection of *Eimeria* species from *Mugil cephalus* using primers and TaqMan probe specific to the ITS1 region of the DNA that code for ribosomal RNA. The technique performed as described by Ogedengbe et al. 2011.

e) Real-Time PCR Master Mix Preparation

Real-Time PCR master mix prepared by one-step Reverse Transcription and Real-Time PCR detection kit (Accu Power Rocket Script RT-qPCR Pre Mix, Bioneer. Korea), and done according to company instructions as following Table (1):

Table 1: Explained the main components of the mix for qRT-PCR technique

qRT-PCR Master mix	Volume
2X Green star master mix	25 µL
DNA template	5µL
ITS1 forward primer 10pmol	1µL
ITS1 reverse primer10pmol	1µL
DEPC water	18µL
Total	50µL

Primer

Primers were designed in this study using the complete sequence of the ITS1 region in the rDNA using the NCBI Gene-Bank and Primer 3 plus online and provided by (Bioneer company, Korea) as shown in Table (2):

Table 2: Explained forward and reverse primers used in the qRT - PCR technique with nucleotide sequence and the size of the resulting DNA

Real-Time Primer	Sequence (ESSP841-CRP999)	PCR SIZE
Eimeria spp	5 GTTCTATTTTGTTGGTTTCTAGGACCA-3 5-CGTCTTCAAACCCCCTACTGTCG-3	174 bp

The reaction components of the qRT-PCR mix listed in Table 1 were added to a standard qPCR tube (8-well strip tubes containing Rocket Script Reverse Transcriptase and TaqMan probe pre-mix) (Fig. 2). Next, all strip tubes were vortexed and centrifuged at 3000 rpm for 3 minutes in an Exispin centrifuge and transferred to a real-time PCR thermal cycler.

Figure 2: Preparation of the mix for real-time PCR

f) Real-Time PCR Thermocycler Conditions

Real-Time PCR thermocycler conditions was set up according to primer annealing temperature and RT-qPCR TaqMan kit instructions as following Table (3):

Table 3: Explained Thermal cycler program or qRTPCR technique

Step	Condition	Cycle
Reverse transcriptase	95°C 15 min	1
Pre-Denaturation	95°C 5 min	1
Denaturation	95°C 20 sec	45
Annealing/Extension	60°C 30 sec	
Detection (Scan)		

Thermal cycles were applied to inspect the Real-Time PCR and relying on instructions AccuPower® 2X Green-StarTM qPCR Master Mix as well as by calculating the degree Tm prefixes using the device MiniOpticon Real-Time PCR system BioRad/USA as in Figure (3) below:

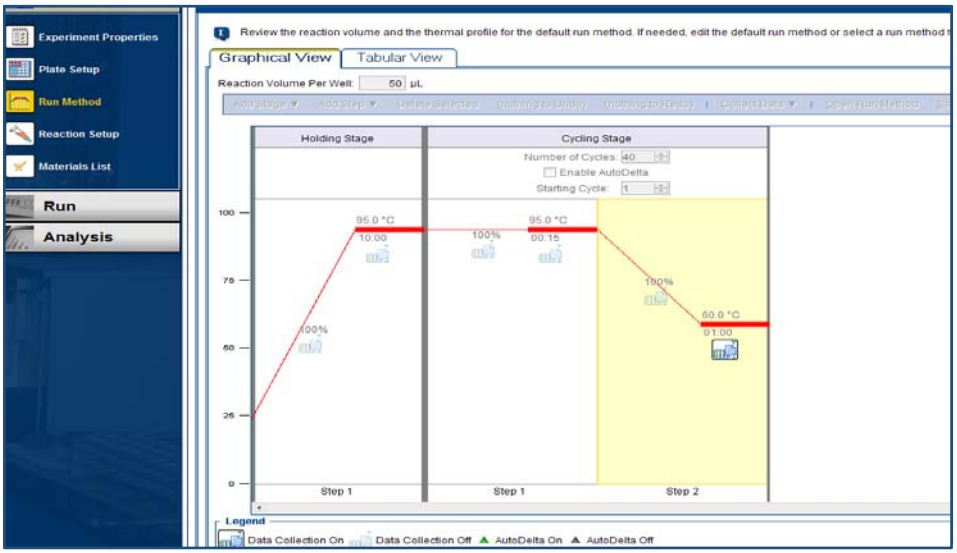


Figure 3: Explained situations of Thermo cycler for REALTIME PCR

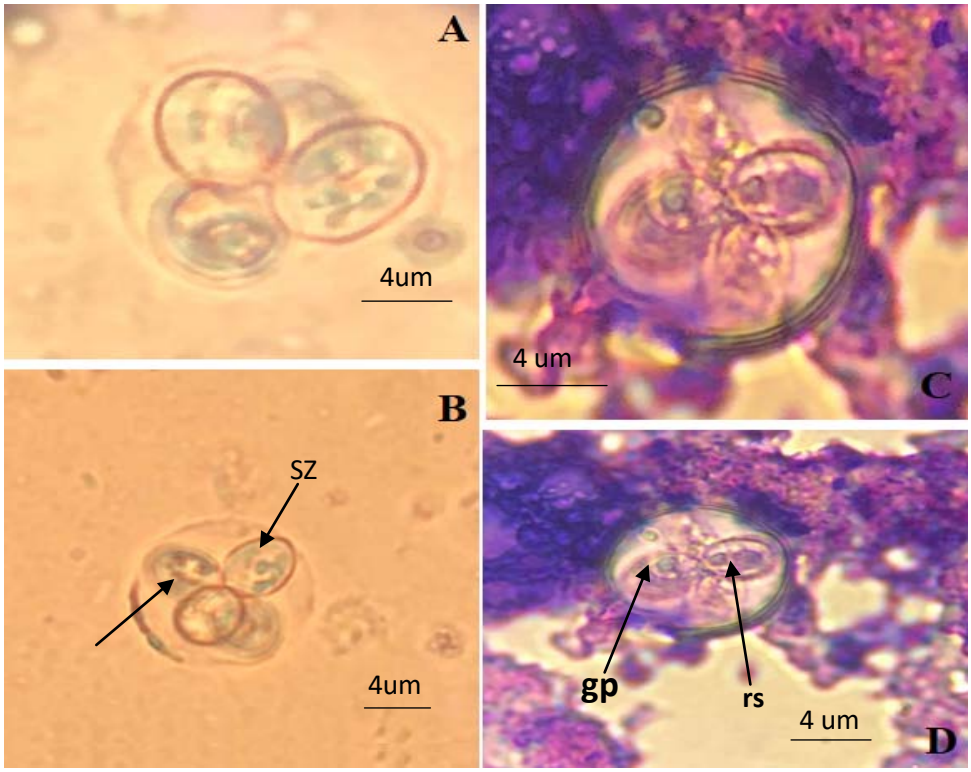
g) Real-Time PCR Data Analysis

qRT-PCR data analysis was performed by calculation the threshold cycle number (CT value) that presented the positive amplification of gene in Real-time cycle number.

III. Results

a) Direct Examination and Staining

In the present study, *Eimeria* oocysts were detected in 378 of 816 (46.3%) gastrointestinal tracts of *Mugil cephalus* examined. This coccidia produces equally spherical oocysts containing sporoblasts and sporocysts (Fig. 4). The oocysts measure 10 ±1.5 um in diameter. The oocyst residue is absent but three polar granules of 2.2 ± 0.5 um diameter each are present (Fig. 4). Each mature oocyst contains four pyriform sporocysts 6.1 ± 0.9 um long and 3.8 ± 0.6 um wide (Fig. 4). At one end of the sporocysts there is a conspicuous projection corresponding to the body of Stieda (Fig. 4). Each sporocyst contains two vermiform sporozoites between which the sporocystic residue is present as three or four refractive granules (Fig. 4).



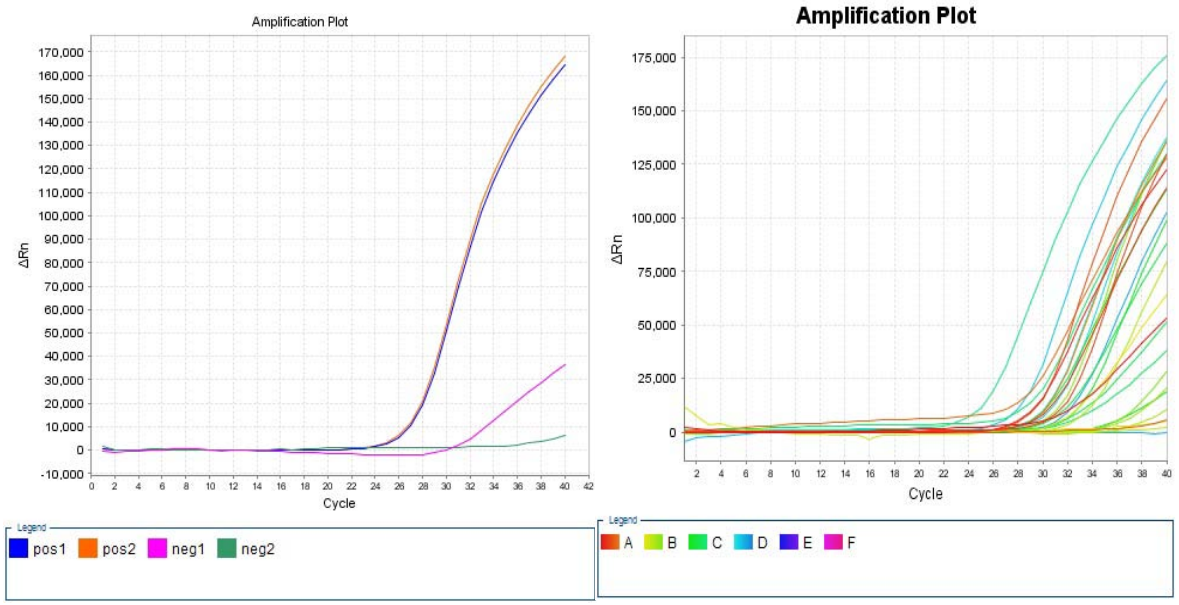
(A) Oocyst containing sporoblasts (bar = 4 um). - (B) Sporulated oocyst. Sporozoites (sz) are visible inside the sporocysts. The arrow indicates the Stieda body (bar = 4 um) - (C) Sporulated oocyst showing three polar granules (gp) and (D) sporocystic residue (rs) in one of the sporocysts (bar = 4 um).

Figure 4: Light microscopy of *Eimeria* sp. oocysts found in *Mugil cephalus*

b) Results of Molecular Examination by qRT-PCR

In the present study, *Eimeria* oocysts were detected in 378 of 816 (46.3%) gastrointestinal tracts of *Mugil cephalus*. Measurements of sporulated oocysts, sporocysts and other morphological characteristics identified the oocysts as *Eimeria* sp. We confirmed by molecular analysis of the small ribosomal RNA subunit (rRNA-SSU) gene, a single sequence of ~174 bp was obtained for all positive samples. The results of the molecular examination using qRT-PCR revealed that of 378 samples collected, 378 (100%) were positive. This complemented and confirmed the results of our microscopic examinations.

The use of qRT-PCR techniques in the specific detection of *Eimeria* sp. showed a fluorescence of the SYBER green dye which was most clearly seen through the formation of an amplification pattern for positive samples from cycle 22 onwards as shown in Figure 5.



A-Positive and negative control profile B-Patient profiles

Figure 5: Amplification graphs of the ITS1 region of *Eimeria* sp. in which the SYBER Green fluorescence represents positive samples above the threshold while control samples are below the threshold

IV. Discussion

The genus *Eimeria* comprises obligate intracellular protozoan parasites belonging to the phylum Apicomplexa. Members of this genus cause enteric disease in a wide range of vertebrate hosts, including fish, reptiles, birds and mammals. A total of 157 species of *Eimeria* that parasitise fish have been described; however, molecular information on these fish parasites is scarce.

In the present study, *Eimeria* oocysts were detected in 378 of 816 (46.3%) gastrointestinal tracts of *Mugil cephalus* in the eastern coast of Algeria. Measurements of sporulated oocysts, sporocysts and other morphological characteristics identified the oocysts as Eimeria sp. By molecular analysis of the small ribosomal RNA subunit gene (rRNA-SSU), by quantitative PCR all direct positive samples came back positive with different Ct's from 22. This confirmed the presence of *Eimeria* sp and complemented the direct examination.

Coccidia of the genus *Eimeria* Schneider, 1875 produce tetrasporidoocysts and dizoicsporocysts. The sporocysts have a Stieda body and sometimes a Stiedasubbody at one end (Lom and Dykovà, 1992). The coccidia described here has these characteristics.

Several studies have used the PCR technique targeting different regions of the *Eimeria* genome, such as the 5S rRNA (the small rRNA subunit (Mushattat and Sukayna (2013), Ogedengbe et al, 2011), the sporozoite antigen gene EASZ240/160 (Qvarnstrom et al., 2005) and the genomic regions ITS-1 (Long and Reid, 1982, Williams, 1998, Lew et al., 2003) and ITS-2 (Lien et al., 2007; Shirley et al., 2005). As the ITS regions are less conserved than the rRNA genes, the wide variation in this region of the DNA sequence between *Eimeria* species makes primer design straightforward and reduces the risk of cross-reactions between different species (Morris and Gasser, 2006). The REAL-TIME test has been shown to be directly comparable in sensitivity and robustness, capable of detecting 10 parasite genomes but not a single one, without being affected by the presence of DNA derived from the host or other species tested (Kirs and Smith, 2007). Each sporulated oocyst contains eight eimerial genomes, suggesting that the DNA equivalent of a single oocyst will be consistently detectable given normal experimental replication (between one and 10 genomes detected per reaction). Mature intracellular stages represent in the order of 10 to 100 eimerial genomes (depending on species and stage (Johnston et al., 2001). This suggests that even a fraction of one can be counted (Damer et al., 2008).

V. Conclusion

This study is the first to characterise *Eimeria* sp in *Mugil cephalus* from the Algerian east coast. Although routine tests such as macroscopic and microscopic diagnosis are important, they are unable to establish a qualitative diagnosis of the *Eimeria* causing the infection in *Mugil cephalus*. The use of molecular methods such as real-time PCR which is characterised by high accuracy, but these methods are expensive compared to routine methods. The use of specific primers for the diagnosis of the ITS1 region is important for the molecular detection of *Eimeria* species that are isolated from the intestines of mules.

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Memberships

Fellows/Associates of Science Frontier Research Council

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20. Think technically: Always think technically. If anything happens, search for its reasons, benefits, and demerits. Think and then print: When you go to print your paper, check that tables are not split, headings are not detached from their descriptions, and page sequence is maintained.

21. Adding unnecessary information: Do not add unnecessary information like "I have used MS Excel to draw graphs." Irrelevant and inappropriate material is superfluous. Foreign terminology and phrases are not apropos. One should never take a broad view. Analogy is like feathers on a snake. Use words properly, regardless of how others use them. Remove quotations. Puns are for kids, not grunt readers. Never oversimplify: When adding material to your research paper, never go for oversimplification; this will definitely irritate the evaluator. Be specific. Never use rhythmic redundancies. Contractions shouldn't be used in a research paper. Comparisons are as terrible as clichés. Give up ampersands, abbreviations, and so on. Remove commas that are not necessary. Parenthetical words should be between brackets or commas. Understatement is always the best way to put forward earth-shaking thoughts. Give a detailed literary review.

22. Report concluded results: Use concluded results. From raw data, filter the results, and then conclude your studies based on measurements and observations taken. An appropriate number of decimal places should be used. Parenthetical remarks are prohibited here. Proofread carefully at the final stage. At the end, give an outline to your arguments. Spot perspectives of further study of the subject. Justify your conclusion at the bottom sufficiently, which will probably include examples.

23. Upon conclusion: Once you have concluded your research, the next most important step is to present your findings. Presentation is extremely important as it is the definite medium though which your research is going to be in print for the rest of the crowd. Care should be taken to categorize your thoughts well and present them in a logical and neat manner. A good quality research paper format is essential because it serves to highlight your research paper and bring to light all necessary aspects of your research.

Informal Guidelines of Research Paper Writing

Key points to remember:

- Submit all work in its final form.
- Write your paper in the form which is presented in the guidelines using the template.
- Please note the criteria peer reviewers will use for grading the final paper.

Final points:

One purpose of organizing a research paper is to let people interpret your efforts selectively. The journal requires the following sections, submitted in the order listed, with each section starting on a new page:

The introduction: This will be compiled from reference matter and reflect the design processes or outline of basis that directed you to make a study. As you carry out the process of study, the method and process section will be constructed like that. The results segment will show related statistics in nearly sequential order and direct reviewers to similar intellectual paths throughout the data that you gathered to carry out your study.

The discussion section:

This will provide understanding of the data and projections as to the implications of the results. The use of good quality references throughout the paper will give the effort trustworthiness by representing an alertness to prior workings.

Writing a research paper is not an easy job, no matter how trouble-free the actual research or concept. Practice, excellent preparation, and controlled record-keeping are the only means to make straightforward progression.

General style:

Specific editorial column necessities for compliance of a manuscript will always take over from directions in these general guidelines.

To make a paper clear: Adhere to recommended page limits.

Mistakes to avoid:

- Insertion of a title at the foot of a page with subsequent text on the next page.
- Separating a table, chart, or figure—confine each to a single page.
- Submitting a manuscript with pages out of sequence.
- In every section of your document, use standard writing style, including articles ("a" and "the").
- Keep paying attention to the topic of the paper.
- Use paragraphs to split each significant point (excluding the abstract).
- Align the primary line of each section.
- Present your points in sound order.
- Use present tense to report well-accepted matters.
- Use past tense to describe specific results.
- Do not use familiar wording; don't address the reviewer directly. Don't use slang or superlatives.
- Avoid use of extra pictures—include only those figures essential to presenting results.

Title page:

Choose a revealing title. It should be short and include the name(s) and address(es) of all authors. It should not have acronyms or abbreviations or exceed two printed lines.

Abstract: This summary should be two hundred words or less. It should clearly and briefly explain the key findings reported in the manuscript and must have precise statistics. It should not have acronyms or abbreviations. It should be logical in itself. Do not cite references at this point.

An abstract is a brief, distinct paragraph summary of finished work or work in development. In a minute or less, a reviewer can be taught the foundation behind the study, common approaches to the problem, relevant results, and significant conclusions or new questions.

Write your summary when your paper is completed because how can you write the summary of anything which is not yet written? Wealth of terminology is very essential in abstract. Use comprehensive sentences, and do not sacrifice readability for brevity; you can maintain it succinctly by phrasing sentences so that they provide more than a lone rationale. The author can at this moment go straight to shortening the outcome. Sum up the study with the subsequent elements in any summary. Try to limit the initial two items to no more than one line each.

Reason for writing the article—theory, overall issue, purpose.

- Fundamental goal.
- To-the-point depiction of the research.
- Consequences, including definite statistics—if the consequences are quantitative in nature, account for this; results of any numerical analysis should be reported. Significant conclusions or questions that emerge from the research.

Approach:

- Single section and succinct.
- An outline of the job done is always written in past tense.
- Concentrate on shortening results—limit background information to a verdict or two.
- Exact spelling, clarity of sentences and phrases, and appropriate reporting of quantities (proper units, important statistics) are just as significant in an abstract as they are anywhere else.

Introduction:

The introduction should "introduce" the manuscript. The reviewer should be presented with sufficient background information to be capable of comprehending and calculating the purpose of your study without having to refer to other works. The basis for the study should be offered. Give the most important references, but avoid making a comprehensive appraisal of the topic. Describe the problem visibly. If the problem is not acknowledged in a logical, reasonable way, the reviewer will give no attention to your results. Speak in common terms about techniques used to explain the problem, if needed, but do not present any particulars about the protocols here.

The following approach can create a valuable beginning:

- Explain the value (significance) of the study.
- Defend the model—why did you employ this particular system or method? What is its compensation? Remark upon its appropriateness from an abstract point of view as well as pointing out sensible reasons for using it.
- Present a justification. State your particular theory(-ies) or aim(s), and describe the logic that led you to choose them.
- Briefly explain the study's tentative purpose and how it meets the declared objectives.

Approach:

Use past tense except for when referring to recognized facts. After all, the manuscript will be submitted after the entire job is done. Sort out your thoughts; manufacture one key point for every section. If you make the four points listed above, you will need at least four paragraphs. Present surrounding information only when it is necessary to support a situation. The reviewer does not desire to read everything you know about a topic. Shape the theory specifically—do not take a broad view.

As always, give awareness to spelling, simplicity, and correctness of sentences and phrases.

Procedures (methods and materials):

This part is supposed to be the easiest to carve if you have good skills. A soundly written procedures segment allows a capable scientist to replicate your results. Present precise information about your supplies. The suppliers and clarity of reagents can be helpful bits of information. Present methods in sequential order, but linked methodologies can be grouped as a segment. Be concise when relating the protocols. Attempt to give the least amount of information that would permit another capable scientist to replicate your outcome, but be cautious that vital information is integrated. The use of subheadings is suggested and ought to be synchronized with the results section.

When a technique is used that has been well-described in another section, mention the specific item describing the way, but draw the basic principle while stating the situation. The purpose is to show all particular resources and broad procedures so that another person may use some or all of the methods in one more study or referee the scientific value of your work. It is not to be a step-by-step report of the whole thing you did, nor is a methods section a set of orders.

Materials:

Materials may be reported in part of a section or else they may be recognized along with your measures.

Methods:

- Report the method and not the particulars of each process that engaged the same methodology.
- Describe the method entirely.
- To be succinct, present methods under headings dedicated to specific dealings or groups of measures.
- Simplify—detail how procedures were completed, not how they were performed on a particular day.
- If well-known procedures were used, account for the procedure by name, possibly with a reference, and that's all.

Approach:

It is embarrassing to use vigorous voice when documenting methods without using first person, which would focus the reviewer's interest on the researcher rather than the job. As a result, when writing up the methods, most authors use third person passive voice.

Use standard style in this and every other part of the paper—avoid familiar lists, and use full sentences.

What to keep away from:

- Resources and methods are not a set of information.
- Skip all descriptive information and surroundings—save it for the argument.
- Leave out information that is immaterial to a third party.

Results:

The principle of a results segment is to present and demonstrate your conclusion. Create this part as entirely objective details of the outcome, and save all understanding for the discussion.

The page length of this segment is set by the sum and types of data to be reported. Use statistics and tables, if suitable, to present consequences most efficiently.

You must clearly differentiate material which would usually be incorporated in a study editorial from any unprocessed data or additional appendix matter that would not be available. In fact, such matters should not be submitted at all except if requested by the instructor.

Content:

- Sum up your conclusions in text and demonstrate them, if suitable, with figures and tables.
- In the manuscript, explain each of your consequences, and point the reader to remarks that are most appropriate.
- Present a background, such as by describing the question that was addressed by creation of an exacting study.
- Explain results of control experiments and give remarks that are not accessible in a prescribed figure or table, if appropriate.
- Examine your data, then prepare the analyzed (transformed) data in the form of a figure (graph), table, or manuscript.

What to stay away from:

- Do not discuss or infer your outcome, report surrounding information, or try to explain anything.
- Do not include raw data or intermediate calculations in a research manuscript.
- Do not present similar data more than once.
- A manuscript should complement any figures or tables, not duplicate information.
- Never confuse figures with tables—there is a difference.

Approach:

As always, use past tense when you submit your results, and put the whole thing in a reasonable order.

Put figures and tables, appropriately numbered, in order at the end of the report.

If you desire, you may place your figures and tables properly within the text of your results section.

Figures and tables:

If you put figures and tables at the end of some details, make certain that they are visibly distinguished from any attached appendix materials, such as raw facts. Whatever the position, each table must be titled, numbered one after the other, and include a heading. All figures and tables must be divided from the text.

Discussion:

The discussion is expected to be the trickiest segment to write. A lot of papers submitted to the journal are discarded based on problems with the discussion. There is no rule for how long an argument should be.

Position your understanding of the outcome visibly to lead the reviewer through your conclusions, and then finish the paper with a summing up of the implications of the study. The purpose here is to offer an understanding of your results and support all of your conclusions, using facts from your research and generally accepted information, if suitable. The implication of results should be fully described.

Infer your data in the conversation in suitable depth. This means that when you clarify an observable fact, you must explain mechanisms that may account for the observation. If your results vary from your prospect, make clear why that may have happened. If your results agree, then explain the theory that the proof supported. It is never suitable to just state that the data approved the prospect, and let it drop at that. Make a decision as to whether each premise is supported or discarded or if you cannot make a conclusion with assurance. Do not just dismiss a study or part of a study as "uncertain."

Research papers are not acknowledged if the work is imperfect. Draw what conclusions you can based upon the results that you have, and take care of the study as a finished work.

- You may propose future guidelines, such as how an experiment might be personalized to accomplish a new idea.
- Give details of all of your remarks as much as possible, focusing on mechanisms.
- Make a decision as to whether the tentative design sufficiently addressed the theory and whether or not it was correctly restricted. Try to present substitute explanations if they are sensible alternatives.
- One piece of research will not counter an overall question, so maintain the large picture in mind. Where do you go next? The best studies unlock new avenues of study. What questions remain?
- Recommendations for detailed papers will offer supplementary suggestions.

Approach:

When you refer to information, differentiate data generated by your own studies from other available information. Present work done by specific persons (including you) in past tense.

Describe generally acknowledged facts and main beliefs in present tense.

The Administration Rules

Administration Rules to Be Strictly Followed before Submitting Your Research Paper to Global Journals Inc.

Please read the following rules and regulations carefully before submitting your research paper to Global Journals Inc. to avoid rejection.

Segment draft and final research paper: You have to strictly follow the template of a research paper, failing which your paper may get rejected. You are expected to write each part of the paper wholly on your own. The peer reviewers need to identify your own perspective of the concepts in your own terms. Please do not extract straight from any other source, and do not rephrase someone else's analysis. Do not allow anyone else to proofread your manuscript.

Written material: You may discuss this with your guides and key sources. Do not copy anyone else's paper, even if this is only imitation, otherwise it will be rejected on the grounds of plagiarism, which is illegal. Various methods to avoid plagiarism are strictly applied by us to every paper, and, if found guilty, you may be blacklisted, which could affect your career adversely. To guard yourself and others from possible illegal use, please do not permit anyone to use or even read your paper and file.

Criterion for Grading a Research Paper (Compilation) by Global Journals

Please note that following table is only a Grading of "Paper Compilation" and not on "Performed/Stated Research" whose grading solely depends on Individual Assigned Peer Reviewer and Editorial Board Member. These can be available only on request and after decision of Paper. This report will be the property of Global Journals.

Topics	Grades		
	A-B	**C-D**	**E-F**
Abstract	Clear and concise with appropriate content, Correct format. 200 words or below	Unclear summary and no specific data, Incorrect form Above 200 words	No specific data with ambiguous information Above 250 words
Introduction	Containing all background details with clear goal and appropriate details, flow specification, no grammar and spelling mistake, well organized sentence and paragraph, reference cited	Unclear and confusing data, appropriate format, grammar and spelling errors with unorganized matter	Out of place depth and content, hazy format
Methods and Procedures	Clear and to the point with well arranged paragraph, precision and accuracy of facts and figures, well organized subheads	Difficult to comprehend with embarrassed text, too much explanation but completed	Incorrect and unorganized structure with hazy meaning
Result	Well organized, Clear and specific, Correct units with precision, correct data, well structuring of paragraph, no grammar and spelling mistake	Complete and embarrassed text, difficult to comprehend	Irregular format with wrong facts and figures
Discussion	Well organized, meaningful specification, sound conclusion, logical and concise explanation, highly structured paragraph reference cited	Wordy, unclear conclusion, spurious	Conclusion is not cited, unorganized, difficult to comprehend
References	Complete and correct format, well organized	Beside the point, Incomplete	Wrong format and structuring

INDEX

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