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Discovering Thoughts, Inventing Future

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A Monte Carlo-based Evaluation of Fishery Management Strategies for Lizardfish (*Saurida undosquamis*) in the Gulf of Suez, Red Sea

By Aly Yousif

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The Biological Reference Points for *S. unodsquamis* are MSY= 1.4*1000ton, B/B_{MSY}= 0.629, Exploitation F/F_{MSY}= 1.29, Carrying Capacity k= 18.2*1000ton, The intrinsic growth rate of the fish population r=0.304 y^{-1}. Such information constructs the base to set up a reliable strategy to rebuild the stock and avoid the management side effects on the community of the fishermen.

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Keywords: stock status, biological reference points, Saurida undosquamis, CMSY++, Gulf of suez.

I. Introduction

Saurida undosquamis is the most commercially important fish dominating the other species from the bottom trawl fishery in the North West Red Sea. Landings of *S. undosquamis* represent the first catch category from the trawl fishery. The landings of the lizard fishes constitute about 30% of the total trawl catch in the Gulf of Suez. Economically, *S. undosquamis* comes in the second order after the large shrimps. Due to lack of scientific management, occasionally open access, unselective fishing methods and fishing in the spawning areas and nursery grounds, fisheries of the Gulf of Suez may experience all types of overfishing, as growth and recruitment overfishing. Seasonal fishing landings from bottom trawl, purse seine and artisanal fisheries of the Gulf suffer deep fluctuations and continuous degeneration. Consequently, the factual fishing season mostly has been reduced to barely six months, ending during March, instead of May, since the fishing operation costs more than profit (GAFRD, 2022). *S. undosquamis* covers all the Gulf zones starting from Suez Bay in the north to strait of Gobal in the south, coming with all tows (Yousif, 2003).

The major obstacle that have prevented assessing the fish stocks in the developing countries and regions, since long time ago is the absence of stock assessment methods suitable for use in data-sparse situations. In addition to, lack of expertise and scarcity of data have contributed in that situation (Palomares *et al*., 2018). The latest development of the simple computer intensive fish stock assessment methods reliant basically on time series of catch data made such defects easy to overcome.

Monte Carlo Catch-Maximum Sustainable Yield (CMSY++) method is the most recent developed by Froese *et al.*, (2017). CMSY++ can estimate fisheries reference points (MSY, F_{MSY}, B_{MSY}) besides relative stock size (B/B_{MSY}) and exploitation (F/F_{MSY}). The above essential reference points can be estimated using simple inputs as catch data, a prior for resilience or productivity (r), and broad priors for the ratio of biomass to unfished biomass (B/k) at the beginning, an intermediate year and the end of the time series. The advanced Bayesian state-space application of the Schaefer surplus production model BSM is included in the (CMSY++) model.

The stock reduction analysis (Kimura & Tagart, 1982; Kimura *et al*., 1984) motivated Martell and Froese (2013) to develop CMSY+ as a Monte-Carlo method. Froese *et al.*, (2017) updated CMSY+ in order to resolve some problems.

The CMSY+ method was first used in Egypt by Yousif to manage the population of *Siganus rivulatus* in Suez Bay, Gulf of Suez (Nafea *et al*., 2022). The main advantage of BSM compared to other implementations of surplus production models is the focus on informative priors and the acceptance of short and incomplete catch-per-unit-of-effort (CPUE) data to estimate biomass (B) and the current status of *Saurida unodsquamis* in the Gulf of Suez. Also, key fisheries reference points such as intrinsic rate of population increase (r), carrying capacity (k), maximum sustainable yield (MSY), and the terminal ratio B/B_{MSY} are estimated. Since a long time ago, it has attracted researchers to study its biological and fisheries parameters using commercial landings and constant parameter fisheries models (e.g., Shenouda, 1969, Sanders & Kedidi, 1984, Sanders & Morgan, 1989, El-Ganainy, 1992 & 2003, Ramadan, 1995, Amin *et al.*, 2007, and El-Etreby *et. al.*, 2013).

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II. Material and Method

a) Study Area

The Gulf of Suez (Fig., 1) is a semi enclosed shallow basin extends westerly from north of the Red Sea with another Gulf (of Aqaba) extends easterly, and Sinai Peninsula separates them. The Gulf of Suez prolongs from nearly Lat. 30^0 & Long. 32.5^0 to southerly Lat. 27.5^0 & Long. 34^0. It is approximately 400 km long and varies in width between 40 km and 80 km. Depth varies between 13m in the far north to 77m in the far south (Yousif, 2003). The surface water temperature in the northwestern part of the Gulf vary from 18℃ in winter to 28℃ in summer (Pears, 1969). It is characterized by high salinity that exceeds 42% in the northern third of the Gulf and drops below 40.4% near the mouth (Abd El-Mongy and El-Moselhy 2015). It is one of the productive areas in the Red Sea where four main fishing ports are found (El-Salakhana, Attaka, Ras-Gharib, and El-Tour harbor). Bottom trawling, purse seine, long line and artisanal gears are used in the Gulf (El-Ganainy et al 2018).

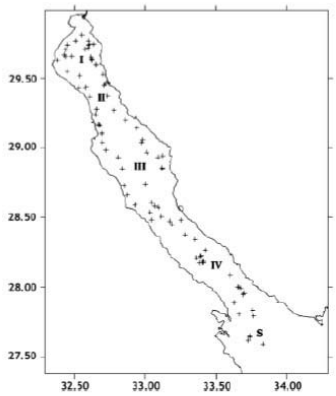

Figure 1: Gulf of Suez, with the distribution of *Saurida undosquamis*, (after Yousif, 2003)

b) Catch Data

Forty-two years (1980–2021) of *S. unodsquamis* comprising annual bottom trawl landings from the Gulf of Suez were used in this study. The series of catch data were taken from the General Authority of the Fisheries Resource, Office in Suez (GFRAD).

The total catch of lizardfish was calculated in tons (t). The estimated average catch of *S. unodsquamis* was about 1115.6t, while the minimum catch was 133.4t in 1991, and the maximum was 2968.7t reported in 1997.

c) Model CMSY++

Estimation of BRPs from catch and resilience data of *S. unodsquamis* was conducted using a Monte Carlo method-based Surplus Production Model SPMs called CMSY++. The CMSY++ can predict biomass using catch time series data.

This research used the CMSY++ approach to assess the Biological Reference Points (BRPs) MSY, B/B_{MSY}, F/F_{MSY}, k (carrying capacity), r (intrinsic growth rate of the fish population) related to *S. unodsquamis*.

$$B_{(t+1)} = B_t + r\,(1 - B_t/k\,)B_t - C_t \qquad (1)$$

The biomass exploited in (t + 1) year is $B_{(t+1)}$, existing biomass is B_t, and catch in t year is C_t. Equation (2) is used when stock sizes are severely depleted, and biomass falls below 1/4 k.

$$B_{(t+1)} = B_t + 4\,(B_t/k)\,r\,(1 - B_t/k\,)B_t - C_t \mid B_t/k < 0.25 \qquad (2)$$

The Fish Baseresilience score for *S. unodsquamis* is "medium" so the prior range for r is 0.2–0.8 used as the input parameter in the CMSY++ (Table, 1). The prior range of k is determined using three assumptions: the unexploited stock size (k) > largest catch in the time series, the maximum sustainable catch (F_{MSY}) is productivity-dependent, and the maximum catch represents a more significant fraction of k in significantly depleted stocks than in lightly depleted stocks. By default, and based on the anticipated degree of depletion, probable biomass ranges (Table, 1) provide prior estimations of relative biomass at the beginning and end of time series data.

The technique of the CMSY+ method, since 2017 (Froese *et al*., 2017) has been continually updated and developed and is currently accessible as CMSY++ (Froese *et al*., 2021).

Table 1: Distributions of the priors for CMSY++ used for *S. unodsquamis*

Input Parameters	Ranges of the Values
Prior initial relative biomass	0.2–0.6
Prior intermediate relative biomass	0.1 - 0.391 in a year (2014) default
Prior final relative biomass	0.128 - 0.451 default
Prior range for r	0.2 - 0.8 expert
Prior range for k*1000	7.13 - 43.4
Prior MSY*1000	1.75
B/k prior used for first year in BSM and intermediate year and last year.	
Prior range of q	0.24 - 5.83
Assumed effort creep	0.02 %.

III. Results

a) CMSY++ Derived Biological Reference Points (BRPs)

The CMSY++ method delivered important stock information and BRPs (Table, 2). The catch fit diagram (Fig., 1A) depicted a general gradual decrease and fluctuation from the year 1980 to 2021. The seasonal landings of *S. unodsquamis* from the Gulf of Suez, Red Sea was as follows: the highest catch (3.0*1000t) was observed in 1987, while the lowest catch (1.3*1000t) was reported in 1991.

Table 2: CMSY++ estimated Biological Reference Points BRPs (k, r & MSY) of *S. unodsquamis* in the Gulf of Suez with 95% confidence intervals (CI), Bayesian Schaefer Model (BSM) using catch & CPUE

k (1000ton), 95%CI	r (year^{-1}), 95%CI	MSY (1000ton), 95%CI	B/k (2021), P. (2.5th -97.5th)	E;{F/(r/2)} (2021), P. (2.5th -97.5th)
18.2, (11.9-30.7)	0.304, (0.185–0.468)	1.4, (1.17–1.73)	0.315, (0.21-0.427)	1.29 (0.779-2.44)

 # Where k is the carrying capacity of the fishery, (r) is the intrinsic rate of the population increase.
 # E: exploitation - # F: Fishing mortality - # P: percentiles.

b) CMSY++ Assessment

Figure (2) shows the CMSY++ assessment graphs;

1. The black curve in **A** shows the time series of catches and the blue curve shows the smoothed data with an indication of the highest and lowest catch in red, as used in the estimation of prior biomass by the default rules. Catch shows higher values during the first two-thirds of the eighteens and then gets down to lower fluctuated values to the end of the investigated catch time series.
2. Panel **B** shows the explored log *r-k* space and in dark grey the *r-k* pairs which were found by the model to be compatible with the catches and the prior information. The dotted rectangle indicates the range of the priors provided in the ID file. The point in the center of the blue cross is the most likely r-k pair predicted by CMSY and horizontal and vertical error bars approximate 95% confidence limits for *r* and *k,* respectively, which are again closer view in Panel **C**. Following BSM analysis, the red cross in panel **B** indicates the best *r-k* estimate of BSM. In panel **C**, the black dots are the viable *r-k* pairs found by BSM, with an indication of a red cross for the best estimate with 95% confidence limits.
3. The blue curve in **D** shows the median of the biomass trajectories estimated by CMSY. The median of the biomass trajectories generally behaves as the estimated catch data in **A.** The dotted lines indicate the 2.5th and 97.5th percentiles. Vertical blue lines indicate the prior biomass ranges. The red curves in panel **D** show the BSM predictions for relative biomass, the dots indicate the CPUE data scaled by BSM and corrected for effort creep, and the green line indicates the uncorrected CPUE.

The biomass trajectories estimated by both models CMSY and BSM fluctuate under 0.5 level of relative biomass. The estimated medians started around 0.5 level of relative biomass and decreased fast to reach about 0.2 in 1990 and then increased to 0.5 level for CMSY estimates, and 0.4 level for BSM estimates. Finally, estimates of both models dropped to near 0.2 level in 2015 and approximately flattened to the end of the studied period.

4. Panel **E** shows the medians of the exploitation (F/F_{MSY}) as a blue curve, with the dotted curves indicating 2.5th and 97.5th percentiles. The steep increase in the upper confidence limit in the last year results from catch relative to the lower confidence limit of biomass in panel **D**. The optimum fishing mortality is the fishing mortality yields the Maximum Sustainable Yield of the fishery, thus ($F=F_{MSY}$, i.e., $F/F_{MSY}=1$). The red curves in panel **E** show the BSM predictions for exploitation, with the dots showing catch per unit of effort CPUE, as scaled by BSM. The BSM predictions for exploitation (red curves) are nearer to the unity than the CMSY predictions (blue curves). Moreover, the medians reached near unity during the periods of 1995 to 2004 and 2014 to 2021.
5. Panel **F** shows the Schaefer equilibrium curve of catch/MSY relative to *B/k*, indented, pointed by the reddish arrow, at $B/k < 0.25$ to account for reduced recruitment at low stock sizes. The blue curve shows the predictions by CMSY, from the first year (square) to the last year (triangle). The red curve shows the BSM predictions for exploitation and relative stock size. The dots are showing the predicted catch per predicted biomass as scaled by BSM. Both predictions of CMSY and BSM models go in the same way parallel and near each other.

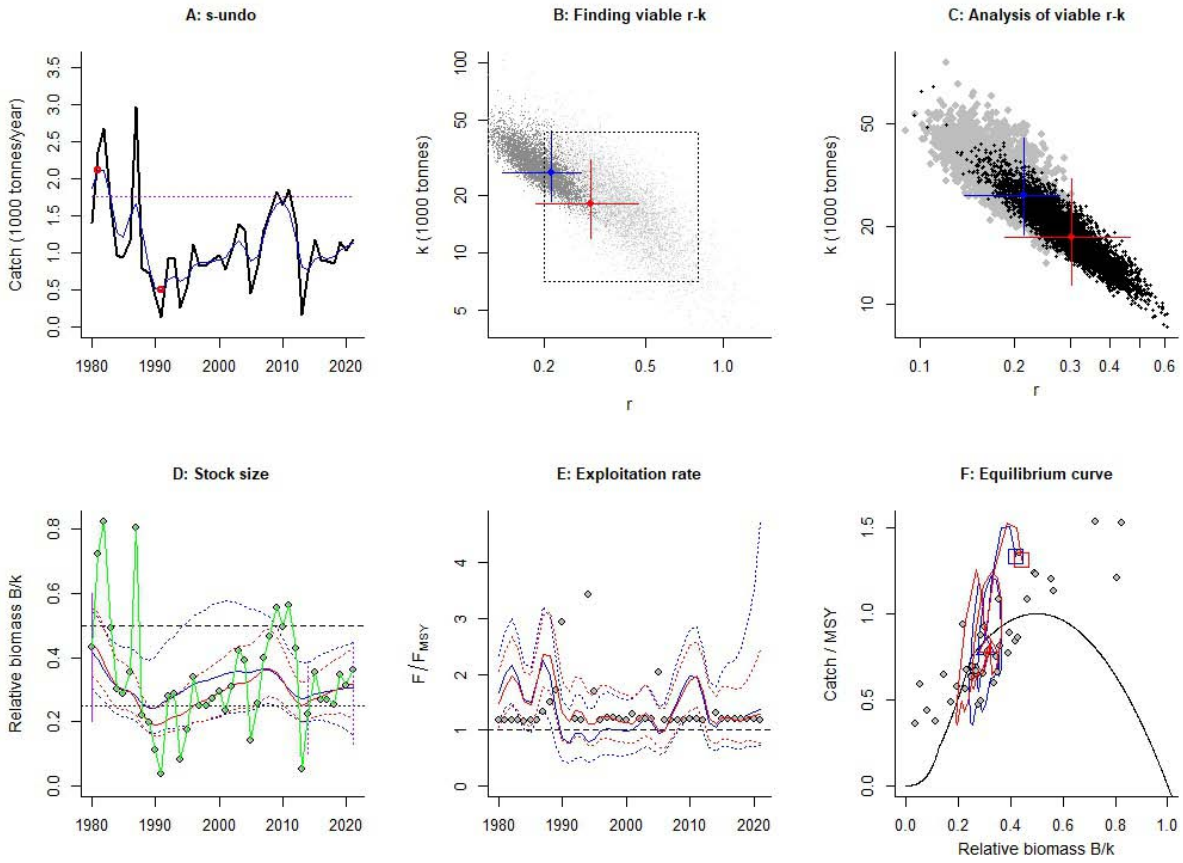

Figure 2: The CMSY and BSM assessment graphs for *S. unodsquamis* in the Gulf of Suez, Red Sea

c) Stock Status of S. unodsquamis Fishery

Table 3: Results from Bayesian Schaefer model (BSM) using catch & CPUE

q = 0.728,	lcl = 0.434, ucl = 1.12 (derived from catch and CPUE)
r = 0.304,	95% CL = 0.185 - 0.468,
k = 18.2 (*1000t),	95% CL = 11.9 - 30.7,
r-k log correlation = -0.919	
MSY = 1.4 (*1000t),	95% CL = 1.17 - 1.73
B (2021) = 0.315 k,	2.5th perc. = 0.21, 97.5th perc. = 0.427
Exploitation F/(r/2) (2021) = 1.29,	2.5th perc. = 0.779 , 97.5th perc. = 2.44

Figure (3) shows that; the upper left panel shows the catch relative to MSY, with an indication of 95% confidence limits in grey. Catch of the first 3 years exceeded the upper limit of the confidence interval of MSY, while in the next 5 years catch fluctuated around MSY. Then the catch dropped lower than the lower limit of the confidence interval of MSY. Catch increased again over the upper limit of the confidence interval of MSY during 2008-2011 and dropped once again under the lower limit of the confidence interval, and continued to the end of the time series. Most of the values of the catch of the studied period fell below the maximum sustainable yield MSY.

The upper right panel shows the development of predicted relative total biomass (B/B_{MSY}), with the grey area indicating uncertainty. The relative biomass of *S. unodsquamis* fluctuated between 1 and 0.5 in the time series except around the nineties it dropped under 0.5.

The lower left graph shows fishing pressure (F/F_{MSY}). Fishing pressure generally fluctuated over unity. The value of fishing pressure exceeded 2.25 in 1987 and 1988 and went just under unity during 2004 and 2005 and increased again over unity and fluctuated to the end of the time series.

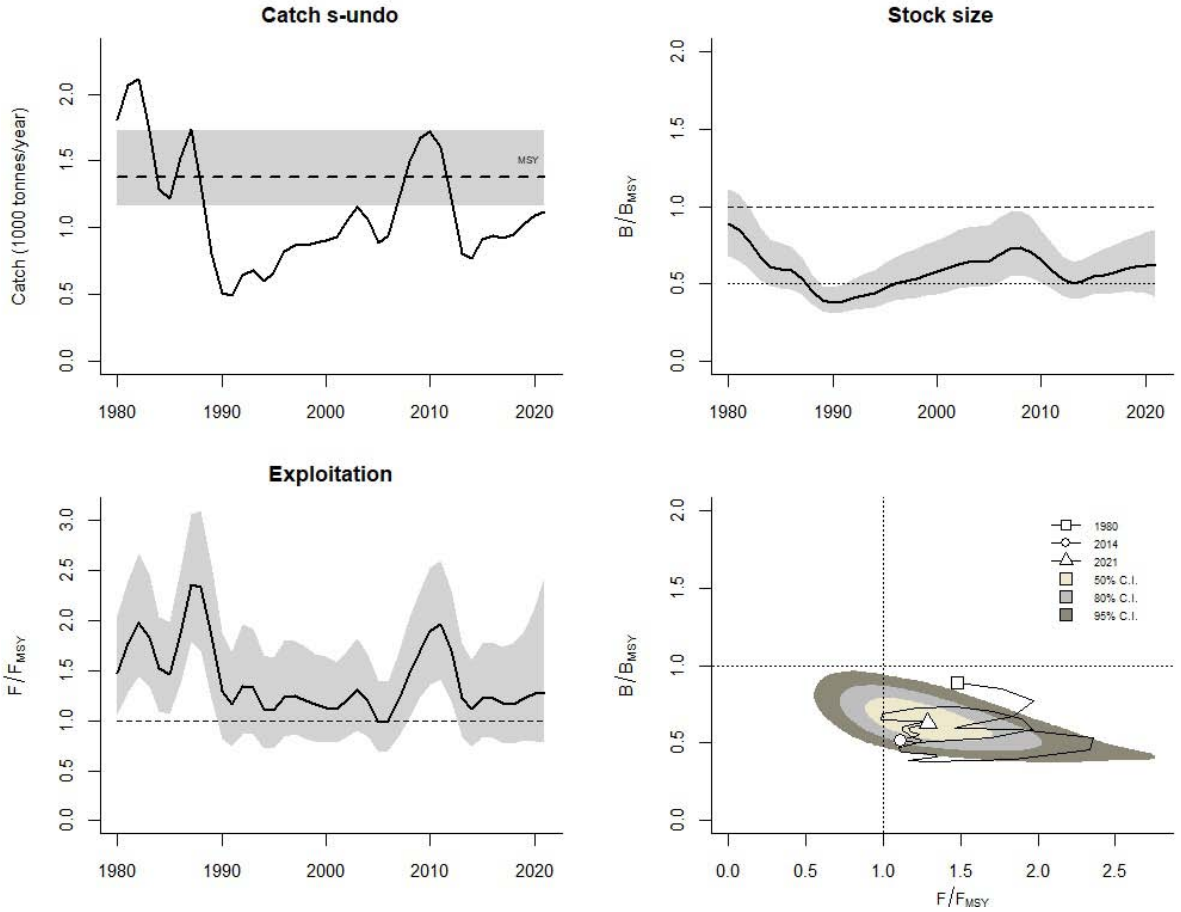

Figure 3: The stock status of *S. unodsquamis* in Suez Bay, Gulf of Suez

The lower-right panel shows the trajectory of relative stock size (B/B_{MSY}) as a function of fishing pressure (F/F_{MSY}). The "banana" shape around the assessment of the final year triangle indicates uncertainty with yellow for 50%, grey for 80%, and dark grey for 95% confidence levels. Most of the studied series, as most of the banana shape, fell in the fourth quarter of the graph which is of high fishing pressure and low relative biomass.

d) Prior and Posterior Distributions of Densities of BRPs for S. undosquamis

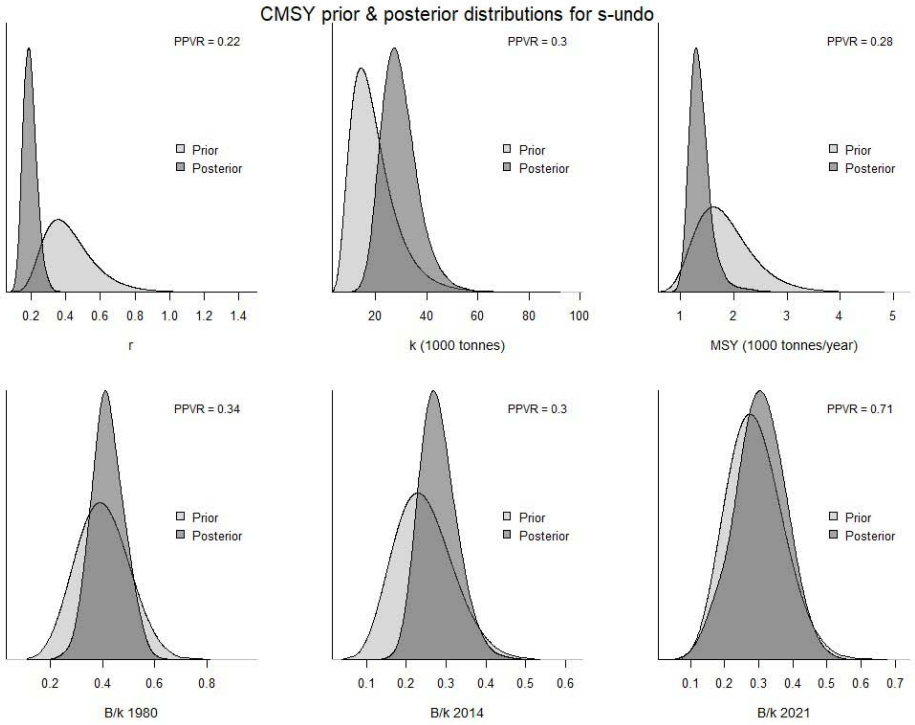

Figure 4: CMSY distributions of prior and posterior densities (same area under curves) for resilience or productivity (r), unexploited stock size (k), maximum sustainable yield (MSY), and relative stock size (B/k) at the beginning, the end, and an intermediate year of the available time series (1980-2021) of catch data, for *S. undosquamis* from Gulf of Suez, Red Sea

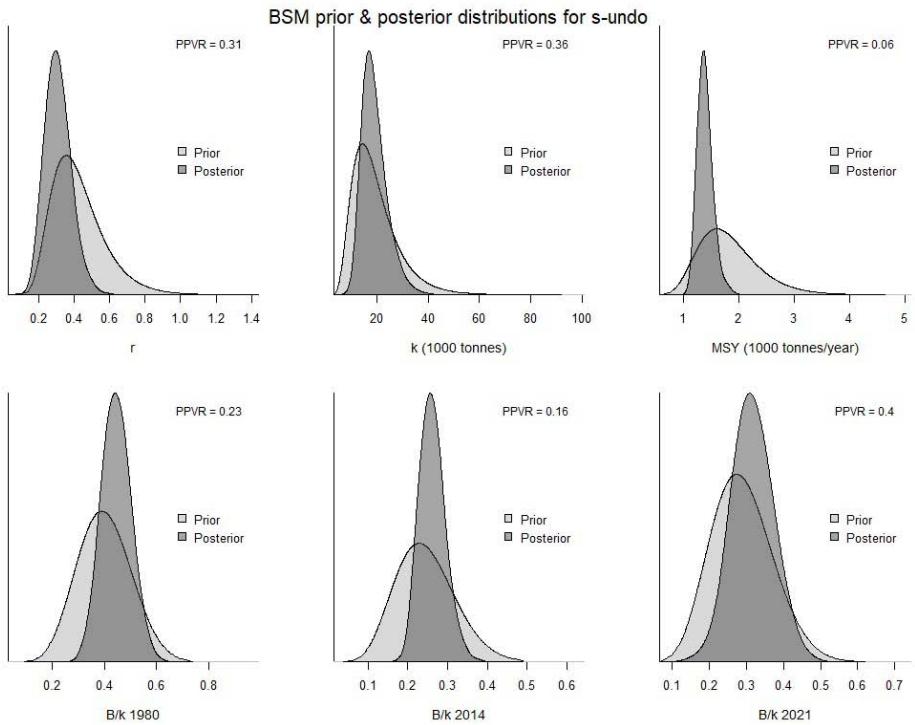

Figure 5: BSM distributions of prior and posterior densities (same area under curves) for resilience or productivity (r), unexploited stock size (k), maximum sustainable yield (MSY), and relative stock size (B/k) at the beginning, the end, and an intermediate year of the available time series (1980-2021) of catch data, for *S. undosquamis* from Gulf of Suez, Red Sea

In the logistic model of population growth, r and k are inversely correlated with k = 4 MSY/r and a slope of -1 in log-space. A random distribution of r-k pairs generated from that consideration is shown with blue dots in Figure (6). A random distribution of r-k pairs taking into account the empirical slope of -0.919 generated dots shown in purple. Orange dots show a distribution of r-k points derived from JAGS modeling based on the priors for r and k. The green dots show the posterior distribution of r-k points using the Bayesian modeling for *S. undosquamis* from the Gulf of Suez, Red Sea.

The dotted rectangle indicates the range of the priors provided in the ID file. The rectangle includes most of the randomly distributed Logistic r-k, Empirical r-k, JAGS r-k, and about the lower second half of the Posterior r-k.

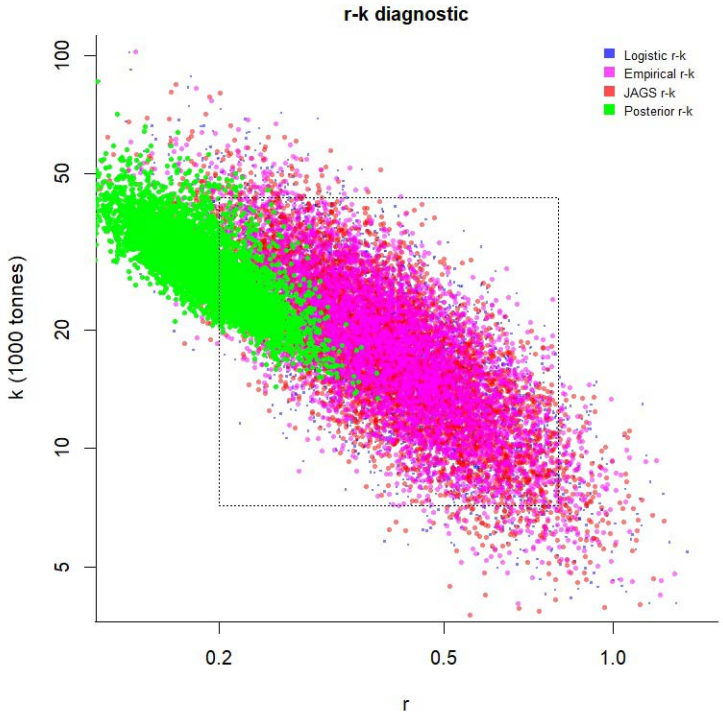

Figure 6: Diagnostic plot of different methods to generate prior distributions for r and k that take into account their negative correlation

e) Management of S. unodsquamis Fishery

Using model BSM analysis, the values of the estimated catch time series are nearly the same as the observed ones (Fig., 7). Regarding the catch per unit effort, 18 values of CPUE are out of the confidence interval of the fitted line. The process variation clarifies the proximity of the estimated and observed values of biomass along the time series. In addition, the figure does not show considerable autocorrelation of residuals.

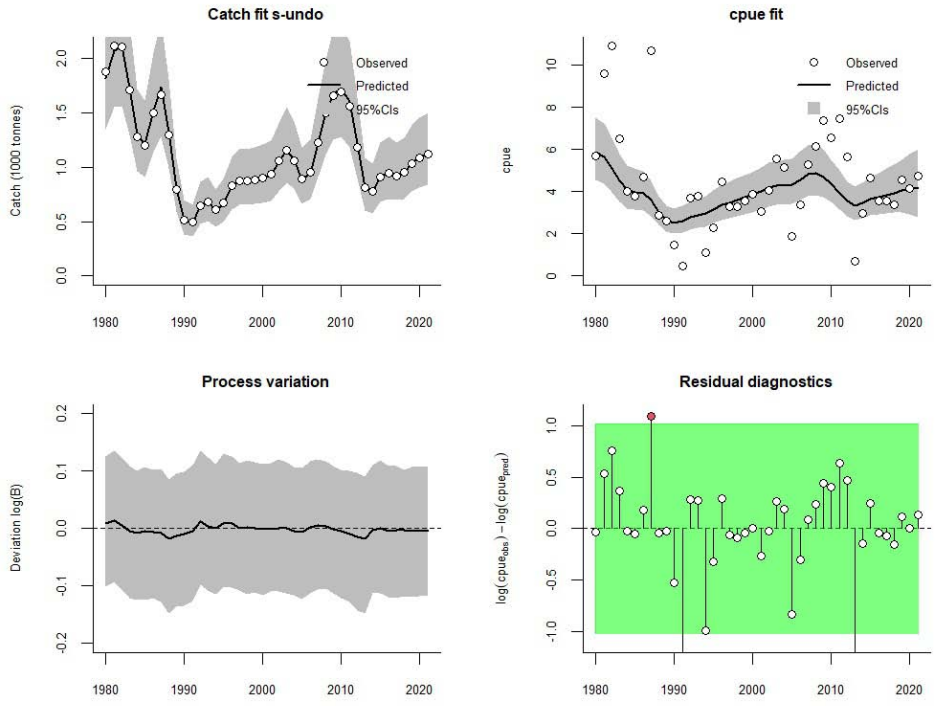

Figure 7: Analytical graph for BSM analysis of *S. unodsquamis*, showing the fit of the predicted to the observed catch, the fit of predicted to observed CPUE, the deviation from observed to predicted biomass, and an analysis of the log-CPUE residuals, with a green background where autocorrelation of residuals is deemed negligible

As long as B is greater than half of B_{MSY} then F_{MSY} equals half (r) as shown in the first line of the table (4). In addition, due to the value of the ratio, B/B_{MSY} being > 0.5, r and F_{MSY} are not linearly reduced.

Table 4: Management information of *S. unodsquamis* in the Gulf of Suez based on BSM

Parameter	P. Value	95% Confidence Intervals (CI) / Percentile
F_{MSY}	0.152,	0.093 - 0.234 (1)
F_{MSY}	0.152,	0.093 - 0.234 (2)
MSY	1.4 (*1000t),	1.17 - 1.73
B_{MSY}	9.1 (*1000t),	5.96 - 15.4
B (2021)	5.7 (*1000t),	2.5th perc = 3.35 , 97.5 perc = 10.2
B/B_{MSY} (2021)	0.629,	2.5th perc = 0.419 , 97.5 perc = 0.853
F (2021)	0.194,	2.5th perc = 0.104 , 97.5 perc = 0.361
Exploitation F/F_{MSY}	1.29,	2.5th perc = 0.779 , 97.5 perc = 2.44

(1) (if $B > 1/2\ B_{MSY}$ then $F_{MSY} = 0.5$ r)
(2) (r and F_{MSY} are linearly reduced if $B < 1/2\ B_{MSY}$)
(P. = Parameter),
*[MSY, B_{MSY} and $B_{(2021)}$]*1000t*

Kobe phase plot (Fig. 7) was used to depict the current stock status and exploitation rate relative to target reference points (TRPs) such as F_{MSY}. The Kobe plot is characterized by four colored quadrants (orange, red, yellow, and green) for F/F_{MSY} on B/B_{MSY}. The orange plot denotes the healthy stock that will be depleted by overfishing. The red color plot indicates the overfished and overfishing status in which the biomass cannot produce the MSY. The yellow color plot indicates very low biomass, but the stock has a chance to recover in a sustainable state if fishing pressure is reduced. The green plot is the management targeted area, signifying healthy stock status and sustainable fishing to produce the MSY. The legend in the plot's upper right corner indicates the probability of the stock falling into one of the colored areas over the last year. The probability of the stock falling into the red area is 83.9% and 16.1% is the probability of the stock falling into the yellow area. The probability of the stock subsiding into the green area and falling into the orange area is 0.0%. All yearly cases of stock of *S. undosquamis* in the Gulf of Suez had fallen in the red quadrate. Except two cases of the

stock of *S.undosquamis* had fallen on the border between red and yellow quadrates.

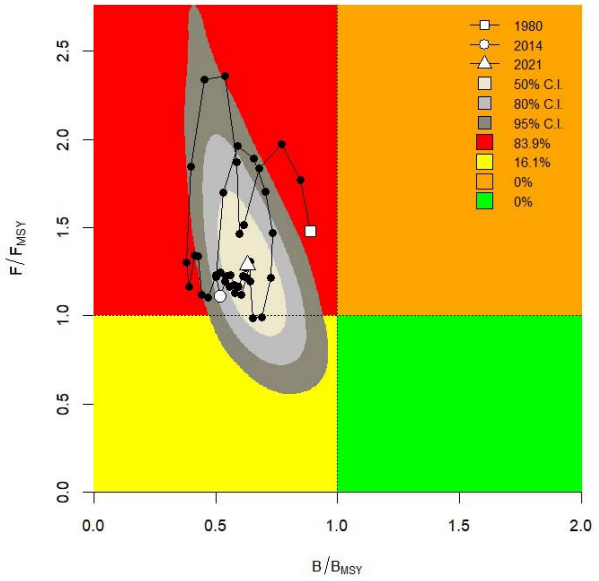

Figure 7: Kobe plot illustrating the concurrent movement of exploitation (F/F_{MSY}) and the relative biomass (B/B_{MSY}) of *S. undosquamis* in the Gulf of Suez

IV. Discussion

Many fishery researchers dealt scientifically with the fish species *Saurida unodsquamis*, using the classical methods to estimate the species' biological reference parameters as; the growth coefficient (k), the theoretical maximum length (L_∞), and the theoretical parameter (t_0) pertained to the growth model of von Bertalanffy. For instance, in the territorial waters of Egypt in the Red Sea some researchers investigated *S. unodsquamis*. Through FAO project, Sanders and Kedidi (1984) studied the population of *S. unodsquamis* in the Gulf of Suez using reasonable monthly sampling for landings from the commercial boats, not scientific marine surveys. They used Thompson and Bell model (1934), and stated a maximum sustainable yield (MSY) of 2500 tons and recommended a decrease in the fishing effort by 10%. In addition, Sanders and Morgan (1989) republished the above results. The Association of the Industrial Fishing Boat Owners has indirect authority to accept or refuse any recommendation in the fisheries committees, so they refused to apply FAO recommendations and others. They manage the fisheries unscientifically following the fishermen opinions.

El-Ganainy (2003) used Pella and Tomlinson time-series model (1969), reported MSY of 1331 tons; and recommended a decrease in the fishing effort by 33.3%. Moreover, she used the Fox model (1975) and estimated MSY as 1007 tons and a decrease in the fishing effort by 35.5%. Sampling methods and used models, particularly constant parameter models might cause the discrepancy in the resulted values. Shenouda (1969), El-Ganainy (1992), Ramadan (1995), Amin *et al.* (2007), and El-Etreby *et. al.,* (2013) studied some other biological parameters for *S. unodsquamis* in the Gulf of Suez.

Actually, these parameters are of individual growth not of population growth. Population growth follows two processes; one for the individual and another for the population. Therefore, in the CMSY++ method; the parameter of the intrinsic increase rate of a population (r) is an important indication to understand a fishery (Anderson *et al*., 2008; Cheung *et al*., 2010). Such parameter (r) includes the changes in the size of the individual along its age besides the changes in the number of recruitments in different years. The same corresponds to the different biological reference parameters of the CMSY++ method. Therefore, this method is mostly objective and the statistical procedure of estimating r-k may give it priority over other statistical methods, that used the size frequency of the fish species.

The statistical methods used for fish size frequency are common in tropical and subtropical regions whereas in temperate zones fishery scientists use hard structures as otoliths to age fish, which are more reliable and avoid the possible terrible uncertainty of the statistical methods. Aging fish using the hard structures gives more accurate results than using the size frequency but in the case of subtropical and tropical cases, the annulus is mostly unclear, and the hard structures, e.g. otolith, may contain chaotic lines and/or ruptures.

The catchability coefficient q (0.73) is relatively high (Table, 3). The catchability coefficient is identified as the proportion of the stock taken by one unit of fishing effort (Hoggarth *et al*., 2006). So, the proportion of the stock taken (q) is determined mainly by the unit of effort used. The intrinsic increase rate of a population (r) is an important indication to understand a fishery (Anderson *et al*., 2008; Cheung *et al*., 2010). The estimated (r) is 0.30 $year^{-1}$, revealing a medium increase rate of *S. unodsquamis* that is able to add above 30% biomass to the standing population per year. When r is 0.1, it is mentioned that population size can increase by 10% in a time interval (Hoggarth *et al*., 2006). The (r) value strongly correlates with fisheries resilience related to natural mortality (Froese & Pauly, 1980; Froese *et al*., 2017). The fisheries resilience value ranges are; 0.015–0.1, categorized as low resilience, 0.2–0.8 as medium resilience, and 0.6–1.5 as high-resilience fishery (Froese *et al*., 2017). This study found the population of *S. unodsquamis* as a "medium-resilience" fishery in the Gulf of Suez, Red Sea.

The value of the intrinsic rate of population growth (r), resulting from CMSY and BSM analyses may not be significantly different where the value of (r) from

CMSY analysis (0.214) lies in the 95% confidence range of (r) from BSM analysis (0.185 - 0.468). Results from CMSY and from BSM models are nearly the same.

The catch mostly dropped under the level of the MSY. Except for 7 years in the first of eighteens and 5 years around 2010, the rest of the studied period catch went down MSY and sometimes deeply alerting biomass overfishing. Moreover, the population might suffer from recruitment overfishing (Fig., 2F), which hinders the fishery for a long time to recuperate.

A common misconception of Bayesian analyses is that the priors determine the results. It is true that if grossly wrong priors are provided as input to CMSY, the results will be wrong. But that is true for any model provided with wrong data. If instead reasonable priors are provided, as Figures 4 & 5show, the priors (light grey) inform the results, with posterior understanding (dark grey) of the stock clearly improved compared to prior perceptions (Froese, *et al.*, 2019). The lower the prior-posterior variance ratio (PPVR), the more the posterior knowledge is improved relative to prior knowledge. Both CMSY and BSM produced the same areas under curves in the graph of distributions of prior and posterior densities. Maximum sustainable yield MSY is 1.4*1000t per year, which is higher than last year's catch (1.2*1000t per year in 2021), indicating the poverty of fish biomass to yield that value of MSY. Therefore, the catch should be increased by more than 14% to attain the maximum sustainable yield MSY. Moreover, the fishery of *S. unodsquamis* in the Gulf of Suez was suffering from overfishing through the time 2012 to 2021.

CMSY++ method assesses whether F/F_{MSY} values and B/B_{MSY} values, both are approaching 1, to ensure safe fishing conditions and healthy stock in which biomass levels are enough to harvest the MSY and accordingly the biomass levels are enough for a sustainable state of *S. unodsquamis* in the Gulf of Suez. Biomass-producing MSY is 9.1*1000t while Biomass of 2021 is 5.7*1000t, therefore Biomass needs to be rebuilt by about 3.3*1000t. Relative Biomass (B/B_{MSY}) fluctuated under the level of unity along the studied time period and even under 0.5 around the nineteens and raised to 0.5 some years after 2010. Consequently, the Biomass of the population of *Saurida undosquamis* in the Gulf of Suez is suffering from overfishing.

Froese, *et al.*, (2021) analyzed observed r-k correlations of 240 stocks and got an empirical slope of -0.76. Analyzing the observed r-k correlation of the stock of *S. undosquamis* from the Gulf of Suez, Red Sea gave a slope of -0.919.

Most of the studied series, as most of the banana shape (Fig., 7), fell in the first quarter of the graph, which is of high fishing pressure and low relative biomass. Consequently, we might conclude that the stock of *S. undosquamis* in the Gulf of Suez has been suffering from overfishing for a long time. The representation of the Kobe phase plot and table (6) suggest that the current level of fishing pressure should be reduced by about 30% to ensure sustainability for the population of *S. unodsquamis* in the Gulf of Suez. Moreover, by decreasing the fishing pressure the ratio of B/B_{MSY} could be increased by about 37% to attain a healthy state.

a) Management of S. undosquamis Fishery in the Gulf of Suez

1. The fishery suffers from recruitment overfishing as shown in Figure (2, F), and the accompanied photo (Fig., 8), taken on 11 September 2019, for juveniles of *S. undosquamis* from the Gulf of Suez.

Figure 8: Photo of premature juveniles of *S. udosquamis* north Gulf of Suez, 11 Sep. 2019

2. The fishery of *S. undosquamis* in the Gulf of Suez has suffered from biomass overfishing and overexploitation since the early 1980s of the last century (Fig., 2D; Fig., 3; Table 2; Table, 3; Fig., 3; Table, 4; & Fig., 7).
3. Additionally, Yousif (2003) reported significant numbers of small fishes (9cm-17cm), shown in the accompanied (Fig., 9) of the area of the northern Gulf of Suez (El-Sukhnna Bay) and the length frequency of *S. undosquamis*. The area extends in the northern Gulf, from Latitudes 29°50\ (Ras Misalla) in the eastern Gulf - 29°49.5\ (Ras Adabiya) in the western Gulf to Latitudes 29°27\ (Ras Matarma) - 29°23\ (Ras Abu Daraq) southward, from Autumn 1998 commercial bottom trawl survey for the Gulf of Suez.
4. The size at which 50% of fishes are mature was 15.0 cm for males and 15.5 cm. for females, which indicates that all individuals over one year of age were sexually mature. Furthermore, the maximum average values of GSI were recorded for both males and females, showing intensive spawning in Spring (El-Etreby *et. al.,* 2013).

El-Ganainy (1992 & 2003) stated that the stock of lizard fishes in the Gulf of Suez has experienced heavy exploitation.

5. Yousif (2003) reported a fishing depth of 20m for lizard fish to ensure fish sizes bigger than 16cm and one spawning time to avoid recruitment overfishing.
6. The population of *S. unodsquamis* in the Gulf of Suez is able to add above 30% biomass to the standing population per year, at healthy stock status.

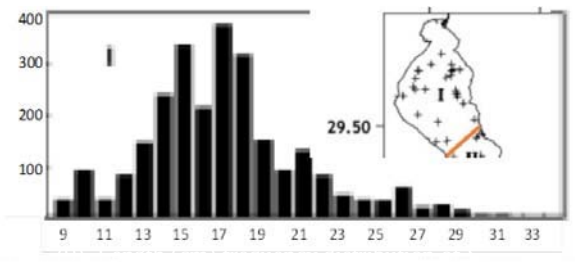

Figure 9: Length (cm) frequency distribution of *S. undosquamis* in the first area (I) of the Gulf of Suez during the trawl survey of Autumn 1998, Yousif (2003)

As a consequence, if we scientifically and carefully select two months to stop fishing we might recover the health of the stock status in three years.

Therefore, I strongly recommend stopping the fishing activities in the month of September to avoid recruitment and biomass overfishing; and starting the fishing season in October. In addition, I indeed recommend seizing the fishing season during April.

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Sustract Effect in the Development on Tubetes of *Sporobolus Virginicus* (L.) Kunth

By Danay Rodríguez Ramos, Daylon Fundora Caballero
& Javier Agustín González García

Abstract- The investigation had objective to reproduce on tubetes the species Sporobolus virginicus, as plantation material for dune ecosystems benefitted with sand dumpings. The variables evaluated were: sprouting percentage, height, number of leaves and number of sprouts. Three substrates (sand, sand+humus and worm humus) was evaluated. In the substrate with more sprouting percentage was evaluated the position of the rhizome segment (horizontal, vertical and in angle of 45°), the angle formed in the cut (90° and 45°), the viability at 24 o'clock, 48, 72 and 96 hours from its prospecting and the survival post-transplant. The rhizomes planted in humus did not sprout. The biggest sprouting percentage with 37% was obtained in the compound by the mixture of sand and humus. The horizontal position of the segments of rhizomes in the tubetes reached the biggest values for the evaluated variables and the number of final sprouts duplicated the vegetable established material initially. The position forming angle of 45° showed increment in the number of sprouts until the 64 days.

Keywords: *propagation, substrates, sandy coast vegetation.*

GJSFR-C Classification: *FOR Code: 069999*

Strictly as per the compliance and regulations of:

Sustract Effect in the Development on Tubetes of *Sporobolus Virginicus* (L.) Kunth

Efecto del sustrato en el desarrollo sobre tubetes de *Sporobolus virginicus* (L.) Kunth

Danay Rodríguez Ramos [α], Daylon Fundora Caballero [σ] & Javier Agustín González García [ρ]

Resumen- La investigación tuvo por objetivo reproducir sobre tubetes la especie *Sporobolus virginicus*, como material de plantación para ecosistemas dunares beneficiados con vertimientos de arena. Las variables evaluadas fueron: porcentaje de brotación, altura, número de hojas y número de brotes. Se evaluaron tres sustratos (arena, arena+humus de lombriz y humus de lombriz). En el sustrato con mayor porcentaje de brotación se evaluó la posición del segmento de rizoma (horizontal, vertical y en ángulo de 45°), el ángulo formado en el corte (90° y 45°), la viabilidad a las 24, 48, 72 y 96 horas desde su prospección y la supervivencia post-trasplante. Los rizomas plantados en humus no brotaron. El mayor porcentaje de brotación con 37% se obtuvo en el sustrato compuesto por la mezcla de arena y humus. La posición horizontal de los segmentos de rizomas en los tubetes alcanzó los mayores valores para las variables evaluadas y el número de brotes finales duplicó el material vegetal establecido inicialmente. La posición formando ángulo de 45° mostró incremento en el número de brotes hasta los 64 días. El ángulo en el corte del rizoma no influyó en las variables evaluadas. A las 96 horas de realizada la prospección, la viabilidad de los segmentos de rizomas se afectó. Es posible la reproducción de la especie en tubetes, con la utilización de humus de lombriz y arena, hasta las 72 horas de ser prospectado el material vegetal y con un 100% de supervivencia post-trasplante.

Palabras Clave: propagación, sustrato, vegetación de costa arenosa.

Abstract- The investigation had objective to reproduce on tubetes the species Sporobolus virginicus, as plantation material for dune ecosystems benefitted with sand dumpings. The variables evaluated were: sprouting percentage, height, number of leaves and number of sprouts. Three substrates (sand, sand+humus and worm humus) was evaluated. In the substrate with more sprouting percentage was evaluated the position of the rhizome segment (horizontal, vertical and in angle of 45°), the angle formed in the cut (90° and 45°), the viability at 24 o'clock, 48, 72 and 96 hours from its prospecting and the survival post-transplant. The rhizomes planted in humus did not sprout. The biggest sprouting percentage with 37% was obtained in the compound by the mixture of sand and humus. The horizontal position of the segments of rhizomes in the tubetes reached the biggest values for the evaluated variables and the number of final sprouts duplicated the vegetable established material initially. The position forming angle of 45° showed increment in the number of sprouts until the 64 days. The angle in the cut of the rhizome did not influenced in the evaluated variables. At the 96 hours of having carried out the prospecting, the viability of the segments of rhizomes was affected. It is possible the reproduction of this species in tubetes, with the use of worm humus and sand, until the 72 hours of being prospected the vegetable material and with 100% of survival post-transplant.

Keywords: propagation, substrates, sandy coast vegetation.

I. Introducción

El papel de las dunas litorales adquiere mayor importancia, ante los desafíos que imponen los efectos del cambio climático entre los que se destacan el incremento del nivel medio del mar y la mayor frecuencia de los eventos meteorológicos extremos. En Cuba el plan de estado para el enfrentamiento al cambio climático (Tarea Vida) incluye entre sus tareas conservar, mantener y recuperar integralmente las playas arenosas del archipiélago cubano, priorizando las urbanizadas de uso turístico. Para el cumplimiento de estas acciones es importante la reproducción de plantas del complejo de vegetación de costa arenosa y la optimización de sus protocolos de reproducción. Estas especies actúan como barrera ante la acción del viento y mareas, al conservarse, retardan los cambios en la dinámica de la costa, brindando así protección a las construcciones en caso de tormentas tropicales y huracanes; al mismo tiempo fijan la duna y permiten la acumulación de materia orgánica y, por ende, la formación de suelo (Espejel, 1992; Durán-García *et al*., 2010).

En este sentido, *Sporobolus virginicus* es tolerante a la sequía y a la sal, por lo que se convierte en una especie útil en la estabilización de las costas. Como otras gramíneas, posee semillas con baja viabilidad por lo que la reproducción asexual es la vía más práctica de propagación (Duvauchelle, 2007). En la actualidad no existen trabajos que aborden la influencia del sustrato humus de lombriz en combinación con la tecnología sobre tubetes para especies del complejo de vegetación de costa arenosa. Situación que implica la búsqueda de nuevos procedimientos que permitan

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obtener el mayor número de plántulas a un bajo costo, en el menor tiempo posible y de esa manera lograr una exitosa propagación vegetativa.

II. Materiales y Métodos

a) Ubicación del área de estudio

El estudio se desarrolló a partir del 12 de marzo de 2020, en un área de vivero experimental sobre tubetes en el Centro de Ingeniería Ambiental y Biodiversidad (CIBA), ubicado en la carretera a la localidad de Patria km 1½ (22°05´59´´N y 78°36´55´´W), municipio Morón, Ciego de Ávila.

b) Material vegetal utilizado

Se utilizaron rizomas estoloníferos colectados en Playa Larga, los que se fraccionaron a 3,5 cm de longitud y fueron colocados en agua libre de cloro hasta el momento de la plantación en el tubete.

c) Envases, sustrato y riego

Se utilizaron tubetes tipo: ALMTB 08145 RPE de factura brasileña con capacidad: 111 cm^3 (8 estrías y diámetro interno de 3.5 cm). Estos contenedores se agruparon en bandejas plásticas con capacidad de alojamiento para 56 tubetes. El llenado se realizó de forma manual con arena, humus de lombriz y la mezcla de ambos sustratos. Se colocaron algas (obtenidas en la limpieza de la arena en la playa) en el fondo del tubete para evitar la pérdida del sustrato.

El riego se realizó de forma manual directamente al sustrato; la frecuencia del mismo estuvo en correspondencia con el estadio de desarrollo de las plantas, siendo más frecuente e intenso durante los primeros 30 días (etapa de establecimiento). Posteriormente, se redujo de forma gradual la frecuencia para contribuir a disminuir el estrés hídrico posterior al trasplante.

Para determinar el efecto del sustrato sobre la brotación de los segmentos de *Sporobolus virginicus* se conformaron los siguientes tratamientos:

Tratamiento 1: arena
Tratamiento 2: 50% arena + 50% humus de lombriz
Tratamiento 3: humus de lombriz

El sustrato donde las plantas mostraron mejores porcentajes de brotación fue utilizado para evaluar el efecto de la posición del segmento en el tubete y se evaluaron tres formas de colocar en el tubete el segmento de rizoma: (horizontal, vertical y en ángulo de 45°). Para la evaluación de la influencia del ángulo en el corte del segmento se evaluaron dos ángulos 90° y 45°. Los cortes se realizaron con una tijera de poda de una mano. La viabilidad de los segmentos se determinó a las 24, 48, 72 y 96 horas a partir de su colecta como material de plantación.

Las variables evaluadas fueron las siguientes:

Porcentaje de segmentos brotados (%): del total de segmentos evaluados cuántos emitieron yemas foliares.

Altura total de la planta (cm.): se midió con una regla graduada, desde la superficie del sustrato hasta el ápice.

Número de hojas por planta: se determinó mediante conteo visual.

Número de brotes por segmento: cantidad de brotes existentes en cada segmento.

Segmentos viables: segmentos que permanecieron vivos y fueron capaces de brotar una vez plantados en los sutratos.

Brotación acumulada: número de brotes en correspondencia con los días de realizada la plantación hasta los 64 días desde la plantación.

Supervivencia post trasplante: macollas que sobrevivieron a los 30 días de realizado el trasplante.

Cada evaluación constituyó un experimento independiente.

La temperatura y la humedad relativa se determinó diariamente en horarios de las 10:00 AM y 3:00 PM. Para esta evaluación se utilizó un medidor meteorológico Kestrel 3000. Para la confección de los gráficos se utilizó la media entre las dos lecturas para ambas variables. Estas variables fueron registradas solo para la evaluación de la influencia de los sustratos en el desarrollo de la especie.

Cada experimento tuvo un diseño completamente aleatorizado. Para la evaluación de las diferentes variables se utilizaron 10 plantas por cada repetición, para un total de 30 plantas por tratamiento (tres repeticiones). Las evaluaciones se realizaron cada 7 días hasta el momento del trasplante (65 días).

d) Análisis estadístico

Los datos se registraron en el tabulador electrónico EXCEL y se procesaron mediante el programa estadístico IBM SPSS Statistic ver. 22. Se realizó un análisis de homogeneidad de varianza (Levenne) y un test de normalidad por Kolmogorov Smirnov. Se utilizó el procedimiento ANOVA de un factor para determinar diferencias significativas entre los tratamientos. En los casos en que el ANOVA fue significativo, la discriminación de medias se realizó mediante la prueba de Tukey. Para el análisis del ángulo en el corte de los segmentos se realizó una Prueba T para muestras independientes.

III. Resultados y Discusión

Al analizar el porcentaje de brotación de los segmentos estoloníferos según el tipo de sustrato utilizado (Figura 3.), estos comenzaron a emerger a partir de los 12 días de ser plantados y alcanzaron un porcentaje similar. Los segmentos plantados en humus de lombriz no brotaron.

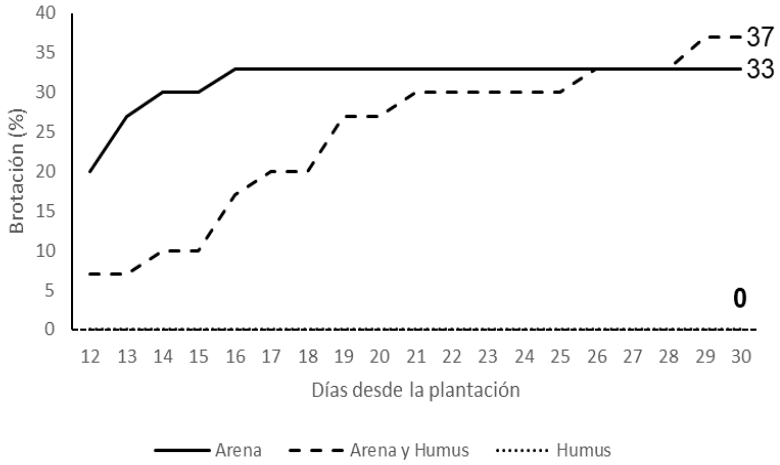

Figura 3: Porcentaje de brotación según sustratos en el desarrollo de *S. virginicus*

Según Avedaño *et al*., 2018 las condiciones del sustrato pueden determinar cambios en el desarrollo de *Sporobolus virginicus*. Asimismo, Reyes *et al*. (2015) refieren que el humus de lombriz facilita el desarrollo radical de las plantas, el crecimiento del tallo y hojas. Estos fenómenos que provocan, dan como resultados plantas más saludables y vigorosas que aumentan la producción y el rendimiento por área de cultivo.

Por su parte Sequeira *et al*., (2004) al evaluar diferentes porcentajes de humus de lombriz y suelo, como sustrato en la producción de posturas de *Capsicum anumm* L en bandejas para transplante, obtuvieron resultados similares y alegan que este efecto puede deberse a que el sustrato se compactaba demasiado, lo que impide el crecimiento del sistema radical y al mismo tiempo a la movilidad - disponibilidad de los nutrientes para las plantas. Domínguez *et al*., 2010 mencionan que los efectos del humus de lombriz podrían no reducirse a los meramente físicos - químicos y señalan la posible existencia de mecanismos biológicos de estimulación de crecimiento vegetal.

Al comparar las variables altura, número de hojas y brotación (Tabla 2) los mejores resultados se alcanzaron para la mezcla de sustratos de arena y humus.

Tabla 2: Influencia de los sustratos en la altura, número de hojas y brotación de *S. virginicus*

Tratamientos	Altura	Hojas	Número de Brotes
T1	4.583b	3.57a	0.73b
T2	6.897a	3.57a	2.60a
T3	0 c	0 b	0 b
Desviación típica	7.2666	4.286	2.429
Error típico	.7660	.452	.256

Letras distintas sobre las columnas indican diferencias significativas entre tratamientos (Tukey, $p \leq 0.05$).

Lo anterior pudiera estar atribuido a que cuando se utiliza el humus de lombriz como sustrato, se aportan colonias microbiales que participan en la transformación de todos los nutrientes minerales necesarios para la nutrición de la planta (Medina y Quezada, 2004).

Estos resultados son similares a los obtenidos por Liriano-González *et al*., 2017 al utilizar el humus de lombriz en la producción de plántulas de *Lycopersicon esculentum* Mill. y obtener para esta especie incrementos de altura y número de hojas, lo que se atribuyen a la gran riqueza y calidad biológica de este abono orgánico, que influye sobre propiedades biológicas tales como: mejora en los procesos energéticos y modificación de la actividad enzimática, favorece la síntesis de ácidos nucleicos y sirve de amortiguador al regular la disponibilidad de los nutrientes según las necesidades de las plantas. Si bien *S. virginicus* se adapta a suelos de baja fertilidad estos resultados coinciden con lo planteado por Leithead, Yarlett & Shiflett, 1976 quienes alegan que enmiendas con nutrientes son beneficiosas para el rápido establecimiento de esta especie.

El humus de lombriz roja californiana (*Eisenia foetida*) presenta un contenido de minerales muy alto, entre los que se encuentra el nitrógeno, fósforo y potasio, que libera lentamente, incrementando la disponibilidad de los elementos ya existentes en el suelo para ser absorbidos por la planta (Canellas & Rocha, 2004). Por su parte Sotelo & Téllez, 2007 obtuvieron resultados similares a los nuestros cuando los valores para las variables altura y número de hojas se vieron afectados al utilizar sustratos con 100 % de humus de lombriz para el cultivo del café.

Al evaluar como influyó la posición del segmento en el sustrato para la variable brotación, el mejor resultado para esta especie se alcanzó en los segmentos que fueron colocados de forma horizontal (Figura 4).

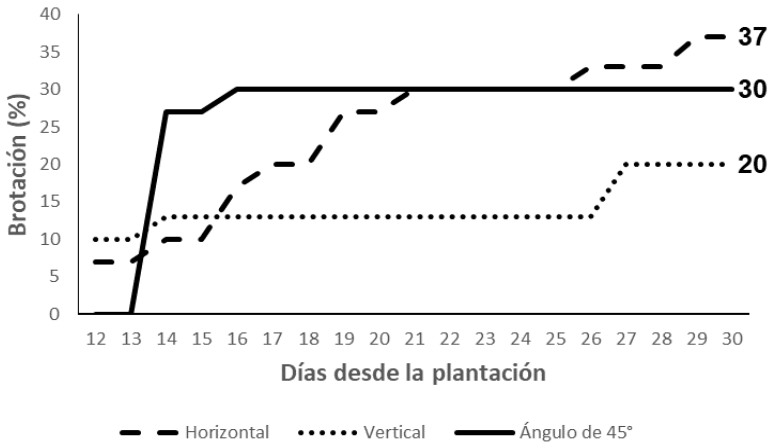

Figura 4: Porcentaje de brotación de *S. virginicus* según posición del segmento en el sustrato conformado por arena y humus

Estos resultados difirieron de los obtenidos por López *et al*., 1995, quienes al evaluar la posición de estacas para algunas especies como *Manihot esculenta* Crantz no obtuvieron diferencia entre la posición vertical, inclinada y horizontal. Sin embargo, estos autores refieren que estos efectos están determinados por la humedad y que cuando en los primeros 30 días existe un período seco para el cultivo, las estacas inclinadas o verticales manifestaron una mayor brotación.

Tabla 3: Posición del segmento en mezcla de arena + humus y su influencia en la altura, número de hojas y brotación de plántulas de *S. virginicus* en tubetes

Tratamientos/ Posición del segmento	Altura	Hojas	Número de Brotes
T1 (horizontal)	6.90a	3.37a	2.6a
T2 (vertical)	4.07c	2.23c	1.4c
T3 (ángulo 45º)	4.97b	2.73b	2.33b
Desviación típica	8.4114	4.424	3.330
Error típico	.8866	.466	.351

Letras distintas en los valores indican diferencias significativas entre tratamientos (Tukey, $p \leq 0.05$).

El comportamiento de las variables morfológicas altura, número de hojas y brotes estuvo relacionado con la forma de colocar el segmento de *Sporobolus* en el tubete (Tabla 3). Sin embargo, los brotes colocados de forma horizontal en su evaluación a partir de los 57 días incrementaron muy poco el número de brotes, mientras que los colocados de forma vertical y formando ángulo de 45 grados mostraron incremento en el número de brotes hasta los 64 días (Figura 5).

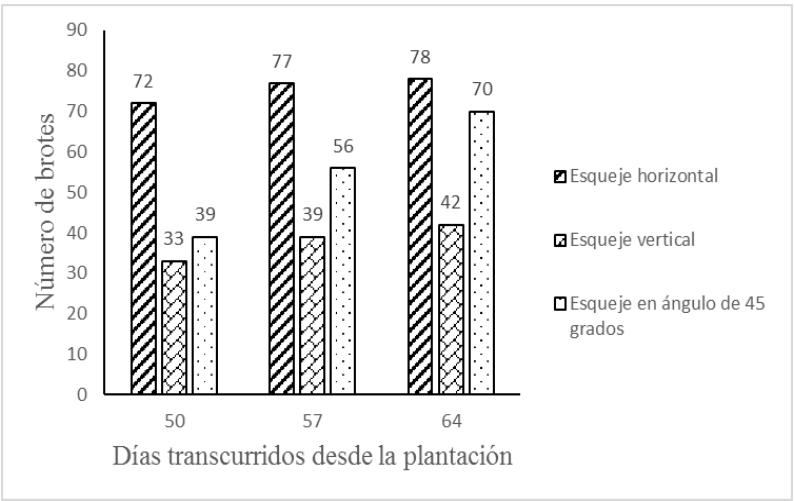

Figura 5: Número de brotes de *S. virginicus* según posición del segmento en mezcla de arena y humus

Sporobolus presentó en su desarrollo sobre tubetes un bajo porcentaje de brotación. Sin embargo, esta situación se revierte al alcanzar un 260 % de brotes finales con respecto a la cantidad de segmento establecidos inicialmente (30). Cuando se colocaron en posición horizontal se incrementaron al doble el número de brotes finales. Este efecto se produjo también en los segmentos colocados formando ángulo de 45°.

Además, cabe resaltar que la especie es autóctona y juega un importante papel en la conservación, por ser un componente esencial en la rehabilitación y recuperación de los ecosistemas primarios de Cuba. Ello se debe a que junto a otras especies, forma parte de la protección natural, al establecer una barrera que se opone a las invasiones (Ricardo y Herrera, 2017).

El ángulo formado al realizar el corte en el extremo del segmento del estolón no influyó en el comportamiento de la brotación (Figura 6). En este sentido Ponce *et al*., 2014 alegan que una las condiciones que garantiza el éxito de la propagación por estacas es la realización del corte transversal para que haya mayor área de producción de raíces. Por su parte Gómez, 2016 para el cultivo de caña de azúcar VARIEDAD CG98-10, propone que el corte de los tallos debe realizarse de forma vertical de manera que quede redondo.

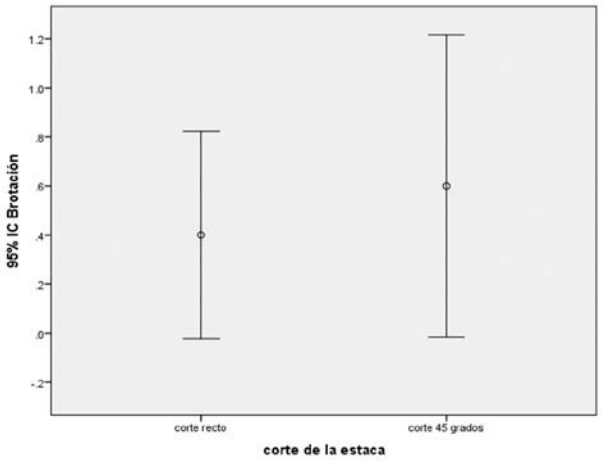

Figura 6: Correlación según el ángulo formado al realizar el corte del segmento de *S. virginicus*

Al analizar el comportamiento de la brotación se aprecia que cuando la plantación se realiza a partir de las 48 horas de colectado el material vegetal ocurre una disminución considerable en esta variable (Figura 7).

Figura 7: Porcentaje de brotación de segmentos en tubetes con arena y humus a las 24, 48, 72 y 96 h desde la prospección

El mantener los segmentos de *Sporobolus* hidratados hasta el momento de su plantación, permitió prolongar su conservación en caso de no ser posible su plantación de forma inmediata. Sin embargo, pasadas 48 horas el efecto beneficioso de la hidratación se modificó pudiendo estar atribuido este suceso a la ocurrencia del desorden fisiológico conocido como hiperhidricidad.

Según Haapala (2005), inmersiones constantes de las raíces en soluciones, pueden generar estos problemas los cuales dañan seriamente los tejidos. Resultados similares también fueron observados por Ramírez *et al*., 2013 en ensayos utilizando segmentos de tallo lateral de *Solanum phureja* donde hubo una mortalidad de segmentos superior al 50% durante este procedimiento.

Las mediciones de temperatura (Figura 8) y humedad relativa (Figura 9) realizadas *in situ* en el vivero arrojaron valores de 30 a 35 °C y de 58 a 69 % respectivamente durante los 58 días del muestreo. Según el sitio http://es.climate-data.org/america-del-norte/cuba/ciego-de-avila/cayo-coco-57554/ en Cayo Coco para el mes de agosto se registraron valores de temperatura mínima de 27.1°C y para la máxima valores

de 29.3°C, registrándose una temperatura media de 28.2°C. Para el mes de septiembre la temperatura mínima fue de 26.7°C y la máxima de 29°C para una media de 27.8. Estos valores difieren de los obtenidos en el microvivero, contradicción atribuida a la distancia existente entre el sitio de propagación y el sitio de establecimiento (más de 62 km) y al equipo de medición utilizado.

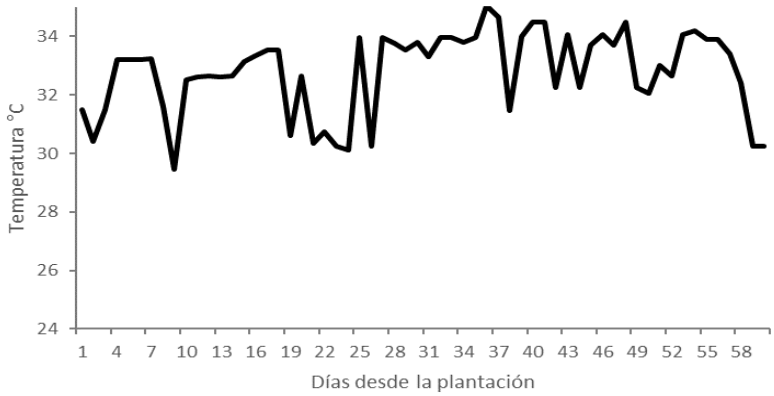

Figura 8: Temperatura durante el desarrollo de *Sporobolus virginicus* sobre tubetes

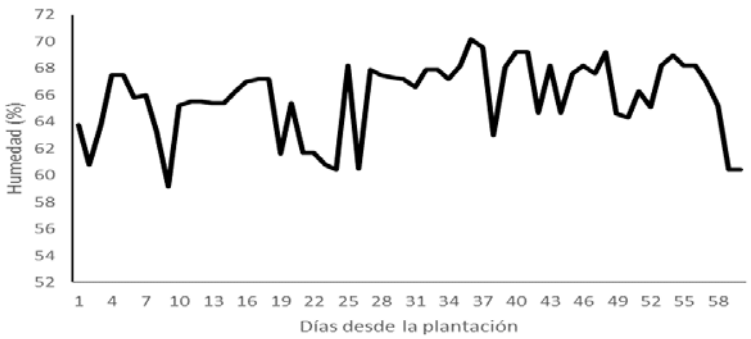

Figura 9: Humedad relativa durante el desarrollo de *Sporobolus virginicus* sobre tubetes

Muñoz *et al*., 2004 determinaron tres condiciones de temperatura para estudios de germinación: 25°C, 25-30°C y 25-35°C los cuales coinciden con los referidos en el estudio. Estos valores se ensayaron para simular las condiciones de temperaturas a que se pudieran ver sometidas los propágulos de *U. paniculata* en su hábitat natural. Estos autores confirmaron que la dormancia está impuesta por la temperatura y la mejor respuesta germinativa fue alcanzada al termoperíodo de 25-30^{0}C. Estos resultados demuestran la importancia de la evaluación de esta variable en la propagación de gramíneas. Lo que coincide con lo planteado por Villalobos *et al*., 2009 quien alega que las temperaturas tienen efecto sobre la germinación, la velocidad de crecimiento, transpiración, respiración, fotosíntesis, y absorción de agua y nutrientes.

El porcentaje de supervivencia de las plantas trasplantadas a la duna fue del 100% (Figura 10). Este resultado está relacionado con su amplia capacidad de distribución en disímiles ambientes costeros como playas, dunas arenosas, herbazales halófilos, praderas costeras o pantanos costeros (Medina *et a*l., 2008).

Figura 10: Establecimiento de *Sporobolus virginicus* en la zona frontal de la duna costera de Playa Las Coloradas, Cuba

Igualmente, este resultado está atribuido a las adaptaciones de la especie las cuales responden a las constantes fluctuaciones de los factores ambientales (Mitsch y Gosselink, 2007), especialmente la salinidad (Sharpe y Baldwin, 2009; Lonard *et al*., 2011) la cual se ve estimulada en los ambientes antes citados. Esto pudiera eliminar el exceso de sal a través de glándulas ubicadas en las hojas de las plantas y evitar la acumulación tóxica de sodio (Lonard *et al*., 2013). Una de las respuestas adaptativas de las plantas ante la variabilidad ambiental de los ecosistemas es la plasticidad fenotípica, que permite la tolerancia a variaciones ambientales, incluidas aquellas asociadas con cambios climáticos (Gratani, 2014).

IV. Conclusiones

Es posible la propagación en tubetes de *S. virginicus* para su establecimiento en ecosistemas dunares.

Para la propagación en vivero de esta especie el humus de lombriz en la mezcla del sustrato propicia un incremento considerable en la emisión de brotes, reponiendo el bajo porcentaje de brotación inicial.

La hidratación de los segmentos estoloníferos previo a la colocación en los tubetes incrementa la brotación.

La plantación de los segmentos debe realizarse ante de las 72 horas de realizada la prospección del material vegetal.

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Musk Melon Root Extract (*Cucumis Melo* L.) Inhibits Mitosis and Growth of Meristematic Cells in the *Allium Cepa* Essay through Chromosomal Abnormalities

By Ngnaniyyi Abdoul & Seino Richard Akwanjoh

University of Dschang

Abstract- *Background:* The Musk melon, *Cucumis melo*, is an essential herbaceous plant species in Cameroon for its nutritional and ethnomedicinal values. Thus, roots of this plant are not edible but are used in treating many local ailments. To date, there has been no report on the cytotoxicity and genotoxicity of the roots of Musk melon. Therefore, this study was to investigate the potential cytotoxic and genotoxic effects of aqueous extracts of the roots of *C. melo* using the *Allium cepa* assay. Allium bulbs were treated with six concentrations (0 µg/ml, five µg/ml, ten µg/ml, 20 µg/ml, 30 µg/ml, and 40 µg/ml) of aqueous extract of the roots of *C. melo* for 96 hours and distilled water was used as the control.

Results: The results showed significant dose-dependent ($p<0.05$) inhibition of sprouting and growth of roots as well as mitodepressive effects on cell division in *A. cepa* root tip cells treated with aqueous extract of the roots of *C. melo*.

Keywords: *Cucumis melo L., aqueous extract, mitotic index, chromosomal abnormalities, Allium cepa L., cancer cell model, mitodepressant.*

GJSFR-C Classification: *LCC Code: PZ7.L9575*

Strictly as per the compliance and regulations of:

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Results: The results showed significant dose-dependent ($p<0.05$) inhibition of sprouting and growth of roots as well as mitodepressive effects on cell division in *A. cepa* root tip cells treated with aqueous extract of the roots of *C. melo*. The results also revealed a significant increase in the dose-dependent frequency of chromosome aberrations (Anaphase bridges, laggards, disorientation, sticky metaphase, and binucleation) in the *A. cepa* root tip cells.

Conclusions: This study revealed that *C. melo* roots have a clastogenic effect on the root tip cells of *A. cepa*. The following research will test this extract on a few cancers induced in Wistar rats.

Keywords: Cucumis melo L., aqueous extract, mitotic index, chromosomal abnormalities, Allium cepa L., cancer cell model, mitodepressant.

I. Background

The Musk melon (*Cucumis melo* L.) is an annual herbaceous plant species native to intertropical Africa and belongs to the family Cucurbitaceae. The fruit, which is fleshy and edible, is gaining productive prominence in Cameroon, especially the North West, West and, Southwest Regions, because of its nutritional and ethnomedicinal values. Melons are naturally low in fat and sodium, have no cholesterol, and provide many essential nutrients such as potassium, in addition to being a rich source of beta-carotene and vitamin C [1, 2]. Chemical analysis has shown that *C. melo* is rich in moisture, carbohydrate, dietary fibers, minerals, carotenoids, folate and flavonoids such as B-carotene lending, xanthin cryptoxanthin, and phenolic compounds. Phenolic compounds are volatile. They are biosynthetically derived from fatty acids, carotenoids, amino acids and terpenes, while non-volatile constituents include constituents such as B-carotenes, flavonoids, carbohydrates, linoleic acid, acid a-linolenic, glycolipids, phospholipids, amino acids, phenolic compounds. glycosides [3, 4, 5, 6]. The total bioactive components of *C. melo* have important health values. Traditionally, *C. melo* is used to treat kidney stones, flatulence, leprosy, fever, jaundice, diabetes, obesity, cough, bronchitis, ascites, anemia, constipation and other abdominal disorders [7, 4, 8]. These total compounds have medicinal value, since the fruits and roots of C. melo are consumed for their therapeutic value [4]. In Cameroon, based on traditional knowledge that has accumulated over centuries, the leaves, the pulp of fruits, and the seeds of C. melo are eaten for their medicinal properties, while the roots are only used for medicinal purposes. The investigating the cytotoxicity and genotoxicity of the sources of *C. melo* will serve as a measure of safety for its continued use for medicinal purposes.

II. Methods

a) Origin of Plant Material and Preparation of Extracts

In this study, the aqueous extract of the roots of *Cucumis melo* was used as the test substance, while the root of *Allium cepa* was used as the test system. The roots of *C. melo* were obtained from a farm in Balessing in the West Region of Cameroon. At the same time, the Violet Galmi variety of onion (*A. cepa* of pure line L78 to eliminate individual variations) was provided to us by the Institute of Agricultural Research for Development (IARD) of Maroua (Far North Region, Cameroon). The roots of *C. melo* were washed in clean water, chopped into small pieces, and then dried in an oven at 48°C for two days. Therefore, dry and brittle roots were ground and used to prepare the aqueous solution using the method of [9]. The *Allium cepa* tests were held at the Applied Biology and Ecology Research Unit (URBEA) Laboratory according to the general description of [10].

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Thus, viable bulbs of *A. cepa* were placed in small transparent disposable cups with their basal ends dipping into various concentrations of the aqueous extract of *C. melo* roots (0 µg/ml, five µg/ml, ten µg/ml, 20 µg/ml, 30 µg/ml and 40 µg/ml and germinated at room temperature (25 - 30°C) for 96 hours. The control group of onion bulbs was dipped into distilled water (0 µg/ml). For each treatment, five bulbs were used per concentration of the test samples.

b) Macroscopic Examination

The onion bulbs were analysed for number of roots germinated and the length of the roots. Total number of roots grown per onion bulb was counted and recorded. To determine root length, ten cores were randomly taken from each treatment and measured with the help of a hardened stainless-steel digital caliper 150mm.

c) Cytogenetic Analysis

Meristematic root tips were obtained from the roots used for the measurement of lengths. The squashes method was prepared as suggested by [11] using 2% aceto-orcein. The chromosome smears thereof were used to determine the Mitotic Index (MI) and the presence of Chromosomal Aberrations (CA) induced by the aqueous extracts of the roots of *C. melo*. Five replicates were performed for each treatment, and scoring was done from the ten sources of each replicate. A minimum of 200 mitotic cells were counted from each slide. Mitotic indices (MI) were calculated for each treatment according to the formula: Number of dividing cells/Total number of cells observed×100. Chromosomal abnormalities were scored in the mitotic cells, and the results are shown in the tables and figures, while the most frequent chromosomal abnormalities are shown in photomicrographs.

d) Statistical Analysis

The data expressed as means ± standard deviation and one-way ANOVA and Tukey's post-test (HSD) were applied to evaluate the significances ($\alpha<0.05$) of between-treatment differences in percentage germination, root length, mitotic index, and frequency of chromosome and nuclear abnormalities. Python software version 3.2, and Pandas packages, were used under the MacOSX operating system.

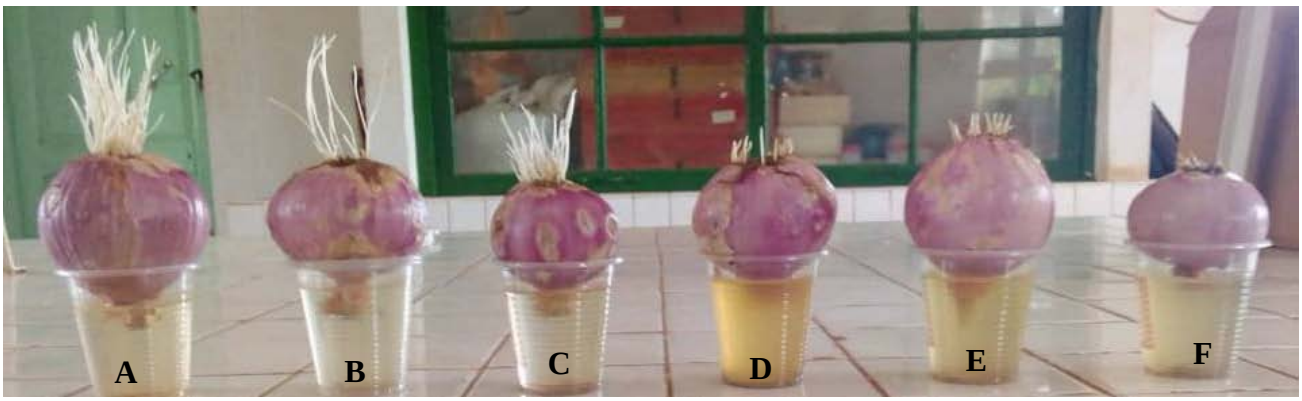
III. Results and Discussions

During the present study, the potential genotoxicity of the aqueous extract of the roots of *C. melo* was evaluated with the *A. cepa* essay. This cytotoxicity and genotoxicity were by analyzing two macroscopic parameters that included the number of roots sprouted and the lengths of the bases, as well as microscopic parameters such as the frequencies of MI and Cas in A. cepa cells plant growth, is an irreversible increase in size resulting from mitotic cell division and cell enlargement. The mitotic process also involves tight control and coordination of proliferative activity and growth in meristematic and differentiated tissues [12]. It is considered that, when the proliferative activity (mitosis) is disrupted, growth inhibition follows. In addition, disruption of mitosis could be the failure to respond appropriately to the stressor signal present in its environment [13]. During this study, the stressor signal was provided by the aqueous extract essential the roots of *C. melo* affected root growth by affecting mitosis in the meristem of the root tip. Therefore, follows that plant root growth inhibition is an indicator of macroscopic cytotoxicity [14, 15, 2].

The mean number of roots and mean lengths of the roots of onion bulbs grown in control (distilled water) and the different concentrations of aqueous extracts of *C. melo* are shown in Table 1. The results obtained for the further concentration of aqueous extracts (5 µg/ml, ten µg/ml, 20 µg/ml, 30 µg/ml, and 40 µg/ml) indicated that the roots grew less when treated with various concentrations of aqueous extracts of *C. melo* (102.10±4.18%, 94.44±5.00%, 91.14±2.02%, 59.02±1.04% and 35.11±0.40% respectively) than in the control group (113.15±6.10) (Table 1). Inhibition of root growth was, therefore, concentration-dependent and was statistically significant ($P < 0.05$) at the test concentrations. For all concentrations of the aqueous extract of *C. melo*, restricted root germination implied toxicity. Mitotic activity expressed here as MI was the first parameter used to evaluate the cytotoxicity of the aqueous extract of *C. melo*. Table 3 carries the MI levels for the control and each treatment concentration. As per the data in Table 3, the cytotoxicity levels were observed to increase with a decreased rate of MI. It is evident from this data (Table 3) that the aqueous extract of the roots of *C. melo* reduced MI compared to the control group. However, the decrease in MI with the extract concentration was extract slight and essential there was stagnation in the mitotic division once the cells entered the Metaphase stage. The extracts reducethe number in cells moving from Metaphase to Anaphase. The data also showed that MI was positively correlated to root length, which decreased with increasing concentration of the aqueous root extracts of *C. melo*. The mean number of dividing cells was lowest in the highest concentration (40 µg/ml) of the aqueous root extract of *C. melo*. Therefore, in this study, aqueous extract of the roots of *C. melo* decreased MI of A cepa root tip cells. This decrease was significant for all treatments concentrations (5 µg/ml, ten µg/ml, 20 µg/ml, 30 µg/ml, and 40 µg/ml) when compared to the control group. These results showed that all treatment concentrations of the aqueous extract of the roots of C. melo were toxic on A. cepa root tip cells. It is suggested here that the bioactive compounds contained in the roots of C. melo have mitodepressant properties, that's to say slow down cell division in the meristematic cells of the root end of

A. cepa however, without killing them. The resumption of cell proliferation is observed when these mitodepressed roots are immersed again in distilled water. It has been variously shown that the mitodepressive effects of some plant extracts have the ability to block the synthesis of DNA and nucleus proteins [16, 17, 18] thereby reducing MI and hence plant growth. In the case of *C. melo*, bioactive substances may not have allowed the formation of spindle proteins hence reducing the movement of cells from metaphase to anaphase.

This study was conducted using chromosome aberrations to detect the clastogenic activity of the aqueous extract of the roots of *C. melo* on the meristematic cells in the root tips of *A. cepa*. A summary of information on aberrant chromosomes in dividing cells of the root tips of *A. cepa* treated with different concentrations of the aqueous extract of the roots of *C. melo* is shown in Table 3. In the meristematic cells of *A. cepa* treated with different concentrations of aqueous extract of the roots of *C. melo*, Anaphase bridges, laggards and disorientation, nuclear vacuoles, sticky metaphase and double nuclei (Fig. 2) were found and their frequencies were higher than in the control group. These chromosomal abnormalities were used to quantitatively and qualitatively evaluate the genotoxic potentials of the aqueous extract of the roots of *C. melo*. Chromosome abnormalities such as recorded in this study probably occurred due to lesions in DNA as well as chromosomal and spindle proteins that cause genetic damage [19]. The genetic damage brings about drastic changes in chromatin, spindle apparatus, and centromere thus preventing alignment at the metaphase plate, and abnormal spindle orientation. This has been shown to occur due to altered quality and quantity of kinetochore heterochromatin [20, 21]. Total chromosome aberration (CA) frequencies in the treated groups were found to be higher than in the control group, and all the differences were statistically significant ($p<0.05$). The total percentage of CAs significantly increased with the concentration of extract. Therefore, CAs were significantly dose-dependent. CA was not found in the control group. Although The frequencies of aberrant chromosomes increased after treatment, a dramatic increase was recorded with the highest concentration of the aqueous extracts of the root of *C. melo* (Table 3). The results of this study revealed that aqueous root extracts of C. melo had a clastogenic effect on the root tip cells of *A. cepa*, since all concentrations tested induced multiple chromosomal abnormalities.



A=0 µg/ml (control); B=5 µg/ml; C=10 µg/ml; D=20 µg/ml; E= 30 µg /ml; F=40 µg/ml.

Figure 1: Examples of series of onions (*A. cepa*) cultivated for 96 h in different concentrations of the aqueous extracts of *Cucumis melo*

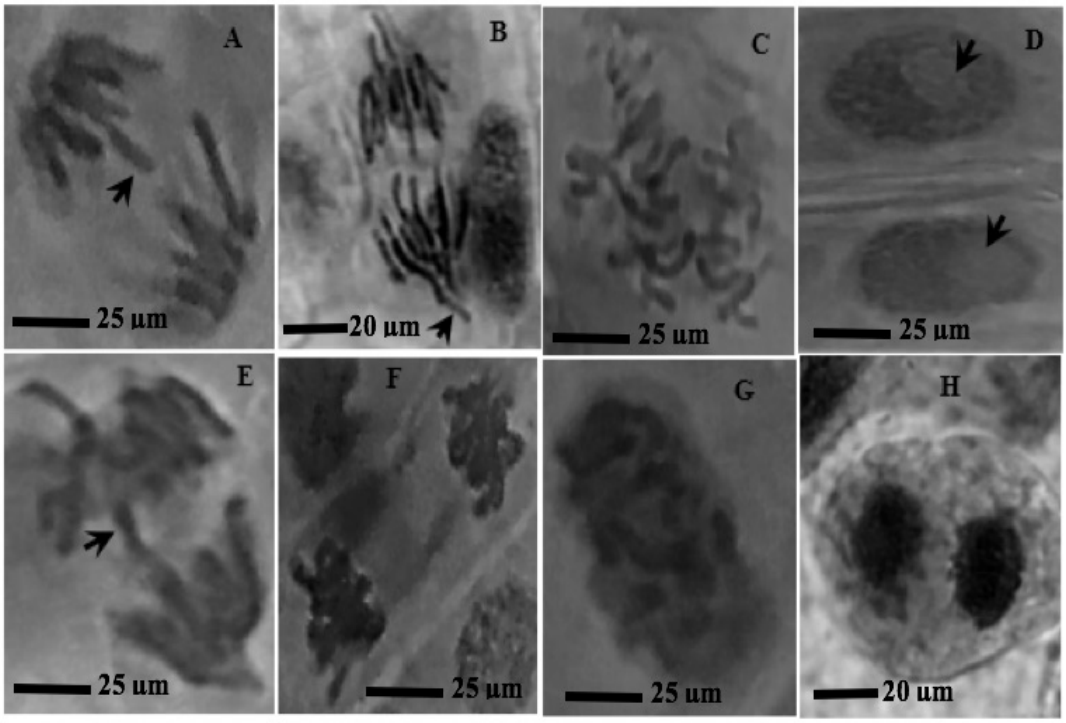


Figure 2: Mitotic and chromosomal aberrations (CA) (arrowed) induced *in Alliumcepa* root tips by aqueous extracts of the roots of *C. melo*. A and B=Anaphase laggard; C= Disoriented Anaphase; D= Nuclear vacuoles; E=Anaphase Bridge; F=Sticky chromosomes; G=Mature cell showing puff; H=binucleated cell

Table 1: Effects of aqueous extract of *Cucumis melo* on the sprouting of *Allium cepa* roots

Concentration (µg/ml)	Mean root number ± SE	% Total root sprouted of NC	Percentage inhibition	95% confidence limit
0	113.15 ± 6.10^{a}	100	0	0.000
5	$102.10\pm4.18^{*b}$	81	19	0.012
10	$94.44\pm5.00^{**cd}$	59	41	0.010
20	$91.14\pm2.02^{***ef}$	49	51	0.017
30	$59.02\pm1.04^{***gh}$	15	85	0.011
40	$35.11\pm0.40^{***i}$	3	97	0.010

*Number of trials n=5, *p<0.05; **p<0.01; *** p<0.001 significantly different from the control group (Distilled water), applying one-way ANOVA followed by Tukey's post-test (HSD). Groups that have no letters in common differ significantly.*

Table 2: Effects of aqueous extract of *Cucumis melo* on the growth of *Allium cepa* roots

Concentration (µg/ml)	Mean root length ± SE	% Total root growth of NC	Percentage inhibition	95% confidence limit
0	$5.77\pm0,5^{a}$	100	0	0.000
5	$4.57\pm0,11^{*b}$	81	19	0.012
10	$4.11\pm0,31^{*cd}$	59	41	0.010
20	$4.06\pm0,13^{**e}$	49	51	0.017
30	$3.88\pm0,26^{**e}$	15	85	0.011
40	$2.89\pm0,24^{**f}$	3	97	0.010

*Number of trials n=5, *p<0.05; **p<0.01 significantly different from the control group (Distilled water), applying one-way ANOVA followed by Tukey's post-test (HSD). Groups that have no letters in common differ significantly.*

Table 3: Cytogenetic analysis of *A. cepa* root tips exposed to different concentrations of aqueous extracts of the roots of *C. melo*

Conc. (µg/ml)	Total cells examined	% Prophase Index	% Metaphase Index	% Anaphase Index	Mitotic Index (MI)	Anaphase					
						Bridges	Laggards	Disoriented	Nuclear vacuoles	Sticky chromosomes	% Total abnormalities
0	454	15.80	14.20	14.40	45.40±1.18[a]	0	0	0	0	0	0[a]
5	439	15.50	15.00	13.40	43.90±1.53[*b]	5	3	6	2	4	3.83±1,47[*b]
10	424	10.40	19.80	12.20	42.40±1.81[*c]	6	5	8	2	6	5.16±2,04[*cd]
20	426	5.70	25.90	11.00	42.60±1.34[***c]	9	6	12	5	9	7.50±3,01[*ef]
30	452	4.60	30.10	10.50	45.20±1.06[**ce]	11	7	14	6	10	8.83±3,43[**g]
40	437	3.30	30.30	10.10	43.70±1.39[***f]	12	9	15	7	11	10.00±3,34[***h]

*Number of trials n=5, *p<0.05; **p<0.01; *** p<0.001 significantly different from control group (Distilled water), applying one-way ANOVA followed by Tukey's post-test (HSD). Groups that have no letters in common differ significantly.*

Abbreviations List
Not used

Declarations

Ethical Approval and Consent to Participate
All experimental studies on plants were complied with relevant institutional, national, and international guidelines and legislation.

Consent to Publication
We declare hereby that this work has not been published or accepted, in whole or in part, and that it is not selected for publication in another journal. All authors have approved the manuscript and agree with its submission.

Availability of Data and Material
Datasets generated during and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Competing Interests
The authors have no competing interests to declare that are relevant to the content of this article.

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Author Contributions
N.A. and S.R.A. conceived and conducted research experiments, N.A. analysed data and conducted statistical analyses, N.A. wrote the original draft article, S.R.A reviewed the manuscript.

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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.

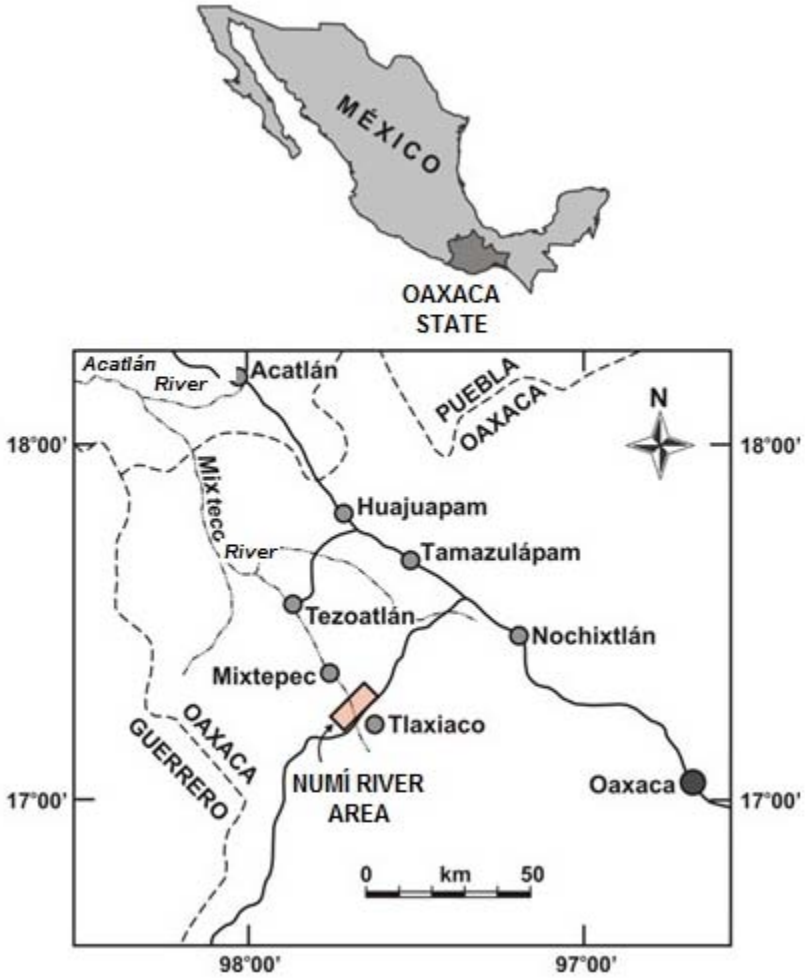

Figure 1: Localization Map of the Numí River area, close to Tlaxiaco, Oaxaca

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.

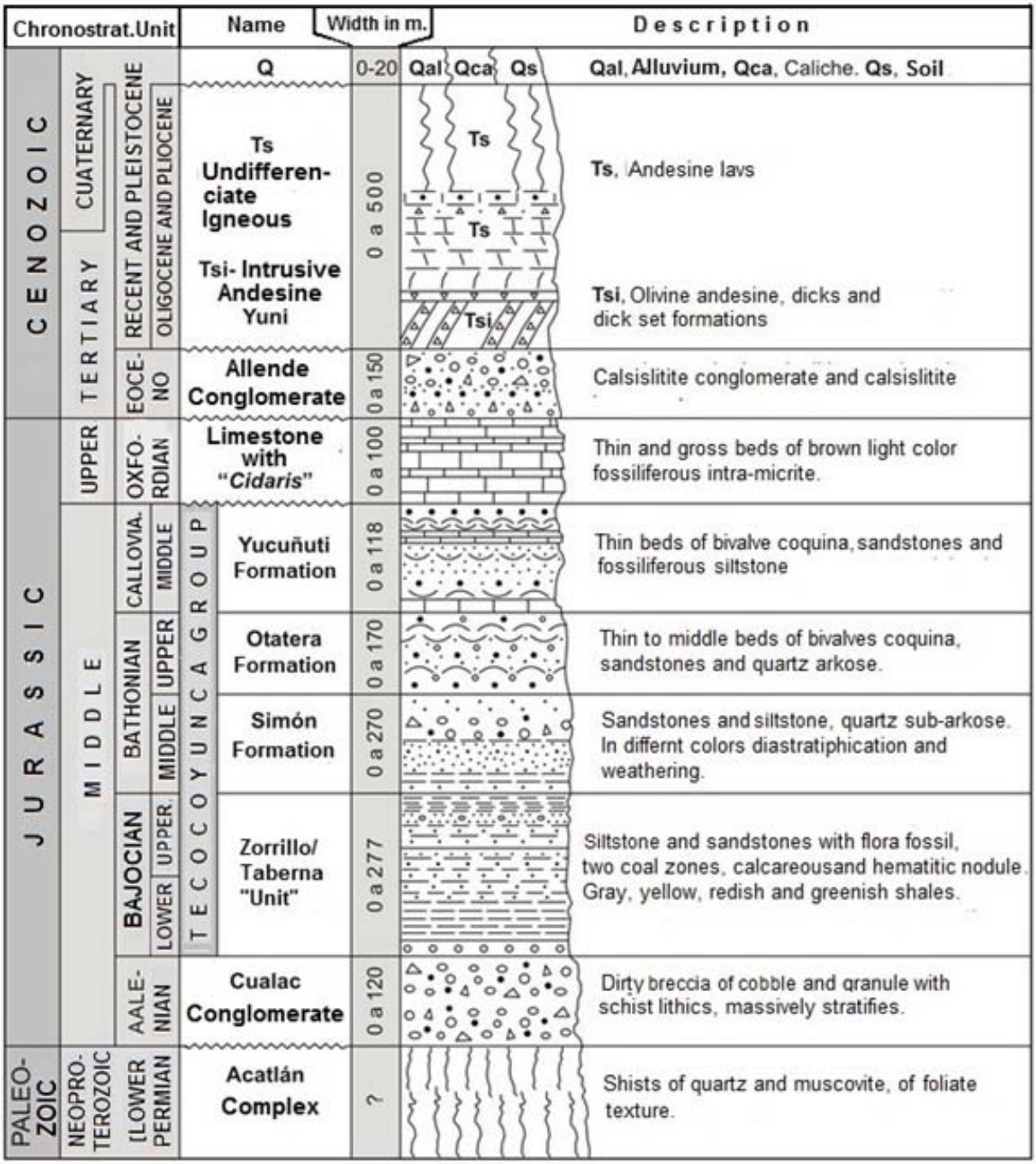

Figure 2: General stratigraphic column of the Numí River Area

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).

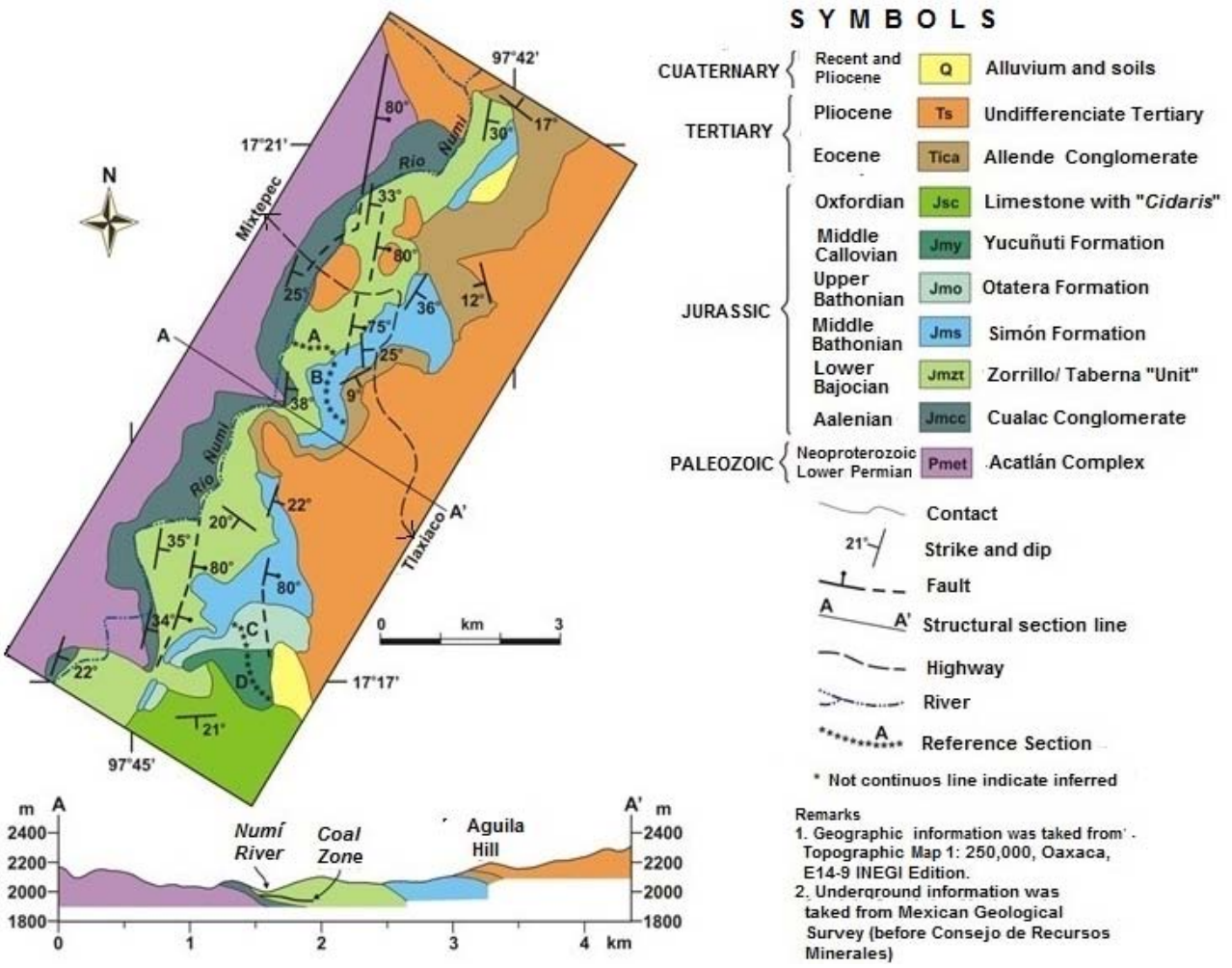

Figure 3: Geologic map and general structural section of the Numí River area, close to Tlaxiaco

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.

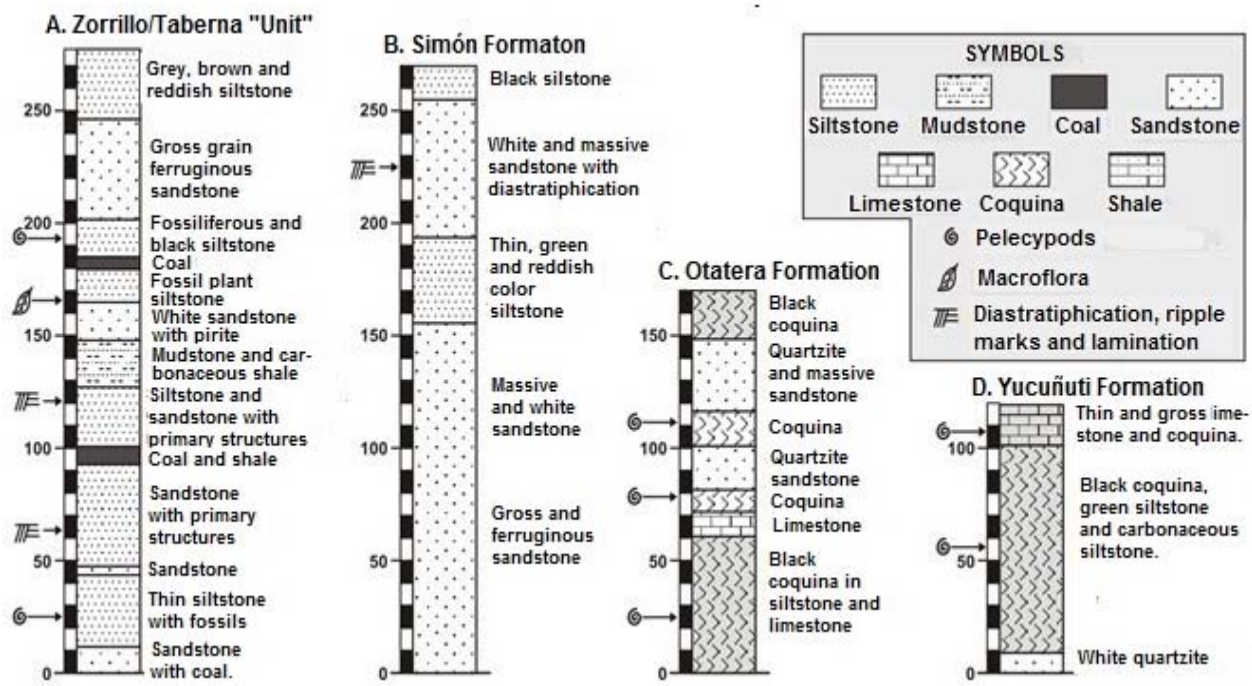

Figure 4: Main Reference Sections of the Tecocoyunca Group formations

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.

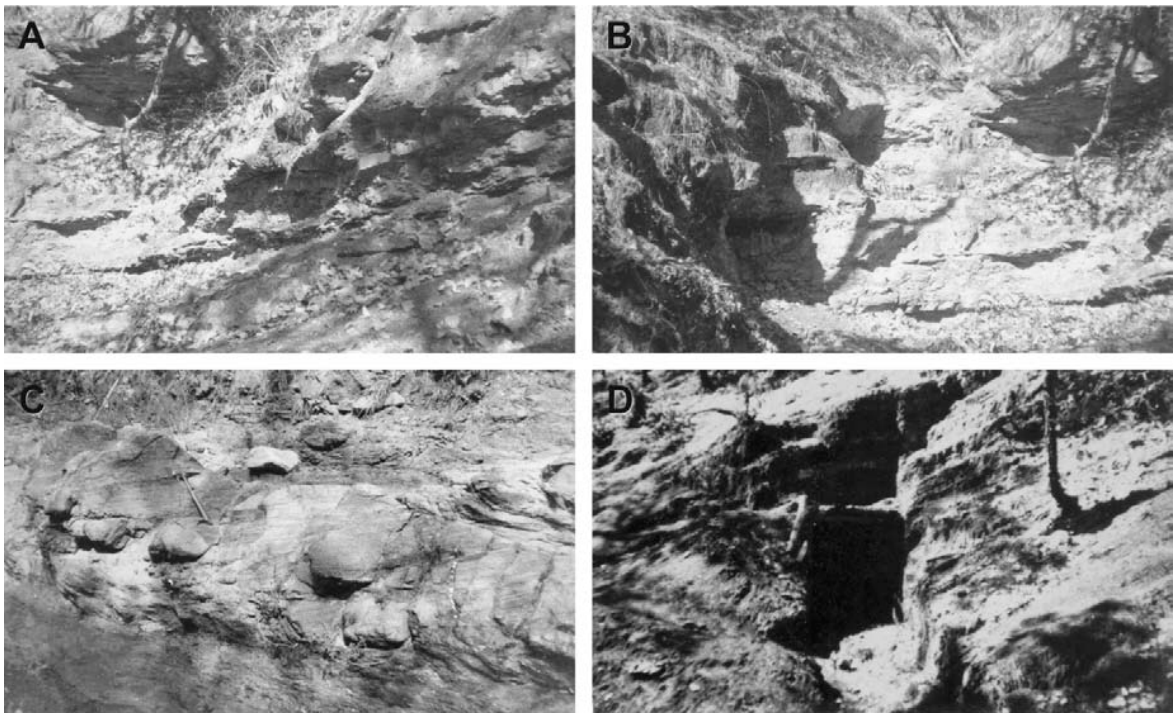

Figure 5: Outcrops of the Zorrillo/Taberna "Unit": A) La Carbonera Stream, show fine grained siltstone and sandstone, road to San Juan Mixtec, Km 6. B) Chicavandicuche Stream, show fine grain siltstone and sandstone, *Idem* km 6 C) Bridge on the Mixtepec road, show siltstone, *Idem*, km 8. D) Small Tunnel,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

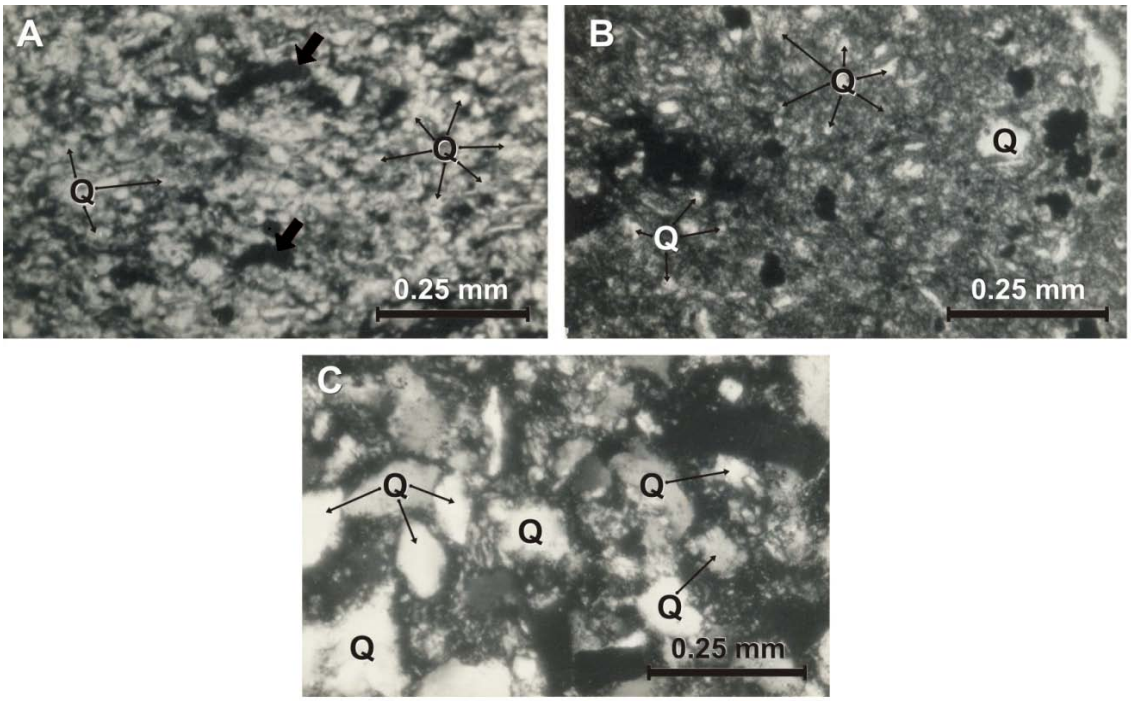

Figure 6: Micro-photographs of the Zorrillo/Taberna "Unit": A) Carbonaceous- phyletic siltstone, at natural light conditions. Clay cement, grains are mainly of quartz (Q) and carbonaceous material is in small forms, pointed out by arrows. B) Quarzite-phylitic mudstone observed by crossed nicol. It is 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).

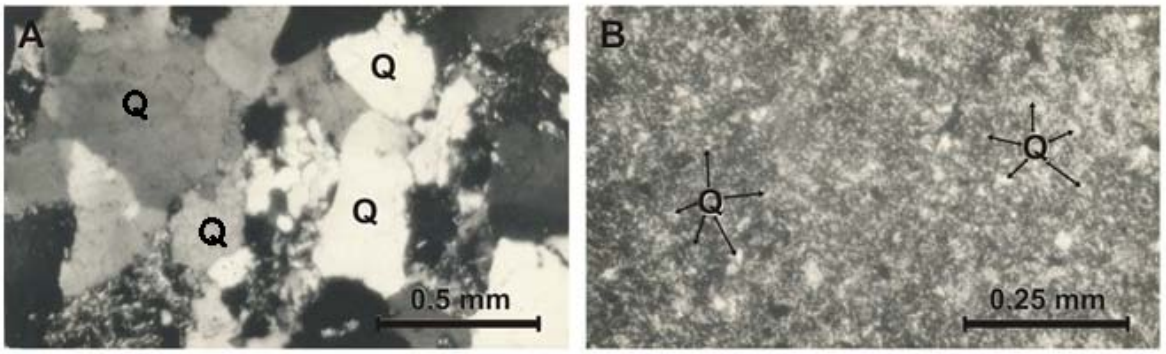

Figure 8: Micro photographs of Simón Formation: A) Quartzite subarkose middle grain sandstone observed with crossed nicole. There are igneous quartz composed (grey) and igneous included in silt. B) Phyletic siltstone parallel light observed. Microcrystals of clay and fine silty size are plentiful with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

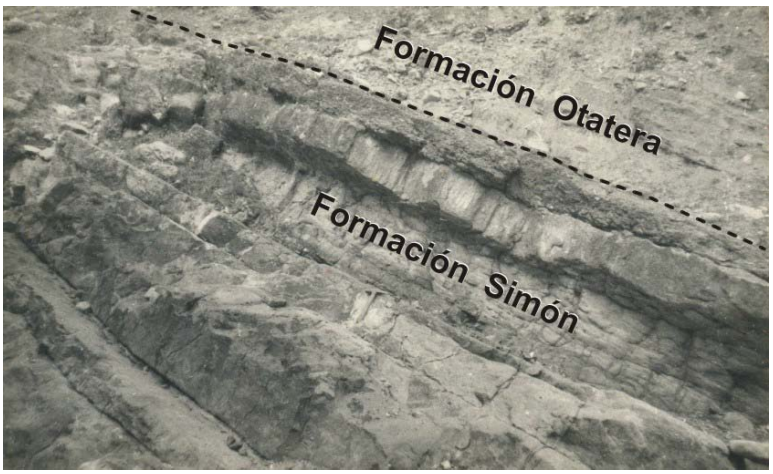

Figure 9: Outcrop show Simón Formation with Otatera Formation contact. It is observed black coquina resting on sandstone

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).

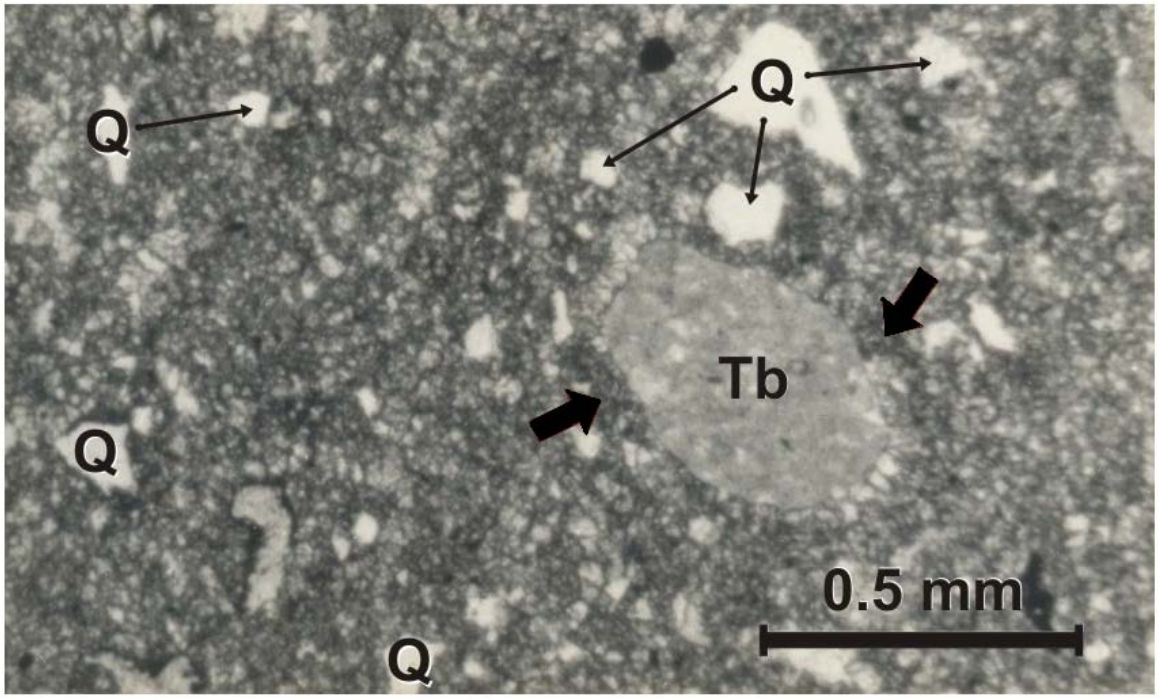

Figure 10: Otatera Formation: Micro photograph using parallel light. There is big tuff grain (Tb) surrounded by spathite (arrows point out)

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.

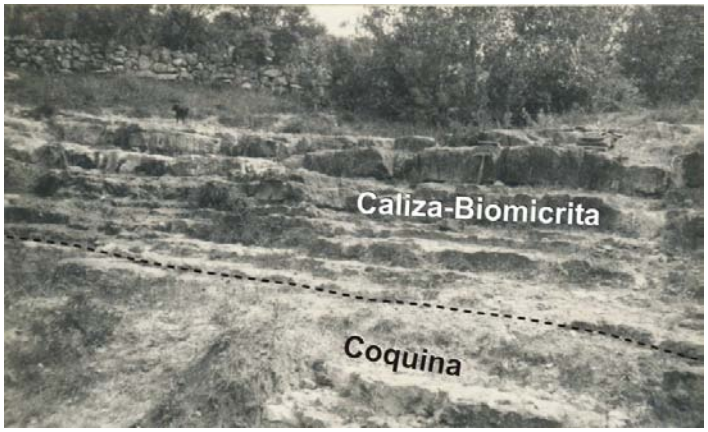

Figure 11: Outcrop of Yucuñuti Formation. Thin beds of light grey biomicrita and coquina

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.

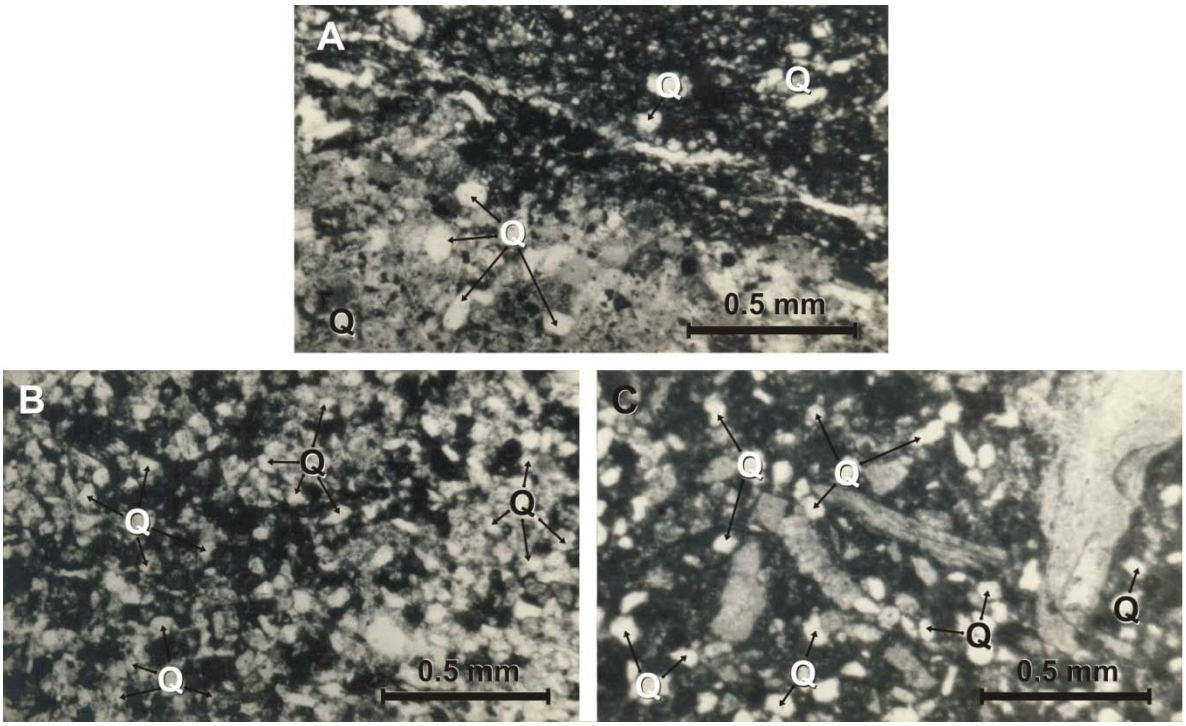

Figure 12: Micro photograph of the Yucuñuti Formation. A) Gross grains of quartz sand siltstone using parallel light. Have a look that grains of quartz are abundant. C) Biomicrite and quartzite

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.

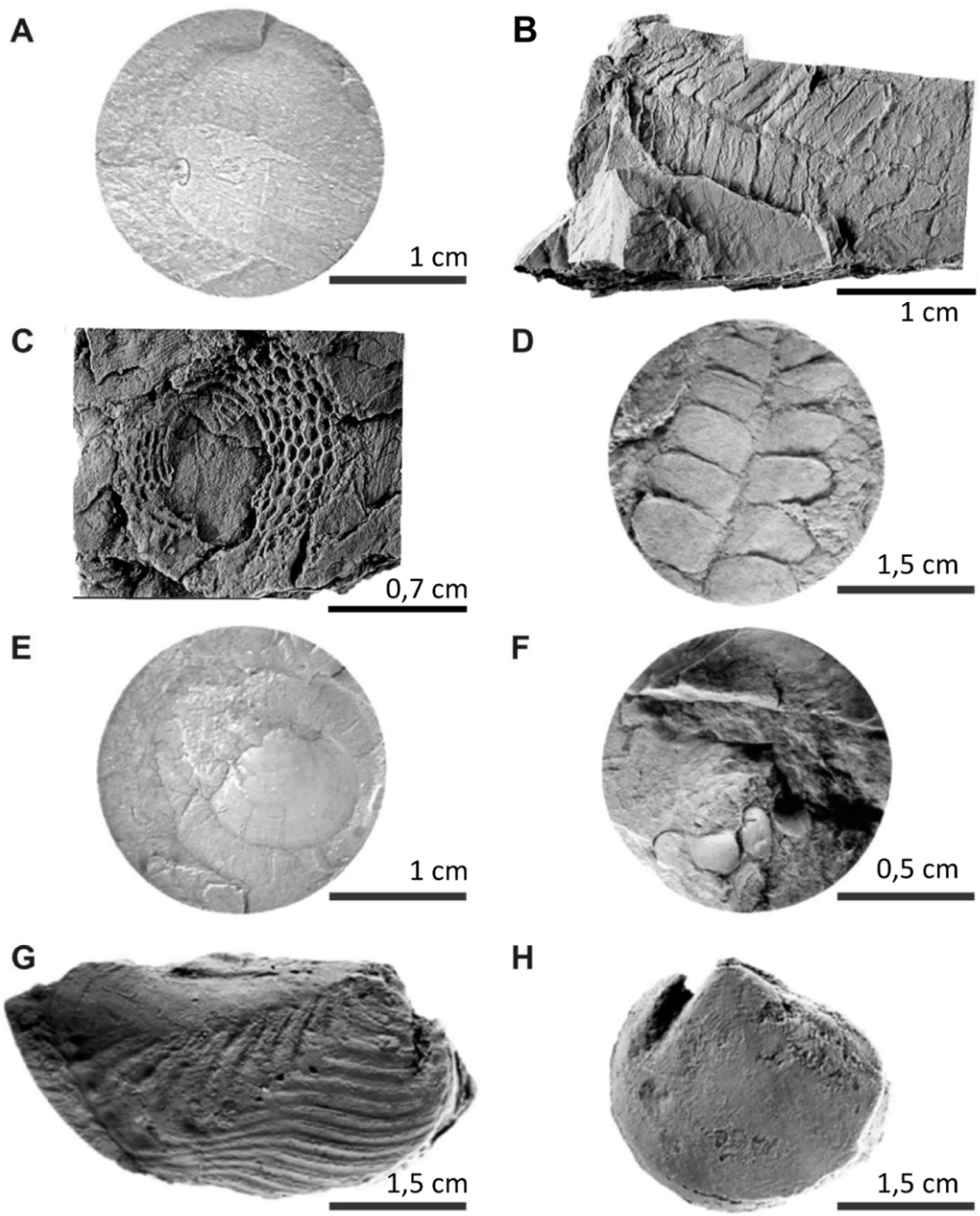

Figure 13: Specimens collected from the Zorrillo/Taberna "Unit". A) *Zamites oaxacencis* B) *Zamites lucerensis,* C) *Williamsonia netzahualcoyotlii*, D) *Ptilophyllum acutifolium*, E) *Lucina* cf. L. *bellona*, F) Astarte sp., G) *Vaugonia* (*Vaugonia*) *v-costata* var. *mexicana*, H) *Trigonia* (*Indotrigonia*)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

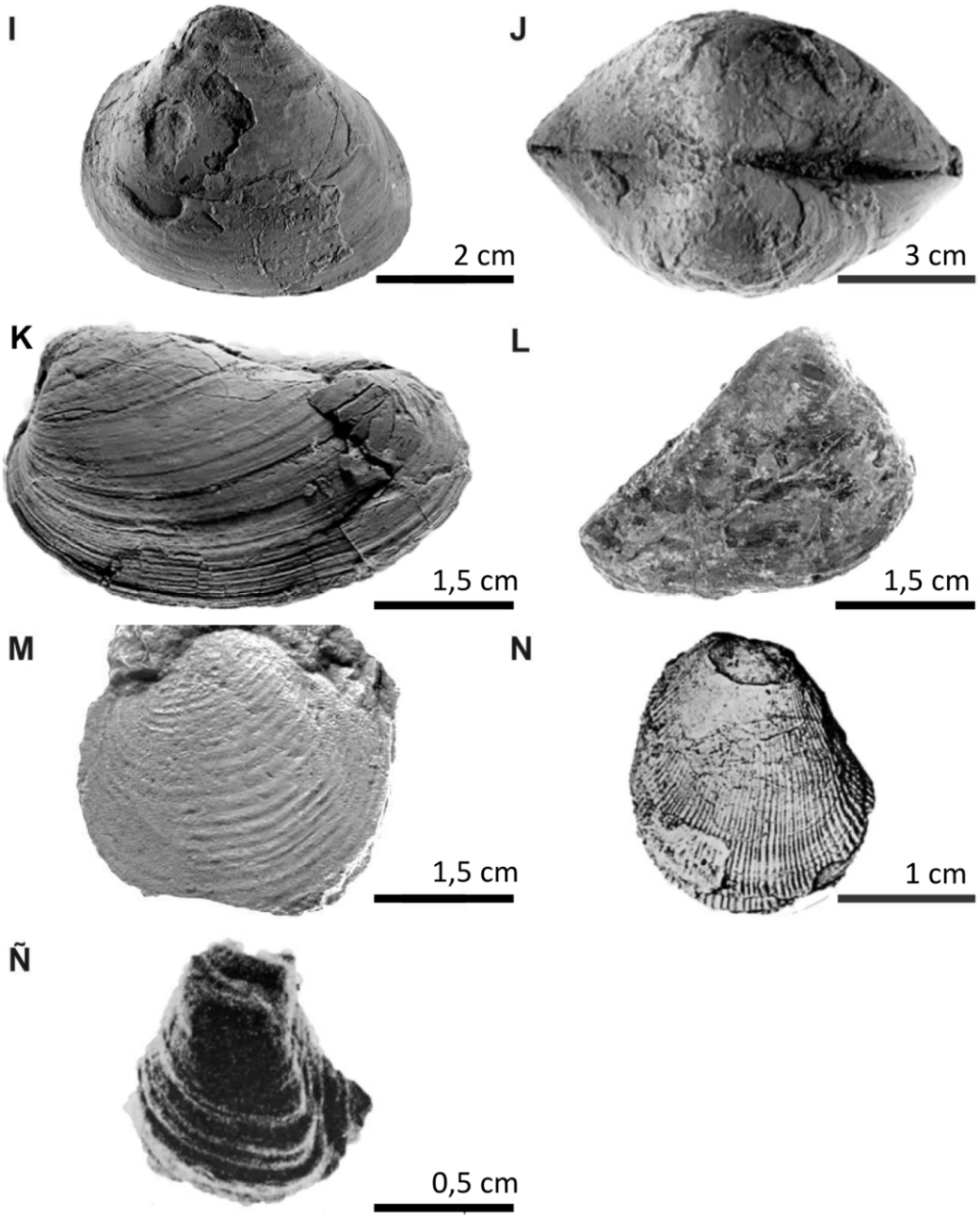

Figure 14: Specimens collected from the Oatera Formation. I) y J) *Eocallista imlayi*, K) *Pleuromya* sp., L) *Crenotrapezium hayam.* Specimens collected from the Yucuñut Formationi. M) *Lucina* sp., N) *Lima* (*Plagiostoma*) sp., Ñ) *Gryphaea mexicana*

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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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Keywords: mugilidae, mugil cephalus, eimeria spp, RT-PCR.

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.

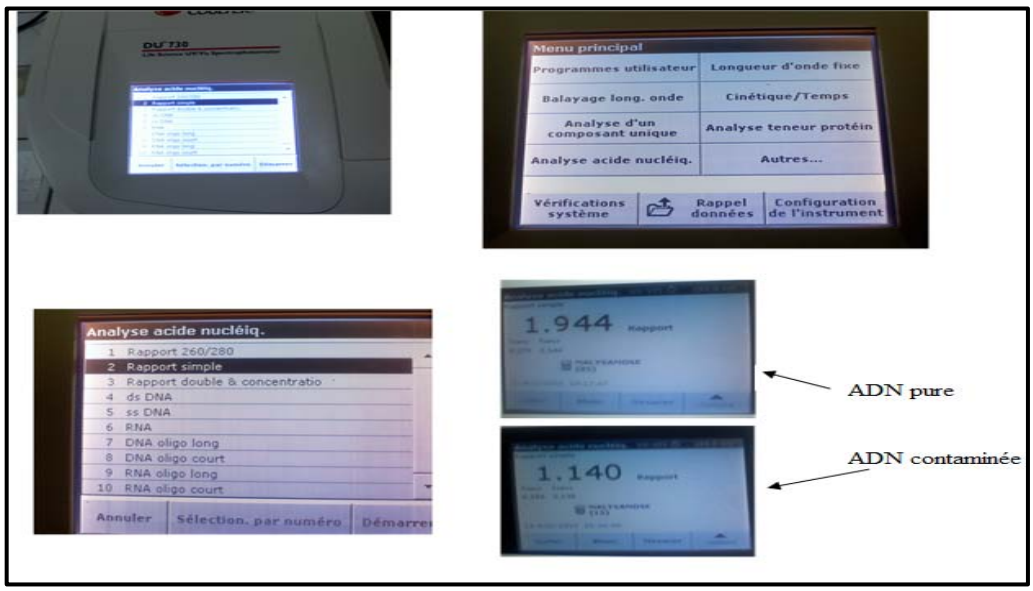


Figure 1: Detection of DNA concentration (ng/μl) and measurement of DNA purity with a Nanodrop spectrophotometer (THERMO. USA)

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:

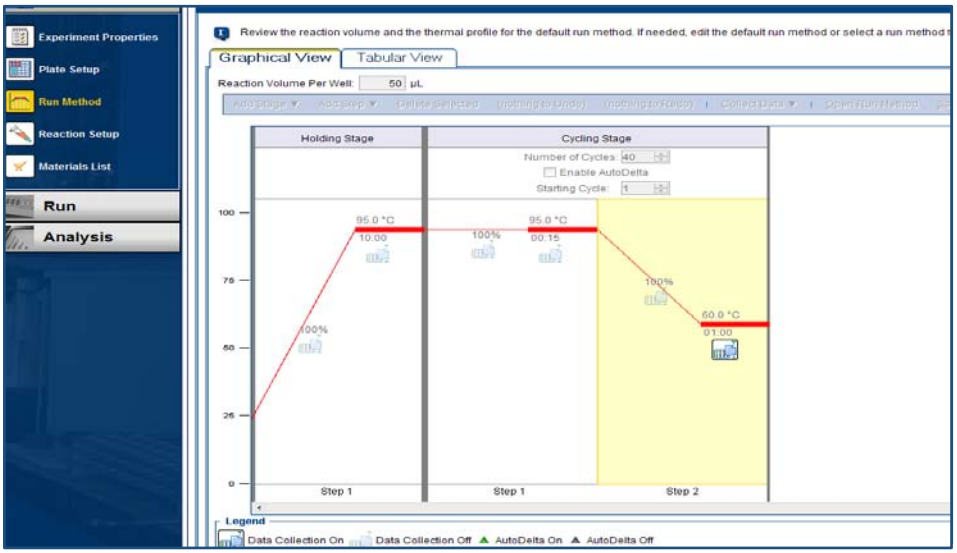


Figure 3: Explained situations of Thermo cycler for REALTIME PCR

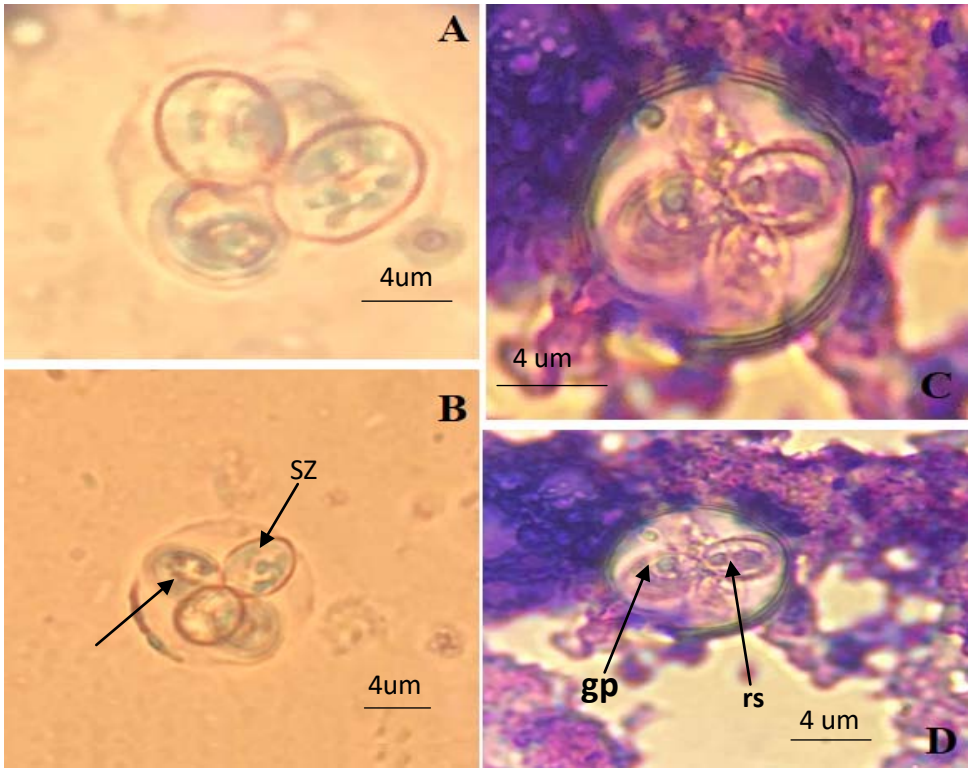
g) Real-Time PCR Data Analysis

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).



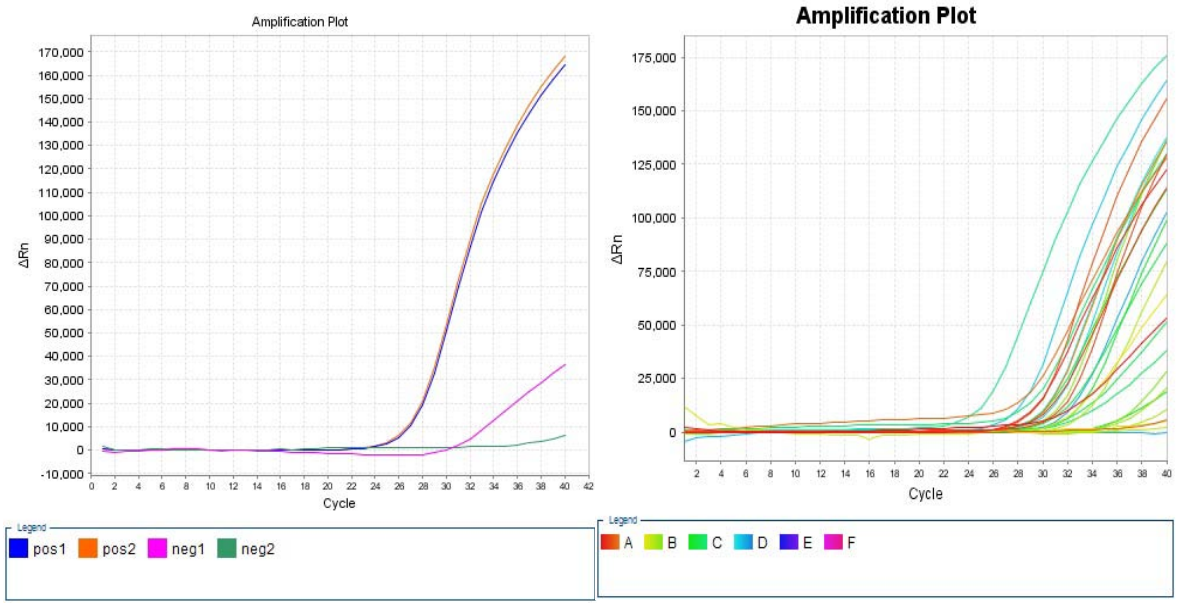
(A) Oocyst containing sporoblasts (bar = 4 um). - (B) Sporulated oocyst. Sporozoites (sz) are visible inside the sporocysts. The arrow indicates the Stieda body (bar = 4 um) - (C) Sporulated oocyst showing three polar granules (gp) and (D) sporocystic residue (rs) in one of the sporocysts (bar = 4 um).

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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Associates of FSFRC/ASFRC are scientists and researchers from around the world are working on projects/researches that have huge potentials. Members support Global Journals' mission to advance technology for humanity and the profession.

FSFRC

Fellow of Science Frontier Research Council

FELLOW OF SCIENCE FRONTIER RESEARCH COUNCIL is the most prestigious membership of Global Journals. It is an award and membership granted to individuals that the Open Association of Research Society judges to have made a 'substantial contribution to the improvement of computer science, technology, and electronics engineering.

The primary objective is to recognize the leaders in research and scientific fields of the current era with a global perspective and to create a channel between them and other researchers for better exposure and knowledge sharing. Members are most eminent scientists, engineers, and technologists from all across the world. Fellows are elected for life through a peer review process on the basis of excellence in the respective domain. There is no limit on the number of new nominations made in any year. Each year, the Open Association of Research Society elect up to 12 new Fellow Members.

Benefit

To the institution

Get letter of appreciation

Global Journals sends a letter of appreciation of author to the Dean or CEO of the University or Company of which author is a part, signed by editor in chief or chief author.

Exclusive Network

Get access to a closed network

A FSFRC member gets access to a closed network of Tier 1 researchers and scientists with direct communication channel through our website. Fellows can reach out to other members or researchers directly. They should also be open to reaching out by other.

Career | Credibility | Exclusive | Reputation

Certificate

Receive a print ed copy of a certificate

Fellows receive a printed copy of a certificate signed by our Chief Author that may be used for academic purposes and a personal recommendation letter to the dean of member's university.

Career | Credibility | Exclusive | Reputation

Designation

Get honored title of membership

Fellows can use the honored title of membership. The “FSFRC” is an honored title which is accorded to a person’s name viz. Dr. John E. Hall, Ph.D., FSFRC or William Walldroff, M.S., FSFRC.

Career | Credibility | Exclusive | Reputation

Recognition on the Platform

Better visibility and citation

All the Fellow members of FSFRC get a badge of "Leading Member of Global Journals" on the Research Community that distinguishes them from others. Additionally, the profile is also partially maintained by our team for better visibility and citation. All fellows get a dedicated page on the website with their biography.

Career | Credibility | Reputation

Future Work

Get discounts on the future publications

Fellows receive discounts on future publications with Global Journals up to 60%. Through our recommendation programs, members also receive discounts on publications made with OARS affiliated organizations.

Career | Financial

GJ Internal Account

Unlimited forward of Emails

Fellows get secure and fast GJ work emails with unlimited forward of emails that they may use them as their primary email. For example,
john [AT] globaljournals [DOT] org.

Career | Credibility | Reputation

Premium Tools

Access to all the premium tools

To take future researches to the zenith, fellows and associates receive access to all the premium tools that Global Journals have to offer along with the partnership with some of the best marketing leading tools out there.

Financial

Conferences & Events

Organize seminar/conference

Fellows are authorized to organize symposium/seminar/conference on behalf of Global Journal Incorporation (USA). They can also participate in the same organized by another institution as representative of Global Journal. In both the cases, it is mandatory for him to discuss with us and obtain our consent. Additionally, they get free research conferences (and others) alerts.

Career | Credibility | Financial

Early Invitations

Early invitations to all the symposiums, seminars, conferences

All fellows receive the early invitations to all the symposiums, seminars, conferences and webinars hosted by Global Journals in their subject.

Exclusive

Publishing Articles & Books

Earn 60% of sales proceeds

Fellows can publish articles (limited) without any fees. Also, they can earn up to 60% of sales proceeds from the sale of reference/review books/literature/ publishing of research paper. The FSFRC member can decide its price and we can help in making the right decision.

Exclusive | Financial

Reviewers

Get a remuneration of 15% of author fees

Fellow members are eligible to join as a paid peer reviewer at Global Journals Incorporation (USA) and can get a remuneration of 15% of author fees, taken from the author of a respective paper.

Financial

Access to Editorial Board

Become a member of the Editorial Board

Fellows may join as a member of the Editorial Board of Global Journals Incorporation (USA) after successful completion of three years as Fellow and as Peer Reviewer. Additionally, Fellows get a chance to nominate other members for Editorial Board.

Career | Credibility | Exclusive | Reputation

And Much More

Get access to scientific museums and observatories across the globe

All members get access to 5 selected scientific museums and observatories across the globe. All researches published with Global Journals will be kept under deep archival facilities across regions for future protections and disaster recovery. They get 10 GB free secure cloud access for storing research files.

ASSOCIATE OF SCIENCE FRONTIER RESEARCH COUNCIL

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Organize seminar/conference

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Exclusive | Financial

Reviewers

Get a remuneration of 15% of author fees

Associate members are eligible to join as a paid peer reviewer at Global Journals Incorporation (USA) and can get a remuneration of 15% of author fees, taken from the author of a respective paper.

Financial

And Much More

Get access to scientific museums and observatories across the globe

All members get access to 2 selected scientific museums and observatories across the globe. All researches published with Global Journals will be kept under deep archival facilities across regions for future protections and disaster recovery. They get 5 GB free secure cloud access for storing research files.

Associate	Fellow	Research Group	Basic
$4800 **lifetime designation**	$6800 **lifetime designation**	$12500.00 **organizational**	APC **per article**
Certificate, LoR and Momento **2** discounted publishing/year **Gradation** of Research **10** research contacts/day **1 GB** Cloud Storage **GJ** Community Access	**Certificate,** LoR and Momento **Unlimited** discounted publishing/year **Gradation** of Research **Unlimited** research contacts/day **5 GB** Cloud Storage **Online Presense** Assistance **GJ** Community Access	**Certificates,** LoRs and Momentos **Unlimited** free publishing/year **Gradation** of Research **Unlimited** research contacts/day **Unlimited** Cloud Storage **Online Presense** Assistance **GJ** Community Access	**GJ** Community Access

Preferred Author Guidelines

We accept the manuscript submissions in any standard (generic) format.

We typeset manuscripts using advanced typesetting tools like Adobe In Design, CorelDraw, TeXnicCenter, and TeXStudio. We usually recommend authors submit their research using any standard format they are comfortable with, and let Global Journals do the rest.

Alternatively, you can download our basic template from https://globaljournals.org/Template.zip

Authors should submit their complete paper/article, including text illustrations, graphics, conclusions, artwork, and tables. Authors who are not able to submit manuscript using the form above can email the manuscript department at submit@globaljournals.org or get in touch with chiefeditor@globaljournals.org if they wish to send the abstract before submission.

Before and during Submission

Authors must ensure the information provided during the submission of a paper is authentic. Please go through the following checklist before submitting:

1. Authors must go through the complete author guideline and understand and *agree to Global Journals' ethics and code of conduct,* along with author responsibilities.
2. Authors must accept the privacy policy, terms, and conditions of Global Journals.
3. Ensure corresponding author's email address and postal address are accurate and reachable.
4. Manuscript to be submitted must include keywords, an abstract, a paper title, co-author(s') names and details (email address, name, phone number, and institution), figures and illustrations in vector format including appropriate captions, tables, including titles and footnotes, a conclusion, results, acknowledgments and references.
5. Authors should submit paper in a ZIP archive if any supplementary files are required along with the paper.
6. Proper permissions must be acquired for the use of any copyrighted material.
7. Manuscript submitted *must not have been submitted or published elsewhere* and all authors must be aware of the submission.

Declaration of Conflicts of Interest

It is required for authors to declare all financial, institutional, and personal relationships with other individuals and organizations that could influence (bias) their research.

Policy on Plagiarism

Plagiarism is not acceptable in Global Journals submissions at all.

Plagiarized content will not be considered for publication. We reserve the right to inform authors' institutions about plagiarism detected either before or after publication. If plagiarism is identified, we will follow COPE guidelines:

Authors are solely responsible for all the plagiarism that is found. The author must not fabricate, falsify or plagiarize existing research data. The following, if copied, will be considered plagiarism:

- Words (language)
- Ideas
- Findings
- Writings
- Diagrams
- Graphs
- Illustrations
- Lectures

- Printed material
- Graphic representations
- Computer programs
- Electronic material
- Any other original work

Authorship Policies

Global Journals follows the definition of authorship set up by the Open Association of Research Society, USA. According to its guidelines, authorship criteria must be based on:

1. Substantial contributions to the conception and acquisition of data, analysis, and interpretation of findings.
2. Drafting the paper and revising it critically regarding important academic content.
3. Final approval of the version of the paper to be published.

Changes in Authorship

The corresponding author should mention the name and complete details of all co-authors during submission and in manuscript. We support addition, rearrangement, manipulation, and deletions in authors list till the early view publication of the journal. We expect that corresponding author will notify all co-authors of submission. We follow COPE guidelines for changes in authorship.

Copyright

During submission of the manuscript, the author is confirming an exclusive license agreement with Global Journals which gives Global Journals the authority to reproduce, reuse, and republish authors' research. We also believe in flexible copyright terms where copyright may remain with authors/employers/institutions as well. Contact your editor after acceptance to choose your copyright policy. You may follow this form for copyright transfers.

Appealing Decisions

Unless specified in the notification, the Editorial Board's decision on publication of the paper is final and cannot be appealed before making the major change in the manuscript.

Acknowledgments

Contributors to the research other than authors credited should be mentioned in Acknowledgments. The source of funding for the research can be included. Suppliers of resources may be mentioned along with their addresses.

Declaration of funding sources

Global Journals is in partnership with various universities, laboratories, and other institutions worldwide in the research domain. Authors are requested to disclose their source of funding during every stage of their research, such as making analysis, performing laboratory operations, computing data, and using institutional resources, from writing an article to its submission. This will also help authors to get reimbursements by requesting an open access publication letter from Global Journals and submitting to the respective funding source.

Preparing your Manuscript

Authors can submit papers and articles in an acceptable file format: MS Word (doc, docx), LaTeX (.tex, .zip or .rar including all of your files), Adobe PDF (.pdf), rich text format (.rtf), simple text document (.txt), Open Document Text (.odt), and Apple Pages (.pages). Our professional layout editors will format the entire paper according to our official guidelines. This is one of the highlights of publishing with Global Journals—authors should not be concerned about the formatting of their paper. Global Journals accepts articles and manuscripts in every major language, be it Spanish, Chinese, Japanese, Portuguese, Russian, French, German, Dutch, Italian, Greek, or any other national language, but the title, subtitle, and abstract should be in English. This will facilitate indexing and the pre-peer review process.

The following is the official style and template developed for publication of a research paper. Authors are not required to follow this style during the submission of the paper. It is just for reference purposes.

Manuscript Style Instruction (Optional)

- Microsoft Word Document Setting Instructions.
- Font type of all text should be Swis721 Lt BT.
- Page size: 8.27" x 11'", left margin: 0.65, right margin: 0.65, bottom margin: 0.75.
- Paper title should be in one column of font size 24.
- Author name in font size of 11 in one column.
- Abstract: font size 9 with the word "Abstract" in bold italics.
- Main text: font size 10 with two justified columns.
- Two columns with equal column width of 3.38 and spacing of 0.2.
- First character must be three lines drop-capped.
- The paragraph before spacing of 1 pt and after of 0 pt.
- Line spacing of 1 pt.
- Large images must be in one column.
- The names of first main headings (Heading 1) must be in Roman font, capital letters, and font size of 10.
- The names of second main headings (Heading 2) must not include numbers and must be in italics with a font size of 10.

Structure and Format of Manuscript

The recommended size of an original research paper is under 15,000 words and review papers under 7,000 words. Research articles should be less than 10,000 words. Research papers are usually longer than review papers. Review papers are reports of significant research (typically less than 7,000 words, including tables, figures, and references)

A research paper must include:

a) A title which should be relevant to the theme of the paper.
b) A summary, known as an abstract (less than 150 words), containing the major results and conclusions.
c) Up to 10 keywords that precisely identify the paper's subject, purpose, and focus.
d) An introduction, giving fundamental background objectives.
e) Resources and techniques with sufficient complete experimental details (wherever possible by reference) to permit repetition, sources of information must be given, and numerical methods must be specified by reference.
f) Results which should be presented concisely by well-designed tables and figures.
g) Suitable statistical data should also be given.
h) All data must have been gathered with attention to numerical detail in the planning stage.

Design has been recognized to be essential to experiments for a considerable time, and the editor has decided that any paper that appears not to have adequate numerical treatments of the data will be returned unrefereed.

i) Discussion should cover implications and consequences and not just recapitulate the results; conclusions should also be summarized.
j) There should be brief acknowledgments.
k) There ought to be references in the conventional format. Global Journals recommends APA format.

Authors should carefully consider the preparation of papers to ensure that they communicate effectively. Papers are much more likely to be accepted if they are carefully designed and laid out, contain few or no errors, are summarizing, and follow instructions. They will also be published with much fewer delays than those that require much technical and editorial correction.

The Editorial Board reserves the right to make literary corrections and suggestions to improve brevity.

Format Structure

It is necessary that authors take care in submitting a manuscript that is written in simple language and adheres to published guidelines.

All manuscripts submitted to Global Journals should include:

Title

The title page must carry an informative title that reflects the content, a running title (less than 45 characters together with spaces), names of the authors and co-authors, and the place(s) where the work was carried out.

Author details

The full postal address of any related author(s) must be specified.

Abstract

The abstract is the foundation of the research paper. It should be clear and concise and must contain the objective of the paper and inferences drawn. It is advised to not include big mathematical equations or complicated jargon.

Many researchers searching for information online will use search engines such as Google, Yahoo or others. By optimizing your paper for search engines, you will amplify the chance of someone finding it. In turn, this will make it more likely to be viewed and cited in further works. Global Journals has compiled these guidelines to facilitate you to maximize the web-friendliness of the most public part of your paper.

Keywords

A major lynchpin of research work for the writing of research papers is the keyword search, which one will employ to find both library and internet resources. Up to eleven keywords or very brief phrases have to be given to help data retrieval, mining, and indexing.

One must be persistent and creative in using keywords. An effective keyword search requires a strategy: planning of a list of possible keywords and phrases to try.

Choice of the main keywords is the first tool of writing a research paper. Research paper writing is an art. Keyword search should be as strategic as possible.

One should start brainstorming lists of potential keywords before even beginning searching. Think about the most important concepts related to research work. Ask, “What words would a source have to include to be truly valuable in a research paper?” Then consider synonyms for the important words.

It may take the discovery of only one important paper to steer in the right keyword direction because, in most databases, the keywords under which a research paper is abstracted are listed with the paper.

Numerical Methods

Numerical methods used should be transparent and, where appropriate, supported by references.

Abbreviations

Authors must list all the abbreviations used in the paper at the end of the paper or in a separate table before using them.

Formulas and equations

Authors are advised to submit any mathematical equation using either MathJax, KaTeX, or LaTeX, or in a very high-quality image.

Tables, Figures, and Figure Legends

Tables: Tables should be cautiously designed, uncrowned, and include only essential data. Each must have an Arabic number, e.g., Table 4, a self-explanatory caption, and be on a separate sheet. Authors must submit tables in an editable format and not as images. References to these tables (if any) must be mentioned accurately.

Figures

Figures are supposed to be submitted as separate files. Always include a citation in the text for each figure using Arabic numbers, e.g., Fig. 4. Artwork must be submitted online in vector electronic form or by emailing it.

Preparation of Eletronic Figures for Publication

Although low-quality images are sufficient for review purposes, print publication requires high-quality images to prevent the final product being blurred or fuzzy. Submit (possibly by e-mail) EPS (line art) or TIFF (halftone/ photographs) files only. MS PowerPoint and Word Graphics are unsuitable for printed pictures. Avoid using pixel-oriented software. Scans (TIFF only) should have a resolution of at least 350 dpi (halftone) or 700 to 1100 dpi (line drawings). Please give the data for figures in black and white or submit a Color Work Agreement form. EPS files must be saved with fonts embedded (and with a TIFF preview, if possible).

For scanned images, the scanning resolution at final image size ought to be as follows to ensure good reproduction: line art: >650 dpi; halftones (including gel photographs): >350 dpi; figures containing both halftone and line images: >650 dpi.

Color charges: Authors are advised to pay the full cost for the reproduction of their color artwork. Hence, please note that if there is color artwork in your manuscript when it is accepted for publication, we would require you to complete and return a Color Work Agreement form before your paper can be published. Also, you can email your editor to remove the color fee after acceptance of the paper.

Tips for Writing a Good Quality Science Frontier Research Paper

Techniques for writing a good quality Science Frontier Research paper:

1. Choosing the topic: In most cases, the topic is selected by the interests of the author, but it can also be suggested by the guides. You can have several topics, and then judge which you are most comfortable with. This may be done by asking several questions of yourself, like "Will I be able to carry out a search in this area? Will I find all necessary resources to accomplish the search? Will I be able to find all information in this field area?" If the answer to this type of question is "yes," then you ought to choose that topic. In most cases, you may have to conduct surveys and visit several places. Also, you might have to do a lot of work to find all the rises and falls of the various data on that subject. Sometimes, detailed information plays a vital role, instead of short information. Evaluators are human: The first thing to remember is that evaluators are also human beings. They are not only meant for rejecting a paper. They are here to evaluate your paper. So present your best aspect.

2. Think like evaluators: If you are in confusion or getting demotivated because your paper may not be accepted by the evaluators, then think, and try to evaluate your paper like an evaluator. Try to understand what an evaluator wants in your research paper, and you will automatically have your answer. Make blueprints of paper: The outline is the plan or framework that will help you to arrange your thoughts. It will make your paper logical. But remember that all points of your outline must be related to the topic you have chosen.

3. Ask your guides: If you are having any difficulty with your research, then do not hesitate to share your difficulty with your guide (if you have one). They will surely help you out and resolve your doubts. If you can't clarify what exactly you require for your work, then ask your supervisor to help you with an alternative. He or she might also provide you with a list of essential readings.

4. Use of computer is recommended: As you are doing research in the field of science frontier then this point is quite obvious. Use right software: Always use good quality software packages. If you are not capable of judging good software, then you can lose the quality of your paper unknowingly. There are various programs available to help you which you can get through the internet.

5. Use the internet for help: An excellent start for your paper is using Google. It is a wondrous search engine, where you can have your doubts resolved. You may also read some answers for the frequent question of how to write your research paper or find a model research paper. You can download books from the internet. If you have all the required books, place importance on reading, selecting, and analyzing the specified information. Then sketch out your research paper. Use big pictures: You may use encyclopedias like Wikipedia to get pictures with the best resolution. At Global Journals, you should strictly follow here.

6. Bookmarks are useful: When you read any book or magazine, you generally use bookmarks, right? It is a good habit which helps to not lose your continuity. You should always use bookmarks while searching on the internet also, which will make your search easier.

7. Revise what you wrote: When you write anything, always read it, summarize it, and then finalize it.

8. Make every effort: Make every effort to mention what you are going to write in your paper. That means always have a good start. Try to mention everything in the introduction—what is the need for a particular research paper. Polish your work with good writing skills and always give an evaluator what he wants. Make backups: When you are going to do any important thing like making a research paper, you should always have backup copies of it either on your computer or on paper. This protects you from losing any portion of your important data.

9. Produce good diagrams of your own: Always try to include good charts or diagrams in your paper to improve quality. Using several unnecessary diagrams will degrade the quality of your paper by creating a hodgepodge. So always try to include diagrams which were made by you to improve the readability of your paper. Use of direct quotes: When you do research relevant to literature, history, or current affairs, then use of quotes becomes essential, but if the study is relevant to science, use of quotes is not preferable.

10. Use proper verb tense: Use proper verb tenses in your paper. Use past tense to present those events that have happened. Use present tense to indicate events that are going on. Use future tense to indicate events that will happen in the future. Use of wrong tenses will confuse the evaluator. Avoid sentences that are incomplete.

11. Pick a good study spot: Always try to pick a spot for your research which is quiet. Not every spot is good for studying.

12. Know what you know: Always try to know what you know by making objectives, otherwise you will be confused and unable to achieve your target.

13. Use good grammar: Always use good grammar and words that will have a positive impact on the evaluator; use of good vocabulary does not mean using tough words which the evaluator has to find in a dictionary. Do not fragment sentences. Eliminate one-word sentences. Do not ever use a big word when a smaller one would suffice.

Verbs have to be in agreement with their subjects. In a research paper, do not start sentences with conjunctions or finish them with prepositions. When writing formally, it is advisable to never split an infinitive because someone will (wrongly) complain. Avoid clichés like a disease. Always shun irritating alliteration. Use language which is simple and straightforward. Put together a neat summary.

14. Arrangement of information: Each section of the main body should start with an opening sentence, and there should be a changeover at the end of the section. Give only valid and powerful arguments for your topic. You may also maintain your arguments with records.

15. Never start at the last minute: Always allow enough time for research work. Leaving everything to the last minute will degrade your paper and spoil your work.

16. Multitasking in research is not good: Doing several things at the same time is a bad habit in the case of research activity. Research is an area where everything has a particular time slot. Divide your research work into parts, and do a particular part in a particular time slot.

17. Never copy others' work: Never copy others' work and give it your name because if the evaluator has seen it anywhere, you will be in trouble. Take proper rest and food: No matter how many hours you spend on your research activity, if you are not taking care of your health, then all your efforts will have been in vain. For quality research, take proper rest and food.

18. Go to seminars: Attend seminars if the topic is relevant to your research area. Utilize all your resources.

19. Refresh your mind after intervals: Try to give your mind a rest by listening to soft music or sleeping in intervals. This will also improve your memory. Acquire colleagues: Always try to acquire colleagues. No matter how sharp you are, if you acquire colleagues, they can give you ideas which will be helpful to your research.

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.

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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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