Contribution to the phylogenetic and phylogeographic study of some limpets species in the Algerian coastline

Karim Mezali[1] and **Zoheir Bouzaza**[1,2]
[1]*Department of Marine Sciences and Aquaculture,* [2]*Department of Biology, Faculty of Natural Science and Life, Abdelhamid Ibn Badis University of Mostaganem, 27000, PO Box 300, Algeria.*
E-mail: riskemall@yahoo.fr

SUMMARY

In ecology, the study of patterns between species is necessary to understand the factors affecting their distribution and the dislocations causing their divergence. The aim of this work is to study the phylogeny and the phylogeography of some Prosobranchia Gastropoda (Patella rustica and Patella ferruginea) to understand the pattern of connectivity between populations by explaining the causes of their differences, their distribution and their responses to environmental change using the paleontological data. After identifying our species morphologically, we performed the molecular work (DNA extraction, PCR, DNA sequencing ...) using the mitochondrial marker cytochrome oxydase I (COI). The results of mitochondrial DNA sequences obtained were treated using some software (Codon Code Align, MEGA 5…) following the method of maximum likelihood "ML". We noted that the divergence and geographic distribution among species was consistent with some paleontological changes, which could be the cause of differences of the studied species.

Keywords: Phylogeography, mitochondrial DNA, paleontological upheaval, Phylogeny, Limpets.

INTRODUCTION

One of the sciences that most intrigued the human being is the study of the relationship between the living being (phylogeny) and their evolution through time and space (The phylogeography) which then gave the idea of the concept of speciation [1]. Natural populations show in the environment where they live many morphological adaptations [2]. Natural selection is a key point of this theory which could influence the life history traits in order to optimize the reproductive success of the species [3]. Marine organisms defy evolutionary models and most common speciation, because they live in an environment that is apparently devoid of dispersal barriers, and often have large numbers of individuals and well-developed dispersal capacities [4]. Speciation in the marine realm defeats the allopatric speciation model, marine species usually show a high dispersal capacity with low barriers, we can say that the absolute dispersion exist in the marine environment [5]. Many marine species, like limpets, release their gametes into the water, where fertilization takes place.
The aim of this work is to know the history of vicariance and evolutions of the species of limpets studied and try to find out when the divergence occurred and what paleogeographic phenomenon has caused it.

STUDY AREA

Our study was performed on the Algerian coast, from east to west; we collected two species of limpets: *Patella rustica*, *Patella ferruginea* (**Fig. 1**). Algeria has a coastline about 1280 km, the Algerian-Moroccan border in the west to the Algerian-Tunisian border to the east [6].

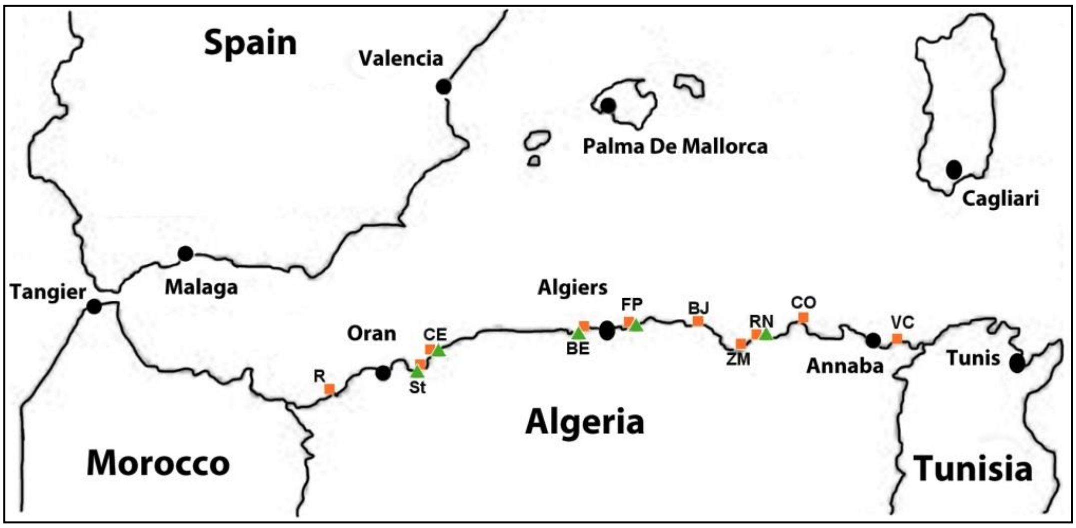

Figure 1: Map showing the sites where the species of limpets have been sampled along the Algerian coastline. The orange squares represent the stations where the species *P. rustica* was collected, and the green triangles represent the stations where the species *P. ferruginea* was collected.

Collection of samples

We chose several samples from the Far East to the extreme west of the Algerian coast as it is shown in Figure 1. Abbreviations and sampling stations are given in Table 1.

Table 1: Abbreviations and details of study sites

Station name	Region name	Geographic situation
VK	Vieille calle (El Kala)	36.9° N, 8.45° E
CO	Collo	36.9° N, 6.53° E
RN	Rocher Noir	36.78° N, 5.63° E
ZM	Ziama Mensouria	36.74° N, 5.56° E
BO	Boulimate	36. 87° N, 4.84° E
FP	Figuier Plage	36.78° N, 3.53° E
BE	Bérard	36.6° N, 2.6° E
CE	Cap Evi	36.12° N, 0.23° E
ST	Stidia	35.83° N, 0° E
R	Rechgoun	35.30° N, 1.46° O

MATERIALS AND METHODS

We chose several samples from the Far East stations to the extreme west of the Algerian coast as it is shown in Figure 1.

Molecular study

The molecular work based on tissue samples taken by scalpel from the podia pulpit of limpets sampled after having carefully washed with distilled water. The samples were stored in 96% Ethanol. Mitochondrial DNA was extracted from tissues and used for PCR amplification [7]. Extraction of mtDNA was made following the "JETQUICK tissue DNA Kit" (This protocol is a modification of the method published by Botwell (1987)) [8]. After extracting mtDNA of our samples, we performed PCR amplification [7]. We have isolated the portion of the mitochondrial COI gene using the sense primer HCO (5'-CATGGATGACCACGACACTC-3') and anti-sense LCO (GGTCAACAAATCATAAAGATATATTGG 5'-3') [9].

Sequencing was performed according to the method of Sanger *et al.* (1977) [10]. We sequenced 584 bp of 24 individuals of *Patella rustica* (Pr), 16 individuals of *Patella ferruginea* (Pf) and four individuals of *Patella caerulea* (Table 2).

Table 2 Table showing the number of sequenced species for each station studied

Species / Stations	*Patella rustica* (Pr)	*Patella ferruginea* (Pf)	*Patella caerulea* (Pc)
VK	6	-	1
RN	3	1	-
ZM	1	-	-
FP	5	5	-
BE	1	2	-
CE	2	4	-
ST	6	4	-
R	-	-	3

Data Analysis

Relationships between individuals or populations are visualized via the construction of phylogenetic trees. After aligned mtDNA sequences scrutinized by the BioEdit program [11] and manually, we proceeded to develop phylogenetic trees, but before that we had to find the best statistical model that could give us the most robust tree. For this, we used the "jModeltest 0.1.1" [12] program. As for the development of phylogenetic trees, "Mega 5.0" program [13] has allowed us to build trees using the method of maximum likelihood [14] "ML". The relationship between haplotypes can be understood through the construction of haplotype networks using the "Median Joining" method [15]. The construction of these networks is based on the theory of coalescence [16].

RESULTS

Phylogenetic and phylogeographic study

The phylogenetic study has been prepared by developing trees by the method of Maximum Likelihood "ML". By using the "jModeltest 0.1.1" [12] program, the "Jukes-Cantor" model was considered as the most suitable to study the distribution parameter and the parameter Gamma invariable site "(G+ I) ". Using a number of replicas bootstrap 100, The Phylogenetic tree of all species sequenced in this study was prepared according to the "ML" method using 584 Pb aligned with the "BioEdit" program [11] and manually.

Sequence analysis revealed, among those species studied, the existence of three strongly divergent groups as shown in **Figure 2** based on the average distances between individual pairs of populations with Log shows (L) = -1349.54 (L being the likelihood). The species *Patella vulgata* (*P. vulgata*), taken from genbank, is used as an "out group" to have a good reference for a better topology.

Between the group of *P. rustica* and *P.caerulea* there is a significant difference of about 14%, however, between *P. ferruginea* and *P. caerulea* there is also a wide divergence of the order of 13.6%. Between *P. rustica* and *P. ferruginea* there is a smaller difference of 9.9%. The significant difference in the three groups is more marked than the average divergence within each group and it is less than 1%.

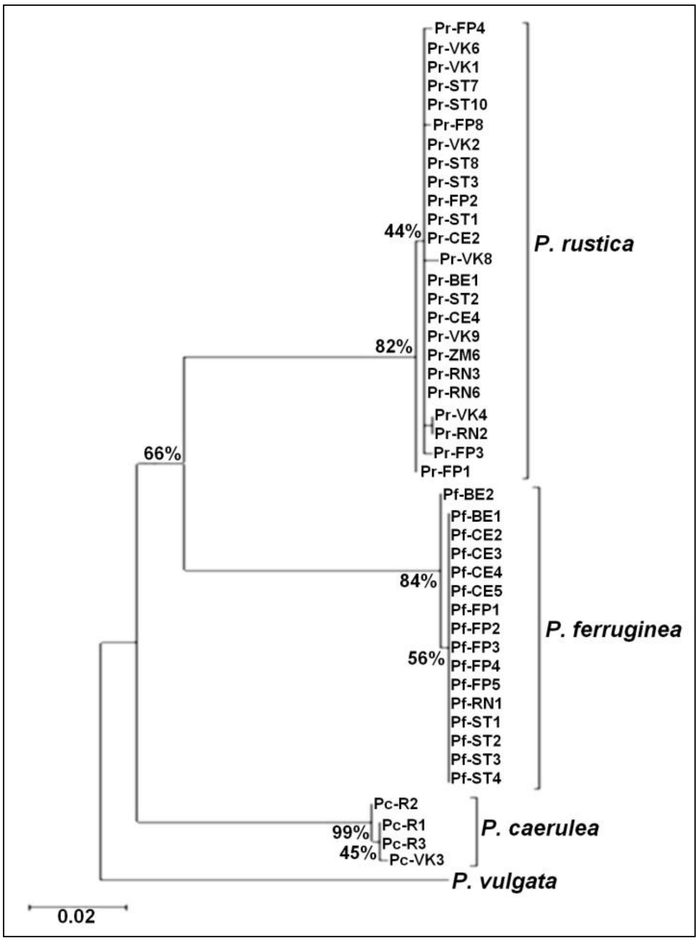

Figure 2: Phylogenetic tree of sequenced species obtained by "ML" method with a number of 100 bootstrap replicas

We used the "relaxed clock" model with a log-normal distribution of substitution rates, with two different mean values (0.6% and 1.2% per million years) [17] and [18] for molluscs, a classically encountered in animals rates [19]. A difference of 9.9% could state that the nearest common ancestor (TMRCA) of the two species (*P.rustica* and *P. ferruginea*) could be between 8.25 and 4.125 MA, for the two species *P. rustica* and *P. caerulea* the nearest common ancestor would be between 11.86 and 5.83 MA, and finally for *P.ferruginea* and *P. caerulea* the TMRCA is between 11.33 and 5.66 MA. Analyses of mitochondrial polymorphism, under the assumption of neutrality, provide interesting ideas about the history of the geographical expansion of each group of species studied. In the absence of selective effect, these features suggest a recent increase in the population size. We can, however, see the representation of minimal relations between haplotypes **(Fig. 3),** there are three distinct groups representing each studied taxa.

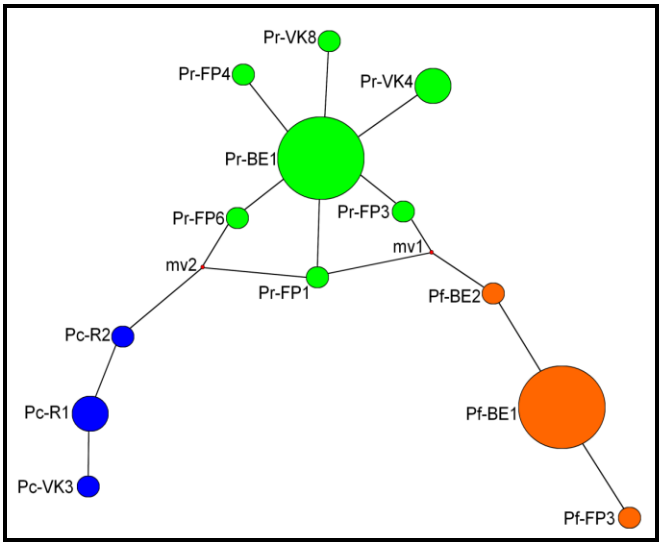

Figure 3: Networks of haplotypes representing minimal relations between haplotypes of three species of limpets present in our study.

DISCUSSION

The divergence between the group of *P. rustica* and *P. caerulea* is the most important, it is between 5.83 and 11.86 Ma, when located on the Upper Miocene (between 11.608 ± 0.005 and 5.332 ± 0.005 Ma) specifically the Tortonian (between 11.6 and 7.2 Ma.). This era also includes the period of divergence between *P. caerulea* and *P. ferruginea*. During this era, Europe as we know it today, was partially submerged by the sea surface and the temperature was higher with significant fauna and flora [20]. For *P. rustica* and *P. ferruginea*, the divergence is smaller, ranging between 8.25 and 4.125 Ma, it might have started during the Messinian by paleontological effects. The sea level in the Mediterranean had fallen to dryness; episode marks the Messinian Salinity Crisis. The Messinian crisis has created new habitats with sporadic distribution, generating an allopatric speciation between the Mediterranean and the Atlantic and the disappearance of many species. The episode ends in the early Pliocene (5.332 ± 0005 to 2.588 ± 0.005 Ma) by the opening of the Strait of Gibraltar [21]. The haplotype network (**Fig. 3**) shows that there is a star distribution. This would explain a recent population explosion, it could be due to the colonization of new territories after the last glacial maximum (20-19 KAAJ) which induced an increase in the sea level and thus the formation of the transition Atlantic waters to the Mediterranean through the Strait of Gibraltar.

REFERENCES

[1] Blondel J. : « Biogéographie : Approche écologique et évolutive ». Collection Ecologie, Masson Ed, 27 : 297 p, Paris. (1995).

[2] Bazzaz F. A., Chiariello N. R., Coley P. D. & Pitelka L. F.: « Allocating resources to reproduction and defence ». BioScience, 37(1): 58 pp. (1987).

[3] Lack D. L., Hodgkin A. L., Venables L. S. V., Mayr E, Hardy A. C., Thorpe W. H. & Tinbergen N.. « My life as an amateur ornithologist ». Ibis, 115(3): 421 (1973).

[4] Muths D. : « Structures génétique et démographique des populations de deux espèces d'ophiures aux caractéristiques biologiques contrastées, Acrocnida brachiata et Ophiothrix fragilis, en Atlantique Nord-Est » Thèse en vue de l'obtention du diplôme de doctorat de l'Université de ParisVI, 256 p. (2006).

[5] Palumbi S. R. :« Genetic divergence, reproductive isolation and marine speciation ». Annual Review of Ecology and Systematics, 25 : 547-572. (1994).

[6] Zeghdoudi E. : « Modélisation bioéconomique des pêcheries méditerranéennes Application aux petits pélagique de la baie de Bou Ismail (Algérie) ». Thèse en vue de l'obtention d'un diplôme de Master de science économique de géstion, Pesquera Barcelone, 60 p. (2006).

[7] Mullis K. B. & Faloona F. A. : « Specific synthesis of DNA in vitro via polymerasecatalyzed chain reaction ». Methods in Enzymology, 155 : 335–350. (1987).

[8] Botwell D. L. L. : « Rapid isolation of eukariotic DNA ». Anal. Biochem. 162: 463 p. (1987).

[9] White T. J., T. Bruns T., Lee S., Taylor J. W. (1990). Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. Pp. 315-322 In: PCR Protocols: A Guide to Methods and Applications, eds. Innis, M. A., D. H. Gelfand, Sninsky J. J., and White T. J.. Academic Press, Inc., New York.

[10] Sanger F., Nicklen S. & Coulson A. R. (1977). « DNA sequencing with chain terminating Inhibitors ». Proceedings of the National Academy of Sciences of the United States of America, 74(12) : 5463–5467.

[11] Hall T. A. : « BioEdit: a user-friendly biological sequence alignment editor and analysis program for windows 95/98/NT ». Nucl. Acids. Symp. Ser. 41: 95-98. (1999).

[12] Posada D. : « jModeltest 0.1.1, Phylogenetic Model Averaging ». Mol. Biol. Evol. 25 : 1253-1256. (2008).

[13] Tamura K., Peterson D., Peterson N., Stecher G.,Nei M., Kumar S. : « MEGA5: Molecular Evolutionary Genetics Analysis ». Software version 5.03, Bioinformatics. http://www.megasoftware.net/ (2011).

[14] Cavalli-Sforza L. L. & Edwards A.W.F. : « Phylogenetic analysis: models and estimation procedures ». American Journal of Human Genetics, 19 : 233-257. (1967).

[15] Bandelt H. J., Forster P. & Rohl A. (1999). « Median-Joining Networks for Inferring Intraspecific Phylogenies ». Mol. Biol. Evol. 16(1):37–48.

[16] Kingman J. F. C. : « The coalescent ». Stochastic Processes and their Applications, 13 : 235-248. (2000).

[17] Hellberg M. E. & Vacquier V. D. : « Rapid evolution of fertilization selectivity and lysine DNA sequences in teguline gastropods ». Molecular Biology and Evolution, 16 : 839–848. (1999).

[18]Marko P. B. : « Fossil calibration of molecular clocks and the divergence times of geminate species pairs separated by the Isthmus of Panama ». Molecular Biology and Evolution, 19 : 2005–2021. (2002).

[19] Rand D. M. : « Thermal habit, metabolic rate and the evolution of mitochondrial DNA ». Trends in Ecology & Evolution 9 : 125-131. (1994).

[20] Bachiri Taoufiq N & Barhoun N. : « La végétation, le climat et l'environnement marin au Tortonien supérieur dans le bassin de Taza-Guercif (corridor rifain, Maroc Oriental) d'après la palynologie ». Geobios, 34(1) : 13-24. (2001).

[21] Liu G., Leopold E. B., Liu Y., Wang W., Yu Z. & Tong G. : « Palynological record of Pliocene climate events in North China ». Review of Palaeobotany and Palynology Vol. 119, Issues 3-4, June 2002, Pages 335-340. (2002).