

LAGOONAL SAND SMELTS (*ATHERINA LAGUNAEA*, TELEOSTEI, *ATHERINIDAE*) INHABIT MARINE SHALLOW WATERS OF THE COASTS OF DJERBA ISLAND (TUNISIA)

Nawzet BOURIGA^{1,5}, S. SELMI², D. AURELLE³, R. BARTHELEMY³, J.-P. QUIGNARD⁴,
M. TRABELSI¹ AND E. FAURE⁵

¹ Unité de Biologie marine. Faculté des Sciences de Tunis. 2092 Campus universitaire. Tunisie.

² Institut National des Sciences et Technologies de la Mer INSTM, La Goulette 2060, Tunisie.

³ UMR 6540 DIMAR, Station Marine d'Endoume, Rue de la batterie des Lions, 13007 Marseille, France.

⁴ Laboratoire d'Ichthyologie, Université Montpellier II, Pl. E. Bataillon, case 102, 34095 Montpellier cedex, France

⁵ LATP, CNRS-UMR 6632, Evolution biologique et modélisation, case 5, Université de Provence, Place Victor Hugo, 13331 Marseille cedex 3, France.

ملخص

التصنيف الجيني لسماك أترينا البحيرات *Atherina lagunae* المتواجد على ضفاف جزيرة جربة : يعتبر سمك الأترينا الذي يعيش في البحر الأبيض المتوسط من الأسماك التي لها قدرة فائقة على التأقلم في العيش في جميع أنواع المياه. أكدت الدراسات الأخيرة أن سمك الأترينا ينقسم إلى 3 فصائل:

* فصيلتان تعيشان في البحار *Atherina punctata* et *Atherina boyeri*.

* فصيلة ثالثة تعيش في البحيرات و على ضفاف جزر قرقنة *Atherina lagunae*.

الهدف من هذه الدراسة هو معرفة إنتماء سمك الأترينا المتواجد بجزيرة جربة إلى أي فصيلة من الفصائل التي وقع ذكرها سابقا. أظهرت دراسة الخاصيات الجينية لقطعة من جين Cyt b إنتماؤها إلى الفصيلة الثالثة *Atherina lagunae* مثلها مثل أسماك جزر قرقنة.

كما أكدت الدراسة الفيلوجينية إنتماء أسماك بحيرة تونس و جزر قرقنة و جزيرة جربة إلى نفس المجموعة الجينية مما يمكننا من تصنيف هذه الأسماك ضمن Metapopulation core satellite.

الكلمات المفتاحية: سمك الأترينا *Atherina lagunae*, Cytochrome b, Metapopulation core satellite, جزيرة جربة.

RÉSUMÉ

Caractérisation génétique d'une population d'athérine lagunaire (*Atherina lagunae*, Teleostei, *Atherinidae*) peuplant les côtes peu profondes de l'île de Djerba (Tunisie). *Atherina boyeri* de la Méditerranée est un complexe hautement polymorphe ayant un génotype capable d'une réponse phénotypique flexible. Elle peut rapidement adapter les traits de sa morphologie à des environnements allant des eaux douces aux eaux saumâtres côtières polyhalines et aux eaux océaniques. Les travaux de recherche réalisés, en Méditerranée, sur ce complexe ont permis de le scinder en trois espèces. Deux espèces marines (*Atherina boyeri* et *Atherina punctata*) et une espèce qui peuplent les lagunes et les côtes des îles Kerkennah (*Atherina lagunae*) (Trabelsi *et al.* 2009).

Dans le présent travail l'analyse d'une portion du gène cytochrome b a été utilisée pour étudier les variations génétiques d'une population d'athérine, prélevée des côtes de l'île de Djerba, afin de situer cette population au sein du complexe *Atherina boyeri*. Nos analyses moléculaires ont montré que les athérines de l'île de Djerba tout comme celles des îles Kerkennah appartiennent à l'espèce *Atherina lagunae*. Toutes les analyses phylogénétiques ont montré que les athérines insulaires de l'île de Djerba constituent avec les athérines du lac de Tunis et celles des îles Kerkennah un clade qui est fortement supporté par les analyses statistiques. D'autre part, les séquences des athérines insulaires de l'île de Djerba et des îles Kerkennah montrent un taux élevé de divergence intrapopulationnel. Cependant les analyses d'une portion du gène cytochrome b des poissons du lac de Tunis présentent un faible taux d'hétérogénéité. Ces résultats pourraient suggérer un modèle de métapopulation avec des structures core-satellites dont les poissons insulaires représentent les cores; alors que ceux du lac de Tunis seraient une population satellite. L'ancêtre des athérines lagunaires pourrait être un poisson marin qui s'est adapté au milieu insulaire en premier lieu et qui a colonisé les lagunes en second lieu ou alors les poissons d'origine lagunaire ont fini par peupler les côtes insulaires. Cependant, le premier scénario pourrait être le plus plausible d'après l'hypothèse du modèle core-satellite, parce qu'il expliquerait la colonisation des milieux lagunaires avec une réduction de la diversité du gène.

Mots clés: *Atherina lagunae* Teleostei; Cytochrome b ; Métapopulation ; Core-Satellite; île de Djerba (Tunisie).

ABSTRACT

The Mediterranean *Atherina boyeri* species complex has been recently divided into three species, two living in marine environments (*A. boyeri* and *A. punctata*) and one found in lagoons and in shallow coasts of the Tunisian Kerkennah islands (*A. lagunae*) (Trabelsi *et al.* 2009). Genetic variation in a portion of the *cytochrome b* gene was examined for two populations belonging to the *A. boyeri* complex which have been caught in shallow waters of another Tunisian southern Island (Djerba). Similarly to the Kerkennah fish, molecular studies have shown that fish from Djerba, inspite of their marine habitat, belong to the *A. lagunae* species. All the phylogenetic analysis showed that the sequences of the Djerba insular fish constitute with the lagoon *Atherina* of the Lake of Tunis and Kerkennah insular fish a clade which is strongly statistically supported. In addition, within this clade, the both sequences from fish caught Djerba and Kerkennah coasts exhibit a great level of intrapopulational divergence between them. Whereas, analysis of partial *cytochrome b* gene of fish from the Lake of Tunis suggests a very low level of sequence heterogeneity. These results could suggest a metapopulation pattern with core-satellite structure in which cores are insular populations; whereas population of Lake of Tunis would be an example of satellite population. The ancestors of the *A. lagunae* can considered marine fish adapted in a first step to shallow waters of the island coasts and in a second step, they have colonized lagoons; or some lagoon fish could have migrated and lived on similar habitats such as island coasts. However, according to the core-satellite hypothesis, the first scenario could be the most realistic according to the fish colonization of lagoon habitats with reduction of its gene diversity.

Keywords: *Atherina lagunae* Teleostei; Cytochrome b; Metapopulation core-satellite; Djerba Island; Tunisia.

INTRODUCTION

Members of the *Atherina boyeri* complex Risso 1810 (Quignard and Pras, 1986; Bucciarelli *et al.* 2002) are extremely euryhaline fish which inhabit coastal and estuarine waters such as lagoon shallow brackish water ecosystems and inland waters. Recent studies coupling biometric and mitochondrial DNA data within this species complex, recognised at least three species: *A. boyeri*, *A. punctata* and *A. lagunae* as respectively non-punctuated marine, punctuated marine and lagoon atherines (Trabelsi *et al.* 2002a, b). Moreover, molecular investigations by mtDNA (Congiu *et al.* 2002; Astolfi *et al.* 2005) and allozyme analysis (Mauro *et al.* 2007) confirmed the Trabelsi *et al.* (2002a, b) hypothesis; by which *A. boyeri* can be considered as a complex of two different species (one marine and one living in lagoons and river mouths), punctuated fish were not present in all these last studies. The distribution of *A. boyeri* species ranges from the Western Atlantic coast of Spain to Mauritania and Madeira, being found throughout the Mediterranean and Black Sea. Some isolated populations have been reported on the coast of England and the Netherlands (Quignard and Pras, 1986). Nowadays, *A. punctata* has been found only on some rocky sea beds in the Western Mediterranean Sea, whereas *A. lagunae* has been found in lagoons along the Mediterranean coasts (Astolfi *et al.* 2005). Moreover, recently, we have shown that *Atherina* populations which inhabit marine shallow waters off shore the Kerkennah Islands coasts are *A. lagunae* (Trabelsi *et al.* 2009), including firstly sympatric speciations followed by a post-Pleistocene

colonisation of the lagoons. In this study, *Atherina* population off shore the Djerba Island coasts has been analysed.

MATERIAL AND METHODS

In total 9 specimens of *Atherina sp.* were collected from a site located on the Djerba Island Coast (33°48'N, 10°44'E). All of the individuals were preserved in 10% formalin. Data for these specimens are given in Table I. Accession numbers are in GenBank; except for Djerba Island fish, data concerning sequences of the *A. boyeri* complex are in Trabelsi *et al.* (2002b, 2004, 2009). For each groups, the number of sequences (seq) used for phylogenetic analyses (when identical sequences have been found, only one sequence has been used in the dataset) are indicated; whereas, the total number of sequences is in brackets. Concerning the outgroup sequences, four of them are from *A. presbiter* (Canary Islands and Madeira Archipelago, Spain; Roscoff and Arcachon Basin, France) and one from *A. hepsetus* (Gulf of Lion, France). More data are in Trabelsi *et al.* (2009).

In this study, 160 previously obtained sequences of Atherids were used, including *Atherina lagunae*, *Atherina boyeri*, and *Atherina punctata* issuing from French and Tunisian coasts (Trabelsi *et al.* 2002b, 2004). As already described in Trabelsi *et al.* (2002b), the procedure is only briefly summarised here. DNA sequences were obtained from specimens collected in Tunisian islands. Total DNA was extracted from approximately 0.25 cm² of caudal fin and a section of 376 bp of mitochondrial (mt) DNA;

Table I. Data concerning the various sequences use for phylogenetic analyses

		Western Mediterranean Sea									
Country		France				Tunisia					
Habitat		Marine		Lagoon	Marine		Lagoon	Islands Coasts			
Species	Location	Gulf of Lion, Thau	Gulf of Lion, Agde	Gulf of Lion, Ayrolle Maughio	Cap Zebib Hergla Monastir	Cap Zebib Hergla	North Tunis Lake	Kerkennah Archipelago			Djerba
		Corsica, Scandola	Corsica, Lavezzi Scandola	Corsica, Biguglia				Sidi Youssouf	Sidi Fraj	El Attaya	
<i>A. boyeri</i>		11seq (28seq)			9seq (28seq)						
<i>A. punctata</i>			12seq (15seq)			7seq (9seq)					
<i>A. lagunae</i>				15seq (39seq)			1seq (12seq)	TKSY 5seq (11seq)	TKSF 7seq (12seq)	TKEA 6seq (10seq)	TD 7seq (9seq)

genome from the *cytochrome b* gene was amplified by polymerase chain reaction (PCR) using published specific primers New-For:

5'-AGCCTACGAAAACCCACCC-3'

and 34-Rev 5'-

AAACTGCAGCCCCTCAGAATGATATTTGTCCTCA-3' (Cantatore *et al.* 1994).

Using the single-stranded DNA as a template, the nucleotide sequence was determined with an automated DNA sequencer. For phylogenetic reconstruction a Maximum likelihood (ML) approach was chosen with the algorithm of Guindon and Gascuel (2003) implemented in the Phyml software. The model of molecular evolution used for reconstruction was chosen with the Modeltest software (Posada and Crandall, 1998) using the FindModel website at Los Alamos National Laboratory (<http://hcv.lanl.gov/content/hcv-db/findmodel/findmodel.html>). This allowed us to choose among 28 nucleotide models with the Akaike Information Criteria (Akaike, 1974). The chosen model was the HKY model with Gamma distribution of rate variation among sites. The parameter of the Gamma distribution as well as the base frequencies and the transition / transversion ratio were estimated by the software. In each case, the robustness of the topologies was tested by using 1000 bootstraps (Felsenstein, 1985). The tree was visualised with the MEGA software version 4 (Tamura *et al.* 2007).

RESULTS

The partial sequence (376 pb) of the mitochondrial *cyt b* gene for 164 French and Tunisian *Atherina boyeri* complex individuals including *A. lagunae* has been previously determined (Trabelsi *et al.* 2002b, 2004). For this study, 9 of individuals from Djerba Island were examined. Preliminary analyses demonstrated that 121 of the 376 positions were variable, whereas, 97 were informative for unweighted parsimony, and that no saturation effects were observed for the different substitution patterns. As no saturation was observed, all the positions were used for the construction of phylogenetic trees. Molecular analysis using three reconstruction methods (MP, ML and NJ) gave the same general topology with the same main groups. In the figure 1, only the topology of the ML analysis has been shown with bootstraps results of the three methods. Bootstraps values (BP) were generally good for inner nodes thereby supporting the main clades.

For the first time, a molecular dataset contains five species of the genus *Atherina*, including all the species present in the Mediterranean Sea. The three phylogenetic methods used (NJ, MP and ML) give similar topologies (Fig. 1). When *A. hepsetus* and *A. presbyter* sequences have been used as outgroups, species of the *A. boyeri* complex form a clade which is supported by high bootstrap (BP) values (100% in ML, NJ and MP).

The topologies of the sequences of the *A. boyeri* complex are similar to those already published (Trablesi *et al.* 2002b, 2004, 2009). The species complex is divided in three clades corresponding to the three species according to Trablesi *et al.* (2002a, b), all of them strongly supported statistically (BP always $\geq 99\%$). Briefly, the *A. boyeri* clade contains sequences from France and Tunisia without

geographical distribution; whereas, the *A. punctata* clade is divided in two groups, one contains the sequences of Tunisian punctuated fish and the other those of French fish. However, if the first clade has strong bootstrap values (ML: 97%, NJ: 99% and MP: 88%), the second group is only statistically supported using the NJ method (93%).

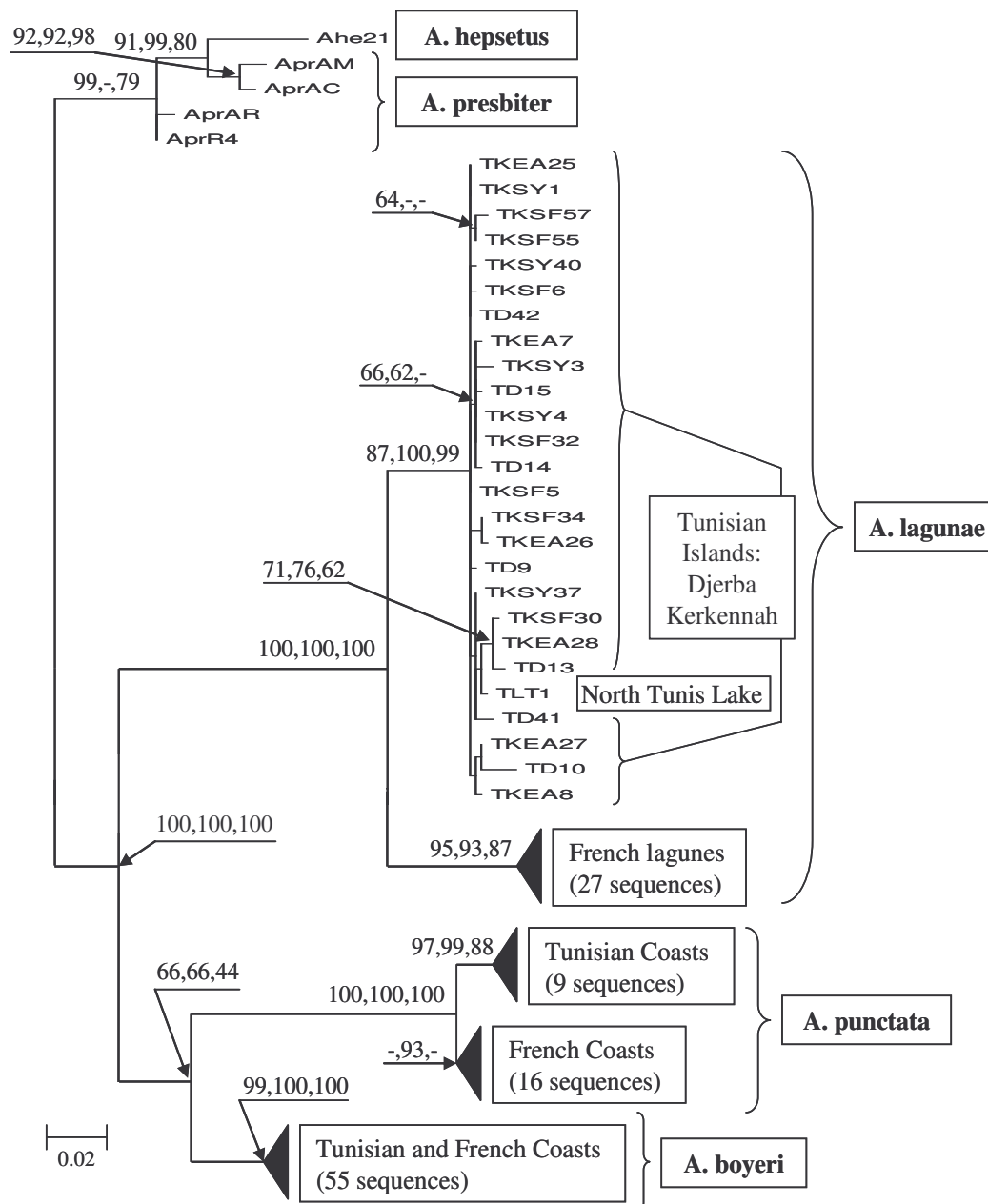


Figure 1. Phylogenetic tree of European *Atherina* species. Sequences of both *A. presbiter* and *A. hepsetus* have been used as outgroups. Bootstrap values (BP in %), carried out with 1000 iterations, are given for each node only if they exceed 60, Maximum Likelihood (left BP), Neighbor Joining (middle BP) and Maximum Parsimony (right BP).

The last group contains all the sequences of lagoon fish added to those of the *A. boyeri* complex caught off shore the coasts of Djerba and Kerkennah Islands. French lagoon fish constitute the sister group of Tunisian lagoon and insular fish, these two groups are strongly supported (BP from 87 to 100%). The most surprising result of this phylogenetic analysis is the grouping of sequences of all insular fish with those of *A. lagunae* from the Lake of Tunis. Twelve individuals caught from this Lake have been sequenced and all of them have strictly the same sequence. Moreover, the phylogenetical analyses do not separate Kerkennah fish from those of Djerba. In this group, the topology of the three trees suggest several subgroups but they were not supported statistically excepted one (BP, ML: 71%, NJ: 76% and MP: 62%) which contains sequences from Djerba Island (TD14) and two from two different sites of the Kerkennah Islands (TKE28 and TKS30) which cluster with sequences from Tunis Lake fish with BP $\leq$ 39%. Moreover, the level of genetical divergence for both fish from Kerkennah and Djerba Islands is similar to those observed between respectively, *A. boyeri*, *A. punctata*, or the French lagoon fish. In addition, some sequences of fish caught from the Kerkennah Coasts are similar to those of fish from Djerba Island.

DISCUSSION

Molecular analysis suggested that insular sand smelts from Djerba are *A. lagunae*; this grouping is supported by high bootstrap values (100%). Some fish from Kerkennah have strictly the same haplotype as some of Djerba fish, suggesting a gene flow around distance of 100 km. In addition, previous analysis have shown a gene flow between the three populations caught on various sites on the coasts of the Kerkennah islands, which are distant to each other by 9 to 19 km (Trabelsi *et al.* 2009). Marine steams and storms could partially allow gene flow within the coasts of Kerkennah islands and within theses ones and the Djerba Coasts.

Moreover, insular sand smelts sequences have shown a remarkable level of divergence between them, which is similar to that observed between French and Tunisian *A. boyeri*, or between French and Tunisian *A. punctata* (Trabelsi *et al.* 2002b, 2004). Island and lagoon *A. lagunae* fish population could constitute a core-satellite metapopulation. In this model, the dominant metapopulation structure is that of a large local population in a large favourable habitat patch, surrounded by relatively unstable populations, which cannot maintain themselves without migration, in smaller habitat patches (Boorman and Levitt, 1973; Harrison, 1991). Moreover, in this bimodal distribution, either movements are unidirectional with smaller sink populations being recolonized or

supplemented from the source population, there is a smaller reciprocal movement or exchange of individuals from the sink populations to the source population (Hanski, 1982; Gotelli, 1991; Hanski and Gyllenberg, 1993). Concerning *A. lagunae* populations, insular fish which have the greatest genetic diversity, suggesting numerous gene flows, are the core populations, whereas lagoon fish constitute the satellite populations. Indeed, satellite populations showed a low level of genetic diversity and all the partial *cyt b* regions from the fish caught in the Lake of Tunis sequenced recently are strictly identical between them. This last data could suggest that the gene flow from islands to lagoon would be broken; moreover, distances between the Lake of Tunis and Kerkennah and Djerba Islands which are respectively 400 and 500 km, could explain the limitation of the gene flow (3 diff/TLT and TKEA28B). However lagoon populations are probably well adapted to their habitats, but, if great changes occur only gene flow from island population(s) could increase genetic diversity and allow responding to environmental stress. The deleterious effect of consanguinity has also been studied by several authors, who have observed that this favours the build-up of anomalies during development (Kincaid, 1976; Paperna, 1978; Piron, 1978).

Very high level of spinal misformation had been found in Tunisian lagoon sand smelts - approximately 10 % of the fish exhibit severe kyphosis – this could be due, at least to possible allelic loss and reduced molecular variability added to recent physical and chemical changes in the habitat (Ayed *et al.* 2007). Contrarily, in the *A. lagunae* individuals from three French lagoons, for which the level of nucleotide variations can reach 4.3% (Trabelsi *et al.* 2004), no spinal misformation were found.

The absence of phylogenetic break between the lineages from Kerkennah and Djerba suggested that there is no barrier to the gene flow between these two populations, for example, 3 sequences issuing from fish caught from the Djerba Coasts (TD43B, TD42B and TD44B) are identical to those from fish caught on the three sites of the Kerkennah coasts (TKEA29E, TKS5, TKS531B, TKS54, TKS533, TKS538, TKS539, TKS536B, TKS52, TKS535B and TKS51). In the “core satellite” metapopulation concept, the source population(s) has a constant and high emigration rate. Contrarily, the high number of divergence between some insular sequences suggests that these fish could be submitted to a rapid evolution. Speciations in shallow-waters were shown or strongly suggested for numerous marine organisms including fish and invertebrates (Hampton *et al.* 1984; Palumbi, 1994; Casanova and Nair, 2002), numerous *A. lagunae* populations differentiation occurred being able to give incipient species.

However, the evolution of these fish is submitted to two opposite forces; indeed, evolution in shallow waters could appear inducing possible speciation but as the habitats are not strongly isolated between them, which partial homogenisation of the populations can occur in the course of time. This scenario could explain the phylogenetic structuration of the insular fish which constitute an undifferentiated group with lagoon Tunisian fish. An ecological feature is flavourous to this hypothesis, in the Kerkennah islands, the very particular type of *Posidonia* fields named "herbier tigré" constitute disrupted habitat which facilitated population fragmentation (Blanpied *et al.* 1979; Boudouresque *et al.* 1990). Moreover, *P. oceanica* meadows are considered to be the most important ecosystem in the Mediterranean and could constitute a nursery for insular sand smelts. It could be also the case for the Bou Grara Lagoon (between the Djerba Island and continental coast), and to a less extent by the Biban Lagoon (less than 70 km to the South far from Djerba Island).

CONCLUSION

The present study suggests strongly a metapopulation structure of Tunisian *A. lagunae* populations. the ancestors of the *A. lagunae* were marine fish adapted in a first step to shallow waters environment of the island coasts and in a second step, they have colonized lagoons, or some lagoon fish could have migrate and live on similar habitats such as those from island coasts. In the future, in order to investigate genetic structure and population history of this species, using microsatellite markers, populations of all other Tunisian lagoons and islands will be studied and compared to those of France.

BIBLIOGRAPHY

- Akaike H. 1974- A new look at the statistical model identification. IEEE Transactions on Automatic Control, 19: 716-723.
- Astolfi L., Dupanloup I., Rossi R., Bisol P.M., Faure E. and Congiu L. 2005- Mitochondrial variability of sand smelt (*Atherina boyeri*, Risso, 1810) populations from North Mediterranean coastal lagoons. Mar. Ecol. Prog. Ser., 297: 233-243.
- Ayed N., Faure E., Quignard J.P., Maamouri F. and Trabelsi M. 2008- Incidence of kyphosis deformities in natural population of *Atherina lagunae* (Trabelsi *et al.* 2002) from the Tunis North Lake, Tunisia. Mar. Biol., 153: 319-25.
- Blanpied C., Burolet P.F., Clairefond P., Shimi M. 1979- Sédiments actuels et holocènes. Ann. Univ. Provence, France, 6:61-82.
- Boorman S.A., and Levitt P.R. 1973- Group selection on the boundary of a stable population. Theo. Pop. Biol., 4:85-128.
- Boudouresque C.F., Ballesteros E., Ben Maiz N. 1990- Livre rouge "Gérard Vuignier" des végétaux, peuplements et paysages marins menacés de méditerranée. MAP Technical Reports Series n°43. Athens, PNUE, IUCN and GIS Posidonie: UNEP publications.
- Bucciarelli G., Golani D., Bernardi G. 2002- Genetic cryptic species as biological invaders: the case of a Lessepsian fish migrant, the hardyhead silverside *Atherinomorus lacunosus*. J. Exp. Mar. Biol. Ecol., 273: 143-9.
- Cantatore P., Roberti M., Pesole G., Ludovico A., Milella F., Gadaleta M.N. and Saccone C. 1994- Evolutionary analysis of cytochrome b sequences in some Perciformes evidence for a slower rate of evolution than in mammals. J. Mol. Evol., 39: 589-97.
- Casanova J.P. and Nair V.R. 2002- A new species of *Sagitta* (Chaetognatha) from a Laccadive lagoon (Indian Ocean) having fan-shaped anterior teeth: phylogenetical implications. J. Nat. Hist. 36: 149-56.
- Congiu L., Rossi R. and Colombo G. 2002- Population analysis of the sand smelt *Atherina boyeri* (Teleostei Atherinidae), from Italian coastal lagoons by random amplified polymorphic DNA. Mar. Ecol. Prog. Ser., 229: 279-89.
- Felsenstein J. 1985- Confidence limits on phylogenies: an approach using the bootstrap. Evolution, 39: 783-91.
- Gotelli N.J. 1991- Metapopulation models: the rescue effect, the propagule rain, and the core-satellite hypothesis, Am. Nat., 138:768-76.
- Guindon S. and Gascuel O. 2003- A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. Syst. Biol., 52:696-704.
- Hampton L., Carson A., and Alan R. 1984- Templeton Genetic Revolutions in Relation to Speciation Phenomena: The Founding of New Populations. Ann. Rev. Ecol. Syst., 15: 97-131.
- Hanski I. 1982- Dynamics of regional distribution: the core and satellite species hypothesis. Oikos., 38:210-21.
- Hanski I. and Gyllenberg M. 1993- Two general metapopulation models and the core-satellite species hypothesis. Am. Nat., 142: 17-41.
- Harrison S. 1991- Local extinction in a metapopulation context: An empirical evaluation. In: M. Gilpin and I. Hanski (eds.), Metapopulation dynamics. Academic Press, Inc., San Diego, pp. 73-88.

- Kincaid H.L. 1976- Inbreeding in rainbow trout (*Salmo gairdneri*). J. Fish. Res. Board. Can., 33: 2420-6.
- Mauro A., Arculeo M., Mazzola A. and Parrinello, N. 2007- Are there any distinct genetic sub-populations of sand smelt, *Atherina boyeri* (Teleostei: Atherinidae) along Italian coasts? Evidence from allozyme analysis. Folia. Zool., 56: 194-200.
- Palumbi S.R. 1994- Genetic divergence, reproductive isolation, and marine speciation. Ann. Rev. Ecol. Syst., 25: 547-72.
- Paperna I. 1978- Swimbladder and skeletal deformations in hatchery bred *Sparus aurata*. J. Fish. Biol., 12: 109-14.
- Piron R.D. 1978- Spontaneous skeletal deformities in the Zebra Danio (*Brachydanio rerio*) bred for fish toxicity tests. J. Fish. Biol. 13: 79-83.
- Posada D. and Crandall K.A. 1998- Modeltest: testing the model of DNA substitution. Bioinformatics. 14: 817-8.
- Quignard J.P. and Pras A. 1986- Atherinidae. In Whitehead PJP, Bauchot M-L, Hureau JC, Nielsen J, Tortonese E (eds) Fishes of the north-eastern atlantic and the mediterranean. Vol. 3. UNESCO, Paris, p 1207-10.
- Tamura K., Dudley J., Nei M. and Kumar S. 2007- MEGA4: Molecular Evolutionary Genetics analysis (MEGA) software version 4.0. Mol. Biol. Evol 10.1093/molbev/msm092.
- Trabelsi M., Bouriga N., Aurelle D., Quignard J.P., Barthelemy R., Faure E. 2009- Some Marine tunisian *Atherina boyeri* populations (Teleostei) have morphological and molecular characteristics of lagoon fishes. Open. Mar. Biol. J., 3: 59-68.
- Trabelsi M., Faure E., Quignard J.P., Boussaid M., Focant B., Maamouri. F. 2002 a- *Atherina punctata* and *Atherina laguna* (Pisces, Atherinidae) new species found in the mediterranean sea, Article 1: morphological investigations of three Atherinid species. C. R. Acad. Sci. III., 325: 967-75.
- Trabelsi M., Gilles A., Fleury C., Quignard J.P., Maamouri F., Faure E. 2002b- *Atherina punctata* and *Atherina lagunae* (Pisces, Atherinidae), new species found in the Mediterranean Sea. Article 2: molecular investigations of three Atherinid species. C.R. Acad. Sci. Paris, Life Sciences., 325:1119-28.
- Trabelsi M, Maamouri F, Quignard JP, Boussaïd M. and Faure E. 2004- Biometric and molecular investigations evidenced allopatric speciation in Western Mediterranean lagoons within the *Atherina lagunae* species (Teleostei, Atherinidae). Estuar. Coast. Shelf. Sci., 61: 713-23.