Significant Genetic Differentiation among meroplanktonic barrel jellyfish *Rhizostoma pulmo* (Cnidaria: Scyphozoa) in the Mediterranean Sea

AR Ben Faleh [a,*], H Allaya[a], A Armani[b] and AAB Shahin[c]

[a]*Marine Biology Unit, Faculty of Sciences of Tunis, University of Tunis El-Manar, Tunisia*

[b]*Fish Lab, Department of Veterinary Sciences, University of Pisa, Via delle Piagge 2, 56124 Pisa, Italy*

[c]*Department of Zoology, Faculty of Science, Minia University, 61519 El Minia, Egypt*

*corresponding author, e-mail: benfalahabdelraouf@yahoo.fr

Molecular data have shown that jellyfishes are more geographically restricted and evolutionarily diverged than previously thought. In the present investigation, we examined the genetic variation and divergence among some sampling sites of the meroplanktonic barrel jellyfish *Rhizostoma pulmo* over the Mediterranean Sea, using sequences of mtDNA cytochrome c oxidase subunit I (*COI*) gene. Sequence analyses were performed on 68 specimens collected from 19 sampling sites along the species distribution range over the Mediterranean Sea water, specifically from the North Adriatic, Western Mediterranean and Tunisian Coast. Of these 68 sequences, 45 (66.2%) were new in specimens collected from nine sampling sites along the Tunisian Coast. A total of 24 haplotypes, with relatively high levels of haplotype diversity (h = 0.866) and low levels of nucleotide diversity (π = 0.004), were obtained. Both of the phylogenetic tree and haplotype network analysis showed presence of three distinct phylogenetic lineages, i.e. populations, with separate geographic distribution. Occurrence of the three lineages was supported by presence of significant and high genetic differentiation between populations (F_{ST} = 0.757; P < 0.001). This significant high genetic differentiation could be attributed either to evolution of both intrinsic and extrinsic barriers to genetic exchange between populations of the three lineages or to local adaptation to variation in environmental conditions.

Keywords: *Rhizostoma pulmo*, mtDNA, genetic differentiation, North Adriatic, Western Mediterranean, Tunisian waters

Introduction

Increasing colonization and number of Scyphozoans in the new habitats has actually gained the attention of scientists to improve our knowledge about the ecology and life cycle of these taxa (Mills 2001). Indeed, the study of their distribution, life history and particularly their ecological parameters are of great interest because of the ecological and social impacts of their outbreaks (Purcell 2005). In addition, the impact of Scyphozoan mass occurrences on the economic and recreational activities has been well documented in several studies and this highlights the importance of these phenomena (Dawson and Hamner 2009, Richardson et al. 2009).

As far as known, the marine environment, in contrast to the terrestrial environment, has no clear geographic barriers and thus it represents a strong challenge for the phylogeography of its own taxa. Kuo and Avise (2005) detected phylogeographic breaks and phylogeographic patterns in many taxa as an evidence of major barriers to gene flow over the sea. In addition, many authors have assumed that organism determinism does not play significant role in divergence (Patarnello et al. 2007, Ayre et al. 2009). However, some others indicated that dispersal ability is an important determinant of phylogeographic patterns (see Ramšak et al. 2012 and references therein). As for example, species with planktonic larvae displayed phylogeographic structure as a response to contemporary oceanographic conditions, while those with restricted dispersion may be more likely to reflect historical processes (Pelc et al. 2009). In the light of these findings, study of scyphozoan species is also important because they have metagenetic life history, with planktonic medusae and benthic phase (meroplanktonic species) (Fuentes et al. 2011, Ramšak et al. 2012).

Interestingly, studies on molecular systematics and phylogeography of jellyfish species over the Mediterranean and European Seas have profoundly increased during the last decades and mitochondrial cytochrome *c* oxidase subunit I (*COI*) gene has been used as a

phylogenetic marker for more than two decades (Holland et al. 2004, Dawson 2005c, Ki et al. 2008, Stopar et al. 2010, Miller et al. 2012, Ramšak et al. 2012, Lee et al. 2013, Glynn et al. 2015). Generally, phylogeographic studies on scyphozoan species have aimed to investigate the genetic differences that characterize organisms inhabiting the boundaries between the marine biogeographic regions (Stopar et al. 2010). In fact, the adaptation to the environmental conditions prevalent within different biogeographic regions is an important factor in maintaining the distribution of taxa (Palumbi 1994, Dawson 2004, 2005b, c). Thus, phylogeographic approaches mostly help to understand the spatial distribution of genetic lineages within and among closely related species occurring in different environments (Stopar et al. 2010, Miller et al. 2012, Ramšak et al. 2012, Lee et al. 2013).

The barrel jellyfish *Rhizostoma pulmo* (Macri 1778) has a meroplanktonic life cycle and occurs in the Northern and Southern Adriatic, the Ionian, the Black, the Eastern and Western Mediterranean Seas (Ramšak and Stopar 2007, Purcell et al. 2012) and in the Mar Menor costal lagoon (South-eastern Spain) of the North Western Mediterranean (Mas 1999, Pagés 2001, Perez-Ruzafa and Aragon 2002, Lilley et al. 2009, Fuentes et al. 2011). In Tunisia, this species has a wide distribution along the coastline as well as in some lagoons (Daly-Yahia et al. 2003, Touzri et al. 2005, Addad et al. 2008).

Recently, Lee et al. (2013) and Prieto et al. (2013) confirmed the validity of the morphological classification of *R. pulmo* as a separate species. As it's known, few demographic (Galil 2000, Kogovšek et al. 2010), ecological (Lilley et al. 2009, Gili et al. 2009) and biological (Fuentes et al. 2011, Purcell et al. 2012, Waryani et al. 2015) studies are reported on *R. pulmo* in the Mediterranean and European Seas. In addition, recent studies based on mitochondrial *COI* gene (Ramšak et al. 2012; Armani et al. 2013) and nuclear (ITS1 and ITS2) regions (Ramšak et al. 2012) ~~are~~ have been carried on for *R. pulmo* along the Mediterranean Sea. However, these investigations ~~are~~ have been carried out only on 13

specimens: eight from North Adriatic, five from South Mediterranean (Tunisian Coast) (Ramšak et al. 2012) and ten from Western Mediterranean (Armani et al. 2013).

Here, phylogenetic analyses of *R. pulmo* were conducted on more samples collected along the Tunisian Coast, using sequences of mtDNA *COI* gene to: 1) describe in details the genetic diversity of *R. pulmo* in this region and 2) compare the present results with that previously published by Ramšak et al. (2012) and Armani et al. (2013) to check whether or not there is a recent evidence of genetic differentiation occurred by this species in the three biogeographic regions, Tunisian Coast, North Adriatic and Western Mediterranean.

Commented [p1]: I suggest many or a wider number of instead of more

Material and methods

Sampling

A total of 45 specimens of the barrel jellyfish *Rhizostoma pulmo* (Macri 1778) were collected from nine different localities along the Tunisian Coast during local blooms between March and September 2013 (Figure 1, Table S1). Medusae specimens were preserved on ice and transported to the laboratory where they were morphologically examined and dissected. Then, tissue samples (bell margin or gonads) were collected from the umbrella and stored in 96 % ethanol for subsequent DNA extraction.

DNA extraction, amplification and sequencing

Total genomic DNA was extracted from the ethanol-preserved tissues using a CTAB-phenol/chloroform based protocol (Dawson et al. 1998, Dawson and Jacobs 2001). The DNA amount was then determined with a NanoDrop ND-1000 spectrophotometer (NanoDrop Technologies, DE, USA). The purity of DNA was evaluated by the ratio of absorbance at 260/280nm. Polymerase chain reaction (PCR) was performed using a PTC-100 thermal cycler (MJ Research Inc., Watertown, MA, USA). The original universal invertebrate primers LCO1490 (5'-GGTCAACAAATCATAAAGATATTGG-3') and

HC02198 (5'-TAAACTTCAGGGTGACCAAAAAATCA-3') (Folmer et al. 1994) were used for the amplification of the *mtCOI* gene. PCRs contained 1 µl of template DNA, 5 µl 10X PCR buffer, 0.2 mM each dNTP (GeneAmp dNTP mix with dTTP, Applied Biosystems), 2.5 mM $MgCl_2$, 0.5 units (U) of Amplitaq (Biogene) and 0.5 µM each primer in a 50 µl volume. The PCR were performed under these conditions: 35 cycles at the following parameters: 1 min at 95°C, 1 min at 40°C, and 1.5 min at 72°C, followed by a final 72°C extension for 10 min. The amplification product (5 µl) was resolved by electrophoresis on 1.5% agarose gel. DNA bands were stained with ethidium bromide in a TBE buffer. The purified products were used as template DNA for cycle sequencing reactions performed by Macrogen (Seoul, Korea). The obtained sequences were analyzed using Clustal W in BioEdit version 7.0.5.2 (Hall 1999). Fine adjustments were manually made after visual inspection. The *COI* gene in protein was translated, and no insertions-deletions or stop codons were revealed, using MEGA (version 3.1) software (Kumar et al. 2004). All new sequences of *R. pulmo* collected in this study were deposited in GenBank under the accession numbers given in Table S1.

Formatted: Font: Italic

Commented [p2]: Suggestion : The nucelotide sequence of the Coi gene was translated in the corresponding aminoacid sequence

Phylogenetic analyses

The 45 Tunisian *COI* sequences of *R. pulmo* obtained in this study were combined with all available *R. pulmo COI* sequences from GenBank. In particular, 23 sequences, eight sequences were obtained from North Adriatic (Ramšak et al. (2012), ten from Western Mediterranean (Armani et al. 2013) and five from Tunisia (Ramšak et al. 2012), were included in the analyses (see Table S1). So, our data set consists of a total of 68 sequences, which were allocated into three separate biogeographic regions: North Adriatic (NA), Western Mediterranean (WM) and Tunisian Coast (T). The sequence of *Nemopilema nomurai* (Kishinouye 1922) (EU373728) and *Rhopilema esculentum* (Kishinouye 1891) (EU373723) were used as outgroups. JMODELTEST version 0.1.1 (Posada 2008) was run to determine the

most suitable model of DNA evolution to be used for our dataset of sequences using hierarchical log-likelihood-ratio tests and the Akaike information criterion. The TIM1+G with a gamma distribution shape parameter equal to 0.120 and the proportion of invariables sites equal to 0.331 were the best evolutionary model for the dataset.

Evolutionary relationships among all haplotypes were inferred by constructing phylogenetic trees using the median-joining method available in NETWORK version 4.2.0.1 (Bandelt et al. 1999) and Bayesian Markov chain Monte Carlo (MCMC) phylogenetic analyses (BI) included in MRBAYES version 3.1 using the default priors (Huelsenbeck and Ronquist 2001, Ronquist and Huelsenbeck 2003). Three heated chains and a single cold chain were employed in all MCMC analyses, and runs were initiated with random trees. Two independent MCMC runs were conducted with two million generations per run and trees and parameters were sampled every 100 generations. Stationary was assessed by examining the average standard deviation of split frequencies and the potential scale reduction factor (Ronquist et al. 2005). For each run, the first 25% of sampled trees were discarded as burn-in. Bayesian posterior probabilities were used to assess branch support of the MCMC tree.

Genetic diversity and geographic structure

Genetic diversity expressed by haplotype (*h*) and nucleotide (π) diversities (Nei 1987), and their standard deviations (± SD) (Tajima 1993), were estimated using DnaSP version 4.10.9 (Rozas et al. 2003). In addition, geographic structure within the three biogeographic regions was estimated with the analysis of molecular variance (AMOVA), using ARLEQUIN version 3.1 (Excoffier et al. 1992) based on the pairwise matrix of distances between haplotypes, which were calculated using the appropriate model of evolution. Haplotypic correlation measure (F_{ST}) was estimated for all possible permutations among samples of the three biogeographic regions. Finally, the significance level of each haplotypic correlation was

tested by conducting a non-parametric permutation procedure 10,000 times in ARLEQUIN version 3.1 (Excoffier et al. 2005).

Results

Haplotype variation

A fragment of 655 base pairs (bp) length of *COI* was sequenced in 45 new specimens of *R. pulmo* collected from nine localities in Tunisia. Thus, we analyzed 68 sequences (23 sequences were used from GenBank and 45 were obtained from this study) and we found a total of 24 distinct mtDNA haplotypes (ten haplotypes were previously identified and 14 were new) with a total of 33 sites, 23 of which were variable and only ten were parsimony informative. Of the 24 haplotypes, 16 (66.66%) were specific to the three sampling sites, 15 (62.5%) were exclusive to the Tunisian Coast samples and five (20.83%) were shared among samples from each biogeographic region. Measures of genetic diversity (haplotype diversity (*h*) and nucleotide diversity (π)) were calculated across the three biogeographic regions (Table1). The overall values of *h* and π were 0.866 ± 0.029 and 0.004 ± 0.007, receptively. Values of *h* and π within the samples of the three biogeographic regions were in the range of 0.644 to 0.857 and 0.001 to 0.003, receptively. Samples of the North Adriatic region showed the highest haplotype and nucleotide diversity (h = 0.857± 0.108 and π = 0.003), while the lowest values (h = 0.644 and π = 0.001) were observed in those of the Western Mediterranean region (Table 1).

Phylogenetic analyses

The phylogenetic analyses generated three lineages corresponding to samples of the three biogeographic regions. The first cluster included the specimens originated from the North Adriatic (NA); the second comprised those of the Western Mediterranean (WM), while the third contained those of the Tunisian Coast (T) (Data not shown). This phylogenetic structure

was also evidenced by the minimum spanning network, which similarly generated three geographic groups of haplotypes corresponding to samples of the three biogeographic regions (Fig. 2).

Genetic diversity and geographic structure

Genetic structure of *R. pulmo* samples from the three biogeographic regions was assessed by the pairwise F_{ST} estimates. Significant high levels of genetic differentiation were found between samples of the three biogeographic regions (F_{ST} = 0.757; P < 0.001). Those of the Western Mediterranean region showed the highest level of differentiation (F_{ST} = 0.763; P < 0.001), while the lowest value was found in those of the North Adriatic region (F_{ST} = 0.752; P < 0.001). More specifically, the F_{ST} values were 0.706 between samples of the Western Mediterranean and North Adriatic regions, 0.722 between those of the latter and Tunisia Cost samples, and 0.788 between those of the latter and Western Mediterranean samples (P < 0.001). In addition, AMOVA analysis showed that 75.74% of variation was explained between samples of the three biogeographic regions (Table 2).

Discussion

In the present investigation, we analyzed 68 specimens of *R. pulmo* collected from 19 sampling sites along the North Adriatic (NA), Western Mediterranean (WM) and Tunisian Coast (T) waters to analyze the genetic diversity as well as to check whether or not there is genetic differentiation occurred by this species in these three biogeographic regions. Mitochondrial DNA cytochrome c oxidase subunit I (*COI*) gene sequences revealed genetic structuring or separation into three phylogenetic lineages, i.e., populations, supported by high and significant F_{ST} value (0.757; P < 0.001).

As previously known, the periods of warmer and cooler climate occurred during the Quaternary have greatly shaped the genetic structure of many extant marine species (Stopar et

al. 2010, Yebra et al. 2011). Such episodes of habitat fragmentation are periods in which gene flow between two basins is extremely limited in numerous marine species including scyphozoan species (Bargelloni et al. 2003, Hellberg, 2009, Dong et al. 2015). These episodes might have triggered the sorting of mtDNA lineages into ancestors of likely possible North Adriatic, Western Mediterranean and Tunisian biogeographic regions.

Recently, phylogeographic studies on several scyphozoan jellyfish species such as *Catostylus mosaicus* (Dawson 2005a), *Cassiopea spp.* (Holland et al. 2004), *Aurelia sp*. (Ramšak et al. 2012), and *R. octopus* (Glynn et al. 2015) revealed different phylogegraphic patterns with deep genetic divergence between lineages in different geographic areas. In more detail, Glynn et al. (2015) investigated the genetic structure of the scyphozoan jellyfish *R. octopus* in the Irish and Celtic Seas using mitochondrial cytochrome oxidase I and revealed prominent genetic differentiation (F_{ST} = 0.300 ; P < 0.001) among populations of the sampling sites. Such intraspecific phylogeographic structuring exhibited by some Semaeostomeae species suggested restricted gene flow (Dawson and Jacobs 2001, Schroth et al. 2002, Dawson 2003, 2005c, Holland et al. 2004), which could be explained by metagenetic life histories including sessile phase (Stopar et al. 2010). Interestingly, two ecological adaptations such as temperature of strobilisation and strobilisation frequency are found to be the main reasons of genetic differentiation displayed by some scyphozoan jellyfishes (Schroth et al. 2002, Stopar et al. 2010). Thus, it is apparent that *R. pulmo* similar to some other scyphozoans belonging to Semaeostomeae and Rhizostomeae which have metagenetic life histories, has a geographically distinct genetic structure. Schroth et al. (2002) reported that the genetic differentiation in *A. aurita* vary with temperature and frequency of strobilisation. Also, Miller et al. (2012) analyzed the genetic structure of *P. noctiluca* populations in Southern Africa and found that there is a significant differentiation between the Northern and Southern Atlantic populations groups. Similarly, Ramšak et al. (2012) reported that *A. aurita*

have different phylogeographic patterns in the Black, North Atlantic and Adriatic Seas and that *Aurelia ssp.* are divided into several cryptic species with no genetic structure between them. Additionally, Ramšak et al. (2012) claimed that *Aurelia sp.* in North Adriatic is adapted to temperate boreal temperatures for strobilation; while in Adriatic Sea it is adapted to specific climatic factors such as minimum winter temperature for strobilation, which occurs during the cold part of year with major differences form other geographic regions. Conversely, Stopar et al. (2010) recorded clear absence of genetic structure between populations of *P. noctiluca* from the Mediterranean Sea and NE Atlantic Ocean and presence of high levels of gene flow between these biogeographic regions.

In the present study, high haplotype diversity (h = 0.866) and low nucleotide diversity (π = 0.004) were observed in the *COI* gene among populations of the three lineages. This significant pattern of genetic differentiation (F_{CT} = 0.757; P < 0.001) could be attributed either to evolution of both intrinsic (specific natural history including dispersal ability and habitat) and extrinsic (ecological, climatic, geological) barriers to genetic exchange between populations of the three lineages (Avise 2000, Goodbody-Gringley et al. 2011, Lerp et al. 2011), or to local adaptation to variation in environmental conditions that causes different individuals of the same species to experience distinct forces of natural selection. These forces can cause local populations to evolve traits that provide an advantage in the local environment regardless of their consequences in other environments (Miller et al. 2012).

In addition, this high genetic differentiation could arise from the natural discontinuity of the habitats occupied, the environmental heterogeneity affecting the biogeographic regions, and the genetic drift (Lee et al. 2013). According to literature, several studies showed that temperature of stobilation (Purcell et al. 2009), salinity and light (Purcell et al. 2007, 2009), food supply (Han and Uye 2010) and oxygen concentration (Ishii et al. 2008) could shape the

genetic differentiation of scyphozoan species such as *Aurelia* species-complex originating in the Mediterranean Sea (Purcell et al. 2015).

Moreover, the genetic diversity of *R. pulmo* populations surveyed here was comparable to the previous data of this species across the North Adriatic and South Mediterranean Seas (h = 0.910; π = 0.48) as well as to other scyphozoan taxa such as *Aurelia aurita* (h = 0.862; π = 0.015) (Ramšak et al. 2012) and *Pelagia noctiluca* (h= 0.962, π = 0.011) (Stopar et al. 2010). Nevertheless, the present finding of genetic structure is inconsistent with that reported on the same species from North Adriatic and Tunisian waters (Ramšak et al. 2012).

Finally, we can conclude that ecological adaptations, phenotypic, and ecological differences may be occurred by divergent selection between populations of of *R. pulmo* collected from the three geographic regions and led to this obvious high genetic differentiation (Ramšak et al. 2012).

Acknowledgments

The authors are grateful to all people who participated in the collection of the samples. We also thank the two referees who reviewed our paper for their very useful and informative comments.

References

Addad S, Daly–Yahia MN, Belghith H, Zamamouch S, Goy J, Daly-Yahia-Kéfi O. 2008. Analyse taxinomique, écologique et caractérisation biochimique des scyphoméduses de la baie de Bizerte et de Tunis (Juillet-Décembre 2004). Systématique et écologie. *Bulletin de la Société Zoologique de France* 133: 33–41.

Armani A, Tinacci L, Giusti A, Castigliego L, Gianfaldoni D, Guidi A. 2013. What is inside the jar? Forensically informative nucleotide sequencing (FINS) of a short mitochondrial

COI gene fragment reveals a high percentage of mislabeling in jellyfish food products. *Food Research International* 54: 1383–1393.

Avise JC, 2000. Phylogeography: The History and Formation of Species. Cambridge, MA: Harvard University Press, p. 447.

Ayre DJ, Minchinton TE, Perrin C. 2009. Does life history predict past and current connectivity for rocky intertidal invertebrates across a marine biogeographic barrier? *Molecular Ecology* 18: 1887–1903.

Bandelt H J, Forster P, Rohl A. 1999. Median-joining networks for inferring intraspecific phylogenies. *Molecular Biology and Evolution* 16: 37–48.

Bargelloni L, Alarcon JA, Alvarez MC, Penzo E, Magoulas A, Reis C, Patarnello T. 2003. Discord in the family Sparidae (Teleostei): divergent phylogeographical patterns across the Atlantic–Mediterranean divide. *Journal of Evolutionary Biology* 16: 1149–1158.

Daly-Yahia MN, Goy J, Daly-Yahia-Kefi O. 2003. Distribution et écologie des Méduses (Cnidaria) du golfe de Tunis (Méditerranée sud occidentale). *Oceanologica Acta* 26: 645–655.

Dawson MN. 2003. Macro-morphological variation among cryptic species of the moon jellyfish, *Aurelia* (Cnidaria: Scyphozoa). *Marine Biology* 143: 369–379.

Dawson MN. 2004. Some implications of molecular phylogenetics for understanding biodiversity in jellyfishes, with emphasis on Scyphozoa. *Hydrobiologia* 531: 249–260.

Dawson NM. 2005a. Incipient speciation of *Catostylus mosaicus* (Scyphozoa, Rhizostomeae, Catostylidae), comparative phylogeography and biogeography in south-east Australia. *Journal of Biogeography* 32: 515–533.

Dawson NM. 2005b. Coupled biophysical global ocean model and molecular genetic analyses identify multiple introductions of cryptogenic species. *Proceedings of the National Academy of Sciences of the United States of America* 102: 11968–11973.

Dawson MN. 2005c. Renaissance taxonomy: integrative evolutionary analyses in the classification of Scyphozoa. *Journal of the Marine Biological Association of the United Kingdom* 85: 733–739.

Dawson MN, Raskoff KA, Jacobs DK. 1998. Field preservation of marine invertebrate tissue for DNA analyses. *Molecular Marine Biology and Biotechnology* 7: 145–152.

Dawson MN, Jacobs DK. 2001. Molecular evidence for cryptic species of *Aurelia aurita* (Cnidaria, Scyphozoa). *Biological Bulletin* 200: 92–96.

Dawson MN, Hamner WM. 2009. A character-based analysis of the evolution of jellyfish blooms: adaptation and exaptation. *Hydrobiologia* 616: 193–215.

Dong Z , Liu Z, Liu D. 2015. Genetic characterization of the scyphozoan jellyfish *Aurelia spp*. in Chinese coastal waters using mitochondrial markers. *Biochemical Systematics and Ecology* 60: 15–23.

Excoffier L, Smouse P E, Quattro J M. 1992. Analysis of molecular variance inferred from metric distances among,DNA haplotypes: application to human mitochondrial DNA restriction data. *Genetics* 131: 479–491.

Excoffier L, Laval G, Schneider S. 2005. ARLEQUIN version 3.0: an integrated software package for population genetics data analysis. *Evolutionary Bioinformatics* 1: 47–50.

Folmer O, Black M, Hoeh W, Lutz R, Vrijenhoek R. 1994. DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Molecular Marine Biology* and *Biotechnology* 3: 294–299.

Fuentes V, Straehler-Pohl I, Atienza D, Franco I, Tilves IU, Gentile M, Acevedo M, Olariaga A, Gili J.M. 2011. Life cycle of the jellyfish *Rhizostoma pulmo* (Scyphozoa: Rhizostomeae) and its distribution, seasonality and inter-annual variability along Catalan coast and the Mar Menor (Spain, NW Mediterranean). *Marine Biology* 158: 2247–2266.

Galil BS. 2000. A Sea under siege - alien species in the Mediterranean. *Biological Invasions* 2: 177–186.

Gili JM, Atienza D, Fuentes V, Lewinsky I. 2009. Medusa project Technical to the Catalan water Agency n° 2, p. 198.

Glynn F, Houghton DRJ, Provan J. 2015. Population genetic analyses reveal distinct geographical blooms of the jellyfish *Rhizostoma octopus* (Scyphozoa). *Biological Journal of the Linnaean Society* 116 : 582–592.

Goodbody-Gringley G, Woollacott RM, Giribet G. 2011. Population structure and connectivity in the Atlantic scleractinian coral *Montastraea cavernosa* (Linnaeus, 1767). *Marine Ecology*: 1–17.

Hall TA. 1999. bioedit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium Series* 41: 95–98.

Hamner WM, Dawson MN. 2009. A review and synthesis on the systematics and evolution of jellyfish blooms: advantageous aggregations and adaptive assemblages. Hydrobiologia 616: 161–191.

Han CH, Uye SI. 2010. Combined effects of food supply and temperature on asexual reproduction and somatic growth of polyps of the common jellyfish *Aurelia aurita* s. l. *Plankton and Benthos Research* 5: 98–105.

Hellberg EM. 2009. Gene Flow and Isolation among Populations of Marine Animals. *Annual Review of Ecology, Evolution, and Systematics* 40: 291–310.

Holland BS, Dawson MN, Crow GL, Hofmann DK. 2004. Global phylogeography of Cassiopea (Scyphozoa: Rhizostomeae): molecular evidence for cryptic species and multiple invasions of the Hawaiian Islands. *Marine Biology* 145: 1119–1128.

Huelsenbeck JP, Ronquist F. 2001. MRBAYES: Bayesian inference of phylogenetic trees. *Bioinformatics* 17: 754-755.

Ishii H, Ohba T, Kobayashi T. 2008. Effects of low dissolved oxygen on planula settlement polyp growth and asexual reproduction of *Aurelia aurita*. *Plankton and Benthos Research* 3: 107–113.

Ki JS, Hwang DS, Shin K, Yoon WD, Lim D, Kang YS, Lee Y, Lee JS. 2008. Recent moon jelly (*Aurelia* sp.1) blooms in Korean coastal waters suggest global expansion: examples inferred from mitochondrial COI and nuclear ITS-5.8S rDNA sequences. *ICES Journal of Marine Science* 65: 443-452.

Kogovšek T, Bogunović B, Malej A. 2010. Recurrence of bloom-forming scyphomedusae: wavelet analysis of a 200-year time series. *Hydrobiologia* 645: 81–96.

Kumar S, Tamura K, Nei M. 2004. MEGA 3: integrated software for molecular evolutionary genetics analysis and sequence alignment. *Brief Bioinformatics* 5: 150–163.

Kuo CH, Avise JC. 2005. Phylogeographic breaks in low dispersal species: the emergence of concordance across gene trees. *Genetics* 124: 179–186.

Lee PLM, Dawson MN, Neill SP, Robins PE, Houghton JDR, Doyle TK, Hays GC. 2013. Identification of genetically and oceanographically distinct blooms of jellyfish. *Journal of the Royal Society Interface* 10: 1–11.

Lerp H, Wronski T, Pfenninger M, Plath M. 2011. A phylogeographic framework for the conservation of Saharan and Arabian *Dorcas gazelles* (Artiodactyla: Bovidae). *Organisms Diversity and Evolution* 11:317–329.

Lilley MKS, Houghton JDR, Hays GC. 2009. Distribution, extent of inter-annual variability and diet of the bloom-forming jellyfish *Rhizostoma* in European waters. *Journal of the Marine Biological Association of the United Kingdom* 89: 39–48.

Mas J. 1999. Estudio de la dinamica de poblaciones de las medusas cotylorhiza tuberculata, Rhizostoma pulmo y *Aurelia aurita en* el mar Menor y de su problematica associada. Instituto Espagnol de Oceanographia, internal report, p. 101.

Miller BJ, Von Der Heyden S, Gibbons M.J. 2012. Significant population genetic structuring of the holoplanktic scyphozoan *Pelagia noctiluca* in the Atlantic Ocean. *African Journal of Marine Science* 34: 425–430.

Miller M. R, Brunelli, J. P., Wheeler P. A., Liu S., Rexroad C. E., Palti Y., Doe C. Q. and Thorgaard G. H. 2012. A conserved haplotype controls parallel adaptation in geographically distant salmonid populations. *Molecular Ecology* 21, 237–249.

Mills CE. 2001. Jellyfish blooms: are populations increasing globally in response to changing ocean conditions? *Hydrobiologia* 451:55–68.

Nei M. 1987. Molecular Euolutionary Genetics. Columbia Univer-sity Press, New York, p 512.

Pagés F. 2001. Past and present anthropogenic factors promoting the invasion, colonization and dominance by jellyfish of a Spanish coastal lagoon. CIESM workshop ser, 14, 69–71.

Palumbi SR. 1994. Genetic divergence, reproductive isolation, and marine speciation. *Annual Review of Ecology, Evolution, and Systematics* 25: 547–572.

Patarnello T, Volckaert FAMJ, Castilho R. 2007. Pillars of Hercules: is the Atlantic–Mediterranean transition a phylogeographical break? *Molecular Ecology* 16: 4426–4444.

Pelc RA, Warner RR, Gaines SD. 2009. Geographical patterns of genetic structure in marine species with contrasting life histories. *Journal of Biogeography* 36: 1881–1890.

Perez-Ruzafa A, Aragon R. 2002. Implicaciones de la gestion y el uso de las aguas subterraneas en el funcionamiento de la red trofica de una laguna costera. In Conflictos entre el desarrollo de las aguas subterraneas y la conservacion de los humedales: litoral mediterraneo, pp. 215–245. Ed. by J. M. Fornes, and M. R. Llamas.

Posada D. 2008. JMODELTEST: phylogenetic model averaging. *Molecular Biology and Evolution* 25: 1253–1259.

Prieto L, Armani A, Macias D. 2013. Recent strandings of the giant jellyfish *Rhizostoma luteum* Quoy and Gaimard, 1827 (Cnidaria: Scyphozoa: Rhizostomeae) on the Atlantic and Mediterranean coasts. *Marine Biology* 160: 3241–3247.

Purcell J .2005. Climate effects on formation of jellyfish and ctenophore blooms: a review. J *Marine Biological Association of United Kingdom* 85:461–476.

Purcell JE, Uye SI, Lo WT. 2007. Anthropogenic causes of jellyfish blooms and their direct consequences for humans: a review. *Marine Ecology Progress Series* 350: 153–174.

Purcell JE, Hoover RA, Schwarck NT.2009. Interannual variation of strobilation by the scyphozoan *Aurelia labiata* in relation to polyp density, temperature, salinity, and light conditions in situ. *Marine Ecology Progress Series* 375: 139–149.

Purcell JE, Atienza D, Fuentes V, Olariaga , Tilves U, Colahan C, Gili JM. 2012. Temperature effects on asexual reproduction rates of scyphozoan species from the northwest Mediterranean Sea. *Hydrobiologia* 690: 169–180.

Pascual M, Fuentes V , Canepa A, Atienza D, Gili JM, Purcell E J. 2015. Temperature effects on asexual reproduction of the scyphozoan *Aurelia aurita* s.l.: differences between exotic (Baltic and Red seas) and native (Mediterranean Sea) populations. *Marine Ecology* 36: 994–1002.

Ramšak, A, Stopar K. 2007. Dispersal ecology and phylogeography of Scyphomedusae in the Mediterranean Sea. *MarBEF Newsletter, Autumn*, 20–21.

Ramšak A, Stopar K, Malej A. 2012. Comparative phylogeography of meroplanktonic species, *Aurelia spp.* and *Rhizostoma pulmo* (Cnidaria: Scyphozoa) in European Seas. *Hydrobiologia* 690: 69–80.

Richardson AJ, Bakun A, Hays GC, Gibbons MJ. 2009. The jellyfish joyride: causes, consequences and management responses to a more gelatinous future. *Tree* 24:312–322.

Ronquist F, Huelsenbeck JP. 2003. MRBAYES 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* 19: 1572-1574.

Ronquist F, Huelsenbeck JP, Van der Mark P. 2005. MrBayes 3.1 Manual. http://mrbayes.csit.fsu.edu/

Rozas J, Sánchez Del Barrio JC, Messeguer X, Rozas R. 2003. DNASP, DNA polymorphism analysis by the coalescent and other methods. *Bioinformatics* 19: 2496–2497.

Schroth W, Jarms G, Streit B, Schierwater B. 2002. Speciation and phylogeography in the cosmopolitan marine moon jelly, *Aurelia sp*. *BMC Evolutionary Biology* 2: 1–10.

Stopar K, Ramšak A, Trontelj P, Malej A. 2010. Lack of genetic structure in the jellyfish *Pelagia noctiluca* (Cnidaria: Scyphozoa: Semaestomae) across European seas. *Molecular Phylogenetics and Evolution* 57: 417–428.

Tajima F. 1993. Simple methods for testing molecular clock hypothesis. *Genetics* 135: 599–607.

Touzri C, Daly-Yahia MN, Goy J. 2005. Le plancton gélatineux de la baie de Sousse (Méditerranée Méridionale). Systématique et écologie. *Bulletin de la Société Zoologique de France* 129: 379–392.

Yebra L, Bonnet D, Harris RP, Lindeque PK, Peijnenburg KTCA. 2011. Barriers in the pelagic: population structuring of *Calanus helgolandicus* and *C. euxinus* in European waters. *Marine Ecology Progress Series 428*: 135-149.

Waryani B, Siddiqui AZ, Khan HS. 2015. Occurrence and temporal variation in the size-frequency distribution of 2 bloom-forming jellyfishes, *Catostylus perezi* (L. Agassiz, 1862) and *Rhizostoma pulmo* (Cuvier, 1800), in the Indus Delta along the coast of Sindh, Pakistan. *Turkish Journal of Zoology* 39: 95–102.

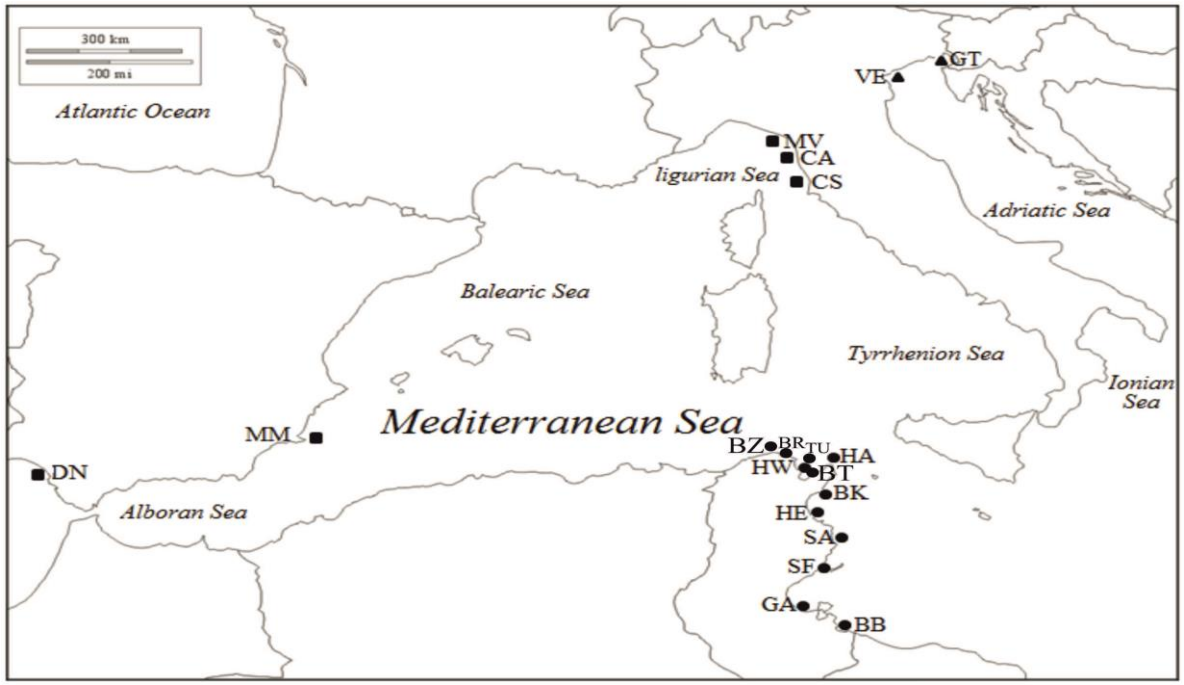

Figure 1: Sites of collection of the specimen of *R. pulmo* used in this study for the phylogenetic analyses. Geographic coordinates, location names and sample sizes are indicated in Table S1. The different symbols correspond to the three clades evidenced by the phylogenetic analyses: triangle (North Adriatic: NA), diamonds (Western Mediterranean: WM) and circles (Tunisia: T). For the locations abbreviations: (Gulf of Trieste, Slovenia (GT), Venice, Italy (VE), Calambrone (CA), Castiglioncello (CS), Marina di Vecchiano (MV), Doñana National Park (DN), Mar Menor (MM), (BT) Bay of Bizerte, (TU) Tunisia : Southern Mediterranean, (BT) Bay of Tunis, Bizerte (BZ), Halk El oued (HW), Hawaria (HA), Benikhiar (BK), Hergla (HE), Salakta (SA), Sfax (SF), Gabes (GA) and Bibane (BB))

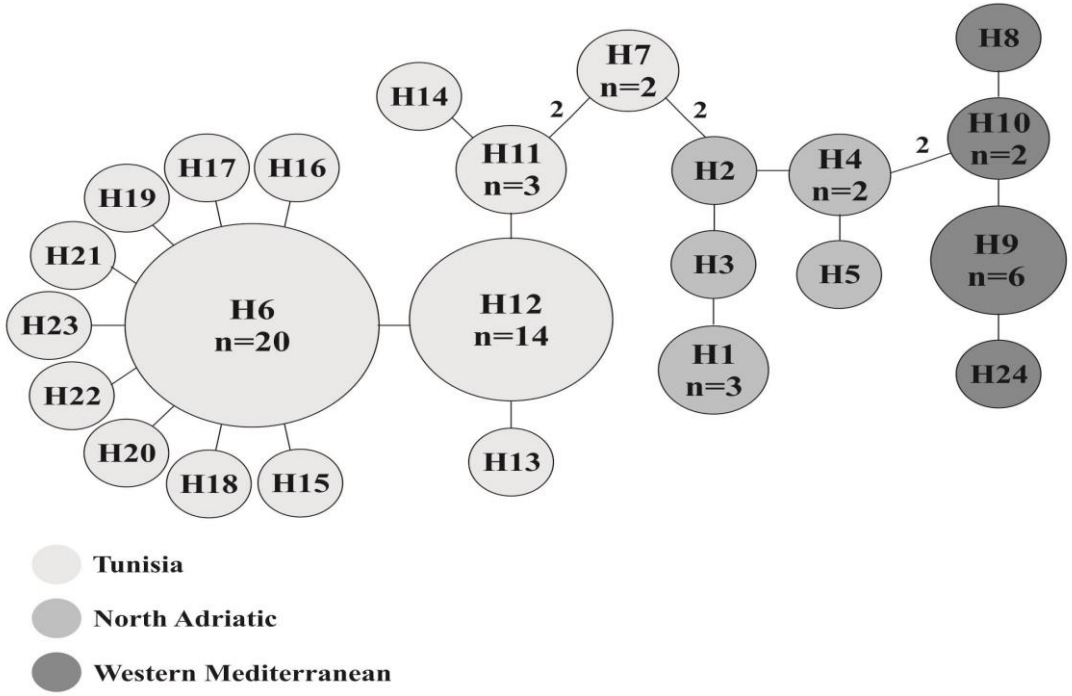

Figure 2: Median-joining network for *COI* haplotypes from *R. pulmo*. Numbers of mutations (greater than one) between haplotypes are indicated near branches, circle sizes are proportional to the number of similar haplotypes observed in the data set. See Table S1 for the haplotype designations

Table 1: Genetic variability in different *R. pulmo* geographic groups

Population	Sample size	No of haplotypes	Haplotype diversity	Nucleotide diversity ($\pi \pm SD$)
North Adriatic	**8**	**5**	0.857± 0.108	0.00289±0.00048
Western Mediterranean	**10**	**4**	0.644± 0.152	0.00132± 0.00041
Tunisia	**50**	**15**	0.767± 0.046	0.00200± 0.00028

Table 2: Analysis of molecular variance (AMOVA) for COI sequences of *R. pulmo* for North Adriatic/Western Mediterranean/Tunisia (*P<0.001)

Structure test	Source of variation	df	Sum of squares	Variance components	Pourcentage of variation	Fixation index
North Adriatic/Western Mediterranean / Tunisia	Among populations	2	60.307	2.0467	75.74	F_{ST}=0.757*
	Within populations	65	42.605	0.655	24.26	
	Total	67	102.912	2.702		

Table S1: Geographical locations, sample abbreviations and haplotypes of *Rhizostoma pulmo* used in this study

Groups	Locality	Geographical coordinates	Sample Code	Haplotype designation	Acession GenBank	Reference
North Adriatic	Gulf of Trieste, Slovenia	45.69444 N, 13.64166 E	RP1403	H1	GQ999568	Ramšak et *al.*, (2012)
			RP1404	H1	HQ902114	
			RP1407	H2	HQ902115	
			RP1408	H1	HQ902116	
			RP2202	H5	HQ902122	
	Venice, Italy	45.48888 N, 12.480055 E	RP1801	H3	HQ902120	
			RP1802	H4	GQ999569	
			RP1804	H4	HQ902121	
Western Mediterranean	Calambrone	43.74633 N 10.38571 E		H9	HF536559	Armani *et al.*, (2013)
				H9	HF536562	
	Castiglioncello	43. 4095 N 10.51085 E		H9	HF536560	
	Marina di Vecchiano	43.89849 N 10.33052 E		H9	HF536561	
	Doñana National Park	36.94758 N -6.4873 W		H24	HF930513	
	Mar Menor	37.67755N 0.957916 W		H10	HF545304	
				H10	HF545305	
		37.80924 N 0.92280 W		H9	HF545306	
				H9	HF545307	
				H8	HF545308	
Tunisia	Bay of Bizerte	37.28333N 9.96666 E	RP1602	H7	GQ999571	Ramšak et *al.*, (2012)
	Tunisia: South Mediterranean	37.28054 N 10.49166 E	RP1502	H6	HQ902117	Ramšak et *al.*, (2012)
			RP1503	H6	HQ902118	
			RP1605	H7	HQ902119	

	Bay of Tunis	36.7-36.88333N 10.28333-10.6166 E	RP1501	H6	GQ999570	
	Bizerte	37.27626 N 9.87307 E	RP1	H11		This study
			RP2	H12		
			RP3	H12		
			RP4	H12		
			RP5	H12		
	Halk El oued	36.8180 N 10.3050 E	RP6	H12		
			RP7	H12		
			RP8	H12		
			RP9	H12		
			RP10	H12		
	Hawaria	37.05000 N 11.016666E	RP11	H13		
			RP12	H12		
			RP13	H12		
			RP14	H12		
	Benikhiar	36.46666 N 10.78333 E	RP15	H11		
			RP16	H12		
			RP17	H11		
			RP18	H14		
			RP19	H12		
	Hergla	36.03333N 10.50000E	RP20	H6		
			RP21	H6		
			RP22	H15		
			RP23	H6		
	Salakta	35.39471 N 11.04006 E	RP24	H6		
			RP25	H6		
			RP26	H16		
			RP27	H6		

		RP28	H6	
Sfax	34.74515 N 10.76130 E	RP29	H6	
		RP30	H6	
		RP31	H6	
		RP32	H17	
Gabes	33.89326 N 10.10291 E	RP33	H18	This study
		RP34	H6	
		RP35	H19	
		RP36	H6	
		RP37	H6	
		RP38	H6	
Bibane	33.25197 N 11.41077 E	RP39	H20	
		RP40	H6	
		RP41	H21	
		RP42	H6	
		RP43	H22	
		RP44	H6	
		RP45	H23	