

Supplementary Material

Drivers of population structure of the bottlenose dolphin (*Tursiops truncatus*) in the Eastern Mediterranean Sea

Stefania Gaspari^a, Aviad Scheinin^{b,c}, Draško Holcer^{d,e}, Caterina Fortuna^f, Chiara Natali^a, Tilen Genov^{g,h}, Alexandros Frantziⁱ, Guido Chelazzi^a, André E. Moura^j

^aDepartment of Biology, University of Florence, Florence, Italy

^bThe Leon Recanati Institute for Maritime Studies and the Department of Maritime Civilizations, the Leon H. Charney School for Marine Sciences, University of Haifa, Haifa, Israel

^cIMMRAC (Israel Marine Mammal Research and Assistance center)

^dDepartment of Zoology, Croatian Natural History Museum, Demetrova 1, Zagreb, Croatia

^eBlue World Institute of Marine Research and Conservation, Kaštel 24, Veli Lošinj, Croatia

^fItalian National Institute for Environmental Protection and Research, Rome, Italy

^gMorigenos, Slovenian Marine Mammal Society, Piran, Slovenia

^hInstitute for Biodiversity Studies, Science and Research Centre, University of Primorska, Koper, Slovenia

ⁱPelagos Cetacean Research Institute, Vouliagmeni, Greece

^jSchool of Life Sciences, University of Lincoln, Lincoln, UK

Corresponding author: Andre E. Moura, amoura@lincoln.ac.uk, Tel: +44 (0)1522886805

Table S1. List of accession numbers used in the comparison with Atlantic mtDNA haplotypes, and corresponding source references.

Genebank Accession Number	Reference
AF268357	Parsons <i>et al.</i> 2002
AY963588-AY963626	Natoli <i>et al.</i> 2005
DQ073641-DQ073673 DQ073675-DQ073728 DQ525357-DQ525388	Quérrouil <i>et al.</i> 2007
FJ68001- FJ68019	Quérrouil <i>et al.</i> 2009a
GQ241419	Quérrouil <i>et al.</i> 2009b
HQ634245-HQ634252 HQ634254-HQ634258	Mirimin <i>et al.</i> 2011
KF650783-KF650837	Louis <i>et al.</i> 2014

Table S2. Pairwise Fst values between: A- the five Mediterranean basins compared in this study; B- within the Adriatic subdivided into sub-regions along its North/South and East/West directions.

A- Mediterranean				
	Adriatic	Ionian	Aegean	Tyrrhenian
Adriatic				
Ionian	0.034**(0.000)			
Aegean	0.002 (0.389)	0.031*(0.039)		
Tyrrhenian	0.062**(0.000)	0.073**(0.000)	0.036 (0.051)	
Levantine	0.081**(0.000)	0.067**(0.000)	0.037*(0.006)	0.146**(0.000)

B- Adriatic			
	Gulf of Trieste	Adriatic North	Adriatic East
Gulf of Trieste			
Adriatic North	0.050* (0.008)		
Adriatic Central-South	0.052* (0.005)	0.000 (0.047)	
Adriatic West			0.024** (0.000)

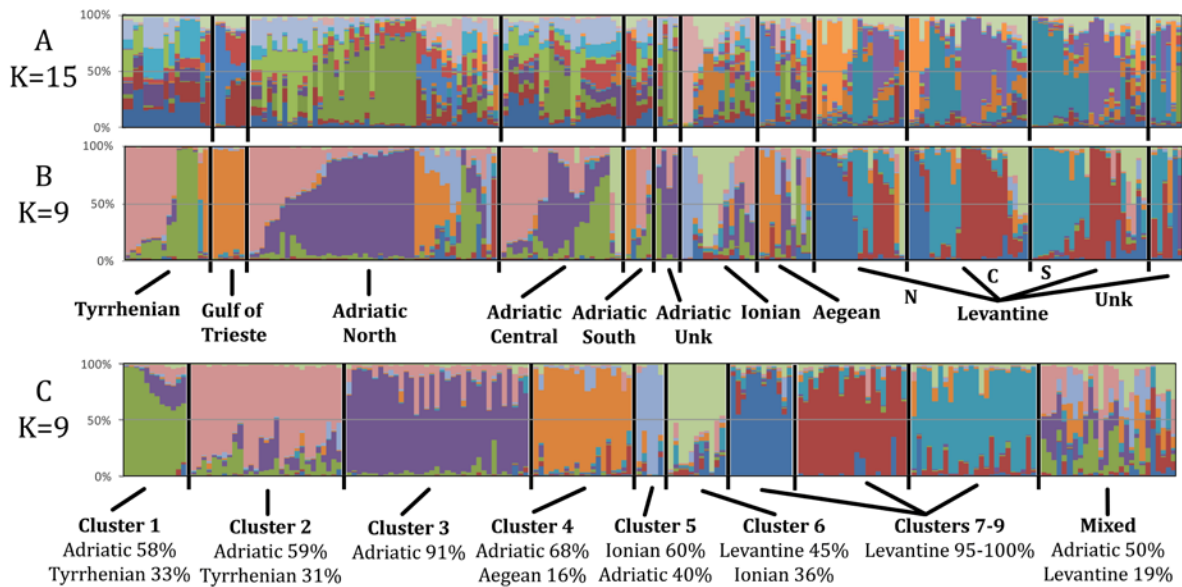


Figure S1. Individual ancestry plots using Bayesian clustering method implemented in the software STRUCTURE (Pritchard *et al.*, 2000), for all Mediterranean *Tursiops truncatus*. **A** - for K=15 with individuals ordered by sampling basin; **B** - for K=9 with individuals ordered by sampling basin; **C** - for K=9 with individuals ordered by cluster assignment. The proportions of the two most represented basins in each cluster, are included below. Unk identifies individuals sampled in a given basin, but whose exact location is unknown.

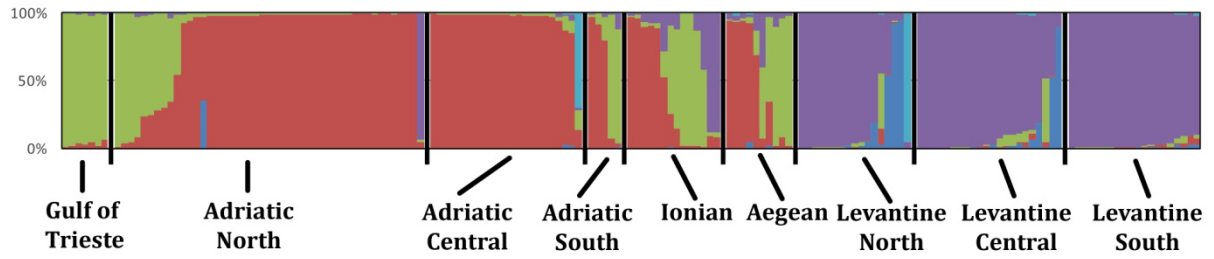


Figure S2. Individual ancestry plots using Bayesian clustering method as implemented in the software TESS (Durand *et al.*, 2011), to investigate local structure within the Eastern Mediterranean Sea (K=5).

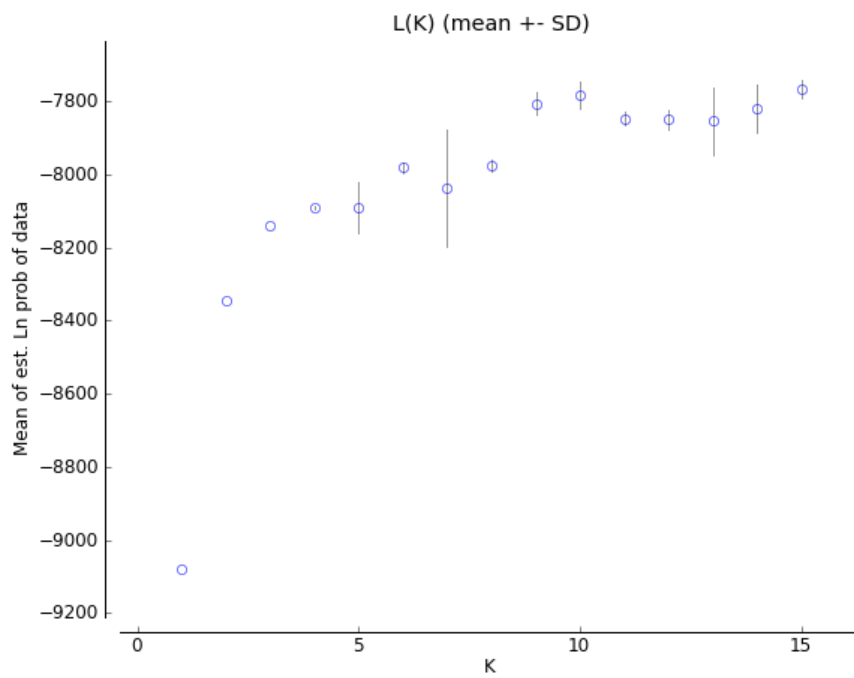


Figure S3. Likelihood plot for all STRUCTURE (Pritchard *et al.*, 2000) runs for K = 1-15. Plot drawn using STRUCTURE-HARVESTER (Earl & vonHoldt, 2012).

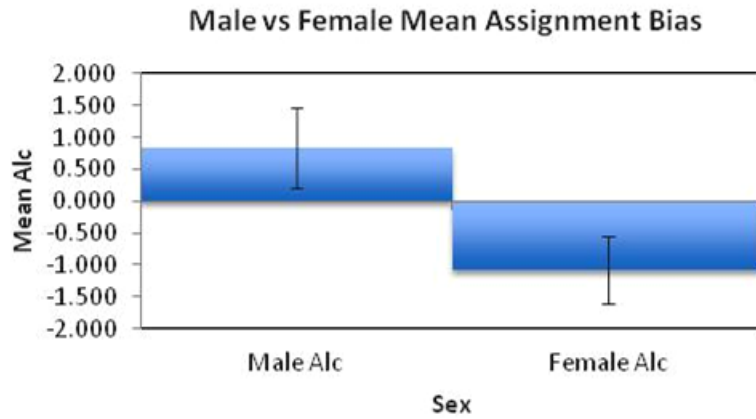


Figure S4. Mean Alc for male and female *Tursiops truncatus* in the Mediterranean sea, calculated based on microsatellite data using GenAlEx (Peakall & Smouse, 2006). Negative values indicate high frequency of migrants, while positive values indicate low frequency of migrants.

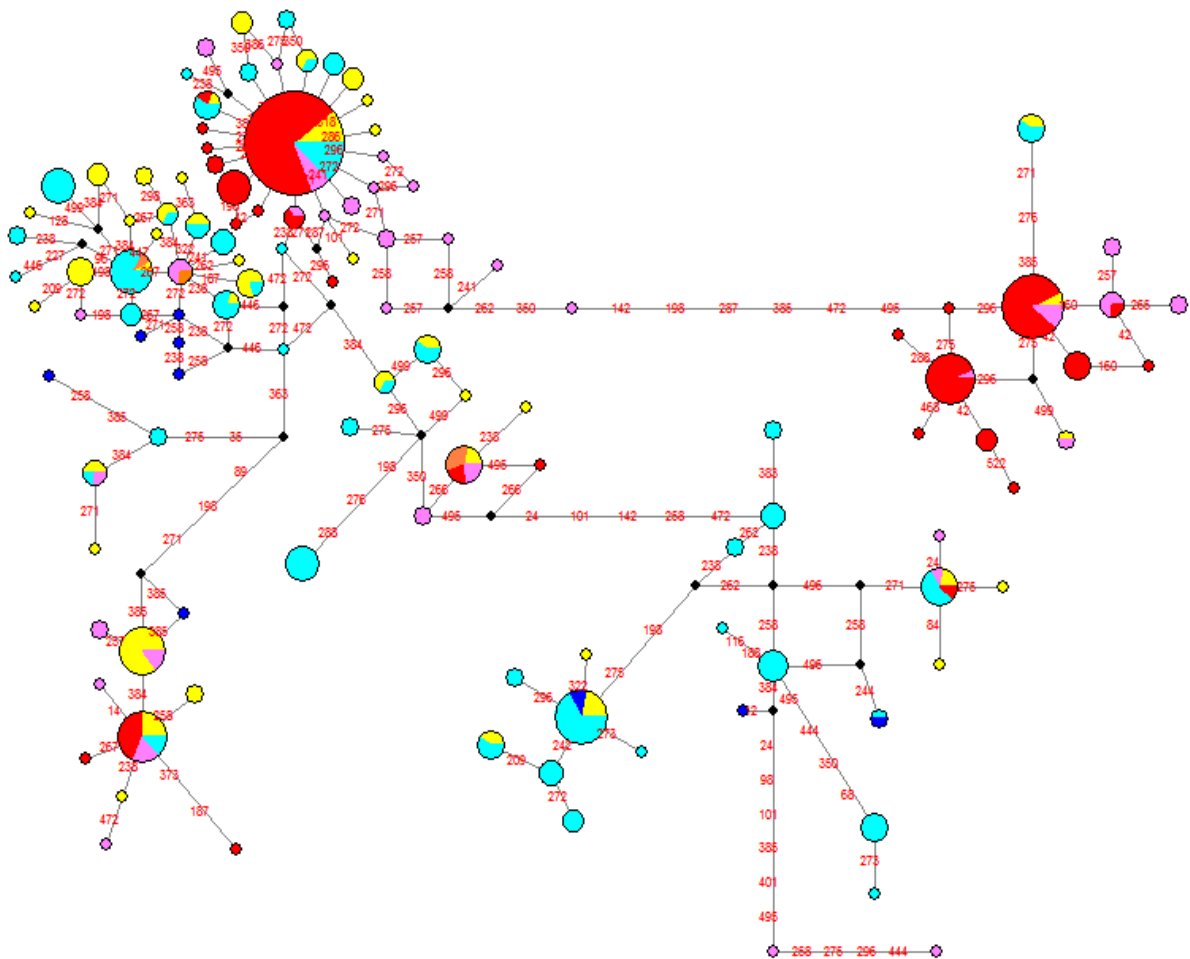


Figure S5. Medium joining network of all *Tursiops truncatus* mtDNA haplotypes produced in this study, together with mtDNA sequences from North Atlantic samples used in previous studies (Table S1). Dark Blue - Western North Atlantic Pelagic ecotype (as defined in Hoelzel et al 1998), data from previous studies; Light Blue - North Atlantic Pelagic (from Azores and Madeira), data previous studies; Yellow - Atlantic "coastal" European, data from previous studies. Pink - Mediterranean, data from previous studies; Red - Mediterranean "coastal", data from this study; Orange - Mediterranean Ionian sea "pelagic", data from this study. Numbers in red refer to single mutational steps.

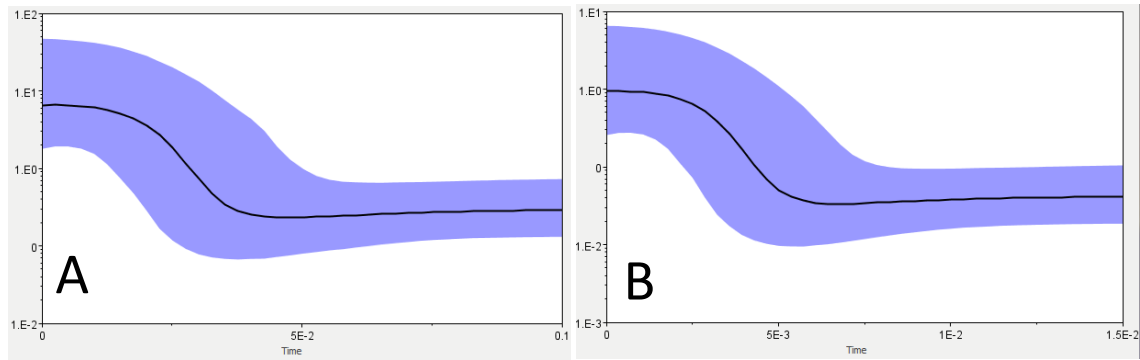


Figure S6. Bayesian skyline plot constructed using all mtDNA sequence obtained from Mediterranean *Tursiops truncatus* in this study, using the software BEAST (Drummond *et al.*, 2012). A fixed mutation rate was used, calculated using a date for the opening of the Bosphorus strait of 10 kya, as described in (Moura *et al.*, 2013). **A** - using the mutation rate calculated for the whole mitogenome; **B** - using the mutation rate calculated for D-Loop region only. See main text for more details.

References

- Drummond, A.J., Suchard, M.A., Xie, D., Rambaut, A. (2012). Bayesian phylogenetics with beauti and the beast 1.7. *Molecular Biology and Evolution*, 29, 1969-1973.
- Durand, E.Y., Patterson, N., Reich, D., Slatkin, M. (2011). Testing for ancient admixture between closely related populations. *Molecular Biology and Evolution*, 28, 2239-2252.
- Earl, D.A., vonHoldt, B. M. (2012). Structure harvester: A website and program for visualizing structure output and implementing the evanno method. *Conservation Genetics Resources*, 4, 359-361.
- Hoelzel, A. R., Potter, C. W., Best, P. B. (1998). Genetic differentiation between parapatric 'nearshore' and 'offshore' populations of the bottlenose dolphin. *Proc. R. Soc. Lond. B*, 265, 1177-1183.
- Louis M., Viricel A., Lucas T., Peltier H., Alfonsi E., Berrow S., Brownlow A., Covelo P., Dabin W., Deaville R. *et al.* (2014a). Habitat-driven population structure of bottlenose dolphins, *Tursiops truncatus*, in the North-East Atlantic. *Molecular Ecology*, 23, 857-874.
- Mirimin, L., Miller, R., Dillane, E., Berrow, S. D., Ingram, S., Cross, T. F., *et al.* (2011). Fine-scale population genetic structuring of bottlenose dolphins in Irish coastal waters. *Animal Conservation*, 14, 342-353.
- Moura, A.E., Nielsen, S. C. A., Vilstrup, J. T., Moreno-Mayar, J. V., Gilbert, M. T. P., Gray, H., *et al.* (2013). Recent diversification of a marine genus (*tursiops* spp.) tracks habitat preference and environmental change. *Systematic Biology*, 62, 865-877.
- Natoli, A., Birkun, A., Aguilar, A., Lopez, A., Hoelzel, A.R. (2005). Habitat structure and the dispersal of male and female bottlenose dolphins (*Tursiops truncatus*). *Proceedings of the Royal Society of London B*, 272, 1217-1226.
- Parsons, K. M., Noble, L. R., Reid, R. J., Thompson, P. M. (2002). Mitochondrial genetic diversity and population structuring of UK bottlenose dolphins (*Tursiops truncatus*): Is the ne scotland population demographically and geographically isolated? *Biol. Conservation*, 108, 175-182.
- Qu erouil S., Silva M. A., Freitas L., Prieto R., Magalh es S., Dinis A., Alves F., Matos J. A., Mendon a D., Hammond P. S. *et al.* (2007). High gene flow in oceanic bottlenose dolphins (*Tursiops truncatus*) of the North Atlantic. *Conservation Genetics*, 8, 1405-1419.
- Qu erouil, S., Silva, M.A., Cascao, I., Freitas, L., Alves, F., Dinis, A., Almeida, J.R., Prieto, R., Borr as, S., Matos, J.A., Mendonca, D. and Santos, R.S. (2009a). Molecular insight on the social organization of small delphinids in the pelagic waters of the Northeast Atlantic. *Genbank direct submission*
- Qu erouil, S. and Silva, M. (2009b). Population genetics of bottlenose dolphins around mainland Portugal. *Genbank direct submission*
- Peakall, R.O.D., Smouse, P.E. (2006). Genalex 6: Genetic analysis in excel. Population genetic software for teaching and research. *Molecular Ecology Notes*, 6, 288-295.
- Pritchard, J.K., Stephens, M., Donnelly, P. (2000). Inference of population structure using multilocus genotype data. *Genetics*, 155, 945-959.