**Table S1.** Haplotype frequencies observed have been reported for each sample locality.

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Genbank Accession Number** | **CLADE** | **Southern Adriatic Sea** | **Aegean Sea** | **Southern Sicily** | **Balearic Sea** | **Tyrrhenian Sea** | **Sardinian Sea** |
| **AY650768** | I | 13 | 4 | 16 | 15 | 3 | 10 |
| **MN652597** | I | 1 | 0 | 0 | 0 | 0 | 0 |
| **AY650778** | I | 2 | 2 | 2 | 6 | 1 | 1 |
| **AY650860** | I | 0 | 0 | 1 | 0 | 0 | 0 |
| **MN652598** | I | 1 | 0 | 1 | 0 | 0 | 0 |
| **MN652599** | I | 0 | 0 | 1 | 1 | 0 | 0 |
| **EU827759** | I | 0 | 0 | 0 | 2 | 0 | 0 |
| **MN652600** | I | 0 | 0 | 0 | 1 | 0 | 0 |
| **MN652601** | I | 0 | 0 | 0 | 0 | 0 | 1 |
| **AY650836** | I | 0 | 0 | 3 | 1 | 1 | 0 |
| **AY650821** | I | 0 | 0 | 0 | 1 | 1 | 0 |
| **MN652602** | I | 1 | 0 | 0 | 0 | 0 | 0 |
| **AY650858** | I | 0 | 0 | 0 | 0 | 0 | 1 |
| **MN652603** | I | 0 | 0 | 0 | 2 | 0 | 0 |
| **MN652604** | I | 0 | 0 | 0 | 0 | 0 | 1 |
| **MN652605** | I | 0 | 0 | 0 | 1 | 0 | 0 |
| **MN652606** | I | 2 | 0 | 0 | 0 | 0 | 0 |
| **MN652607** | I | 0 | 1 | 0 | 0 | 0 | 0 |
| **MN652608** | I | 0 | 0 | 0 | 1 | 0 | 0 |
| **AY650781** | I | 8 | 2 | 6 | 6 | 1 | 2 |
| **MN652609** | I | 2 | 0 | 0 | 1 | 0 | 0 |
| **AY650763** | I | 10 | 4 | 15 | 14 | 0 | 12 |
| **MN652595** | I | 0 | 0 | 1 | 1 | 0 | 0 |
| **AY650855** | I | 0 | 1 | 0 | 0 | 0 | 0 |
| **MN652596** | I | 0 | 0 | 0 | 0 | 1 | 0 |
| **AY650861** | I | 0 | 0 | 0 | 0 | 0 | 2 |
| **AY650809** | II | 1 | 0 | 0 | 1 | 0 | 1 |
| **MN652610** | II | 0 | 0 | 0 | 1 | 0 | 0 |
| **AY650762** | II | 16 | 2 | 12 | 24 | 2 | 17 |
| **AY650814** | II | 2 | 0 | 0 | 1 | 0 | 0 |
| **AY650805** | II | 0 | 0 | 0 | 2 | 1 | 0 |
| **AY650761** | II | 0 | 1 | 0 | 1 | 0 | 0 |
| **AY650829** | II | 0 | 0 | 1 | 0 | 1 | 0 |
| **EU827771** | II | 3 | 0 | 0 | 0 | 2 | 1 |
| **MN652611** | II | 0 | 0 | 0 | 1 | 0 | 0 |
| **EU827787** | II | 0 | 0 | 0 | 0 | 2 | 0 |

**Table S2.** GenBank accession numbers of the mtDNA CR haplotypes, clade assignment and frequencies observed in each temporal sample.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Haplotype Accession Number** | **Clade** | **Viñas et al., 2010** | **This study** | **Total** |
|
| AY650761 | II | 2 | 2 | 4 |
| AY650762 | II | 33 | 73 | 106 |
| AY650763 | I | 19 | 55 | 74 |
| AY650764 | I | 1 | 0 | 1 |
| AY650768 | I | 36 | 61 | 97 |
| AY650776 | I | 1 | 0 | 1 |
| AY650777 | I | 1 | 0 | 1 |
| AY650778 | I | 13 | 14 | 27 |
| AY650781 | I | 19 | 25 | 44 |
| AY650782 | I | 1 | 0 | 1 |
| AY650793 | I | 1 | 0 | 1 |
| AY650794 | I | 1 | 0 | 1 |
| AY650805 | II | 10 | 3 | 13 |
| AY650806 | II | 1 | 0 | 1 |
| AY650809 | II | 4 | 3 | 7 |
| AY650810 | I | 2 | 0 | 2 |
| AY650812 | I | 2 | 0 | 2 |
| AY650813 | II | 1 | 0 | 1 |
| AY650814 | I | 3 | 3 | 6 |
| AY650815 | II | 2 | 0 | 2 |
| AY650816 | II | 2 | 0 | 2 |
| AY650817 | II | 1 | 0 | 1 |
| AY650820 | II | 1 | 0 | 1 |
| AY650821 | II | 1 | 2 | 3 |
| AY650822 | I | 1 | 0 | 1 |
| AY650823 | I | 4 | 0 | 4 |
| AY650825 | I | 3 | 0 | 3 |
| AY650826 | I | 1 | 0 | 1 |
| AY650827 | I | 1 | 0 | 1 |
| AY650828 | I | 1 | 0 | 1 |
| AY650829 | I | 6 | 2 | 8 |
| AY650831 | I | 1 | 0 | 1 |
| AY650832 | II | 1 | 0 | 1 |
| AY650833 | I | 1 | 0 | 1 |
| AY650834 | I | 1 | 0 | 1 |
| AY650835 | II | 1 | 0 | 1 |
| AY650836 | I | 2 | 5 | 7 |
| AY650837 | I | 1 | 0 | 1 |
| AY650839 | I | 1 | 0 | 1 |
| AY650840 | I | 1 | 0 | 1 |
| AY650841 | II | 1 | 0 | 1 |
| AY650842 | I | 1 | 0 | 1 |
| AY650843 | I | 1 | 0 | 1 |
| AY650844 | I | 1 | 0 | 1 |
| AY650846 | I | 1 | 0 | 1 |
| AY650847 | I | 1 | 0 | 1 |
| AY650849 | II | 1 | 0 | 1 |
| AY650851 | I | 1 | 0 | 1 |
| AY650852 | I | 1 | 0 | 1 |
| AY650853 | II | 1 | 0 | 1 |
| AY650854 | II | 1 | 0 | 1 |
| AY650855 | I | 3 | 1 | 4 |
| AY650856 | II | 1 | 0 | 1 |
| AY650857 | I | 1 | 0 | 1 |
| AY650858 | I | 1 | 1 | 2 |
| AY650859 | II | 1 | 0 | 1 |
| AY650860 | I | 3 | 1 | 4 |
| AY650861 | II | 1 | 2 | 3 |
| AY650862 | I | 1 | 0 | 1 |
| AY650863 | I | 1 | 0 | 1 |
| AY650864 | II | 1 | 0 | 1 |
| AY650865 | II | 1 | 0 | 1 |
| AY650866 | II | 1 | 0 | 1 |
| EU827759 | I | 1 | 2 | 3 |
| EU827762 | II | 1 | 0 | 1 |
| EU827763 | I | 1 | 0 | 1 |
| EU827764 | I | 4 | 0 | 4 |
| EU827765 | I | 1 | 0 | 1 |
| EU827766 | I | 1 | 0 | 1 |
| EU827767 | II | 6 | 0 | 6 |
| EU827768 | II | 1 | 0 | 1 |
| EU827769 | I | 1 | 0 | 1 |
| EU827770 | I | 1 | 0 | 1 |
| EU827771 | II | 1 | 6 | 7 |
| EU827772 | I | 1 | 0 | 1 |
| EU827773 | II | 1 | 0 | 1 |
| EU827774 | II | 1 | 0 | 1 |
| EU827775 | I | 1 | 0 | 1 |
| EU827776 | I | 1 | 0 | 1 |
| EU827777 | I | 1 | 0 | 1 |
| EU827784 | I | 1 | 0 | 1 |
| EU827785 | I | 1 | 0 | 1 |
| EU827786 | I | 1 | 0 | 1 |
| EU827787 | II | 2 | 2 | 4 |
| EU827788 | I | 1 | 0 | 1 |
| EU827789 | I | 1 | 0 | 1 |
| EU827790 | I | 1 | 0 | 1 |
| EU827791 | I | 1 | 0 | 1 |
| EU827792 | II | 1 | 0 | 1 |
| EU827794 | II | 1 | 0 | 1 |
| EU827795 | I | 1 | 0 | 1 |
| EU827796 | I | 1 | 0 | 1 |
| EU827798 | II | 1 | 0 | 1 |
| MN652595 | I | 0 | 2 | 2 |
| MN652596 | I | 0 | 1 | 1 |
| MN652597 | I | 0 | 1 | 1 |
| MN652598 | I | 0 | 2 | 2 |
| MN652599 | I | 0 | 2 | 2 |
| MN652600 | I | 0 | 1 | 1 |
| MN652601 | I | 0 | 1 | 1 |
| MN652602 | I | 0 | 1 | 1 |
| MN652603 | I | 0 | 2 | 2 |
| MN652604 | I | 0 | 1 | 1 |
| MN652605 | I | 0 | 1 | 1 |
| MN652606 | I | 0 | 2 | 2 |
| MN652607 | I | 0 | 1 | 1 |
| MN652608 | I | 0 | 1 | 1 |
| MN652609 | I | 0 | 3 | 3 |
| MN652610 | II | 0 | 1 | 1 |
| MN652611 | II | 0 | 1 | 1 |
| Total |  | 251 | 287 | 538 |

S3 Virtual restriction analysis

Computer-simulated RFLP analysis of a 290 bp long portion of the hypervariable L-domain of the mitochondrial control region was performed on the sequences available in Genbank (AY650743–AY650893 and EU827744–EU827798). The sequenced were aligned in Bioedit to identified conserved regions in each clade. The virtual restriction analysis performed in Restrictionmapper v.3 for all commercial enzymes revealing that double-digestion using PacI and VspI restriction enzymes allowed us to classify individuals to one of the two clades. Specifically, four RFLP profile were identified: one for Clade II and three related to Clade I. The predicted restriction fragment sizes are listed in Table S3.

**Table S3.** Predicted fragment sizes (bp) of a 290 bp long portion of the mitochondrial control region virtually double-digested with VspI and PacI.

|  |  |  |  |
| --- | --- | --- | --- |
| **Clade** | **Profile** | **Enzyme (no. of Restriction Sites)** | **Predicted Fragment Sizes** |
| I | a | VspI (2), PacI (1) | 95, 86, 36, 70 |
| I | b | VspI (1), PacI (1) | 181, 36, 70 |
| I | c | VspI (2) | 95, 122, 70 |
| II |  | VspI (1) | 214, 70 |