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New mitochondrial and nuclear primers for the Mediterranean marine bivalve *Pinna nobilis*

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Supplementary Data

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Table S1. Description of the primers used in this study. They were designed on the basis of the sequences available on GenBank for the most related Pteriomorpha species (reported in the last column).

Region	Primer	Primer sequences (5'-3')	Pteriomorpha GenBank sequences
Mitochondrion			
COI	PNCOI-L	L: GGTTGAACTATHATCCNCC	<i>Pinna bicolor</i> (JN182725), <i>P. nobilis</i> (DQ448218), <i>P. muricata</i> (GQ166589), <i>Atrina chinensis</i> (JN944111), <i>A. fragilis</i> (EF536852), <i>A. pectinata</i> (AB059423), <i>A. vexillum</i> (JN944113), <i>Streptopinna saccata</i> (AB076919), <i>Pteria hirundo</i> (AF120647), <i>P. penguin</i> (JN560939), <i>P. sterna</i> (GQ355874), <i>Ostrea denselamellosa</i> (HM015199), <i>Crassostrea gigas</i> (7212445), <i>C. virginica</i> (170675697), <i>C. iredalei</i> (FJ841967), <i>C. hongkongensis</i> (281190775), <i>C. nippona</i> (326536090), <i>C. sikamea</i> (FJ841966), <i>C. angulata</i> (FJ841965), <i>C. ariakensis</i> (FJ841964), <i>Saccostrea mordax</i> (292496236), <i>Mytilus edulis</i> (51830027), <i>M. galloprovincialis</i> (62161117), <i>M. trossulus</i> (DQ198231).
	PNCOI-H	H: GAAATCATYCCAAAAGC	
nad3	PNND3-L	L: CCTTATGARTGYGGBTTT	<i>Ostrea denselamellosa</i> (HM015199), <i>Crassostrea angulata</i> (FJ841965), <i>C. ariakensis</i> (FJ841964), <i>C. gigas</i> (7212445), <i>C. hongkongensis</i> (281190775), <i>C. iredalei</i> (FJ841967), <i>C. nippona</i> (326536090), <i>C. sikamea</i> (FJ841966), <i>C. virginica</i> (170675697), <i>Saccostrea mordax</i> (292496236), <i>Mytilus edulis</i> (51830027), <i>M. galloprovincialis</i> (62161117), <i>M. trossulus</i> (DQ198231).
	PNND3-H	H: TCHATAAGYTCATARTA-YARCCC	
12S	PN12S-L	L: ACTACGAGCAACTGCTTAA-AACTC	<i>Pinna muricata</i> (GQ166555), <i>Atrina pectinata</i> (118596079), <i>Pteria hirundo</i> (JF496750), <i>Ostrea denselamellosa</i> (HM015199), <i>Crassostrea angulata</i> (FJ841965), <i>C. ariakensis</i> (FJ841964), <i>C. gigas</i> (7212445), <i>C. hongkongensis</i> (281190775), <i>C. iredalei</i> (FJ841967), <i>C. nippona</i> (326536090), <i>C. sikamea</i> (FJ841966), <i>C. virginica</i> (170675697), <i>Saccostrea mordax</i> (292496236), <i>Mytilus edulis</i> (51830027), <i>M. galloprovincialis</i> (62161117), <i>M. trossulus</i> (DQ198231).
	PN12S-H	H: ATGTTACGACTTACCTC	
16S	PN16S-L	L: TGCTCAATGCCCAAGGGG-TAAAT	<i>Pinna bicolor</i> (JN182779), <i>P. carnea</i> (313587052), <i>P. muricata</i> (GQ166570), <i>P. nobilis</i> (DQ663473), <i>Atrina chinensis</i> (JN969049), <i>A. fragilis</i> (DQ663474), <i>A. pectinata</i> (HQ829226), <i>A. rigida</i> (313587018), <i>A. vexillum</i> (JN944101), <i>Pteria hirundo</i> (HQ329433), <i>P. lata</i> (HQ329435), <i>P. penguin</i> (AB591841), <i>Ostrea denselamellosa</i> (HM015199), <i>Crassostrea angulata</i> (FJ841965), <i>C. ariakensis</i> (FJ841964), <i>C. gigas</i> (7212445), <i>C. iredalei</i> (FJ841967), <i>C. hongkongensis</i> (281190775), <i>C. nippona</i> (326536090), <i>C. sikamea</i> (FJ841966), <i>C. virginica</i> (170675697), <i>Saccostrea mordax</i> (292496236), <i>Mytilus edulis</i> (51830027), <i>M. galloprovincialis</i> (62161117), <i>M. trossulus</i> (DQ198231).
	PN16S-H	H: AACTCAGATCACGTAGGG	
18S	PN18S-F	For: GACATAGTGAAAC-CGCGAAT	<i>Pinna bicolor</i> (AB377663), <i>P. carnea</i> (HQ329375), <i>P. muricata</i> (AJ389636), <i>Atrina chinensis</i> (JN182743), <i>A. pectinata</i> (EF613241), <i>A. rigida</i> (L78850), <i>A. seminuda</i> (HQ329324), <i>A. vexillum</i> (HQ449252), <i>Pteria hirundo</i> (HQ329383), <i>P. lata</i> (HQ329379), <i>P. penguin</i> (AB591837), <i>P. sterna</i> (HQ329394), <i>Ostrea edulis</i> (JN797505), <i>Crassostrea gigas</i> (AB064942), <i>C. virginica</i> (EU660793).
	PN18S-R	Rev: CGTTACCCGTTACAACCAT	
28S	PN28S-F	For: CAAGGAGTCTAACATGT-GCG	<i>Pinna bicolor</i> (AB105359), <i>P. carnea</i> (HQ329489), <i>P. nobilis</i> (HE965802), <i>P. muricata</i> (AB102754), <i>Atrina chinensis</i> (HM015780), <i>A. fragilis</i> (AF120593), <i>A. pectinata</i> (HM015783), <i>A. pectinata japonica</i> (DQ343846), <i>A. rigida</i> (HQ329438), <i>A. seminuda</i> (HQ329439), <i>A. vexillum</i> (AB594404), <i>Pteria hirundo</i> (AF120592), <i>P. lata</i> (HQ329492), <i>P. penguin</i> (AB594407), <i>P. sterna</i> (HQ329508), <i>Saccostrea saccata</i> (AB102753).
	PN28S-R	Rev: CTATACCCAAAGTTTGAC-GATC	
Nucleus			

Table S2. COI-16S-nad3 dataset: polymorphic sites in the 992 bp long DNA alignment.

	63336678911112222222222333344445555555666667777777888888888888888888999999999999999999
	0470214626780111144455601124012712225778000221256678223333444445578999011112223355566778
	6889103563672523589971388146546925780947589304801261278927550395123935689013507479
OSR1	TTCGCGTTTCTTATTAAACAAGGCTCTTGACCCGCCCTCACTCTGCGTCGCGTCAACACACACCTATCACCTAATTTTGCGCCCATTTACT
OSR2A.....T.....T..A...T.....A
OSR3A.....T.....T.....A
OSR4A.....T.....TT.....A
OSR5T.....T.....CT.....A
OSR6C.....C.....T.....T.....A
BPC25T.....CTT.....A
BPC1T.....T.....CCT.....G.....A.....A
BPC28T.....T.....CTT.....A
BPC51T.....CTT.....A
BPC81T.....CTT.....T.....G.....C.....AT.....A
BPC45T.....CTT.....T.....G.....C.....AT.....A
POR11A.....T.....CT.....G
POR12A.....T.....G.....CTT.....G
LAZ151	...T...T...T.....T...G.....CTT.....A
LAZ152A.....T..C.T.....CTT.....A
OSM2C.....C.....T.....CTT.....A
OSM4C.....A.....T.....CTT.....G
OSM5A.....T.....CTT.....A
OSM18C.....C.....T.....C.T.....A
OSM27A.....T.....C.T.....A
OSM3A.....T.....CTT.....A
MOL1T.....A.....CTT.....CT.....G
MOL3T..G.....CTT.....A
MOL4	...T.T...T.....T...G.....CTT.....A
MOL7T.....CTT.....CT.....A
MOL9	...T...T.....T..GT.....T.....T.....G.....C.....A...T.....A
MOL11	C...T...T.....T...GT.....CT...T...G...C...AT.....A
CCE1A.....T.....CTT.....A
CCE2T.....CT.....T.....A
CCE3A.....T.....A.....CTT.....A
CCE5A.....T.....CTT.....A
CCE4C.....C.....T.....A.....CTT.....CT.....G..
SAL1C.....C.....T.....CTT.....G
SAL2	...A...C.....C.....T.....A.....CTT.....A
SAL3C.....C.....T.....A.....CTT.....G
SAL4T.....A.....CTT.....T.....A
SAL5C.....C.....T.....A.....C.T.....A
MPE1G.A.....T..T...T.....CTT.....A
MPE1bG.A.....T..T...T.....CTT.....A
MPE2A.....T.....CTT.....A
MPE3C.....CC.....T.....CTT.....C.....A
MPE21A.....T.....C.T.....A
OTT1C.....A...C.....T.....A.....CTTCAC.....G
OTT2T.....A.....CTT.....A
OTT4T.....C.T.....C.....A
OTT5C.....C.....T.....A.....CTT.....A
ORI12T.....CT.....T.....A
ORI13T.....CTT.....A
ORI11C...G.....T.....CTT.....A
ORI14	.C.....TA.....CTT.....A
ORI15C.....T.....CTT.....A
ORI21T.....CTT.....A
ORI22C.....T.....CT.....A
MAR31	...A...C...A...C...T...T...T...A
MAR31b	...A...C...A...C...T...C...T...T...A
MAR32C.....A.....T.....CTT.....G...A
MAR34C.....C.....T.....CTT.....G...A
MAR33	...AA...C...C...T...C.T...
MAR35C.....C.....T.....T...C.T...A
IMV41	...A...C...C...T...A...CTT...G...C...A...A
IMV42	...A...C...C...T...A...CTT...G...A
IMV44	...A...C...C...T...G...A...CTT...T...G...C...A...A
IMV43	...A...C...C...T...T...CTT...A
IMV43b	...A...C...C...T...G...CTT...A
VSM1	...A...C...C...T...C...CT...T...A

(continued)

Table S2 (continued)

VSM2			A		T		CTT		G		G																				
VSM3		A		C		C		T		A	CTT		T																		
VSM4								T		A		CT			A																
CPA1		T		T				T		GT		CTT		T	G	C	A		G												
CPA2						A		T				CTT							A												
CPA3						A		T		C		CTT							A												
CPA4				G				T				C						T	G	A											
CPA5						A		T			T		CTT						A												
MAD1						A		T			T	T		CTT					A												
MAD2						A		T					CTT						A												
MAD3				T		T				T		GT		CTT		T		G	C	A	T	C		A							
MAD4						A		T					CTT							A											
MAD5						A		T					CTT							A											
CPC1			A			C				C				TT						A											
CPC2						A				TT			T		TT					A											
CPC4								T						TT						A											
CPC7						C				C			T				T		G	C	A		A								
CPC8						A				T				T						T			A								
CPC9						G								TT							A										
IPI1				CC				A				T			TT			C	G				A								
IPI2						C				C				T						TT			A								
IPI3						C				C				T						TT			G		A						
IPI10						T				T				G						TT					A						
IPI11						T				T				G						T					A						
IPI12						T				T				G						T					A						
SVC1						A				T								T		CT					A						
SVC2						A				T										CTT					A						
SVC3						A				T										CTT				T		A					
SVC4										T										CTT						A					
SVC5						A				T										CTT						A					
MON1						A				T			C							CTT				C		A					
MON2						A				T										CTT						A					
MON3						C				C			T		T					CTT						A					
MON4						A				T										CTT						A					
MON5						G								T						CT				T		A					
MLZ1																				CT						A					
MLZ2																				CTT				CT		A					
MLZ3						T				T										CTT				T	G	C	A		A		
MLZ4										C										CTT								A			
MLZ5																				CTT				CT				A			
PAC2										A										CTT								A			
PAC3						T				T										CTT				T	G	C	A	T		A	
PAC4										G										CT							T			A	
PAC5						CC														CTT									A		
PAC6										AT										CTT									A		
OGN1										A										CTT									A		
OGN2										C										CTT							G			A	
OGN3										A										C	T			T				A	C	A	
OGN4										A										CTT					T					A	
OGN7										A										CTT										A	
ELB1						T				T										CTTG				T		G	C	A	T		A
ELB2										T										CCT						G		A			A
ELB3										A										CTT						C					A
ELB4										A										CTT											A
ELB5										C										CTT											A
OAL11										A										CTT											A
OAL12						AA														CTT											A
OAL13										A										CTT											A
OAL14										T										CTT						A	A				A
OAL15										A										CT											A
OAL17										A										CTT											A
SML24										T										CTT											A
SML21										A	A									CT								T			A
SML22																				CTT											A
SML23																				CTT											A
SML25																				CT											A
SML26																				CTT								G			A
CYP1																				TT											A
CYP2										A										--TT											A
BIZ1																				CTT								C			A

Table S3. COI-16S-nad3 dataset: haplotype frequencies in 136 individuals of *P. nobilis* from 28 sites. Abs. freq.: absolute frequencies; Rel. freq.: relative frequencies (%). Populations are labelled as in Figure 1.

Clades	Abs. freq.	Rel. freq.	Sites
PN 1	1	0.74	ORS
PN 2	1	0.74	ORS
PN 3	1	0.74	ORS
PN 4	1	0.74	ORS
PN 5	1	0.74	ORS
PN 6	1	0.74	ORS
PN 7	5	3.68	BPC, ORI, SVC
PN 8	1	0.74	BPC
PN 9	1	0.74	BPC
PN 10	2	1.47	BPC
PN 11	1	0.74	POR
PN 12	1	0.74	POR
PN 13	1	0.74	LAZ
PN 14	1	0.74	LAZ
PN 15	3	2.21	OSM, MLZ, ELB
PN 16	1	0.74	OSM
PN 17	16	11.76	OSM,CCE, MPE, CPA, MAD, SVC, MON, PAC, OGN, ELB
PN 18	1	0.74	OSM
PN 19	2	1.47	OSM, MPE
PN 20	1	0.74	MOL
PN 21	1	0.74	MOL
PN 22	1	0.74	MOL
PN 23	3	2.21	MOL, MLZ
PN 24	1	0.74	MOL
PN 25	1	0.74	MOL
PN 26	2	1.47	CCE, ORI
PN 27	1	0.74	CCE
PN 28	1	0.74	CCE
PN 29	1	0.74	SAL
PN 30	2	1.47	SAL, OAL
PN 31	1	0.74	SAL
PN 32	1	0.74	SAL
PN 33	1	0.74	SAL
PN 34	2	1.47	MPE
PN 35	1	0.74	MPE
PN 36	1	0.74	OTT
PN 37	1	0.74	OTT
PN 38	1	0.74	OTT
PN 39	3	2.21	OTT
PN 40	1	0.74	OTT, SML
PN 41	1	0.74	ORI
PN 42	1	0.74	ORI
PN 43	1	0.74	ORI
PN 44	1	0.74	MAR
PN 45	1	0.74	MAR
PN 46	1	0.74	MAR
PN 47	1	0.74	MAR
PN 48	1	0.74	MAR
PN 49	1	0.74	MAR
PN 50	1	0.74	IMV
PN 51	1	0.74	IMV
PN 52	1	0.74	IMV

(continued)

Table S3 (continued)

Clades	Abs. freq.	Rel. freq.	Sites
PN 53	1	0.74	IMV
PN 54	1	0.74	IMV
PN 55	1	0.74	VSM
PN 56	1	0.74	VSM
PN 57	1	0.74	VSM
PN 58	1	0.74	VSM
PN 59	1	0.74	CPA
PN 60	1	0.74	CPA
PN 61	1	0.74	CPA
PN 62	1	0.74	CPA
PN 63	1	0.74	MAD
PN 64	1	0.74	MAD
PN 65	1	0.74	CPC
PN 66	1	0.74	CPC
PN 67	1	0.74	CPC
PN 68	1	0.74	CPC
PN 69	1	0.74	CPC
PN 70	1	0.74	CPC
PN 71	1	0.74	IPI
PN 72	2	1.47	IPI, CYP
PN 73	1	0.74	IPI
PN 74	1	0.74	IPI
PN 75	1	0.74	IPI
PN 76	1	0.74	IPI
PN 77	1	0.74	SVC
PN 78	1	0.74	SVC
PN 79	1	0.74	MON
PN 80	1	0.74	MON
PN 81	2	1.47	MON, PAC
PN 82	1	0.74	MLZ
PN 83	1	0.74	MLZ
PN 84	1	0.74	PAC
PN 85	1	0.74	PAC
PN 86	1	0.74	PAC
PN 87	1	0.74	OGN
PN 88	1	0.74	OGN
PN 89	1	0.74	OGN
PN 90	1	0.74	OGN
PN 91	1	0.74	ELB
PN 92	1	0.74	ELB
PN 93	1	0.74	ELB
PN 94	1	0.74	OAL
PN 95	1	0.74	OAL
PN 96	1	0.74	OAL
PN 97	1	0.74	OAL
PN 98	1	0.74	OAL
PN 99	1	0.74	SML
PN 100	1	0.74	SML
PN 101	1	0.74	SML
PN 102	1	0.74	SML
PN 103	1	0.74	CYP
PN 104	1	0.74	BIZ

Table S4. COI-16S-nad3 dataset: pairwise Φ_{ST} values among sampling localities. Significance was assessed by permutation test. Significant values (significance level: $P < 0.05$) after correction for multiple testing are reported in bold. Populations are labelled as in Figure 1.

	STL	BPC	OSM	ORI	MAR	IMV	CPA	CPC	MPE	SAL	CCE	MOL	IPI	SVC	MON	MLZ	MAD	ELB	OGN	PAC	SML	OAL	
STL	0.000																						
BPC	0.378	0.000																					
OSM	0.230	0.260	0.000																				
ORI	0.364	0.171	0.139	0.000																			
MAR	0.286	0.324	0.179	0.228	0.000																		
IMV	0.523	0.423	0.432	0.461	0.167	0.000																	
CPA	0.183	0.020	-0.005	0.078	0.204	0.346	0.000																
CPC	0.020	0.234	0.166	0.241	0.246	0.396	0.122	0.000															
MPE	0.274	0.272	0.016	0.220	0.255	0.452	0.029	0.213	0.000														
SAL	0.438	0.418	0.290	0.336	0.140	0.199	0.282	0.326	0.367	0.000													
CCE	0.230	0.179	-0.021	0.062	0.169	0.377	-0.054	0.177	0.041	0.201	0.000												
MOL	0.331	0.101	0.271	0.219	0.290	0.331	0.007	0.268	0.268	0.333	0.154	0.000											
IPI	0.011	0.259	0.157	0.221	0.201	0.355	0.093	-0.027	0.192	0.279	0.129	0.168	0.000										
SVC	0.372	0.304	0.048	0.256	0.369	0.591	-0.017	0.283	0.074	0.545	0.014	0.277	0.239	0.000									
MON	0.264	0.169	-0.048	0.030	0.189	0.417	-0.071	0.157	0.020	0.315	-0.090	0.195	0.147	-0.026	0.000								
MLZ	0.261	0.033	0.125	0.028	0.165	0.304	-0.039	0.163	0.161	0.254	0.006	-0.069	0.098	0.186	0.052	0.000							
MAD	0.276	0.098	0.046	0.182	0.293	0.414	-0.148	0.177	0.047	0.379	0.011	0.080	0.148	-0.026	-0.011	0.025	0.000						
ELB	0.258	0.008	0.090	0.133	0.199	0.268	-0.113	0.148	0.111	0.265	0.055	-0.060	0.083	0.123	0.041	-0.065	-0.081	0.000					
OGN	0.237	0.233	-0.079	0.159	0.225	0.437	-0.025	0.172	-0.005	0.339	-0.029	0.243	0.150	-0.033	-0.060	0.117	-0.015	0.069	0.000				
PAC	0.229	-0.018	0.016	0.006	0.141	0.281	-0.159	0.110	0.053	0.230	-0.052	-0.004	0.078	0.036	-0.094	-0.085	-0.088	-0.139	0.001	0.000			
SML	0.438	0.400	0.322	0.300	0.164	0.253	0.281	0.342	0.366	-0.035	0.181	0.306	0.281	0.490	0.292	0.240	0.365	0.254	0.333	0.208	0.000		
OAL	0.473	0.426	0.362	0.375	0.146	0.077	0.332	0.354	0.397	0.007	0.270	0.339	0.316	0.513	0.348	0.277	0.392	0.273	0.378	0.261	0.004	0.000	

Table S5. COI-16S-nad3 dataset: results of the analysis of molecular variance (AMOVA). Six combinations of groups (1-6) were defined according to the biogeographic subdivision of Mediterranean suggested by Bianchi *et al.* (2007) (see main text for details on populations). d.f.: degrees of freedom; SSD: sum of squared deviations; var. comp.: variance component; % var: percentage of variation; ^{NS}: not significant.

Source of variation	d.f.	SSD	Var. comp.	% var	Fixation indices	P-value
1) a) Sardinian Sea ; b) Tyrrhenian Sea; c) Strait of Messina; d) Ionian Sea; and e) northern Adriatic Sea						
Among groups	4	32.007	0.12646	4.07	0.04069	0.075 ^{NS}
Among populations within groups	17	92.848	0.54900	17.67	0.21736	< 0.001
Within populations	99	240.784	2.43216	78.26	0.18416	< 0.001
2) a) Sardinian Sea ; b) Tyrrhenian Sea; c) Strait of Messina; and d) Ionian Sea, northern Adriatic Sea						
Among groups	3	18.135	0.00725	0.24	0.00236	0.426 ^{NS}
Among populations within groups	18	106.720	0.63408	20.63	0.20866	< 0.001
Within populations	99	240.784	2.43216	79.13	0.20679	< 0.001
3) a) Sardinian Sea ; b) Tyrrhenian Sea, Strait of Messina; c) Ionian Sea; and d) northern Adriatic Sea						
Among groups	3	29.415	0.17146	5.48	0.05484	< 0.05
Among populations within groups	18	95.439	0.52270	16.72	0.22204	< 0.001
Within populations	99	240.784	2.43216	77.80	0.17690	< 0.001
4) a) Sardinian Sea ; b) Tyrrhenian Sea; c) Strait of Messina, Ionian Sea; and d) northern Adriatic Sea						
Among groups	3	28.929	0.15787	5.06	0.05063	< 0.05
Among populations within groups	18	95.925	0.52775	16.93	0.21991	< 0.001
Within populations	99	240.784	2.43216	78.01	0.17830	< 0.001
5) a) Sardinian Sea ; b) Tyrrhenian Sea, Strait of Messina; and c) Ionian Sea, northern Adriatic Sea						
Among groups	2	15.543	0.05147	1.67	0.01666	0.171 ^{NS}
Among populations within groups	19	109.312	0.60506	19.59	0.21256	< 0.001
Within populations	99	240.784	2.43216	78.74	0.19922	< 0.001
6) a) Sardinian Sea ; b) Tyrrhenian Sea, Strait of Messina, Ionian Sea; and c) northern Adriatic Sea						
Among groups	2	27.491	0.24260	7.66	0.07660	< 0.01
Among populations within groups	19	97.363	0.49246	15.55	0.23209	< 0.001
Within populations	99	240.784	2.43216	76.79	0.16838	< 0.001