

Additional file 1 – List of primers and PCR conditions used in the sequencing of *Ochogaster lunifer* mtDNA (Salvato et al)

Primer Forward		Primer Reverse		AT			
name	5'- sequence-3'	name	5'- sequence-3'	MIX	°C	EXT	CYCL
TFJ6400	TAA TAT CTT CAA TRT YAA RCT CT	071105_Rev3	TTT TTA GCC GGA TTT TAT TCT	1	54	2'	35
TFJ6400	TAA TAT CTT CAA TRT YAA RCT CT	OC_nad5r	TTA TGG CTG GTA TTT CTG CT	1	54	2'	40
TNJ6172	AGA GGT ATA TCA CTG TTA ATG A	HCnad5r2	GCT GGG ATA TTA ACG GCT TT	1	61	2'	40
N5J7806	GAA ACT AAA CCC AAA CCA TCT CA	N4N8727	AAA TCT TTR ATT GCT TAT TCW TC	2	62	2'	35
HCnad4f1	ATC TAT ACA CTC CGC AAT AA	HCnad6r1	AAA GTT CGT TAG AAG CAA TAC	2	60	2'	35
n4n6for	CCA ATT AAC ATG GGT AAA GA	n4n6rev	TTG GGA TAT GAT GGT TTT TG	2	58	1'30"	35
Oc3dfor	GTC TTG TAA AYC AAA AWT AAG A	Oc3adrv	TAG CAA TAA TAA AGG GGA AA	2	50	2'	35
N4J9172	CGC TCT GGY TGR TAA CCY CA	CBN11010	TAT CTA CAG CAA ATC CCC CTC A	2	62	2'	35
CBJ10933	TAT GTA CTA CCA TGA GGA CAA ATA TC	CBN11367	ATT ACA CCT CCT AAT TTA TTA GGA AT	2	52	1'	30
CBJ10933	TAT GTA CTA CCA TGA GGA CAA ATA TC	TS1N11683	TAT TTC TTT ATT ATG TTT TCA AAA C	1	58	3'	30
cobfor	TTC CAC CCA TAT TTT TCC TTT A	N1N12595	GTA GCA TTT TTA ACT TTA TTA GAA CG	1	58	3'	30
OC8ff_For	CTA AAG CCA ATC TTA CTT CG	OC8rr_Rev	AAG TCT AAT CTG CCC ACT G	2	58	3'	35
16Sfor	GGT CGC AAA CTC TCT CTT TTA T	12Srev	ATG ATT TGG CGG TAT TTT AGT T	1	52	1'30"	35
OC10ff_For	GCC GAA TTC CTC AAA ATT A	OC10rr_Rev	TTA AAT CAA ATC AAG ATG CAG A'	2	58	3'	35
SRJ14197	GTA CCY CTA CTT TGT TAC GAC TT	TMN200	TCC TTT ATA TTT GAG GTA TGA RCC	2	62	2'	35
OC2ff_For	TTT GTA TAA CCG CAA CTG CT	OC2rr_Rev	TGA AGC AAT AGA TTG GGT TAA	2	58	3'	35
Simon 12Sfor	TAG GGT ATC TAA TCC TAG TT	SimonMetrev	TGG GGT ATG AAC CCA AAA GC	1	56	1'30"	35
T1J34	GCC TGA ATA AAG GRT TAY YCT GAT A	TYN1433	GGC TGA ATA ATA AGC GAT AAA TTG TAA A	2	54	2'	35
n2c1for	GGA TTT TTC CCT AAA TGA ATT G	n2c1rev	AAA AGC ATG TGC AGT TAC AA	2	62	2'	35
LCO1490	GGT CAA CAA ATC ATA AAG ATA TTG G	HCO2198	TAA ACT TCA GGG TGA CCA AAA AAT CA	1	48	1'	35
c1for	CCT CTC ATT CGA CCA AAT ACC	c1rev	TCG GGG TAA TCT GAA TAA CGA	2	62	2'	35

AT, annealing temperature; EXT, extension time; CYCL, number of PCR cycles; **azure primer**, primer located in the α strand of mitochondrial genome; **red primer**, primer located in the β strand of mitochondrial genome.

Primer Forward		Primer Reverse		TM			
name	5'- sequence-3'	name	5'- sequence-3'	MIX	°C	EXT	CYCL
c1L2for	TCA GAT TAC CCC GAC TCA TA	c1L2rev	GGT TAG ATC AAG TAG CCA TTT C	2	58	2'	35
c2a6for	GGA GTA AAA GTA GAC GCA AAC C	c2a6rev	AAA AGT GGA AAC GAT TAG GG	2	62	2'	35
TKJ3790	CAT TAG ATG ACT GAA AGC AAG TA	C3N4908	CGT GAA AYA TCT CGT CAT CAT TG	2	62	2'	35
HCatp6f1	ACT CTC AAG GAT CAA CAA TC	HCcox3r1	ATA ACG TGT AAA CCG TGG A	2	58	1'30"	35

AT, annealing temperature; EXT, extension time; CYCL, number of PCR cycles; **azure primer**, primer located in the α strand of mitochondrial genome; **red primer**, primer located in the β strand of mitochondrial genome.

	Mix1	Mix2
buffer 10X	4µl	4µl
MgCl ₂ 25mM	2µl	1,5µl
dNTP's 2mM	1µl	1µl
primer fw 10µM	1µl	0,8µl
primer rw 10µM	1µl	0,8µl
Taq (5U/µl)	0,2µl	0,2µl
DNA sample	2µl	2µl
H ₂ O	8,8µl	9,7µl
TOTAL	20µl	20µl