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VIRGINIA RECREATIONAL FISHING DEVELOPMENT FUND
SUMMARY PROJECT APPLICATION*

NAME AND ADDRESS OF APPLICANT:	PROJECT LEADER (name, phone, e-mail):
Virginia Institute of Marine Science P.O. Box 1346 Gloucester Point, VA 23062	John E. Graves 804.684.7352 graves@vims.edu
PRIORITY AREA OF CONCERN:	**PROJECT LOCATION:**
Research	Lower Chesapeake Bay and three reference sites along the U.S. Atlantic coast

DESCRIPTIVE TITLE OF PROJECT:

A genetic assessment of the potential for local depletion of Atlantic menhaden (*Brevoortia tyrannus*) within Chesapeake Bay

PROJECT SUMMARY:

To assess the possibility of local depletion of menhaden within Chesapeake Bay through the reduction fishery, the genetic basis of stock structure of the species will be determined along the U.S. Atlantic coast. Variation of the mitochondrial (mt) DNA control region and nuclear microsatellites will be surveyed in young-of-the-year (YOY) and age-1 (yearling) Atlantic menhaden collected from four broad geographic areas (New England, mid-Atlantic (Chesapeake Bay), southern Atlantic, and Gulf of Mexico) in each of two years.

EXPECTED BENEFITS:

Menhaden constitute an important prey item for many species of sportfish within Chesapeake Bay. The species is also targeted by a commercial fishery. With the consolidation of the Atlantic menhaden reduction fishery to a single fish factory located in the Virginia portion of Chesapeake Bay, the proportion of landings from inside the Bay has increased. Recent assessment modeling activities indicate that Atlantic menhaden are not overfished and overfishing is not occurring; but these models assume that Atlantic menhaden comprise a single unit stock along the U.S. east coast. However, there is very limited population genetics data to support this assumption. This project will provide the molecular genetic data necessary to evaluate the stock structure of menhaden, information critical to determining the interdependence of menhaden within Chesapeake Bay with those along the Atlantic coast.

COSTS:

VMRC Funding:	$57,172
Recipient Funding:	$17,447
Total Costs:	**$74,619**

Detailed budget must be included with proposal.

Updated 6/1/05

***This form alone does not constitute a complete application, see application instructions or contact Sonya Davis at 757-247-8155 or sonya.davis@mrc.virginia.gov : Due dates are June 15 (Jul. – Nov. Cycle) and December 15 (Jan. – May Cycle)**

BUDGET
YEAR 1

MENHADEN

Personnel	**RFAB**	**VIMS**	**TOTAL**
Graves, 5%/2.5%	6,215	3,107	9,322
McDowell, 5%/2.5%	2,801	1,400	4,201
Latour, 3%	2,090		2,090
Graduate Research Assistant	17,500		17,500
Fringe, 30% salaries	3,332	1,217	4,549
Supplies	12,000		12,000
See attached budget explanation			
Sample Procurement	1,800		1,800
Facilities & Administrative Costs	11,434	11,723	23,158
Total	57,172	17,447	74,619

Facilities and Administrative Costs:
F&A Costs capped at 25% for funds requested from RFAB program. Approved rate of 45%.

Budget Justification

Supplies: The figure of $1,000 per month is based on the analysis of 500 samples per year (this includes an expected 10% reanalysis). For each sample costs include DNA isolation, PCR amplification of the mtDNA control region and up to 12 different microsatellite loci, capillary-based sequencing of the mtDNA control region sequences (forward and reverse directions), and gel-based elecrophoresis of microsatellite amplifications. Expenses include DNA isolation kits, PCR kits, PCR primers, and supplies for the capillary-based and gel-based automated sequencers.
Sample Procurement: To reduce costs, we will rely on efforts of collaborators at other institutions to collect YOY and yearling menhaden at the three sites outside of Chesapeake Bay. This provide a significant reduction on travel costs (transportation and lodging). The procurement costs per sample ($300) include labor and shipping charges.

Proposal Submission to

Recreational Fishing Advisory Board
Virginia Marine Resources Commission

by

The Virginia Institute of Marine Science
College of William and Mary

A genetic assessment of the potential for local depletion of Atlantic menhaden (*Brevoortia tyrannus*) within Chesapeake Bay

Proposed starting date: 1 January 2007
Proposed duration: 24 months

Dr. John E. Graves
Principal Investigator
Chair, Department of Fisheries Science

Dr. Jan R. McDowell
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Dr. Robert J. Latour
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Ms. Abigail Lynch
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Department of Fisheries Science

Ms. Jane A. Lopez
Director, Sponsored Programs

Dr. Roger L. Mann
Director for Research and
Advisory Services

Project Need:

Atlantic menhaden (*Brevoortia tyrannus*) play a critical role in the ecology of Chesapeake Bay. Menhaden are filter feeders that primarily ingest phytoplankton. As such, the species has an impact on water quality in the Bay and provides a direct link between primary productivity and the availability of forage fish for larger piscivorous predators. Menhaden are also the target of a reduction fishery.

Over the past several years the menhaden reduction fishery has been consolidated to a single fish factory located in the Virginia portion of Chesapeake Bay. As a result, the proportion of menhaden landings from inside the Bay has increased from a 47% (1985-1995 average) to 58% (1996-2004 average). Despite this relative increase in reduction removals, the actual removals from the Bay have decreased by 28% over the same time period. Recent assessment modeling activities indicate that Atlantic menhaden are not overfished and overfishing is not occurring; but these models assume that Atlantic menhaden comprise a single unit stock along the U.S. east coast. However, there is very limited population genetics data to support this assumption.

The intensified harvest of the reduction fishery within and peripheral to Chesapeake Bay has raised concern about the 'localized depletion' of Atlantic menhaden in the Bay. In 2004, this concept was formalized by the Atlantic States Marine Fisheries Commission (ASMFC) in Addendum II to Amendment 1 to the Interstate Fishery Management Plan for Atlantic Menhaden. Contained in Addendum II are a number of research priorities, several of which involve examining rates of exchange of menhaden between Chesapeake Bay and coastal systems through the use of tagging, otolith microchemisty and genetic markers. While tagging and otolith microchemistry studies

will provide valuable information regarding movements of menhaden, they will not address issues of genetic connectivity. If menhaden have significant genetically-based stock structure, localized depletion resulting from intense fishing pressure may result in the loss of unique genetic variation.

We propose to delineate the underlying genetic basis of stock structure on the menhaden along the US east coast. Specifically, we address NMFS-CBPO-2006-2000487 program priority 1) Ecosystem-based Fisheries Research, Monitoring Modeling, and Assessment, a) Monitoring and Assessing Fisheries Stocks – Monitoring and assessment of forage fish populations (particularly Atlantic menhaden and bay anchovy) - ...and descriptions of stock structure.

Along the US Atlantic coast, menhaden range from central Florida in the south to Nova Scotia in the north (Hildebrand 1963). The closely related Gulf menhaden (*B. patronus*), which differs from Atlantic menhaden at several meristic and morphometric characters, occurs primarily in the Gulf of Mexico, although the two species may overlap along the southeast coast of Florida (Hildebrand 1963, Dahlberg 1970). Atlantic menhaden undertake considerable movement on a seasonal basis. Spawning occurs offshore, primarily in winter months, although the incidence of early life history stages suggests a protracted spawning period (Ahrenholz 1991). Larvae are transported along-shelf and cross-shelf to estuarine nursery areas, primarily along the US mid-Atlantic coast (Lewis et al. 1972, Checkley et al. 1988). There is considerable interannual variation in the timing of estuarine recruitment as well as the size of the larvae that recruit to an estuary (Quinlan et al. 1999). As water temperatures cool in the fall, there is a general movement of juveniles and adults from the estuaries to southern coastal waters,

and in the spring, there is a reverse movement up the coast and into estuarine waters (Nicholson 1972).

In general, one would not expect significant population structuring within a broadly distributed marine species that exhibits high vagility or has the potential for significant dispersal through a pelagic early life history stages (Graves 1998; Waples 1998). However, there are many exceptions to this trend. To cite an extreme example, tag and recapture studies have demonstrated that striped marlin are capable of undertaking movements of thousands of kilometers. However, analysis of mitochondrial and nuclear genes revealed that the species exhibits considerable population structuring in the Pacific Ocean (Graves and McDowell, 1994; McDowell and Graves, in press). In this case, management of striped marlin as a single, Pacific-wide stock (based on tagging information), could result in the loss of unique genetic variation if the fishery were concentrated in a single geographical area.

There is little genetic information on the population structure of Atlantic menhaden. Bowen and Avise (1990) employed restriction fragment length polymorphism (RFLP) analysis of whole mitochondrial (mt) DNA to investigate phylogeographic patterns in the Atlantic and Gulf menhaden. Their analysis of 31 individuals indicated a very high degree of genetic variation (31 of 33 individuals had unique haplotypes), and individuals differed from one another by a mean nucleotide sequence divergence of 2.4%. Two major lineages of mtDNA genotypes were evident, one that was represented in a subset of Atlantic Menhaden, and one that occurred in both Atlantic and Gulf menhaden. The frequencies of the two clades differed between the

Atlantic and Gulf menhaden, but no attempt was made to investigate spatial structuring within Atlantic menhaden due to the small sample sizes.

We propose to examine population structuring within the Atlantic menhaden. Our analysis will focus on the rapidly evolving molecular markers; the mtDNA control region, and nuclear microsatellite loci. We will address the following null hypotheses:

(1) There is no genetic difference between Atlantic menhaden larvae recruiting to Chesapeake Bay early and late in the season.

(2) There is no genetic difference between young-of-the-year (YOY) menhaden recruiting to each of four nursery areas along the US east coast in different years

(3) There is no genetic difference between YOY and age 1 (yearling) menhaden at each of four nursery areas along the US east coast

(4) There is no genetic difference among YOY (or yearling) menhaden from four nursery areas along the US east coast.

Project Objectives:

Sample Collection: At least 50 YOY and yearling menhaden will be collected from each of four locations (including Chesapeake Bay) along the US east coast during 2006 and 2007. The Chesapeake Bay YOY collection for each year will include 100 individuals, 50 early arrivals (larger YOY) and 50 later arrivals (smaller YOY).

mtDNA Analysis: Isolate genomic DNA from all samples, amplify and sequence the mtDNA control region.

Microsatellite Analysis: Screen existing primers used to amplify hypervariable microsatellite loci from other clupeids to find those that reveal variation and work reliably on menhaden. Screen all samples for selected microsatellite loci.

Expected Results or Benefits:

This study will provide high resolution molecular genetic data enabling us to critically evaluate the stock structure of menhaden within Chesapeake Bay and along the U.S. Atlantic coast. This information will allow us to determine the genetic connectivity of Bay menhaden with those along Atlantic coast and to assess the potential for local depletion of menhaden within Chesapeake Bay by a concentrated reduction fishery.

Project Approach:

Sample collection. YOY and yearling menhaden will be collected by beach seine from four geographic locations: New England, Chesapeake Bay, US south Atlantic coast, and the Gulf of Mexico. Samples of 50 individuals (both YOY and yearling) will be collected from each location. In Chesapeake Bay 100 YOY will be colleted each year representing earlier and later recruits (age and size are correlated in menhaden (Maillet and Checkley 1990)). Collections from New England will be facilitated by Massachusetts Division of Marine Fisheries and collections in Chesapeake Bay will be made in conjunction with the VIMS Seine Survey. Colleagues at the Florida Marine Research Institute will assist with collections from the US south Atlantic coast, and Gulf of Mexico collections will be taken in collaboration with Dr. Jay Rooker at Texas A&M

University Galveston. Specimens will be captured by beach seine and either placed in 95% ethanol or frozen upon capture.

Molecular analyses. Total genomic DNA will be extracted from tissue samples by proteinase K/chelex extraction (Estoup et al., 1996) or by phenol-chloroform extraction (Sambrook et al., 1989). The complete mtDNA control region will be amplified by using the CB3R-5' and 12SAR5' PCR primers of Martin & Palumbi (1993). If amplifications are not consistent, new primers will be designed based on control region sequences available for other clupeids (Genbank accession numbers U95925-32-44, AF10449-69, AY21605-13, DQ018339-42, AY48564, AY309498). After amplification, reactions will be purified using the QIAquik PCR purification kit (Qiagen, Valencia, CA) and the amount of DNA present in each sample will be quantified using a Biomate 3 spectrophotometer (Thermo Spectronic, Rochester, NY). Purified PCR products will be cycle-sequenced using the Applied Biosystems BigDye sequencing protocol, loaded onto an ABI 3100 capillary sequencer (Applied Biosystems, Foster City, CA) and analyzed using the program Sequencing Analysis 5.1.1. Standard chromatographic curves of forward and reverse sequences will be imported into the program Sequencher 4.2.2 (Gene Codes Corp., Ann Arbor, MI), aligned and edited. The consensus of the forward and reverse sequence will be exported to the program MacVector 8.1.1 (Oxford Molecular Ltd., Madison, WI) and aligned to other sequences using the CLUSTALW algorithm (Thompson et al., 1994) and visually adjusted.

Microsatellite primers have been developed for several members of the Clupeidae, including *Alosa alosa* (Faria et al. 2004), *Sardinops sagax sagax* (Pereyra et al. 2004), *Clupea pallasi* (Olsen et al. 2002, Miller et al. 2001) and *Clupea harengus*

(McPherson et al. 2001) (Table 1). The 57 published primer pairs will be assessed for their ability to consistently amplify polymorphic loci in menhaden. Primer pairs meeting the above criteria will used to amplify a subset of 30 YOY menhaden from Chesapeake Bay. To test for null alleles, conformance to the expectations of Hardy-Weinburg equilibrium will be evaluated using exact tests following Weir and Cockerham (1984), as implemented in Genepop 3.4 (Raymond and Rousset, 1995). Up to 12 polymorphic microsatellite loci will used to characterize population structure in Menhaden. Following DNA extraction, microsatellite loci will be amplified by PCR (polymerase chain reaction). One primer from each primer pair will be labeled with either IRD-700 or IRD-800 fluorescent dye (LiCor, Lincoln, NE) and visualized on polyacrylamide gels using a LiCor 4200 global sequencer (LiCor). The resulting products will be scored using the GeneImagIR 4.03 software (Scanalytics, CSP, Inc). To ensure repeatability of allele scoring approximately 25% of the samples will be re-run.

Data Analysis. The following statistical approaches will be used for analyzing the microsatellite data. Allele frequencies will be analyzed in respect to deviations from the expectations of Hardy-Weinberg equilibrium (exact tests, Guo & Thompson, 1992) and estimations of observed (H_O) and expected (H_E) heterozygosity as well as tests for genotypic linkage disequilibrium (Fisher's exact test) will be performed by using the GENEPOP 3.1b software package (Raymond & Rousset, 1995). The total observed variation will be broken down into variation between cohorts and variation between locations using hierarchical F_{ST} (AMOVA) analysis as calculated using the ARLEQUIN V3.0 software package (Schneider et al., 2000). We will also use the software

STRUCTURE (Pritchard et al., 2000) to detect how many populations are included in each sample.

The total observed variation based on mitochondrial DNA sequences will be broken down into variation between cohorts and variation between locations using hierarchical Φ_{ST} (AMOVA) analysis as calculated using the Tamura-Nei algorithm (Tamura & Nei, 1993), which corrects for multiple mutations at a single site by taking into account substitutional rate differences between nucleotides and unequal nucleotide frequencies in the ARLEQUIN V3.0 software package (Schneider et al., 2000). The ARLEQUIN software package will also be used to calculate haplotype diversity (h), nucleotide diversity (π) and the demographic parameters θ,τ,F_s, Harpending's raggedness index, and mismatch distributions. Significance will be assessed using a parametric bootstrap approach with 10,000 replicates. DNASP 4.0 (Roszas et al., 2004) will be used to calculate alternate measures of population structure such as the nearest-neighbor statistic, S_{nn}, (Hudson, 2000), which measures how often nearest neighbors in sequence space (closely related sequences) are from the same locality in geographic space. PAUP* 4.0 (Swofford, 2000) will be used to draw both phenetic and maximum likelihood treesto visualize the relationship among sequences

Milestone Table:

	Year 1 (2007)				Year 2 (2008)			
Quarter:	1	2	3	4	1	2	3	4
Sample collection	XXXX				XXXX			
DNA isolation	XXXXX				XXXXX			
Control region amplification/sequencing		XXXXXX				XXXXXX		
Evaluation of clupeid microsatellite primers	XXXX							
Microsatellite amplification/electrophoresis			XXXX				XXXXXX	
Data analysis				XX				XX

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Table 1. Microsatellite primers developed for Clupeidae.

Locus	Primer Sequence (5'-3;)	Repeat motif	Genbank Accession	Reference
Alosa alosa				
Aa14	F: GAGAAGAGGGCATTCG	(GT)8	AY617109	Faria et al. 2004
	R: ATTTAGTGTGTGCCCAGC			
Aa16	F: TTGACCGAGCGCAAACTG	(CA)4AA(CA)3AA(CA)8	AY617110	Faria et al. 2004
	R: TGACACTGACTCATCATGC			
Aa20	F: GGTGTAATGCCCGTCCG	(GT)16	AY617111	Faria et al. 2004
	R: CAGTGTGCAGACCAGCC			
Af6	F: AGGAGATGTTTATCCTGCC	(CA)4AT(CT)5(CA)16AA(CA)8	AY617112	Faria et al. 2004
	R: CACAGAGGCATAAATGTGG			
Af11	F: CGAGTACAATCAAAAGCC	(CA)5CT(CA)4	AY617113	Faria et al. 2004
	R: AGCTTCCTCAGACTGG			
Af13	F: AGGATACATAGTCTCCC	(CA)17	AY617114	Faria et al. 2004
	R: CAAGTTGGAGTGTCACG			
Af15	F: CCCATTCACTCTTTTTCTC	(CA)5TA(CA)12	AY617115	Faria et al. 2004
	R: GAGAGGAGTTGAGTATGG			
Af20	F: AATGGACATATCTGCTGG	(CA)11	AY617116	Faria et al. 2004
	R: ATGGAGGGCCATATTTCG			
Sardinops sagax sagax				
SarB-A07	F: CTCCTCACTCAGCCGCTAAGGA	(GA)12	AY636114	Pereyra et al. 2004
	R: GGGTAACATTTCGGCAAGTGCT			
SarB-A08	F: GTGATACTCTCTGCCTTGGA	(CA)26	AY636115	Pereyra et al. 2004
	R:GCACTTTGTCCTAGTAAATAGC			
Sar1-A11	F:GAGCTGGAAATCTGGTGATATTTAG	(GATA)2GCTA	AY636120	Pereyra et al. 2004
	R:CCTGTTCACAAGTTAGAGCATTC	(GATA)5GCTA(GATA)8		
SarB-CO5	F:GAACGCAGACATAAAAGGGTC	(TC)5TT(TC)4	AY636116	Pereyra et al. 2004
	R:GGGTATGTGGTGATTATCGTTC			
Sar1-D01	F:GCTCTGGTCGGAGGCTCTATC	(CA)29GG(CA)3	AY636121	Pereyra et al. 2004
	R:GGTGTTCACGTGGGCTGGTA			
Sar1-D06(B)	F:CGGCTATTCTTAGACTAGGTG	(TG)18	AY636123	Pereyra et al. 2004
	R:CCCCATCAGCAATGAATAAG			
SarB-D09	F:GGTCATCTGCTTCAACAACAC	(CA)9(GA)8	AY636117	Pereyra et al. 2004
	R:GCAGCCTGTCTGAAACTCTG			
SarB-G09	F:GGTGGAAAGAACACTGGTCA	(GA)6GT(GA)36		

	AY636118	Pereyra et al. 2004	
	R:GGTTCACTATGCAGGCTATG		GT(GA)3
SarB-H04	F:CGAGTTTGTCCCACACCTGGAG		(GT)9
	AY636119	Pereyra et al. 2004	
	R:CTCCAAGCACCGAGAGCATC		
SarB-H04(F	F:CTCTCGGTGCTTGGAGAGGAA		(TG)18
	AY636119	Pereyra et al. 2004	
	R:GGAGGAGGGGAGAAAAGATG		
Sar1-H11(B)	F:CACGGCACGTTACGTTTCAG		(TG)11TA(TG)6
	AY636122	Pereyra et al. 2004	
	R:CCAGCGTGTCATGAAATGATG		

Clupea pallasi

Cpa101	F: CATTGCCACCTACTGACCTG		ATCT13
	AF406937	Olsen et al. 2002	
	R: CACCCTGAAGATGATGAGGA		
Cpa102	F: TTGCACCCAGTCAGCTAAAC		ATCT15
	AF406938	Olsen et al. 2002	
	R: GCGGCAAAGTCATAACCTG		
Cpa103	F: GACTCACAGGTTCTCCTCAACA		TAGA13
	AF406939	Olsen et al. 2002	
	R: TGGAGGGATTGGAACATTT		
Cpa104	F: TGATTGGGTCCTTTTGAACAT		ATCT13
	AF406940	Olsen et al. 2002	
	R: GCAATGACTGACACAGCAAA		
Cpa105	F: CAATCTGTGCTCACTCTTCCA		ATCT16 ATCC8
	AF406941	Olsen et al. 2002	
	R: CACTGGGTCTTCTCCTCTGC		
Cpa106	F: CCATCCTCATCAAGAAAGCA		ATCT10N4 ATCT10
	AF406942	Olsen et al. 2002	
	R: GGTACTTTGACCTCTCCTCTCC		
Cpa107	F: ATGATTTTTCGCCTTTTGCT		ATCT19
	AF406943	Olsen et al. 2002	
	R: CCCAGAAACAAGAGCTAGGC		
Cpa108	F: TTGTGTATGTATGTCGGTGAGG		TAGA12`
	AF406944	Olsen et al. 2002	
	R: CAGTATGTAGGGAGGGTGGTC		
Cpa109	F: TGCCCGAACTCATCAGAATA		ATCT6N36 ATCT8
	AF406945	Olsen et al. 2002	
	R: AGACTGTTGTTGTGGAGTAGGC		
Cpa110	F: CTGACAACCCTCGACATACAT		TAGA7
	AF406946	Olsen et al. 2002	
	R: ACAATTTGCACTGGTTTGTAGTAG		
Cpa111	F: TGTCCAGTAAAACATGCCTGA		TAGA8
	AF406947	Olsen et al. 2002	
	R: GCTCCGTTCTCTTTCTTGCT		
Cpa112	F: GAGAGGGAGTTAAAATTGACAGC		TAGA7
	AF406948	Olsen et al. 2002	
	R: GGCACAAGATGAGAGTGCAG		
Cpa113	F: TGTCCATCTGTCCATTCAGC		ATCT17
	AF406949	Olsen et al. 2002	
	R: ACCACACAGCACATTTACAGG		
Cpa114	F: GCGTTTGTCCATACCACATT		ATCT10
	AF406950	Olsen et al. 2002	
	R: CAGCTCTGAAAACCCAGACA		

Clupea harengus

1005	F:TGCAAGATAGAGTCACAG		GACA
	AF304359	McPherson et al. 2001	
	R:GGGGACAGAACCAACTTCAC		
1014	F:TCCTAAACCAACCCCTGTGA		GATA
	AF304360	McPherson et al. 2001	
	R:ATTATTGTGTTAAATTTGACAGACC		
1017	F:GGTCTCATTATCTTCTCACTCTTTTG		GATA
	AF289096	McPherson et al. 2001	
	R:TCTCCCTATGTGTATTGTTTTACTGTG		
1020	F:CCTGGAGAGACAGATAGAAAA		GACA
	AF289095	McPherson et al. 2001	
	R:GAGTTTAGCAGACGCTTTA		
1027	F:ATTCAACCCCCTACAAGC		GACA
	AF290885	McPherson et al. 2001	
	R:TGAGGCAGCAGACGATACAC		
1045	F:CATTAGGGATGGCTCTGC		GATA
	AF304361	McPherson et al. 2001	
	R:CCAGAAAAGAAGTCCCAGATG		
1059	F:CATCTACCACCTCCGACTCC		GACA
	AF289094	McPherson et al. 2001	
	R:AATCTAAAGGAAGCCCACTC		
1202	F:TTTCCGTTACACTTTCACATCG		GACA
	AF304363	McPherson et al. 2001	
	R:GTGCCTCAGTTTTCACATACA		
1235	F:GCCCCTCCCTCTGTCTTTTA		GACA/GATA
	AF304362	McPherson et al. 2001	
	R:ACGATGGAGGTAGTGTGTGC		

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*Cpa*4	F:CTTATCTGTCTGACTGCCTATTTG		(GACA)10
	AF309800	Miller et al. 2001	
	R:**GTTT**CTTCTCTGCTCCACCCAGAA		
*Cpa*6	F:GTGTGAGTTTGCTCCAAA		(GATA)14
	AF309801	Miller et al. 2001	
	R:**GTTT**GTACCAATGAATGATTACAA		
*Cpa*7	F:GGTATTGTGTTTGACAAACT		(GATA)14
	AF309803	Miller et al. 2001	
	R:**GTTT**GTAAGTGTATAAGCTACTA		
*Cpa*8	F:GATCCTTCTTTTAAGGAAAA		(GACA)27
	AF309804	Miller et al. 2001	
	R:**GTTT**GACAGAACTTACTATCTCAGA		
*Cpa*27	F:5CACATTTATCAATTTCTTTG		(GACA)15
	AF309799	Miller et al. 2001	
	R:**GTTT**CAGAAAGAGAATCTAACCTCT		
*Cpa*100	F:GCCTGGGCTATATATGTA		(CTA)5(ATT)2 (TACA)10(AT)3
	AF309790	Miller et al. 2001	
	R:**GTTT**CATTTTGTCTCACTAACCTAATA		
*Cpa*104	F:ACGTAGGCGCAGACAT		(TG)54
	AF309791	Miller et al. 2001	
	R:**GTTT**GCTCAAGTCAATGTGATTTTTA		
*Cpa*107	F: GCATTACACAGAGAGGAAT		(TC)33
	AF309792	Miller et al. 2001	
	R:**GTTT**AGATACGCCTCTCTCTTT		
*Cpa*113	F: CAGTCAGAAAGAAGGAGA		(CT)30

	AF309793	Miller et al. 2001	
	R: **GTTT**CCTCCTCGTGCTCTTT		
*Cpa*115	F: GTTCTGTTGACTTGTGCAT		(GA)49(GGGA)4
	AF309794	Miller et al. 2001	
	R: **GTTT**CTGCTTATCTCTGTTGCAAT		
*Cpa*125	F: GCAAGAAAGAGCAGCAGA		(GA)32i(GT)26
	AF309796	Miller et al. 2001	
	R:**GTTT**CGACTCAACAGCTGGAA		
*Cpa*134	F:CATTCTCTACAAAGGGCATATA		(CA)57
	AF309798	Miller et al. 2001	
	R:**GTTT**CATACCATTGAATCCAGCTA		
*Cpa*67	F:CAGCTTTTAACCTTTTGCCAA		(AC)11(GC)22
	AF309802	Miller et al. 2001	
	R: **GTTT**ATGTGAACCACTGTCGTCAC		
*Cpa*108	F:CTTGACATACAGTATGTTCAAAT		(CA)42
	AF318286	Miller et al. 2001	
	R:**GTTT**CTGTGAGCTGTACACCA		
*Cpa*120	F:ACACGCTTGCCTTGAGAT		(CA)41
	AF309795	Miller et al. 2001	
	R: **GTTT**GATCTGATTATCTTGAAAATTTG		