Caspian J. Env. Sci. 2015, Vol. 13 No.2 pp.167~171

CJES
Caspian Journal of Environmental Sciences

[Short Communication]

Cross-species amplification of Clupeidae microsatellite DNA markers in common kilka, *Clupeonella cultriventris* from the Caspian Sea

M. Norouzi*, M. H. Samiei
Dept of Marine Biology and Fisheries Sciences, Tonekabon Branch, Islamic Azad University, Tonekabon, Iran.

** Corresponding author's E-mail: mnoroozi@toniau.ac.ir*

(Received: Dec. 02.2014 Accepted: May. 03.2015)

ABSTRACT

Common kilka *Clupeonella cultriventris* (Nordmann, 1840) is a brackish water and small pelagic fish species and is one of the most abundant fishes that live gregariously in the Caspian Sea. A total of 60 specimens of adult common kilka were sampled from two seasons. Fifteen pairs of microsatellites previously developed for *A.sapidissima, C. pallasi, C.harengus*, and *S. pilchardus* were tested for cross-species amplification on the common kilka. In this study, only five primer pairs were used successfully. Analyses revealed that the average of alleles per locus was 14.4. The average observed and expected heterozygosity was 0.153 and 0.888, respectively. All loci significantly deviated from H–W equilibrium. These results together with significant Rst. values for genotypic differences support the existence of different genetic populations along the Caspian Sea coast (Guilan Province).

Keywords: Population genetics, South Caspian Sea, microsatellite, Clupeonella cultriventris

INTRODUCTION

Common kilka, *Clupeonella cultriventris*, that belongs to the family Clupeidae lives in the Caspian Sea, and feeds on zooplankton and crustaceans such as copepods and cladocerans (http://www.fishbase). They spawn in spring. The Caspian stocks of common kilka face challenges resulting from overfishing and the invader Ctenophore *Mnemiopsis leidyi* (Velikova *et al.*, 2012; Rowshantabari *et al.*, 2012; Nasrollahzadeh, 2010). Microsatellites are abundantly distributed across the genome, demonstrate high levels of allele polymorphism, and can be easily amplified by PCR (Sekar *et al.*, 2009). Microsatellite genotypes are particularly helpful to identify the genetic structure in closely related populations, regardless of whether they are in an evolutionary equilibrium (Chistiakov *et al.*, 2005). In addition, primers designed for one species can often be used for other related species (Chistiakov *et al.*, 2005). However, the development of new species-specific microsatellites by traditional methods requires substantial amount of time and money. One possible shortcut on the path of describing new microsatellite makers is to cross-amplify markers from other species, based on the fact that the microsatellite flanking regions may be conserved in closely related species (Hulák *et al.*, 2010).

MATERIALS AND METHODS

This study represents the population genetic analysis of common kilka in the South Caspian Sea (Guilan Province, Anzali Port). A total of 60 specimens of adult common kilka were sampled from a single sampling location, but the fish were caught during different seasons (spring and summer) and preserved in 95% ethanol. Genomic DNA was extracted from the fin tissue using High Pure PCR Template Preparation Kit (Roche Applied Science, Germany) according to manufacturer's instructions. The quality and concentration of DNA were assessed by 1% agarose gel electrophoresis and spectophotometry (CECIL model CE2040)

Table 1. Microsatellite loci used for cross-species PCR amplifications in common kilka, GenBank Accession no., Reaction consistence, Cycling condition, PCR Amplification.

Locus	GenBank Accession no.	Reaction consistence	Cycling condition	PCR Amplification	Refrence
Cpa6	AF309801	0.2 mM each dNTPs; 0.4µM each primer; 200 ng template DNA; 0.4 U/Hot-StarTaq™ DNA polymerase; 1x HotStarTaq™ PCR buffer ; 4.5 mM $MgCl_2$	95 °C/10m [95°C/30s;52 °C/2m ;72 °C/1m]^{2} [95°C/1m;51 °C/1m ;72 °C/1m]^{38} 72 °C/5m	Yes	
Cpa8	AF309804	0.2 mM each dNTPs; 0.4µM each primer; 200 ng template DNA; 0.4 U/Hot-StarTaq™ DNA polymerase; 1x HotStarTaq™ PCR buffer ; 4.5 mM $MgCl_2$	95 °C/10m [95°C/30s;52 °C/2m ;72 °C/1m]^{2} [95°C/1m;51 °C/1m ;72 °C/1m]^{38} 72 °C/5m	Yes	Miller et al.(2001)
Cpa100	AF309790	2mM $MgCl_2$	[58 °C]^{40}	No	
Cpa104	AF309791	0.2 mM each dNTPs; 0.4µM each primer; 200 ng template DNA; 0.3 U/Hot-StarTaq™ DNA polymerase; 1x HotStarTaq™ PCR buffer ; 4.5 mM $MgCl_2$	95 °C/10m [95°C/30s;51.5 °C/45s ;70 °C/45s]^{2} [95°C/40s;51.5 °C/45s ;72 °C/45s]^{38} 72 °C/5m	Yes	
Cpa107	AF309792	1mM $MgCl_2$	[49 °C]^{40}	No	
Cpa120	AF309795	1mM $MgCl_2$	[61 °C]^{40}	No	
Cpa134	AF309798	1mM $MgCl_2$	[61.2 °C]^{40}	No	
Cpa125	AF309796	0.2 mM each dNTPs; 0.2µM each primer; 200 ng template DNA; 0.3 U/Hot-StarTaqTM DNA polymerase; 1x HotStarTaqTM PCR buffer ; 4.5 mM MgCl2	95 oC/10m [95oC/30s; 59oC/40s ;70 oC/45s]2 [95oC/40m;59 oC/40s ;72 oC/45s]38 72 oC/5m	Yes	Miller et al.(2001)
AsaC051	EF014992	0.2 mM each dNTPs; 0.2µM each primer; 200 ng template DNA; 0.3 U/Hot-StarTaqTM DNA polymerase; 1x HotStarTaqTM PCR buffer ; 4.5 mM MgCl2	95oC/10m [94oC/30s;54 oC/40s ;72 oC/2m]38 72 oC/5m	Yes	
AsaC059	EF014993	1.25mM MgCl2	[58 oC]40	No	Julian and Barton,2007
AsaC249	EF014994	2.5mM MgCl2	[59 oC]40	No	
AsaC334	EF014995	2.5mM MgCl2	[56 oC]40	No	
SAR1.12	EF012617	2.5mM MgCl2	[49 oC]40	No	Gonzalez and Zardoya, 2007
1235	AF304362	2.5mM MgCl2	[58 oC]40	No	McPherson et al., 2001
1014	AF304360	2.5mM MgCl2	[58.5 oC]40	No	

and stored at −20°C until use. Fifteen pairs of microsatellites previously developed for American shad (*Alosa sapidissima*), Pacific herring (*Clupea pallasi*), Atlantic herring (*Clupea harengus*), and sardine (*Sardina pilchardus*) were tested for cross-species amplification on the common kilka. For all primer pairs, amplification was performed in a reaction volume of 25 µL containing 0.2 mM of dNTPs, 0.2–0.4 µM of each primer, 200 ng of template DNA; 0.3–0.4 unit of HotStarTaqTM DNA polymerase; 1×HotStarTaqTM PCR buffer, and 2.5–4.5 mM MgCl2 (Table 1). PCR products were separated on 10% polyacrylamide gels (29:1 acrylamide: bis-acrylamide; 1×TBE buffer) followed by silver staining. Gels were run at 40 mA for 14h. Alleles were sized using Uvitec software, and each gel contained an allelic ladder (100bp) to assist in consistent scoring of alleles.

Data analysis via codominant data were computed in the GenAlex 6 software (Peakall and Smouse, 2006) and Arlequin 3.5 (Excoffier and Lischer, 2010).

RESULTS

Of the 15 pairs of microsatellite primers, 10pairs did not show any flanking sites on the common kilka genome. Five pairs of primers (Cpa6, Cpa8, Cpa104, Cpa125 and AcaC051) were amplified successfully and they showed polymorphic pattern in the 60 individuals assayed. All microsatellite primers that were able to produce DNA bands displayed a characteristic disomic banding pattern.

The average number of alleles found in the seasons was 14.4 (±1.7) and ranged at each locus from 5 (AcaC051) to 21 (Cpa8) alleles. The effective number of alleles (Ne) per locus ranged from 4 to 17.8, with an average of 10.7 (±1.03). Allelic richness per locus and population ranged from 7.4 to 23.5 (Cpa104, 23.5; Cpa6, 16.9; Cpa8, 13.4; Cpa125, 17; and AcaC051, 7.4).

All sampled populations contained a significant number of private alleles, none of which was found in other seasons (Table 2). The Nm and Fst via frequency, ranged from 25.14 to 5.64 and from 0.010 to 0.026, respectively (Tables 2, 3).

Table 2. Number of privet allele, actual size (bp) and Allele frequency in spring and summer seasons.

		Cap6	Cap8	Cap104	Cap 125	Asac051	Total
Number of privet	Spring	1(164)	1(144)	2(334,364)	3(216,230,248)	-	7
allele (actual size)	Summer	2(120,124)	1(188)	1(388)	1(260)	1(180)	6

Table 3. F-Statistics and Estimates of Nm over All Populations for each locus using 5 sets of microsatellite primers

	Cap6	Cap8	Cap104	Cap 125	Asac051	Average
Fst	0.025	0.016	0.013	0.026	0.010	0.018
Nm	9.66	15.08	19	9.4	25.14	05.64

DISCUSSION

The results of the present study showed that at least five microsatellite primers could be used to investigate population genetics of species in the Caspian Sea. There is initial indication of population differentiation of common kilka stocks in the Caspian Sea. Because the common kilka is a shared stock between 5 Caspian countries, it is highly recommended to develop a joint research project on this species, which would cover the entire Caspian Sea. In summary, this study provides preliminary evidence for the existence of at least two differentiated populations in the South Caspian Sea (Guilan Province, Anzali Port). The existing private alleles and significant Fst and Rst confirm that spring and summer populations in Anzali Port. Probably, extra populations are present in the Caspian Sea; therefore, a comprehensive investigation using more samples and primer sets from the entire Caspian Sea may confirm this hypothesis.

ACKNOWLEDGMENT

The study was supported by Islamic Azad University, Tonekabon Branch and was performed in Molecular Genetic Lab. We would like to thank Dr. Ali Nazemi, Amin Ravai and Mohhamad Eskandari (Dept. of Biology).

REFERENCES

Brown, B.L., Gunter, T.P., Waters, J.M. and Epifanio, J.M. (2000). Evaluating genetic diversity associated with propagation-assisted restoration of American shad. *Conservation Biology*, 14: 294–303.

Chistiakov, D.A., Hellemans, B., Haley, C.S., Law, A.S., Tsigenopoulos, C.S., Kotoulas, G., Bertotto, D., Libertini A. and Volckaert, F.A. (2005). A microsatellite linkage map of the European sea bass Dicentrarchus labrax L. *Genetics,* 170: 1821–1826.

Excoffier, L., Laval G. and Schneider, S. (2005). Arlequin ver. 3.0: An integrated software package for population genetics data analysis. *Evolutionary Bioinformatics Online,* 1: 47-50.

Gonzalez, E. G. and Zardoya, R. (2007). Isolation and characterization of polymorphic microsatellites for the sardine *Sardina pilchardus* (Clupeiformes: Clupeidae). *Molecular Ecology Notes*, 7: 519–521.

Hulák, M., Kašpar, V., Kozák P., Bu¢ri, M., Filipová, L. and Petrusek A. (2010). Cross-species amplification of microsatellite markers in the invasive spiny-cheek crayfish (*Orconectes limosus*): assessment and application. J*ournal of Applied Genetics*, 51: 73–78.

Julian, Sh. E., Barton, M. L., (2007). Microsatellite DNA markers for American shad (*Alosa sapidissima*) and cross-species amplification within the family Clupeidae. *Molecular Ecology Notes,* 7: 805-807.

Laloei, F., Rezvani Gilkolaei, S., Nirani, M. and Taqavi, M. T. (2006). The PCR-RFLP investigation of Clupeonella cultriventris from the South Caspian Sea, Iran. *Iranian Scientific Fisheries Journal*, 15: 119-128.

Miller, K. M., Laberee, K., Schulze, A. D. and Kaukinen K. H. (2001). Development of microsatellite loci in Pacific herring (Clupea pallasi). *Molecular Ecology Notes*, 1, 131-132.

McPherson, A. A., O'Reilly, P. T., McParland, T. L., Jones, M. W. and Bentzen P. (2001). Isolation of nine novel tetranucleotide microsatellites in Atlantic herring (Clupea harengus). *Molecular Ecology Notes*, 1: 31-32.

Nasrollahzadeh, A. (2012). Caspian Sea and its Ecological Challenges. *Caspian Journal Environment Science*, 8 (1): 97-104.

Peakall, R. and Smouse, P.E. (2006). GENALEX 6: genetic analysis in excel. Population genetic software for teaching and research. *Molecular Ecology Notes,* 6: 288-295.

Rowshantabari, M., Finenko, G.A., Kideys, A. E. and Kiabi, B. (2012). Effect of temperature on clearance rate, daily ration and digestion time of Mnemiopsis leidyi from the southern Caspian Sea. *Caspian Journal Environment Science,* 10 (2): 157-167.

Sekar, M., Suresh, E., Kumar, N.S., Nayak, S.K., and Balakrishna, C. (2009). Microsatellite DNA markers a fisheries perspective Part 1: The nature of microsatellites. *Genetics and Biodiversity,* pp. 27-29.

Velikova, V.N., Shaudanov, A.K., Gasimov, A., Korshenko, A., Abdoli, A., Morozov, B., Katunin, D. N., Mammadov, E., Bokova, E. B., Emadi, H., Annachariyeva, J., Isbekov, K., Akhundov, M., Milchakova, N., Muradov, O., Khodorevskaya, R., Shahifar, R., Shiganova, T., Zarbaliyeva, T. S., Mammadli, T., Velikova, V., Barale, V. and Kim, Y. (2012). Review of the environment and bioresources in the Caspian Sea ecosystem 2000-2010. *CaspEco Report*, P: 423.

بهینه سازی بین گونه ای نشانگرهای میکروستلایت خانواده شگ ماهیان در ماهی کیلکای معمولی *Clupeonella cultriventris* دریای خزر

م. نوروزی*، م. سمیعی

(تاریخ دریافت: ۹۳/۹/۱۱ - تاریخ پذیرش: ۹۴/۲/۱۳)

چکیده

ماهی کیلکای معمولی، *Clupeonella cultriventris* یک ماهی کوچک سطح زی و ساکن آب لب شور است که در دریای خزر به فراوانی پراکنده است. در این بررسی ۶۰ نمونه ماهی کیلکای معمولی بالغ از دو فصل جمع آوری شدند. پانزده جفت نشانگر میکروستلایت که پیشتر برای *A. sapidissima* ، *C. pallasi* و *C. harengus* طراحی شده بود، برای استفاده بین گونه ای برای ماهی کیلکای معمولی بهینه سازی شدند. در این مطالعه، فقط ۵ جفت از نشانگرها تکثیر شدند. میانگین اللی در جایگاهها ۱۴/۴ بدست آمد. میانگین هتروزیگوسیتی مشاهده شده و قابل انتظار به ترتیب ۰/۱۵۳ و ۰/۸۸۸ بود. تمامی جایگاهها انحراف از تعادل هاردی- وینبرگ را نشان دادند. این نتایج به همراه Rst معنی دار نشان دهنده وجود جمعیتهای ژنتیکی متفاوت در سواحل دریای خزر (استان گیلان) می باشد.

* مولف مسئول