Egyptian Journal of Aquatic Biology & Fisheries
Zoology Department, Faculty of Science,
Ain Shams University, Cairo, Egypt.
ISSN 1110 – 6131
Vol. 23(4): 323 – 334 (2019)
www.ejabf.journals.ekb.eg

Genetic divergence in *Trachurus indicus* (Nekrasov, 1966) and *Decapterus maruadsi* (Temminck & Schlegel, 1843) , Family: Carangidae, dwelling the Gulf of Suez, Red Sea, Egypt.

Mohamed E. Megahed[1], Manal M. Sabrah[1*], Tasneem E. Abo-El-Maaty[2], Assar S. El-Sherbiny[1] and Alaa M. Younis[2]

1- National Institute of Oceanography and Fisheries (NIOF), Suez & Aqaba Gulfs Branch, Suez, Postal code: 43511, Egypt.
2- Faculty of Fish Resources, Fisheries Dep., Suez Univ., Egypt.

* Corresponding Author: manal_sabrah@yahoo.co.uk

ARTICLE INFO

Article History:
Received: Sept.15, 2019
Accepted: Oct. 14, 2019
Online: Oct. 20, 2019

Keywords:
Carangidae
Trachurus indicus
Decapterus maruadsi
Gulf of Suez
Microsatellite
genetic divergence

ABSTRACT

Family Carangidae is widely distributed in the Atlantic, Indian and pacific Oceans. Two morphologically similar species, *Trachurus indicus* and *Decapterus maruadsi*, were collected and recognized from the Gulf of Suez. The two species have no distinct differences morphologically. Microsatellite DNA markers revealed that the populations of the two species represented distinct genetic divergence. This genetic diversity study showed that the populations of the two species are not related to each other. Overall, this study reveals high genetic diversity of *T. indicus* and *D. maruadsi* populations. Further studies on the genetic structure of these two species, are needed not only to understand the evolutionary history of the species, but also to improve the knowledge-based fishery management programs of this important biological resources.

INTRODUCTION

Carangids are found in all tropical and subtropical marine waters of the world, and some occur in temperate regions **(**Smith, 1986**).** Family Carangidae are the most dominant fish group in the Gulf of Suez, Red Sea fisheries. *Trachurus indicus* and *Decapterus maruadsi* are pelagic marine species constituting the most commercially-important Carangidae in the Suez Gulf (Sabrah, 2015). Kijima *et al*. (1986, 1988) stated that the subfamily Caranginae could be divided into two large group on the basis of genetic variation by analyzing the relationship within a certain taxonomic level. The species in family Carangidae is morphologically somewhat highly specialized, adapting to long migration around offshore water (Vergara, 1972). Although a lot of morphological and ecological studies among species were published (Gushiken, 1983), taxonomic relationships among species have not yet been thoroughly estimated. Very little information is available concerning fish genetic identification. The identification process through DNA barcoding was under taken by many authors (ex. Kijima *et al*. 1988; Mat Jaafar *et al*. 2012; Mat Jaafar, 2014; Ahmad *et al*. 2016). It would be necessary to analyze relationships among them by using genetic markers such as microsatellites. The Wahlund effect was first described by (Wahlund, 1928).

It is an explanation for the observation of a deficit of heterozygotes or excess of homozygotes at the majority of loci in a sample from a naturally occurring population. If heterozygote deficit occurs in the minority of loci, other explanations are more likely (e.g. natural selection, null alleles, and inbreeding). Heterozygote deficit occurs at nuclear markers such as allozymes and microsatellite loci. When there are two alleles at a locus, the robust Hardy-Weinberg principle allows to calculate expected frequency of heterozygous individuals, as Hardy-Weinberg equilibrium postulates balanced frequencies of different populations in equally-mixed alleles.

The objectives of the present study are to estimate the degrees of genetic divergence and richness among and within *T. indicus* and *D. maruadsi* populations in the Gulf of Suez on the basis of Microsatellites markers.

MATERIALS AND METHODS

Sample collection and DNA extraction

Trachurus indicus and *Decapterus maruadsi* samples were randomly collected from the commercial purse seine boats adopted in the Gulf of Suez (Fig. 1) during the period from September 2018 to April 2019. In this study, 12 specimens of *T. indicus* and 12 of *D. maruadsi* were selected for comparison of their molecular data. The fin clips from each specimen were preserved in 95% ethanol immediately after collection from the landing site of Gulf of Suez, Attaka fishing port landing site at the Gulf of Suez.

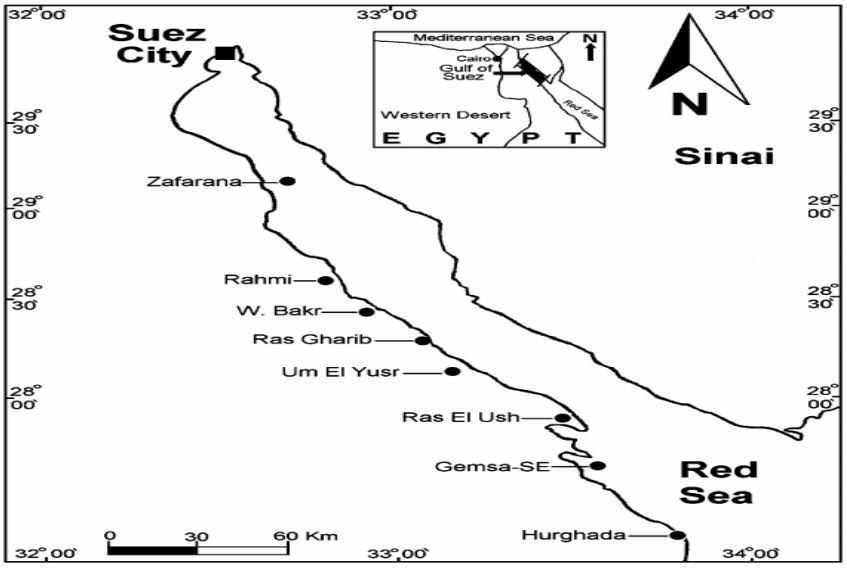

Fig. 1: Gulf of Suez, showing the fishery ground of *T. indicus* and *D. maruadsi.*

Total genomic DNA was extracted using two extraction methods: CTAB extraction method (Mirimin and Roodt-Wilding, 2015) and using a commercial PureLink Genomic DNA Mini Kit (ThermoFisher Scientific, USA), from fin clips (10-15 mg) of the preserved fin clips. The extracted DNA was kept at -20°C for further analyses. The reason to use two methods for DNA extraction is to find out the cheapest methods for DNA extraction in routine work in fisheries population genetics.

The quality and quantity of the extracted DNA was checked using agarose gel electrophoresis and UV spectrophotometer (Beckman, USA) by taking the optical density (OD) at 260 nm and 280 nm. The value between 1.7 - 1.8 indicates good quality DNA without protein/RNA contamination. DNA quantification was done according to the following calculation: sample showing 1 OD at 260 nm is equivalent

to 50 μg of DNA/ml. The OD of each DNA sample at 260 nm was measured and quantified accordingly.

Microsatellite analysis

Two microsatellite loci, TmurA115 $TG_{(28)}$ Genbank (FJ668658) F: GTCACTGGAGCAATCAATAGAC R: TGCAATGTTACATGACTCAGAG and TmurB104 F: TGAAGCACAAGTTTCCAAATC $ATC_{(14)}$ Genbnk (FJ668661) R: AAAGGTCAGAGAGAGAACAACG developed by Cristian *et al*. (2009) were used in this study. Mendelian inheritance mode of these loci in all families was studied. PCR amplifications used with the following parameters: 94^{o}C for 3 min, followed by 35 cycles of 94^{o}C for 40 s, 57^{o}C for 30 s, 72^{o}C for 30 s, and a final extension at 72^{o}C for 5 min. All loci successfully amplified under the same conditions. Amplified samples were run on a polyacrylamide gel with 7.6% acrylamide/bis acrylamide (19:1), 7.6 M urea, lxTBE (89 mM tris, 89 mM boric acid, 2.5 mM EDTA). The gel was pre-run for 30 min and followed by electrophoresis for 45-60 min in a BioRad Sequi-Gen GT DNA sequencing cell at 50°C and 110 W. The bands were visualized using the silver staining technique modified from Merril *et al.* (1979) and the fragment sizes were estimated using the Gel Imaging Analyzing System (Kodak Digital Science, EDAS-120) with a standard 30-330 bp DNA ladder (Invitrogen). A single set of stutter bands was regarded as a single allele and the strongest band was consistently scored for size determination (Tautz, 1989; Xu *et al*. 2001).

A double-band pattern was treated as a heterozygous pair, while a single band or a single set of stutter bands was considered as a homozygous (or null) allele. Genetic diversity in each locality was estimated from the average and effective number of alleles, the allelic and genotypic frequencies, and the expected and observed heterozygosities. Departure from Hardy-Weinberg equilibrium was detected using an exact test (Guo and Thompson, 1992) with the Markov chain algorithm with 3000 dememorization steps and 100000 randomizations using Arlequin (Schneider *et al*. 2000). F_{IS} values were estimated to determine the genetic variation within population (Weir and Cockerham, 1984). Probabilities of significance for these values were tested based on 480 permutations by using FSTAT version 2.9.3.2 software (Goudet, 1995). Genetic differentiation was determined by performing exact test on allelic frequency and distribution between population pairs (Raymond and Rousset, 1995a) using GENEPOP version 3.4 (Raymond and Rousset, 1995b). F_{ST} values were estimated from the pairwise distance based on an infinite-alleles model (Reynold, *et al.* 1983; Weir and Cockerham 1984; Slatkin, 1985) and the analogous R_{ST} values were calculated from the sum of squared size differences based on a stepwise-mutation model (Slatkin, 1985; Michalakis and Excoffier, 1996; Rousset, 1996). The significance of each estimate was tested by performing 10 000 permutations with Arlequin and R_{ST} CALC (Goodman, 1997). Mantel test (Mantel, 1967; Sokal and Rohlf, 1995) was performed using Arlequin to test genetic isolation by distance. An assignment test was also performed to clarify the identity of specimens from the sympatric zone by using the software Whichrun version 4.1 (Banks and Eichert, 2000).

RESULTS

Morphological comparison

The morphological comparison between *D. Maruadsi and T. Indicus* was illustrated in Table (1). Most of the morphological characters are shared by the two species to the degree that is could be difficult to distinguish between them visually.

Table 1: Morphological comparison between *D. Maruadsi and T. Indicus* according to Dalyan and Eryilmaz (2009).

Items	*D. maruadsi*	*T. indicus*
Photo		
Body	Body elongate, fusiform, and moderately compressed	Body elongate, slightly compressed, with upper and lower profiles similar.
Eye	Moderate eye with adipose eyelid well developed usually covering most of eye except for a vertical oval centered on pupil.	Moderate eye with adipose eyelid well developed usually covering most of eye except for a vertical oval centered on pupil.
Jaw	Upper Jaw reaching to just below front margin eye.	Upper jaw moderately broad and extending to below anterior margin of eye.
Teeth	In single series, those in upper jaw confined to anterior	Teeth small, in a single row in upper and lower jaws.
Dorsal Fin	First dorsal fin: 7-8 spines; second dorsal fin: one spine and 30-33 soft rays.	First dorsal fin: 7-8 spines; second dorsal fin: one spine and 30-33 soft rays.
Anal Fin	3 spines, followed by 23-29 soft rays.	3 spines, followed by 23-29 soft rays.
Caudal Fin	Forked	Forked
Finlet	A single finlet behind dorsal and anal fins.	-
Lateral Line	Lateral line slightly arched, becoming straight. Curved portion longer than straight portion; 32 to 38 moderate scutes.	Lateral line arched , the scutes on the anterior to the curved part of the lateral line not shorter than those on the posterior part of the line
Scutes On Curved Part Of Lateral Line	38:40	38:40
Scutes On Straight Part Of Lateral Line	36:39	36:39
Color	Body: Colour green to blue-green above, silvery white below. Fins: dorsal, pectoral and caudal fins pale yellow, anterior apex of 2nd dorsal fin with a black spot on edge of operculum, pupil black.	Black opercular spot on edge near upper margin; body and head dorsally dusky to nearly black or grey to bluish green; lower two-thirds of body and head usually paler, whitish to silvery; posterior margin of caudal fin blackish.

Microsatellites analyses

Allelic distribution and population differentiation

DNA quality was assessed using two extraction methods (Fig. 2). The two methods, CTAB and commercial PureLink Genomic DNA Mini Kit (ThermoFisher Scientific, USA), gave high quality DNA templates for the microsatellites PCR reaction.

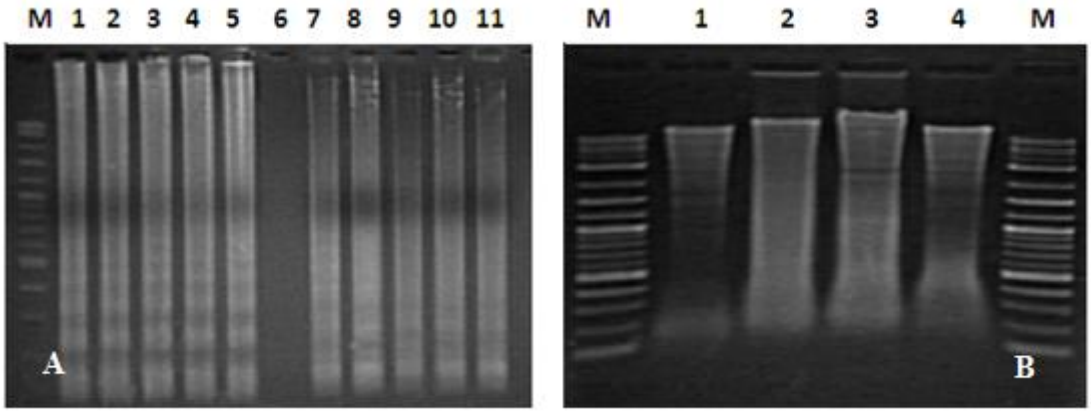

Fig. 2: DNA extraction gel electrophoresis of *T. indicus* and *D. maruadsi* using two DNA extraction methods. A) M, molecular markers; lane 6, sterilized water, lanes 1-5 DNA extraction from *T. indicus* and Lanes 7-11, DNA extraction from *D.maruadsi* using CTAB extraction method. B) M, molecular markers; lanes 1-2 DNA extraction from *T. indicus* and Lanes 3-4, DNA extraction from *D. maruadsi* using PureLink Genomic DNA Mini Kit (ThermoFisher Scientific, USA).

The two microsatellite loci used in this study were highly polymorphic. A total number of 139 and 126 Alleles were obtained from *T. indicus* and *D. maruadsi* using microsatellite marker TmurA115, *respectively* (Table 2). Allele sizes ranged from118-199 and 118-180 in *T. indicus* and *D. maruadsi* using microsatellite marker TmurA115, respectively (Table 2). Using microsatellite marker TmurB104, a total of 124 and 105 alleles were obtained from *T. indicus* and *D. maruadsi*, respectively. Alleles sized ranged from 99 – 200 and 105-198 in *T. indicus* and *D. maruadsi*, respectively (Table 2).

Average gene diversity over the two loci of the *T. indicus* population (0.875-0.967) was greater than that of the *D. maruadsi* (0.835-0.975), *(P*<0.05). For TmurA115, alleles with sizes 199 were found only in the *T. indicus* population. The most frequent allele was 119 which only accounted for 41.66% of the alleles in the *T. indicus* and *D. maruadsi* population. For TmurB104, alleles with sizes <200 were observed only in the *T. indicus* populations and absent in *D. maruadsi* populations. The most abundant allele (99) accounted for 25% of the alleles in the two populations. Across the two loci, observed heterozygosity (H_0) for each population ranged from 0.854 to 0.953 and 0.785 to 0.975 in *T. indicus* and *D. maruadsi* populations, respectively. The expected heterozygosity (H_s) ranged from 0.934 to 0.993 and from 0.933 to 0.977 in *T. indicus* and *D. maruadsi* populations, respectively. The F_{is} values indicated significant reduction in the average proportion of homozygous genotypes in *T. indicus* and *D. maruadsi* populations from Gulfs of Suez for the combined loci. Allelic distribution revealed significant difference in the alleles found in *T. indicus* was also found in *D. maruadsi* (Table 2). Pairwise F_{st} and R_{st} revealed that the *T. indicus* populations were genetically distinct from *D. maruadsi* (F_{st} = 0.001-0.077; F_{st} =0.001- 0.063, *P*<0.05) (Table 3).

Table 2: Allelic variability of two microsatellite loci of *T. indicus* and *D. maruadsi* populations.

Loci		*T. indicus*						*D. maruadsi*					
Tmur A115	Allele size	121-179	118-187	120-199	119-198	119-199	119-199	116-180	116-169	116-169	119-168	119-170	118-170
	No. of alleles *(A)*	19	22	23	27	23	25	23	20	22	20	20	21
	a_e	13.0	5.3	16.2	21.2	19.7	19.3	17.8	17.9	13.4	11.4	11.2	11.2
	H_0	0.903	0.953	0.895	0.874	0.831	0.915	0.906	0.963	0.897	0.901	0.908	0.847
	H_e	0.946	0.951	0.969	0.974	0.976	0.984	0.957	0.946	0.983	0.978	0.938	0.946
	D	-0.014	0.013	-0.074	-0.119	-0.145	-0.042	0.008	-0.024	-0.158	-0.055	-0.147	-0.235
		<0.0001	1.000	0.093	<0.0001	0.691	0.166	0.961	1.000	0.083	0.963	0.994	0.091
	F_{is}	0.016	-0.015	0.078	0.119	0.165	0.047	-0.013	-0.050	0.175	0.067	0.159	0.559
Tmur B104	Allele size	101-200	99-197	99-219	95-200	98-198	99-212	105-198	119-195	101-150	115-142	115-142	106-142
	No. of alleles *(A)*	17	19	22	26	20	18	19	11	16	19	19	21
	a_e	17.3	18.2	19.3	17.5	17.2	19.8	16.8	17.21	19.3	17.21	17.3	19.7
	H_0	0.879	0.952	0.975	0.898	0.793	0.898	0.897	0.885	0.897	0.818	0.567	0.870
	H_e	0.985	0.989	0.993	0.967	0.964	0.986	0.866	0.798	0.834	0.875	0.819	0.857
	D	-.048	-0.097	0.017	-0.088	-0.184	-0.096	0.015	-0.086	0.055	-0.035	-0.353	0.066
	P	0.098	0.676	0.855	0.987	<0.0001	0.065	0.169	0.697	0.193	1.000	0.097	0.786
	F_{is}	0.053	0.122	-0.018	0.095	0.155	0.88	-0.069	0.064	-0.076	-0.054	0.326	-0.083
Two loci	H_o	0.923	0.953	0.921	0.854	0.912	0.946	0.957	0.889	0.822	0.864	0.785	0.895
	H_e	0.993	0.984	0.934	0.974	0.986	0.958	0.976	0.933	0.995	0.959	0.938	0.997
	D	-0.074	-0.088	-0.083	-0.127	-0.187	-0.079	-0.046	-0.126	-0.119	-0.097	-0.236	-0.198
	P	<0.0001	0.762	0.025	<0.0001	<0.001	0.468	0.135	<0.001	0.057	0.239	<0.0001	<0.001
	F_{is}	0.0376	0.067	0.074	0.099	0.147	0.064	0.046	0.089	0.069	0.086	0.954	0.089
	Gene diversity	0.967	0.965	0.965	0.943	0.9474	0.875	0.835	0.856	0.856	0864	0.975	0.876

[1]ae effective number of alleles; H0, observed heterozygosity; He expected heterozygosity; [2]D, deficit or excess of heterozygosity; P, probability of deviation from Hardy-Weinberg equilibrium; FIS, genetic variation within population.[1] ae: Calculated according to the formula: $= 1/\sum Xi^2$ (Crow and Kimura 1965); and [2]D = (H0 - He)/ He, P<0.05.

Table 3: Pairwise comparison of genetic differentiation between *T. indicus* and *D. maruadsi* populations based on the combined data of the two microsatellite loci.

	T. indicus						*D. maruadsi*					
Population	1	2	3	4	5	6	7	8	9	10	11	12
1		-0.053	0.015	-0.019	0.009	-0.008	0.119	0.170	0.131	0.065	0.097	0.264
2	0.001		-0.011	0.063	0.006	0.008	0.323	0.393	0.350	0.246	0.241	0.329
3	-0.004	0.00		0.025	-0.002	-0.006	0.239	0.294	0.236	0.151	0.132	0.275
4	0.003	0.003	0.004		0.008	0.024	0.079	0.141	0.117	0.025	0.023	0.235
5	0.005	0.004	0.004	-0.005		0.001	0.164	0.225	0.148	0.055	0.090	0.200
6	0.009	-0.001	0.002	0.003	0.005		0.215	0.272	0.248	0.130	0.082	0.322
7	0.062	0.043	0.035	0.022	0.032	0.039		0.009	-0.012	-0.014	0.143	0.363
8	0.081	0.087	0.061	0.057	0.060	0.077	0.048		0.012	0.055	0.285	0.442
9	0.065	0.063	0.053	0.042	0.044	0.057	0.008	0.054		0.017	0.201	0.346
10	0.061	0.052	0.053	0.026	0.020	0.046	0.007	0.021	0.027		0.119	0.467
11	0.072	0.091	0.094	0.047	0.065	0.075	0.056	0.137	0.068	0.058		0.924
12	0.058	0.063	0.052	0.038	0.041	0.040	0.063	0.123	0.133	0.096	0.154	

P < 0.05

Fst (below diagonal) and Slatkin's analogous Rst (above diagonal). Significance of genetic distances tested by 10100 permutations was calculated using Arlequin.

Pairwise values among *T. indicus* populations were generally not greater than zero, revealing a high level of gene flow among populations (F_{st} = 0.001-0.077, P<0.05). The *D. maruadsi* populations was significantly diverged from the *T. indicus* (F_{st} = 0.007-0.154, R_{st} = 0.025-0.924; P<0.00001). Genetic heterogeneity was observed in comparisons between *D.maruadsi* and *T. indicus* (*P<0.05*), suggesting that *T. indicus* and *D. maruadsi* populations were not related. The two geographically close populations showed unexpected divergence (F_{st} = 0.001-0.154, F_{st} = 0.001-0.924, *P<0.05*). Exact tests of population differentiation revealed that *T. indicus* population was genetically diverged from *D.maruadsi* (*P<0.005*). The test also supported genetic divergence of the *T. indicus* and *D. maruadsi* population *(P*<0.00001). However, genetic heterogeneity was observed in comparisons between the populations of *T. indicus* and *D. maruadsi (P* = 0.742 and 0.347, respectively).

The two geographically distant *T. indicus* and *D.maruadsi* populations showed unexpected divergence *(P*=0.017). Mantel tests of geographical distance versus genetic divergence were not significant in *(P*>0.05) except between the *D.maruadsi* populations *(P*<0.05) (Table 4).

Table 4: Exact test of population differentiation among *T. indicus* and *D. maruadsi* populations.

	T. indicus						***D. maruadsi***					
Population	1	2	3	4	5	6	7	8	9	10	11	12
1		0.104	0.742	0.209	0.026	0.203	<0.00001	<0.00001	<0.00001	<0.001	<0.00001	<0.00001
2	0.231 *0.228*		0.021	0.644	0.012	0.657	<0.00001	<0.00001	<0.00001	<0.00001	<0.00001	<0.00001
3	0.600 *0.352*	0.004 *0.912*		0.150	0.052	0.253	<0.00001	<0.00001	<0.00001	<0.00001	<0.00001	<0.00001
4	0.079 *0.441*	0.409 *0..342*	0.151 *0.268*		0.416	0.349	<0.001	<0.001	<0.00001	0.009	<0.00001	<0.00001
5	0.010 *0.319*	0.004 *0.400*	0.035 *0.326*	0.329 *0.796*		0.079	<0.00001	<0.001	<0.00001	0.008	<0.00001	<0.00001
6	0.015 *0.524*	0.228 *0.628*	0.102 *0.765*	0.153 *0.713*	0.043 *0.277*		<0.00001	<0.00001	<0.00001	<0.00001	<0.00001	<0.00001
7	0.001 *<0.00001*	0.014 *<0.00001*	0.002 *< .00001*	0.107 *0.001*	0.008 *< .00001*	0.003 *< .00001*		0.033	0.029	0.286	<0.00001	<0.00001
8	0.024 *<.00001*	0.011 *<.00001*	0.011 *< .00001*	0.033 *0.001*	0.053 *< 0.0001*	0.006 *< .00001*	0.224 *0.012*		0.016	0.347	<0.00001	<0.00001
9	<0.0001 *<0.00001*	0.005 *<0.00001*	<0.001 *< .00001*	0.006 *< 0.0001*	0.009 *< .00001*	0.001 *<0.00001*	0.123 *0.142*	0.017 *0.058*		0.015	<0.00001	<0.00001
10	0.030 *0.0002*	0.023 *<0.00001*	0.007 *< 0.0001*	0.414 *0.022*	0.352 *0.001*	0.003 *< 0.001*	0.135 *0.472*	0.420 *0.138*	0.047 *0.035*		0.017	<0.00001
11	0.164 *<0.00001*	0.031 *<0.00001*	<0.001 0.012	0.030 *<.00001*	*< .00001* *< .00001*	0.003 *<0.00001*	<0.001 *0.002*	0.007 *< .00001*	<0.0001 *<* *0.00001*	0.450 *0.003*		<0.00001
12	<0.00001 <0.001	<0.00001 <0.001	<0.00001 <0.001	<0.00001 0.002	<0.00001 0.001	<0.00001 *<0.00001*	*<* *0.00001* *< 0.0001*	0. 003 *< 0.0001*	*<* *0.00001* *<0.00001*	0. 006 *<0.00001*	*<0.00001* *<0.00001*	

P<0.05 The results based on 10 000 Markov steps for each locus (TmurA115 and TmurB104) and Fisher's method across the two loci of microsatellite data (below diagonal: TmurA115 in normal font and TmurB104 in italic; above diagonal: both loci).

DISCUSSION

Analyses of allelic frequency and distribution of microsatellite loci offer strong evidence for insignificant gene flow between the two *T. indicus* and *D. maruadsi* species. The result shows the presence of a large proportion of shared microsatellite alleles among the two species as a result from the retention of ancestral polymorphism rather than hybridization. Thus the two microsatellite loci used in this study are effective in clarifying the issue of reproductive isolation between *T. indicus* and *D. maruadsi*. Breeding experiments are needed to elucidate whether the two species are reproductively compatible. No significant deviation from Hardy-Weinberg equilibrium (HWE) indicates a homogenous deficit in two microsatellite loci in the 12 population studied. No significant departure from HWE and this was not showing

Wahlund effect (Wahlund, 1928). The homogenous deficit in *T. indicus* and *D. maruadsi* population may be resulting from the non mixing of individuals from the two populations homogenous that shows they are genetically different due to their occurrence in a single locality (Gulf of Suez) (Johnson and Black, 1984; Dixon *et al*. 1993). The Wahlund effect has been suggested as a cause for heterozygous deficiency, (Johnson and Black, 1984; Pritchard *et al.* 2000; Valles-Jimenez *et al.* 2005; Zelenina & Rastorguev, 2010; Buryakova & Glubokov, 2011; Bekkevold *et al*. 2011; Afanaziev, *et al.* 2012 and Ovenden, 2013). The results shows the presence of null alleles as indicated by the low F_{is} values (homozygous deficit) and this is a common problem in population studies using microsatellites, (Hauser *et al*. 2002; Ball and Chapman 1998; Burridge and Smolenski, 2003; Cristian *et al*. 2009; Tzeng *et al.* 2009; Bekkevold *et al*. 2011; .Nugroho *et al*. 2011). Assortative mating and reproductive success (Supungul *et al*. 2000) could lead to inbreeding and HW disequilibrium. In this case, all loci are supposed to be significantly correlated (linkage disequilibrium) (Ayre *et al*. 1997; Pritchard *et al.* 2000; Castric *et al*. 2002; Burridge and Smolenski, 2003; Cárdenas *et al.* 2005; Bekkevold *et al*. 2011) and this has not been shown from the present results indicating the two species are separated and not mixed. The present study did not show linkage disequilibrium between the two microsatellites loci used, except for population from *T. indicus* and *D. maruadsi* (Pritchard *et al*. 2000). Thus there are no inbreeding detected using microsatellites markers used in this study. The moderate to high genetic diversity and increase in the effective numbers of alleles in the *T. indicus* and *D. maruadsi* population may reveal a sufficient and high effective population size and shows the reproductive success of the two populations (Okazaki *et al*., 1996; Ayre *et al.*, 1997; Brooker *et al*,. 2000; Bekkevold *et al*., 2011).

This study provides strong evidence for phylogeographic structuring of *T. indicus* and *D. maruadsi* in the Gulf of Suez. Investigations on seasonal variations in population structure and migratory behaviors of the *T. indicus* and *D. maruadsi* population in the Gulf of Suez are required. The migratory behavior of the two studied species may have different habitat preferences **(**Knowlton, 1993; Okazaki *et al.*, 1996). The geographical distribution of the two species may be limited by environmental tolerances such as temperature (Okazaki *et al.*, 1996; Chu *et al.*, 2005). This also shows that the species might have evolved independently, driven by different selective pressure (such as temperature), establishing the two cryptic species. *T. indicus* and *D. maruadsi* have successfully adapted to the Gulf of Suez environment. This may explain the genetic differentiation between the two populations. The population genetic structure of the two species observed today in the Gulf of Suez is likely attributed to environmental adaptations. Selective forces may shift the gene frequencies rapidly and contribute significantly to reproductive isolation (Barton, 1989; Ayre *et al*., 1997; Pritchard *et al.*, 2000; Bekkevold *et al.*, 2011; Ovenden, 2013). Studies of their environmental tolerances and reproductive biology can provide us with new insights into the biology and evolutionary history of these two species. This study shows that *T. indicus* and *D. maruadsi* should not be considered as a single taxon. Thus all relevant biological studies of these two species, with their implications in fisheries and conservation should be revaluated. Concerning microsatellite markers, the existing results shows that there are some differences between the two species, as it appears in the genetic differences in the two species over the whole distribution area (Cárdenas *et al*., 2005; Ovenden, 2013). There are no discrete borders between the different spawning and population areas in the Gulfs of Suez and its shows the existence of at least two separate "groups" of *T. indicus* and *D.*

maruadsi along the Gulf of Suez. In general, molecular methods have a higher reproducibility than morphometrics. These advantages as a result of their ability to detect minor genome differences compared with that of phenotypic profiles for some species (Tenover *et al*., 1997).

CONCLUSION

The results elucidate the geographic distribution of the two species (*T. indicus* and *D. maruadsi*) in the Gulf of Suez with a population structure. The *T. indicus* and *D.maruadsi* are species complex comprising two morphologically similar but genetically distinct species in the Gulf of Suez and they are characterized by reproductive isolation and high level of genetic divergence. The two species are genetically diverged from the others. This information is crucial for elucidating the taxonomy and evolutionary history of these species. The understanding of *T. indicus* and *D.maruadsi* phylogeography is important in formulating knowledge-based fishery management and development programs for these important marine biological resources. Further studies on the genetic structure of the Gulf of Suez population and the biology of the two species are crucial for elucidating the taxonomy and evolutionary history of these species.

REFERENCES

Ahmad, M. I.; Sabrah, M. M.; Heneish, R. A. and Alwany, M. E. (2016). DNA barcoding uncover Cryptic diversity in Goat fishes (Family: Mullidae) across the Egyptian coastal waters. Pakistan J. Bio. Sci. 19:65-70.

Afanaziev, P.K.; Rabchun, M.A. and Glubokov, A.I. (2012). Russian population genetics stidues of jack mackerel in the South Pacific. SWG-11-JM-11, 6 pp.

Ayre, D.J.; Hughes, T.P. and Standish, R.S.(1997). Genetic differentiation, reproductive mode, and gene flow in the brooding coral pocillopora damicornis along the Great Barrier Reef, Australia. Marine Ecology-Progress Series, 159: 175-187.

Ball, A.O.; Leonard, S. and Chapman, R. W. (1998). Characterization of (GT) in microsatellites from native white shrimp (*Penaeus setiferus*). Mol. Ecol., 7: 1251-1253.

Banks, M.A. and Eichert, W. (2000). WHICHRUN (Version 3.2) a computer program for population assignment of individuals based on multilocus genotype data. J Hered. 91: 87-89.

Barton, N.H.(1989). Founder effect speciation. In: Otte D, Endler JA (eds.) Speciation and Its Consequences, Sinauer, Sunderland.

Bekkevold, D.; Clausen, L.A.W. and Mariani, S. (2011). Genetic mixed-stock analysis of Atlantic herring populations in a mixed feeding area. Marine Ecology Progress Series, 442: 187-199.

Brooker, A.L.; Benzie, J.A.H.; Blair, D. and Versini, J. J. (2000). Population structure of the giant tiger prawn *Penaeus monodon* in Australian waters, determined using microsatellite markers. Mar. Biol., 136: 149-157.

Burridge, C.P. and Smolenski, A.J. (2003). Lack of genetic divergence found with microsatellite DNA markers in the tarakihi Nemadactylus macropterus. New Zealand Journal of Marine and Freshwater Research, 37: 223-230.

Buryakova, M.E. and Glubokov, A.I. (2011). Russian population genetics study of jack mackerel in the South Pacific. SWG-10-JM-05, 2011: 6 pp.

Cárdenas, L.; Hernandez, C.E. and Poulin, E. (2005). Origin, diversification, and historical biogeography of the genus Trachurus (Perciformes: Carangidae). Molecular Phylogenetics and Evolution, 35: 496-507.

Castric, V.; Bematchez, L.; Belkhir, K. and Bonhomme, F. (2002). Heterozygote deficiencies in small lacustrine populations of brook charr Salvelinus fontinalis Mitchill (Pices, Salmonidae): a test of alternative hypotheses. Heredity, 89: 27-35.

Chu, P.; Chen, Y. and Kuninaka, A. (2005). Seasonal variability of the Yellow Sea/East China Sea surface fluxes and thermohaline structure. Adv Atmos Sci., 2: 11-24.

Cristian, B.; Canalesm, A.; Sandra, F. and Ricardo Galleguillos. (2009). Isolation and characterization of microsatellite loci for the jack mackerel (*Trachurus murphyi* Nichols, 1920). Conserv Genet. DOI 10.1007/s10592-009-9932-y

Dixon, P.I.; Worland, L.J. and Chan, B.H.Y. (1993). Stock identification and discrimination of pilchards in Australian waters, using genetic criteria.

Dalyan, C. and Eryilmaz, L. (2009). The Arabian scad Trachurus indicus, a new Indo-Pacific species in the Mediterranean Sea. J. Fish Biology, 74: 1615–1619.

Goodman, S.J. (1997). Rst CACL: A collection of computer programs for calculating unbiased estimates of genetic differentiation and determining their significance for microsatellite data. Mol. Ecol., 6: 881-885.

Goudet, J. (1995). FSTAT version 1.2: a computer program to calculate F-statistics. J Hered, 86: 485-486.

Guo, S. and Thompson, E.(1992). Performing the exact test of Hardy-Weinberg proportion for multiple alleles. Biometrics, 48: 361-372

Gushiken, S. (1983). Revision of the carangid fishes of Japan. Galaxea, 2: 135-264.

Hauser, L.; Adcock, G. J.; Smith, P. J.; Ramirez, J. H. B. and Carvalho, G. R. (2002). Loss of microsatellite diversity and low effective population size in an overexploited population of New Zealand snapper (*Pagrus auratus*). Proceedings National Academy Science, USA 99, 11742-11747.

Johnson, M.S. and Black, R. (1984). The Wahlund effect and the geographical scale of variation in the intertidal limpet Siphonaria sp. Marine Biology 79, 295-302.

Kijima, A.; Taniguchi, N. and Ochiai, A. (1986). Genetic relationships in the family Carangidae. Proceedings of the Second International Conference on Indo-Pacific Fishes, 840-848

Kijima, A.; Taniguchi, N. and Ochiai, A. (1988). Genetic divergence among fifteen species of genera Trachurus, Decapterus, Selar and Selaroides. Jap. J. Ichthyol, 35: 167-175

Knowlton, N. (1993). Sibling species in the sea. Ann Rev Ecol. Syst, 24: 189-216.

Mantel, N.(1967). The detection of disease clustering and a generalized regression approach. Cancer Res., 27: 209-220.

Mat Jaafar, T. N. A.; Taylor, M. I.; Mohd Nor, S. A.; de Bruyn, M. and Carvalho, G. R. (2012). DNA Barcoding Reveals Cryptic Diversity within Commercially Exploited IndoMalay Carangidae (Teleosteii: Perciformes). PLoS ONE 7(11): e49623. doi:10.1371/journal.pone.0049623.

Mat Jaafar, T. N. A. (2014). DNA barcoding and population genetic structure of malaysian marine fishes. Ph D Thesis, School of Biological Sciences Bangor University, Wales United Kingdom 2014, 383 p.

Michalakis, Y. and Excoffier, L. (1996). A generic estimation of population subdivision using distances between alleles with special reference to microsatellite loci. Genetics, 142: 1061-1064.

Merril, C. R.; Switzer, R. C. and Van Keuren, M. L. (1979). Trace polypeptides in cellular extracts and human body fluid detected by two-dimensional electrophoresis and a highly sensitive silver stain. Proc Natl Acad Sci USA., 76: 4335-4339.

Mirimin, L. and Roodt-Wilding, R. (2015). Testing and validating a modified CTAB DNA extraction method to enable molecular parentage analysis of fertilized eggs and larvae of an emerging South African aquaculture species, the dusky kob *Argyrosomus japonicus*. J Fish Biol., 86 (3):1218-23.

Nugroho, E. (2011). Genetic diversity evaluation on giant gourami strains based on DNA marker. Jurnal Perikanan J. Fish. Sci., 13 (2): 86-89. [Indonesian]

Okazaki, T.; Kobayashi, T. and Uozumi, Y. (1996). Genetic relationships of pilchards (genus: *Sardinops*) with anti-tropical distributions Marine Biology, 126: 585-590.

Ovenden, J. R. (2013). Crinkles in connectivity: combining genetics and other types of biological data to estimate movement and interbreeding between populations. Marine and Freshwater Research, 64: 201.

Pritchard, J.K.; Stephens, M. and Donnelly, P. (2000). Inference of population structure using multilocus genotype data. Genetics, 155: 945-959.

Raymond, M. and Rousset, F. (1995a). An exact test for population differentiation. Evolution 49: 1280-1283.

Raymond, M. and Rousset, F. (1995b). GENEPOP (version 1.2): population genetics software for exact test and ecumenicism. J. Hered, 86: 248-249.

Reynolds, J.; Weir, B.S. and Cockerman, CC. (1983). Estimation for the coancestory coefficient: basis for a short term genetic distance. Genetics, 105: 767-779.

Rousset, F. (1996). Equilibrium values of measure of population subdivision for stepwise mutation processes. Genetics, 142: 1357-1362.

Sabrah, M. M. (2015). An overview on the fisheries population of Arabian scad, Trachurus indicus Nekrasov, 1966 (F: Carangidae) caught by two different fishing methods from the Gulf of Suez, Egypt. Egypt. J. Aqua. Biol. & Fish., 19(2): 137-148.

Schneider, S.; Roessli, O. and Excoffier, L. (2000). Arlequin Ver 2.000: A Software for Genetics Data Analysis. Genetics and Biometry Laboratory, University of Geneva, Switzerland.

Slatkin, M. (1985). Gene flow in natural populations. Ann Rev Ecol Syst 16: 393-430.

Smith-Vaniz, W. F. (1986). Carangidae. p. 638-661. In M.M. Smith and P.C. Heemstra (eds.) Smiths' sea fishes. Springer-Verlag, Berlin.

Sokal, R. R. and Rohlf, F. J. (1995). Biometry. W. H. Freeman and Company, New York.

Supungul, P.; Sootanan, P.; Klinbunga, S.; Kamonrat, W.; Jarayabhand, P. and Tassanakajon, A. (2000). Microsatellite polymorphism and the population structure of the black tiger shrimp (Penaeus monodon) in Thailand. Mar Biotechnol, 2: 339-347.

Tautz, D. (1989). Hypervariablity of simple sequences as a general source of polymorphic DNA markers. Nucleic Acids Res., 17: 6463-6471.

Tenover, F.C.; Arbeit, R.D. and Goering, R.V. (1997). How to select and interpret molecular strain typing methods for epidemiological studies of bacterial infections: A review for healthcare epidemiologists. Infect. Control Hosp. Epidemiol., 18: 426-439.

Tzeng, C.H.; Chen. C.S.; Tang, P.C. and Chui, T-S. (2009). Microsatellite and mitochondrial haplotype differentiation in blue mackerel (Scomber australasicus) from the western North Pacific., 66: 816-825.

Valles-Jimenez, R.; Cruz, P. and Perez-Enriquez, R. (2005). Population genetic structure of Pacific white shrimp (Litopenaeus vannamei) from Mexico to Panama: microsatellite DNA variation. Mar Biotechnol, 6: 475-484.

Vergara, R. (1972). Analysis taxonomico y consideraciones filogeneticas sobre las especies Cubanas del genero Caranx. (Teleostei, Perciformes, Carangidae). Cent. Invest. Pseq. Cuba, Contrib., 34: 1-138.

Wahlund, S. (1928). Zusammensetzung von Populationen und Korrelationsers-chinungen von Standpunkt der Vererbungslehre aus betrachtet. Hereditas 11: 65-106.

Weir, B. S. and Cockerham, C. C. (1984). Estimating F-statistics for the analysis of population structure. Evolution, 38: 1358-1370.

Xu, Z.; Primavera, J. H.; Pena de la, L. D.; Pettit, P.; Belak, J. and Alcivar Warren, A. (2001). Genetic diversity of wild and cultured black tiger shrimp (*Penaeus monodon*) in the Philippines using microsatellites. Aquaculture, 199: 13-40.

Zelenina, D. A. and Rastorguev, S. M. (2010). Russian population genetics study of jack mackerel in the South Pacific., SWG-09-INF-07, 2010: 7 pp.