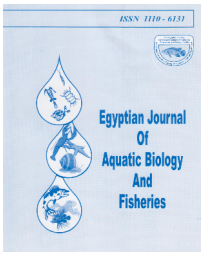
Egyptian Journal of Aquatic Biology & Fisheries
Zoology Department, Faculty of Science,
Ain Shams University, Cairo, Egypt.
ISSN 1110 – 6131
Vol. 22(5): 363- 375 (2018)
www.ejabf.journals.ekb.eg



Restriction Fragment Length Polymorphism (RFLP) of Two Morphologically Confused Crabs from Abu Quir, Alexandria, Egypt.

Tarek G. Ali
Zoology Department, Faculty of Science, Ain Shams University, Cairo, Egypt.
tarekali80@yahoo.com

ARTICLE INFO

Article History:
Received: Oct. 22,2018
Accepted:Dec. 19, 2018
Online: Dec. 30, 2018

Keywords:
Callinectes spidus
Callinectes ornatus
RFLP
Morphometry
Abu Quir
Alexandria
Mediterranean Sea.

ABSTRACT

Two types of morphologically confused crabs were collected from Abu Quir, Alexandria, Egypt, *Callinectes spidus* and *Callinectes ornatus*. The carapace of these crabs was first removed, and the abdominal muscles were fixed in absolute ethyl alcohol. DNA was extracted and restricted by three enzymes, and RFLP technique was conducted. Molecular weight, optical density, flowing bands and Gel-Pro-Analyzer technique were also applied. Morphometric analysis of the two species was applied on the carapace width, carapace length and body weight.

The estimation of similarity index applied on the two species was 0.59. In addition, morphometric studies showed no significant difference in the body weight and carapace length of all samples of both males and females. On the other hand, the mean width of carapace of 16.7% of the male samples showed significant difference, while the rest of male and female samples (83.3%) showed no significant difference. In conclusion, the result of RFLP together with the morphometric data may suggest that the two studied crabs belong to one species.

INTRODUCTION

Crabs form important economically group among invertebrates as edible food species, and may be considered as cosmopolitan species (Fuseya and Watanabe, 1996). Variability of morphological characters of the *crab Portunus trituberculatus* was studied in Japan, using the RFLP technique, where 234 individual of eight populations were surveyed with 4 restricted enzymes (Imai and Numachi, 2002). The same technique was applied on shore crab *Carcinus maenas* and *C. aestuarii* in USA (Darling and Tepolt, 2007). The identification of four species of genus *Maja* on the European coast was investigated using RFLP analysis (Guero, *et al*. 2011). 16 potential species of the Indian Mangrove Mub crab of genus *Scylla* were also studied using the same technique (Mandal, *et al*., 2014). Devi *et al*. (2017) applied the same of technique on *Scylla* crab from south Indian water and found two species of *Scylla*.

Silva and Paula (2008) and Silva *et al*. (2010) studied the morphometric analysis of the shore crab *Carcinus maenas,* using the crab chela, for differentiation between populations of *C. maenas*. In 2004, Smith investigated claw size to differentiate between population of the same species. The patterns of morphometric data for *C. maenas* was also studied by Briam *et al*. (2006).

Callinectes spidus and *Callinectes ornatus* are very similar to each other except that the number of frontal teeth on the carapace in *Callinectes spidus* is four, while this number in *Callinectes ornatus* is six (Rothschild, 2004). In the present study, RFLP technique together with morphometric studies were used to differentiate between these two species, to investigate whether they are one species, two separate species, or two subspecies.

MATERIALS AND METHODS

Biological Material

Adult crab of the two species, *Callinectes spidus* and *Callinectes Ornatus,* were collected from shores of Abu-Quir, Alexandria, Egypt. Their carapace was first removed, and the abdominal muscles of two species were dissected out and placed in absolute ethyl alcohol for molecular analysis in the Molecular biology unit at Entomology Department, Faculty of Science, Ain Shams University.

DNA Extraction (using phenol chloroform isoamyl method)

Pieces of muscle was grinded in an Epindorf tube, then 7ml buffer I, and 7000µl buffer II were added to 1gm of muscle. Protinase K (0.1µg) was also added. The tube was placed in water bath for ⅓ hour at 65°C, then chloroform was added, and the tube was centrifuged at 8000r/min. Supernatant was mixed by vortex, then 70% absolute alcohol was added until the tube was 2/3 full. The mixture was centrifuged at 8000r/10min for 10 seconds. Alcohol was removed and the DNA pellet was centrifuged at 8000r/min for 5 min. 30-50µl water were added to the DNA pellet, and then incubated for further work at 37^{o}C.

RFLP technique

According to Darling and Tepolt (2007), RFLP technique was performed using a series of steps briefly outlined below:

- The total reaction volume (25 µl) was prepared using 15 µl of purified DNA, 3 µl of each restriction endonuclease, 2.5 µl buffer and 4.5 µl distilled H_2O
- Three restricted enzymes (*Eco*RI, *Hin*dIII and *Hae*III) were used as indicated in Table 1:

Table 1: Restriction endonucleases engaged in the current work with their sources, recognition sequences and cut ends.

Enzyme	Source	Recognition Sequence	Cut	Conc.
*Eco*RI	*Escherichia coli*	5'GAATTC 3'CTTAAG	5'---G AATTC---3' 3'---CTTAA G---5'	1 µl/1Ngm **DNA**
*Hin*dIII	*Haemophilusinfluenzae*	5'AAGCTT 3'TTCGAA	5'---A AGCTT---3' 3'---TTCGA A---5'	1 µl/1Ngm **DNA**
*Hae*III	*Haemophilusaegyptius*	5'GGCC 3'CCGG	5'---GG CC---3' 3'---CC GG---5'	1 µl/1Ngm **DNA**

The DNA fragments that have been specifically cut by restriction endonucleases were run in 1% agarose gel. Bands of DNA fragments were photographed by a Polaroid^{TM}-Snap 10.0-Megapixel Digital Camera.
Model: POLSP01W. Bands Patterns of DNA fragments were analyzed by Gel-Pro-Analyzer program .

Statistical analysis

Genetic distance or similarity index (S) was calculated by gel analysis using the simplest estimation equation (Nei and Li, 1979).

$$S = \frac{2\,N1,2}{N1+N2}$$

N1,2 is the total common number of bands between *C. spidus* and *C. ornatus*.
N1 is bands of *C. spidus*,
N2 is bands of *C. ornatus*

N.B. *According to Nei and Li (1979), if the S value is above 0.5, then the tested DNA samples are for one species. On the other hand ,if the S value is lower than (0.5), then the DNA samples are for two separate species or for subspecies.*

Morphometric Studeis

Morphometric studies were applied on the body weight (b.wt.), carapace width (c.w.), and carapace length (c.l.) of 50 males and 50 females of each species. Mean, standard deviation and T test were estimated.

Statistical Analysis

The data was expressed as mean±SD of the crab body weight, carapace width, and carapace length. These data were used to elucidate the difference between the measured values. Significance was defined as $P<0.05$ (Field *et al.*, 2000). Statistical analysis of data was carried out using 'SPSS (Version 23) incorporated within the Microsoft Excel 2010 (Microsoft® Windows 2010) software program.

RESULTS

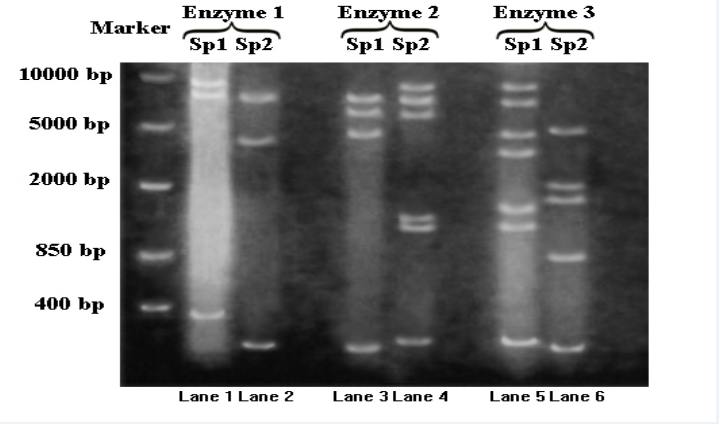


Fig. 1: Photograph of agarose gel showing PCR-RFLP pattern applied on extracted DNA of *C. spidus* (Sp1) and *C. Ornatus* (Sp2), using 3 restricted enzymes (1: *Eco*RI, 2: *Hin*dIII, and 3: *Hae*III).

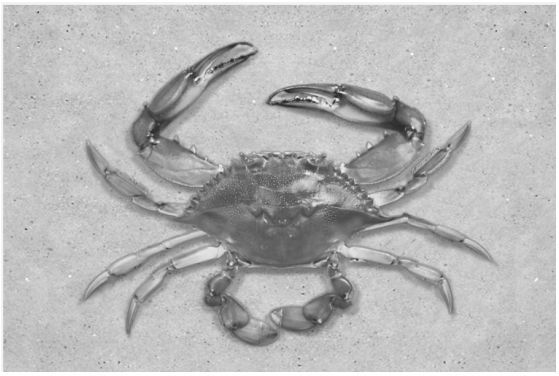


Fig. 2: Dorsal view of *C. spidsus*

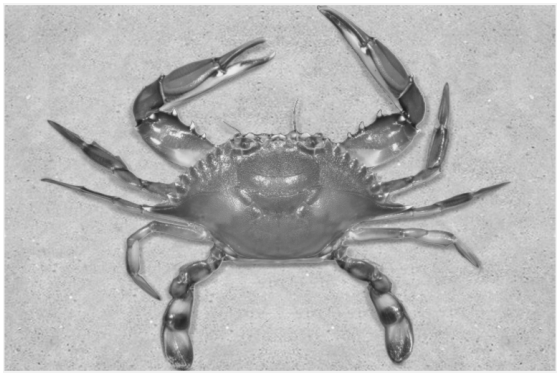


Fig. 3: Dorsal view of *C. ornatus.*

Table 2: Base pair (bp) of DNA restricted segments of *C. spidus* and *C. ornatus*.

Lanes / Rows	Marker bp	Lane 1 *(C. spidus)*	Lane 2 *(C. ornatus)*	Lane 3 *(C. spidus)*	Lane 4 *(C. ornatus)*	Lane 5 *(C. spidus)*	Lane 6 *(C. ornatus)*
		bp of *Eco*RI- restricted DNA segment		bp of *Hin*dIII-restricted DNA segment		bp of *Hae*III-restricted DNA segment	
1		9821			9210	9210	
2		8229	8229				
3				7594	7473	7354	
4				6063	6063		
5	5000						4764
6			3928	4396		4396	
7						3345	
8	2000						2000
9						1509	1509
10					1352		
11					1226	1226	
12	850						850
13							
14	400	359					
15			239	225	254	243	225

The maximum mol.wt was 9821.8 bp. in lane 1, and the minimum was 225.56 bp. in lane 3 and lane 6, and the number of common bands was 8 (Table 2).

Table 3: The rate of flow (Rf) or relative mobility of bands

Lanes:	**Lane 1**	**Lane 2**	**Lane 3**	**Lane 4**	**Lane 5**	**Lane 6**
Rows	**(Rf)**	**(Rf)**	**(Rf)**	**(Rf)**	**(Rf)**	**(Rf)**
r1	0.0862			0.1	0.1	
r2	0.123	0.123				
r3			0.14	0.14	0.147	
r4			0.187	0.187		
r5						0.238
r6		0.279	0.255		0.255	
r7					0.313	
r8						0.421
r9					0.5	0.5
r10				0.531		
r11				0.559	0.559	
r12						0.66
r13						
r14	0.852					
r15		0.943	0.957	0.93	0.94	0.957

The maximum rate of flow was 0.957 in lane 3 and lane 6, and the minimum was 0.0862 in lane 1 (Table 3).

Table 4: The Optical Density (OD) of bands.

Lanes:	Marker	Lane 1	Lane 2	Lane 3	Lane 4	Lane 5	Lane 6
Rows	(max OD)	(max OD)	(max OD)	(max OD)	(max OD)	(max OD)	(max OD)
r1		142.4			108.07	104.57	
r2		143.93	115.07				
r3				111.03	113.63	104.37	
r4				110.83	104.37		
r5	106.43						96.1
r6			101	110		104.2	
r7						107.97	
r8	102.57						96.367
r9						116.83	99.833
r10					93.767		
r11					95.467	120.3	
r12	101.23						109.07
r13							
r14	91.933	114.2					
r15			87.433	96.667	93.3	109.47	98

The maximum percentage (%) amount of bands is 6.9209 in lane 4 and the minimum was 2.6145 in lane 2 (Table 4).

Table 5: The DNA percentage (%) in each band of the six lanes.

Lanes:	Marker	Lane 1	Lane 2	Lane 3	Lane 4	Lane 5	Lane 6
Rows	DNA %	DNA %	DNA %	DNA %	DNA %	DNA %	DNA %
R1		3.5376			3.3494	4.3863	
R2		5.022	6.8441				
R3				5.008	6.9209	3.757	
R4				4.734	3.255		
R5	5.8649						4.4952
R6			3.3837	5.4195		3.1463	
R7						4.227	
R8	4.7032						3.2147
R9						3.5224	4.6434
r10					2.9456		
r11					3.6301	3.9873	
r12	8.2703						3.6535
r13							
r14	6.0173	2.8488					
r15			2.6145	3.8003	3.2414	4.6167	3.2531
Sum	24.856	11.408	12.842	18.962	23.342	27.643	19.26
In Lane	100	100	100	100	100	100	100

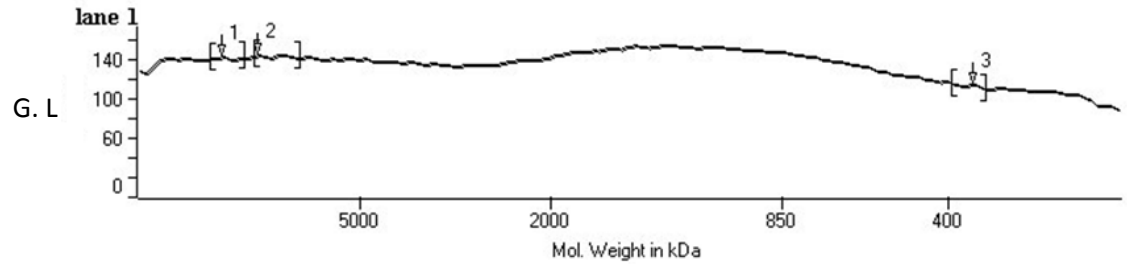


Fig. 4: Showing mol. w. of bands against gel length (G.L) for each lane. Lane 1 showing 3 bands only.

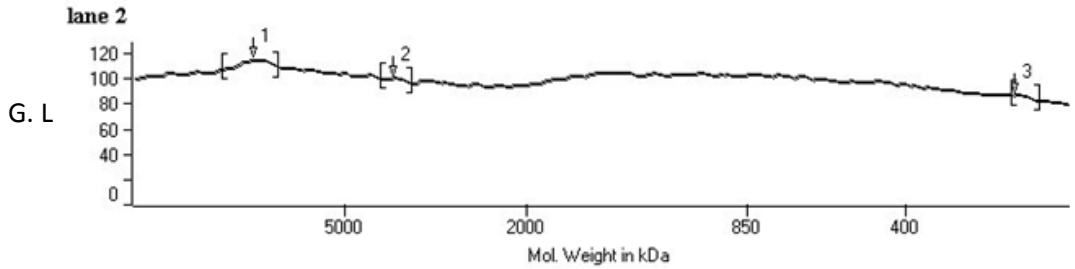


Fig. 5: Showing mol. w. of bands against gel length (G.L) for each lane. Lane 2 showing 3 bands only.

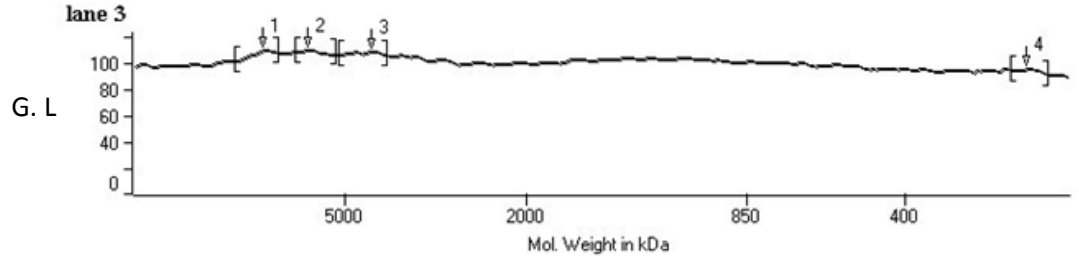


Fig. 6: Showing mol. w. of bands against gel length (G.L) for each lane. Lane 3 showing 4 bands only.

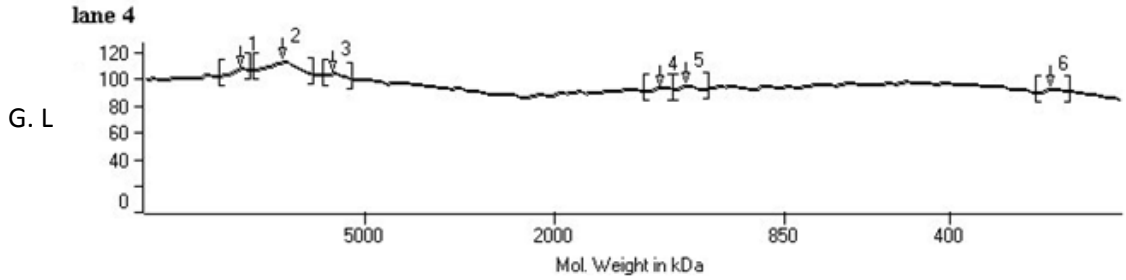


Fig. 7: Showing mol. w. of bands against gel length (G.L) for each lane. Lane 4 showing 6 bands only.

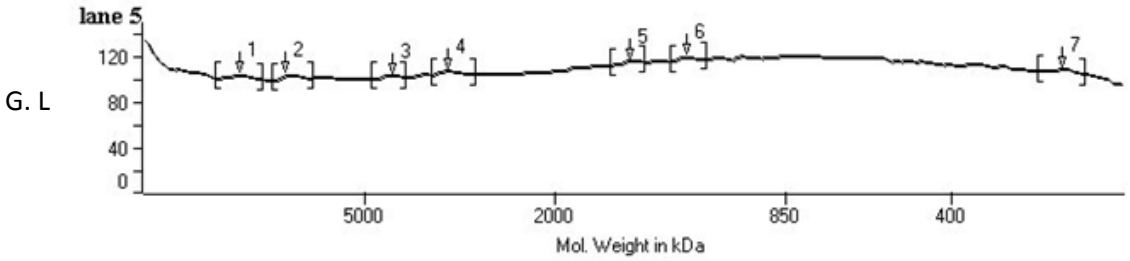


Fig. 8: Showing mol. w. of bands against gel length (G.L) for each lane. Lane 5 showing 7 bands only.

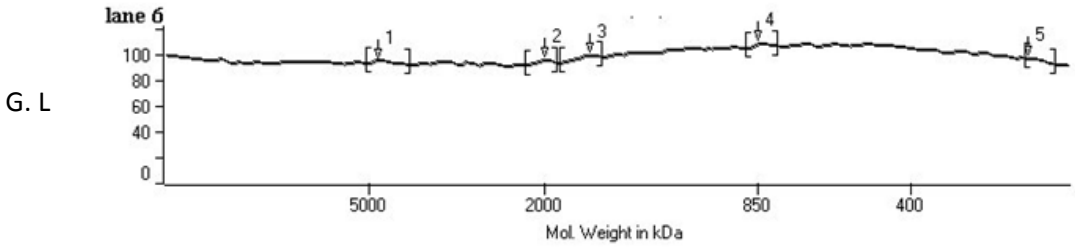


Fig. 9: Showing mol. w. of bands against gel length (G.L) for each lane. Lane 6 showing 5 bands only.

In the current study, RFLP analysis was performed on the two species of *C. spidus* and *C. ornatus*, the results were analyzed using the agarose gel electrophoresis (Fig. 1) with three restricted enzymes. All bands were ranging between 225-9821 base pairs (bp). The first enzyme showed three bands with species of *C. spidus*: (9821, 8229 and 359bp) and three bands with *C. ornatus* species (8229, 3928 and 239bp). The second enzyme resulted in four DNA bands with specice of *C. spidus*:

(7594, 6063, 4396 and 225bp) and six bands with *C. ornatus*: (9210, 549, 6063, 1352, 1226 and 254bp). The third enzyme gave seven bands with *C. spidus*: (9210, 7354, 4396, 3345, 1509, 1226 and 243bp) and only five bands with *C. ornatus*: (4764, 2000, 1509, 850 and 225bp). The total number of bands for the two crab species was 28 bands.

The estimation equation of similarity index for each enzyme gave the following results:

For *Eco* R1 enzyme (lane 1, lane 2): N1 = 3, N2 = 3, N1, 2= 2, S = $\frac{4}{6}$ = 0.67

For *Hin*dIII enzyme (lane 3, lane 4): N1 = 4, N2 = 6, N1, 2= 4, S = 0.8

For *Hae*III enzyme (lane 5, lane 6): N1 = 7, N2 = 5, N1, 2= 2, S = 0.3

The average value of the previous results = 0.59. Since the S value was above 0.5, then the tested DNA samples of the studied crabs are thought to be for one species.

Table 6: The mean±SD (standard deviation) of measured parameters off males and females *C. spidus*.

	Male	**Female**
Parameter	**mean ± SD**	**mean ± SD**
c. w	54.62 ± 1.83 (mm)	53.66 ± 2.35 (mm)
c. l.	65.66 ± 6.57 (mm)	62.53 ± 2.70 (mm)
b. wt	162.14 ± 4.97 (gm)	163 ± 4.22 (gm)

Table 7: The mean±SD (standard deviation) of measured parameters of males and females *C. orantus*.

	Male	**Female**
Parameter	**mean ± SD**	**mean ± SD**
c. w.	53.6 ± 3.26 (mm)	51.96 ± 2.70 (mm)
c. l.	63.99 ± 9.29 (mm)	60.54 ± 2.80 (mm)
b. wt	161.1 ± 7.09 (gm)	159 ± 4.38 (gm)

Table 8: T test of measured parameters of males and females of *C. spidus* and *C. ornatus*.

	Male		**Female**	
c. w	Significant	P<0.05	Not Significant	P>0.05
c. l.	Not Significant	P>0.05	Not Significant	P>0.05
b. wt	Not Significant	P>0.05	Not Significant	P>0.05

The mean width of carapace of 16.7% of the male samples of the two species (*C. spidus* and *C. ornatus*) showed significant difference, while the rest of male and female samples (83.3%) showed no significant difference(P<0.05). On the other hand, the difference was not significant when applied on the carapace length and body weight of the two species (P>0.05).

DISCUSSION

Attempts to identify species may led to much confusion because of the stable morphological differences between individuals of the same species (Keenan *et al.,* 1998). Within species, variation for many important diagnostic characters is large and often overlapping between species. Ontogenetic changes also make identification of juvenile stages difficult.

Although they are morphologically similar except that the number of frontal teeth on the carapace in *Callinectes spidus* is four, while this number in *Callinectes ornatus* is six, these two species were previously described as two separate species

(Rothschild, 2004). However, by applying the estimation equation of similarity index of PCR-RFLP pattern in the present study, the S value of the two species was 0.59. According to Nei and Li (1979), if the S value is above 0.5, then the tested DNA samples are for one species. On the other hand, if the S value is lower than (0.5), then the DNA samples are for two separate species or for subspecies. Since the S value of the two tested species is above 0.5, therefore there is no genetic distance between the two species, and consequently they most likely belong to only one species.

PCR-RFLP is an ideal approach for detecting multiple target species, and its utility has been repeatedly demonstrated (Weathersbee *et al.,* 2003). Weathersbee *et al.* (2003) adopted PCR-RFLP to distinguish between morphologically cryptic eggs of two closely related root weevils, the regulated invasive *Diaprepes abbreviatus* and the minor native pest *Pachnaeus litus*. They described PCR-RFLP as an ideal approach for detecting the genetic distance between different species. In some cases, underlying variation has been sufficient even to target populations from specific geographic origins. Saltonstall (2003), for instance, were able to develop a rapid and inexpensive means of distinguishing invasive and noninvasive haplotypes of the common reed *Phragmites australis* in North America. In another study, species specific restriction sites and genus-specific PCR primers allowed identification of both European and Asian varieties of introduced gypsy moths *Lymantria dispar* (Pfeifer *et al*., 1995).

Darling and Tepolt (2007) also adopted PCR-RFLP to highly sensitive detection of invasive shore crab (*Carcinus maenas* and *Carcinus aestuarii*) larvae in mixed plankton samples. In fact, RFLP pattern has been extensively used as a confirmatory tool to resolve the taxonomic ambiguity of the genus. For instance, this technique was carried out by Fuseya and watanabe (1996) to differentiate *S. serrata* and *S. olivacea* from two other species; *S. paramamosain* and *S. reanquebarica.* Moreover, these authors identified all four species using RFLP by double digestion with *Dra*I and *Hind*III. In addition, Shaji *et al*. (2006) and Mandal *et al*. (2014) used the RFLP pattern to differentiated between the two crab species of the genus *Scylla* (*S. serrata* and *S. olivacea*), and proved that there were a great genetic distance between them. PCR-RFLP was also utilized to distinguish between four species of the genus *Maja* on European coast (Sotelo *et al*., and 2009 Guero *et al*., 2011). Recently, Balasubramanian *et al*. (2014) used RFLP technique to distinguish between green crabs and brown crabs of the genus *Scylla* inhabiting the costal region of India, and they found that this genus could be divided into two genetically separate species; green *S. serrate* and brown *S. olivacea*.

Several morphometric studies of decapod crustaceans have shown significant relationships among some morphometric characters to carapace or total length (e.g., Oh and Hartnoll 1999; Sampedro *et al.* 1999; Conan *et al*. 2001; Wardiatno and Tamaki 2001; Mashar and Wardiatno 2013a,b; Wardiatno and Mashar 2013; Muzammil *et al*. 2015). Variations of some morphometric characters within the same species from different sites or different species from the same family at the same site would be interesting and useful from a biological or taxonomical perspective (Pramithasari *et al*., 2017). Wardiatno and Tamaki (2001) revealed that variations in the size of the cornea and rostrum angle of *Nihonotrypaea japonica* and *Nihonotrypaea harmandi* were effective characters to separate the two species. Wardiatno and Mashar (2013) speculated that variations in claw size between two mantis shrimp species (*Harpiosquilla raphidea* and *Oratosquillina gravieri*) may be a factor behind their competitive abilities.

In the present work, morphometric analysis of the two species *C. orantus* and *C. spidus* was applied on the carapace width, carapace length and body weight. The analyses of covariance were able to detect significant variation in the carapace width of males, while the rest of data for both males and females were insignificant. Some researchers have indicated that the variation in morphometric characters of any species may be caused by various factors, such as geographic region (Hepp *et al.* 2012) including elevation and latitude, environmental conditions (Waldman *et al.* 1988; Hausch *et al.* 2013, Wahidah *et al*. 2015), as well as genetic factors (Waldman *et al.* 1988; Bissaro *et al.* 2012). Qonita *et al.* (2015) showed that variations in morphology were due to environment conditions in the pile ark cockle (*Anadara pilula*), and this finding strengthened the argument of Barria *et al.* (2011) who hypothesized that morphological variations were brought about by adaptive responses to environmental conditions.

Pramithasari, *et al*. (2017) studied the variation in morphometric data of sand crab *Albmea symmysta* populations in Benghulu, and found that two populations are considered as one population. Three populations of Asian horseshoe crabs (*Tychypleus gigas)* were morphometrically analyzed by Razak and Nassim (2018). They found no significant difference between males weight in all populations, but there was significant difference in females weight in three populations. Silva and Paula (2008) and Silva *et al*. (2010) studied the morphometric analysis of the shore crab *Carcinus maenas,* using the crab chela, for differentiation between populations of *C. maenas*. In 2004, Smith investigated claw size to differentiate between population of the same species.

In conclusion, the result of RFLP together with the morphometric data may suggest that the two studied crabs most likely belong to the same species.

REFERENCES

Balasubramanrian, C. P.; Mohanlal, D. I.; Ponniah, G. A.; Raj, K. L.; Bineesh, K.K.; Ravichandran, P. S.; Gopalakrishran, A. R.; Mandal, A. M and Jena, J. K. (2014). DNA sequnce and RFLP resolves taxonomic ambiguity of common crab (Genus *Scylla*) in Indian water http: //informahealthcare.Com/mdn.

Barría, E. M.; Sepúlveda, R. D. and Jara, C. G. (2011). Morphologic variation in *Aegla* Leach (Decapada: Reptantia: Aeglidae) from central-southern Chile: interspecific, sexual dimorphism, and spatial segregation. *J. Crust. Biol.*, 31(2): 231–239. https://doi.org/10.1651/10-3324.1

Bissaro, F. G.; Gomes-JR, J. L. and Di Benedito, A. P. M. (2012). Morphometric variation in the shape of the cephalothorax of shrimp *Xiphopenaeus kroyeri* on the east coast of Brazil. *J. Mar. Biol. Ass. United Kingdom*, 93(3): 683–691. https://doi.org/ 10.1017/S0025315412000409.

Briam, J. V.; Fernande, J. l. and Todd, P.J. (2006). Patternes of morphological and gentic variability in UK populations of Carcinus maenes, J. exp. Mar. Boil. Ecol., 329: 47-54.

Conan, G. Y.; Comeau, M. and Moriyasu, M. (2001). Are morphometrical approaches to establish size at maturity for male American lobster, *Homarus americanus*? *J. Crust. Biol.*, 21(4): 937–947. https://doi.org/10.1651/0278-0372.

Darling, A.A. and Tepolt, R.C. (2007). Highly sensitive Detection of shore crab *Carcinus maenas* and *C. aestuarii* asing (PCR-RFLP). Aquatic Inv., 3(2): 141-152.

Devi, L. A.; Aneykutty, J. J; Mandal, A. A. and Alphy, K. R. (2017). On the systematic of Genus *Scylla* De Haan, 1833 of backwaters, a south Indian estuary, J. Bio. Env. Sci., 10(2):1-15.

Field; J. P. ; Prima S. G. and Subsol N. R. (2000). Statistical analysis of normal and abnormal dissymmetry in volumetric medical images, Med. Image Anal., 4(2):111-121.

Fuseya, R.L. and Watanabe, S.C. (1996). Geneti variability in the mud crab genus *Scylla* Fish. Sci. 62(5): 705-709.

Guero, G.G.; Karl, B.S.; Carlo,I. F. and Guiomar, R.R. (2011). Scientla Marina. 75: 129-134.

Hausch, S.; Shurin, J. B. and Matthews, B. (2013). Variation in body shape across species and populations in a radiation of diaptomid copepods. *PloS ONE,* 8(6): e68272. https://doi.org/10.1371/journal.pone.0068272

Hepp, L. U.; Fornel. R.; Restello, R. M.; Trevis, A, and Santos, S. (2012). Intraspecific morphological variation in a freshwater crustacean *Aegla plana* in Southern Brazil: effects of geographical isolation on carapace shape. *J. Crust. Biol.*, 32(4):511–518. https://doi.org/ 10.1163/ 193724012 X630660.

Imai, H.R. and Numachi,L. K. (2002). Genetic Variability of swimming crab Based on RFLP Analysis DNA. Suisanzoshku. 50(1): 1-7.

Keenan, C.P.; Davie, P.F. and Mann, D.I. (1998). A revision of Genus *Scylla* de Haan, The Raffles. Ball. Of Zool., 46(1): 217-245.

Mandal, A.A.; Mathews, V.L.; Anjali, K.C.; Sobba, S.B. and Yohannan, C.M. (2014). Identification of Indian Mangrove Mud crab *Scylla* spp. using RAPD and RFLPs Markers. J. of shellfish. Res. 33(2): 503-5010.

Mashar, A. Wardiatno, Y.; Boer, M.; Butet, N. A. and Farajallah, A. (2014). The diversity and abundance of sand crabs in south coast of Central Java. *Ilmu Kelautan*, 19(4): 226–232 [in Indonesian].

Mashar, A. and Wardiatno, Y. (2013a). Growth aspect of the mole crab, *Emerita emeritus* of Kebumen District sandy beach. *J. Biol. Tropis*, 13(1): 29–38 [in Indonesian].

Mashar, A. and Wardiatno, Y. (2013b). Growth aspect of the mole crab, *Hippa adactyla* of Kebumen District sandy beach. *J. Biol. Tropis*, 13(2): 119–127 [in Indonesian].

Muzammil, W.; Wardiatno, Y. and Butet, N. A. (2015). Carapace length-width ratio, growth pattern, condition factor, and relative condition factor of the sand crab (*Hippa adactyla*) in sandy beach of Cilacap and Kebumen. *J. Ilmu Pertanian Indonesia*, 20(1):78–84. [in Indonesian].

Nei, M.L. and Li, S. W. (1979). Mathematical model for studying genetic variation in terms of restriction endonucleases proc. Nath. Acad. Sci. USA. 76(10): 5269-5273.

Oh, C.W. and Hartnollm, R. G. (1999). Size at sexual maturity, reproductive output, and seasonal reproduction of *Philocheras trispinosus* (Decapoda) in Port Erin Bay, Isle of Man. *J. Crust. Biol.*, 19(2): 252–259. https://doi.org/10.2307/1549231.

Pfeifer, T.A.; Humble, L.M.; Ring, M. and Grigliatti, T. A. (1995). Characterization of gypsy moth populations and related species using a nuclear-DNA marker. *Canad. Entom.*, 127: 49-58 http://dx.doi.org/ 10.4039/Ent12 749-1

Pramithasari, F. A.; Butet, N. A.and Wardiatno, Y.M. (2017). Variation in Morphometric characters in four sund crab (Albunce symmysta) populations collected from Sumatra and Jara islcrnd Indonesia., Trop life sci. Res. 28(1):103-115.

Qonita, Y.; Wardiatno, Y. and Butet, N. A. (2015). Morphological variation in three populations of the pill ark cockle, *Anadara pilula* (Mollusca: Bivalve) of Java, Indonesia. *AACL Bioflux*, 8(4): 556–564.

Razak, A. M. and Hassim, Z.M. (2018). Comparison of hoesehoe crabs (Tachypleus gigas) morphometry between different population using allometric analysis, AACL Bioflux. 11(1): 143-156.

Rothschild, S. B. (2004). "Sandy beaches". Beachcomber's Guide to Gulf Coast Marine Life: Texas, Louisiana, Mississippi, Alabama, and Florida (3rd ed.). Taylor Trade Publications. pp. 21–38.

Saltonstall, K. (2003). A rapid method for identifying the origin of North American *Phragmites* populations using RFLP analysis. *Wetlands* 23: 1043-1047, http://dx.doi.org/10. 1672/0277-5212(2003)023[1043:ARMFIT]2.0.CO;2

Sampedro, M. P.; González, G. E.; Freire, J. and Muiño, R. (1999). Morphometry and sexual maturity in the spider crab *Maja squinado* (Decapoda: Majidae) in Galicia, Spain. *J. Crust. Biol.*, 19(3): 578–592. https://doi.org/10.2307/1549263.

Shaji,M.M.; Quinitio,F.L.;Thampi,K. C.; Kandan,L. S.and Ganesh, K.L. (2006).Seed production of mud crab *Scylla* at Rajiv Gandhi center for aquaculture, Tami Nadu, India, Aquacul. Asia Mag., 57:17-20.

Silva, I. C.; Alves, M., J.and Hawhins, J. S. (2010). Populations differentiations of shore crab Carcinus maenus on the south west English coast based on genetic and morphometric analyzes. Sci. Mar. 74: 435- 444.

Silva, I.C and Paula, M.N. (2008). is there a better chela to use for morphometric differentiation in Carcinns maenes. J. Mar. Biol. ASS. UK., 88: 941-953.

Smith, L. D. (2004). Biogeographic difference in claw size of Carcinns maenes, Mar. Ecol. Prog. Ser., 276: 209-222.

Sotelo, G. P.; Moran, P.L. and Posada, D.E. (2009). Molecular phylogeny and biogeographic history of the European *Maja*. Mol. Phylogenet. Evol., 53: 314-319.

Wahidah, S. B.; Andy, O.; Trijuno, D. D. and Nugroho, E. (2015). Morphometric variance of South Sulawesi's freshwater prawn *Macrobrachium rosenbergii* and *Macrobrachiumidae*. *Inter. J. Sci. Res.*, 5(4): 1–5.

Waldman, J. R.; Grossfield, J. and Wrigin, I. (1988). Review of stock discrimination techniques for striped bass. *North American Journal of Fisheries Management* 8(4): 410– 425. https://doi.org/10.1577/1548-8675.

Wardiatno, Y. and Mashar, A. (2013). Morphometric study of two Indonesian mantis shrimp *(Harpiosquilraphidea* and *Oratosquillina gravieri*). *Buletin PSP*, 21(1): 19–30.

Wardiatno, Y. and Tamaki, A. (2001). Bivariate discriminant analysis for the identification of *Nihonotrypaea japonica* and *N. hardmani* (Decapoda: Thalassinidea: Callianassidae). *J. Crust. Biol.*, 21(4): 1042–1048. https://doi.org/10.1163/20021975-99990196

Weathersbee, A. A.; Bullock, R.L.; Panchal, T. D. and Dang, P. M. (2003). PCR-RFLP and species specific PCR amplification of 18s rDNA products. Annals of the Entom. Soc. Amer., 96: 637-642.

ARABIC SUMMARY

استخدام تقنية التعدد الشكلي لطول الشظية الناتجة عن القطع المتخصص (RFLP) ودراسات مورفومترية لنوعين من الكابوريا المتداخلين مورفولوجيا من شاطئ أبو قير، الاسكندرية - مصر.

طارق غريب على
قسم علم الحيوان – كلية العلوم – جامعة عين شمس

تم في هذه الدراسة جمع عينات من نوعين من الكابوريا المتداخلين مورفولوجيا من شواطئ أبو قير بالأسكندرية بجمهورية مصر العربية، وقد تم فصل درقة هذه القشريات قبل التشريح والحصول على عضلات البطن لوضعها بالكحول الإيثلي المطلق ثم استخلاص الـ د ن أ وأستخدام ثلاثة انزيمات قاطعة والحصول على التعدد الشكلي، ثم أستخدام برنامج (جيل – برو – أناليزر) لتحليل النتائج.

تم الحصول على الوزن الجزيئي والكثافة البصرية وكذلك درجة الأنسياب الجيني. كما استخدمت معادلة التقارب والتباعد الجيني والتى أعطت نتيجة (٠.٥٩) وبالتالي فالنوعين قد يكونا نوع واحد. و قد استخدمت أيضا القياسات المورفومتريه للنوعين من حيث الطول والعرض للدرقة، وكذلك وزن الجسم، وقد أظهرت القياسات المورفومتريه أهمية مقدارها ١٦.٧% هي عرض الدرقة للذكور فقط وهو ما يطابق إتجاة الدراسة الجزيئية (RFLP)، وهذا يدل على أنهما نوع واحد. تقع أهمية هذة الدراسة في أن الفروق المرفولوجية البسيطة بين الأنواع قد لا تعكس فروق جينية حقيقية بينها.