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Comparative Molecular Identification of Genus *Dicentrarchus* Using Mitochondrial Genes and Internal Transcribed Spacer Region

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ABSTRACT

DNA barcoding is one of the powerful DNA-based identification tools that are used for accurate identification of a species. Despite several DNA barcodes were used, the accuracy and suitability of these barcodes depend on species-specific variations.The current study was conducted to compare the efficiency of two mitochondrial genes to the nuclear Internal Transcribed Spacer (ITS) region in the molecular identification of genus Dicentrarchus. A total of 80 fish samples for *Dicentrarchus labrax* and *Dicentrarchus punctatus* were randomly collected from two different locations in Egypt; Alexandria and Bardawil Lagoon. All samples were morphologically characterized. For species barcoding, the ITS region was firstly employed to carry out the PCR amplifications. Additionally, two mitochondrial genes; cytochrome b (Cyt b) and cytochrome oxidase subunit I (COI) were also used for species barcoding. To evaluate the efficiency of each marker, three different approaches were used. Firstly, phylogenetic relationship was constructed between the collected samples and a reference species using each genetic marker. Secondly, Automated Barcode Gap Discovery (ABGD) method was used for each marker in order to assign the samples into presumed species without priori species assumption. Lastly, the Poisson Tree Processes (PTP) model was used which relies on Bayesian support values to delimit species on the input tree. The two species of genus Dicentrarchus exhibited nearly similar ITS sequences, leading to an ambiguous identification of the two species. However, the two mitochondrial COI and Cyt b genes were able to accurately distinguish between the two species. The three approaches, phylogeny, ABGD and PTP presented consistent results. Overall, COI and Cyt b outperformed ITS in assigning species accurately. Mitochondrial barcodes could provide a leading guide for fish species identification. ITS should be abandoned in favor of COI and Cyt b as primary DNA barcode markers for fish species in general and Dicentrarchus genus, in particular.

INTRODUCTION

The two species of genus *Dicentrarchus* are close relatives in their morphological features, particularly, in the early stages of development (Merlo *et al.*, 2010; Ali and Mamoon, 2019). The use of multiple identification tools can aid in the

accurate identification of a species. Generally, morphological characteristics are a primary tool for species identification. Such morphological characteristics can not differentiate between two closly related species either at their early stages of development or at their adult stages (Fischer *et al.*, 1987). In such cases, DNA-based tools are significant methods to achieve an accurate identification. DNA barcoding is one of the superior DNA-based identification tools relating to the ecology and evolution of natural systems (Kress *et al.*, 2015). DNA barcodes are short standard parts of the genome that are amplified to identify species (Kress *et al.*, 2015; Hebert *et al.*, 2003). Perfect DNA barcode allows rapid, reliable, automatable, and cost-effective species identification for users with little taxonomic experience (Hebert *et al.*, 2003; Hajibabaei *et al.*, 2005; Hebert and Gregory, 2005). Moreover, such barcodes should be easily amplified from most species in a target group using universal primers and expected to show high inter-specific divergence (Luo *et al.*, 2011). Species identification via DNA barcoding is usually performed by comparing the unknown sequence to reference DNA barcodes, mainly via alignment searching [e.g., Basic Local Alignment Search Tool (BLAST) (Altschul *et al.*, 1990; Altschul *et al.*, 1997), or other known methods such as distance-based tree construction (Hebert *et al.*, 2003), the characteristic attribute organization system (CAOS) (Hebert *et al.*, 2004), and the back-propagation neural network (BP based species identification) (Zhang *et al.*, 2008). Recently few methods have been developed which rely on different statistical models. For instance, ABGD (Puillandre *et al.*, 2012) uses prior intraspecific divergence among species to infer species phylogeny, whereas, PTP uses Bayesian statistics to assign species into groups (Zhang *et al.*, 2013).

Nuclear and mitochondrial barcodes are usually used for DNA barcoding purposes. Mitochondrial (mt) genome barcode is the most effective single-locus marker, as it possesses small size when compared to the nuclear genome. The mt genome carries various protein coding genes (PCGs). These PCGs encode for proteins involved in oxidative phosphorylation including cytochrome oxidase subunits, cytochrome b, NADH dehydrogenase subunits, ATPases 6 and 8, 16S and 12S ribosomal RNA genes, and 22 transfer RNA genes (Luo *et al.*, 2011). Mitochondrial genome barcodes have several desirable features that are superior to the nuclear DNA barcodes. These features include limited exposure to recombination, rapid evolution, high copy number, and lack of introns. Additionally, such features facilitate the routine amplification by polymerase chain reaction and the use of the mitochondrial molecular marker (Xu, 2005; Waugh, 2007). Among the PCGs, the cytochrome oxidase I (COI) gene locus is the most widely used DNA barcode locus for animal taxa, as it appears to fulfill the criteria for most groups (Hebert *et al.*, 2003; Vences *et al.*, 2005; Ward *et al.*, 2005; Smith *et al.*, 2005). For fish, the Fish Barcode of life initiative (FISH-BOL) campaign, (Ward *et al.*, 2009) has utilized the sequence of the COI gene "648 bp region" for more than 100,000 specimens representing more than 10,000 species. With these resources, DNA barcoding has embloyed COI to trace fish species in the Egyptian aqua-feed formulations (Galal-Khallaf *et al.*, 2016), differentiate between members of the Sparidae species (Armani *et al.*, 2015; Abbas *et al.*, 2017), and verify the labels on seafood products commercialized in Southern Brazil marketplaces (Carvalho *et al.*, 2015), and other studies on fish in Europe (Di Pinto *et al.*, 2013; Vandamme *et al.*, 2016; Helyar *et al.*, 2014). Cytochrome b (Cyt b) is another mitochondrial gene widely used for animal species differentiation (Tsai *et al.*, 2007). This gene is also used for the dentification of ~50 European marine fish species (Kochzius *et al.*, 2010); and fish mislabeling in Italy (Filonzi *et al.*, 2010). *D.*

*labra*x was identified using two mitochondrial genes barcodes, Cyt b and COI and microarrays (Kochzius *et al.*, 2010).

On the other hand, the eukaryotic rRNA cistron consists typically of the 18S, 5.8S linked to 25-28S rRNA genes separated by external and internal transcribed spacer. Two internal spacers can separate between 18S, 5.8S rRNA genes (ITS1) and 5.8S, 28S rRNA gene (ITS2) (Hillis and Dixon, 1991). The two spacers, with the 5.8S gene, are commonly referred to as "ITS region". The 18S nuclear ribosomal small subunit rRNA gene (SSU) is commonly used as a DNA barcode in species identification and taxonomy (Fonseca *et al.*, 2014; Šlapeta *et al.*, 2005; Lie *et al.*, 2014; Zhan *et al.*, 2014). The 28S nuclear ribosomal large subunit rRNA gene can discriminate species in isolation or integrated with ITS. Such ITS region is variable in the rRNA cistron among genera and species and its sequence comparisons are popular tools for phylogenetic analysis and populations studies (Paul, 2001). Similarly, ITS region is used in the barcoding of fungi (Dentinger *et al.*, 2011; Stern *et al.*, 2012). However, the direct use of ITS region, ITS1 and ITS2 as a universal barcode for barcoding fish species is not common and COI is still the primary and the most efficient barcode used for fish identification. In addition, there are many conflicting views about the exact ITS locus that should be used as a barcode, (complete ITS region, ITS1 or ITS2) (Yao *et al.*, 2010; Wang *et al.*, 2015; Tahir *et al.*, 2018). ITS2 is known to be a satisfactory barcode for medicinal plants families identification such as Fabaceae and Poaceae (Tahir *et al.*, 2018), and it is more effective than the complete ITS region for barcoding of plants (Han *et al.*, 2012). On the other hand , according to (Wang *et al.*, 2015; Yao *et al.*, 2010), ITS1 over performed ITS2 in barcoding number of eukaryotic taxa using sequences from the Genebank on the bases of the sequences CG content. The sequence of ITS4 primer described by White *et al.* (1990) has been modified by Elmosallamy *et al.* (2015) to be more efficient in amplifying the ITS region in animals (ITS4-A). Using the latter modified primer with ITS1 primer (White *et al.*, 1990), helped in successful amplification of ITS region in *Posthodiplostomum sp.*, directly from Nile Tilapia (*Oreochromis niloticus*) that acts as its second host. Therefore, it was promising to apply the modified primer (ITS4-A) for fish identification. Furthermore, there are limited number of studies that compared fish barcodes; for example (Kochzius *et al.*, 2010) used mitochondrial barcodes to confirm the efficiency of COI and Cyt b compared to 16S rRNA in fish species identification.

For the previously mentioned reasons, the objective of the herein study is to conduct a comparison between the efficiency of the mitochondrial Cyt b and COI, and the nuclear ITS region for identification of genus *Dicentrarchus* to specify an accurate and reliable barcode for fish species identification.

MATERIALS AND METHODS

Samples collection:

Fifty-five samples of *Dicentrarchus labrax*, and twenty-five samples of *D. punctatus* were collected in July 2016 from, Alexandria city and Bardawil Lagoon in Egypt with approximate coordinates 31°11' 26" N, 33° 09' 44" E and 31° 12' 56 " N 29° 57' 19" E, respectively. The samples were morphologically identified by their external features described by the FishBase (Whitehead *et al.*, 1986; White *et al.*, 1990).

Molecular examination:

DNA extraction:

The molecular examination was performed at the Genetics Laboratories of the National Institute of Oceanography and Fisheries (NIOF, Egypt), between July and November 2016. After examining the morphological characteristics of all samples, caudal fins of *D. labrax* samples

were preserved in -20˚C in absolute ethanol. DNA was extracted as described by Asahida *et al.* (1996) with some modifications as described by (Ali *et al.*, 2017). Briefly, 700µL of TNES-Urea Buffer and 30 µL of proteinase K (10 mg/ mL) were added. Phenol-Chloroform-Isoamy Alcohol (25:24:1) was added twice with an equal volume and centrifuged. Finally, DNA precitation was done using two volumes of ice cold 100% EtOH. DNA pellets were dissolved in Tris EDTA (TE) buffer. DNA concentrations were spectrophotometrically quantified (eppendorf, Hamburg, Germany) and stored at 4˚C for further analysis.

PCR amplification for ITS region:

The amplifications of the ITS region and other target regions (COI, Cyt b) were performed in a 30 µL volume using BIOLINE master mix (2X My Taq^{TM} Red Mix) according to the manufacturer′s instructions. PCR mixture contained 15 µL BIOLINE master mix, 2 µL DNA template (final concentration 20 mg), and 1 µL of each primer (final concentration 0.25 µM). The ITS region amplification and sequencing were performed using ITS1 forward primer described by White *et al.* (1990) and ITS4-A reverse primer modified by Elmosallamy *et al.* (2015) Table 1. PCR was performed using BIO-RAD PCR System (BIO-RAD, T100 96-well Thermal Cycler, USA). Amplifications for ITS region were performed as followed: i) initial denaturation for 4 min at 95°C, ii) 35 cycles of 94°C for 50 s, 55°C for 50 s, and 72°C for 90 s, and iii) final extension for7 min at 72°C.

PCR amplification for mitochondrial genes:

The target region of mitochondrial gene COI was amplified using the primer pairs described by Ward *et al.* (2005), Table 1. Amplifications were performed using the following thermal profile: i) initial denaturation for 4 min at 95°C, ii) 30 cycles of 94°C for 30 s, 57°C for 30 min, and 72°C for 30 s, and iii) final extension at 72°C for 7 min. The target region of mitochondrial gene, Cyt b was amplified using the primer pairs described by Patarnello *et al.* (1993), Table 1. Amplifications were performed with the following thermal profile: i) initial denaturation at 95°C for 4 min, ii) 35 cycles of 94°C for 30 s, 56°C for 50 min, and 72°C for 50 s, and iii) 7 min final extension at 72°C. Five µL of the PCR products were loaded to 2.5% agarose gel containing 2 µL of Eth Br (100 mg/mL), and electrophoresed. The acceptable bands sizes (~750 base pairs) were purified using the Gene JET Gel Extraction Kit (Catalog no. K0961, Thermo Scientific). The purified PCR products of all samples were then sequenced using the Applied Bio-systems 3500 Genetic Analyzer Sequencer (Hitachi, Japan).

Table 1. Primer sequences for the three markers used in the study.

Primer code	Primer sequence (5′-3′)	Reference
ITS1-F	CTTGGTCATTTAGAGGAAGTAA	**(White *et al.*, 1990**;
ITS4-A-R	TGCCGGTATTTAGCCTTAGATGGAG	**Elmosallamy *et al.*, 2015)**
COI-Fish-F	TTCTCAACTAACCAYAAAGAYATYGG	**(Ward *et al.*, 2005)**
COI-Fish-R	TAGACTTCTGGGTGGCCRAARAAYCA	
Cyt b-F	TCGCAAATCATGCACTTGTT	**(Patarnello *et al.*, 1993)**
Cyt b-R	CCCCTCAAATCCACTGAACT	

Sequence alignment and data analysis:

Prior to sequence analysis, the obtained sequences for each studied marker were compared to the available reference sequences on GenBank using BLAST algorithm available from http://www.ncbi.nlm.nih.gov/blast. A representative sample of the obtained sequences were then deposited in GenBank database (accession numbers LC387524- LC387528 for ITS region; LC387519- LC387523, for COI,; and LC384918- LC384922 for Cyt b). The obtained sequences were aligned and edited using BioEdit (Hall, 1999). Following sequence alignment, consensus sequences from forward and reverse sequences were obtained and then contigs were assembled across each species for each studied marker using BioEdit to be used in phylogenetic reconstruction. For each studied marker, a reference sequence, and an out-group sequence (*Oreochromis niloticus*) were retrieved from NCBI for further inclusion in the data analysis (Table 2). Three independent approaches were employed to determine the efficiency of ITS, COI, and Cyt b markers in the identification of *Dicentrarchus* species. First, genetic relationship among the studied species and their reference sequence was obtained based on Kimura 2-parameter (K2P) genetic distance (Kimura, 1980) as implemented in MEGA X (Kumar *et al.*, 2018). Phylogenetic relationship among species using contig sequences was then obtained according to unweighted pair group method with arithmetic mean (UPGMA) as implemented in MEGA X. The relative robustness of individual branches was estimated by bootstrapping (Felsenstein, 1985), in which 10000 bootstrapped trees were generated from the re-sampled data. Second, ABGD method was used (Puillandre *et al.*, 2012) with the ITS, Cyt b, and COI datasets for all species sequences (80 samples). This method uses a repetitive process to assign sequences into presumed species without a priori species assumption even when two species distributions overlap (Puillandre *et al.*, 2012). The ABGD was used with K2P model to calculate pairwise distances (K80 option with TS/TV = 2.0), 10 recursive steps, X (relative gap width) = 1.5 and the remaining parameters set to default values (Pmin= 0.001, Pmax = 0.1, Nb bins = 20). Prior distribution (*P*) values obtained from ABGD were plotted using SigmaPlot (Systat Software, San Jose, CA) which were adapted from original program output. Third, the PTP model was used (Zhang *et al.*, 2013). This approach uses Bayesian support values (BS) to delimited species on the input tree; the higher BS values on any given node support the notion that all sequences descendent from this node are more likely from one species (Zhang *et al.*, 2013).

Table 2. Reference and out-group sequences for the three markers used in the study.

Molecular marker	Reference sequence	Gene Bank accession number	Out-group sequence	Gene Bank accession number
ITS	*D. labrax (Ref)*[*]	HQ291516.1	*Oreochromis niloticus*	MF460358.1
	D. punctatus (Ref)	HM014387.1		
COI	*D. labrax (Ref)*	KY176457.1		MG428624.1
	D. punctatus (Ref)	LC317272.1		
Cyt b	*D. labrax (Ref)*	KU168693.1		MH041447.1
	D. punctatus (Ref)	EU107385.1		

[*] Ref indicates reference sequence.

RESULTS

Molecular identification:

All tested samples showed positive PCR amplifications for the ITS region, and COI, Cyt b mitochondrial genes. The amplification of the target regions in the two mitochondrial genes produced 610 bp and 370 bp fragments for COI and Cyt b,

respectively. BLAST analysis of all sequences of the morphologically identified *D. punctatus* and *D. labrax* samples confirmed 100% match with both *D. Labrax* and *D. punctatus* species. The identity ranged between 97% and 100% with the maximum identity of 99% and 100% for COI and Cyt b sequences, respectively. However, the analysis of ITS fragment (~ 900 bp) showed inconsistent results for the species identification. These results came in agreement with the aligned ITS sequences that showed 97% - 100% similarity with both species (*D. punctatus* and *D. labrax*), during BLAST searches. Moreover, by retrieving and aligning the submitted ITS sequences for *D. punctatus and D. labrax* from the GenBank database, no observed differences were detected among the ITS sequences. Following sequence alignment, slight differences were observed between the studied and the reference ITS sequences of *D. labrax* and *D. punctatus* indicating that, this barcode region was not able to differentiate between these two closely related species. On the other hand, clear differences were found between the COI and Cyt b sequences for two species on the GenBank database.

Phylogeny, barcocde gap discovery and Bayesian analysis:

Using ITS marker, the phylogenetic relationship of *D. punctatus*, *D. labrax* and its reference sequences based on K2P genetic distance clearly showed one main group (Fig. 1A). This group included *D. labrax*, *D. labrax (Ref)*, *D. punctatus* and *D. punctatus (Ref)*. On the other hand, when using COI and Cyt b markers to infer species phylogeny, both markers showed similar grouping pattern with two distinct groups (Fig. 1B) and (Fig. 1C) for COI and Cyt b, respectively. In both phylogenetic trees, *D. labrax* and *D. labrax (Ref)* formed one group while *D. punctatus* and *D. punctatus (Ref)* formed a second group. Alike, ABGD analysis was able to infer two putative groups according to the ITS and COI and three putative groups according to Cyt b (Fig. 2).

However, based on ITS marker the, assignment of studied species into two putative groups were not clear. For instance, at number of inferred groups = 2, prior intraspecific divergence (P = 0.005) (Fig. 2A), all 55 *D. labrax* sequences, 14 *D. punctatus* sequences, *D. labrax (Ref) and D. punctatus (Ref)* sequences were assigned to one group whereas the remaining 11 *D. punctatus* sequences were assigned to the other putative group, Table 3. On the other hand, COI and Cyt b showed consistent results; *D. labrax* sequences and its *D. labrax (Ref)* sequence, were assigned to one group while *D. punctatus* and *D. punctatus (Ref)* were assigned into the second group. However using Cyt b, only 17 of *D. labrax* sequences were assigned into sparet group (Table 3).

Table 3: Number of identified groups and its assigned sequences according to ABGD.

	Assigned sequences		
Molecular Marker	**Group 1**	**Group 2**	**Group 3**
ITS	*D.labrax* **55** *D.punctatus* **14** *D.punctatus (Ref)* *D.labrax (Ref)*	*D.punctatus* **11**	-
COI	*D.labrax* **55** *D.labrax (Ref)*	*D.punctatus* **25** *D.punctatus (Ref)*	-
Cyt b	*D.labrax* **38** *D.labrax (Ref)*	*D.labrax* **17**	*D.punctatus* **25** *D.punctatus (Ref)*

Numbers in bold represent number of sequences assigned in each defined group.
- Not defined.

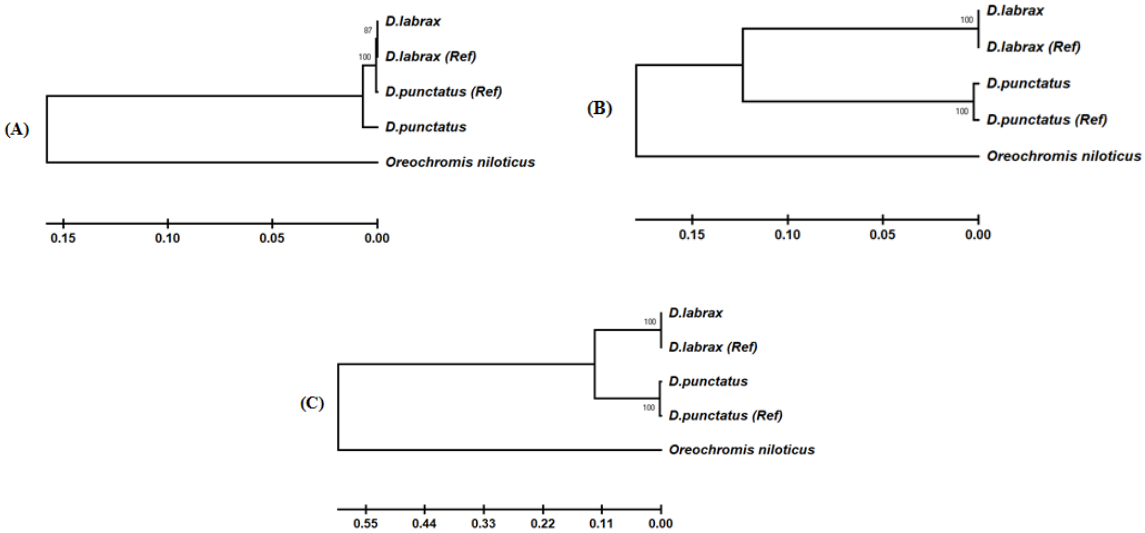

Fig. 1: Phylogenetic relationship of *Dicentrarchus* species and its outgroup using UPGMA method: (A) ITS, (B) COI, and (C) Cyt b.

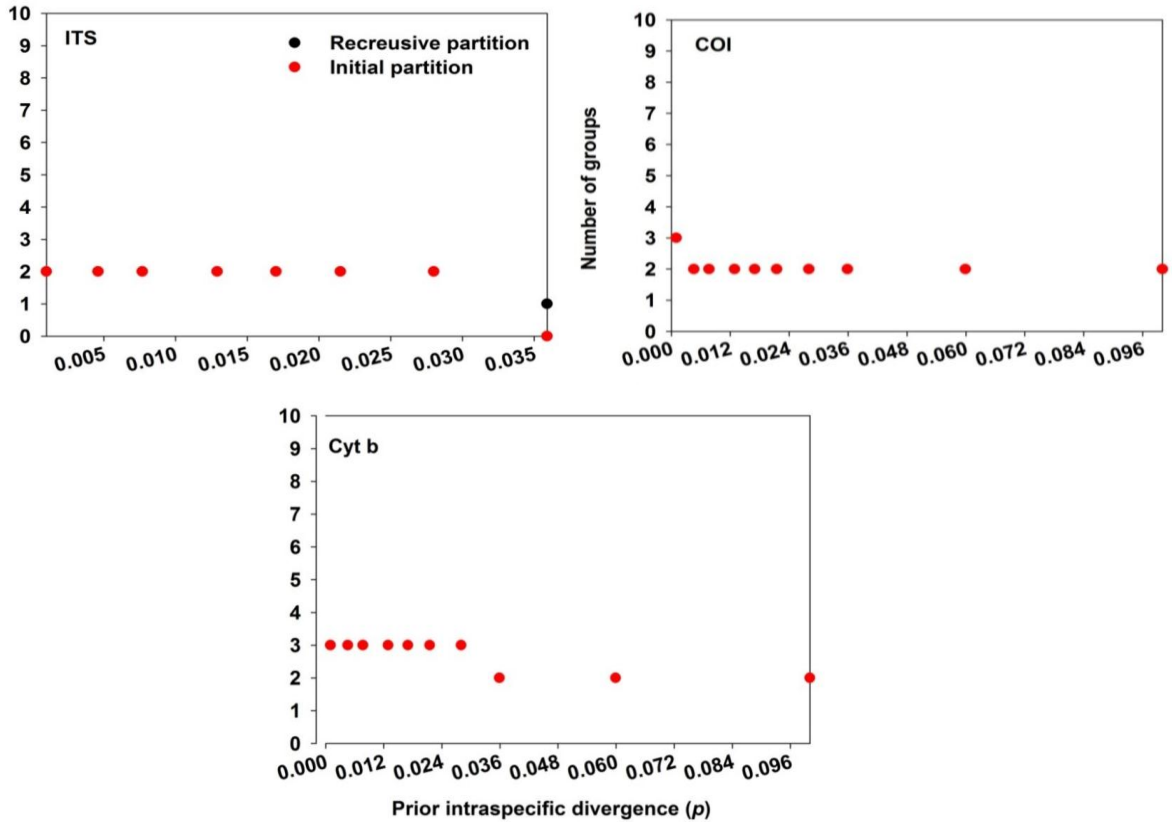

Fig. 2: The number of groups inferred from ABGD analysis according to prior intraspecific divergence (*P*)

According to PTP model and based on ITS marker, *D. labrax* and *D. punctatus* sequences and their reference sequences were assigned into six groups (Table 4). The studied sequences of both species were assigned randomly to the identified groups. For instance in group 5, a total of 18 *D. labrax* sequences were assigned into one group along with *D. punctatus (Ref)* with BS value of 53%. Contrarily, consistent results were obtained using COI and Cyt b where PTP was able to assign all 55 *D. labrax* sequences and its reference sequence *D. labrax (Ref)* into one group and all 25

D. punctatus sequences and its reference sequence *D. punctatus (Ref)* into a second group with BS value of 83% and 100% COI and Cyt b, respectively (Table 4).

Table 4: Number of identified groups and its assigned sequences according to PTP.

	Assigned sequences					
Molecular Marker	**Group 1**	**Group 2**	**Group 3**	**Group 4**	**Group 5**	**Group 6**
ITS	*D.punctatus* (0.920)* **13**	*D.punctatus* (0.563) **12**	*D.labrax* (0.284) **16**	*D.labrax (Ref)* (0.284)	*D.labrax* **18** *D.punctatus (Ref)* (0.535)	*D.labrax* (0.535) **21**
COI	*D.labrax* **55** *D.labrax (Ref)* (0.838)	*D.punctatus* **25** *D.punctatus (Ref)* (1.000)	-	-	-	-
Cyt b	*D.labrax* **55** *D.labrax (Ref)* (0.838)	*D.punctatus* **25** *D.punctatus (Ref)* (1.000)	-	-	-	-

*Bayesian support values associated with PTP.
Numbers in bold represent number of sequences assigned in each defined group.
Not define.

DISCUSSION

Overall, the primer combinations used for amplifying the target regions of different barcodes were successful. The ITS4-A primer (Elmosallamy *et al.*, 2015) increased the efficiency of amplification quality and specificity at standard annealing temperature (55˚C). However, the performance of the mitochondrial genes (COI, Cyt b) and ITS as barcode markers were different for the identification of *Dicentrarchus* species. Based on sequence similarity, both mitochondrial genes COI and Cyt b were accuarte in distinguishing between the two species. Such identification was supported by the results of the morphological examination and the analysis of the employed approaches; PTP, ABGD and the classical phylogenetic approach. These results are in agreement with similar findings in different animal taxa barcoding using COI (Smith *et al.*, 2005); in Australian fish, (Ward *et al.*, 2005); in Canadian fish, (Hubert *et al.*, 2008); in Taiwanese fish, (Bingpeng *et al.*, 2018). Collectivley, these reports display the efficiency of COI gene barcode for identification of fish species. Additionally, Cyt b barcode region was successfully used in barcoding the animal taxa in general (Tsai *et al.*, 2007) and fish barcoding in particular (Kochzius *et al.*, 2010; Filonzi *et al.*, 2010). The recorded efficiency of these mitochondrial barcodes could be explained on the basis of their features that make them suitable markers for fish barcoding which include limited exposure to recombination, lack of introns, and rapid evolution (Xu, 2005; Waugh, 2007).

On the other hand, Using the classical phylogenetic approach based on genetic distance, the ITS region was not able to accurately differentiate between *D. labrax* and *D. punctatus*. Additionally, using both ABGD and PTP which rely on two different analytical models, ITS showed weakness in the assignment of species into its specific group (Tables 2 & 3). Liu *et al.* (2012) compared the efficiency of the ITS region as a multiple-copy region to the single-copy ribosomal protein S7 gene intron 1 (rpS7), and they proved the effectiveness of the RPS-1st intron as a successful barcode for *Coilia nasus* (Clupeiformes: Engraulidae).

The retrieved ITS sequences covered a partial sequence of 18S ribosomal RNA gene, the complete sequence of internal transcribed spacer 1 (ITS1), and a partial

sequence of 5.8S ribosomal RNA gene (Merlo *et al.*, 2010). Several studies had reported the conserved nature of both 18S and 5.8S regions sequences (Dentinger *et al.*, 2011; Bulygin *et al.*, 2003; Pánek *et al.*, 2013). This theory can explain the inefficiency of the amplified sequence to distinguish two closely related species (Merlo *et al.*, 2010). The presence of these invariable sequences in the amplified region can affect the efficiency of this region as a barcode for most species. Whereas, the primer pairs employed in this study amplified the ITS region (ITS1, 5.8S rDNA, and ITS2) which is considered the most hypervariable region in the DNA cistron (Paul, 2001). However, this variable region could not perform as an accurate barcode to identify *D. labrax*. These findings probably related to the presence of the invariable 5.8S DNA sequence in this region, that can decrease the comparative divergence, and increase sequence similarity between individuals (Dentinger *et al.*, 2011). Even though ITS barcode marker is considered the best barcode for most fungi (Dentinger *et al.*, 2011; Stern *et al.*, 2012); it failed to accurately distinguish fish species.

Generally, the success of DNA barcoding technique for species identification relies on two main conditions, first; the selection of a suitable barcode target and, second; the previous knowledge of reference sequences for specific species (Ward *et al.*, 2009). The establishment of an online database can improve the reliability and facilitate the use of barcoding technique (Ekrem *et al.*, 2007). For example, the FISH-BOL campaign (Ward *et al.*, 2009), has already collected the COI sequence for more than 10,000 species. Using these reference sequences, COI based DNA barcoding has been extensively used to barcode fish species (Hubert *et al.*, 2008), study different fish populations (Wang *et al.*, 2017), and to evaluate the fish product mislabeling (Helyar *et al.*, 2014; Vandamme *et al.*, 2016).

CONCLUSION

Our results revealed that the mitochondrial barcode markers (COI and Cyt b) are more constant and over performed ITS region in the molecular identification of *D. labrax*. We suggest applying COI and/or Cyt b compared to ITS region which has very limited power as barcode in the molecular identification of the fish species. Nonetheless, more studies are encouraged where more samples to be included in order to further support this suggestion.

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ARABIC SUMMARY

التعريف الجزيئي المقارن لجنس *Dicentrarchus* باستخدام جينات الميتوكوندريا ومنطقة ITS

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يعد التعريف الجينى (DNA barcoding) من أدق الوسائل لتعريف الأنواع. وبالرغم من أن هناك العديد من المعرفات الجينية المستخدمة (DNA barcodes) في التعريف الجيني الا أن هذه الأنواع تختلف في كفاءتها في التعريف. وقد أجريت هذه االدراسة لمقارنة كفاءة كل من: بعض الجينات الخاصة بالميتوكندريا (COI وCyt b) وأحد الجينات النووية (Nuclear Internal Transcribed Spacer, ITS) في التعريف الجيني لجنس *Dicentrarchus*. لذلك فقد تم تجميع ٨٠ عينه من *Dicentrarchus labrax* و *Dicentrarchus punctatus* من منطقتين متباعدتين بمسافة ٣١٣ كم في مصر. وبعد التعريف المورفولوجي للنوعين ، تم استخدام (ITS region) أولا في التعريف الجيني للنوعين، ثم تم استخدام الجينات الخاصة بالميتوكندريا (COI وCyt b) في تعريف النوعين محل الدراسة . ولتقييم كفاءة كل منطقة مستخدمة، تم استخدام ٣ طرق: شجرة القرابة الوراثية و Automated Barcode Gap Discovery (ABGD) و Poisson Tree Processes (PTP). أظهرت النتائج أن كلا النوعين لهم تسلسل حمض نووى متشابه تقريبا في منطقة ITS ، مما أدي الي عدم القدرة علي تمييز النوعين باستخدام منطقة ITS. بينما كانت تتابعات كلا من (COI و Cyt b) مميزه لكل نوع علي حده ولم يوجد أي تداخل في تسلسل الحمض النووى لجينات الميتوكوندريا بين النوعين. واستنادا الى هذه النتائج ، اتضح ان جينات الميتوكوندريا (COI و Cyt b) هي الأدق والأفضل في التعريف الجيني لجنس *Dicentrarchus* بصفة خاصة وللأسماك بصفة عامة. وتعد هذه الدراسة من الدراسات القليله التي تتناول المقارنة بين كفاءة الانواع المختلفة للتعريف الجينى.