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Identification of Sex-specific Molecular Markers in Barbel and Nile Carp using SCoT and ISSR Markers

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Abstract

The present study aimed to investigate molecular sex differences between males and females of two members of family Cyprinidae in Egypt namely Benni and Lebeis using two different molecular markers i.e., start codon targeted polymorphism (SCoT) and inter simple sequence repeats (ISSR). The bulked DNA sample for each gender of the tested species was screened with eight primers from each marker. However, SCoT marker was more efficient than ISSR marker by showing higher number of possible sex-specific bands in the two tested species. SCoT primers were able to generate 7 and 4 male-specific bands, along with two and 5 female-specific bands for *B. bynni* and *L. niloticus*, respectively. Whereas, ISSR primers generated 4 and two male-specific bands, vis-à-vis two and three female-specific bands for *B. bynni* and *L. niloticus*, respectively. Interestingly, SCoT-01 primer generated unique common female specific band (690bp) which appeared only in the females of Benni and Lebeis. This band is more possible to be female sex-specific. The results obtained in this study could serve as a keystone for molecular sex differentiation studies in the two tested species and other fish species. However, increasing the number of analyzed individuals is highly recommended.

Keywords: *SCoT, ISSR, Cyprinidae, sex differentiation.*

Introduction

Family Cyprinidae is considered as one of the most widely distributed freshwater fishes in Africa (Winfield and Nelson 1991). It represents in Egypt by more than 10 species, where both of *Barbus bynni* and *Labeo niloticus* (Forsskål, 1775) are considered the most important species for commercial use. The two species *B. bynni* and *L. niloticus* belong to the large fish species that have a maximum length of 82 and 47cm, respectively. The two species are distributed along Nile River and Lake Nasser (Bishai and Khalil., 1997).

With increasing the world population, a big effort was directed into fish farming and breeding. In this regard, it is very important to differentiate between males and females in many fish species at an early stage of development, because they are varying in their developmental nature, where in some fish species one sex grows faster than the other. In common carp, females grow faster than males with an average weight of 30% higher than that of some same-aged males. However, in tilapia species, males grow faster, so they are of greater value (Mei and Gui 2015).

In majority of fish species, it is very difficult to discriminate between males and females at early stages according to their morphological features. Subsequently, using sex-specific molecular markers is a suit-

able alternative tool. Identification of sex specific DNA markers in fishes is very useful in fish farming and hatchery management (Durna, 2009). Also, it is important for investigating sex determination system, identifying sex chromosomes and sex-related genes (Mei and Gui 2015 and De Rosa *et al.,* 2017). Also, it could serve in determining the effect of environmental factors on sex differentiation (De Rosa *et al.,* 2017).

Interestingly, in the last decades, many molecular markers have been developed and successfully used in molecular sexing studies in many species including fish, such as random amplified polymorphic DNA (RAPD) (Wuertz *et al.,* 2006; Chen *et al*., 2009; Durna, 2009; Xia *et al.,* 2011; Silva *et al.,* 2012; Al-Qurainy *et al.,* 2018 and Mohamed *et al.* 2019), start codon targeted (SCoT) polymorphism (Mohamed and Sami, 2015 and Mohamed *et al*. 2019) and inter simple sequence repeat (ISSR) (Wuertz *et al.,* 2006; Adhikari *et al.,* 2014 and Mohamed et al. 2019).

It is not possible to discriminate morphologically between male and female of *B. bynni* and *L. niloticus* at their early stage of development. Moreover, to date, there is no available information about molecular sex differences between males and females of the two species. Therefore, the objective of this study was to use SCoT and ISSR markers to identify sex-specific molecular markers based on DNA samples collected from recognized male and female individuals of *B. bynni* and *L. niloticus* fishes.

Material and Methods

Specimens' collection

Fifteen specimens (ten males and five females) of *Barbus bynni* and eleven specimens *(*five males and six females) of *Labeo niloticus* were collected from Nile River at Assiut city, Egypt. The live specimens were transported into the Molecular Biology Laboratory, Genetics Department, Faculty of Agriculture, Assiut University for molecular analysis. Where, a piece of the dorsal fin (1 cm^2) from each specimen was preserved in absolute ethanol and kept at -20˚C until the extraction of genomic DNA.

DNA extraction

Total genomic DNA was extracted from dorsal fin from each individual following the protocol of Youssef *et al.* (2015) with slight modifications. The concentration and purity of the isolated DNA were checked by spectrophotometer and agarose gel electrophoresis. Subsequently, equal quantities of genomic DNA from males or females' samples of the same species were mixed to prepare the bulked DNA sample for each gender.

Molecular markers assay

Ten primers of start codon targeted polymorphism (SCoT) and ten primers of inter simple sequence repeats (ISSR) markers were used for investigating molecular sex differences between male and female of *B. bynni* and *L. niloticus*; out of them eight primers were selected for good amplification (Table 1). PCR mixtures and programs of SCoT and ISSR were carried out as previously described by Collard and Mackill, (2009) and Zhigileva *et al.,* (2013), respectively. PCR products of SCoT and ISSR were separated on 1.5 and

2% agarose gel, respectively and then gels were visualized by UV transilluminator after staining with ethidium bromide.

Table 1. Codes and sequences of SCoT and ISSR primers tested

SCoT primers			ISSR primers		
No.	**Code**	**Sequence (5`→3`)**	**No.**	**Code**	**Sequence (5`→3`)**
1	SCoT 01	CAACAATGGCTACCACCA	1	UBC 807	AGAGAGAGAGAGAGAGT
2	SCoT 02	CAACAATGGCTACCACCC	2	UBC 808	AGAGAGAGAGAGAGAGC
3	SCoT 18	ACCATGGCTACCACCGCC	3	UBC 810	GAGAGAGAGAGAGAGAT
4	SCoT 22	AACCATGGCTACCACCAC	4	UBC 811	GAGAGAGAGAGAGAGAC
5	SCoT 28	CCATGGCTACCACCGCCA	5	UBC 826	ACACACACACACACACC
6	SCoT 32	CCATGGCTACCACCGCCT	6	UBC 834	GAGAGAGAGAGAGAGAGAGAT
7	SCoT 34	ACCATGGCTACCACCGCA	7	UBC840	GAGAGAGAGAGAGAGATT
8	SCoT 35	CATGGCTACCACCGGCCC	8	UBC 846	CACACACACACACACAT

Molecular data analysis

Gel profiles of SCoT and ISSR were analyzed by scoring only clear bands as present (1) or absent (0) between males and females of each species. Male- or female-sex specific bands were recorded if the band appears only in male or female bulked samples, respectively.

Results

Start Codon Targeted Polymorphism (SCoT)

Ten SCoT primers were used in the present study; out of them eight were selected for good amplification (Fig. 1). A total of 49 and 46 bands were amplified in the two species *B. bynni* and *L. niloticus*, respectively. In *B. bynni*, the number of generated bands using the tested primers ranged from one (SCoT-34) to 10 bands (SCoT-35), with an average of 6.13 bands per primer.

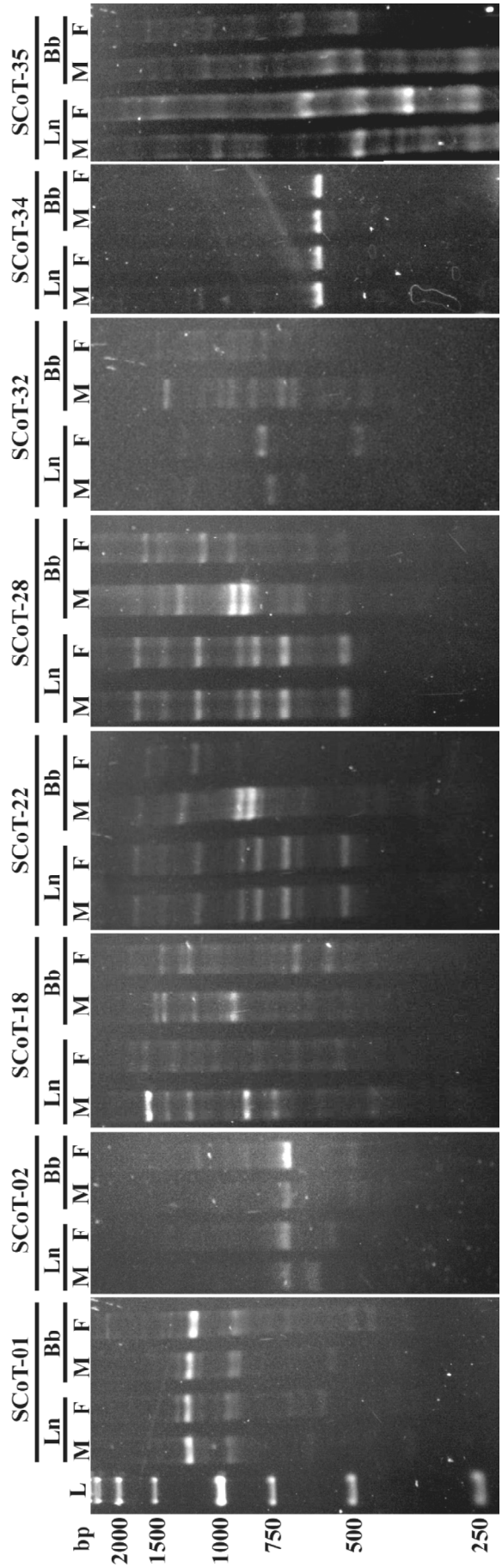

Figure 1. SCoT profiles differentiating males (M) and females (F) of *Labeo niloticus* (Ln) and *Barbus bynni* (Bb).

However, five primers could successfully generate nine sex-specific bands. Out of them, seven bands (14.29%) were specific for male (SCoT-18, 1654 and 949bp; SCoT-22, 861bp; SCoT-28,622bp and SCoT-35,476,319 and 206bp) and two bands (4.08%) were specific for female (SCoT-01,690bp and SCoT-28, 1139bp). Whereas, in *L. niloticus,* the number of generated bands using the tested primers ranged from one (SCoT-34) to 9 bands (SCoT-18), with an average of 5.75 bands per primer. SCoT marker showed nine sex-specific bands in *L. niloticus* that were revealed by five primers, where four bands (8.70%) were specific for male (SCoT-02, 612bp; SCoT-18, 872 and 743bp and SCoT-32, 774bp) while five bands were sex-specific for female (SCoT-01, 690bp; SCoT-02, 556bp; SCoT-32, 829 and 432bp and SCoT-35, 303bp) (Tables 2 & 3).

Table 2. Bands generated by SCoT primers in *B. bynni*

Primers	TNB	NMB	NPB	%P	SB size	Male	Female
SCoT-01	5	4	1	20.00	690	0	1
SCoT-18	9	7	2	22.22	1654	1	0
					949	1	0
SCoT-22	5	4	1	20.00	861	1	0
SCoT-28	8	6	2	25.00	1139	0	1
					622	1	0
SCoT-35	10	7	3	30.00	476	1	0
					319	1	0
					206	1	0
SCoT-02	2	2	0	0.00	---	---	---
SCoT-32	9	9	0	0.00	---	---	---
SCoT-34	1	1	0	0.00	---	---	---
Total	**49**	**40**	**9**	**18.37**	**---**	**---**	**---**

TNB: Total number of bands; NMB: Number of monomorphic bands; NPB: Number of polymorphic bands; % P: Percentage of Polymorphism and SB: Specific bands.

Table 3. Bands generated by SCoT primers in *L. niloticus*

Primers	NB	NMB	NPB	%P	SB size	Male	Female
SCoT-01	6	5	1	16.67	690	0	1
SCoT-02	3	1	2	66.67	612	1	0
					556	0	1
SCoT-18	9	7	2	22.22	872	1	0
					743	1	0
SCoT-32	4	1	3	75.00	829	0	1
					774	1	0
					432	0	1
SCoT-35	8	7	1	12.50	303	0	1
SCoT-22	8	8	0	0.00	---	---	---
SCoT-28	7	7	0	0.00	---	---	---
SCoT-34	1	1	0	0.00	---	---	---
Total	**46**	**37**	**9**	**19.57**	**---**	**---**	**---**

TNB: Total number of bands; NMB: Number of monomorphic bands; NPB: Number of polymorphic bands; % P: Percentage of Polymorphism and SB: Specific bands.

Inter Simple Sequence Repeats (ISSR)

Among the ten ISSR primers used in this study, eight were selected due to their good amplification (Fig. 2). A total of 82 and 89 bands were scored in the two species *B.bynni* and *L. niloticus*, respectively. In *B. bynni*, the number of generated bands ranged from 5 (UBC-826) to 17 bands (UBC-808), with an average of 10.25 bands per primer. However, four primers successfully generated six sex-specific bands; out of them, four (4.88%) bands were specific for male (UBC-807, 799 and 341 bp; UBC-834, 319bp and UBC-840, 445bp) and two (2.44%) bands were specific for female (UBC-834, 633bp and UBC-846,543bp). Whereas, in *L. niloticus*, the number of bands generated ranged from 7 (UBC-826) to 14 (UBC-808 and UBC-840) bands with an average of 11.12 bands per primer. However, five ISSR primers (UBC-807, UBC-808, UBC-834, UBC-840 and UBC-846) showed five sex-specific bands in *L. niloticus*, among which two (2.25%) bands were specific for male (UBC-807, 516bp and UBC-840, 219bp), while three (3.37%) bands were specific for female (UBC-808,1131bp, UBC-834,858bp and UBC-846, 712bp), (Tables 4 & 5).

Table 4. Bands generated by ISSR primers in *B.bynni.*

Primers	TNB	NMB	NPB	%P	SB size	Male	Female
UBC-807	11	9	2	18.18	799	1	0
					341	1	0
UBC-834	6	4	2	33.33	633	0	1
					319	1	0
UBC-840	10	9	1	10.00	445	1	0
UBC-846	11	10	1	9.09	543	0	1
UBC-808	17	17	0	0.00	---	---	---
UBC-810	14	14	0	0.00	---	---	---
UBC-811	8	8	0	0.00	---	---	---
UBC-826	5	5	0	0.00	---	---	---
Total	**82**	**76**	**6**	**7.32**	**---**	**---**	**---**

TNB: Total number of bands; NMB: Number of monomorphic bands; NPB: Number of polymorphic bands; % P: Percentage of Polymorphism and SB: Specific bands.

Table 5. Bands generated by ISSR primers in *L. niloticus*

Primers	TNB	NMB	NPB	%P	SB size	Male	Female
UBC-807	13	12	1	7.69	516	1	0
UBC-808	14	13	1	7.14	1131	0	1
UBC-834	11	10	1	9.09	858	0	1
UBC-840	14	13	1	7.14	219	1	0
UBC-846	11	10	1	9.09	712	0	1
UBC-810	11	11	0	0.00	---	---	---
UBC-811	8	8	0	0.00	---	---	---
UBC-826	7	7	0	0.00	---	---	---
Total	**89**	**84**	**5**	**5.62**	**---**	**---**	**---**

TNB: Total number of bands; NMB: Number of monomorphic bands; NPB: Number of polymorphic bands; % P: Percentage of Polymorphism and SB: Specific bands.

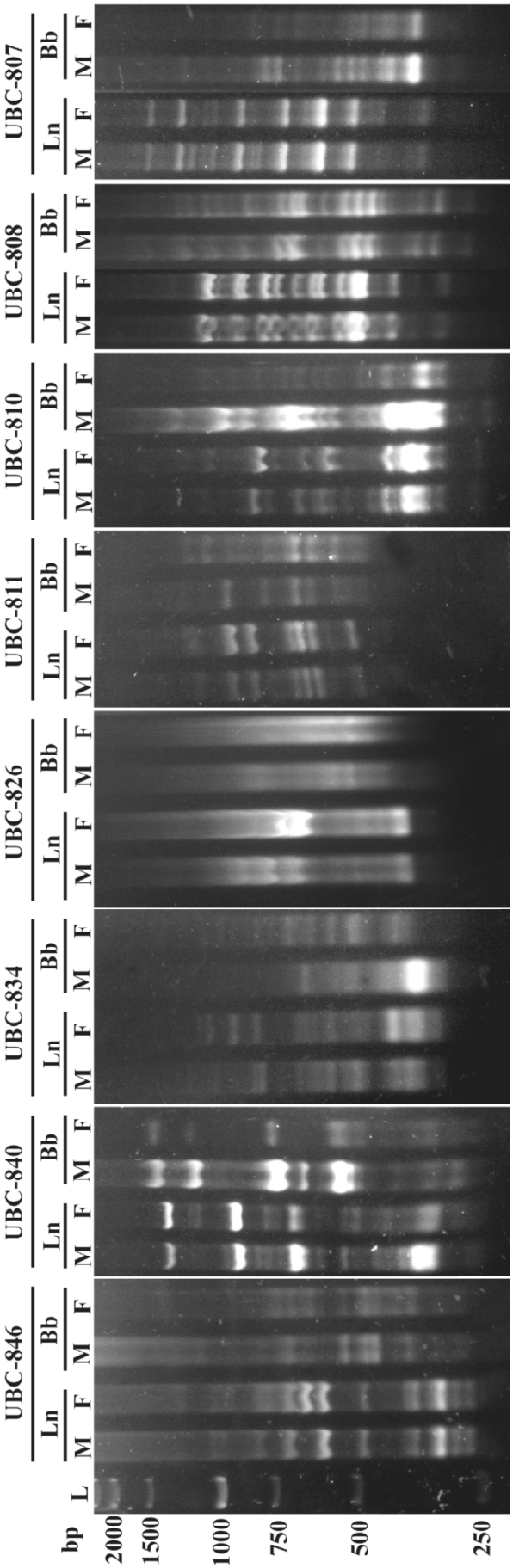

Figure 2. ISSR profiles differentiating males (M) and females (F) of *Labeo niloticus* (Ln) and *Barbus bynni* (Bb).

Discussion

To the best of our knowledge, this is the first time to investigate sex determination based on molecular markers in *B. bynni* and *L. niloticus*. In majority of fish species, sex determination based on the differences in morphological features between male and female before maturity or even out of the spawning season in the mature individual is very difficult. On the other hand, distinguishing between the sex chromosomes and autosomes in fishes are difficult because of fish chromosomes are so numerous and some are so small that it's hard to count them reliably (Martínez *et al.,* 2014). Accordingly, it is difficult to differentiate between the immature individuals by traditional cytogenetic techniques. Consequently, using alternative methods such as molecular marker assays can solve this problem. In this study, two markers assay i.e., SCoT and ISSR were used to identify any possible sex-specific molecular markers in *B. bynni* and *L. niloticus* based on the bulked DNA sample from each gender with eight primers from each marker.

The first marker used in the present study was SCoT. It is a novel, efficient and lower cost with high repeatability technique. SCoT marker was firstly developed by Collard and Mackill (2009) based on the short-conserved region in plant genes surrounding the ATG translation initiation codon. It has been widely used to assess genetic diversity in many crop plant species including rice (Collard and Mackill, 2009), mango (Luo *et al.,* 2010 and 2011) and date palm (Al-Qurainy *et al.,* 2015). However, it was also used to assess genetic diversity in animals such as camel (Al-Soudy *et al*., 2018) and fish (Marie and Allam, 2017). Also, SCoT marker was efficient to identify molecular sex marker in date palm (Adawy *et al.* 2014 and Mohamed and Sami 2015) and when used with fish it successfully discriminated between male and female of *Brycinus nurse* using bulked and individual samples (Mohamed *et al*. 2019).

The set of primers analyzed in this study successfully bound with a complementary regions of the nuclear genomic DNA of the two species tested and amplified suitable number of bands in *B.bynni* and *L. niloticus*. Furthermore, most of the tested primers could successfully generate possible male and/or female specific bands which refer to the efficiency of SCoT marker for molecular sexing in the present study. Interestingly, there is one female specific band in both species. It seems that this band is related to sex determination in Cyprinidae family. This band is more possible to be sex-specific, thus its isolation and sequencing is highly recommended.

The second marker used in the present study was ISSR. It is easy to use, low-cost, and methodologically less demanding compared to other dominant markers, making it an ideal genetic marker for organisms whose genetic information is lacking (Ng and Tan, 2015). Herein, the set of ISSR primers produced a number of bands higher than those generated by SCoT primers in the two species tested. While many primers produced male and/or female possible specific bands, ISSR primers produced lower number of molecular possible sex

specific bands and it is efficiency in molecular sexing was lower compared to SCoT marker. ISSR was a powerful marker and extensively used in assessment genetic diversity in many fish species (Liu *et al*., 2006; Li *et al*., 2009; Li *et al.,* 2013; Vitorino *et al.,* 2015 and Kumla *et al.,* 2012). Also, it has been widely used and was successful tool in molecular sexing studies in plants (Sharma *et al.,* 2008; Younis *et al.,* 2008; Sarmah and sarma 2011; Nanda *et al.,* 2013 and Sarmah *et al.,* 2017).

In the present study, the two molecular markers, i.e., ISSR and SCoT tested showed their efficiency in sex determination in two Cyprinidae species (*B. bynni and L. niloticus*). However, SCoT marker was more efficient than ISSR marker by showing higher number of sex-specific bands in the two species more than that of ISSR marker.

Conclusion

Both of SCoT and ISSR markers used in the present study showed their efficiency in sex determination in two Cyprinidae species i.e. *B. bynni* and *L. niloticus* and produced many possible sex specific bands. However, SCoT marker was more efficient than ISSR marker by showing higher number of possible sex-specific bands in the two species tested. Results herein are very important and could help in sex differentiation in the two tested species at early developmental age. However, confirmation of these bands should be performed by increasing the number of analyzed individuals of both species.

References

Adawy, S.S., Jiang, J. I. M. I. N. G., & Atia, M.A. (2014). Identification of novel sex-specific PCR-based markers to distinguish the genders in Egyptian date palm trees. Int J Agric Sci Res, 4(5), 45-54.

Adhikari, S., Saha, S., Bandyopadhyay, T.K., & Ghosh, P. (2014). Identification and validation of a new male sex-specific ISSR marker in pointed gourd (Trichosanthes dioica Roxb.). The Scientific World Journal, 2014.

Al-Qurainy, F., Khan, S., Nadeem, M., & Tarroum, M. (2015). SCoT marker for the assessment of genetic diversity in Saudi Arabian date palm cultivars. Pakistan Journal of Botany, 47(2), 637-643.

Al-Qurainy, F., Al-Ameri, A.A., Khan, S., Nadeem, M., Gaafar, A. R. Z., & Tarroum, M. (2018). SCAR marker for gender identification in date palm (*Phoenix dactylifera* L.) at the seedling stage. International journal of genomics, 2018.

Al-Soudy, A., El-Sayed, A., El-Itriby, H., & Hussein, E. (2018). Assessment of the genetic diversity, breeds structure and genetic relationships in four Egyptian camel breeds using microsatellite and start codon targeted (SCoT) markers. Journal of Biodiversity & Endangered Species, 6, S2.

Bishai, H. M., & Khalil, M.T. (1997). Freshwater fishes of Egypt. Egyptian Environmental Affairs Agency.

Chen, J., Wang, Y., Yue, Y., Xia, X., Du, Q., & Chang, Z. (2009). A novel male-specific DNA sequence in the common carp, Cyprinus carpio. Molecular and cellular probes, 23(5), 235-239.

Collard, B. C., & Mackill, D. J. (2009). Start codon targeted (SCoT) polymorphism: a simple, novel DNA marker technique for generating gene-targeted markers in plants. Plant molecular biology reporter, 27(1), 86-93.

De Rosa, L.; V.P. Cruz; E.A. Serrano; N.J. Mendes; A.P. Wasko; J.A. Senhorini; B.G. Pardo; P. Martínez; C. Oliveira and F. Foresti (2017). Identification of a sex-specific molecular marker in *Salminus brasiliensis* (Characiformes) based on SCAR marker. Journal of Applied Ichthyology., 33: 1024-1026.

Durna, S.(2009). The determination of sex-linked molecular markers with random amplified polymorphic DNA (RAPD) technique in Aphanius danfordii (Cyprinodontidae) species. Fen Bilimleri Dergisi, 30(2).

Forskål, P. (1775). Descriptiones animalium avium, amphibiorum, piscium, insectorum, vermium; quæ in itinere orientali observavit Petrus Forskål. Post mortem auctoris edidit Carsten Niebuhr. Adjuncta est materia medica Kahirina atque tabula maris rubri geographica. - pp. 1-20, I-XXXIV [= 1-34], 1-164, 1 map. Hauniæ. (Möller).

Kumla, S., Doolgindachbaporn, S., Sudmoon, R., & Sattayasai, N. (2012). Genetic variation, population structure and identification of yellow catfish, Mystus nemurus (C&V) in Thailand using RAPD, ISSR and SCAR marker. Molecular Biology Reports, 39(5), 5201-5210.

Li, J. L., Wang, G. L., & Bai, Z. Y. (2009). Genetic diversity of freshwater pearl mussel (*Hyriopsis cumingii*) in populations from the five largest lakes in China revealed by inter-simple sequence repeat (ISSR). Aquaculture International, 17(4), 323-330.

Li, W., Sun, W. X., Fan, J., & Zhang, C.C. (2013). Genetic diversity of wild and cultured swamp eel (*Monopterus albus*) populations from central China revealed by ISSR markers. Biologia, 68(4), 727-732.

Liu, Y.G., Chen, S.L., Li, J., & Li, B.F. (2006). Genetic diversity in three Japanese flounder (Paralichthys olivaceus) populations revealed by ISSR markers. Aquaculture, *255*(1-4), 565-572.

Luo, C., He, X. H., Chen, H., Ou, S.J., & Gao, M.P. (2010). Analysis of diversity and relationships among mango cultivars using start codon targeted (SCoT) markers. Biochemical Systematics and Ecology, 38(6), 1176-1184.

Luo, C., He, X. H., Chen, H., Ou, S. J., Gao, M. P., Brown, J. S., ... & Schnell, R. J. (2011). Genetic diversity of mango cultivars estimated using SCoT and ISSR markers. Biochemical Systemat-

ics and Ecology, 39(4-6), 676-684.

Mar'ie, Z.A., & Allam, M. (2017). Using start codon targeted (SCoT) polymorphism for genetic diversity analysis of three Red Sea fishes (Family: Carangidae). IOSR Journal of Pharmacy and Biological Sciences, 12, 50-56.

Martínez, P., Viñas, A. M., Sánchez, L., Díaz, N., Ribas, L., & Piferrer, F. (2014). Genetic architecture of sex determination in fish: applications to sex ratio control in aquaculture. Frontiers in genetics, 5, 340. https://doi.org/10.3389/fgene.2014.00340

Mei, J., & Gui, J. F. (2015). Genetic basis and biotechnological manipulation of sexual dimorphism and sex determination in fish. Science China Life Sciences, 58(2), 124-136.

Mohamed, A. A., & Sami, A. S. (2015). Novel set of sexs pecific PCR-based markers reveals new hypothesis of sex differentiation in date palm. Journal of Plant Sciences, 3(3), 150-161.

Mohamed, E. A., Zohry, N.N., Gamal, M., & Abdo, R. F. (2019). Karyotyping and Molecular Sexing in Brycinus nurse (Characiformes, Alestidae) from Nile River in Egypt. Journal of Agricultural Chemistry and Biotechnology, 10(7), 127-131.

Nanda, S., Kar, B., Nayak, S., Jha, S., & Joshi, R. K. (2013). Development of an ISSR based STS marker for sex identification in pointed gourd (*Trichosanthes dioica Roxb*.). Scientia Horticulturae, 150, 11-15.

Ng, W.L., & Tan, S.G. (2015). Inter-simple sequence repeat (ISSR) markers: are we doing it right. ASM Sci J, 9(1), 30-39.

Sarmah, P., & Sarma, R.N. (2011). Identification of a DNA marker linked to sex determination in *Calamus tenuis Roxb*., an economically important rattan species in northeast India. Molecular breeding, 27(1), 115-118.

Sarmah, P.; V.J. Barua; J. Nath; R.N. Sarma; B. Kurian; A.S. Hemanthkumar & K.K. Sabu (2017). ISSR and SSR markers reveal sex-specific DNA sequences in three *Calamus* species from India. Agroforestry Systems, 91: 509-513.

Sharma, K., Agrawal, V., Gupta, S., Kumar, R., & Prasad, M. (2008). ISSR marker-assisted selection of male and female plants in a promising dioecious crop: jojoba (*Simmondsia chinensis*). Plant Biotechnology Reports, 2(4), 239-243.

Vitorino, C.A., Oliveira, R.C., Margarido, V.P., & Venere, P.C. (2015). Genetic diversity of Arapaima gigas (Schinz, 1822) (Osteoglossiformes: Arapaimidae) in the Araguaia-Tocantins basin estimated by ISSR marker. Neotropical Ichthyology, 13(3), 557-568.

Winfield, I.J. & Nelson, J. S. (eds) (1991). Cyprinid fishes. London: Chapman & Hall.

Wuertz, S., Gaillard, S., Barbisan, F., Carle, S., Congiu, L., Forlani, A., ... & Grillasca, J. P. (2006).

Extensive screening of sturgeon genomes by random screening techniques revealed no sex-specific marker. Aquaculture, 258(1-4), 685-688.

Xia, X., Zhao, J., Du, Q., Zhi, J., & Chang, Z. (2011). Cloning and identification of a female-specific DNA marker in Paramisgurnus dabryanus. Fish physiology and biochemistry, 37(1), 53-59.

Younis, R.A., Ismail, O. M., & Soliman, S.S. (2008). Identification of sex-specific DNA markers for date palm (*Phoenix dactylifera L.*) using RAPD and ISSR techniques. Research Journal of Agriculture and Biological Sciences, 4(4), 278-284.

Youssef, M., Valdez-Ojeda, R., Ku-Cauich, J.R., & Escobedo-GraciaMedrano, R.M. (2015). Enhanced protocol for isolation of plant genomic DNA. Journal of Agriculture and Environmental Sciences, 4(2), 172-180.

Zhigileva, O.N., Baranova, O.G., Pozhidaev, V.V., Brol, I.S., & Moiseenko, T.I. (2013). Comparative analysis of using isozyme and Issr-Pcr markers for population differentiation of cyprinid fish. Turkish Journal of Fisheries and Aquatic Sciences, 13(1), 59-168.

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تحديد الواسمات الجزيئية الخاصة بالجنس في أسماك الليبس النيلي والبني الأصيل باستخدام واسمات SCoT و ISSR

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قسم الوراثة – كلية الزراعة – جامعة أسيوط – أسيوط – مصر

الملخص

كان الهدف من الدراسة الحالية دراسة الفروق الجنسية بين الذكور والإناث في نوعين من الأسماك التابعين للعائلة Cyprinidae في مصر المعروفين باسم البني والليبس، وذلك باستخدام نوعين من الواسمات الجزيئية الـــ SCoT والـــ ISSR. تم تحليل المادة الوراثية المجمعة من عدة أفراد لكل جنس من كلا النوعين بواسطة ثماني بادئات من كل واسم جزيئي. إتضح أن، الواسم SCoT كان أكثر فعالية من الواسم ISSR من خلال إظهار عدد أكبر من الحزم المحتمل أنها خاصة بالجنس في كلا النوعين تحت الدراسة. حيث أنتجت البادئات الخاصة بالـــ SCoT عدد سبعة حزم وأربعة حزم خاصة بالذكور وأيضاً عدد حزمتين وخمسة حزم خاصة بالإناث في السمك البني والليبس على الترتيب. بينما أظهر الواسم ISSR عدد أربعة حزم وحزمتين خاصة بالذكور بالإضافة إلى عدد حزمتين وثلاث حزم خاصة بالإناث في نوعي البني والليبس على الترتيب. وجدير بالذكر تمكن البادئ (SCoT-01) من إنتاج حزمة فريدة خاصة فقط بالإناث من كلا النوعين (بحجم ٦٩٠ زوج قاعدة نيتروجينية). ومن المرجح أن تكون هذه الحزمة متخصصة بجنس الإناث وبالتالي فيجب عزلها وتحديد التتابع الخاص بها. تعتبر النتائج المتحصل عليها في هذه الدراسة بغاية من الأهمية، حيث يمكن اعتبارها حجر أساس لدراسات تمييز الجنس في نوعي السمك محل الدراسة والأنواع السمكية الأخرى. ولكن، من الموصي به زيادة عدد الأفراد الواجب تحليلهم في الدراسات المقبلة.