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Evaluation of genetic markers for assessing sex-related differences in the hawksbill turtle (*Eretmochelys imbricata*)

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Abstract

Objective

The hawksbill sea turtle (*Eretmochelys imbricata*) is a critically endangered species that has a temperature-dependent sex determination (TSD) system. Given the impact of climate change on the sex ratio of this species and the lack of information on genetic differences associated with sex, this study aimed to evaluate the utility of four genetic markers 6299, 6299-T, 14749, and 14749-T (introduced in previous genomic studies on the Chinese softshell sea turtle (*Pelodiscus sinensis*)) to investigate whether these markers can reveal potential genetic differences between males and females of *E. imbricata*.

Materials and methods

Sampling was conducted during the nesting season from three main nesting habitats of the hawksbill sea turtle in the Persian Gulf, including Nakhiloo, Ommolgorm Islands, and Naiband Coasts. The samples consisted of one adult female turtle and fifteen hatchlings. Genomic DNA was extracted from organ tissue and its quality and quantity were assessed by agarose gel electrophoresis and spectrophotometry. The PCR reaction was performed using four primer pairs

introduced in the study of Zeng et al. (2024). The PCR products were electrophoresed on 2% agarose gel and the banding patterns between individuals, populations and sexes were compared qualitatively and semiquantitatively.

Results

The results showed that markers 6299 and 6299-T produced significant genetic variation between individuals and populations, but these differences were not uniformly associated with sex. Marker 14749 revealed differences at the population level, such that the banding patterns varied between islands but were uniform within each population. In contrast, marker 14749-T showed almost identical banding patterns in all samples, indicating low genetic diversity in this genomic region in E. imbricata. None of the markers showed a definitive and stable pattern of male-female segregation.

Conclusion

This study shows that genetic markers introduced for species with genetic sex determination cannot be used as definitive sex determination markers in a species with a temperature-dependent sex determination system such as *Eretmochelys imbricata*. However, some of these markers can be valuable tools for investigating genetic diversity, population structure, and conservation studies. The results emphasize the need to develop and validate species-specific markers and conduct genomic, epigenetic, and gene expression studies to better understand the molecular mechanisms associated with sex in sea turtles.

Keywords: genetic diversity, genetic markers, hawksbill turtle (*Eretmochelys imbricata*), sea turtle conservation, sex determination

Paper Type: Research Paper.

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Introduction

One of the ancient and valuable vertebrates in marine ecosystems is the sea turtle. This animal plays an important role in the sustainability of coastal and oceanic habitats. The hawksbill turtle (*Eretmochelys imbricata*) is one of these turtles. This species of turtle is critically endangered. This is because its shells are illegally exploited, and its nesting habitats have been destroyed (Lovich et al., 2018). In addition, marine pollution and climate change have also contributed to this issue, and all these factors have caused the population of this species to decline sharply worldwide. This species plays a very important ecological role in coral ecosystems (Patel et al., 2022). For example, it controls the population of sponges, and maintains the biodiversity of coral reefs. Therefore, it is of great conservation importance and must be studied from various aspects of biology, reproduction, and population genetics in order to design effective conservation programs for it, because implementing conservation plans for this animal is very essential (dei Marcovaldi et al., 2014). The mechanism of sex determination in sea turtles, including E. imbricata, is the Temperature-Dependent Sex Determination (TSD). This mechanism is one of the important biological features of sea turtles. In this system, unlike species with genetic sex determination, the sex of embryos is determined by the temperature of egg incubation during a critical period of embryonic development (Miller et al., 2017). Usually, male babies are produced at lower temperatures. However, female babies are produced at higher temperatures. While if the temperature is in the middle range, that is, at the "axial temperature", the same number of males and females are produced and a balanced sex ratio is created (Chatting et al., 2021). This type of mechanism causes the number of males and females in sea turtle populations to be strongly affected by environmental conditions. Therefore, climate change and increased temperatures of nesting beaches have important effects on the sex determination of these animals. Unfortunately, in recent years, global temperatures have increased and the physical conditions of the coasts have also changed. This has caused a significant shift in the sex ratio towards females in many sea turtle populations. Such an imbalance can reduce the reproductive capacity of the populations in the long term. As a result, the survival of this species is at risk (Sani et al., 2024). Although sex determination in this species is influenced by temperature, increasing evidence suggests that the process of sexual differentiation in reptiles with TSD is also controlled by a network of genes, molecular pathways and epigenetic regulations, the expression of which can differ between males and females. Genes such as DMRT1, SOX9, FOXL2 and CYP19A1 (aromatase) are among the key elements in this process. Various reports have shown that these elements play an important role in the regulation of sexual differentiation in reptiles. Recent genomic studies in some turtle species, including the Chinese softshell turtle (*Pelodiscus sinensis*), have been conducted by Zeng et al. (2024). They identified and reported sex-associated genetic markers and candidate genes

involved in sexual differentiation. Although this species has a genetic sex determination system and the findings are not directly generalizable to sea turtles, the markers and genomic regions introduced in such studies can be evaluated as tools to investigate patterns of sex-associated genetic differences in species with TSD. Examining the utility of these markers in non-target species could help to better understand the molecular similarities and differences in sex-related processes. Moreover, genetic variety is crucial for promoting the development of more sophisticated genes, safeguarding existing populations, advancing evolutionary processes, and enabling adaptation to changing conditions in the natural environment (Javanmard et al., 2008; Mohammadabadi et al., 2021a). Conversely, the identification of gene polymorphisms is crucial in the process of detecting and treatment of diseases (Mohammadabadi, 2016; Saadatabadi et al., 2023). Moreover, the study of populations and breeds, using molecular techniques is very important and useful for their characterizing (Mohammadifar and Mohammadabadi, 2017; Noori et al., 2017; Mohammadinejad et al., 2022). Conservation of genetic diversity requires the proper performance of conservation superiorities and sustainable handling plans that should be based on universal information on population structures, including genetic diversity resources among and between populations and breeds (Mohammadifar & Mohammadabadi, 2018; Mohammadabadi et al., 2024a). Genetic diversity is an essential element for genetic improvement, preserving populations, evolution and adapting to variable environmental situations, and sex determination (Sulimova et al., 2007; Mohammadabadi et al., 2024b). On the other hands, determination of gene polymorphism is important in characterizing of various populations (Mohammadabadi et al., 2010; Mohammadabadi et al., 2024c) in order to define genotypes of individuals and their associations with immune system, resistance or susceptibility to diseases, and sex determination (Mohammadabadi et al., 2021b; Shokri et al., 2023). Unfortunately, there is very little information on the genetic differences between males and females in the Hawksbill turtle. Therefore, the aim of this study was to investigate the use of four genetic markers 6299, 6299-T, 14749, and 14749-T introduced in previous genomic studies to assess the presence or absence of genetic differences between males and females of *Eretmochelys imbricata*. In this study, without assuming that these markers are sex-determining, an attempt has been made to investigate the potential association of these markers with sex in order to pave the way for further studies in the field of reproductive genetics, population structure, and conservation of this endangered species.

Materials and methods

This study was conducted on the coast of the Persian Gulf. The Persian Gulf is a shallow, semi-enclosed sea. It is located in the subtropical northwest Indian Ocean. The Persian Gulf is connected to the open waters of the Sea of Oman and the Indian Ocean through the Strait of

Hormuz. The main currents enter the Gulf through this strait. These currents curve around the Iranian coast at the northern end of the Strait of Hormuz. They then pass the coasts of the surrounding Arab countries and exit the Strait of Hormuz. The Persian Gulf covers an area of about 251,000 square kilometers. It is about 1,000 kilometers long and between 200 and 300 kilometers wide. The Persian Gulf is located between latitudes 24°N and 30°N and longitudes 48°E and 57°E. Various nesting sites for the Hawksbill turtle have been reported in different habitats of the Persian Gulf (Tabib et al., 2016; Askari-Hesni et al., 2016; Sinaei et al., 2018). In this study, sampling was conducted during the nesting season (March to June), from late evening until before sunrise, when the hatchlings were emerging from their nests. Three to four nests (one sample from each nest) were sampled on three islands. Nests with emerging hatchlings were examined, and dead embryos were immediately collected. Sampling was conducted in three green turtle nesting habitats: Nakhiloo, Ommolgorm Islands, and Naiband Coasts (Figure 1). Nakhiloo and Ommolgorm Islands are part of the Deir-Nakhiloo National Park located in the Mand River Delta. All of these areas are designated as marine protected areas by the Iranian Department of Environmental Protection (DOE) (Jafari & Rahnama, 2018). This study was conducted in accordance with the standards of the Ethics Committee of Shahid Bahonar University of Kerman, and all experimental protocols and sampling techniques were approved by this committee. In addition, the ARRIVE 2.0 guidelines were followed in conducting this study. Since dead turtle hatchlings were used in this study, no turtles were killed or injured. Shahid Bahonar University of Kerman complied with relevant laws and regulations in this study. All sampling procedures involving wild sea turtles were carried out in accordance with relevant national and institutional guidelines and regulations. A sampling permit was obtained from the Bushehr Provincial Environmental Protection Organization under permit number 2/1403/1224. Sampling was done from a female hawksbill turtle from Nakhilooo Island and fifteen hawksbill turtle hatchlings. Sampling was done in such a way that 5 samples were taken from Ommolgorm Island, 4 samples from Naiband Coasts, and 6 samples from Nakhilooo Island. The samples were packaged and labeled. Then, they were stored on ice and transported to the laboratory. A part of the isolated organ tissue was ground into a fine powder in liquid nitrogen. The powdered tissue was lysed with 500 µl of buffer. This solution contained proteinase K (200 mg/ml), 100 mM NaCl, 10 mM Tris-HCl, 13 mM EDTA, 35 mM SDS, and 3.5 mM sodium citrate. Standard kits were used for DNA extraction. Electrophoresis on 1% agarose gel and spectrophotometry using a NanoDrop Onec device were used to evaluate the quantity and quality of extracted DNA. In this study, 4 pairs of primers introduced by Zeng et al. (2024) were used. These genetic markers were introduced for the Chinese softshell turtle (*Pelodiscus sinensis*) and were used for the species E. imbricata in this study without changing the primer sequences. These markers were introduced in

the aforementioned study as sex-related markers or candidate genes in the process of sexual differentiation. The specifications of these primers are given in Table 1.

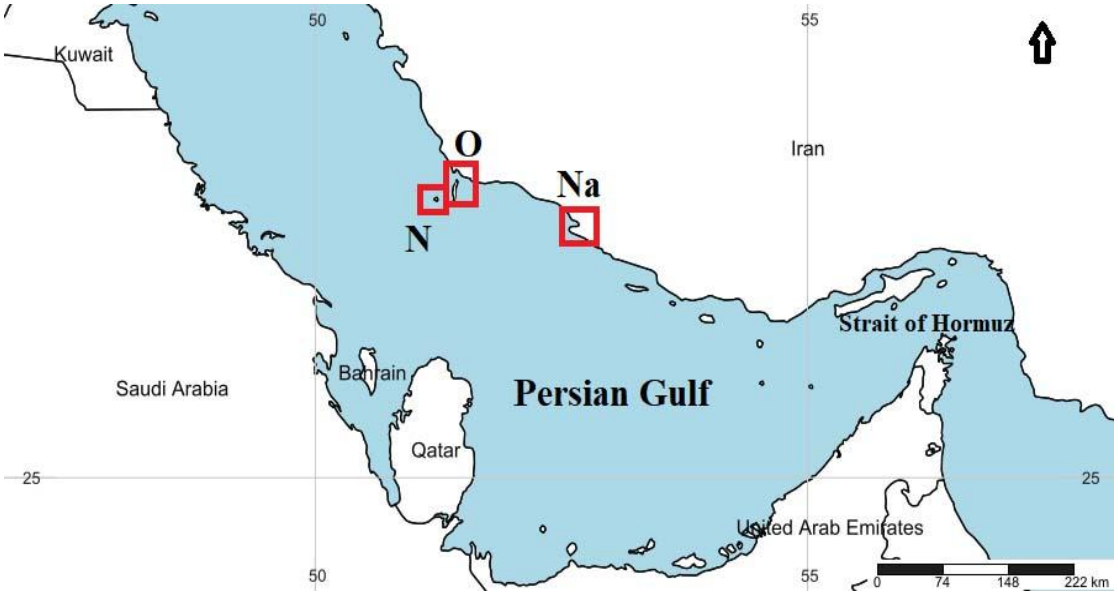

Figure 1. Map of locations along the northern shores of the Persian Gulf, Iran, where sampling of hawksbill turtles (*Eretmochelys imbricata*) has taken place (Na, Naiband Coasts; O, Ommolgorm Island; N, Nakhiloo Island). The map produced in https://www.simplemappr.net/ and modified in Adobe Photoshop 2022 v23.5.4.981.

Table 1. Characteristics of used primers for studying hawksbill turtle

Primer name		Primer sequence (5'-3')	Tm (°C)	Reference
6299	F	ATGGGCAGGGAGGTGTGAATGG	58	Zeng et al.
	R	GCAGTTCGCTGGTCGCTTCCTT		(2024)
6299-T	F	TTGCATAGGAACTTGGGCAAACGG	62	Zeng et al.
	R	GCAGTTCGCTGGTCGCTTCCTT		(2024)
14749	F	GCTGCTGCTGTAGTTCATCTCC	58	Zeng et al.
	R	GCTATCATGGTCACCGCTCTGT		(2024)
14749-T	F	AGCAGGATTGCCCCCAGGT	63	Zeng et al.
	R	GCTATCATGGTCACCGCTCTGT		(2024)

PCR reactions were performed in a final volume of 25 µL. Each reaction contained template DNA (50-100 ng), $MgCl_2$, dNTPs, 1X PCR buffer, forward and reverse primers (each at a final concentration of 0.2-0.5 µM), and Taq DNA polymerase. PCR thermal conditions included an initial denaturation step at 95°C for 5 min, followed by 35 cycles of denaturation (95°C, 30 s), primer annealing (specific annealing temperature for each primer according to Table 1, 45 s), and elongation (72°C, 60 s), and finally a final elongation step at 72°C for 10 min. PCR products were

electrophoresed on a 2% agarose gel prepared in TBE buffer. Gel staining was performed using ethidium bromide. DNA bands were visualized and imaged using a Gel Documentation System. Band sizes were determined using a 100 M molecular weight marker. The PCR band patterns for each primer were examined and compared between males and females to assess the presence or absence of potential sex-related genetic differences. The results were analyzed qualitatively and semiquantitatively, providing a basis for investigating the possible association of these markers with sex in the Hawksbill Turtle.

Results and discussion

For the extracted DNA, the absorbance ratio 260/280 was 1.9, indicating its high purity. Also, the DNA bands observed on the agarose gel were smear-free and clear. The results of PCR with primer 6299 for a female hawksbill turtle from Nakhiloo Island and for 5 hawksbill turtle hatchlings from Ommolgorm Island, 4 from Naiband Coasts and 6 from the Nakhiloo island are shown in Figure 2.

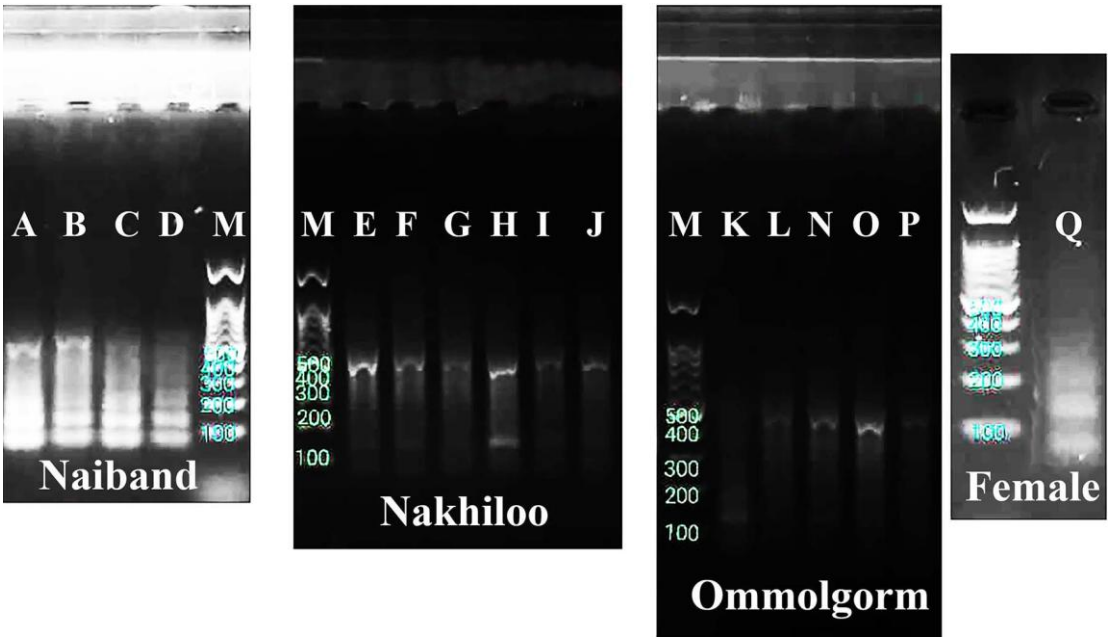

Figure 2. Results of PCR with primer 6299 for a female hawksbill turtle (Q) from Nakhiloo Island and for 4 hawksbill turtle hatchlings from Naiband Coasts (A, B, C and D), 6 from Nakhiloo Island (E, F, G, H, I and J) and 5 from Ommolgorm Island (K, L, N, O and P)

As is clear from Figure 2, this marker showed completely different patterns in different animals. For example, individual H on Nakhiloo Island is completely different from the rest of the individuals on the island and produced two completely distinct bands. Also, the Naiband Coasts specimens have completely different banding patterns from those from Nakhiloo Island.

On Ommolgorm Island, individual K also showed a different banding pattern from the rest of the individuals on the island. On the other hand, all the patterns on this island are completely different from the Naiband Coasts banding patterns. The female individual's banding pattern is similar to the banding patterns of individuals C and D on Nakhiloo Island. This marker showed high genetic diversity in Hawksbill, so it can be used for individual identification, population structure, genetic diversity, or intraspecific phylogenetic studies. Also, since differences were observed between males and females for this marker, it can be said that it can be introduced as a potential genetic marker for sex determination. However, this marker cannot currently be considered as a definitive sex determination marker in E. imbricata and requires further validation. Although this marker has been introduced by Zeng et al. (2024) as a genetic marker for sex determination in the Chinese softshell turtle (*Pelodiscus sinensis*). Of course, this sex determination in this turtle is different from that of the hawksbill turtle. This result is consistent with previous studies in species with temperature-dependent sex determination (TSD), in which it is challenging to find "sex-definitive markers" through classical genetic methods; for example, in the turtle Chrysemys picta (TSD), no evidence of distinct sex chromosomes or sex-definitive markers has been found, and previous attempts to discover such markers have been unsuccessful (Valenzuela et al., 2014). Figure 3 shows the results of PCR with primer 6299-T for a female hawksbill turtle from Nakhiloo Island and for 4 hawksbill turtle hatchlings from Naiband Coasts (A, B, C, and D), six samples from Nakhiloo Island (E, F, G, H, I, and J), and 5 samples from Ommolgorm Island (K, L, N, O, and P). The 6299-T marker also exhibited different banding patterns in different individuals. Female turtle (Q) showed a completely different banding pattern from all individuals on Naiband Coasts and from some turtles on Nakheel and Ommolgorm islands. Turtle J from Nakheel produced a completely different pattern from all individuals on the three islands and the female. This marker produced completely different patterns for males and females in the Chinese softshell turtle (*Pelodiscus sinensis*) (Zeng et al., 2024). Although this species has a genetic sex determination system, its sex determination is not the same as in the hawksbill turtle. In species with TSD, it is less likely that stable and stable genetic markers that directly determine sex exist; however, the observed genetic differences could reflect intraspecific genetic variation, population structure, or regulatory differences associated with molecular pathways of sexual differentiation. The observation of unique patterns in some individuals, such as female Q and individual J from Nakhiloo Island, may indicate individual or population genetic differences that are not necessarily limited to sex. Therefore, the results of this study suggest that marker 6299-T, although acting as a sex-dependent marker in a GSD species such as P. sinensis, can be interpreted more as a polymorphic marker in E. imbricata with the ability to reveal genetic variation between individuals and populations. This finding emphasizes the need for species-specific validation of

genetic markers, especially when transferring them between species with different sex determination systems.

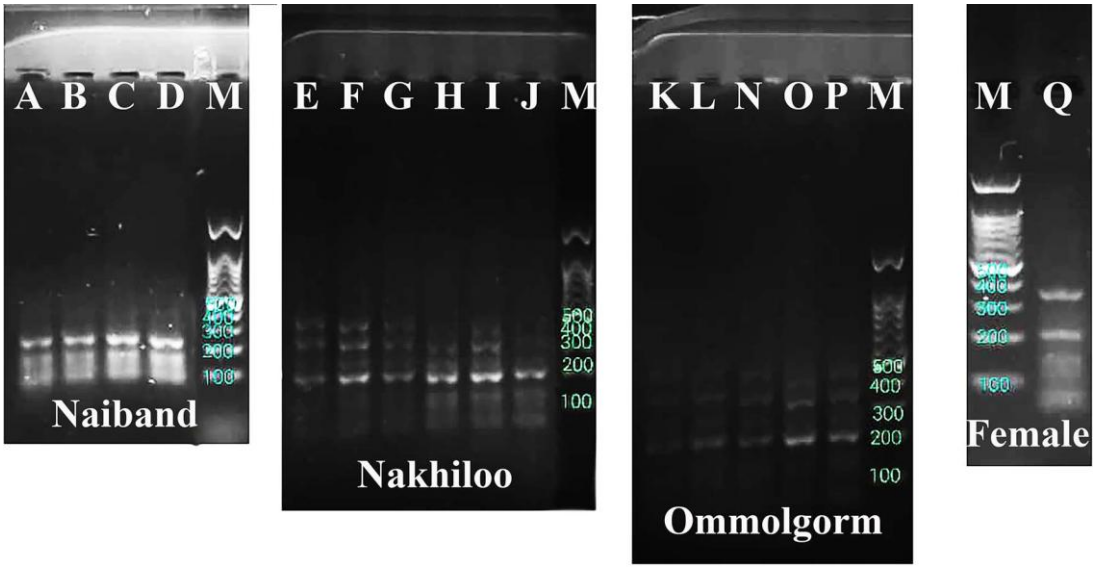

Figure 3. Results of PCR with primer 6299-T for a female hawksbill turtle (Q) from Nakhiloo Island and for 4 hawksbill turtle hatchlings from Naiband Coasts (A, B, C, and D), six samples from Nakhiloo Island (E, F, G, H, I, and J), and 5 samples from Ommolgorm Island (K, L, N, O, and P)

The results of PCR with primer 14749 for a female hawksbill turtle from Nakhiloo Island and for 4 hawksbill turtle hatchlings from Naiband Coasts (A, B, C, and D), six samples from Nakhiloo Island (E, F, G, H, I, and J), and 5 samples from Ommolgorm Island (K, L, N, O, and P) are shown in Figure 4. For marker 14749, large differences were also observed between the turtles studied. The banding patterns of Naiband Coasts were similar to those of the female. However, they were different from the banding patterns of turtles from Nakheel and Ommolgorm Islands. No differences were observed between turtles in each population. Within each of the studied populations, no differences in banding patterns were observed, and all individuals in each population showed a uniform pattern. These results indicate the existence of genetic differentiation at the population level for marker 14749. The observed patterns for marker 14749 indicate that this marker reflects genetic differences at the population level rather than sex-related differences. The lack of intrapopulation differences for this marker could indicate the relative fixation of this genomic region within each population, such that differences appear mainly between populations rather than between individuals within the same population. Such a pattern has been reported in genetic studies of sea turtle populations and could reflect the limitation of gene flow, fidelity to nesting sites, and spatial structure of populations.

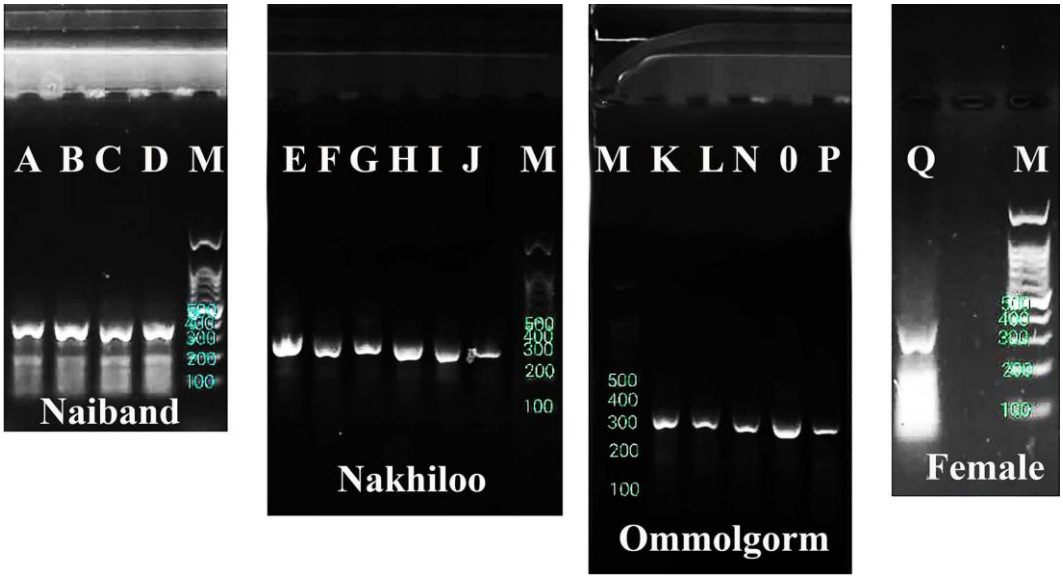

Figure 4. Results of PCR with primer 14749 for a female hawksbill turtle (Q) from Nakhiloo Island and for 4 hawksbill turtle hatchlings from Naiband Coasts (A, B, C, and D), six samples from Nakhiloo Island (E, F, G, H, I, and J), and 5 samples from Ommolgorm Island (K, L, N, O, and P)

Given that marker 14749 has been reported as a sex-linked marker in the genetically sexed species (*Pelodiscus sinensis*), the lack of repetition of the sex-linked pattern in *Eretmochelys imbricata* once again emphasizes the importance of fundamental differences in the sex determination system between species. In species with temperature-dependent sex determination, genetic markers are expected to reflect genetic diversity, population structure, or indirect regulatory differences rather than sex determination. Therefore, the results obtained with marker 14749 indicate that this marker can be useful for investigating genetic differences between populations of hawksbill turtles, but do not provide sufficient evidence for its use as a marker to distinguish between males and females. Figure 5 shows the results of PCR with primer 14749-T for a female hawksbill turtle from Nakhiloo Island and for 4 hawksbill turtle hatchlings from Naiband Coasts (A, B, C, and D), six samples from Nakhiloo Island (E, F, G, H, I, and J), and 5 samples from Ommolgorm Island (K, L, N, O, and P). As can be seen, the 14749-T marker showed almost similar banding patterns for all the islands studied. The uniformity of the banding patterns observed for marker 14749-T suggests that this genomic region in the hawksbill turtle has a low level of genetic diversity. Such a pattern could indicate evolutionary conservation of this region or the absence of selective pressures leading to diversity in the populations studied. The lack of individual or population differences observed for this marker suggests that 14749-T, unlike some of the other markers examined in this study, does not have the ability to genetically segregate between individuals, populations, or sexes.

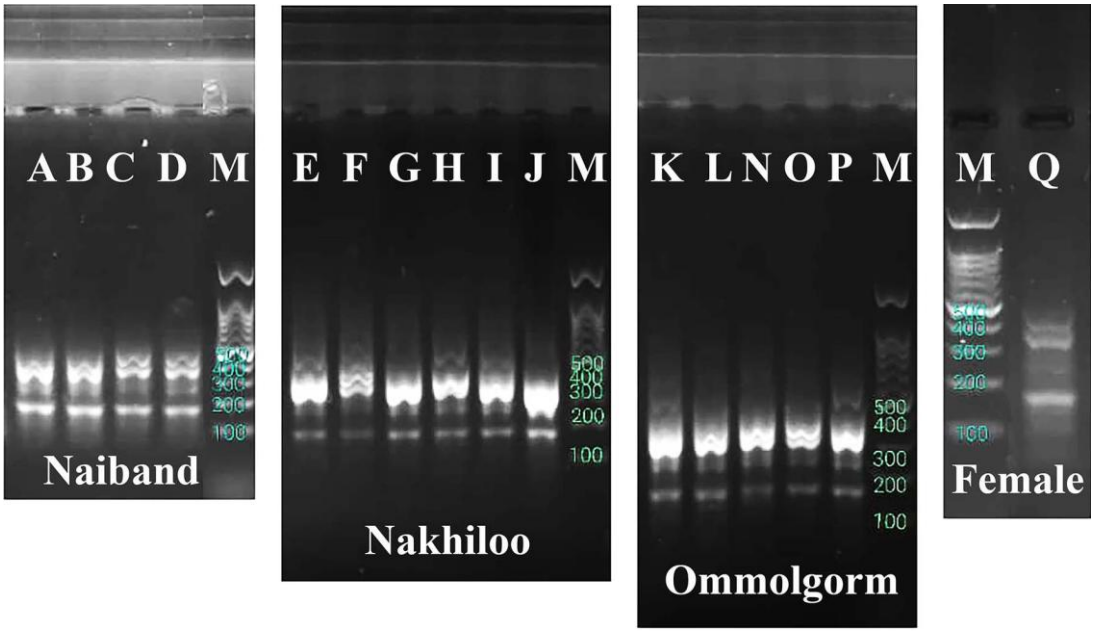

Figure 5. Results of PCR with primer 14749-T for a female hawksbill turtle (Q) from Nakhiloo Island and for 4 hawksbill turtle hatchlings from Naiband Coasts (A, B, C, and D), six samples from Nakhiloo Island (E, F, G, H, I, and J), and 5 samples from Ommolgorm Island (K, L, N, O, and P)

In the reference study conducted on the Chinese softshell turtle (*Pelodiscus sinensis*), this marker was introduced as one of the sex-linked markers; however, the lack of such a pattern in E. imbricata once again emphasizes the fundamental differences between species with genetic sex determination and species with temperature-dependent sex determination. In species with TSD, some genomic regions associated with sexual differentiation are expected to either lack sufficient diversity or play different roles in species with GSD. From a practical perspective, the results from marker 14749-T indicate that this marker is not suitable for studies of sex segregation or population structure in hawksbill turtles, but can be used as a relatively stable genomic region for genetic comparisons or data quality control in molecular studies. Genomic studies in species with a genetic sex determination (GSD) system, such as *Pelodiscus sinensis*, have shown that the development of sex-specific markers can be carried out with high precision and even lead to the ability to definitively distinguish males and females; so that in this species, markers with complete and accurate sex discrimination have been developed and specific banding patterns are observed in males and females (Liang et al., 2019). Overall, the differences reported in the markers studied in *Eretmochelys imbricata* in this study are in stark contrast to the results in GSD species such as P. sinensis. While in GSD species, the discovery of sex-defining and sex-specific markers has been a reliable scientific method, in TSD species such as sea turtles, attempts to determine sex

based on superficial genetic differences have not always yielded conclusive results, and the observed genetic variation is likely to be more dependent on population differences, gene patterns unrelated to sex determination, and population structure (Zhu et al., 2024).

Conclusion: Overall, the results of this study indicate that although some of the markers studied are capable of revealing genetic diversity in the hawksbill turtle, they do not provide sufficient evidence to use them as definitive markers for sex differentiation in this species. These findings are expected given the fundamental difference in the sex determination system between species with genetic sex determination and species with temperature-dependent sex determination, and emphasize the need for species-specific validation of genetic markers before their use in sex determination studies. Finally, this study indicates that genetic markers adapted from species with genetic sex determination may provide valuable information on genetic diversity and population structure in species with TSD such as E. imbricata, but further studies are required for molecular sex determination, including candidate gene expression studies, epigenetic analyses, and the integration of genetic data with environmental information. These results can be used as a basis for future research in the field of reproductive genetics and sea turtle conservation.

Author Contributions

N.P.: Methodology, investigation, data curation, and validation. M.M.: Conceptualization, methodology, software, validation, formal analysis, investigation, resources, data curation, writing-original draft preparation, writing-review and editing, visualization, supervision, project administration, and funding acquisition. M.A.H.: Resources, supervision, writing-review and editing, and funding acquisition. M.A.F.: Methodology, and project administration. All authors have read and agreed to the published version of the manuscript.

Data Availability Statement

Data available on request from the authors.

Acknowledgment

The authors thank the local communities of Dayer City and Bonood village for their assistance in coastal monitoring. We are especially grateful to DOE Bushehr for their invaluable collaboration in sample collection.

Ethical considerations

This study was conducted in accordance with the standards of the Ethics Committee of Shahid Bahonar University of Kerman, and all experimental protocols and sampling techniques were approved by this committee. In addition, the ARRIVE 2.0 guidelines (https://arriveguid.elines.org/) were followed in conducting this study. Since dead turtle hatchlings were used in this study, no turtles were killed or injured.

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Conflict of Interest

The authors declare no conflict of interest.

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ارزیابی مارکرهای ژنتیکی برای بررسی تفاوت‌های مرتبط با جنسیت در لاک‌پشت منقار عقابی (*Eretmochelys imbricata*)

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چکیده

هدف: لاک‌پشت منقار عقابی (*Eretmochelys imbricata*) یکی از گونه‌های به‌شدت در معرض خطر انقراض است که دارای سیستم تعیین جنسیت وابسته به دما (TSD) می‌باشد. با توجه به تأثیر تغییرات اقلیمی بر نسبت جنسی این گونه و کمبود اطلاعات در زمینه تفاوت‌های ژنتیکی مرتبط با جنسیت، این مطالعه با هدف ارزیابی کاربرد چهار مارکر ژنتیکی 6299، 6299-T، 14749 و 14749-T (معرفی‌شده در مطالعات ژنومی پیشین بر روی لاک‌پشت نرم‌لاک چینی (*Pelodiscus sinensis*)) انجام شد تا بررسی شود آیا این مارکرها می‌توانند تفاوت‌های ژنتیکی بالقوه‌ای بین نرها و ماده‌های *E. imbricata* آشکار سازند یا خیر.

مواد و روش‌ها: نمونه‌برداری در طول فصل تخم‌گذاری از سه زیستگاه اصلی تخم‌گذاری لاک‌پشت منقار عقابی در خلیج فارس شامل جزایر نخیلو، و ام‌الگورم و سواحل پارک ملی دریایی نایبند انجام شد. نمونه‌ها شامل یک لاک‌پشت ماده بالغ و پانزده جوجه لاک‌پشت بودند. DNA ژنومی از بافت اندامی استخراج و کیفیت و کمیت آن با الکتروفورز ژل آگارز و اسپکتروفتومتری ارزیابی شد.

واکنش PCR با استفاده از چهار جفت پرایمر معرفی‌شده در مطالعه Zeng et al. (2024) انجام شد. محصولات PCR روی ژل آگارز دو درصد الکتروفورز شده و الگوهای باندی بین افراد، جمعیت‌ها و جنس‌ها به‌صورت کیفی و نیمه‌کمی مقایسه گردید.

نتایج: نتایج نشان داد که مارکرهای 6299 و 6299-T تنوع ژنتیکی قابل توجهی بین افراد و جمعیت‌ها ایجاد می‌کنند، اما این تفاوت‌ها به‌طور یکنواخت با جنسیت مرتبط نبودند. نشانگر 14749 تفاوت‌هایی در سطح جمعیتی آشکار ساخت، به‌گونه‌ای که الگوهای باندی بین جزایر متفاوت ولی درون هر جمعیت یکنواخت بودند. در مقابل، نشانگر 14749-T الگوهای باندی تقریباً یکسانی در تمامی نمونه‌ها نشان داد که بیانگر تنوع ژنتیکی پایین این ناحیه ژنومی در *E. imbricata* است. هیچ‌یک از مارکرها الگوی قطعی و پایدار تفکیک نر و ماده را نشان ندادند.

نتیجه‌گیری: این مطالعه نشان می‌دهد که مارکرهای ژنتیکی معرفی‌شده برای گونه‌های دارای تعیین جنسیت ژنتیکی، در گونه‌ای با سیستم تعیین جنسیت وابسته به دما مانند *Eretmochelys imbricata* نمی‌توانند به‌عنوان مارکرهای قطعی تعیین جنسیت مورد استفاده قرار گیرند. با این حال، برخی از این مارکرها می‌توانند ابزارهای ارزشمندی برای بررسی تنوع ژنتیکی، ساختار جمعیتی و مطالعات حفاظتی باشند. نتایج بر ضرورت توسعه و اعتبارسنجی مارکرهای گونه‌اختصاصی و انجام مطالعات ژنومی، اپی‌ژنتیکی و بیان ژن برای درک بهتر مکانیسم‌های مولکولی مرتبط با جنسیت در لاک‌پشت‌های دریایی تأکید می‌کند.

کلمات کلیدی: تعیین جنسیت، تنوع ژنتیکی، حفاظت از لاک‌پشت‌های دریایی، لاک‌پشت منقار عقابی (*Eretmochelys imbricata*)، مارکرهای ژنتیکی

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