RESEARCH ARTICLE Agricultural Science Digest, Volume 45 Issue 3: 548-554 (June 2025)

Length-weight Relationship and Study using RAPD Technique to Determine the Genetic Variation of Yellow Catfish (*Mystus nemurus,* Valenciennes 1840) in Nong Han lake, Sakon Nakhon Province, Thailand

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10.18805/ag.DF-674

ABSTRACT

Background: Yellow catfish, one of Southeast Asia's most economically significant fish, is found in natural water sources in Thailand. Research on genetic diversity is essential for effective aquatic species management. The purpose was to assess the length-weight relationship (LWR) and genetic diversity of yellow catfish (*Mystus nemurus*) from Nong Han Lake, Thailand.

Methods: 330 samples were collected from ten subpopulations: Ban Tha Rae (n = 31), Don Ang (n = 30), Wat Phon Bok (n = 30), Don Sakhan (n = 32), Don Sao Aey (n = 30), Don Sawan (n = 40), Nguyen Don (n = 32), Lum Nam Phung (n = 40) and Ban Tha Sala (n = 35). Surasawadi floodgate (n = 30). The LWR and feeding intensity were described by the equation $W = aTL^b$ and the condition factor (KF) was calculated as $KF = 100\ W/TL^b$ and RAPD technique was used to evaluate the populations.

Result: The results showed b and KF values ranged from 2.7841-3.6942 and (KF) values from 0.1706-2.0943. 102 DNA bands were identified from RAPD with sizes ranging from 200 to 2,500 bp. Polymorphic loci were found in 71.57% to 98.04%. Nei's gene diversity ranged from 0.24 to 0.36. The genetic distance between reproducible bands of amplified DNA fragments varied from 200 to 2,500 bp. The UPGMA dendrogram of the RAPD-PCR patterns revealed two groups of genetic relationships. The average genetic distance across all samples ranged from 0.020 to 0.245.

Key words: Genetic distance, Genetic diversity, LWR, *Mystus nemurus,* RAPD.

INTRODUCTION

Yellow catfish (*Mystus nemurus*, Valenciennes 1840), one of Southeast Asia's most economically significant fish, is found in a range of wild habitats, including rivers, lakes and reservoirs in Thailand, Malaysia and Indonesia (Rainboth 1996; Kumla *et al.,* 2012). Known for its excellent palatability and desirable flesh texture, this catfish is common in domestic and international markets. However, its presence in freshwater has diminished due to water contamination, which hampers its survival and reproduction (Usmani *et al.,* 2003). Overfishing is depleting *M. nemurus* populations in natural habitats (Kumla *et al.,* 2012). Consequently, the current numbers are inadequate for both consumption and conservation, prompting the development of catfish farming systems to enhance production.

Nong Han Lake is the largest freshwater marsh in Sakon Nakhon Province, northeastern Thailand, covering an area of 12,322.56 ha (Settacharnwit, 2003). The lake is nourished by the Lam Nam Pun stream and receives excess water from the "Surasawadi" floodgate in the south, where it connects the Khong River of That Phanom District, Nakhon Phanom Province (Thomas, 1962). It is an aquatic ecosystem supporting agriculture, traditional fisheries and abundant aquatic breeding.

The application of molecular markers facilitates identification, leading to enhanced accuracy of information and understanding evolutionary relationships. Genome wide association study (GWAS) is a powerful tool to dissect the genetic basis of complex traits, there was conducted to identify candidate SNPs and genes related to body length (BL) and body weight (BW) in female yellow catfish (Huang *et al.,* 2022) and the genetic diversity and the phylogenetic relationships within the Indonesian catfish by using mitochondrian ND6 gene (Widayanti *et al.,* 2023). The study of genetic variation and structure in *M. nemurus* underscores

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How to cite this article: Phromthep, A., Prisingkorn, W., Wongmaneeprateep, S., Kumla, S. and Doolgindachbaporn, S. (2025). Length-weight Relationship and Study Using RAPD Technique to Determine the Genetic Variation of Yellow Catfish (*Mystus nemurus,* Valenciennes 1840) in Nong Han lake, Sakon Nakhon Province, Thailand. Agricultural Science Digest. 45(3): 548-554. doi: 10.18805/ag.DF-674.

Submitted: 14-09-2024 **Accepted:** 13-11-2024 **Online:** 12-12-2024

the importance of preserving genetic diversity (Leesa-Nga *et al.,* 2000). RAPD (Random Amplified Polymorphic DNA) has been extensively used in population genetic studies to characterize genetic divergence within and among populations or species of various animals for molecular genetic characterization (Saini *et al.,* 2011). RAPD successfully identified six bagrid catfish species and assessed genetic diversity in *Clarias batrachus*, *Horabagrus brachysoma*, *Horabagrus nigricollaris*, *M. nemurus* and Indian major carps (*Labeo rohita*, *L. calbasu*, *Catla catla* and *Cirrhinus mrigala*), as well as population structure in *Horabagrus brachysoma* and *Paralichthys olivaceus* (Khedkar *et al.,* 2010; Muneer *et al.,* 2011; Leesa-Nga *et al.,* 2004; Barman *et al.,* 2003; Liu *et al.,* 2007). Also, a wild population of walking catfish in Sylhet, Bangladesh was investigated. Four RAPD markers were used to assess genetic variability among 8 individuals of *Clarias batrachus* genotypes (Miah *et al.,* 2020). However, there are scarce studies on the genetic variation, including diversity and phylogenetic dendrograms, as well as the length-weight relationship (LWR) and condition factor of yellow catfish. Such research is essential for foundational knowledge, management, conservation and commercial farming of catfish. Therefore, this study employed the RAPD technique to investigate the population structure and genetic variation of *M. nemurus* in Nong Han Lake.

MATERIALS AND METHODS

Experimental site and sampling

The study was conducted in 2020 to 2021 at Sakon Nakhon Rajabhat University, Thailand. Using basic random sampling, 330 yellow catfish were collected from Nong Han Wetland in Sakon Nakhon Province, Thailand, from 2020 to 2021. The fish were obtained from ten subpopulations located at Ban Tha Rae (n = 31), Don Ang (n = 30), Wat Phon Bok (n = 30), Don Sakhan (n = 32), Don Sao Aey (n = 30), Don Sawan (n = 40), Nguyen Don (n = 32), Lum Nam Phung (n = 40), Ban ThaSala (n = 35) and Surasawadi floodgate (n = 30).

Data collection

The total length (cm) and wet body weight (g) of each fish were recorded. A small tissue sample from the upper section of the caudal fin was collected from each specimen. For DNA extraction, specimens were preserved in 100% ethanol at -20°C.

Length-weight relationship

The length-weight relationship (LWR) of fish was determined by the method of Ricker (1975). For both sexes, the relationship was expressed as $W = aL^b$ or in a logarithmic equation as.

$$\log (W) = \text{Log } a + b \log (L)$$

Where,
W = Wet body weight (g).
L = Total length (TL) (cm).
a = The intercept (constant).
b = The slope (growth coefficient and R^2 = coefficient of determination).

This equation, represented as $W = aTL^b$, was used to assess fish growth. Allometric growth is indicated by $< 3 <$ b and isometric growth indicates b = 3. Additionally, Fulton's condition factor (KF) was used to compare the health of fish across populations. KF was calculated using the equation $KF = 100\ W/TL^3$ (Fulton 1904) to determine feeding intensity and environmental conditions. In this study, the equation $KF = 100\ W/TL^b$ was also presented.

DNA extraction

DNA was extracted using a modified version of the method described by Asahida *et al.* (1996). DNA quality and quantity were assessed using 0.8% agarose gel electrophoresis and a spectrophotometer at wavelengths of 260 and 280 nm, respectively. The DNA solution was stored at 4°C.

Genetic variation

Genetic variation was investigated using (RAPD) with nine primers (OPB-07, OPB-10, OPC-02, OPC-05, OPC-11, OPC-13, OPC-15, OPC-16 and UBC122). The reactions were prepared in 10 µl containers with the following components: 20 µl of DNA template, 5 µl of Go Taq® Green Master Mix (Promega, Madison, USA), 1.5 mM MgCl, , 1.8 mM primer and 2.9 µl of nuclease-free water. The experiments were conducted using a Gene Amp PCR System 9700. The cycling program was set as follows: 1) pre-denaturation at 94°C for 5 min; 2) denaturation at 94°C for 30 sec; 3) annealing at 40°C for 1 min; 4) extension at 72°C for 2 min and 5) final extension at 72°C for 10 min.

Data collection and analysis

The Gene Tools software (Syngene, USA) was used to analyze growth and weight across different populations of *M. nemurus*, as well as to compare genetic variation based on microsatellite markers. Genetic variation and gene differentiation were assessed using several metrics: Nei's gene diversity (H), Nei's gene diversity among populations (HT), Nei's gene diversity within subpopulations (HS), coefficient of differentiation (GST) and gene flow (Nm). These parameters were estimated using Popgene 1.32 software (Wachira *et al.,* 2001). Genetic distance (D) was calculated using NTSYS-PC 2.1 software and the Arithmetic Mean method was employed for constructing the unweighted pair group (UPGMA) (Adams and Rohlf, 2000).

RESULTS AND DISCUSSION

Length-weight relationship (LWR)

The results are shown in Table 1 and 2, the total body length ranged from 9.1 to 27.79 cm and the wet body weight from 8.0 to 225.64 g. The ten subpopulations of yellow catfish showed similar average values for total length and body weight. The relationships between total length (TL) and weight (W) were consistent, indicating that weight gain is associated with increasing TL.

Table 1: Fish number (N), total length (TL) (cm) and body weight (W) (g) of *M. nemurus.*

Sampling sites	N	TL ± SD			W ± SD		
		Min	Max	Average	Min	Max	Average
A	31	11.3	27.54	18.98±3.63	12.7	211.55	82.31±44.15
B	30	9.1	27.37	18.32±4.41	12.3	185.39	71.28±48.45
C	30	10.3	27.79	18.36±4.25	8.0	220.86	70.14±49.34
D	32	10.5	24.03	17.40±3.88	8.2	144.34	65.19±43.07
E	30	9.2	26.52	18.63±4.92	8.1	225.22	81.71±52.69
F	40	11.0	27.15	19.00±3.28	12.2	185.35	76.27±44.23
G	32	10.8	26.37	17.85±4.01	8.4	151.04	65.97±41.78
H	40	11.2	26.22	18.67±3.84	12.2	225.64	74.08±48.17
I	35	9.4	25.05	17.25±4.35	12.5	186.78	58.19±42.50
J	30	10.3	23.42	17.19±3.40	8.2	147.69	62.38±46.10

Table 2: Regression analyses of LWR and K_F.

Sampling sites	$W = aTL^b(n, R^2)$	(K_F)
A	$W = 0.0136TL^{2.9188}(31, 0.8363)$	1.2037
B	$W = 0.0094TL^{3.0081}(30, 0.9378)$	1.1599
C	$W = 0.0039TL^{3.2970}(30, 0.9560)$	1.1340
D	$W = 0.0024TL^{3.5064}(32, 0.9117)$	1.2368
E	$W = 0.0103TL^{3.0003}(30, 0.9533)$	1.2637
F	$W = 0.0034TL^{3.3486}(40, 0.8803)$	1.1120
G	$W = 0.0061TL^{3.1602}(32, 0.8867)$	1.1593
H	$W = 0.0042TL^{3.2824}(40, 0.9023)$	1.1385
I	$W = 0.0173TL^{2.7841}(35, 0.8837)$	1.1323
J	$W = 0.0013TL^{3.6942}(30, 0.9593)$	1.2288

Genetic variation

102 DNA bands were identified, representing 100% of the bands observed. The percentage of polymorphic loci ranged from 71.57% to 98.04%. Nei's gene diversity (H) varied among the ten subpopulations, with Wat Phon Bok (C), Don Sakhan (D) and Don Sawan (F) exhibiting the highest diversity values of 0.36. The lowest gene diversity was observed in the Surasawadi floodgate (J), with a value of 0.24 (Table 3).

Gene differentiation

Table 4 shows the findings on gene differentiation of yellow catfish. OPC-16 exhibited the highest gene diversity among populations (HT), while OPC-13 had the highest gene diversity within subpopulations (HS). The values for HT and HS were 0.27 and 0.24, respectively. Additionally, the average gene flow (Nm) across all populations was 5.22 and the GST value for the entire population was 0.08.

Genetic distance among pair group

The *M. nemurus* samples were two distinct groups: one group comprising Ban Tha Rae (A), Don Ang (B), Wat Phon Bok (C), Don Sakhan (D), Don Sao Aey (E), Don Sawan (F), Nguyen Don (G), Lum Nam Phung (H) and Ban ThaSala (I) and the other comprising Surasawadi floodgate (J). The Nei's genetic distances between pairs of *M. nemurus* subpopulations, determined by RAPD analysis, ranged from 0.020 to 0.245. The lowest genetic distance was observed between Ban Tha Rae (A) and Don Ang (B), while the highest distance was between Wat Phon Bok (C) and Surasawadi floodgate (J) (Fig 1). The genetic distance between *Hemibagrus nemurus* and *Osteochilus hasselti* from the Nam Kam River, as determined by a genetic differentiation test based on microsatellite frequencies, ranged from 0.1077 to 0.2395 (Hanpongkittikul, 2017).

Due to the availability of feed, enhanced feeding practices and suitable environmental conditions, the growth of *M. nemurus* was positively impacted, especially during the rainy season. Both sexes exhibited isometric and allometric growth patterns to their length-weight relationship (LWR). Specifically, isometric growth was observed at the Don Ang (B) and Don Sakhan (D) stations, while allometric growth was detected at the remaining stations. The condition factors (KF) in this study varied from 1.120 to 1.2368, with an average of KF =1.1769 for the combined sexes. Similarity, negative allometric growth was observed in the population of *Eutropiichtys vacha* and *Rita kuturnee*, inhabiting the Godavari River, South India (Rao *et al.,* 2024).

In comparison, Ayo-Olalusi (2014) investigated LWR and condition factors for African mud catfish (*Clarias gariepinus*) in a flow-through system at a stocking density of 400 fish per cubic meter over three months. Their study found condition factors ranging from 0.1706 to 2.0943. Similarly, Ujjania *et al.* (2012); Asadi *et al.* (2017) and Rao *et al.* (2024) reported that condition factors more than 1 indicated good feeding levels and proper environmental conditions. The LWR and KF findings from this study provide valuable insights for fisheries management, marking the first presentation of these metrics for *M. nemurus* in Nong Han Lake. Najmudeen *et al.* (2019) studied the length-weight relationships (LWRs) of the three species of pelagic sharks belonging to the family Carcharhinidae *viz.*, the silky shark *Carcharhinusf alciformis*, the graceful shark *C. amblyrhynchoides* and Alopidae *viz*, the pelagic thresher *Alopiaspe lagicus* and reported allometric coefficient b values ranging from 2.687

(*A. pelagicus*, N = 122) to 3.11 (C., N = 295). Their coefficient of determination scores ranged from 0.901 (*A. pelagicus*) to 0.984 (*C. amblyrhynchoides*, N = 108), indicating robustness of the analyzed sample.

In stations A, B, E and I, results indicate a good feeding and proper environmental conditions. The findings revealed that weight gain was associated with increasing total length (TL). One limitation of the study was the relatively small

Table 3: Genetic diversity (H= Nei's gene diversity) in ten subpopulations of *M. nemurus* with RAPD technique.

Population stations	Total number of bands	Number of polymorphic bands	Percentage of polymorphic (%)	H
A	96	94	92.16	0.33
B	96	94	92.16	0.33
C	97	97	95.10	0.36
D	98	98	96.08	0.36
E	95	93	91.18	0.34
F	100	100	98.04	0.36
G	87	87	85.29	0.27
H	90	89	87.25	0.31
I	93	93	91.18	0.34
J	78	73	71.57	0.24
Total	102	102	100.00	0.35

Table 4: Primers codes and sequences used for RAPD analysis along with genetic differentiation and population genetic structure.

Primer codes	Sequences (5′-3′)	H_T	H_S	G_{ST}	N_m
OPB-07	GGTGACGCAG	0.28	0.25	0.09	4.76
OPB-10	CTGCTGGGAC	0.24	0.23	0.06	6.71
OPC-02	GTGAGGCGTC	0.24	0.22	0.06	6.84
OPC-05	GATGACCGCC	0.26	0.24	0.09	5.04
OPC-11	AAAGCTGCGG	0.27	0.24	0.07	6.04
OPC-13	AAGCCTCGTC	0.28	0.26	0.08	5.23
OPC-15	GACGGATCAG	0.24	0.21	0.10	4.22
OPC-16	CACACTCCAG	0.33	0.30	0.09	4.68
UBC122	GTAGACGAGC	0.28	0.25	0.09	4.73
Average		0.27	0.24	0.08	5.36
Overall populations		0.36	0.32	0.08	5.22

The parameters reported include: (H_T = Nei's gene diversity among populations, H_S = Nei's gene diversity within sub populations, G_{ST} = Coefficient of differentiation and N_m = Gene flow).

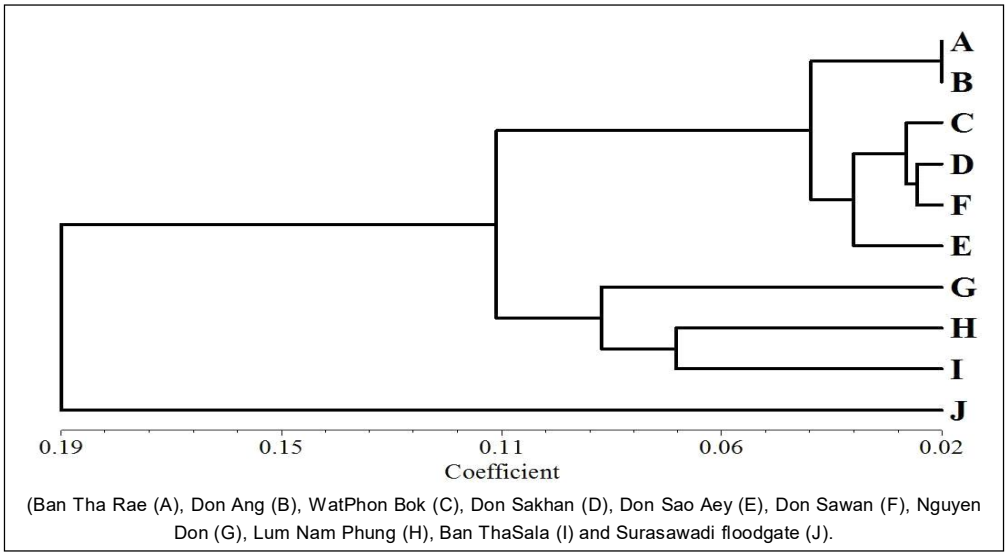

Fig 1: UPGMA dendrogram using Nei's unbiased genetic distance of fish ten stations.

sample size. The investigation identified 102 DNA bands, with sizes ranging from 200 to 2,500 bp. Polymorphic loci were observed in 71.57% to 98.04% of the yellow catfish subpopulations. Nei's gene diversity among *M. nemurus* populations ranged from 0.24 to 0.36, indicating low genetic variation and differentiation. However, gene flow was high due to the fish's freedom of movement. The genetic linkages among *M. nemurus* subpopulations were grouped into two clusters, each showing high polymorphism. The Surasawadi floodgate likely facilitated movement, resulting in closely related phylogenetic relationships among the yellow catfish from Lam Nam Gum. Without such water connections, these relationships would be less apparent. According to Kumla *et al.* (2012), the highest gene diversity (H) was 0.20 and the lowest was 0.11. Similarly, Kumla *et al.* (2016) reported a maximum gene diversity (H) of 0.24 and a minimum of 0.17 in a study of *M. nemurus* genetic variation in Nam Oun Dam, Sakon Nakhon Province. In that study, Nei's gene diversity among populations was 0.31 and within subpopulations 0.28, reflecting lower gene diversity likely due to widespread breeding or escapes from aquaculture and crossbreeding with other species.

Kumla *et al.* (2012) investigated the genetic variation of *M. nemurus* in Thailand with seven primers. The results indicated 83 brands for overall DNA. Using seven primers, Kumla *et al.* (2012) looked at the genetic variation of *M. nemurus* in Thailand. The total DNA findings showed 83 brands. The remaining 80 fragments (96.39%) were found to be polymorphic loci, which was lower than this study because of the number of primers and population differentiation. Kumla *et al.* (2016) investigated the gene flow of *M. nemurus* was 1.60 in Nam Oun Dam, Sakon Nakhon province. Kumla *et al.* (2012) used seven primers to investigate *M. nemurus* genetic variation in Thailand, identifying 83 DNA bands, with 96.39% polymorphic loci. This result was lower than in the current study, which may be attributed to the number of primers used and the degree of population differentiation. Kumla *et al.* (2016) found that gene flow for *M. nemurus* was 1.60 in Nam Oun Dam, Sakon Nakhon Province. Similarly, a genetic differentiation test using microsatellite frequencies assessed the genetic distance between *Hemibagrus nemurus and Osteochilus hasselti* from the Nam Kam River. The results indicated a range of 0.1077-0.2395 (Hanpongkittikul, 2017). The high gene flow observed can be attributed to the unrestricted movement of *M. nemurus* within Nong Han Lake, facilitated by the waterway from NumPung and the fish ladder at the Surasawadee floodgate. This connectivity allows fish from NumPung to migrate freely into Nong Han Lake, suggesting that gene flow may also be influenced by human activities related to fish breeding. Additionally, catfish (*Clarias batrachus*) in Sylhet, Bangladesh was investigated by RAPD markers. Total of 132 bands were detected and all the markers were shown different levels of polymorphism. Intra-specific polymorphisms were observed at a rate of 60% in primer OPB-12, with an average polymorphism recorded at 46.81%. The genetic distance of experimental samples varied from 0.6 to 1 (Miah *et al.,* 2020). Genetics has advanced significantly, revealing that the mitochondrial ND6 gene exhibits a genetic variation among populations ranging from 0.9% to 25%, with an average evolutionary divergence of 14% across catfish groups (Widayanti *et al.,* 2023). Gene flow for *H. brachysoma*, reported by Abdul-Muneer *et al.* (2009), ranged from 0.0275 (in OPAH-09) to 2.0968 (in OPAH-01), with a mean value of 0.6731. In this study, the average value of Nm across all primers among populations was 0.4880. Several RAPD fragments exhibited fixed frequencies within specific populations, which can be utilized as stock-specific markers to differentiate between populations. Eight RAPD fragments were identified using five primers, were unique to particular populations. This information is valuable for managing genetic diversity, preserving it and advancing the *M. nemurus* breeding program.

The results of this study demonstrate that RAPD is highly effective for investigating the genetic diversity of yellow catfish, as evidenced by the high number of polymorphic bands. However, due to the low repeatability of random primers, results vary under different conditions. To address this issue, Sequence Characterized Amplified Regions (SCARs), as outlined by Araneda *et al.* (2005), should be utilized. SCARs provide consistent DNA bands and use specific primers with good repeatability, offering more reliable results than random primers. Despite the high polymorphism observed in this study, employing SCARs would enhance the accuracy and consistency of genetic analyses derived from RAPD techniques.

CONCLUSION

A total of ten subpopulations, including 330 samples, can conclude that both sexes have isometric and allometric development connections between the sexes and their LWR. Isometric growth was seen in the stations Don Ang (B) and Don Sakhan (D). Allometric growth was detected at the rest stations and ten subpopulations of yellow catfish using the RAPD technique. The genetic diversity of catfish in Nong Han Lake was low, as indicated by genetic diversity, variation and distance. They were grouped into two distinct clusters: Group 1 included Ban Tha Rae (A), Don Ang (B), Wat Phon Bok (C), Don Sakhan (D), Don Sao Aey (E), Don Sawan (F), Nguyen Don (G), Lum Nam Phung (H) and Ban ThaSala (I), while Group 2 consisted of Surasawadi floodgate (J).

ACKNOWLEDGEMENT

The present study was supported by Sakon Nakhon Rajabhat University, Thailand and Department of Fisheries, Faculty of Agriculture, Khon Kaen University, Thailand for facilities. Thankful to Asst. Prof. Krongjai Somrug helped to translate in molecular results.

Disclaimers

The views and conclusions expressed in this article are solely those of the authors and do not necessarily represent

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Informed consent

All animal procedures for experiments were approved by the Committee of Experimental Animal care and handling techniques were approved by the University of Animal Care Committee. Ethics Statement: The Institute of Animals for Scientific Purpose Development, National Research Council of Thailand, under license number U1-08815-2563.

Conflict of interest

The authors declare that there are no conflicts of interest regarding the publication of this article. No funding or sponsorship influenced the design of the study, data collection, analysis, decision to publish, or preparation of the manuscript.

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