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Molecular, Morphological, and Production Performance Analysis of Introduced Black Tilapia *Oreochromis* sp. Strains

Dwi Hany Yanti[1,2], Dinar Tri Soelistyowati[1], Odang Carman[1], Dian Hardiantho[2], Hasan Nasrullah[1], & Alimuddin Alimuddin[1]*

[1]Department of Aquaculture, Faculty of Fisheries and Marine, IPB University, Bogor, West Java, Indonesia
[2]Sukabumi Freshwater Aquaculture Center, Selabintana, Sukabumi, West Java, Indonesia
*Corresponding author, email: alimuddin@apps.ipb.ac.id
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ABSTRACT Evaluating the introduced black tilapia strains from the molecular to production performance levels is essential in aquaculture to ensure genetic purity, reproductive efficiency, and sustainable improvements in production. However, uncontrolled hybridization and limited molecular evaluation of introduced strains have often led to inconsistent performance in hatcheries and grow-out systems. This study aimed to identify and characterize introduced black tilapia strains from Thailand (MAG NIN, BIG NIN, and GIFT) compared to the locally developed SAKTI strain from Indonesia based on molecular, morphological, and production performance aspects. Molecular characterization was performed using cytochrome oxidase subunit I (COI) gene sequences from three fish per strain. Morphological aspects were assessed based on body dimensions and proportions from ten fish per strain. Production performance focused on reproductive and growth parameters. Reproductive parameters, including fecundity, egg size, hatching rate, and larval survival rate, while growth performance parameters comprised specific growth rate, aquaculture productivity, feed conversion ratio, sex ratio, and survival rate. Results revealed that all introduced strains belonged to the same species as the *Oreochromis niloticus* SAKTI strain. Morphologically, BIG NIN had a significantly longer body shape than other strains ($p < 0.05$). BIG NIN also demonstrated superior production performance ($p < 0.05$). These findings highlight the importance of integrating molecular and performance-based evaluations to support selective breeding and strain improvement programs that enhance tilapia productivity.

Keywords: Black tilapia; broodstock; morphological analysis; production performance; selective breeding

INTRODUCTION

Tilapia (*Oreochromis* sp.) is a freshwater fish of significant economic importance in global aquaculture due to its adaptability to diverse aquatic environments (El-Sayed, 2019; Nobrega *et al*., 2020; Moses *et al*., 2021) and rapid growth rates (El-Hack *et al*., 2022). National tilapia production in Indonesia increased by 11%, from 1.21 million tons in 2021 to 1.36 million tons in 2022 (FAO, 2023), underscoring its economic value. Despite this achievement, the sector still faces challenges related to the limited availability of high-quality broodstock and fry, primarily caused by inbreeding, unplanned crossbreeding, and the use of unverified genetic strains (Kwikiriza *et al*., 2023). These issues lead to reduced genetic diversity, slower growth, and inconsistent performance in growth and production. Addressing these challenges requires the development and evaluation of superior strains through selective breeding programs supported by molecular, morphological, and production assessments. In this context, Indonesia's Ministry of Marine Affairs and Fisheries introduced three tilapia strains from Thailand in 2022: GIFT (Genetically Improved Farmed Tilapia), BIG NIN, and MAG NIN. GIFT is widely recognized for its disease resistance, while BIG NIN (a GIFT-derived crossbreed) and MAG NIN (a hybrid of GIFT × BIG NIN) are noted for fast growth and high fillet yield, respectively (Tilapiathai.com, accessed January 1, 2023). Although these strains have shown superior performance in their country of origin, their adaptability, stability, and genetic integrity under Indonesian aquaculture conditions remain insufficiently studied.

Over the past decade, most studies on tilapia improvement have focused on either molecular identification (e.g., DNA barcoding and genetic diversity) or phenotypic and production traits separately. However, few have integrated molecular, morphological, and production performance analyses to assess the suitability of introduced strains comprehensively. This gap limits the understanding of how genetic variation corresponds to morphological differentiation and production outcomes under local farming conditions.

Therefore, a comprehensive evaluation combining these three aspects is urgently needed to ensure that the introduced black tilapia strains possess both genetic authenticity and superior production performance in Indonesian environments. The findings will provide scientific evidence for selective breeding and strain management programs, ultimately supporting the sustainable development of national aquaculture.

This study specifically aimed to (1) identify the genetic characteristics of black tilapia strains introduced from Thailand using molecular markers, (2) evaluate their morphological differentiation, and (3) assess their production performance compared with the locally developed SAKTI strain. The SAKTI strain, released by the Ministry of Marine Affairs and Fisheries in 2023 (Decree No. 182), was developed through family selection by the Freshwater Aquaculture Development Center in Sukabumi, West Java, and is known for its rapid growth and *Streptococcus agalactiae* resistance. Comprehensive evaluations are expected to help optimize breeding strategies and provide superior strain options to enhance tilapia productivity in Indonesia.

MATERIALS AND METHODS

Study duration, location, and fish strains

The study was conducted from March 2023 to August 2024 at the Sukabumi Freshwater Aquaculture Center (BBPBAT), West Java, Indonesia, located at 6°54'25.6"S,

106°56'12.7"E, and an altitude of 700 m above sea level. The experimental fish included three introduced strains of Nile tilapia from Thailand—GIFT, BIG NIN, and MAG NIN—along with the SAKTI strain, developed locally by BBPBAT, as a control.

Methods

Experimental design

This study was conducted using a completely randomized design with four black tilapia strains as treatments: GIFT, BIG NIN, MAG NIN and SAKTI. Each treatment consisted of three replicates, except for the GIFT strain, which had one available replicate for growth data.

Preparation of material and tools

A total of 100 broodstock (BW ≥ 50 g) were selected and sexed before the experiment. Fish were maintained in 200 m^2 concrete tanks for maturation. Experimental facilities included concrete ponds, hapas (2 m^2 each), circular tanks (10 m^3), an aquarium (96 L), and analytical tools such as digital scales (0.01 g accuracy), microscopes, and a portable multiparameter device (Lutron WA-2017SD, Taiwan).

Fish maintenance

Broodstock conditioning and spawning

Broodstock were maintained from March 28 to May 4, 2023, and fed commercial pellets containing 28-30% protein at 2-3% of body weight daily. Water quality was maintained at 25-30 °C, pH 6.5-8.5, and dissolved oxygen >5 mg/L (SNI, 2009). Mature males were identified by reddish pointed genital papillae, while females exhibited swollen abdomens (Kwikiriza *et al*., 2023).

Spawning was conducted from May 27 to September 9, 2023, using 20 fish pairs per strain (male: female = 1:1) in 2 m^2 hapas placed in a 400 m^2 pond. Daily observations were conducted to identify mouthbrooding females, and their eggs were collected for measurements of fecundity, egg diameter, and hatching rate.

Egg incubation

Eggs were incubated in 96 L aquarium at 28-30 °C for 5-7 days. The harvested eggs were then observed to collect data on fecundity, hatching rate and egg diameter. Thirty eggs from each strain were measured for diameter using a microscope (ZEISS SteREO Discovery.V12, Germany) at 10× magnification (mm). The fecundity was calculated, and the hatching rate was calculated according to Smalås *et al*. (2017), as follows:

$$\text{Fecundity (egg/kg)} = \frac{Total\ number\ of\ eggs}{Female body\ weight\ (kg)}$$

$$\text{HR (\%)} = \frac{No.of\ hatched\ eggs}{Total\ number\ of\ eggs} \times 100$$

Nursery phase

Larvae were reared in 2 m^2 hapas at a density of 100 larvae/m^2 for 30 days (September 13-October 13, 2023). During this period, larvae were fed commercial powdered feed (40% protein) at 20% of biomass daily. Juveniles (3-5 cm) were then transferred to 16 circular tanks (10 m^3) at 50 fish/m^3 and reared for 42 days (October-November 2023) with 30% protein feed at 10% biomass daily. The survival rate of the larvae was calculated on the 30th day after hatching based on Abaho *et al*. (2022), where No is the number of larvae at the beginning of rearing and Nt is the number of larvae at the end of rearing, as follows:

$$\text{Survival rate (\%)} = \frac{Nt}{No} \times 100$$

Grow-out phase

Fingerlings (body length: 3-5 cm) were transferred to circular tanks and reared for 42 days until they reached 6-8 cm in length. Fish were fed commercial pellets containing 30% protein at a daily rate of 10% biomass. Fingerlings (body length: 6-8 cm) were further reared to an average body weight of 300 g for 148 days (ending July 23, 2024) in circular tanks at 10 fish/m^3. Fish were fed commercial pellets (30% protein) at a daily rate of 5% biomass.

Body weight, standard length, and total length of 30 fish from each strain were measured every month during rearing. Body measurements were taken before feeding in the morning. The specific growth rate (SGR) and feed conversion ratio (FCR) were calculated following Bostock *et al*. (2022), the survival rate (SR) was calculated using the formula from Abaho *et al*. (2022), the production yield (PY) was calculated based on Ricker (2009), and the percentage of males was calculated using the formula from Atriani *et al*. (2024), as follows:

$$\text{SGR (\% per day)} = \frac{\ln Wt - \ln Wo}{t} \times 100$$

$$\text{FCR} = \frac{Total\ feed\ given\ (g)}{Biomass\ gained\ (g)}$$

$$\text{SR (\%)} = \frac{Nt}{No} \times 100$$

$$\text{Productivity (kg/ton)} = \frac{Final\ biomass\ (kg)}{Tank\ volume\ (ton)}$$

$$\text{Male (\%)} = \frac{Number\ of\ males}{Total\ number\ of\ fish} \times 100$$

Where:

Wt: final body weight (g)

Wo: initial body weight (g)

t: rearing duration (days)

N_o: number of fish at the start of rearing

N_t: number of fish at the end of rearing

Water quality parameters, including dissolved oxygen (2.82-9.21 mg/L), temperature (24.78-27.72 °C), and pH (6.99–8.99), were measured monthly at 9:00 AM using a portable multiparameter device (Lutron WA-2017SD, Taiwan).

Molecular analysis

Molecular analysis aimed to identify the species of introduced tilapia strains using the mitochondrial DNA barcoding method targeting the cytochrome oxidase subunit I (COI) gene (Iyiola *et al*., 2018). Genomic DNA was extracted from tail fin tissue samples (5–10 mg) from three fish per strain using the Puregene Tissue Kit (Qiagen, Germany), following the protocol by Petersons *et al*. (2023). PCR amplification of the COI gene employed primers FishF2-F (5'-TCGACTAATCATAAAGATATCGG-

CAC-3') and FishR2-R (5'-ACTTCAGGGTGACCGAAGAATCAGAA-3') (Ward *et al*., 2005). The PCR reaction volume (20 µL) included 10 µL 2× My-Taq HS Red Mix (Bioline, UK), 0.8 µL of each primer (20 µM), and one µL genomic DNA template (20 ng/µL). PCR was performed using a Veriti Thermal Cycler (Applied Biosystem, USA) under the following conditions: initial denaturation at 95°C for 5 min, 40 cycles of 95°C for 30 s, annealing at 55°C for 30 s, extension at 72°C for 30 s and a final extension at 72°C for 5 min.

Amplified fragments (600-700 bp) were visualized on 2% agarose gel electrophoresis with gel dye (Biotium, USA), purified using a PCR purification kit (Applied Biosystem, USA), and sequenced using a Genetic Analyzer 3500 (Applied Biosystems, USA). Sequence editing and alignment were performed with MEGA XI (Tamura *et al*., 2021). Species identification was based on Basic Local Alignment Search Tool (BLAST) comparison against the GenBank database (https://blast.ncbi.nlm.nih.gov/Blast.cgi, accessed on August 26, 2024).

Morphological observation

Morphological measurements were performed on 10 broodstock per strain, covering body weight, standard length, total length, body height, and head length (dos Santos *et al*., 2019). Body proportions were calculated as the ratios of standard length to body height (SL/BH), standard length to head length (SL/HL) and head length to standard length (HL/SL). These data were used to compare morphological differentiation among strains.

Data analysis

All data were expressed as mean ± standard deviation. Normality of data distribution was assessed using the Shapiro-Wilk test and homogeneity of variance was evaluated with Levene's test. One-way ANOVA followed by Tukey's post hoc test was used to determine differences among strains ($p<0.05$). Growth performance data for the GIFT strain, which had only one replicate, were excluded from the statistical analysis. Statistical analyses were performed using IBM SPSS Statistics for Windows, version 25.0 (IBM Corp., USA), and data were managed in Microsoft Excel 10 (Microsoft, USA).

RESULTS AND DISCUSSION

This study evaluated the molecular, morphological, and production performance characteristics of three introduced black Nile tilapia strains and the SAKTI strain. Insights from these analyses can support the optimization of breeding strategies and provide superior strain options to enhance Nile tilapia productivity for farmers.

Molecular species identification

Mitochondrial DNA (mtDNA) barcoding amplification was successfully conducted on 12 Nile tilapia samples, yielding fragments of 648–757 bp (Table 1). BLAST analysis revealed high similarity (96.08-99.86%) between all strains, including GIFT, MAG NIN, BIG NIN, SAKTI and *Oreochromis niloticus* reference sequences (GenBank accession number no. KU565859.1 and MF280061.1). Sequence alignment showed no base substitutions at diagnostic sites within the COI gene region (positions 1-686), indicating high genetic conservation among the strains. These findings confirm species traceability and align with the intended development of *O. niloticus* strains while emphasizing the importance of maintaining genetic purity to prevent unintentional introgression of non-native species.

The cytochrome c oxidase subunit I (COI) gene is widely recognized as a reliable DNA barcoding marker for species differentiation across taxa (Iyiola *et al*., 2018; Kasayev & Arisuryanti, 2022), including closely related species such as Nile tilapia (Ordoñez *et al*., 2016; Nascimento *et al*., 2023) and gourami (Nuryanto *et al*., 2018). Comparable studies using the COI gene also demonstrated high genetic similarity among *O. niloticus* populations, 99.67–100% (Ordoñez *et al*., 2016), further supporting the validity of COI as a robust molecular marker. The genetic resemblance between Thailand and Indonesian strains can be attributed to their shared breeding history derived from the GIFT strain lineage, which has been widely distributed and used as a base for selective breeding in Asia. Moreover, the mitochondrial COI gene evolves relatively slowly, making it less sensitive to geographic variation and thus suitable for species-level identification (Nascimento *et al*., 2023).

Morphological characterization

Morphological measurements revealed slight differences in standard length and the standard length-to-body height ratio (SL/BH) among the introduced black Nile tilapia strains and the SAKTI strain. BIG NIN exhibited the longest body length ($p < 0.05$) (Table 2). The SL/BH ratio of the introduced strains (MAG NIN: 2.38 ± 0.20, BIG

Table 1. Species identification based on CO1 gene.

Strain	Base pairs	BLAST results				
		Query Cover (%)	E-value	Percent Identification (%)	Accession Number	Identified Species
MAG NIN	649	100.00	0.15	99.69	KU565859.1	*Oreochromis niloticus*
MAG NIN	658	100.00	0.15	99.70	KU565859.1	*Oreochromis niloticus*
MAG NIN	677	100.00	0.00	99.60	KU565859.1	*Oreochromis niloticus*
BIG NIN	703	99.00	0.14	99.72	MF280061.1	*Oreochromis niloticus*
BIG NIN	662	99.00	0.15	99.70	KU565859.1	*Oreochromis niloticus*
BIG NIN	693	99.00	0.00	99.86	MF280061.1	*Oreochromis niloticus*
GIFT	664	99.00	0.00	96.08	KU565865.1	*Oreochromis niloticus*
GIFT	648w	100.00	0.00	99.85	KU565859.1	*Oreochromis niloticus*
GIFT	684	100.00	0.00	99.56	MF280061.1	*Oreochromis niloticus*

SAKTI	689	99.00	0.00	99.71	MF280061.1	*Oreochromis niloticus*
SAKTI	757	99.00	0.00	99.86	MF280061.1	*Oreochromis niloticus*
SAKTI	723	98.00	0.00	99.85	KU565865.1	*Oreochromis niloticus*

NIN: 2.40 ± 0.17, and GIFT: 2.26 ± 0.11) was significantly higher than that of the SAKTI strain ($p < 0.05$).

These findings align with Moses *et al*. (2021), who reported that BIG NIN demonstrated the greatest standard length in both freshwater and brackish water environments, resulting in a relatively elongated body shape compared to other strains. This feature suggests that BIG NIN could yield a higher fillet percentage due to its broader body area, an economically valuable trait in aquaculture. Fish body shape is a critical economic characteristic influenced by selective breeding and contributes to aquaculture productivity (de Oliveira *et al*., 2016; Dee *et al*., 2021). Body shape also impacts fish aesthetics consumer preferences (Murphy *et al*., 2024) and market demands, which can vary between regions (Mehar *et al*., 2019). Additionally, differences in body morphology among introduced strains can be attributed to their diverse genetic origins (Montoya-López *et al*., 2019; Dee *et al*., 2021).

In this study, molecular analysis was conducted to determine genetic relationships among tilapia strains, while morphological analysis focused on describing body shape variation. Although the molecular results indicated genetic differentiation among strains, this study did not directly investigate the specific genetic mechanisms or expression pathways responsible for morphological formation. Future developmental pathways analyses would provide deeper insights into the mechanisms linking genetic variation and morphological traits in Tilapia.

Production performance

Reproductive performance was evaluated by observing spawning success rates across strains. Among 20 pairs, BIG NIN and SAKTI showed the highest spawning success (40%, 8 pairs), followed by GIFT (25%, five pairs) and MAG NIN (20%, four pairs). Only successful spawners were sampled to assess reproductive performance (Table 3).

Table 2. Fish body weight and morphological analysis after rearing.

Parameter	Strain Tilapia			
	MAG NIN	BIG NIN	GIFT	SAKTI
Body weight; BW (g)	202.81 ± 83.58^{a}	271.11 ± 70.98^{a}	234.14 ± 58.51^{a}	274.52 ± 100.14^{a}
Standard length; SL (cm)	17.14 ± 1.99^{a}	20.01 ± 1.64^{b}	18.61 ± 1.48^{a}	19.04 ± 2.30^{a}
Body height; BH (cm)	7.29 ± 1.31^{a}	8.38 ± 0.81^{a}	8.23 ± 0.68^{a}	8.65 ± 1.29^{a}
Head length; HL (cm)	7.43 ± 1.23^{a}	8.39 ± 0.61^{a}	8.23 ± 0.50^{a}	8.30 ± 1.00^{a}
BW/HL	26.48 ± 6.30^{a}	32.11 ± 7.38^{a}	28.22 ± 5.65^{a}	32.27 ± 8.70^{a}
SL/BH	2.38 ± 0.20^{b}	2.40 ± 0.17^{b}	2.26 ± 0.11^{b}	2.21 ± 0.13^{a}
SL/HL	2.32 ± 0.14^{a}	2.39 ± 0.19^{a}	2.26 ± 0.13^{a}	2.30 ± 0.11^{a}
HL/SL	0.43 ± 0.03^{a}	0.42 ± 0.03^{a}	0.44 ±0.03^{a}	0.44 ± 0.02^{a}

*Different superscript letters in the same row indicate the significant difference ($p < 0.05$).

MAG NIN exhibited the highest fecundity (5843 ± 1077 eggs/kg), followed by SAKTI (5348 ± 815 eggs/kg) ($p < 0.05$). Conversely, BIG NIN and GIFT achieved the highest hatching rates (91.52 ± 10.18% and 88.25 ± 8.89%, respectively) ($p < 0.05$). The SAKTI strain produced the largest egg size (26.20 ± 0.32 mm) compared to other strains ($p < 0.05$).

High spawning success indicates the adaptability of introduced strains to their new environment (Teletchea, 2021). However, elevated aggression, particularly in GIFT and MAG NIN strains, led to mortality during spawning, likely due to pair incompatibility and resulting conflicts. This aggressive behavior explains their lower spawning success compared to BIG NIN and SAKTI. Although MAG NIN and SAKTI showed higher fecundity, BIG NIN and GIFT achieved better hatching rates. The large standard deviations suggest high individual variation within strains, which may have obscured differences in larval production among them.

In grow-out trials conducted in circular tanks without biofloc, BIG NIN displayed the highest specific growth rate (3.28 ± 0.34%/day) ($p < 0.05$) (Table 4). No significant differences were observed among strains for average productivity, feed conversion ratio, sex ratio, or survival rate (Table 4). These results underscore BIG NIN's superior growth performance, highlighting its potential as an

Table 3. Fecundity, hatching rate, and egg size.

Tilapia Strain	Sampel (n)	Female Body weight (g)	Fecundity (no. egg/kg)	Hatching rate (%)	Egg size (mm)
MAG NIN	4	189.36 ± 58.53^{a}	5843 ± 1.077^{b}	66.33 ± 13.61^{a}	24.82 ± 0.68^{a}
BIG NIN	2	296.49 ± 111.78^{a}	2592 ± 731^{a}	91.52 ± 10.18^{b}	24.66 ± 1.10^{a}
GIFT	3	268.63 ± 82.84^{a}	2697 ± 760^{a}	88.25 ± 8.89^{b}	24.82 ± 0.94^{a}
SAKTI	2	305.96 ± 113.25^{a}	5348 ± 815^{b}	47.71 ± 8.28^{a}	26.20 ± 0.32^{b}

*Different superscript letter in the same column shows the significant difference ($p < 0.05$).

optimal strain for selective breeding programs. Similar findings have been reported that BIG NIN is an improved strain with proven growth potential across a wide range of environments (Moses *et al.,* 2021). This trial was conducted in round tanks without biofloc to evaluate each strain's performance, free from interference from other factors. Hence, the selection of superior Nile tilapia strains for development is based solely on production performance.

Variations in production performance among strains may be attributed to differences in physiological conditions, environmental adaptability, and genetic background (Sallam *et al.,* 2024; Debes *et al*., 2025). Strains that are newly introduced, such as GIFT in this study, may still experience physiological stress and incomplete adaptation to local environmental cues, including temperature, photoperiod, and feed type, which influence gonadal maturation and spawning behaviour (Teletchea, 2021). Moreover, genotype-environment interactions (G x E) play a significant role in shaping phenotyping expression, where the same genetic potential may result in different production performance outcomes under varying environmental conditions (Moses *et al*., 2021; Mengistu *et al.,* 2022; Debes *et al*., 2025).

Several genes are known to be growth-related in teleosts, such as growth hormone (GH), insulin-like growth factor I (IGF-1), and myostatin (MSTN). Upregulation of GH and IGF-1 enhances somatic growth and feed efficiency (Islam *et al*., 2025), whereas suppression of MSTN promotes muscle development (Wang *et al*., 2023). Variations in fecundity, egg size, and hatching success in teleosts are influenced by the expression of key genes regulating gonadal development, such as follicle-stimulating hormone receptor (FSHR) and luteinizing hormone receptor (LHR), both of which are G protein-coupled receptors expressed in fish gonads and participate in regulating the reproductive activities of fish (Yu *et al*., 2022).

CONCLUSION AND RECOMMENDATION

Conclusion

This study confirmed that all introduced black Nile tilapia strains belong to the same species as the SAKTI strain, *Oreochromis niloticus*. Differences in body morphology, reproductive traits, and growth performance suggest varying adaptability and production potential under local conditions. These findings highlight the value of molecular, morphological, and production assessments to ensure strain authenticity and to support selective breeding strategies for sustainable tilapia aquaculture.

Table 4. Specific growth rate, productivity, feed conversion ratio, male ratio and survival rate.

Tilapia strain	Specific growth rate (%/day)	Productivity (kg/ton)	Feed conversion ratio	Male ratio (%)	Survival rate (%)
MAG NIN	2.70 ± 0.12[a]	2.71 ± 1.21[a]	1.58 ± 0.59[a]	56.84 ± 23.24[a]	84.41 ± 22.05[a]
BIG NIN	3.28 ± 0.34[b]	3.40 ± 0.23[a]	1.10 ± 0.08[a]	59.43 ± 4.46[a]	93.82 ± 5.15[a]
SAKTI	2.74 ± 0.05[a]	3.26 ± 0.25[a]	1.14 ± 0.09[a]	51.78 ± 7.07[a]	97.58 ± 0.54[a]

*Different superscript letters in the same column indicate the significant difference ($p < 0.05$).

Recommendation

Further studies should examine the performance of black tilapia strains across diverse environmental conditions to understand genotype-environment interactions better. Combining molecular identification with long-term production evaluation is recommended to strengthen broodstock selection, maintain genetic integrity, and enhance productivity in Indonesian aquaculture programs.

AUTHOR'S CONTRIBUTION

DHY: Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. AA: Ideas, Writing – review & editing, Methodology, Conceptualization. DTS: Writing – review & editing, Methodology, Conceptualization. OC: Writing – review & editing, Methodology, Conceptualization. DH: Ideas, Writing – review & editing, Methodology, Supervision, Conceptualization. HN: Writing – review & editing, Data curation.

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