

Morphological and molecular identification, length–weight relationship, and growth pattern of *Glyptothorax hainanensis* from Vietnam

Le Hoang Duy Minh¹, Nguyen Thao Nguyen¹, Vo Quoc Vu¹, Nguyen Hoang Nhat Minh¹, Nguyen Thi Diem Quynh¹, Nguyen Thi Hao¹, Nguyen Thi Thuy¹, Tran Van Giang^{1,2,*}

¹University of Education, Hue University, 34 Le Loi St., Hue City, Vietnam

²Hue University, 03 Le Loi St., Hue City, Vietnam

*Correspondence to: Tran Van Giang <tvgiang@hueuni.edu.vn, tranvangiang@dhsphue.edu.vn>

Abstract. *Glyptothorax hainanensis* is a sisorid catfish inhabiting fast-flowing freshwater systems in Southeast Asia. However, biological and genetic information on this species remains limited. In this study, we combined morphological examination, length-weight relationship (LWR) analysis, and mitochondrial COI sequence analysis to support species identification and provide basic biological information about this species in Vietnam. Morphological observations revealed typical external characteristics of the genus *Glyptothorax*, including a dorsoventrally compressed body, an inferior mouth with well-developed barbels reflecting adaptation to fast-flowing environments. The LWR was described by $W = 0.02 \times TL^{2.6067}$ ($R^2 = 0.92$), indicating a strong length-weight relationship and negative allometric growth. Molecular analysis based on COI sequencing further provided consistent molecular evidence. BLAST comparisons showed the highest sequence similarity with *G. hainanensis*, and phylogenetic reconstruction placed the studied specimens in the well-supported *G. hainanensis* clade, clearly distinct from other closely related species. The agreement between morphological, biometric, and molecular supported the identification the classification and highlighted the effectiveness of integrated methods in identifying species in morphologically conserved fish groups. The results of this study provide valuable baseline data on the biology and genetic identity of *G. hainanensis*, contributing to a better understanding of freshwater fish diversity in Southeast Asia.

Keywords: Cytochrome c oxidase I, DNA barcoding, freshwater fish Sisoridae, *Glyptothorax hainanensis*, length–weight relationship

1 Introduction

The genus *Glyptothorax* (Siluriformes: Sisoridae) comprises more than 100 described species of benthic catfishes distributed primarily throughout South and Southeast Asia [1, 2]. Members of this genus exhibit remarkable adaptations to fast-flowing environments through specialized morphological features, including a dorsoventrally flattened body, enlarged paired fins, and a thoracic adhesive apparatus that enables individuals to maintain position on rocky substrates under strong currents [2, 3]. These ecological and morphological adaptations render

Glyptothorax an important component of upland riverine fish assemblages.

Glyptothorax hainanensis was originally described from Hainan Island in southern China and northern Vietnam [4]. However, comprehensive information regarding its distribution, genetic diversity, and phylogenetic placement remains limited, particularly for populations within Vietnam and the broader Southeast Asian region, where molecular data in public databases such as GenBank and FishBase are critically scarce. Most existing records of *G. hainanensis* are based solely on morphological identification, whereas molecular data for this species are scarce in public databases such as

GenBank and FishBase. Moreover, the species shares close morphological similarity with congeners, including *G. sinensis* and *G. honghensis*, further complicating reliable identification based exclusively on external characters [4, 5].

In Vietnam, the genus *Glyptothorax* is represented by several species inhabiting mountainous river systems; however, molecular evidence for species identification remains limited [6]. Accurate species delimitation is crucial not only for taxonomic clarification but also for biodiversity assessment, biogeographic inference, and conservation planning in freshwater ecosystems that are increasingly threatened by habitat alteration and hydropower development [7, 8]. Therefore, integrative taxonomic approaches combining morphological and molecular data are strongly recommended to resolve species identification issues within this group [6].

Mitochondrial DNA markers, such as cytochrome c oxidase subunit I (COI) and 16S rRNA, have been widely applied in fish taxonomy due to their relatively high mutation rates, maternal inheritance, and the availability of comparative sequence data in global reference databases [9, 10]. These markers have proven effective in discriminating closely related *Glyptothorax* species and in reconstructing phylogenetic relationships within the family Sisoridae [2, 11]. Phylogenetic analyses based on mitochondrial sequences not only facilitate accurate species identification but also provide valuable insights into evolutionary relationships and historical biogeographic patterns within the genus. The present study aims to contribute new molecular data for *G. hainanensis* based on specimens collected in Vietnam. The results provide molecular evidence supporting the occurrence of *G. hainanensis* in Vietnam and add valuable genetic information to support future systematic, ecological, and conservation studies of *Glyptothorax* species in Southeast Asia.

2 Materials and Methodology

2.1 Sample collection

Specimens of *Glyptothorax hainanensis* were collected from the Dong Chau – Khe Nuoc Trong Nature Reserve, Quang Tri Province, Vietnam. Following collection, tissue samples were preserved in 95% ethanol and stored at low temperature until molecular analyses were conducted. Voucher specimens were photographed in fresh condition to document external morphological characteristics (Figure 1).



Figure 1. Specimens collected from the Dong Chau – Khe Nuoc Trong Nature Reserve, Quang Tri Province, Vietnam.

2.2 Length–weight relationship analysis

The length–weight relationship (LWR) was estimated using the equation: $W = a \times TL^b$, where W represents body weight (g), TL is total length (cm), a is the intercept, and b is the regression coefficient of the LWR [12]. The parameters a and b were calculated through linear regression analysis based on natural logarithm transformation: $\ln(W) = \ln(a) + b \times \ln(TL)$. The 95% confidence limits (CL) for parameters a and b were estimated following the recommended procedure using 1,000 bootstrap iterations.

2.3 DNA extraction and gene sequencing.

Fin tissue was separated and placed in an Eppendorf tube and genomic DNA was extracted and purified using the DNeasy Blood and Tissue

Kit (QIAGEN, Hilden, Germany) following the manufacturer’s instructions. The extracted DNA was eluted in nuclease-free water and stored at -20°C prior to downstream analyses. Polymerase chain reaction (PCR) was used to amplify the mitochondrial cytochrome c oxidase subunit I (COI) gene using primers LCO1490/HCO2198 [13]. PCR conditions included initial incubation at 94 °C

for 1 minute, followed by five cycles of 94 °C for 40 seconds, 45 °C for 40 seconds, and 72 °C for 60 seconds; 35 cycles of 94 °C for 40 seconds, 51 °C for 40 seconds, and 72 °C for 60 seconds; and a final extension step of 72 °C for 5 minutes. PCR products were separated by electrophoresis using a 1.5% agarose gel (ABT, Vietnam).

Table 1. Primers used to amplify and sequence

Locus	Primer	primer sequence	Reference
COI	LCO1490	GGTCAACAAATCATAAAGATATTGG	Folmer <i>et al.</i> (1994)
	HCO2198	TAAACTTCAGGGTGACCAAAAAATCA	

The purified PCR products were sequenced by the Sanger method at a sequencing facility in Malaysia. The obtained COI sequences were edited and assembled using BioEdit software, then analyzed, and verified using the BLAST program. Phylogenetic reconstruction was performed using the Maximum Likelihood (ML) method based on the Kimura 2-parameter (K2P) substitution model in MEGA X software. The reliability of the phylogenetic tree was assessed using 1,000 bootstrap replicates. Reference sequences include published COI sequences of *Glyptothorax hainanensis* (PX556454.1, HQ593584.1), *G. sinensis* (DQ514360.1, PP514561.1), *G. honghensis* (PV878808.1), *G. lampris* (OK077085.1), *G. plicolabris* (DQ514344.1) and *G. buchanani* (OK077106.1). While multiple specimens were collected for morphological and biometric assessments, a representative specimen (MG2212) was selected for COI sequencing to establish the initial molecular identity of the species in this region

2.4 Statistical analysis.

All statistical analyses, including length–weight relationship assessments, were performed using Microsoft Excel 2016. The significance of the difference between the estimated regression coefficient (b) and the isometric value (b = 3) was

tested using a t-test. Homologous nucleotide sequences were aligned with Clustal W. Phylogenetic reconstruction was performed in MEGA X Software.

3 Results

3.1 Morphological characteristics

The specimens were identified as *G. hainanensis* based on morphological features consistent with the original description provided by Nichols and Pope (1927). While they exhibited typical external characteristics of the genus *Glyptothorax*, including a distinctly dorsoventrally flattened body, flattened head, and inferior mouth adapted to fast-flowing waters (Figure 2), specific diagnostic characters support this species identification. Notably, the dorsal fin originated slightly anterior to the pelvic fin insertion, and the maxillary barbels extended posteriorly to approximately the base of the pectoral fin. Furthermore, the coloration pattern brown to dark gray dorsally with irregular dark blotches along the body and fins matches the diagnostic pigmentation described for *G. hainanensis*. A developed thoracic adhesive apparatus, a characteristic of the genus, was also present on the ventral surface.

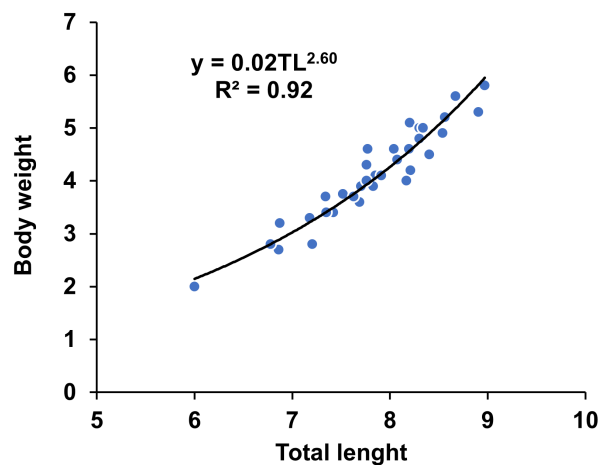


Figure 2. External morphology of *G. hainanensis*.

The dorsal fin was short, bearing a strong and smooth spine followed by soft rays, and originated slightly anterior to the insertion of the pelvic fins. The pectoral fins were large and horizontally expanded, contributing to stability in fast-flowing water. The pelvic fins were ventrally

positioned and moderately developed, whereas the anal fin was relatively short. The caudal fin was deeply forked, with pointed lobes. In fresh specimens, the overall coloration ranged from brown to dark gray dorsally, gradually becoming paler ventrally. Irregular dark blotches were observed along the body and fins, providing effective camouflage against the rocky substrates of mountain streams. The combination of body shape, fin morphology, barbel arrangement, and the presence of a well-developed thoracic adhesive apparatus confirmed the assignment of the specimens to the genus *Glyptothorax* (Figure 2).

3.2 Length–weight relationship



Species	n	a	b	95% CI of b	R ²	Growth type
<i>G. hainanensis</i>	34	0.02	2.607	2.34 – 2.87	0.92	Negative allometry

Figure 3. Length–weight relationship of *G. hainanensis*

A total of 34 specimens were used to estimate the length–weight relationship (LWR). The mean total length (TL) was 7.83 ± 0.64 cm, and the mean body weight (W) was 4.12 ± 0.87 g. The length–weight relationship (LWR) was described by the equation: $W=0.02 \times TL^{2.60}$. Regression

parameters derived from log-transformed data were expressed as $\ln(W) = -3.96 + 2.60\ln(TL)$. The coefficient of determination was relatively high ($R^2 = 0.92$), indicating a strong correlation between total length and body weight. The exponent b (2.60 ± 0.13 SE) was significantly lower than the isometric value of 3.0 ($p < 0.05$). The 95%

confidence interval for b ranged from 2.34 to 2.87, confirming a negative allometric growth pattern. This suggests that individuals increase in length at a proportionally faster rate than in body mass as they grow. The relatively high R^2 value indicates that the model adequately explains the variation in body weight based on total length.

3.3 Molecular analysis

Genomic DNA extracted from the muscle tissue of the sample was found to be of good quality and integrity. Agarose gel electrophoresis

showed clear and distinct DNA bands, with no signs of degradation or excessive blurring, indicating successful DNA extraction and suitability for subsequent molecular analyses (Figure 4). PCR amplification of the target mitochondrial gene segment produced a single, robust band of the expected size, confirming the specificity and efficiency of the amplification process. The absence of non-specific bands or primer pairs further demonstrates the reliability of the PCR conditions used.

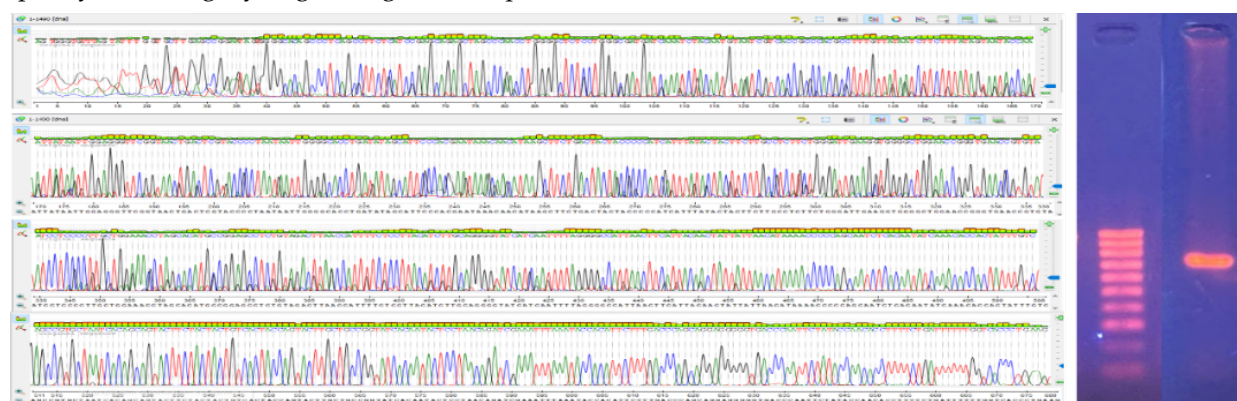


Figure 4. Genomic DNA extracted from *Glyptothorax hainanensis* visualized on agarose gel electrophoresis.

The Sanger sequencing chromatograms exhibited sharp, clear peaks with minimal background noise throughout most of the sequence length. No evidence of unclear base signals, mixed signals, or double peaks was observed, indicating that the resulting sequence was clean and reliable. The overall sequencing quality allowed for confident base identification and subsequent sequence alignment and phylogenetic analysis.

3.4 Phylogenetic analysis

The mitochondrial DNA sequence obtained from specimen MG2212 was successfully compared with available sequences in the GenBank database using BLAST. The BLAST analysis revealed the highest similarity to *G. hainanensis*, with a sequence identity of 96.08% (589/613 bp) and a high query coverage of 96%.

Although this similarity value is slightly lower than the conventional threshold for species-level barcoding in some fish groups, it is consistent with the levels of intraspecific genetic variation often reported in *Glyptothorax* species. When considered alongside our morphological assessments and the robust phylogenetic clustering of the specimen within the well-supported *G. hainanensis* clade, these results provide consistent molecular evidence for assigning the examined specimen to *G. hainanensis*. Other closely related species within the genus *Glyptothorax* showed lower levels of sequence similarity. In particular, sequence identity with *G. sinensis* and *G. fokiensis* was below 94%, indicating clear genetic divergence between the studied specimen and these congeners (Figure 5).

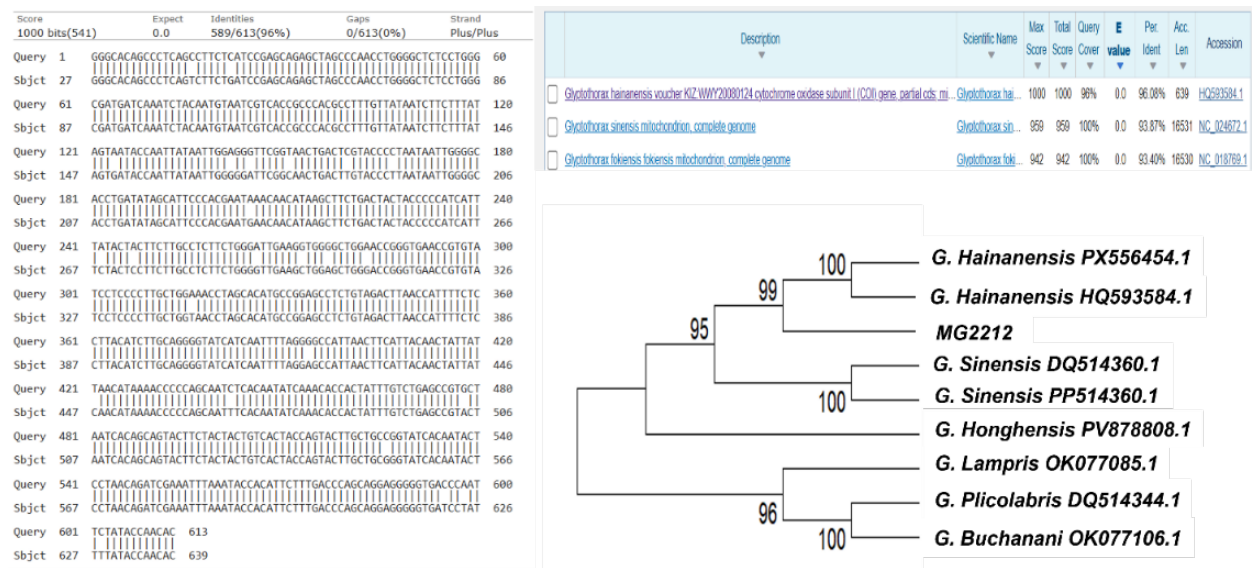


Figure 5. Phylogenetic analysis

Phylogenetic analysis based on aligned mitochondrial gene sequences further corroborated the BLAST results. In the reconstructed phylogenetic tree, the sequence of specimen MG2212 clustered with reference sequences of *G. hainanensis*, forming a well-supported clade with high bootstrap values ($\geq 95\%$). This clade showed distinct divergence from the sampled other *Glyptothorax* species, including *G. sinensis* and *G. fokiensis*, which formed distinct and independent lineages within the tree. The high bootstrap support observed at major nodes (95–100%) indicates strong confidence in the inferred phylogenetic relationships (Figure 5). Overall, both sequence similarity analysis and phylogenetic reconstruction supported the identification that the examined specimen belongs to *G. hainanensis*.

4 Discussion

The present study provides integrative morphological and molecular evidence supporting the occurrence of *G. hainanensis* in Vietnam. The combination of traditional morphological examination, length–weight relationship (LWR)

analysis, and mitochondrial DNA data offers a robust framework for species identification and biological characterization within the genus *Glyptothorax*, a group well known for its taxonomic complexity and high degree of morphological similarity among congeners.

Morphological assessment of the examined specimens revealed a suite of diagnostic characters typical of the genus *Glyptothorax*, including a dorsoventrally flattened body, depressed head, and an inferior mouth with well-developed barbels. These features are widely recognized as key adaptations to torrential mountain stream habitats and have been consistently reported in previous taxonomic studies of Sisoridae catfishes [1, 4]. Notably, the origin of the dorsal fin slightly anterior to the pelvic fin insertion, the length of the maxillary barbels reaching the pectoral fin base, and the specific coloration pattern featuring irregular dark blotches serve as key diagnostic characters that support the identification of *G. hainanensis* and distinguish it from other locally occurring congeners. Although external morphology alone may not be sufficient for reliable species-level identification within *Glyptothorax* due

to overlapping characters and intraspecific variation [1, 4, 9], the observed traits clearly support the generic placement of the examined specimens. Therefore, molecular evidence was necessary to confirm their taxonomic identity

The estimated length–weight relationship of *Glyptothorax hainanensis* was expressed as $W = 0.02 \times TL^{2.60}$, indicating a strong relationship between total length and body weight, as reflected by the high coefficient of determination ($R^2 = 0.92$). This high R^2 value suggests that total length is a reliable predictor of body mass in the studied population and reflects a well-fitted regression model, comparable to values commonly reported for freshwater fishes [12]. The regression coefficient b (2.60 ± 0.13 SE) was significantly lower than the isometric value of 3.0. The 95% confidence interval (2.34 – 2.87) indicates a negative allometric growth pattern, suggesting that individuals increase in length at a proportionally faster rate than in body weight as they grow [12, 14]. Such growth patterns are frequently observed in rheophilic catfishes inhabiting fast-flowing environments, where a more slender and elongated body form may enhance locomotor efficiency and adaptation to strong current conditions [14]. Liang and Cai (2020) reported a length–weight relationship for *G. hainanensis* from the Nandu and Wanquan rivers on Hainan Island as $W = 0.01 \times TL^{2.82}$ [14]. Differences in LWR parameters among studies may be attributed to variations in environmental conditions, food availability, seasonal sampling, ontogenetic composition, and sample size [4, 7]. In the present study, the relatively moderate sample size ($n = 34$) may also have contributed to minor deviations in parameter estimates. Nevertheless, the obtained b value falls within the biologically expected range (2.5 – 3.5) for teleost fishes [14], supporting the reliability of the results. The LWR parameters generated in this study provide baseline information for future ecological

assessments, biomass estimation, and conservation management of *G. hainanensis* in central Vietnam.

Molecular analysis based on the COI sequence facilitated the identification of the examined specimen as *G. hainanensis*. BLAST comparisons revealed the highest sequence similarity with reference sequences of *G. hainanensis*, whereas substantially lower similarity was observed with other congeners, including *G. sinensis* and *G. fokiensis* [9]. These findings are consistent with previous studies demonstrating the effectiveness of mitochondrial markers in discriminating closely related freshwater fish species [2, 15]. Phylogenetic reconstruction further reinforced the BLAST results, as the studied sequence clustered within a well-defined *G. hainanensis* clade clearly separated from other *Glyptothorax* species. The high bootstrap support values (95–100%) indicate strong confidence in the inferred phylogenetic relationships and substantiate the taxonomic assignment. Similar phylogenetic patterns have been reported in molecular studies of Sisoridae catfishes and other rheophilic fish groups [9]. It should be noted that the COI sequence identity of 96.08% is slightly below the conventional 98–99% threshold often suggested for species-level barcoding in fishes. However, this value likely reflects intraspecific genetic variation caused by geographic divergence between the populations of *G. hainanensis* in Hainan Island and those in the isolated river systems of Central Vietnam. Furthermore, the limited number of *G. hainanensis* sequences currently available in public databases (e.g., GenBank) may restrict the accuracy of BLAST comparisons. While these molecular results provide robust support for the taxonomic identification when considered alongside the strong phylogenetic clustering and consistent morphological traits, we acknowledge that this identity value warrants a degree of caution. The confirmation of *G. hainanensis* based on integrated

morphological and molecular evidence contributes to a clearer understanding of the species' distribution in mainland Southeast Asia. Given the limited number of molecularly verified records for *Glyptothorax* species in the region, these findings provide additional baseline data that may complement future research on the species. The confirmation of *G. hainanensis* based on integrated morphological and molecular evidence contributes to a clearer understanding of the species' distribution in mainland Southeast Asia. Given the limited number of molecularly verified records for *Glyptothorax* species in the region, the present findings provide valuable reference data for future taxonomic revisions and biodiversity assessments.

5 Conclusion

This study provides new morphological, biometric, and molecular data for *G. hainanensis* from Vietnam. Morphological examination and COI-based molecular analyses consistently confirmed the taxonomic identity of the examined specimens, while length–weight analysis indicated a negative allometric growth pattern. The agreement between morphological and molecular evidence highlights the value of an integrative approach for species identification in morphologically similar taxa such as *Glyptothorax*. These findings provide important baseline information on the biology and genetic identity of *G. hainanensis* and support future taxonomic, ecological, and conservation studies in Southeast Asia.

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