

Research Article

Karyotype analysis and Ag-NOR localization in the Asian swamp eel (*Monopterus albus*) from Ban Pong District, Ratchaburi Province, Thailand

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Abstract - This study aimed to analyze the karyotype and standard idiogram of the Asian swamp eel (*Monopterus albus*) collected from Ban Pong District, Ratchaburi Province, Thailand. A total of nine specimens, comprising four males and five females, were examined. Chromosomes were prepared directly from kidney tissue using the direct method and stained with conventional staining and the Nucleolar Organizer Regions (NORs) banding techniques. The karyotype and standard idiogram were constructed based on computer-assisted image analysis. The results revealed that *M. albus* possessed a diploid chromosome number of 24 ($2n = 24$) and a fundamental number (NF) of 26, comprising one pair of acrocentric chromosomes and 11 pairs of uni-armed chromosomes. The karyotype consisted of 2 large acrocentric, 2 large telocentric, and 20 medium telocentric chromosomes, respectively. No chromosomal differences were observed between males and females. Ag-NOR banding analysis demonstrated a single signal located at the telomeric region of the long arm of a telocentric chromosome no. 7 (7a). This study provides supportive cytogenetic evidence contributing to the taxonomic classification of *M. albus*. Furthermore, the findings serve as baseline information for the conservation of swamp eel genetic resources and future studies on chromosomal evolution. The karyotype formula of *M. albus* is $2n (24) = L_2^a + L_2^t + M_{20}^t$

Received: 28th January 2026,

Revised: 27th May 2026,

Accepted: 28th May 2026

Citation: Sribenja, K., & Getlekha, N. (2026). Karyotype analysis and Ag-NOR localization in the Asian swamp eel (*Monopterus albus*) from Ban Pong District, Ratchaburi Province, Thailand. *Food Agricultural Sciences and Technology (FAST)*, 12(3), 290-299. <https://doi.org/10.14456/fast.2026.20>

Keywords: *Monopterus albus*, chromosome, karyotype, idiogram

1. Introduction

The Asian swamp eel (*Monopterus albus*), a member of the family Synbranchidae, is a widespread tropical freshwater fish distributed throughout Southeast Asia and regarded as both an ecologically significant and economically important species in local fisheries and aquaculture (Maneechot et al., 2020; Chetrakran et al., 2021). Previous cytogenetic studies have demonstrated that *M. albus* exhibits notable karyotypic variation among populations, suggesting complex chromosomal evolution within the species and the potential existence of cryptic diversity (Supiwong et al., 2019). However, despite these findings, it remains unclear whether such karyotypic variation reflects geographically structured chromosomal polymorphism or the presence of distinct evolutionary lineages within the species. In particular, conventional staining and molecular cytogenetic techniques have revealed differing diploid chromosome numbers (e.g., $2n = 18$ and $2n = 24$) and chromosomal structures, yet no study has explicitly evaluated which karyomorph predominates in specific local populations or tested hypotheses regarding their distribution patterns, highlighting the need for localized chromosomal characterization to better understand intra-specific variation and evolutionary dynamics.

Cytogenetic analyses, such as karyotyping and nucleolar organizer region (NOR) banding, are valuable techniques used to identify chromosomal polymorphisms and structural variations that may not be detected by conventional staining alone, and provide essential insights into genomic organization, chromosomal rearrangements, and

genetic differentiation that are not evident through morphological observations alone. These techniques have been widely used to assess chromosomal patterns in teleost fish, contributing to taxonomic clarification, identification of population structure, and conservation management strategies (Maneechot et al., 2025). In addition to studies conducted in Thailand, broader cytogenetic and evolutionary investigations of Synbranchidae have highlighted the role of chromosomal rearrangements in speciation processes, underscoring the importance of integrating regional data into a wider comparative framework. In *M. albus*, several studies have reported a diploid number of 24 chromosomes with an all telocentric/acrocentric karyotype in Thai populations, although variations have been observed among different geographic localities, emphasizing the need for expanded sampling and cytogenetic documentation.

Ratchaburi Province, located in Western Thailand, encompasses diverse freshwater ecosystems within the Mae Klong River Basin, where *M. albus* is frequently encountered. Despite its local importance and potential as a biological model for cytogenetic research, there remains a paucity of data on the chromosomal characteristics of swamp eel populations in this region. To address this gap, the present study aimed to conduct a comprehensive chromosomal analysis of *M. albus* from Ban Pong District, Ratchaburi Province, using conventional staining and Ag-NOR banding to establish baseline cytogenetic information. Such data are critical for improving our understanding of the genetic structure of *M. albus*, informing taxonomic classification, and supporting future conservation and evolutionary studies.

2. Materials and methods

2.1 Sampling collection

Specimens of the Asian swamp eel (*M. albus*) (Figure 1) were collected from Ban Pong District, Ratchaburi Province, Thailand, using various fishing gears, including fish nets, fish traps, fishing rods, and hand nets. The specimens were transported alive to the laboratory at the Faculty of Science and Technology, Muban Chombueng Rajabhat University. A total of nine individuals (4 males and 5 females) were examined, and sex was determined by gonadal dissection following established criteria

for this protogynous hermaphroditic species. Species identification followed Nelson (2006) and related taxonomic references, including Vidthayanon (2008), Saenjundaeng (2014), Phanitwong (2020), and Suvarnaraksha and Utsugi (2023). Although the sample size is limited, it is adequate for preliminary cytogenetic characterization; however, interpretation of intra-population chromosomal variation should be made with caution. Voucher specimens were preserved and deposited in the institutional collection with accession numbers to ensure reproducibility.



Figure 1. Characteristics of the Asian swamp eel (*M. albus*). Scale bar: 5 cm.

2.2 Cytogenetic investigation

The procedures were conducted in accordance with established ethical guidelines. Anesthesia was induced by soaking the specimens on ice prior to euthanasia. The study protocol was approved by the Ethics Committee of Muban Chombueng Rajabhat University and the Ethics Committee for Animal Experimentation of the National Research Council of Thailand (License No. U1-04484-2559). Chromosome preparations were performed using the direct method, modified from Sumner (1990) and Getlekha et al. (2022). Four male and five female specimens were injected

intraperitoneally with 0.02% colchicine solution (1 ml/100 g body weight) and left for 1 h to arrest cells at metaphase. Kidney tissue was excised, minced, and treated with 0.075 M KCl hypotonic solution for 30 min.

Cells were fixed with Carnoy's fixative (methanol : acetic acid, 3:1) through repeated centrifugation at 2,500 rpm for 8 min, and the supernatant was carefully discarded. The cell pellet was then resuspended in freshly prepared cold fixative and centrifuged under the same conditions. This fixation process was repeated several times until a cloudy white cell suspension was obtained,

indicating adequate fixation. The final cell suspension (3–4 ml) was dropped onto clean slides, air-dried on a hot plate at 75 °C, and stained using conventional staining with 20% Giemsa and Ag-NOR banding (50% AgNO₃ : 2% gelatin solution). Chromosomes were examined and photographed under a light microscope at 1,000× magnification.

Karyotype analysis followed the protocol of [Chaiyasut \(1989\)](#). Metaphase plates were selected based on well-spread chromosomes, appropriate chromosome length, complete diploid chromosome number, and clearly distinguishable centromeres. Chromosome measurements were obtained from 20 well-prepared metaphase cells from both male and female specimens. Parameters measured included the short arm length (Ls), long arm length (Ll), total length (LT), centromeric index (CI), and relative length (RL), which were used to classify chromosome types and sizes following the criteria of [Levan et al. \(1964\)](#) (Table 1). A standard idiogram was subsequently constructed using computer-assisted analysis (Adobe Illustrator), with chromosomes arranged according to their morphological characteristics and relative lengths.

3. Results and discussion

3.1 Chromosome number and karyotype structure

Cytogenetic analysis of the Asian swamp eel (*M. albus*) collected from Ban Pong District, Ratchaburi Province, revealed a diploid chromosome number of $2n = 24$ and a fundamental number (NF) of 26 in both male and female specimens. No sex-related chromosomal differences were observed (Figure 2), indicating the absence of morphologically differentiated sex chromosomes in this

population. This finding is consistent with previous cytogenetic studies of *M. albus* populations in Thailand, which also reported a diploid number of 24 chromosomes (Table 2) and no detectable heteromorphic sex chromosomes ([Donsakul & Maktoon, 1994](#); [Navarro & Barton, 2003](#); [Lowry & Willis, 2010](#); [Twilprawat et al., 2013](#); [Srisukonrat, 2017](#); [Jay et al., 2018](#); [Supiwong et al., 2019](#); [Maneechot et al., 2025](#)).

Previous studies have reported that some *M. albus* populations exhibit a diploid chromosome number of $2n = 18$ ([Donsakul & Maktoon, 1994](#); [Navarro & Barton, 2003](#); [Supiwong et al., 2019](#)), indicating karyotypic variation within *M. albus*. Differences in karyotype composition among individuals have allowed the classification of two distinct karyomorphs, designated as karyomorph A ($2n = 24$) and karyomorph B ($2n = 18$), both sharing the same fundamental number (NF = 24). The identical NF combined with divergent karyotype formulas suggests a close evolutionary relationship between these karyomorphs. It has been proposed that karyomorph B originated from karyomorph A, which is composed predominantly of uni-armed chromosomes, through centric fusion events, resulting in the formation of six metacentric chromosomes. Nevertheless, an alternative hypothesis involving centric fission cannot be excluded, whereby the fragmentation of metacentric chromosomes may have generated the additional acrocentric chromosomes observed in karyomorph A. Such chromosomal rearrangements are consistent with previous reports indicating that chromosomal evolution in fishes frequently involves heterochromatic regions, particularly at the centromeres ([Supiwong et al., 2019](#)).

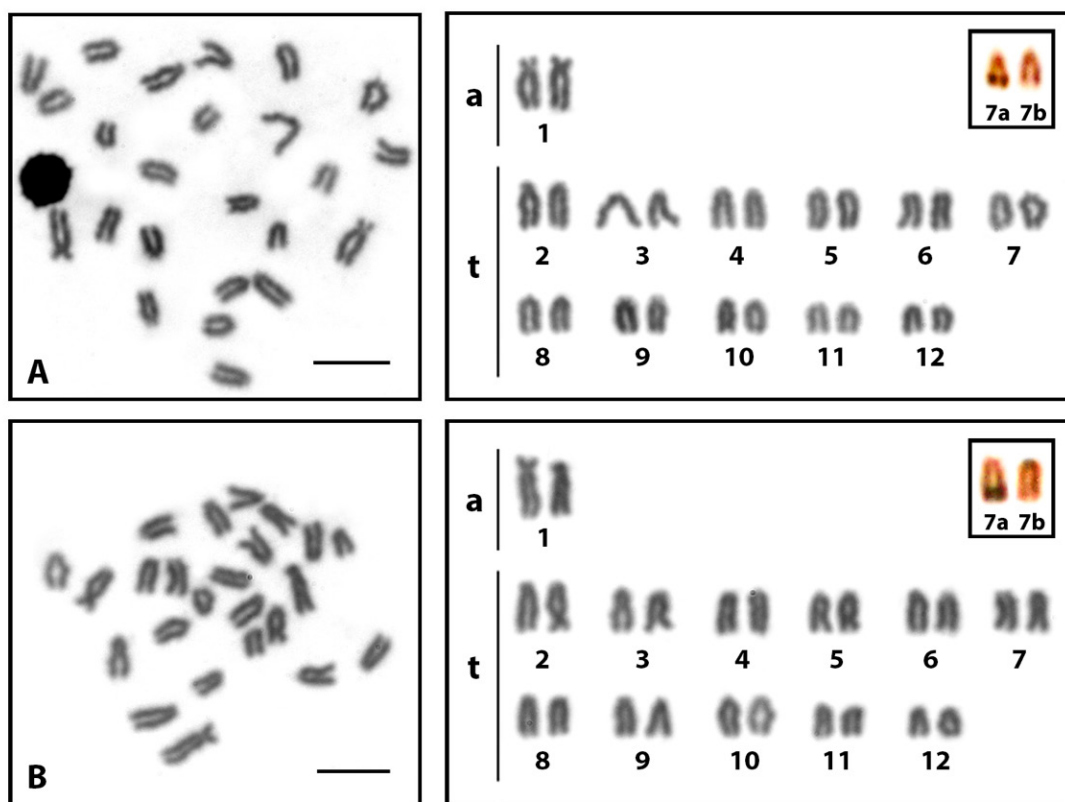


Figure 2. Metaphase cells and karyotypes of male (A) and female (B) Asian swamp eels (*M. albus*, $2n = 24$) using conventional staining. Abbreviations: a = acrocentric chromosome; t = telocentric chromosome. Boxes indicate the Ag-NOR sites on chromosome pair no. 7. Scale bar = 2 μ m.

In the present study, the karyotype of *M. albus* consisted of 2 large acrocentric, 2 large telocentric, and 20 medium telocentric chromosomes (Table 1, Figures 2 and 3). The predominance of telocentric chromosomes suggests a relatively conserved karyotype structure, a feature commonly observed in members of the family Synbranchidae (Supiwong et al.,

2019). The presence of a limited number of bi-armed chromosomes suggests that historical chromosomal rearrangements, such as centric fusion, pericentric inversion, or Robertsonian translocation, may have played a role in shaping the karyotype evolution of *M. albus* populations.

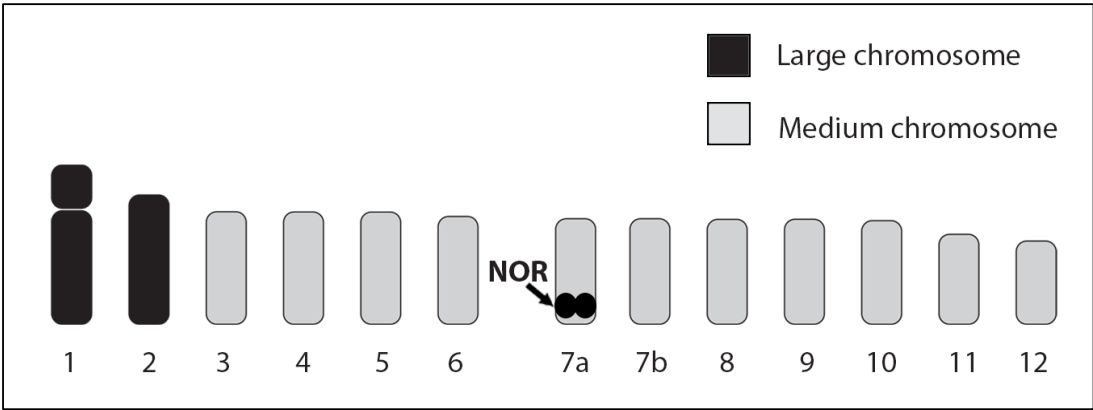


Figure 3. Standard idiogram of the Asian swamp eel (*M. albus*, 2n = 24). The arrow indicates the Ag-NOR site (no. 7a).

Table 1. The mean lengths of 20 metaphase chromosomes were determined, including the short chromosome arm (Ls), long chromosome arm (Ll), length of each chromosome pair (LT) in micrometers, centromeric index (CI), relative length (RL), and standard deviation (SD) of CI and RL of the Asian swamp eel (*M. albus*, 2n = 24).

Pairs	Ls	Ll	LT	CI ± SD	RL ± SD	Size	Type
1	0.433	1.136	1.570	0.724±0.012	0.118±0.003	Large	Acrocentric
2	0.000	1.294	1.294	1.000±0.000	0.098±0.002	Large	Telocentric
3	0.000	1.126	1.126	1.000±0.000	0.085±0.002	Medium	Telocentric
4	0.000	1.124	1.124	1.000±0.000	0.085±0.004	Medium	Telocentric
5	0.000	1.123	1.123	1.000±0.000	0.085±0.004	Medium	Telocentric
6	0.000	1.079	1.079	1.000±0.000	0.081±0.002	Medium	Telocentric
7	0.000	1.056	1.056	1.000±0.000	0.080±0.003	Medium	Telocentric
8	0.000	1.051	1.051	1.000±0.000	0.079±0.001	Medium	Telocentric
9	0.000	1.050	1.050	1.000±0.000	0.079±0.002	Medium	Telocentric
10	0.000	1.037	1.037	1.000±0.000	0.078±0.002	Medium	Telocentric
11	0.000	0.900	0.900	1.000±0.000	0.068±0.002	Medium	Telocentric
12	0.000	0.833	0.833	1.000±0.000	0.063±0.002	Medium	Telocentric

Note: The unit of measurement is micrometers (µm).

3.2 Ag-NOR localization

Ag-NOR banding analysis revealed a single signal located at the telomeric region of the long arm of a telocentric chromosome no. 7a (Figure 2). The presence of NORs on only one chromosome of a homologous pair is consistent with previous reports in several fish species. Such variation suggests that

fish populations, even those inhabiting small or geographically isolated areas, may exhibit karyotypic differences. Therefore, the use of NOR localization in explaining evolutionary relationships or phylogenetic affinities depends largely on the degree of stability and variability of this characteristic within each taxonomic group (Supiwong et al., 2017). Comparable

Ag-NOR localization has been documented in other Thai populations of *M. albus* (Supiwong et al., 2019; Maneechot et al., 2025), indicating limited intraspecific variation in Ag-NOR distribution despite geographic separation. The presence of a single Ag-NOR-bearing chromosome pair is a common feature among teleost fishes and has been reported in several eel and catfish species (Supiwong et al., 2017; Supiwong et al., 2019). This conserved Ag-NOR pattern suggests chromosomal stability and supports the hypothesis that *M. albus* maintains a relatively simple organization of ribosomal DNA clusters.

3.3 Comparative cytogenetics and taxonomic implications

The karyotypic characteristics observed in this study are largely consistent with previously reported cytogenetic data for *M. albus* from other regions of Thailand (Donsakul & Maktoon, 1994; Twilprawat et al., 2013; Srisukonot, 2017; Supiwong et al., 2019; Maneechot et al., 2025). However, variation in chromosome morphology reported among populations may partly reflect differences in the cytogenetic classification criteria adopted by different authors rather than true biological divergence. In cytogenetic studies, chromosome morphology is commonly classified according to centromere position; nevertheless, the terminology and classification systems used are not always consistent among studies.

In the present study, chromosome classification followed the criteria of Levan et al. (1964), in which chromosomes were categorized into four morphological types: metacentric, submetacentric, acrocentric, and telocentric. Under this criterion, chromosomes possessing only a visible long arm and lacking a distinguishable short arm were classified as telocentric chromosomes. Consequently, such chromosomes were considered uni-

armed and contributed one chromosomal arm to the fundamental number ($NF = 1$ per chromosome). In contrast, some previous studies adopted an alternative classification system in which chromosomes with an extremely short or undetectable short arm were grouped as acrocentric rather than telocentric chromosomes. Under those criteria, acrocentric chromosomes were likewise interpreted as uni-armed chromosomes and therefore also contributed one chromosomal arm to the NF value ($NF = 1$ per chromosome).

These differences in classification criteria may lead to apparent inconsistencies when comparing karyotype formulas among studies, particularly regarding the distinction between acrocentric and telocentric chromosomes. Therefore, chromosome morphology should be interpreted together with the reported NF value and the classification criteria employed in each study. In the present study, the predominance of uni-armed chromosomes and the corresponding NF calculation support the interpretation that these chromosomes are telocentric rather than acrocentric. Thus, discrepancies between the present findings and previous reports most likely reflect methodological and terminological differences in chromosome classification rather than substantial chromosomal divergence among populations.

Nevertheless, variation in chromosome morphology and diploid number ($2n = 18$ and $2n = 24$) reported among different populations (Donsakul & Maktoon, 1994; Supiwong et al., 2019) may indicate the occurrence of chromosomal rearrangements during the evolutionary history of *M. albus*. Such cytogenetic variation could reflect population differentiation or the presence of cryptic diversity within the species complex, emphasizing the importance of further regional cytogenetic and molecular investigations.

Table 2. Reviews of papers on cytogenetics in *M. albus* (2n = diploid number, NF = fundamental number, NORs = nucleolar organizer regions, m = metacentric, a = acrocentric, t = telocentric, – = not available).

Species	2n	NF	Formula	NORs	Reference
<i>Monopterus albus</i>	18	24	6m+12a	-	Donsakul and Maktoon (1994)
	24	24	24a	-	Donsakul and Maktoon (1994)
	18	24	6m+12a	-	Navarro and Barton (2003)
	24	24	24a	-	Navarro and Barton (2003)
	24	24	24a	-	Lowry and Willis (2010)
	24	24	24a	-	Twilprawat et al. (2013)
	24	24	24a	-	Srisukonat (2017)
	24	24	24a	-	Jay et al. (2018)
	18	24	6m+12a	2	Supiwong et al. (2019)
	24	24	24a	2	Supiwong et al. (2019)
	24	24	24t	2	Maneechot et al. (2025)
	24	26	2a+22t	2	Present study

The karyotypic pattern observed in the Ratchaburi population supports the occurrence of chromosomal variation within *M. albus*. However, interpretations regarding chromosomal evolution, lineage divergence, or cryptic diversity remain preliminary and require broader comparative sampling and molecular phylogenetic analyses. Nevertheless, the cytogenetic data presented here provide useful baseline information for future comparative and evolutionary studies within Synbranchidae.

Cytogenetic data, particularly karyotype structure and Ag-NOR distribution, provide informative chromosomal markers for comparative analysis and may contribute to taxonomic interpretation in morphologically conserved taxa such as swamp eels. In this context, the present study adds baseline cytogenetic information for *M. albus* from the Mae Klong River Basin, supporting its inclusion in broader comparative and evolutionary frameworks within Synbranchidae.

The chromosomal information presented here may also serve as a

reference for future assessments of genetic variation at the population level, which is relevant to understanding patterns of diversity. Integration of cytogenetic data with complementary molecular approaches would further enhance its applicability in studies of genetic resources and population structure. Moreover, the cytogenetic characteristics described here provide a foundation for future investigations employing molecular cytogenetic techniques, such as fluorescence *in situ* hybridization (FISH), to further elucidate chromosomal evolution and genome organization in *M. albus*.

4. Conclusion

This study provides cytogenetic characterization of the Asian swamp eel (*M. albus*) from Ratchaburi Province, Thailand, using conventional staining and Ag-NOR banding techniques. The results revealed a conserved karyotype with 2n = 24 and NF = 26, characterized predominantly by uni-armed chromosomes, no detectable sex-related

chromosomal differences, and a single Ag-NOR signal located at the telomeric region of telocentric chromosome pair no. 7a. These findings are consistent with previous reports from other regions of Thailand and suggest overall karyotypic stability in *M. albus* despite regional chromosomal variation across its distribution range. The conserved karyotype observed in the central Mae Klong River Basin indicates that major chromosomal rearrangements are not uniformly distributed among populations.

Nevertheless, the relatively limited sample size examined in the present study may not fully represent the chromosomal diversity throughout the species' distribution range. Therefore, interpretations regarding population differentiation, chromosomal evolution, or potential cryptic diversity should be considered with caution. Overall, this study provides essential baseline cytogenetic data that support taxonomic clarification, conservation of genetic resources, and future investigations into chromosomal evolution and potential species complexes in *M. albus*.

Acknowledgements

This research was financially supported by the Research and Development Institute, Muban Chombueng Rajabhat University. The authors also gratefully acknowledge the Department of Biology, Faculty of Science and Technology, Muban Chombueng Rajabhat University, for providing research facilities, technical support, and assistance in verifying the accuracy of the data.

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