Establishing quality standards for Kohaku Koi Fish (*Cyprinus rubrofuscus*): integration of truss morphometrics, red color area, and pigmentation intensity

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Abstract. This study aims to quantify the quality standards for Kohaku koi fish (*Cyprinus rubrofuscus*) by integrating truss morphometric measurements, red color coverage area, and pigmentation intensity analysis. Twenty-four juvenile Kohaku specimens were selected from the results of selective crossbreeding between Nidan female parents and Sandan Kohaku male parents. Morphometric analysis measured 21 truss morphometric characters of koi fish, and digital image analysis using ImageJ software measured the area of coverage and intensity of red color. Principal component analysis and hierarchical clustering were employed to classify phenotypic patterns of individual fish characteristics. Results demonstrated significant morphometric variation with coefficients ranging from 10.76% to 27.91% within the population. Red color coverage area varied extensively from 4,200 to 442,851 pixels, while intensity values ranged from 85.2 to 160.0 on a 0-255 scale. Kohaku specimens with larger red areas show higher color intensity, wider tail fins, and greater body depth. Size distribution analysis revealed 41.7% large, 37.5% medium, and 20.8% small fish, demonstrating successful selective breeding outcomes. This integrated quantitative approach provides a scientific foundation for standardized quality evaluation, replacing subjective assessment methods and supporting data-driven breeding programs to enhance the aesthetic and economic value of Kohaku koi in commercial production.

1 Introduction

Kohaku koi fish (*Cyprinus rubrofuscus*) are one of the koi fish species that have significant economic value. The beauty of Kohaku koi fish lies in their red backs with white bodies. The color of Kohaku koi fish has not yet been genetically deciphered, but it is thought to be due to complex color inheritance in koi fish [1]. The pigmentation mechanism of koi fish has been successfully elucidated in recent molecular genetic research through the identification of mRNA associated with color patterns and pigmentation in the skin [2;3]. This research has successfully complemented conventional breeding processes by providing molecular insights into the genetic basis of color inheritance in koi fish strains. Color quality and brightness are key factors determining the selling price of koi fish. The low quality of koi fish color remains

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a major challenge for breeders, while the demand for high-quality koi fish continues to rise annually. A common issue in a single koi fish farming cycle is the low percentage of koi fish fry with superior quality that will be used for the next growth phase, which affects the selling value.

According to [4], body color is an indicator of beauty in ornamental fish, especially in koi fish, where it is one of the main determinants of aesthetic value and attractiveness. The brighter the color of the fish, the more attractive it is and the higher its selling price. Recent research by [5] has measured this relationship, showing that digital image analysis can objectively measure color quality parameters that are directly correlated with market value and competitive success. Factors influencing the color quality of koi fish include genetics, diet, and environment. Genetic factors influence the inheritance of color patterns in koi fish, for example, in Kohaku, the inheritance of red color patterns on the body. Diet and environmental factors influence the brightness and intensity of red color in Kohaku. Examples of environmental factors influencing the intensity of Kohaku color include water quality, temperature, and sunlight. Recent targeted metabolomics studies have revealed that seasonal variations significantly influence skin color plasticity in ornamental koi fish, providing new insights into environmental regulation of pigments [3].

In addition to color quality, the body shape of koi fish is also a consideration in determining the quality standards for good koi fish. However, to date, there are no standardized criteria for determining such quality. Therefore, information regarding morphological variations and their relationship with color quality is a fundamental aspect that requires further research. The traditional reliance on subjective assessments by expert judges is increasingly being questioned by the need for objective and repeatable quality assessment methods. Recent developments in digital image processing and computer-assisted analysis have opened up new avenues for standardized koi assessment, overcoming the high subjectivity and low efficiency of manual classification methods [2]. Therefore, information regarding morphological variations and their relationship with color quality is a fundamental aspect that requires further research.

According to [6,3], morphometric studies are one way to classify fish populations, in addition to being used for identification. Morphometric characteristics in population classification are more influenced by genetic factors. Morphometric characteristics have a genetic basis, but environmental components can modify the expression of these characteristics during larval development, which can also affect their offspring. Contemporary morphometric analyses in aquaculture have evolved beyond traditional measurements to incorporate advanced statistical techniques such as geometric morphometrics and multivariate analysis, providing more comprehensive characterization of phenotypic variation [7;8]. The benefit of this research is to produce a standard formula for determining the quality of superior Kohaku koi fish through the analysis of morphometric diversity, coverage area, and red color intensity. The integration of truss morphometric analysis with digital color quantification represents a paradigm shift from subjective to objective quality assessment in the ornamental fish industry. Recent studies have successfully applied similar integrated approaches to various fish species, demonstrating the effectiveness of combining morphological and color parameters for quality standardization [6].

Furthermore, the development of deep learning-based classification systems, such as KRS-Net, has shown promising results in automated koi variety recognition, achieving high accuracy even for varieties with high morphological similarity [2]. These technological advances support the urgent need for standardized, data-driven approaches to koi quality evaluation. The economic significance of establishing robust quality standards extends beyond individual breeding programs. The global ornamental fish market, valued at billions of dollars, increasingly demands transparent and objective quality metrics that can support fair trade practices and consumer confidence.

The purpose of this study was to determine the characterization of morphometric diversity, coverage area, and red color intensity, and their relationship with the color quality of Kohaku koi fish in order to determine the standards for high-quality Kohaku koi fish.

2 Materials and methods

2.1 Sample collection

Twenty-four Kohaku strain koi fish were obtained from the Omah Koi Farm Indonesia breeding facility in Banyuwangi, East Java. Fish were selected based on the clear phenotypic expression of the characteristics of the Kohaku strain, namely, a red color pattern with a white body color base. All test fish were juvenile fish with a total length of 15-20 cm to ensure a consistent comparison of developmental stages of the red color pattern and intensity. Fish were maintained under standardized conditions with water temperature of 25±28°C, pH of 7.0±0.5, and dissolved oxygen levels above 6 mg/L prior to analysis.

2.2 Truss morphometric analysis

Morphometric measurements of koi fish were analyzed using the truss morphometric method [9], which has been applied to koi fish. The purpose of truss morphometric measurement of koi fish is to quantitatively evaluate the body shape and morphological variation of fish. Morphometric analysis aims to control production quality and contest standards, broodstock selection, quality improvement, population classification, and species or strain identification. The Kohaku koi fish measured were 6 months old, with a total of 24 fish. Morphometric measurements were made by directly measuring the length of certain body parts using a ruler. Fish were positioned on a laminated millimeter block paper with the left side facing forward and measured by following the morphometric characteristics guide. The morphometric parameters measured can be seen in Fig 1.

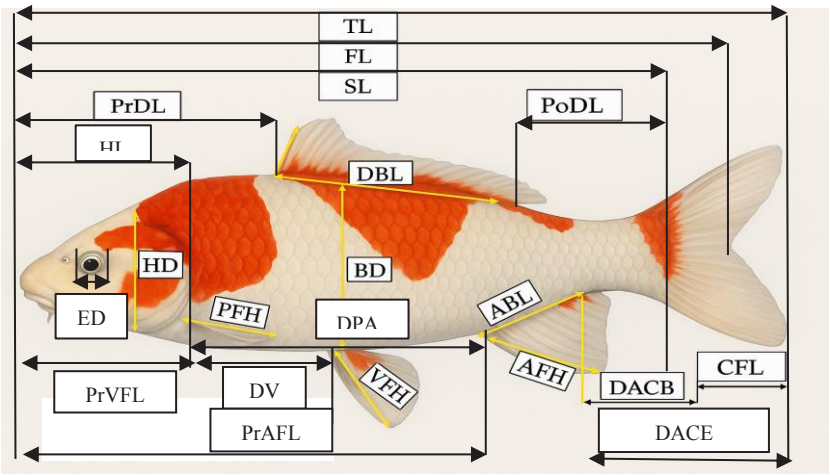

Fig. 1. Measurement of morphometric characteristics of Kohaku koi fish. TL (total length), FL (fork length, SL (standard length), HL (head length), PrDL (pre-dorsal length), PoDL (post-dorsal length), DBL (dorsal fin base length), CFL (caudal fin length), DACB (distance from anal fin to caudal base), AFH (anal fin height), ABL (anal fin base length), VFH (ventral fin height), BD (body depth), PFH (pectoral fin height), HD (head depth), PrVFL (pre-ventral fin length, PrAFL (pre-anal fin length), ED (eye diameter), DVP (distance between ventral and pectoral

fin, DPA (distance between pectoral and anal fin), DACE (distance from anal fin to caudal fin end).

2.3 Quantification of red color rea

Red color quantification was performed using digital image analysis techniques adapted from a recent study on koi color assessment [10,11]. High-resolution digital photographs were taken using standard photographic protocols under controlled lighting conditions to minimize color variations due to environmental factors.

Images were processed using ImageJ software equipped with a color analysis plugin. In ImageJ, an RGB (Red, Green, Blue) image is a type of color image that consists of three channels: red, green, and blue. Each channel has an 8-bit bit depth, which means each pixel has a color intensity value for each channel. In ImageJ, RGB images are used to represent true colors, as seen in actual specimens [11,12].

Two key color metrics were calculated: (1) red color coverage area expressed as a percentage of the total body area, and (2) average red color intensity measured on a standard scale. The calculation of red color coverage area excluded the head region in the anterior part of the operculum to focus on the body pattern, which is the main evaluation criterion in Kohaku assessment.

2.4 Pigmentation intensity scoring

The intensity of the red color is evaluated using a quantitative scoring system based on the intensity values of pixels extracted from digital images. The intensity scoring system uses a 0-255 scale corresponding to the digital pixel values, with higher values indicating a more intense red color.

Regional analysis was performed to assess the variation of red color intensity in the red color pattern in the body area. The standard body region divisions are: anterior dorsal, middle dorsal, posterior dorsal, and lateral regions. This regional approach allows the identification of pigmentation distribution patterns that may correlate with quality assessment.

2.5 Data analysis

All statistical analyses were performed using SPSS version 22.0. Data on red color coverage area, pigmentation intensity, truss morphometrics, and phenotypic characteristics were analyzed using Analysis of Variance (ANOVA) to ensure comprehensive evaluation.

3 Result and discussion

3.1 Morphometric characteristics of Kohaku Koi Fish

This study focuses on quantitative research related to the standardization of Kohaku koi fish (*Cyprinus rubrofuscus*) quality through the integration of truss morphometric analysis, red color coverage area, and red color intensity. A total of 24 juvenile Kohaku koi fish from the selective breeding of Kohaku koi fish collected from Omah Koi Farm Indonesia were analyzed using digital measurements with ImageJ software to quantify the red color coverage area and intensity. Meanwhile, morphometric measurements were taken using a ruler with a millimeter grid paper base to obtain 21 morphometric truss characteristics of Kohaku koi fish.

The objective parameters established are supported by recent research, which confirms the importance of morphological, genetic, and environmental aspects, as well as the aesthetic superiority of Kohaku koi fish, which are the stars of every international contest and market [11,12]. The development of digital-based measurement methods and the integration of multivariate statistical analysis are the latest trends in the field of quantitative genetics [11,13,14]. The results of the morphometric truss measurements of Kohaku koi fish are presented in Table 1 as follows.

Table 1. Morphometric characteristics (cm) of Kohaku koi fish.

Parameter	Description	N	Mean ± SD	Min	Max	CV (%)
TL	Total Length	24	21.39 ± 3.37	15.50	28.00	15.73
FL	Fork Length	24	18.78 ± 3.46	11.50	25.00	18.40
SL	Standard Length	24	17.25 ± 2.81	12.00	22.20	16.31
PrDL	Pre-Dorsal Length	24	8.27 ± 1.33	6.00	11.00	16.10
HL	Head Length	24	5.03 ± 1.14	3.50	9.50	22.74
DBL	Dorsal Fin Base Length	24	6.35 ± 1.18	4.50	8.00	18.54
PoDL	Post-Dorsal Length	24	3.13 ± 0.75	2.00	5.00	23.95
ED	Eye Diameter	24	1.06 ± 0.11	0.80	1.20	10.76
HD	Head Depth	24	3.83 ± 0.64	2.60	5.00	16.74
PFH	Pectoral Fin Height	24	3.02 ± 0.65	2.00	4.50	21.70
BD	Body Depth	24	5.60 ± 0.83	4.00	7.00	14.74
DPA	Dorsal to Pelvic Distance	24	8.45 ± 1.11	6.00	10.00	13.12
ABL	Anal Fin Base Length	24	1.68 ± 0.47	1.00	2.50	27.91
DACB	Distance from Anal to Caudal Base	24	2.91 ± 0.68	1.50	4.50	23.41
CFL	Caudal Fin Length	24	4.70 ± 0.72	3.30	6.00	15.30
PrVFL	Pre-Ventral Fin Length	24	8.80 ± 1.33	6.20	11.00	15.13
DVP	Distance from Dorsal to Ventral Pelvic	24	4.07 ± 0.66	3.00	5.30	16.27
PFH	Pectoral Fin Height	24	2.93 ± 0.48	2.00	4.00	16.48
AFH	Anal Fin Height	24	2.84 ± 0.51	2.00	4.00	17.79
DACE	Distance from Anal to Caudal End	24	7.34 ± 1.45	3.50	9.70	19.76
PrAFL	Pre-Anal Fin Length	24	13.14 ± 2.06	8.50	16.60	15.65

Notes: N = number of samples; SD = standard deviation; CV = coefficient of variation; Min = minimum value; Max = maximum value. All measurements are in cm unless otherwise stated.

Table 1 presents comprehensive morphometric data from 24 Kohaku koi test fish, measuring 21 different morphometric body characteristics that are important for phenotypic characterization. Morphometric parameters exhibit significant phenotypic variation within the studied population, with coefficients of variation ranging from 10.76% (eye diameter) to 27.91% (anal fin base length). The average total length measurement of 21.39 ± 3.37 cm indicates a uniform size class suitable for comparative analysis. The relatively high coefficient of variation for certain parameters, particularly anal fin base length (27.91%) and post-dorsal length (23.95%), indicates significant morphological diversity within the Kohaku strain. The standard length measurement (17.25 ± 2.81 cm) showed a strong correlation with total length, confirming the reliability of morphometric assessment. The body depth measurement (5.60 ± 0.83 cm) indicated a robust body conformation, characteristic of high-quality koi specimens. Morphometric data indicate that head length accounts for approximately 23.5% of standard length, consistent with the proportional relationships established in cyprinid fish.

Based on the research data, several key parameters were identified that can be used to characterize high-quality Kohaku koi fish, namely truss morphometry, red color coverage area, red color intensity, and the correlation between morphometry and pigmentation.

Morphometric measurements are obtained by measuring 21 standard truss distances used as geometric projections of the body. Statistical analysis shows that the average total length is 21.39±3.37 cm, body height, tail base width, and other parameters are closely related to the evaluation criteria used in koi contests. Measurement of red color coverage on the body using ImageJ resulted in an average coverage area of 141,426±112,847 pixels, with a high range of coverage between individuals. The extensive red color coverage on the back and sides is highly valued in determining the quality of superior Kohaku koi. The average red color intensity in ImageJ ranges from 113.8±19.2 (scale 0–255), with a minimum value of 85.2 and a maximum value of 160.0. The combination of high red intensity with a large red area is a characteristic of superior Kohaku. High-quality Kohaku koi have a thicker body (high body depth), wider tail fins, and a large red coverage area and intensity, which aligns with conventional evaluation criteria in international koi contests.

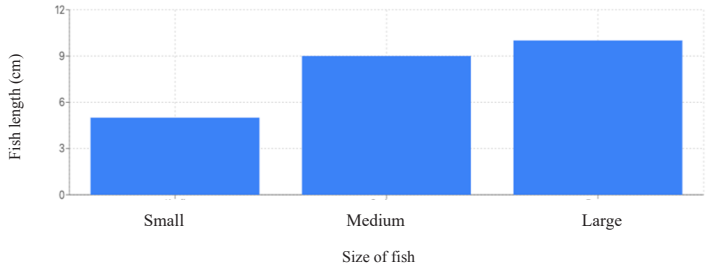

Fig. 2. Size distribution of Kohaku Koi Fish based on Total Length (TL).

Fig 2**.** shows the size distribution of Kohaku koi fish based on total length (TL) classification in the form of a bar chart. The size classification is divided into three categories: small (<18 cm), medium (18-23 cm), and large (>23 cm). Based on measurements from the 24 fish analyzed, the size distribution is uneven, with medium and large sizes dominating. There are 5 small fish (20.8% of the total sample), 9 medium fish (37.5% of the total sample), and 10 large fish (41.7% of the total sample). This distribution indicates a balanced population with a tendency toward medium to large sizes, suggesting the success of the selection program.

The dominance of medium (37.5%) and large (41.7%) size categories indicates that the F1 population resulting from the selective breeding of Kohaku koi carp has achieved consistent and uniform growth rates. Recent research by [10] shows that F1 Koi carp offspring from controlled breeding exhibit significant and consistent growth during the rearing period, both in terms of length and body weight. The average length of F1 after 60 days of rearing increases rapidly, and the distribution of individual sizes becomes relatively uniform, as a result of selecting superior parents and a controlled breeding environment. [6] Confirm that selection techniques and intensive breeding programs successfully produce Kohaku Koi populations with uniform growth performance and body quality, as reflected in proportional size distribution. Other studies confirm that under optimal nutritional management and density conditions, the F1 population resulting from crossbreeding will dominate in the medium-to-large size class [10].

Fig. 3. Relationship of main morphometric parameters.

Fig 3. shows the relationship between the main morphometric parameters. Panel A shows the relationship between total length (TL) and standard length (SL). The analysis results indicate a positive correlation between TL and SL with a strong linear correlation coefficient ($r > 0.90$) and proportional consistency. A proportional relationship is observed between total length (TL) and body depth (BD), with an optimal TL:BD ratio of (0.26 ± 0.03). The presence of morphometric variation indicates genetic diversity within the Kohaku F1 koi fish population. This data supports a selection program for Kohaku koi fish based on morphometric characteristics.

Research related to the growth performance of juvenile koi fish by [11] shows that the parameters of total length and standard length increase over time with a highly significant statistical correlation, supporting that both are closely related as measures of growth. [6] In their evaluation of Kohaku koi fish quality using image analysis and truss morphometry noted that total length and standard length have a very high correlation ($r > 0.90$), forming the basis for morphometric classification of koi fish for breeding selection and quality.

Analysis of size distribution and morphometric correlations indicates that the Kohaku F1 fish population possesses advantageous characteristics for advanced breeding programs. The size distribution, dominated by medium-to-large categories, combined with strong and proportional morphometric correlations, suggests genetic and phenotypic stability that supports consistent production quality. The strong linear relationship between key morphometric parameters provides a scientific basis for standardizing evaluations and enables the development of more efficient selection protocols. These findings contribute to the development of a data-driven breeding program that can enhance the quality and economic value of Indonesian Kohaku koi fish in the international market.

3.2 Red Coloration Parameters

The results of measuring the coverage area and red color intensity of Kohaku koi fish are presented in Table 2 as follows:

Table 2. Red coloration coverage area and intensity measurements in fish specimens (n=24).

Specimen ID	Coverage Area (pixels)	Red Channel Intensity Values				CV (%)
		Mean ± SD	Min	Max	Range	
Fish_01	147.873	122.4 ± 27.3	0	205	205	22.3
Fish_02	442.851	137.0 ± 51.2	14	255	241	37.4
Fish_03	103.776	91.8 ± 20.5	0	194	194	22.3
Fish_04	177.150	123.4 ± 32.4	0	221	221	26.3
Fish_05	147.582	109.5 ± 27.3	3	199	196	24.9
Fish_06	141.933	118.9 ± 31.5	13	205	192	26.5
Fish_07	119.508	117.6 ± 36.2	31	216	185	30.8
Fish_08	218.625	133.7 ± 46.0	0	243	243	34.4
Fish_09	193.874	128.5 ± 45.8	15	251	236	35.6
Fish_10	12.618	94.6 ± 27.5	0	158	158	29.1
Fish_11	100.107	121.8 ± 27.6	39	221	182	22.7
Fish_12	385.209	153.2 ± 44.2	4	251	247	28.8
Fish_13	223.454	114.6 ± 35.9	22	212	190	31.3
Fish_14	61.842	109.6 ± 23.1	49	208	159	21.1
Fish_15	144.207	99.7 ± 27.2	0	204	204	27.3
Fish_16	25.299	85.2 ± 12.5	56	140	84	14.7
Fish_17	77.182	125.5 ± 35.6	15	213	198	28.4
Fish_18	179.539	101.1 ± 31.6	13	192	179	31.3
Fish_19	89.599	93.8 ± 25.3	14	186	172	27.0
Fish_20	4.200	104.4 ± 20.6	49	159	110	19.7
Fish_21	66.006	114.9 ± 20.4	50	200	150	17.8
Fish_22	100.028	110.0 ± 26.3	23	188	165	23.9
Fish_23	150.279	92.2 ± 22.5	26	146	120	24.4
Fish_24	128.877	160.0 ± 41.2	37	255	218	25.8

CV = Coefficient of Variation. Values represent mean ± standard deviation. Specimen Fish_24 showed the highest red intensity value (highlighted). Coverage area measured in pixels; intensity values range from 0-255 (8-bit scale).

Table 2 presents a quantitative analysis of the characteristics of red pigments in all test fish. Measurements of red color coverage area showed remarkable variation, ranging from 4,200 pixels (Fish_20) to 442,851 pixels (Fish_02), indicating a 105-fold difference in red pigment area. The average red intensity values showed moderate variation (85.2 to 160.0),

with Fish_24 exhibiting the highest intensity (160.0 ± 41.2). The coefficient of variation for the average red intensity was 26.2%, indicating a consistent measurement protocol that captures natural biological variation. Fish_12 exhibited exceptional characteristics with a large coverage area (385,209 pixels) and high intensity (153.2 ± 44.2), representing optimal phenotypic expression. The statistical distribution shows a positive skew for coverage area (1.42), indicating that most test fish have relatively small red color coverage areas, with a few individuals exhibiting extensive red color coverage areas.

Table 3. Descriptive statistics for red coloration parameters.

Parameter	Mean ± SD	Median	Min - Max	95% CI	Skewness	Kurtosis
Coverage Area (pixels)	141.426 ± 112.847	133.405	4.200 – 442.851	93.822 – 189.030	1.42	1.89
Red Intensity	113.8 ± 19.2	114.8	85.2 - 160.0	105.6 - 122.0	0.89	0.76
Standard Deviation	30.7 ± 10.4	27.5	12.5 - 51.2	26.3 - 35.1	0.52	-0.31
Coefficient of Variation (%)	26.2 ± 5.8	26.0	14.7 - 37.4	23.7 - 28.7	0.11	-0.89

CI = Confidence Interval. Statistical analysis performed using standard descriptive methods. Normal distribution assessed using the Shapiro-Wilk test ($p > 0.05$ for all parameters except coverage area).

Table 3 summarizes the statistical properties of color parameters, providing important metrics for establishing quality standards. The 95% confidence interval for red intensity (105.6–122.0) sets the initial threshold for high-quality test fish. The distribution of red color coverage area shows a significant positive skewness (1.42) and high kurtosis (1.89), indicating a normal distribution. The median coverage area (133,405 pixels) differs significantly from the mean (141,426 pixels), confirming a skewed distribution. The standard deviation value indicates a normal distribution (skewness = 0.52), confirming the consistency of individual variation measurements. These statistical parameters form the basis for developing standard evaluation criteria in the commercial evaluation system for high-quality Kohaku koi.

4. Conclusion

The quantitative quality standards for Kohaku koi fish (*Cyprinus rubrofuscus*) through analysis of body morphometry, red color coverage area, and red color intensity in 24 juvenile Kohaku koi fish revealed phenotypic variation with coefficients of variation ranging from 10.76% to 27.91% in size class distribution of 41.7% large fish, 37.5% medium fish, and 20.8% small fish. A strong positive correlation was observed superior Kohaku fish, which exhibited a larger red color coverage area (up to 442,851 pixels) with higher red color intensity (maximum 160.0 on a 0-255 scale), wider tail fins, and greater body depth. The results of this study provide a measurable scientific basis as a selection criterion in koi breeding programs to replace subjective assessment methods and valid data-based determination of aesthetic value in the koi industry.

Acknowledgement

The authors gratefully acknowledge research funding through the 2024 Doctoral Dissertation Program scheme by the Directorate General of Higher Education, Research and Technology,

Ministry of Education, Culture, Research and Technology, Number: 027/E5/PG.02.00.PL/2024. We are very grateful to Omah Koi Farm Indonesia, Banyuwangi, East Java, Indonesia, for permitting us to use the place for this study.

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