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Research Article

Genetic diversity of the migratory Mekong endemic catfish species *Pangasius macronema* populating along the Hau and Tien Rivers

Mai Thy Le Nguyen, Yen Thuy Duong*

ABSTRACT

Pangasius macronema is an endemic and important species with high economic value for fisheries in the Mekong River basin. The rapid increase in fishing levels can have an impact on genetic diversity. This study evaluated the genetic diversity of three wild populations of *P. macronema* using intersimple sequence repeat (ISSR) markers. Six primers were used to analyze 72 samples from three locations (populations) along the Hau and Tien Rivers in An Giang (n=20), Soc Trang (n=29), and Dong Thap (n=23). The results showed that all populations of *P. macronema* had relatively high genetic diversity (effective number of alleles, Ne, from 1.381 to 1.454 and unbiased heterozygosity, uHe, from 0.243 to 0.286), with the Soc Trang population having the highest level. As a result, Nei's unbiased genetic distances among the three populations were low, ranging from 0.015 to 0.035, with the smallest genetic distance between the An Giang and Dong Thap populations. The genetic differences among the three populations may be influenced by the species' migration patterns and hydrological connectivity.

Key words: fish population, genetic diversity, ISSR markers, Pangasius macronema

Can Tho Unversity, Viet Nam

Correspondence

Yen Thuy Duong, Can Tho Unversity, Viet Nam

Email: thuyyen@ctu.edu.vn

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INTRODUCTION

Pangasius macronema (Bleeker 1851) is a small migratory catfish belonging to the Pangasiidae family. This species is endemic to the Mekong River basin and can be found across the region, including Thailand, Cambodia, Laos, and Vietnam [1]. The distribution of this fish generally occurs throughout the year, but it is more abundant during the migration period [2]. Poulsen et al. (2004) reported that *P. macronema* migrates from the mainstream of the Mekong River into smaller tributaries and rivers from April to June [3]. Migration can generate gene flow, inevitably affecting the genetic diversity of fish species [4]. On the other hand, seasonal migration provides resources for fisheries in the area.

P. macronema also plays an important role in local fisheries with high yield and economic value, although it is a small fish [2,5]. In the Mekong Delta, the main catch season for this species is from March to May [5]. The catch production in Tien Giang Province – one branch of the Mekong River – is approximately 78.86 tons/year [6]. Nevertheless, the diversity of wild populations in the Mekong Delta can currently be threatened by serious effects such as overexploitation, water pollution, and food shortages [7]. Therefore, the size of wild fish populations tends to decrease rapidly, which can be associated with the decline in genetic diversity [8].

In recent years, *P. macronema* has been cultured in the Mekong Delta using wild-caught fingerlings. They can be raised in earthen ponds and cages along the Tien and Hau Rivers [9]. The domestication process began because of an increase in seed demand for farming of this species. A good base broodstock population for domestication should be chosen based on genetic information.

Based on the aforementioned factors, it is necessary to investigate the genetic diversity of *P. macronema*. Previous genetic studies of this species, however, have been limited. Among various molecular markers used to determine the genetic diversity of fish species, ISSR (intersimple sequence repeat) is a simple but high-efficiency marker [10]. This study aimed to evaluate the genetic diversity of wild *P. macronema* populations along the Hau and Tien Rivers using ISSR markers. The results of this study provide preliminary genetic information for the management of wild populations and domestication program of this species.

MATERIALS AND METHODS

Fish sampling

P. macronema samples were collected from fishermen at three sites herein called three populations, including Thanh Binh district, Dong Thap province (DT), Chau Thanh district, An Giang province (AG), and

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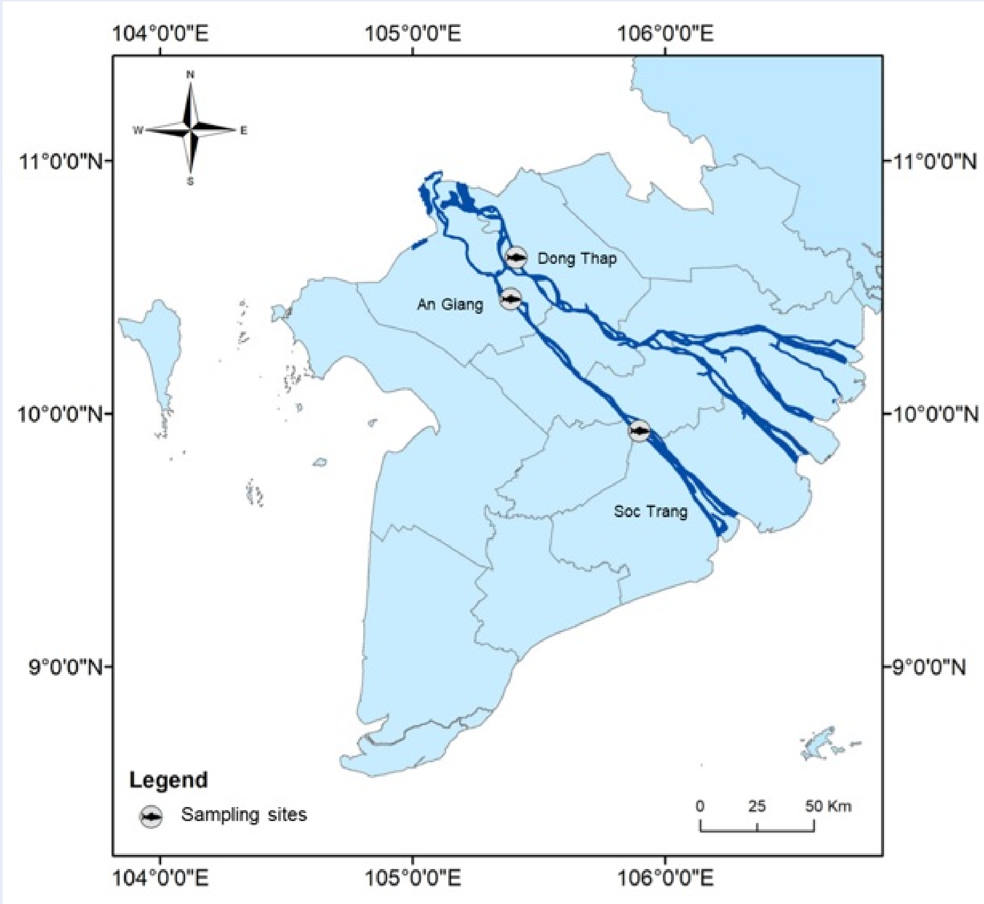

Figure 1: Sampling sites of three populations

Ke Sach district, Soc Trang province (ST). These sampling sites (Figure 1) are located at the two main river branches, Tien (DT) and Hau branch (AG and ST), of the Mekong River. The fish were identified based on morphological keys [11,12]. This pangasiid species is characterized by a small body size (18-20 cm), large eyes, and long barbels reaching the pectoral-fin base. Samples from the three populations (n=23 for DT, n=20 for AG, and n=29 for ST) were chosen for genetic analysis. Fin clips of these samples were preserved in 95% ethanol until DNA analysis.

Genetic diversity analysis methods

DNA extraction methods

DNA was extracted from fin clips by using the ammonium acetate protocol [13]. Approximately 25 mg of fin clip from each fish was first cut and then placed in an Eppendorf tube containing 650 μl of lysis buffer solution and 5 μl of Proteinase-K. After being incubated overnight at 55°C, all samples were added to 240 μl ammonium acetate and then incubated at 4°C for 30 minutes to precipitate the protein. Next, the tube was centrifuged at 10,000 rpm for 15 minutes two times, and 650 μl of the above solution was pipetted into a new tube. Then, 100% ethanol was added to the tube, incubated, and centrifuged to obtain DNA, which remained at the bottom of the tubes. Finally, the tube was air-dried, and 100 μl TE was added to obtain DNA.

Screening primers and PCR

Two samples from each population were selected randomly for primer screening. A total of 15 primers from ISSR libraries were screened. Six primers that had high polymorphisms and were visible on gels were chosen (Table 1). PCR amplifications were conducted in a 10 μL mixture containing 5 μL Promega PCR Master Mix (including Taq DNA polymerase supplied in a proprietary reaction buffer (pH 8.5), 400 μM dNTPs, and 3 mM $MgCl_2$), 0.4 μL primer (10 μM),

Table 1: Sequences and annealing temperatures of the six primers used in the study

Primers	Sequence (5' – 3')	Ta	References
Micro11	[GGAC]4	44^{o}C	Fernandes Matioli et al. (2000)[14]
Chiu-SSR1	[GGAC]3A	46^{o}C	Pazza et al. (2007)[15]
Chiu-SSR2	[GGAC]3C	46^{o}C	Pazza et al. (2007)[15]
HB10	[GTG]5GC	46^{o}C	Paterson et al. (2009)[16]
ISSR15	[TCC]5	46^{o}C	Tiwari et al., (2009)[17]
17898A	[CA]6AC	50^{o}C	Paterson et al. (2009)[16]

Ta: Annealing temperature

1.0 μL DNA, and 3.6 μL nuclease-free water. Thermal conditions for PCRs included one denaturing cycle at 95°C for 5 minutes, exceeded by 38 repeated cycles of 95°C for 30 seconds, annealing temperature (Ta) from 44°C to 50°C (Table 1) for 40 seconds, extension at 72°C for 1 minute; and one final extension has one cycle at 72°C for 5 minutes.

Electrophoresis and band scoring

PCR results after amplification were checked with 1.2% agarose electrophoresis. Each gel contained a 1 kb DNA ladder (ABM, Canada) and individuals representative of the three populations. The electrophoresis process was carried out at 50 V for 80 minutes. Then, the gels were dipped in ethidium bromide (5 μg/mL) solution for staining for 15 minutes. The bands were visualized by scanning in a UV transilluminator, and band sizes were estimated based on the DNA ladder.

ISSR bands of each gel were scored by using a binary data matrix, '1' for the presence of the band and '0' for the absence of the band. To minimize errors in band scoring, only clear and intensified bands were scored (low-intensity bands were not scored[18], and scored bands were compared between gels of the same primers). Furthermore, scoring was performed independently by two lab technicians, and the results were then discussed to finalize the data.

Data analysis

Genetic diversity parameters, including the effective number of alleles (Ne), percentage of polymorphism, private alleles, unbiased expected heterozygosity (uHe), and Shannon index (I), were estimated for each population by using GenAlEx 6.5 software[19]. Genetic differences among the three populations were evaluated based on the overall genetic difference (G_{ST}), Nei's genetic distance, and principal coordinates analysis (PCoA). In addition, a dendrogram was constructed based on the unweighted pair-group method with arithmetic average (UPGMA) method using Popgene version 1.3.1[20] and MEGA 6.0[21].

RESULTS

ISSR variability

There were 72 samples from three populations using six primers (Table 1) for ISSR analysis. A total of 67 bands were generated ranging from 390 bp (17898A) to 3000 bp (Chiu-SSR1, Chiu-SSR2, and Micro11) (Figure 2). The number of bands of each primer fluctuated from 7 (ISSR15) to 15 (Micro11). Band numbers from each population ranged from 65 bands (AG) to 66 bands (DT and ST), in which one private band was found in the ST population.

Genetic diversity parameters of the three *P. macronema* populations

The genetic diversity parameters of *P. macronema* are presented in Table 2. The percentage of polymorphic loci varied from 80.6% to 95.03%. The number of effective alleles (Ne) fluctuated from 1.381 to 1.454; the Shannon index (I) ranged from 0.369 to 0.435, and the unbiased expected heterozygosity (uHe) ranged from 0.243 to 0.286. The ST population had the highest genetic diversity values (Ne=1.454±0.038, I=0.435±0.024, and uHe=0.286±0.019), and the lowest was the AG population (Ne=1.381±0.040, I= 0.369±0.029, and uHe=0.243±0.021).

Genetic differences among the three *Pangasius macronema* populations

The overall genetic difference among populations (G_{ST}) was 0.053, and the number of migrants per generation (Nm) was 8.90. The values of Nei's unbiased genetic distance and genetic identity among the three *P. macronema* populations ranged from 0.015 to 0.035 and from 0.966 to 0.985, respectively (Table 3). The genetic distance between the ST and AG populations

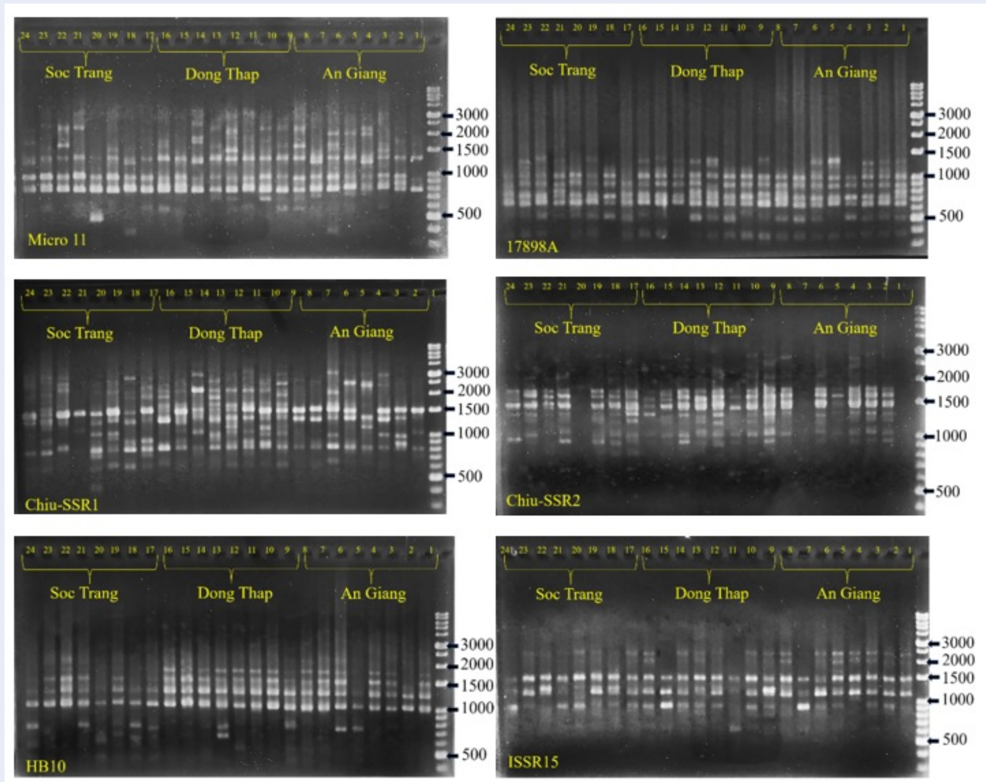

Figure 2: ISSSR bands of *P. macronema* samples from three populations (Dong Thap, An Giang, and Soc Trang) using six primers (Micro11, 17898A, Chiu-SSR1, Chiu-SSR2, HB10, and ISSR15)

Table 2: Genetic diversity parameters of three *P. macronema* populations from Dong Thap, An Giang, and Soc Trang

Population	Number of samples	P (%)	Ne	I	uHe
Dong Thap	23	86.57	1.423 ± 0.039	0.404 ± 0.028	0.268 ± 0.021
An Giang	20	80.60	1.381 ± 0.040	0.369 ± 0.029	0.243 ± 0.021
Soc Trang	29	94.03	1.454 ± 0.038	0.435 ± 0.024	0.286 ± 0.019

Note: P: polymorphism, Ne: number of effective alleles, I: Shannon's information index, uHe: unbiased expected heterozygosity

was the largest, and the AG - DT pair was the lowest. In addition, the genetic relationship among populations of this fish was shown by the UPGMA dendrogram using Nei's unbiased genetic distance (Figure 3). The AG and DT populations are more genetically similar than the ST population.

The molecular analysis of variance (AMOVA) showed that the genetic variation within populations (97%) was larger than that among populations (3%). Principal coordinates analysis (PCoA) indicated the genetic difference among the three populations in a two-axis (coordinate) plot, with 8.54% and 6.51% of the total genetic variation explained by coordinates 1 and 2,

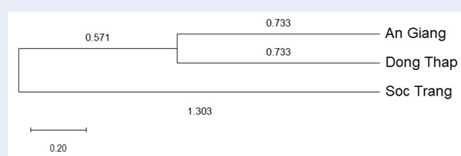

Figure 3: UPGMA dendrogram constructed using Nei's unbiased genetic distances among three populations of *P. macronema* from Dong Thap, An Giang, and Soc Trang

Table 3: Nei's unbiased genetic distance (below diagonal) and identity (above diagonal) among *P. macronema* populations

Populations	Dong Thap	An Giang	Soc Trang
Dong Thap	*	0.985	0.983
An Giang	0.015	*	0.966
Soc Trang	0.017	0.035	*

respectively (Figure 4). The AG and the majority of individuals from DT populations were clustered together in the same region, whereas most individuals from the ST population were distributed further apart from the plot. The ST population, followed by the DT population, was more widely scattered on the coordinate plot than the AG population, indicating that these two (ST and DT) populations had larger levels of genetic diversity.

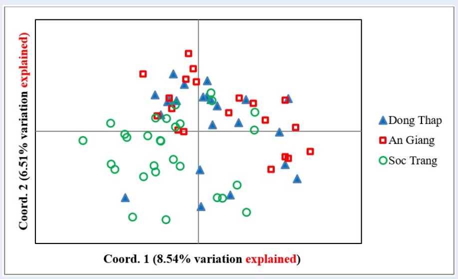

Figure 4: Principal coordinates analysis (PCoA) of *P. macronema* from Dong Thap, An Giang, and Soc Trang populations in the Mekong Delta

DISCUSSION

Levels of genetic diversity of *P. macronema* populations

The study found that all populations of *P. macronema* had high levels of genetic diversity (with Ne from 1.381 to 1.454, I from 0.369 to 0.435, and uHe from 0.243 to 0.286) in comparison with other fish species based on ISSR markers. A study on *Pangasius krempfi* showed lower values of genetic diversity parameters, with Ne from 1.352 to 1.376, I from 0.299 to 0.320, and uHe from 0.208 to 0.222 [22]. In yellow catfish (*Mystus nemurus*) [23], the results of Ne ranged from 1.225 to 1.284, lower compared to the present study. On the other hand, *P. macronema* has a higher and equivalent level of genetic diversity than some scale fish species. Kissing gourami (*Helostoma temminckii*) had less genetic diversity than *P. macronema* (Ne from 1.295 to 1.387, I from 0.269 to 0.386) [24]. In addition, genetic parameters based on eight ISSR primers of three *Channa lucius* populations in the Mekong Delta were comparable to *P. macronema,* with Ne ranging from 1.422 to 1.458, I from 0.364 to 0.395, and uHe from 0.250 to 0.271 [25]. Although the AG population of *P. macronema* had lower genetic parameters than the other two populations, the magnitudes of the differences were small, ranging from 3% to 5% for Ne and from 9% to 15% for I or uHe.

The genetic diversity of a population depends on internal factors of the population, such as population size and random genetic drift, and external factors of the gene exchange process due to the migration of individuals among populations [4]. The level of genetic diversity has a positive correlation with population size (or the number of individuals) [26]. Compared to other *Pangasius* species, *P. macronema* can be found in abundance, especially during the flooding season (from July to November) in tributaries of the Tien and Hau Rivers [6,27]. Based on the catch data, 78.86 tons/year in Tien Giang Province, it can be predicted that *P. macronema* has a large population size in the Mekong Delta, resulting in the high level of genetic diversity of this fish. In small populations, random genetic variation is powerful, causing some genes to be completely lost [28]. In kissing gourami (*Helostoma temminckii*), a low level of genetic diversity was observed in a closed population resulting from a small population size [24]. Moreover, the ability to exchange genes during migration is also a factor that can increase the genetic diversity of the population [4]. *Hypostomus ancistroides* in Brazil was detected to have low genetic diversity, which might be related to the sedentary habits of the species, reducing gene flow between populations of different locations [29].

Genetic differentiation among *P. macronema* populations

The genetic differentiation of the *P. macronema* populations was low, as indicated by the low values of G_{ST} (0.053) and Nei's unbiased genetic distances and the high value of the number of migrants per generation (Nm=8.9). These parameters are indicative of genetic exchange among populations[4]. Nei's unbiased genetic distances among *P. macronema* populations (from 0.015 to 0.035) were lower than those of other freshwater species in the Mekong Delta. The Nei's values ranged from 0.023 to 0.102 for kissing gourami *Helostoma temminckii*[24] and from 0.022 to 0.057 for *Channa lucius* (with G_{ST} = 0.09)[25]. Baird et al. (2001) reviewed different hypotheses about the migration patterns of *P. macronema*[1]. The fish can migrate upstream from the Mekong River floodplain for spawning and from the main river to smaller streams and tributaries. Because of this migratory behavior, genetic flow occurs strongly. Consequently, gene flow reduces genetic differences between populations and increases the variation within populations[4]. An example is Malayan leaf fish (*Pristolepis fasciata*), which migrate during the flooding season from the Mekong River into wetland areas and then migrate back to the river in the dry season and have low genetic differences among populations[30]. In addition, another study found that the low genetic distance and weak genetic structure of *P. krempfi* were affected by their migration behavior[22].

The genetic differences among the three populations of *P. macronema* may be affected not only by their migratory behavior but also by the geographical distance and the ecological conditions of the rivers. The geographical distance of the ST population and the other two populations was furthest, corresponding to larger genetic differences of ST-AG and ST-DT. Meanwhile, sampling sites in AG and DT are located in the Hau and Tien Rivers, but they are connected by the Vam Nao River. This hydrological connectivity allows gene flow between the AG and DT populations, resulting in their low genetic difference. Similarly, in wild *Channa lucius* populations in the Mekong Delta, a population with the greatest hydrological distance had the largest genetic distance from the other populations[25]. On a geographically regional scale, Duong et al. (2019) reported that genetic differences among wild striped snakehead (*Channa striata*) populations in Vietnam and Cambodia were positively correlated with hydrological distances[31]. Moreover, the distribution of river systems and ecological conditions can affect the migration of fish and ultimately the population structure[32]. The differences in river systems and associated ecological factors were determinants of the genetic differentiation of striped snakehead populations[33] and climbing perch strains in Thailand[34].

The findings of the present study have implications for the domestication and wild population management of *P. macronema*. In the Mekong Delta, domestication of *P. macronema* has been in progress with preliminary results of artificial reproduction (unpublished information). Because of their high genetic diversity, the three *P. macronema* populations, of which the ST population is relatively better, could be good resources for domestication. Furthermore, combining these groups would boost genetic variety in a base population, allowing for more effective domestication programs[35]. In terms of wild fish management, low genetic differentiation among the three populations (G_{ST}=0.053) or large gene flow (Nm=8.9) implies that they can be considered a "panmictic population", in which free genetic exchange occurs among individuals from different locations[36]. In this case, it is suggested that they can be managed as one population[37].

CONCLUSIONS & RECOMMENDATIONS

The results from the ISSR markers showed that the three wild *P. macronema* populations had high levels of genetic diversity, of which the ST population had the highest level. The genetic differentiation among populations was low, as indicated by the low values of G_{ST} and Nei's unbiased genetic distances and the high gene flow value.

It is recommended that the three populations be managed as the entire population of the Lower Mekong River system. These populations can be utilized to generate a base population for domestication efforts (either individually or better in combination).

LIST OF ABBREVIATIONS

AMOVA: analysis of molecular variance
G_{ST}: overall genetic difference among populations
I: Shannon index
ISSR: intersimple sequence repeat
Ne: number of effective alleles
Nm: number of migrants per generation
P: polymorphism
PCoA: principal coordinates analysis
PCR: polymerase chain reaction
uHe: unbiased expected heterozygosity
UPGMA: unweighted pair-group method with arithmetic average

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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AUTHORS' CONTRIBUTIONS

Le Nguyen Mai Thy contributed to the investigation, data analysis and writing.

Duong Thuy Yen contributed to conceptualization, funding, writing and editing.

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Tạp chí Phát triển Khoa học và Công nghệ
Đại học Quốc gia Tp. Hồ Chí Minh

Tạp chí Phát triển Khoa học và Công nghệ
ISSN: 1859-0128
Hình thức xuất bản: In và trực tuyến
Hình thức truy cập: Truy cập mở (Open Access)
Ngôn ngữ bài báo: Tiếng Anh
Tỉ lệ chấp nhận đăng 2021: 72%
Phí xuất bản: Miễn phí
Thời gian phản biện: 43 ngày
Lập chỉ mục (Indexed): Google Scholar, Scilit

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Tạp chí Phát triển Khoa học và Công nghệ - Khoa học Tự nhiên
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Hình thức truy cập: Truy cập mở
Ngôn ngữ bài báo: Tiếng Việt
Tỉ lệ chấp nhận đăng 2021: 75%
Phí xuất bản: Miễn phí
Thời gian phản biện: 30-45 ngày
Lập chỉ mục (Indexed): Google Scholar, Scilit

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Tạp chí Phát triển Khoa học và Công nghệ - Kĩ thuật và Công nghệ
ISSN: 2615-9872
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Hình thức truy cập: Truy cập mở
Ngôn ngữ bài báo: Tiếng Việt
Tỉ lệ chấp nhận đăng 2021: 61%
Phí xuất bản: Miễn phí
Thời gian phản biện: 50 ngày
Lập chỉ mục (Indexed): Google Scholar, Scilit

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Tạp chí Phát triển Khoa học và Công nghệ - Kinh tế-Luật và Quản lý
ISSN: 2588-1051
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Hình thức truy cập: Truy cập mở
Ngôn ngữ bài báo: Tiếng Việt
Tỉ lệ chấp nhận đăng 2021: 65%
Phí xuất bản: Miễn phí
Thời gian phản biện: 45 ngày
Lập chỉ mục (Indexed): Google Scholar, Scilit

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Tạp chí Phát triển Khoa học và Công nghệ - Khoa học Xã hội và Nhân văn
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Hình thức truy cập: Truy cập mở
Ngôn ngữ bài báo: Tiếng Việt
Tỉ lệ chấp nhận đăng 2021: 62%
Phí xuất bản: Miễn thu phí đối với tác giả là CBVC của ĐHKHXHNV, ĐHQG-HCM; Tác giả khác: 500.000 VNĐ/bài
Thời gian phản biện: 75 ngày
Lập chỉ mục (Indexed): Google Scholar, Scilit

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Tạp chí Phát triển Khoa học và Công nghệ - Khoa học Trái đất và Môi trường
ISSN: **2588-1078**
Hình thức xuất bản: In & trực tuyến
Hình thức truy cập: Truy cập mở
Ngôn ngữ bài báo: Tiếng Việt và tiếng Anh
Tỉ lệ chấp nhận đăng 2021: 87%
Phí xuất bản: liên hệ tòa soạn
Thời gian phản biện: 45 ngày
Lập chỉ mục (Indexed): Google Scholar, Scilit

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Tạp chí Phát triển Khoa học và Công nghệ - Khoa học Sức khỏe
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Hình thức truy cập: Truy cập mở
Ngôn ngữ bài báo: Tiếng Việt
Tỉ lệ chấp nhận đăng 2021: 70%
Phí xuất bản: Miễn phí
Thời gian phản biện: 30 ngày
Lập chỉ mục (Indexed): Google Scholar, Scilit

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