



# **Protein-Based Genetic Diversity Assessment of *Tilapia guineensis* and *Sarotherodon melanotheron* Populations from South-West Nigerian Coastal Waters**

**E. A. Ukenye<sup>1\*</sup>, I. Megbowon<sup>1</sup>, M. M. A. Akinwale<sup>1</sup>, M. A. Fowora<sup>2</sup>, I. Chidume<sup>1</sup>,  
N. C. Eze<sup>1</sup>, R. O. Adeleke<sup>1</sup> and C. C. Omatah<sup>1</sup>**

<sup>1</sup>*Department of Biotechnology, Nigerian Institute for Oceanography and Marine Research, Victoria Island, Lagos, Nigeria.*

<sup>2</sup>*Department of Molecular Biology and Biotechnology, Nigerian Institute of Medical Research, Yaba, Lagos, Nigeria.*

## **Authors' contributions**

*This work was carried out in collaboration among all authors. Author EAU designed the study, involved in sample collection, performed laboratory analysis and wrote the first draft of the manuscript.*

*Authors IM and MMAA carried out field survey and reviewed the first draft of the manuscript. Author MAF wrote the protocol and performed the data analysis. Authors IC and NCE managed the literature searches while authors ROA and CCO participated in laboratory analysis. All authors read and approved the final manuscript.*

## **Article Information**

DOI: 10.9734/AJFAR/2020/v8i230134

### Editor(s):

(1) Dr. Pinar Oguzhan Yildiz, Ataturk University, Turkey.

### Reviewers:

(1) Karan Veer Singh, ICAR-NBAGR, India.

(2) Mojgan Bandehpour, Shahid Beheshti University of Medical Sciences, Iran.

Complete Peer review History: <http://www.sdiarticle4.com/review-history/59568>

**Original Research Article**

**Received 20 May 2020**  
**Accepted 27 July 2020**  
**Published 06 August 2020**

## **ABSTRACT**

The present study was carried out to investigate the genetic differences in the Protein banding pattern of *Tilapia guineensis* and *Sarotherodon melanotheron* populations in Southwest Nigeria using Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis (SDS-PAGE). Four populations of *Tilapia guineensis* and three populations of *Sarotherodon melanotheron* from Ondo and Lagos states were considered for the study. The sarcoplasmic protein of the studied Cichlid species resolved on 12% SDS-PAGE revealed variations in their genetic diversity indices (number of

\*Corresponding author: Email: eekwelem@yahoo.com;

alleles, shanon information index, heterozygosity and percentage polymorphism). *T. guineensis* had more proteins and higher genetic diversity as was revealed by the genetic diversity parameters and was found to be more polymorphic with a percentage polymorphism of 78.57% than *S. melanotheron* (57.14%). The two species had similarity coefficient of 0.82 indicating high genetic similarity between them. UPGMA (Unweighted Pair Group Method with Arithmetic Mean) dendrogram also revealed some level of genetic similarity between the studied populations and among the two species. Analysis of molecular variance (AMOVA) confirmed the low genetic variation among the populations of the cichlid species and demonstrated that genetic variation was mostly within populations in both species. It is established from the study that *Tilapia guineensis* had higher genetic diversity than *Sarotherodon melanotheron* and the two species are closely related. Further study involving molecular markers is encouraged to complement this finding.

**Keywords:** Protein profile; genetic diversity; *Tilapia guineensis*; *Sarotherodon melanotheron*.

## 1. INTRODUCTION

*Tilapia guineensis* (Bleeker, 1862) and *Sarotherodon melanotheron* (Ruppell, 1852) are among the most abundant Cichlid species commonly found in creeks, lagoons, estuaries and other coastal waters of West Africa [1]. They are key fish species of lagoon fisheries with good aquaculture potentials and are continually contributing greatly to nutritional and economic development of many West African countries including Nigeria. Cichlids constitute a significant percentage of inland fish catch in reservoirs and lakes [2] and most coastal waters in Nigeria. *T. guineensis* and *S. melanotheron* are among the lagoon and estuarine Tilapias that represent important genetic resources in terms of genetic diversity which made them potential aquaculture species [3]. They share much the same salinity range and habitat and have been successfully raised in ponds, enclosures, cages, and tanks. However, *T. guineensis* and *S. melanotheron* like other Tilapias present some challenges to fish culturist due to their capacity to over breed leading to relatively slow growth rates as reported by many workers. A better understanding of the genetic diversity of these species is required for sustainable aquaculture practices.

According to [4], protein profile and molecular markers analysis reveal better genetic variations which are usually free from genotype and environment interactions. [5] stated that electrophoretic technique of SDS-PAGE can discriminate variants by their charge or mass and does not depend on prior information of fish genomes. Fish geneticists have been using protein electrophoresis as their primary tool to characterize population-level and species genetic variation [6]. This technique has been utilized severally and is still relevant.

Previously, [1] assessed the morphological variation of *T. guineensis* and *S. melanotheron* from South-west Nigeria. [7] also studied the morphological variation of Cichlids from Kainji Lake, Nigeria. However, despite the importance of this conventional method in species identification and characterization, it is still insufficient for genetic improvement and sustainable aquaculture production. Thus, protein electrophoresis can be effectively used to identify and discriminate fish species as to complement their morphological findings. Therefore, the present study was carried out to investigate the genetic diversity among *T. guineensis* and *S. melanotheron* from South-west Nigerian coastal waters using their protein profile for efficient breeding and conservation program.

## 2. MATERIALS AND METHODS

### 2.1 Source of Fish and Specimens Collection

Sample collection was carried out in four locations (Pepe, Ugbonla, Badagry and Epe) from two coastal states (Ondo and Lagos; two locations per state) in South-west Nigeria for *T. guineensis* and three (Pepe, Ugbonla and Badagry) out of the four locations for *S. melanotheron* species. Table 1 shows the geographical location (longitudes and latitudes) of the sampling stations. Identification of these cichlid species was done using [8]. After identification, the fish were procured from the fishermen at the landing site of every station and were immediately transported to the laboratory live and acclimatized. A total of seventy (70) samples; forty (40) for *T. guineensis* and thirty (30) for *S. melanotheron* were collected from both locations (Pepe, Ugbonla, Badagry and Epe). Ten (10) specimens from each species

**Table 1. Geographical location of the sampling stations**

| Location | Latitude                               | Longitude                               | State |
|----------|--|---|-------|
| Pepe     | N06° 10 <sup>1</sup> 01.3 <sup>1</sup> | E02° 52.988 <sup>1</sup>                | Ondo  |
| Ugbonla  | N06° 08 <sup>1</sup> 31.1 <sup>1</sup> | E004° 47 <sup>1</sup> 39.8 <sup>1</sup> | Ondo  |
| Badagry  | N04° 25.012 <sup>1</sup>               | E02° 52.988 <sup>1</sup>                | Lagos |
| Epe      | N04° 27.200 <sup>1</sup>               | E007° 19.618 <sup>1</sup>               | Lagos |

were randomly selected from each location for protein profiling. They were filleted and skinned with a stainless steel knife and then the muscle meat was used for protein extraction.

## 2.2 Protein Extraction

One gram of minced fish meat was homogenized by grinding it in a mortar with 1 ml of phosphate buffer saline (PBS) containing protease inhibitor cocktail. The homogenate was centrifuged at 10,000 rpm for 15 min at room temperature and the supernatant (sarcoplasmic protein) was used for electrophoresis.

## 2.3 Qualitative Analysis of Proteins by SDS-PAGE

SDS-PAGE (12%) was carried out as outlined by [9] using a vertical gel electrophoresis unit to resolve the protein (SCIE-PLAS Model #TV 50, SCIE-PLAS Ltd., UK). For determination of molecular mass of each protein, a molecular weight marker kit was purchased from Fermentas, Lahore, Novagen by Merck (10–250 kDa). The gels were stained with Coomassie blue R-250, destained with destaining solution (methanol, acetic acid and distilled water), and were photographed with a digital camera.

## 2.4 Data Analysis

The scoring of protein data across the studied species was done by the presence of protein bands as 1 or their absence as 0 for each category. The binary data so obtained were used

to determine number of polymorphic bands, level of polymorphisms, Nei's Pairwise similarity and dissimilarity matrices. A dendrogram was constructed by using the un-weighted pair group method with arithmetic average (UPGMA) with NTSYS software.

## 3. RESULTS

### 3.1 Species Dynamics

Sarcoplasmic proteins of *T. guineensis* and *S. melanotheron* populations both from the wild were characterized by 12% SDS-PAGE (Plate 1). The average number of protein bands detected among the populations of *T. guineensis* and *S. melanotheron* were 29 and 23 respectively. *T. guineensis* had higher number of alleles (1.57) and mean effective alleles (1.57) than *S. melanotheron* that recorded 1.43 numbers of alleles and 1.35 mean effective alleles respectively. Shannon information index was observed higher (0.47) in *T. guineensis* than in *S. melanotheron* (0.31). The value recorded for mean heterozygosity across the species ranged from 0.21 in *S. melanotheron* to 0.33 in *T. guineensis* as shown in Table 2. The result suggests that *T. guineensis* is the most variable species while *S. melanotheron* is the least. Percentage value of polymorphism across populations was 78.57% in *T. guineensis* and 57.14% in *S. melanotheron* (Table 2).

Table 3 shows the genetic similarity and distance between *T. guineensis* and *S. melanotheron* using sarcoplasmic protein profiling. The

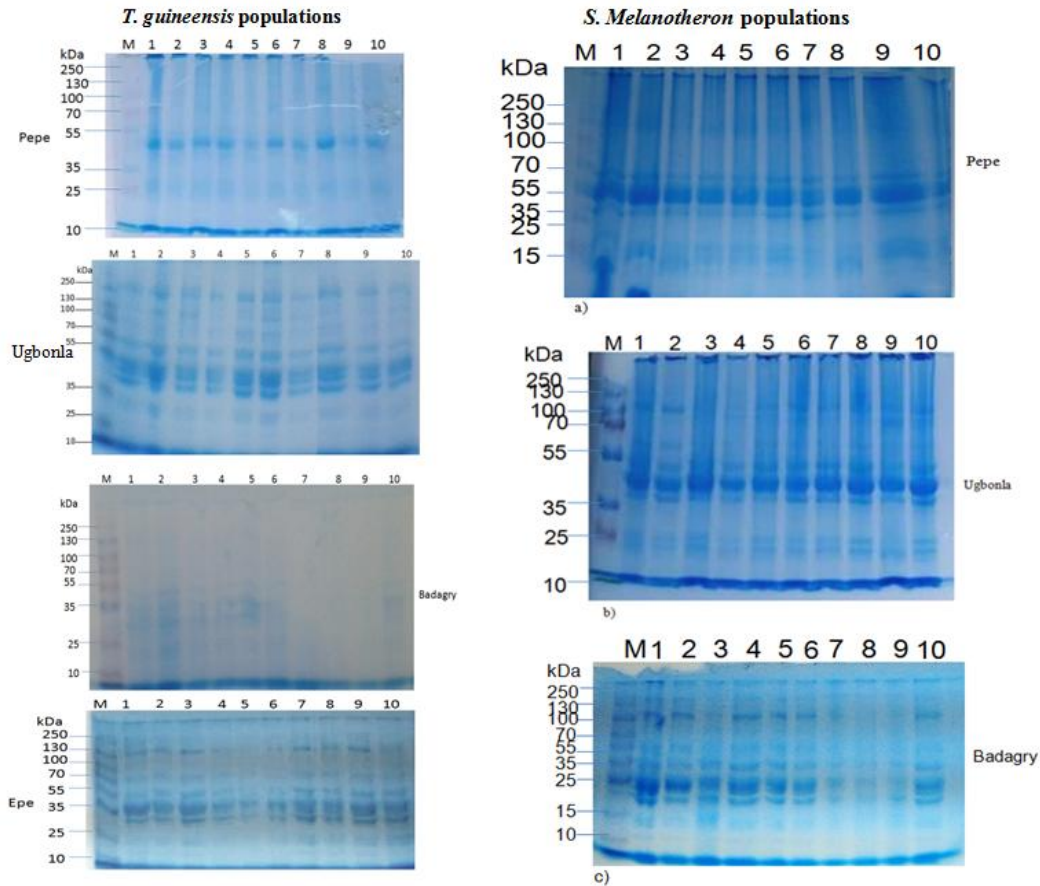
**Table 2. Summary of the species dynamics**

| Parameters                  | <i>Tilapia guineensis</i> | <i>Sarotherodon melanotheron</i> |
|-----------------------------|---------------------------|----------------------------------|
| nbp                         | 29                        | 23                               |
| Number of alleles           | 1.57±0.23                 | 1.43±0.20                        |
| Effective number of alleles | 1.57±0.10                 | 1.35±0.10                        |
| Shannon's information index | 0.47±0.07                 | 0.31±0.08                        |
| Heterozygosity              | 0.33±0.05                 | 0.21±0.05                        |
| Number of polymorphic loci  | 11                        | 12                               |
| Percentage polymorphism±    | 78.57%                    | 57.14%                           |

nbp- number of protein bands

similarity coefficient between them was 0.820 while their genetic distance is 0.198. The UPGMA dendrogram based on the genetic distances revealed three major clusters; the clusters obtained showed that the seven populations of the two species clustered into three groups (Fig. 1). Association among all populations of the two species was also

resolved by using principle coordinate analysis (PCA) (results not shown). The PCA showed a similar clustering pattern of the populations with that of the dendrogram. Analysis of molecular variance (AMOVA) revealed 30% genetic variation among populations of the species and 70% within populations of the species (Table 4).



**Plate 1. Protein profile of *Tilapia guineensis* and *Sarotherodon melanotheron* populations on 12% SDS-PAGE (Lane [1–10] fish sample and marker [M])**

**Table 3. Genetic similarity and distance between *T. guineensis* and *S. melanitheron* species**

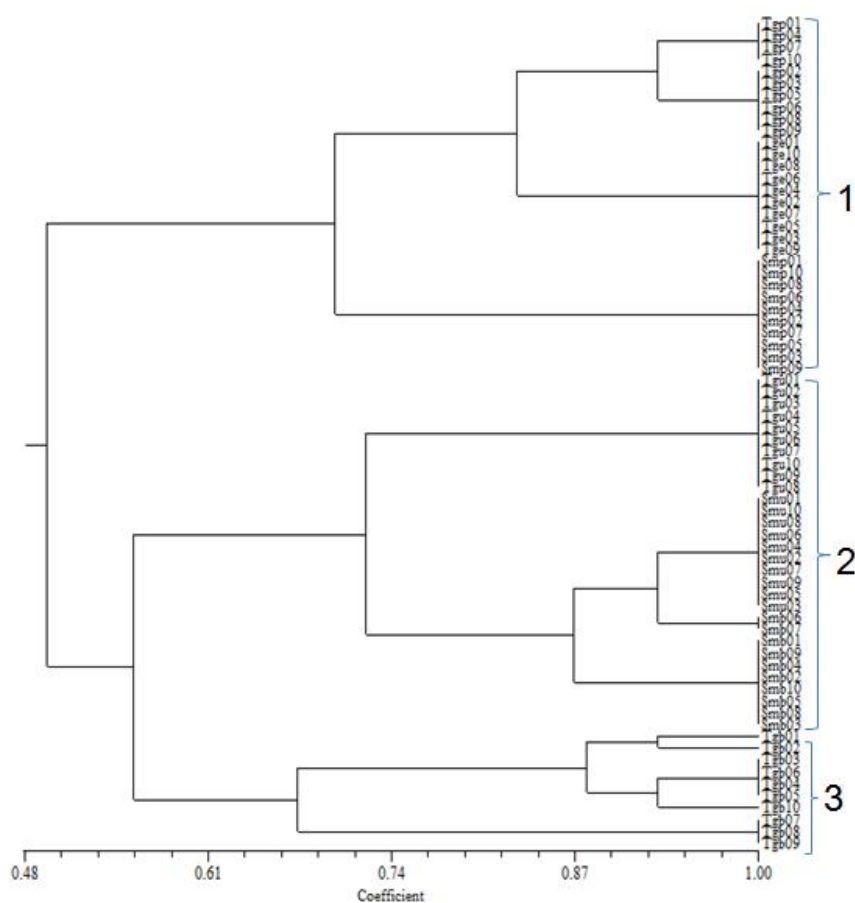
|                        | <i>T. guineensis</i> | <i>S. melanitheron</i> |
|------------------------|----------------------|------------------------|
| <i>T. guineensis</i>   | -                    | 0.820                  |
| <i>S. melanitheron</i> | 0.198                | -                      |

*Nei's genetic similarity index (above diagonal) and genetic distance index (below diagonal)*

**Table 4. Analysis of molecular variance (AMOVA)**

| Source      | df | MS     | Est. var. | %Var |
|-------------|----|--------|-----------|------|
| Among Pops  | 1  | 33.160 | 0.905     | 30%  |
| Within Pops | 68 | 2.144  | 2.144     | 70%  |
| Total       | 69 |        | 3.048     | 100% |

*Df*: degree of freedom; *MS*: mean square; *Est. Var.*: estimated variation; *%Var*: percentage variation



**Fig. 1. UPGMA Dendrogram showing the genetic relationships among species populations based on Nei's genetic distance**

#### 4. DISCUSSION

The current study was undertaken to get a better understanding of genetic diversity among *T. guineensis* and *S. melanotheron* in South-west Nigerian coastal waters from their protein profile. [10] stated that muscle protein is commonly used to assess the polymorphism among fish species. The sarcoplasmic protein profile resolved through 12% SDS-PAGE was able to reveal low interspecies variation, as the population number per species was not uniform. In the case of *T. guineensis*, data revealed overall of 29 proteins ranging from 10 to 250kDa from the protein profile while in the case of *S. melanotheron*, 23 proteins were revealed. This varied considerably from the number of protein bands in the plasma and muscle of Tilapia as observed by [11]. The higher number of protein bands and genetic diversity indices revealed in *T. guineensis* reflects high genetic diversity than in *S. melanotheron* in the present study. This could be

as a result of low sample size of *S. melanotheron* species since [12] stated that there are significant positive correlations between genetic diversity and population size both in different species and different populations of the same species. In their finding, they observed that *Oreochromis niloticus* exhibited higher levels of genetic diversity than *O. aureus*. High similarity coefficient that was recorded between the species is an indication of low genetic diversity that existed between *T. guineensis* and *S. melanotheron*. This could be attributed to the species belonging to the same family (Cichlidae). This close genetic relatedness between the species might lead to high chances of hybridization. This is in line with the finding of [13] in protein profile expression of two Clariids- *C. gariepinus* and *H. bidorsalis*. A similar result was also reported by [14] who obtained a similarity coefficient of 78% between *T. guineensis* and *S. melanotheron* from the wild. High percentage polymorphism observed in both

species indicates genetic polymorphism in all the studied populations though, *T. guineensis* species is more polymorphic than *S. melanotheron* species.

The high mean heterozygosity observed in *T. guineensis* confirmed high genetic variability in *T. guineensis* species. This agreed with the report of [1] who observed significant differences in the morphometrics of *Tilapia guineensis* and *Sarotherodon melanotheron* from Badagry and Lagos lagoon water bodies. Clustering based on the genetic distance gave three major clusters indicating some level of genetic similarity between the studied populations and among the two species. This is in line with the report of [15] who found a close phylogenetic relationship among snakehead species. This genetic similarity that exists between *T. guineensis* and *S. melanotheron* shows that the species have a common ancestor.

## 5. CONCLUSION

The current study revealed high similarity coefficient between the two Cichlid species reflecting low genetic diversity among them. *T. guineensis* exhibited higher genetic diversity than *S. melanotheron*. Nevertheless, more sample size and sensitive molecular markers should be considered in further studies to support the efficiency of this finding.

## ACKNOWLEDGEMENTS

We appreciate our Head of Department, Dr. Oresgun, A. and the entire management team of Nigerian Institute for Oceanography and Marine Research (NIOMR) Lagos, Nigeria in funding this project. The cooperation of other Biotechnology Department staff of NIOMR in conducting this research is well acknowledged.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

## REFERENCES

1. Kuton MP, Adeniyi BT. Morphological variations of *Tilapia guineensis* (Bleeker 1862) and *Sarotherodon melanotheron* (Ruppell 1852) (Pisces: Cichlidae) from Badagry and Lagos Lagoon, South-West, Nigeria. *Journal of Fisheries and Livestock Production*. 2014;2:112.
2. Mireku KK, Blay J, Yankson K. Reproductive biology of Blackchin tilapia, *Sarotherodon melanotheron* (Pisces: Cichlidae) from Brimsu Reservoir, Cape Coast, Ghana. *International Journal of Fisheries and Aquaculture*. 2016;8(4):42-54.
3. Falk TM, Teugels GG, Abban EK. Genetic diversity of West African Lagoon Tilapias and its implications for fisheries, aquaculture and biodiversity conservation: Case studies on *Sarotherodon melanotheron*, *Sarotherodon nigripinnis* and *Tilapia guineensis*. World Fish Center, Biodiversity, Management and Utilization of West African Fishes; 2004.
4. Torkpo EY, Danquah EY, Offei SK, Blay ET. Esterase, total protein and seed storage protein diversity in Okra (*Abelmoschus esculentus* L. Moend). *West African Journal of Applied Ecology*. 2006;9:177-183.
5. Verspoor E, Beardmore JA, Consuegra S, DeLeaniz CG, Hindar K, Jordan WC, Koljonen ML, Mahkrov AA, Paaver T, Sánchez JA, Skaala, Titov S, Cross TF. Population structure in the Atlantic salmon: Insights from 40 years of research into genetic protein variation. *Journal of Fish Biology*. 2005;67:3-54.
6. Tewari Grishma, Singh IJ, Mohd. Danish, Barat AK. Genetic diversity analysis of *Labeo gonius* (Hamilton, 1822) in three different reservoirs of Uttarakhand by using allozyme marker. *African Journal of Biotechnology*. 2013;12(19):2532-2539.
7. Olufeagba SO, Aladele SE, Okomoda VT, Sifau MO, Ajayi DA, Oduoye OT, Bolatito OA, Nden DS, Fabunmi-Tolase AS, Hassan T. Morphological variation of cichlids from Kainji Lake, Nigeria. *Journal of Fisheries Sciences*. 2015;9(3):070-080.
8. Daget J. Chromidotilapia. In: Check-list of the freshwater fishes of Africa (CLOFFA) (Eds. J. Daget, J., Gosse, J. P., Teugels, G. G. and Thysvan Audenaerde, D. F. E.). MRAC, Tervuren; and ORSTOM, Paris; 1991.
9. Laemmli UK. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature*. 1970;227:680-685.
10. Rashed MA, Tantawi TMA, Atta AH, Salah SH, El-Ganal AA. Plasma and muscle protein electrophoretic locality variations in catfish (*Clarias gariepinus* L.). *Egyptian*

- Journal of Genetics and Cytology. 2000;29(2):127-146.
11. El-Serafy SS, Abdel-Hamide NAH, Awwad MH, Azab MS. Comparative study on electrophoretic protein pattern characterization of *Tilapia* species in the River Nile, Egypt. Egypt. J. Aqual. Biol. Fish. 2006;10:147-178.
  12. Dan-en Gu, Xi-dong Mu, Hong-mei Song, Du Luo, Meng Xu, Jian-ren Luo, Yin-chang Hu. Genetic diversity of invasive *Oreochromis spp.* (tilapia) populations in Guangdong province of China using microsatellite markers. Journal of Biochemical Systematics and Ecology. 2014;55:198-204.
  13. Agbebi OT, Sofela SO, Olowofeso O, Idouiu AA. Protein profile expression of *Clarias gariepinus*, *Heterobranchus bidorsalis* and their reciprocal hybrids in Southwest Nigeria. Proceedings of 28<sup>th</sup> Fison Annual Conference. 2013;224.
  14. Usman BA, Agbebi OT, Bankole MO, Oguntade OR, Popoola MO. Molecular characterisation of two cichlids populations (*Tilapia guineensis* and *Sarotherodon melanotheron*) from different water bodies in Lagos State, Nigeria. International Journal for Biotechnology and Molecular Biology Research. 2013;4(5):71-77.
  15. Haniffa MA, Jeya Sheela P, James Milton M, Kavitha K. Protein profiling for phylogenetic relationship in snakehead species. Iranian Journal of Fisheries Sciences. 2017;16(4):1178-1186.

© 2020 Ukenye et al.; This is an Open Access article distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/4.0>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

*Peer-review history:*  
*The peer review history for this paper can be accessed here:*  
<http://www.sdiarticle4.com/review-history/59568>