Zootaxa 5550 (1): 305–313
https://www.mapress.com/zt/

Article

ISSN 1175-5326 (print edition)
ZOOTAXA
ISSN 1175-5334 (online edition)

https://doi.org/10.11646/zootaxa.5550.1.30
http://zoobank.org/urn:lsid:zoobank.org:pub:76AD88C5-7579-46E6-8657-6C65448124E3

Molecular evidence for clear speciation of *Tachysurus adiposalis* complex in northern and central Taiwan

HUE MAN TRAN[1] & I-SHIUNG CHEN[1,2,*]
[1]*Institute of Marine Biology, National Taiwan Ocean University, Keelung, 202301, Taiwan, R.O.C.*
madisontran1403@gmail.com; https://orcid.org/0009-0000-9300-8885
[2]*Center for Excellence of the Oceans, National Taiwan Ocean University, Keelung, 202301, Taiwan, R.O.C.*
iscfish@gmail.com; https://orcid.org/0000-0002-4190-7720
**Corresponding author*

Abstract

Tachysurus adiposalis (Oshima, 1919) belongs to the bagrid catfish genus *Tachysurus* in Taiwan. To understand the phylogenetic status of this species, this study was planned to use the ND2 gene and D-loop sequences. A total of 14 samples were used in phylogenetic reconstruction under both method of Neighbor-Joining and Maximum-Likelihood. In the molecular phylogenetic tree shows good results in distinguishing each single haplotype for its own represented species. By this study, which indicates that the *Tachysurus adiposalis* from Nothern Taiwan are the sister clade of the *Tachysurus* sp.1 from the Central Taiwan with specific mitogenetic distance for further explorations.

Key words: Bagridae, *Tachysurus adiposalis*, Taiwan, molecular evidence, molecular phylogenetics

Introduction

Catfish group of Order Siluriformes (catfish) is an important member of freshwater fish except for the mainly marine Ariidae and Plotosidae families, but which also include representatives in brackish and freshwater (Malabarba & Malabarba 2020). This order is one of the largest orders of teleosts containing over 4200 species in the world (Fricke *et al*. 2023). The freshwater catfish family Bagridae, commonly known as bagrid catfish, is one of the rich-species family and complicated groups within Siluriformes, with about 228 species in 19 genera and widely distributed in the tropics of South America, Africa and Asia, (Nelson *et al.* 2016; Zhang *et al*. 2022; Fricke *et al*. 2023). Among the members of this family, the genus *Tachysurus* is known as a relatively small group of catfish, which inhabits reservoirs, lakes, and large rivers. The genus currently consists of 53 recognized species, distributed throughout China, Japan, Korea, Vietnam, and Taiwan (Ng 2009; Cheng *et al.* 2021; Shao & Zhang 2022; Froese & Pauly 2023). In Southeast Asia countries, *Tachysurus* is primarily distributed in China but has also been found in other places such as Japan, Korea, Taiwan, and Vietnam**.** In Taiwan, the bagrid catfish genus *Tachysurus* is represented by *Tachysurus adiposalis* (Oshima, 1919) belongs to the bagrid catfish genus *Tachysurus* in Taiwan, which was described as *Pseudobagrus adiposalis*. The *Tachysurus adiposalis*, the species that are geographically distributed in the North and Central of the island (Watanabe 2007).

Several phylogenetic using molecular approaches have been conducted on freshwater fish of Taiwan. These genetic markers have demonstrated success in population genetic structure in a variety of fish species and suggested that Taiwan provides an excellent opportunity for examining phylogeographic patterns. The current study aims to analyze the phylogenetic of *Tachysurus adiposalis* with that of other Bagrid catfishes and provide insights into their relationships.

Accepted by S.-C. Wang: 7 Nov. 2024; published: 10 Dec. 2024

Materials and Methods

Sample collection and DNA extraction

Fish specimens of *Tachysurus adiposalis* complex used in this study were collected from Tamsui River, Daxi district, Taoyuan city (location: 24°53'02.1"N 121°17'01.0"E) (Northen Taiwan), and Wu River, Nantou County, Guoxing township (location: 24°01'36.5"N 120°49'27.5"E) (Central Taiwan), using long fishing trap, and were recognized by traditional morphology. Fin clip samples were preserved in 95% ethanol and stored at -20ºC in the refrigerator at the laboratory until DNA extraction, while the rest of the specimen was fixed in 10% formaldehyde for at least 48 hours, washed in water, and then transferred to 70% ethanol for long term storage at National Taiwan Ocean University — Taiwan (NTOU). Genomic DNA was extracted from ethanol-preserved fin tissue using the Tissue and Cell Genomic DNA Purification Kit (GeneMark, Taichung, Taiwan).

TABLE 1. List of PCR and sequencing primers used to analyze ND2 and D-loop of *Tachysurus adiposalis* from Taiwan.

Name	Sequence (5’ to 3’)
TACHY-DL-23F	5’-AGCGC.CGGTC.TTGTA.ATCCG-3’
TACHY-DL-1203R	5’-ACTTG.CATGT.ATAAA.TTG-3’
TACHY-ND2-101F	5’-GCTTT.TGGGC.CCATA.CCCCA.AAAA-3’
TACHY-ND2-1235R	5’-AAGCT.TTGAA.GGCTT.TTGGT.CT-3’

PCR amplification and sequencing

To sequence the D-loop and ND2 of *Tachysurus adiposalis*, polymerase chain reaction (PCR) primers were designed based on the mitogenome sequence of 5 available *Tachysurus* mitogenomes downloaded from GeneBank (Table 2): *Tachysurus fulvidraco* (Liang *et al*. 2012), *Tachysurus intermedius*, *Tachysurus ussuriensis* (Wan *et al*. 2013), and *Tachysurus vachellii*. Additionally, *Hemibagrus spilopterus* (Htun *et al*. 2019) was used as an outgroup. Table 1 shows the primer, TACHY-23F-5'AGCGCCGGTCTTGTAATCCG3' and TACHY-1203R-5'ACTTGCATGTATAAATTG3' was used for the amplification of the D-loop gene. The PCR was performed using Applied Biosystem 2720 Thermal Cycler followed as thermocycling condition: initial denaturation at 94ºC in 5 minutes, followed by 45 cycles of denaturation at 94ºC in 1 minute, annealing at 50ºC in 1 minutes, extension at 72ºC in 2 minutes, final extension at 72ºC for 7 minutes and hold at 4ºC. The primer, TACHY-101F- 5'GCTTTTGGGCCCATACCCCAAAAA3' and TACHY-1235R- 5'AAGCTTTGAAGGCTTTTGGTCT3' was used for the amplification of the ND2 gene. The thermal regime consisted of an initial step of denaturation at 94ºC in 5 minutes, followed by 45 cycles of denaturation at 94ºC in 50 seconds, annealing at 55ºC in 80 seconds, extension at 72ºC in 2 minutes, final extension at 72ºC for 7 minutes and hold at 4ºC.

TABLE 2. The catfish species employed for molecular phylogenetic analysis and GeneBank number.

Species	Family	GenBank Acc. No.	Citation
Tachysurus fulvidraco	Bagridae	NC015888	Liang *et al.,* 2012
Tachysurus intermedius	Bagridae	MK335935	unpublished
Tachysurus ussuriensis	Bagridae	NC020344	Wan *et al.,* 2013
Tachysurus vachellii	Bagridae	MW841469	unpublished
Hemibagrus spilopterus	Bagridae	NC023222	Htun *et al.,* 2019

To confirm PCR efficiency and the integrity of the genomic DNA, all PCR products, and DNA Ladder (PROTECH) were evaluated via 1.5% of agarose gel in 0.5xTBE buffer with 5% ClearView DNA Stain (PROTECH, Taiwan). The sequence process was performed in ABI 3730XL DNA ANALYZER (Thermo Fisher Scientific Inc; Waltham, MA, USA) using Big-Dye Terminator (Bigdye kit) at the DNA Sequencing Core Facility of the Institute of Biomedical Sciences, Academia Sinica, Nangang District, Taipei City, Taiwan. Chromatographs from forward and reverse reads were aligned through CLUSTAL W (Thompson *et al*. 1994), then compiled into consensus sequences after checked manually using the BIOEDIT v.7.2.5 (Hall 2001).

TABLE 3. The divergent site of the aligned mitochondrial DNA (mtDNA) D-loop sequences from three species of *Tachysurus*.

	5	1 3	6 8	7 7	8 4	9 2	1 1 0	1 2 7	1 2 8	1 4 9	1 6 5	2 0 5	2 0 6	2 5 4	2 8 2	2 8 4	2 9 9	3 0 0	3 0 3	3 0 8	3 2 4	3 2 9	3 5 4	3 5 5	5 7 2	5 9 6	6 0 4	6 1 1	6 2 4	6 2 6	6 2 8	6 2 9
T. ussuriensis	C	T	C	G	C	G	G	A	C	C	T	A	C	C	A	A	C	C	G	C	G	C	G	A	C	T	T	G	A	C	T	G
T. adiposalis TSR1	.	C	.	A	T	.	A	T	T	T	C	G	T	T	G	C	T	.	A	T	A	.	A	G	T	.	.	A	C	T	G	C
T. adiposalis TSR2	.	C	.	A	T	.	A	T	T	T	C	G	T	T	G	C	T	.	A	T	A	.	A	G	T	.	.	A	C	T	G	C
T. adiposalis TSR3	.	C	.	A	T	.	A	T	T	T	C	G	T	T	G	C	T	.	A	T	A	.	A	G	T	.	.	A	C	T	G	C
T. adiposalis TSR4	.	C	.	A	T	.	A	T	T	T	C	G	T	T	G	C	T	.	A	T	A	.	A	G	T	.	.	A	C	T	G	C
T. adiposalis TSR5	.	C	.	A	T	.	A	T	T	T	C	G	T	T	G	C	T	.	A	T	A	.	A	G	T	.	.	A	C	T	G	C
T. adiposalis TSR6	.	C	.	A	T	.	A	T	T	T	C	G	T	T	G	C	T	.	A	T	A	.	A	G	T	.	.	A	C	T	G	C
T. sp.1 WUR1	T	C	T	.	T	A	A	T	T	T	C	G	T	T	.	C	T	T	A	T	.	T	A	G	T	C	C	.	C	T	G	C
T. sp.1 WUR2	T	C	T	.	T	A	A	T	T	T	C	G	T	T	.	C	T	T	A	T	.	T	A	G	T	C	C	.	C	T	G	C
T. sp.1 WUR3	T	C	T	.	T	A	A	T	T	T	C	G	T	T	.	C	T	T	A	T	.	T	A	G	T	C	C	.	C	T	G	C
T. sp.1 WUR4	T	C	T	.	T	A	A	T	T	T	C	G	T	T	.	C	T	T	A	T	.	T	A	G	T	C	C	.	C	T	G	C
T. sp.1 WUR5	T	C	T	.	T	A	A	T	T	T	C	G	T	T	.	C	T	T	A	T	.	T	A	G	T	C	C	.	C	T	G	C
T. sp.1 WUR6	T	C	T	.	T	A	A	T	T	T	C	G	T	T	.	C	T	T	A	T	.	T	A	G	T	C	C	.	C	T	G	C

TABLE 3. (Continued)

	6 3 0	6 3 2	6 3 4	6 3 5	6 3 7	6 3 8	6 3 9	6 4 0	6 4 1	6 4 3	6 4 4	6 4 5	6 4 6	6 4 7	6 4 8	6 5 3	6 6 6	6 7 9	6 8 2	6 8 3	6 8 4	6 8 5	6 8 6	6 8 8	6 9 1	6 9 3	7 1 3	7 3 3	8 5 7
T. ussuriensis	C	T	A	A	G	A	T	A	G	T	T	A	T	G	C	T	T	G	T	A	C	A	C	T	G	A	T	T	G
T. adiposalis TSR1	T	G	T	T	A	T	A	T	T	A	A	T	G	C	A	C	C	A	C	C	A	C	A	.	A	G	.	A	.
T. adiposalis TSR2	T	G	T	T	A	T	A	T	T	A	A	T	G	C	A	C	C	A	C	C	A	C	A	.	A	G	.	A	.
T. adiposalis TSR3	T	G	T	T	A	T	A	T	T	A	A	T	G	C	A	C	C	A	C	C	A	C	A	.	A	G	.	A	.
T. adiposalis TSR4	T	G	T	T	A	T	A	T	T	A	A	T	G	C	A	C	C	A	C	C	A	C	A	.	A	G	.	A	.
T. adiposalis TSR5	T	G	T	T	A	T	A	T	T	A	A	T	G	C	A	C	C	A	C	C	A	C	A	.	A	G	.	A	.
T. adiposalis TSR6	T	G	T	T	A	T	A	T	T	A	A	T	G	C	A	C	C	A	C	C	A	C	A	.	A	G	.	A	.
T. sp.1 WUR1	T	G	T	T	A	T	A	G	T	A	A	T	G	C	A	C	.	.	.	C	.	.	.	A	.	.	A	A	A
T. sp.1 WUR2	T	G	T	T	A	T	A	G	T	A	A	T	G	C	A	C	.	.	.	C	.	.	.	A	.	.	A	A	A
T. sp.1 WUR3	T	G	T	T	A	T	A	G	T	A	A	T	G	C	A	C	.	.	.	C	.	.	.	A	.	.	A	A	A
T. sp.1 WUR4	T	G	T	T	A	T	A	G	T	A	A	T	G	C	A	C	.	.	.	C	.	.	.	A	.	.	A	A	A
T. sp.1 WUR5	T	G	T	T	A	T	A	G	T	A	A	T	G	C	A	C	.	.	.	C	.	.	.	A	.	.	A	A	A
T. sp.1 WUR6	T	G	T	T	A	T	A	G	T	A	A	T	G	C	A	C	.	.	.	C	.	.	.	A	.	.	A	A	A

TABLE 4. The divergent site of the aligned mitochondrial DNA (mtDNA) ND2 sequences from three species of *Tachysurus*.

	2 0	7 5	9 0	1 2 9	1 4 4	1 4 7	1 6 8	2 1 1	2 8 6	2 8 7	2 9 4	3 3 0	3 7 2	3 8 1	3 9 3	3 9 9	3 3 9	4 4 3	4 7 7	4 9 5	5 0 4	5 0 7	5 0 8	5 1 9	5 4 3	5 5 2	5 5 5	6 2 9	6 3 0	6 6 0	6 6 1	6 8 4	6 8 7	6 8 8
T. ussuriensis	C	C	A	C	C	C	T	C	A	T	T	A	C	G	T	A	C	C	T	G	C	T	T	C	C	C	T	T	T	G	T	T	A	T
T. adiposalis TSR1	T	T	G	T	T	.	C	T	.	C	C	C	T	A	C	G	T	T	C	A	A	C	C	T	T	T	C	C	C	A	C	C	G	C
T. adiposalis TSR2	T	T	G	T	T	.	C	T	.	C	C	C	T	A	C	G	T	T	C	A	A	C	C	T	T	T	C	C	C	A	C	C	G	C
T. adiposalis TSR3	T	T	G	T	T	.	C	T	.	C	C	C	T	A	C	G	T	T	C	A	A	C	C	T	T	T	C	C	C	A	C	C	G	C
T. adiposalis TSR4	T	T	G	T	T	.	C	T	.	C	C	C	T	A	C	G	T	T	C	A	A	C	C	T	T	T	C	C	C	A	C	C	G	C
T. adiposalis TSR5	T	T	G	T	T	.	C	T	.	C	C	C	T	A	C	G	T	T	C	A	A	C	C	T	T	T	C	C	C	A	C	C	G	C
T. adiposalis TSR6	T	T	G	T	T	.	C	T	.	C	C	C	T	A	C	G	T	T	C	A	A	C	C	T	T	T	C	C	C	A	C	C	G	C
T. sp.1 WUR1	T	T	G	T	T	T	C	T	G	C	C	.	T	A	C	G	T	T	C	A	A	C	C	T	T	T	C	.	C	A	C	C	.	C
T. sp.1 WUR2	T	T	G	T	T	T	C	T	G	C	C	.	T	A	C	G	T	T	C	A	A	C	C	T	T	T	C	.	C	A	C	C	.	C
T. sp.1 WUR3	T	T	G	T	T	T	C	T	G	C	C	.	T	A	C	G	T	T	C	A	A	C	C	T	T	T	C	.	C	A	C	C	.	C
T. sp.1 WUR4	T	T	G	T	T	T	C	T	G	C	C	.	T	A	C	G	T	T	C	A	A	C	C	T	T	T	C	.	C	A	C	C	.	C
T. sp.1 WUR5	T	T	G	T	T	T	C	T	G	C	C	.	T	A	C	G	T	T	C	A	A	C	C	T	T	T	C	.	C	A	C	C	.	C
T. sp.1 WUR6	T	T	G	T	T	T	C	T	G	C	C	.	T	A	C	G	T	T	C	A	A	C	C	T	T	T	C	.	C	A	C	C	.	C

.....continued on the next page

TABLE 4. (Continued)

	7 0 2	7 1 7	7 2 3	7 3 2	7 3 3	7 4 1	7 4 7	7 5 1	7 5 9	7 6 5	7 6 8	7 8 0	7 9 3	7 9 5	7 9 9	8 1 5	8 2 2	8 2 9	8 3 4	8 5 5	8 7 9	8 8 8	8 8 9	9 0 3	9 0 9	9 7 2	9 7 3	9 7 6	9 8 9	9 8 6	1 0 2 6	1 0 3 3	1 0 3 6
T. ussuriensis	A	T	T	C	G	T	T	T	T	T	A	C	T	A	T	C	T	C	T	C	T	A	T	T	T	C	T	A	T	T	G	C
T. adiposalis TSR1	G	C	A	T	A	C	A	.	C	C	.	T	C	G	C	T	C	T	C	T	C	G	C	C	C	A	C	G	C	C	A	T
T. adiposalis TSR2	G	C	A	T	A	C	A	.	C	C	.	T	C	G	C	T	C	T	C	T	C	G	C	C	C	A	C	G	C	C	A	T
T. adiposalis TSR3	G	C	A	T	A	C	A	.	C	C	.	T	C	G	C	T	C	T	C	T	C	G	C	C	C	A	C	G	C	C	A	T
T. adiposalis TSR4	G	C	A	T	A	C	A	.	C	C	.	T	C	G	C	T	C	T	C	T	C	G	C	C	C	A	C	G	C	C	A	T
T. adiposalis TSR5	G	C	A	T	A	C	A	.	C	C	.	T	C	G	C	T	C	T	C	T	C	G	C	C	C	A	C	G	C	C	A	T
T. adiposalis TSR6	G	C	A	T	A	C	A	.	C	C	.	T	C	G	C	T	C	T	C	T	C	G	C	C	C	A	C	G	C	C	A	T
T. sp.1 WUR1	G	C	A	T	A	C	A	C	A	C	G	T	C	G	C	T	C	T	C	T	C	G	C	C	C	A	C	G	C	C	A	T
T. sp.1 WUR2	G	C	A	T	A	C	A	C	A	C	G	T	C	G	C	T	C	T	C	T	C	G	C	C	C	A	C	G	C	C	A	T
T. sp.1 WUR3	G	C	A	T	A	C	A	C	A	C	G	T	C	G	C	T	C	T	C	T	C	G	C	C	C	A	C	G	C	C	A	T
T. sp.1 WUR4	G	C	A	T	A	C	A	C	A	C	G	T	C	G	C	T	C	T	C	T	C	G	C	C	C	A	C	G	C	C	A	T
T. sp.1 WUR5	G	C	A	T	A	C	A	C	A	C	G	T	C	G	C	T	C	T	C	T	C	G	C	C	C	A	C	G	C	C	A	T
T. sp.1 WUR6	G	C	A	T	A	C	A	C	A	C	G	T	C	G	C	T	C	T	C	T	C	G	C	C	C	A	C	G	C	C	A	T

TABLE 5. The genetic distance based on D-loop sequence.

		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
1	*T. fulvidraco*																			
2	*T. intermedius*	0.067																		
3	*T. ussuriensis*	0.144	0.135																	
4	*T. vachellii*	0.048	0.095	0.134																
5	*T. adiposalis* TSR1	0.129	0.135	0.060	0.124															
6	*T. adiposalis* TSR2	0.129	0.135	0.060	0.124	0.000														
7	*T. adiposalis* TSR3	0.129	0.135	0.060	0.124	0.000	0.000													
8	*T. adiposalis* TSR4	0.129	0.135	0.060	0.124	0.000	0.000	0.000												
9	*T. adiposalis* TSR5	0.129	0.135	0.060	0.124	0.000	0.000	0.000	0.000											
10	*T. adiposalis* TSR6	0.129	0.135	0.060	0.124	0.000	0.000	0.000	0.000	0.000										
11	*T.* sp1 WUR1	0.122	0.129	0.057	0.116	0.026	0.026	0.026	0.026	0.026	0.026									
12	*T.* sp1 WUR2	0.122	0.129	0.057	0.116	0.026	0.026	0.026	0.026	0.026	0.026	0.000								
13	*T.* sp1 WUR3	0.122	0.129	0.057	0.116	0.026	0.026	0.026	0.026	0.026	0.026	0.000	0.000							
14	*T.* sp1 WUR4	0.122	0.129	0.057	0.116	0.026	0.026	0.026	0.026	0.026	0.026	0.000	0.000	0.000						
15	*T.* sp1 WUR5	0.122	0.129	0.057	0.116	0.026	0.026	0.026	0.026	0.026	0.026	0.000	0.000	0.000	0.000					
16	*T.* sp1 WUR6	0.122	0.129	0.057	0.116	0.026	0.026	0.026	0.026	0.026	0.026	0.000	0.000	0.000	0.000	0.000				
17	*T. brevianalis*	0.086	0.109	0.159	0.116	0.141	0.141	0.141	0.141	0.141	0.141	0.131	0.131	0.131	0.131	0.131	0.131			
18	*T. brevianalis taiwanensis*	0.084	0.104	0.156	0.115	0.138	0.138	0.138	0.138	0.138	0.138	0.132	0.132	0.132	0.132	0.132	0.132	0.004		
19	*H. spilopterus*	0.658	0.683	0.674	0.696	0.658	0.658	0.658	0.658	0.658	0.658	0.653	0.653	0.653	0.653	0.653	0.653	0.694	0.692	

TABLE 6. The genetic distance based on ND2 sequence.

		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
1	*T. fulvidraco*																			
2	*T. intermedius*	0.084																		
3	*T. ussuriensis*	0.147	0.143																	
4	*T. vachellii*	0.013	0.094	0.154																
5	*T. adiposalis* TSR1	0.143	0.137	0.063	0.152															
6	*T. adiposalis* TSR2	0.143	0.137	0.063	0.152	0.000														
7	*T. adiposalis* TSR3	0.143	0.137	0.063	0.152	0.000	0.000													
8	*T. adiposalis* TSR4	0.143	0.137	0.063	0.152	0.000	0.000	0.000												
9	*T. adiposalis* TSR5	0.143	0.137	0.063	0.152	0.000	0.000	0.000	0.000											
10	*T. adiposalis* TSR6	0.143	0.137	0.063	0.152	0.000	0.000	0.000	0.000	0.000										
11	*T.* sp1 WUR1	0.142	0.138	0.064	0.150	0.008	0.008	0.008	0.008	0.008	0.008									
12	*T.* sp1 WUR2	0.142	0.138	0.064	0.150	0.008	0.008	0.008	0.008	0.008	0.008	0.000								
13	*T.* sp1 WUR3	0.142	0.138	0.064	0.150	0.008	0.008	0.008	0.008	0.008	0.008	0.000	0.000							
14	*T.* sp1 WUR4	0.142	0.138	0.064	0.150	0.008	0.008	0.008	0.008	0.008	0.008	0.000	0.000	0.000						
15	*T.* sp1 WUR5	0.142	0.138	0.064	0.150	0.008	0.008	0.008	0.008	0.008	0.008	0.000	0.000	0.000	0.000					
16	*T.* sp1 WUR6	0.142	0.138	0.064	0.150	0.008	0.008	0.008	0.008	0.008	0.008	0.000	0.000	0.000	0.000	0.000				
17	*T. brevianalis*	0.098	0.087	0.133	0.108	0.139	0.139	0.139	0.139	0.139	0.139	0.140	0.140	0.140	0.140	0.140	0.140			
18	*T. brevianalis taiwanensis*	0.095	0.091	0.137	0.105	0.140	0.140	0.140	0.140	0.140	0.140	0.141	0.141	0.141	0.141	0.141	0.141	0.010		
19	*H. spilopterus*	0.232	0.231	0.230	0.235	0.238	0.238	0.238	0.238	0.238	0.238	0.238	0.238	0.238	0.238	0.238	0.238	0.245	0.252	

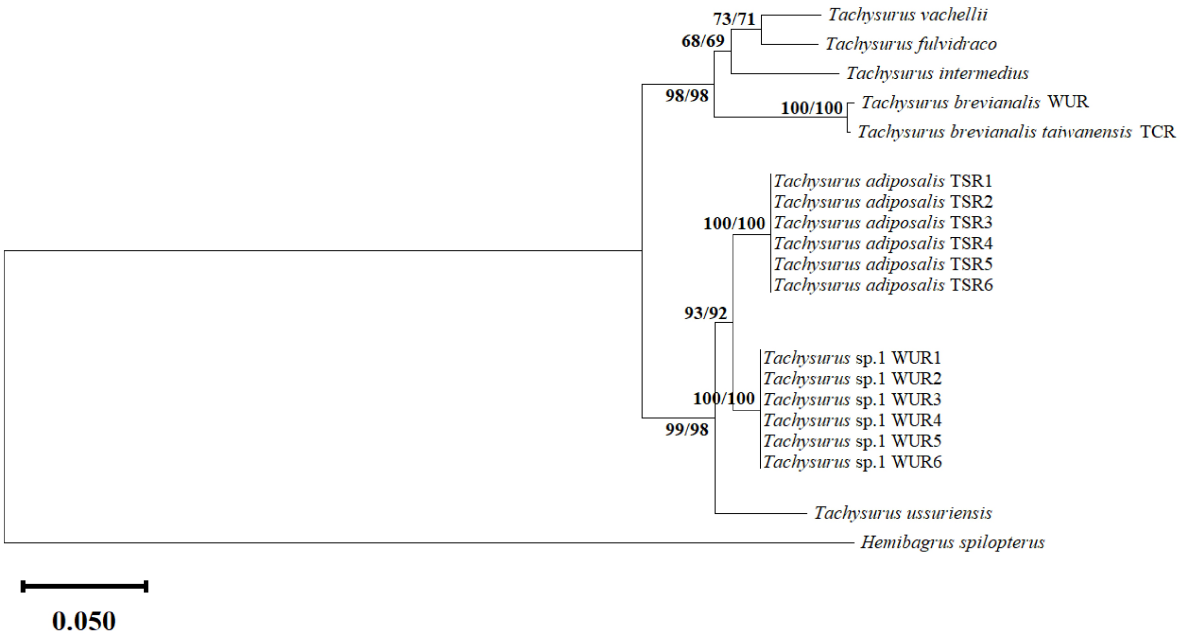

FIGURE 1. D-loop phylogenetic tree of *Tachysurus adiposalis*. The numbers at the nodes separated by "/" indicate the bootstrap value.

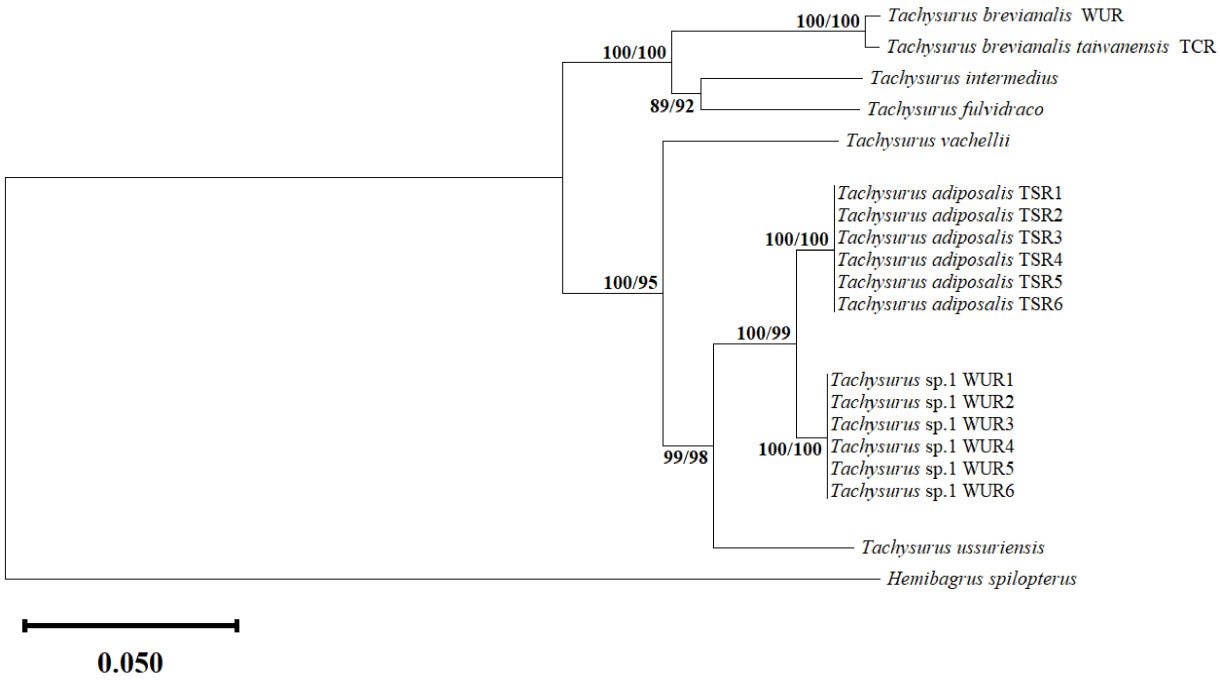

FIGURE 2. The molecular phylogenetic tree of *Tachysurus adiposalis* inferred from ND2 gene and D-loop sequences. The numbers at the nodes separated by "/" indicate the bootstrap value.

Phylogenetic analysis

The sequence fragments were checked, edited manually, and assembled using the BIOEDIT v7.2.5 (Hall 2001). The newly generated sequences for molecular marker mitochondrial DNA genes (D-loop and ND2) were aligned through CLUSTALW (Thompson *et al.* 1994) after verified using BIOEDIT version 7.2.5 (Hall 2001). A total of 6 species were used in phylogenetic reconstructions under maximum likelihood (ML) and Neighbor-Joining (NJ) criteria which performed in Molecular Evolutionary Genetics Analysis (MEGA X) (Kumar *et al*. 2018). Branch support was established via bootstrap analysis (1,000 replications). Among them, 1 species were our own data, the remaining 5 species data were retrieved from the GenBank.

Results and Discussion

Bagrid catfishes constitute a very important group among siluriform having immense commercial importance from inland fisheries and aquaculture farming in south-east countries. The control region (D-loop) is a common feature in fish mitochondrial genome which includes the origin sites of transcription and replication (Noack *et al*. 1996; Taanman 1999). Many studies have been focused on this region because of its supposedly rapid rate of evolution (Saccone 1987; Lee *et al*. 1995; Guo *et al*. 2004). This control region has been amplified, sequenced, and assembled for *Tachysurus adiposalis* was 892 bp, located between tRNA-Pro and tRNA-Phe. The complete protein-coding gene (ND2) was 1045 bp, located between tRNA-Met and tRNA-Trp. The phylogenetic position of *Tachysurus adiposalis* was inferred by a phylogenetic tree using MEGA X based on the combined of ND2 and D-loop for 5 published Bagridae species, with *Hemibagrus spilopterus* (Htun *et al*. 2019) weas used as outgroup. Both ND2, D-loop, and ND2 and D-loop were constructed with different OTUs from Genebank and an outgroup from *Hemibargus spilopterus*.

The D-loop phylogenetic tree analysis reveals bagrid catfishes could be separated into two clades excluding *Hemibagrus spilopterus* (Htun *et al*. 2019) with strong support (Figure 1). Clade I included only *Tachysurus* species, and was composed of *Tachysurus adiposalis* from Tamsui river are in the same subclade, on the other hand, the second subclade shows *Tachysurus* sp.1 from Wu River, which indicates that the *Tachysurus adiposalis* from Tamsui River are the sister of the *Tachysurus* sp.1 from Wu River. Clade I also show that the subclade of *Tachysurus fulvidraco*, *Tachysurus vachellii*, and *Tachysurus intermedius* species are more distantly related to the *Tachysurus adiposalis* and *Tachysurus* sp.1, while *Tachysurus brevianalis* from Wu River and *Tachysurus brevianalis taiwanensis* from Touchian River are even more distantly related. Nevertheless, clade II shows only the species *Hemibagrus spilopterus*, and this species was paraphyletic. In addition, D-loop phylogenetic trees showed that *Tachysurus brevianan lis* and *Tachysurus brevianalis taiwanensis* clustered together forming a group. The ND2 phylogenetic tree also shows 2 clades, the tree looks quite the same as the D-loop phylogenetic tree, also included in the first clade with *Tachysurus* species. The phylogenetic tree of the combine between ND2 and D-loop sequence (Figure 2) looks like ND2 and D-loop phylogenetic tree, with 2 separated clades, and clade 1 was only *Tachysurus* species, but the combined between ND2 and D-loop phylogenetic tree gives a better figure. In addition, the phylogenetic trees showed that *Tachysurus adiposalis* are more distance related to other 6 species of *Tachysurus* species. This result supports molecular evidence that in the genetic relationship, *Tachysurus adiposalis* species from the Tamsui River and *Tachysurus* sp.1 from Wu River are sister species.

The phylogenetic tree, including the ND2, D-loop, and combined ND2 and D-loop region, yields good results in distinguishing each haplotype for their own species for *Tachysurus adiposalis* complex. Moreover, the combined ND2 and D-loop analysis offers superior results, indicating that it provides more comprehensive information compared to the other analysis. The findings of current molecular analysis contribute to a better understanding of *Tachysurus adiposalis* and provide insights into the relationships within other Bagrid catfishes.

Acknowledgments

This study was partially funded by NAMR, Kaohsiung and Water Resources Planning Branch, Water Resources Agency, Ministry of Economic Affairs, MOEA, Wufong, Taichung. The project is also partially supported by CEO, NTOU, Keelung, Taiwan.

References

Cheng, J.L., Shao, W.H., López, J. & Zhang, E. (2021). *Tachysurus lani*, a new catfish species (Teleostei: Bagridae) from the Pearl River basin, South China. *Ichthyological Exploration of Freshwaters*, IEF-1156, 1–17.

Fricke, R., Eschmeyer, W.N., & Van der Laan R. (2023) Eschmeyer's catalog of fishes: genera, species, references.

Froese, R. & Pauly, D. (2023) "*Tachysurus*" in FishBase. Available from: https://www.fishbase.se/identification/SpeciesList.php?genus=Tachysurus (accessed 10 April 2023)

Guo, X., Liu, S. & Liu, Y. (2004) Comparative analysis of the mitochondrial DNA control regions in cyprinids with differents ploidy level. *Aquaculture,* 224, 25–38.
https://doi.org/10.1016/S0044-8486(03)00168-6

Hall, T.A. (2001) Bioedit: a user-friendly biological sequence alignment editor and analysis, version 5.09. Department of Microbiology, North Carolina State University.

Htun, K.K., Pan, H. & Peng, Z. (2019) The complete mitochondrial genome of *Hemibagrus spilopterus* (Teleostei: Siluriformes). *Mitochondrial DNA Part B*, 4, 1566–1567.
https://doi.org/10.1080/23802359.2019.1601526

Kumar, S., Stecher, G., Li, M., Knyaz, C. & Tamura, K. (2018) MEGA X: Molecular Evolutionary Genetics Analysis across Computing Platforms. *Molecular Biology and Evolution*, 35, 1547–1549.
` https://doi.org/10.1093/molbev/msy096

Lee, W.J., Conroy, J., Howell, W.H. & Kocher, T.D. (1995) Structure and evolution of teleost mitochondrial control regions. *Journal of Molecular Evolution,* 41, 54–66.
https://doi.org/10.1007/BF00174041

Liang, H.W., Hu, G.F., Li, Z., Zou, G.W. & Liu, X.L. (2012) Mitochondrial DNA sequence of yellow catfish (*Pelteobagrus fulvidraco*). *Mitochondrial DNA*, 23, 170–172.
https://doi.org/10.3109/19401736.2012.668899

Malabarba, LR. & Malabarba, M.C. (2020) Phylogeny and classification of Neotropical fish. In: Nelson, J.S., Grande, T.C. & Wilson, M.V. (Eds.), *Biology and Physiology of Freshwater Neotropical Fish.* John Wiley and Sons, New York (NY), pp. 1–19.
https://doi.org/10.1016/B978-0-12-815872-2.00001-4

Ng, H.H. (2009) *Tachysurus spilotus*, a new species of catfish from central Vietnam (Teleostei: Bagridae). *Zootaxa*, 2283, 16–28.
https://doi.org/10.11646/zootaxa.2283.1.2

Noack, K., Zardoya, R. & Meyer, A. (1996) The complete mitochondrial DNA sequence of the Bichir (*Polypterus ornatipinnis*), a basal ray-finned fish: Ancient establishment of the consensus vertebrate gene order. *Genetics*, 144, 1165–1180.
https://doi.org/10.1093/genetics/144.3.1165

Oshima, M. (1919) Contributions to the study of the freshwater fishes of the island of Formosa. *Annals of the Carnegie Museum*, 12, 169–328, pls. 48–53.
https://doi.org/10.5962/p.34608

Saccone, C., Attimonelli, M. & Sbisa, E. (1987) Structural elements highly preserved during the evolution of the D-loop-containing region in vertebrate mitochondrial DNA. *Journal of Molecular Evolution,* 26, 205–211.
https://doi.org/10.1007/BF02099853

Shao, W.H. & Zhang, E. (2022) *Tachysurus latifrontalis*, a new bagrid species from the Jiulong-Jiang basin in Fujian Province, South China (Teleostei: Bagridae). *Ichthyological Research*, 70, 110–122.
https://doi.org/10.1007/s10228-022-00867-0

Taanman, J.W. (1999) The mitochondrial genome: structure, transcription, translation and replication. *Biochimica et Biophysica Acta*, 1410, 103–123.
https://doi.org/10.1016/S0005-2728(98)00161-3

Thompson, J.D., Higgins, D.G. & Gibson, T.J. (1994) CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Research,* 22, 4673–4680.
https://doi.org/10.1093/nar/22.22.4673

Wan, Q., Tao, G., Cheng, Q., Chen, Y. & Qiao, H. (2013) The complete mitochondrial genome sequence of *Pseudobagrus ussuriensis* (Siluriformes: Bagridae). *Mitochondrial DNA*, 24, 333–335.
https://doi.org/10.3109/19401736.2012.760079

Watanabe, K., JiangLiaw, N.H., Zhang, C.G., Jeon, S.R. & Nishida, M. (2007) Comparative phylogeography of bagrid catfishes in Taiwan. *Ichthyological Research,* 54, 253–261.
https://doi.org/10.1007/s10228-007-0398-y

Zhang, R., Deng, L., Lv, X. & Tang, Q. (2022) Complete mitochondrial genomes of two catfishes (Siluriformes, Bagridae) and their phylogenetic implications. *Zookeys,* 1115, 103–116.
https://doi.org/10.3897/zookeys.1115.85249