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A PRELIMINARY STUDY OF GENETIC VARIATION OF *Galaxias olidus* (SALMONIFORMES : GALAXIIDAE) IN WESTERN VICTORIA, AUSTRALIA

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ABSTRACT

Arisuryanti, T. 2000. A preliminary study of genetic variation of *Galaxias olidus* (Salmoniformes : Galaxiidae) in western Victoria, Australia. *Biologi* 2 (9): 487–498.

Four populations (two western populations : Fitzroy River and Scrubby Creek, and two eastern populations : Matthews Creek and Barwon River) of the mountain galaxias, *Galaxias olidus*, were investigated for genetic variation at 38 loci encoding electrophoretically detectable proteins recorded from 23 system enzymes and general proteins using allozyme electrophoresis methods. Genetic variation was concentrated at only three loci : Malate dehydrogenase (*Mdh*), Glycerol-3-phosphate dehydrogenase-1 and 2 (*Gpdh-1* and *Gpdh-2*). Fixed allozyme differences were found between the eastern populations and the western populations at *Mdh* and *Gpdh-2* loci while within the western populations were observed at one locus (*Gpdh-1*). On the basis of genetic similarity values, these four populations are still considered to be conspecific (I=0.95) nonetheless leading to incipient speciation.

Key words: *genetic variation – **Galaxias olidus** – allozyme electrophoresis*

INTISARI

Arisuryanti, T. 2000. Studi awal variasi genetik ikan *Galaxias olidus* (Salmoniformes: Galaxiidae) di bagian barat Victoria, Australia. *Biologi* 2 (9): 487–498.

Tiga puluh delapan lokus gen yang berasal dari 23 sistem enzim dan protein diinvestigasi dengan menggunakan metode allozim elektroforesis untuk mengetahui variasi genetik 4 populasi ikan *Galaxias olidus* yang terdiri dari 2 populasi bagian barat (Fitzroy River dan Scrubby Creek) dan 2 populasi bagian timur (Matthews Creek dan Barwon River). Variasi genetik difokuskan pada 3 lokus yang polimorfik yaitu Malate dehydrogenase (*Mdh*), Glycerol-3-phosphate dehydrogenase –1 dan 2 (*Gpdh-1*

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lan *Gpdh*-2). Hasil penelitian menunjukkan bahwa antara populasi bagian timur dengan bagian barat terdapat perbedaan variasi genetik pada lokus *Mdh* dan *Gpdh*-2. Khusus pada populasi bagian barat, yaitu antara populasi Fitzroy River dengan Scrubby Creek, perbedaan genetik dijumpai pada lokus *Gpdh-1*. Berdasarkan nilai kesamaan genetik, keempat populasi tersebut masih tetap dipertimbangkan sebagai satu species (I = 0,95) meskipun telah menunjukkan perbedaan genetik yang nyata. Tampaknya perbedaan genetik yang ada telah menunjukkan indikasi terjadinya proses spesiasi awal.

Kata kunci: *variasi genetik* – ***Galaxias olidus*** – *allozim elektroforesis*

INTRODUCTION

The mountain galaxias, *Galaxias olidus* (Gunther), is one of a widespread freshwater species in eastern Australia ranging from North-east Coast in southern Queensland to Murray Darling drainage divisions in south-eastern South Australia. The fish are commonly present at medium to high altitudes (up to 1800 m) where temperature are cooler and the streams are smaller with rock, gravel or sand substrates (Merrick & Schmida, 1984; McDowall, 1996). This species is between 90 and 150 mm, but it is usually found around 100 mm (Merrick and Schmida, 1984).

As one of non-diadromous species, its life history is confined entirely to freshwater (no migration to the sea and lacking a marine 1988). The fish usually spawn during spring and the fertilized eggs take 2-3 weeks to hatch. A consequence of this life cycle is therefore reproductive isolation between one and another population occuring genetic isolation and over sufficiently long periods of time this can lead to speciation. Indeed this life cycle is thought to be important factor to account for high diversity of galaxiid fishes in Australia (Johnson *et al.*, 1983; Watts *et al.*, 1995; Waters and White, 1997).

The widespread distribution of this species, combined with the effect of local environment on morphological characters including morphometric, meristic and colouration (McDowall & Frankenberg, 1981; Merrick & Schmida, produced an exceed-

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ingly confusing taxonomy with proliferation named species. Historically, this species has been described many times under several names including *G.schomburgkii* Peters, *G. kayi* Ramsay and Ogilby, *G. ornatus* Castelnau, *G. bongbong* Macleay, *G. findlayi* Macleay, *G. occonori* Ogilby, *G. coxii* Walford, *Lyragalaxias oconnori* Whitley and *G. fuscus* Mack (McDowall & Frankenberg, 1981; McDowall, 1996). Due to the controversy regarding the taxonomic status of this species, some researches assigned tentatively that this species should be considered as a species complex and *G. olidus* should be applied until completed data including detail of morphological, chromosome, allozyme and molecular information are collected to re-evaluate the taxonomy of this species (McDowall & Frankenberg, 1981; McDowall, 1996, Waters *et al.*, 1999).

The investigations of genetic variation of this species in eastern Australia, notably in western Victoria using allozyme electrophoresis have not been done. Therefore, in this study the author surveyed allozyme characters at 23 enzyme systems and general proteins representing 38 presumptive gene loci as the primary means to determine genetic variation among 4 populations (2 eastern populations and 2 western populations) of this species in western Victoria, Australia. This investigation is needed to examine whether genetic divergence found in these 4 populations reveals cryptic speciation which should be considered to be separate species or still recognised as conspecific based on genetic similarity values. The findings of this research are therefore expected to add information concerning the clarity of the taxonomic status of this species together with further studies.

MATERIALS AND METHODS

1. Specimen collection and storage

Specimens of *G.olidus* from Fitzroy River (141°32¹E, 38°07¹S; 5 samples), Scrubby Creek (142°24¹E, 37°10¹S; 3 samples), Matthews Creek (143°47¹E, 38°20¹S; 5 samples) and Barwon River (143°50¹E, 38°18¹S; 5 samples) were caught using either baited fish traps or a seine net. The collection of specimens was made under Permit No. FSP/CW/225 (2-3) from the Depart-

ment of Natural Resources and Environment, Victoria, Australia. Procedures for handling of live fish and their euthanasia used in this research were approved by Deakin University's Annual Ethics Committee.

Based on morphological characters identified from McDowall and Frankenberg (1981) and McDowall (1996), all individuals collected in this study are *G. olidus*. Next, upon capture fish were placed on ice and either frozen whole in liquid nitrogen in the field or transported live to the laboratory and frozen at –20° C. Subsequently, muscle, liver and gill tissue were dissected from each partially thawed fish and placed into 1.5 ml screw cryogenic vials and maintained at –80°C until required for allozyme electrophoresis.

2. Sample preparation

Approximately 0.5 grams of tissue sample was homogenised with grinding solution containing 10% (w/v) sucrose, 0.1% (w/v) bromophenol blue and 0.1% (v/v) mercaptoethanol using a Microson Ultrasonic Cell Disruptor (Model XL 2005) operated at ap- second. Homogenised extracts were then centrifuged at 13,000 rpm to pellet tissue debris. Next, the supernatant was absorbed onto Whatman (no. 1) filter paper rectangles (5x3 mm in size) for starch gel electrophoresis. For cellulose acetate gel electrophoresis, the supernatant was applied to gel plates using the Helena applicator system described by Hebert and Beaton (1993).

3. Allozyme electrophoresis procedures

Allozyme eletrophoresis procedures followed those described in Shaklee and Keenan (1986) and Murphy *et al.* (1996) for starch gel electrophoresis. The method of Richardson *et al.* (1986) and Hebert and Beaton (1993) was used for cellulose acetate gel electrophoresis. Enzyme are referred to by their Enzyme Commision (EC) Number and enzyme loci are referred to use a standardized nomenclature following the recommendation of Richardson *et al.* (1986). Buffer systems and volumes, running conditions and staining procedures were essentially those of Richardson *et al.* (1986), Shaklee and Keenan (1986) and Murphy *et*

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Table 1. Stains and buffers used in the electro phoretic analysis of *Galaxias* samples

Enzyme name	Locus	E.C.[1] number	No. of loci	Buffer[2]	Tissue[3]	Electrophoresis[4]
Alcohol dehydrogenase	*Adh*	1.1.1.1	1	TG	L	CA
Creatine kinase	*Ck*	2.7.3.2	1	TC8	L	S
Esterase	*Est*	Non-specific	2	TC8	L	S
Fructose biphosphate	*Fbp*	3.1.3.11	2	TC8	L	S
Fructose biphosphate aldolase	*Fba*	4.1.2.13	2	TC8	M	S
General proteins	*Gp*	Non-specific	6	Poulik	M	S
Glucose-6-phosphate dehydrogenase	*G6pdh*	1.1.1.49	1	TC6	G	S
Glutamate oxaloacetate	*Got*	2.6.1.1	2	TC8	L	S
Glutamate dehydrogenase	*Gdh*	1.4.1.2	1	TC8	L	S
Glycerol-3-phosphate dehydrogenase	*Gpdh*	1.1.1.8	2	TG	L	CA
Isocitrate dehydrogenase	*Idh*	1.1.1.42	1	TC8	L	S
Lactate dehydrogenase	*Ldh*	1.1.1.27	1	TC8	L	S
Malate dehydrogenase	*Mdh*	1.1.1.37	1	TC8	L	S
Mannose-6-phosphate isomerase	*Mpi*	5.3.1.8	1	TG	L	CA
Peptidase L-leucyl-glycyl-glycine	*Pep-LGG*	3.4.11.4	2	Poulik	L	S
Peptidase L-leucyl-proline	*Pep-LP*	3.4.13.9	1	Poulik	L	S
Peptidase L-leucyl-L-tyrosine	*Pep-LT*	3.4.13.11	2	Poulik	M	S
Peptidase glycyl-L-leucine	*Pep-GL*	3.4.x.x	2	Poulik	M	S
Phosphoglucomutase	*Pgm*	5.4.2.2	2	TC8	L	S
Phosphogluconate hydrogenase	*6pgd*	1.1.1.44	1	TC6	G	S
Glucose-6-phosphate isomerase	*Pgi*	5.3.1.9	2	TC8	M	S
L-iditol-dehydrogenase	*Sdh*	1.1.1.14	2	TC8	L	S
Superoxide dismutase	*Sod*	1.15.1.1	1	TG	L	CA

1 EC = Enzyme Commision Number
2 See Murphy et al. (1996) and Hebert and beaton (1993) for buffer recipes
3 Tissue : L=Liver; M=Muscle; and G=Gill
4 Electrophoresis ; S=Starch gel; and CA=Cellulose acetate gel

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phoresis and Hebert and Beaton (1993) for cellulose acetate gel electrophoresis (Table 1). Staining procedures utilised agar overlays for all systems except Esterase (EST), Glutamate oxaloacetate (GOT) and General protein (GP). For EST and GOT enzyme gels were presoaked in substrate solution before adding fast blue BB. General protein (GP) resolved by staining gels with a solution of amido black followed by a series of washes in a methanol : distilated water : acetic acid (5:5:1) solution. In order to determine the identical zymograms, the other galaxiid fishes (*G. maculatus*, *G. truttaceus* and *G. occidentalis*) were included as an output group and used as comparative purposes.

4. Data analysis

Electrophoretic banding patterns were interpreted using standard procedures (Richardson *et al.*, 1986) and converted to genotypes. Genotype data were then analysed using the BIOSYS-1 program (Swofford & Selander, 1981) which demonstrated levels of genetic variability within and between populations of this species. From this program, the frequen-

for each population were also determined.

Genetic relationships among populations were computed using genetic identity (Nei, 1978; 1987) and Rogers' genetic distance (Rogers, 1972) and summarised by clustering using the unweight pair group arithmetic average method (UPGMA) (Richardson *et al.*, 1986). UPGMA clustering was carried out using the NEIGHBOUR program from the PHYLIP (v. 3.57) package (Felsentein, 1995) and dendogram were manipulated and edited using the Tree View (v.1.5) program.

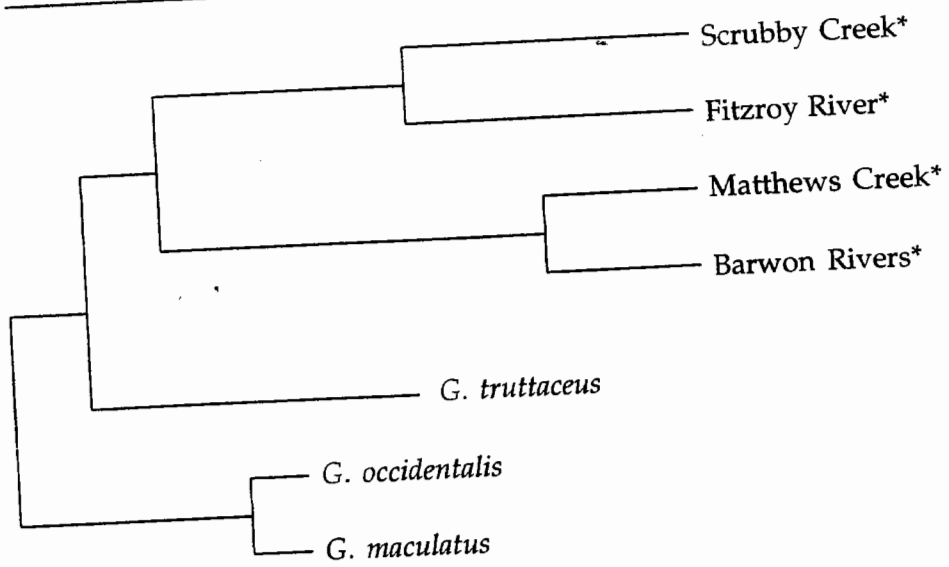
RESULTS AND DISCUSSION

In this study, initially a total of 41 loci scored from 23 enzymes and general protein stains were judged to be under independent genetic control for all *Galaxias* species. However, from the 41 enzymatic loci, 3 loci (*Fbp-1*, *Gp-5* and *Gp-6*) of *G. olidus* had blank bands which could not be scored. The remaining 38 loci were then screened for all *G. olidus* samples and the genetic basis of variation within these loci is inferred as follows. In 35 loci, all individuals exhibited single bands of identical mobility. This is assumed that these proteins are encoded by single, monomorphic loci at which every individual is homozygous for the same allele. In addition, analysis of allozyme data revealed that single and three banded phenotypes were found at *Gpdh-1*, *Gpdh-2* and *Mdh*. Such phenotypes indicate that the active enzyme or protein is a dimer and the typical three banded phenotypes represent heterozygotes.

The 38 allozyme loci are symbolised by the italicised abbreviation given in Table 2, with a numerical suffix where needed to identify particular zone. Alleles encoding the most common allozyme in each zone are designated 100. Each other allele is designated by subtracting or adding to 100 the number of millimeters by which the variant differ in mobility from the most common allele (in this study, the author used allozyme data of *G. maculatus* as a marker to determine each allele). From an inspection of this table, it can be seen that variation at the *Gpdh-2* and *Mdh* loci is principally responsible for genetic differences between the two genetic groups identified in the preceding analyses. Two alleles were observed at the *Gpdh-2* locus with the Gpdh-2*52 allele occuring at high frequency ranging from 0.90 to 1.00 within the predominately western group. At the *Mdh* locus, the Mdh*80 allele is found within all of this group individuals, but is absent from all the eastern samples. In contrast, the eastern populations are characterized by the alternative Gpdh-2*74 allele which ranges in frequency from 0.80 to 1.00. Further, in this group the Mdh*80 allele is absent, all individuals are homozygous for Mdh*88 allele. Within the western populations, genetic divergence is also found between Fitzroy River and Scrubby Creek populations at the *Gpdh-1* locus. High frequency allele appears in Scrubby Creek population (Gpdh-1*90; 1.00) and low in Fitzroy River population (Gpdh-1*90; 0.10). Conversely, the Gpdh-1*100 allele exhibited in Fitzroy River samples at high frequency (0.90) is absent in Scrubby Creek population. A possible explanation of this genetic differences is that the exchange genetic material between these populations is likely to be restricted. This was also supported by the level of variation of these populations which was found to be lower than those of diadromous galaxias populations (*G. maculatus* and *G.*

Table 3. Summary of genenic similarity and genetic distance values among 4 populations of *G. olidus*. Below diagonal gives Nei's (1978) unbiased genetic identity and above diagonal gives Rogers (1972) genetic distance

Sample	FITZ	SCRU	MATH	BARW
Fitzroy River (FITZ)	*	0.024	0.044	0.049
Scrubby Creek (SCRU)	0.980	*	0.068	0.068
Matthews Creek (MATH)	0.964	0.936	*	0.010
Barwon River (BARW)	0.956	0.936	0.999	*



* *G. olidus*

Figure 1. UPGMA phenogram derived from Rogers' (1972) genetic distance for samples of *G. olidus*. Samples of *G. maculatus*, *G.*

patterns are also required to support this genetic data.

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THE EFFECTS OF CCC AND SAWDUST OF COCONUT FIBRES AS MULCH ON THE GROWTH AND RHIZOME YIELD OF EDIBLE CANNA *(Canna edulis* Ker.)

Kumala Dewi*, L. Hartanto Nugroho* and B. Twitchin**

ABSTRACT

Dewi, K., L. H. Nugroho, and B. Twitchin. 2000. The effects of CCC and sawdust of coconut fibres as mulch on the growth and rhizome yield of edible canna (*Canna edulis* Ker.). *Biologi* 2(9): 499–513.

The effects of CCC and sawdust of coconut fibre as mulch on the growth and rhizome yield of edible canna (*Canna edulis* Ker.) has been observed. Edible canna was grown at 0.5m x 0.5m spacing in the experimental field. Split-Plot was used as an experimental design. The main plot is CCC application which consists of 4 levels of concentration (C_0 = 0 ppm, C_1 = 500 ppm, C_2 = 1000 ppm and C_3 = 2500 ppm), the sub-plot is mulch application (M_0 = without mulch and M_1 = with mulch).

CCC application of 500 ppm on plants grown with mulch (treatment C_1M_1) gave the highest harvest index, starch content and reducing sugar content in rhizome. Increasing concentration of CCC applied, however, reduced the plant height, internode length and reducing sugar in leaf. Total leaf area decreased by CCC application both in M_0 and M_1 plants. The greater concentration of CCC applied the greater reduction in leaf area observed. On the other hand, total chlorophyll content increased by increasing CCC concentration.

The number of vascular bundle in stem (internode) was not affected by CCC application but the stem diameter and trachea diameter was smaller in plants treated with higher CCC concentration (1000 ppm and 2500 ppm).

The chromatograms of leaf and rhizome extract gave an indication that endogenous GA_{19}, GA_{20} and GA_1 occurred in edible canna. The level of active GA_1 seems influenced by the CCC application at 1000 ppm and 2500 ppm. The results demonstrate that hormones metabolism (Gibberellin) have role in assimilates alocation proccess.

Key word : *Edible canna (**Canna edulis** Ker.), rhizome, saw-dust of coconut fibre, mulch*

Abbreviation : CCC (2-chloroethyl trimethylammonium chloride), GAs (Gibberellins), HPLC (High Performance Liquid Chromatography), GC-MS-SIM (Gas Chromatography-Mass Spectrometry-Selected Ion Monitoring)

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