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IDENTIFICATION OF NINE SPECIES OF MORAY EEL BY SDS-PAGE

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***ABSTRACT.* –** Moray eels are commercially important fish species in Taiwan. These fishes are distributed in the tropical and subtropical coral reef and are edible. To obtain useful information for identifying the species of moray eel that might be used in processed fish products, nine fresh moray eels including *Gymnothorax favagineus, G. fimbriatus, G. flavimarginatus, G. meleagris, G. pseudothyrsoideus, G. undulatus, G. albimarginatus, G. javanicus* and *G. kidako* were assayed using the electrophoresis method of SDS-PAGE. The SDS-PAGE patterns of myofibrillar and sarcoplasmic proteins for the nine fresh moray eel species showed species-specific protein bands of 30 kD. The species of moray eel meat that may occur in processed fish might therefore be identified through comparison of the myofibrillar and sarcoplasmic proteins revealed by SDS-PAGE patterns.

***KEY WORDS.* –** Moray eel, species identification, sarcoplasmic protein, myofibrillar protein, SDS-PAGE.

INTRODUCTION

Moray eels are distributed throughout tropical and subtropical coral reefs and are edible. They form a commercially-important and expensive category of fish in markets in Taiwan and are usually cut into several fish fillets before their sale. Once the fillets are cut and separated it becomes difficult for species identification of moray eel by examining only the 'fillet morphology'.

Recently, in April 2004, a poisoning incident occurred through the ingestion of processed moray eel meat at Fugil fishing port, Taipei, Taiwan. This food poisoning is well known as 'ciguatera'. The major toxic component is ciguatoxin, which has been isolated from the moray eel *Gymnothorax javanicus* (Lewis, 2001). In order to prevent the sale of such a toxic moray eel species it has therefore been crucial to develop analytical methods for indentifying the species of fresh moray eel fillets for sale at fish markets.

Several electrophoretic methods have been employed to differentiate species of seafood or seafood products (AOAC, 1990). Other feasible methods have been subsequently applied in identifying fish species; these include: sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) (An et al., 1988; Chen & Hwang, 2002; Chen et al., 2002; Mackie, 1997; Rehbein et al., 1999; Pineiro et al., 1999), urea-isoelectric focusing (urea-IEF) (Chen et al., 2003), two-dimensional gel electrophoresis (2D) (Martinez & Friis, 2004), high performance liquid chromatography (HPLC) (Wang et al., 2005), immunoassay (Lopata et al., 2002), capillary electrophoresis (CE) (Vallejo-Córdoba & Cota-Rivas, 1998) and DNA techniques (DeSalle & Birstein, 1996). The aims of this study are to establish basic data of SDS-PAGE patterns and densities of sarcoplasmic and myofibrillar proteins in nine commercial moray eel species in Taiwan. The species of causative fresh moray eel fillet might then be identified when comparing the SDS-PAGE patterns of sarcoplasmic and myofibrillar proteins with those of commercial moray eel species.

MATERIALS AND METHODS

Samples of nine moray eel species, *Gymnothorax favagineus* (GFA), *G. fimbriatus* (GFI), *G. flavimarginatus* (GFL), *G. meleagris* (GME), *G. pseudothyrsoideus* (GPS), *G. undulatus* (GUN), *G. albimarginatus* (GAL), *G. javanicus* (GJA) and *G. kidako* (GKI) were purchased from seafood markets in Keelung, Taiwan. Each species was represented by at least three specimens. The body weight and body length of the tested moray eel samples are shown in Table 1. All specimens and the causative sample (CS) of moray eel fillet (250 g) provided by the poisoning victims were stored at −20° until use.

Two reagents were used to extract the fish proteins. Two phosphate buffers were used for extracting sarcoplasmic and myofibrillar proteins: 15.6 mM Na_2HPO_4 – 3.5 mM KH_2PO_4 (ionic strength, I = 0.05, pH 7.5) and 0.45 M KCl – 15.6 mM Na_2HPO_4 – 3.5 mM KH_2PO_4 (I = 0.50, pH 7.5), respectively. All extracts were supplemented by 0.1 mM phenylmethylsulfonyl fluoride (PMSF), 10 mM EDTA, and 0.01% (w/v) sodium azide to inhibit proteases and microbial growth.

The extracts of fish sarcoplasmic and myofibrillar proteins were prepared according to the procedure described by Hashimoto et al. (1979). All the operations were performed at 4°C. A 2 g sample was homogenized with 5 volumes of phosphate buffer (I = 0.05, pH 7.5) for 1 min using a Polytron-Aggregate® (setting 4.0, Kinenatica® Littau/Luzern, Switzerland). The homogenate was centrifuged at 10,000 *g* for 15 min, using Himac CF15D2 with rotor RT15A6 (Hitachi, Japan). The precipitate was extracted by the same procedure again. The 2 supernatants were combined and used as the sarcoplasmic proteins fraction. The residue from the above was homogenized with 5 volumes of 0.45M KCl-phosphate buffer (I = 0.50, pH 7.5) in a Waring blender equipped with a baffle to prevent frothing and centrifuged. The precipitate was similarly homogenized and centrifuged again. Both supernatants were combined and used as the myofibrillar proteins fraction.

The protein concentration was determined by bicinchoninic acid method (Smith et al., 1985). The protein extracts were adjusted with sample buffer to 1 mg/ml for Coomassie blue staining.

SDS-PAGE was performed according to the modified procedure of Laemmli (1970) and O'Farrell (1975), using a Mini-Protein unit (Bio-Rad, Richmond, Calif.). Slab gels consisted of a separating gel (15.0%), which was polymerized for 1 hr, and a stacking gel (4.0%), which was poured 30 min before sample application. The 5 µl of protein standard and 10 µl of samples were applied in the wells of the gel. Electrophoresis was carried out at a constant voltage of 150 volts when the tracking dye reached the separating gel. Electrophoresis was completed when the dye front reached the bottom of the gel.

Gels were stained either with Coomassie blue reagent, 0.1% (w/v) Coomassie blue R-250 in 40% methanol and 10% acetic acid. After staining, gels were destained in 10% methanol and 10% acetic acid. Molecular weights were determined by comparing relative mobilities of protein bands to standard proteins (Weber et al., 1972). Protein standards were obtained from Bio-Rad (Broad range kit, myosin 211.0 kD; β-galactosidase 121.0 kD; bovine serum albumin 100.0 kD; ovalbumin 54.4 kD; carbonic anhydrase 38.7 kD; soybean trypsin inhibitor 29.8 kD; lysozyme 20.0 kD; aprotinin 7.3kD).

The gels were scanned by Gel Dox® and the acquired images were analyzed with the Quantity One® (Bio-Rad). Images of protein profiles were stored in computer memory and molecular weights were estimated by comparing Rf values on the gel with those of the protein standards. The relative amount of each protein band in SDS-PAGE pattern was the optical density of each protein band to total optical densities of all protein bands in the same lane. The protein amounts applied in analysis were approximately 20 µg for Coomassie blue staining.

RESULTS AND DISCUSSION

The concentrations of sarcoplasmic protein and myofibrillar protein extracted from moray eel muscle are shown in Table 2. Judging from the high molecular weight region (> 30.0 kD) of two protein extracts with Comassie blue staining, there were no species-specific protein bands among these moray eel species (Figs. 1, 2). In addition, the density of high molecular weight region (> 30.0 kD) were too high to differentiate moray eel species. The bands at the high molecular weight region are apparently not useful in identifying moray eel species.

All tested moray eel species could be differentiated by using the SDS-PAGE patterns of sarcoplasmic proteins in the low molecular weight region (< 30.0 kD) (Fig. 1). The characteristic protein bands were as follows: 7.3, 7.7, 9.3, 13.1, 14.2 kD for GFA; 7.3, 9.3, 13.1 kD for GFI; 6.2, 7.3, 9.3, 12.2 kD for GFL; 7.1, 11.1, 12.2 kD for GME; 5.7, 7.1, 7.7, 10.5, 12.2, 13.1 kD for GPS; 7.7, 8.9, 10.2, 14.2 kD for GUN; 7.1, 10.2 kD for GAL; 8.1, 12.2, 13.1 kD for GJA; 7.1, 8.9, 12.2, 14.2 kD for GKI and 8.1, 12.2, 13.1 kD for CS. However, the protein bands in the region of 6.1 to10.0 kD were blurred and clustered together so that they could not be used for moray eel species identification.

The causative moray eel established the same SDS-PAGE patterns of sacroplasmic proteins as *G. javanicus*. Therefore, SDS-PAGE patterns of sarcoplasmic proteins stained with Coomassie blue, if carefully prepared, could be useful in identifying moray eel species.

The low molecular weight region (< 30.0 kD) of the SDS-PAGE patterns of myofibrillar proteins, comprising a number of faint bands including myosin light chains,

Table 1. The body weight and length of the tested moray eel samples.

Sample	Body weight (g)	Body length (cm)
Gymnothorax favagineus (GFA)	541–1,313* (939 ± 387)	72–90 (82 ± 9)
Gymnothorax fimbriatus (GFI)	1,169–1,359 (1,280 ± 99)	87–90 89 ± 2
Gymnothorax flavimarginatus (GFL)	938–2,617 (1,679 ± 857)	79–105 (92 ± 13)
Gymnothorax meleagris (GME)	1,054–1,363 (1,213 ± 155)	72–81 (77 ± 5)
Gymnothorax pseudothyrsoideus (GPS)	541–823 (641 ± 158)	67–74 (70 ± 4)
Gymnothorax undulates (GUN)	1,022–1,303 (1,205 ± 159)	79–89 (84 ± 5)
Gymnothorax albimarginatus (GAL)	173–980 (526 ± 413)	49–103 (73 ± 27)
Gymnothorax javanicus (GJA)	554–3,350 (1,554 ± 1,558)	71–107 (86 ± 19)
Gymnothorax kidako (GKI)	983–1,189 (1,072 ± 106)	79–87 (84 ± 4)

* Data represent range and mean ± SD in the parentheses (n = 6).

troponins, and parvalbumins (7.0 to 30.0 kD), showed some species-specific protein patterns (Fig. 2). Inspection of the percentage compositions of myofibrillar proteins extracted by phosphate buffers with the low molecular weight (< 30.0 kD) are shown in Fig. 2. Inspection of the 10.3 kD to 27.0 kD protein band region shows that the characteristic protein bands for each moray eel were as follows: 13.3, 19.2, 23.1, 24.7 kD for GFA; 13.3, 19.2, 23.1 kD for GFI; 10.3, 13.3, 17.0, 19.2, 23.1 kD for GFL; 10.3, 11.6, 13.3, 15.6, 19.2, 23.1, 14.7 kD for GME; 10.3, 13.3, 15.6, 19.2, 23.1 kD for GPS; 10.3, 13.3, 17.0, 19.2, 23.1 kD for GUN; 13.3, 15.6, 17.0, 19.2, 23.1, 24.7 for GAL; 10.3, 13.3, 19.2, 23.1, 24.7 kD for GJA; 11.6, 13.3, 15.6, 19.2, 23.1, 24.7 kD for GKI and 10.3, 13.3, 19.2, 23.1, 24.7 kD for CS.

Therefore, the moray eel species could be identified by the pattern of the low molecular weight region in the myofibrillar proteins when band density was taken into account. Because the species-specific proteins of myofibrillar proteins were more obvious than those of sarcoplasmic proteins, all the tested moray eel could be more easily differentiated by myofibrillar protein patterns. The causative moray eel established similar to SDS-PAGE pattern of myofibrillar proteins to that of *G. javanicus.* Hence, the species of the causative moray eel was identified as *G. javanicus.*

This result was similar to other reports (Chen & Hwang, 2002; Chen et al., 2003; Barai et al., 1992). In this study, the individual mobility variation between fish of the same species was not measured. Protein band density appeared somewhat variable and this may have had a slight effect on our ability to discriminate moray eel species.

Besides the use of phosphate buffers as extractants when comparing the protein bands, other extractants may be used; 1% SDS and 8M urea are reported to be more suitable for processed seafood (Mackie, 1997; Rehbein et al., 1999; Pineiro et al., 1999). Therefore, in order to establish best method to identify the species of processed moray eel products, further study is required.

CONCLUSION

Species identification of all tested moray eels could be achieved by examining the lower molecular weight protein bands following SDS-PAGE of any of the above two extracts along with the densities of the characteristic protein bands using staining. The protein bands below 30.0 kD in SDS-PAGE pattern from two different protein extracts were thus more useful for identifying moray eel species than those of higher molecular weight proteins (> 30.0 kD). The phosphate buffers (I = 0.05 and I = 0.50) were suitable extractants to use in order to differentiate the SDS-PAGE patterns of different moray eel species. Moreover, the causative moray eel fillet implicated in the food poisoning was identified as *G. javanicus.*

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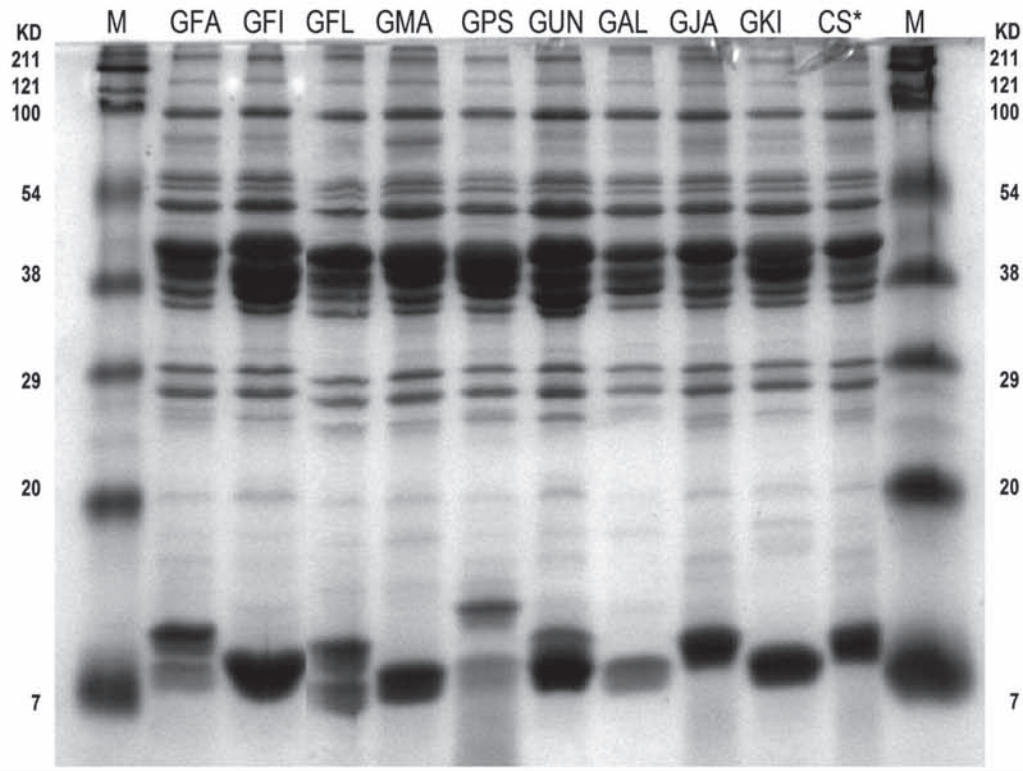
Table 2. The concentrations of sarcoplasmic protein and myofibrillar protein extracted from moray eel muscle.

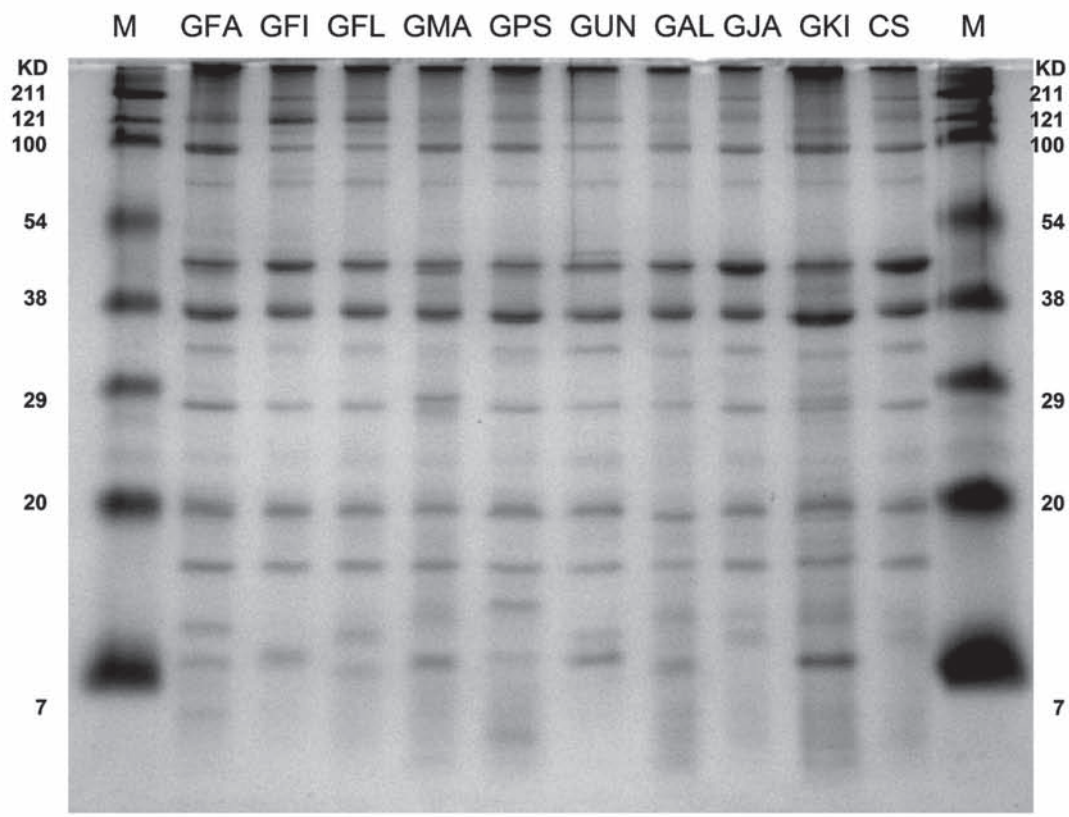
	GFA*	GFI	GFL	GME	GPS	GUN	GAL	GJA	GKI	CS
Sarcoplasmic protein (mg/ml)	2.88 ± 0.01	3.47 ± 0.02	2.81 ± 0.06	3.58 ± 0.04	2.98 ± 0.01	3.49 ± 0.03	2.34 ± 0.03	2.37 ± 0.05	3.45 ± 0.12	2.41 ± 0.06
Myofibrillar protein (mg/ml)	4.32 ± 0.03	5.44 ± 0.03	4.66 ± 0.00	4.36 ± 0.03	4.05 ± 0.03	4.86 ± 0.04	4.64 ± 0.04	5.10 ± 0.20	4.35 ± 0.07	4.58 ± 0.09

* GFA, *Gymnothorax favagineus*; GFI, *G. fimbriatus*; GFL, *G. flavimarginatus*; GME, *G. meleagris*; GPS, *G. pseudothyrsoideus*; GUN, *G. undula*tes; GAL, *G. albimarginatus*; GJA, *G. javanicus*; GKA, *G. kidako*; CS (causative sample) was the moray eel fillet provided by the victims.



MW (kD)	GFA	GFI	GFL	GME	GPS	GUN	GAL	GJA	GKI	CS
29.2	3.56±0.21	3.30±0.23	3.18±0.16	3.92±0.18	3.19±0.10	3.39±0.12	3.65±0.10	3.72±0.09	3.78±0.05	3.68±0.17
27.3	4.00±0.05	3.90±0.02	4.36±0.22	4.34±0.14	3.91±0.11	4.68±0.19	3.63±0.13	4.55±0.18	4.42±0.17	4.37±0.12
25.7	4.77±0.08	4.38±0.20	3.14±0.17	4.61±0.09	5.93±0.08	4.76±0.26	4.61±0.17	5.67±0.05	4.09±0.03	5.62±0.05
22.9	2.18±0.01					1.07±0.09				
20.3	2.13±0.20	4.22±0.19	3.12±0.15	3.57±0.10	2.16±0.13	2.56±0.21	1.53±0.21	1.90±0.03	3.03±0.09	1.39±0.14
16.6	1.54±0.11	1.52±0.17		3.41±0.21	1.24±0.15	1.96±0.11	2.09±0.10	2.26±0.18	1.79±0.10	2.35±0.08
14.2	2.21±0.12					2.72±0.16			3.04±0.18	
13.1	1.30±0.09	3.24±0.13			1.51±0.23			2.26±0.22		2.83±0.16
12.2			12.26±0.11	2.04±0.10	2.31±0.11			1.54±0.05	1.74±0.21	1.59±0.04
11.1				1.50±0.20						
10.5					8.95±0.11					
10.2						3.01±0.12	2.27±0.02			
9.3	10.99±0.17	4.94±0.16								
8.9			11.83±0.10			3.56±0.00			3.33±0.13	
8.1								17.94±0.20		17.01±0.00
7.7	3.04±0.27				4.55±0.17	17.48±0.05				
7.3	9.47±0.25	18.71±0.04	4.59±0.02							
7.1				18.66±0.19	5.37±0.14		16.67±0.13		19.07±0.19	
6.2			5.95±0.02							
5.7					3.17±0.08					
5.4								5.40±0.29		4.74±0.22

Fig. 1. SDS-PAGE patterns of sarcoplasmic proteins extracted with phosphate buffer from nine moray eel species with Coomassie blue staining. M, protein standards; GFA, *Gymnothorax favagineus*; GFI, *G. fimbriatus*; GFL, *G. flavimarginatus*; GME, *G. meleagris*; GPS, *G. pseudothyrsoideus*; GUN, *G. undulates*; GAL, *G. albimarginatus*; GJA, *G. javanicus*; GKI, *G. kidako*; CS: causative sample.



MW (kD)	GFA	GFI	GFL	GME	GPS	GUN	GAL	GJA	GKI	CS
29.7								2.61±0.08		2.54±0.32
28.9	1.75±0.22	1.02±0.08				1.09±0.09	1.69±0.23		2.41±0.01	
27.1	4.20±0.01	3.80±0.16	4.10±0.31	7.10±0.02	3.94±0.10	3.98±0.33	4.42±0.30	3.95±0.32	4.84±0.09	4.40±0.21
24.7	0.83±0.21			1.23±0.04			2.58±0.08	1.24±0.12	2.48±0.18	1.24±0.21
23.1	3.07±0.10	3.24±0.22	4.26±0.10	2.87±0.07	3.37±0.03	5.96±0.21	3.53±0.21	3.78±0.18	3.62±0.19	3.53±0.29
19.2	10.12±0.53	8.61±0.22	6.18±0.17	6.78±0.15	7.46±0.20	7.21±0.02	2.47±0.09	7.97±0.21	5.94±0.04	7.78±0.22
17.0			2.90±0.02			2.53±0.02	3.83±0.05			
15.6				2.52±0.08	1.29±0.19		2.56±0.02		2.33±0.28	
13.3	4.93±0.32	4.40±0.28	4.67±0.11	4.00±0.19	3.64±0.14	4.80±0.08	3.45±0.10	5.50±0.02	3.70±0.09	5.49±0.23
11.6				1.35±0.13					1.34±0.11	
10.3			3.18±0.04	6.55±0.18	1.53±0.01	1.10±0.09		2.06±0.09		2.05±0.15
9.1	4.94±0.28	2.33±0.15				1.13±0.14	4.33±0.24	2.75±0.20	6.25±0.03	2.53±0.18
8.7			4.66±0.03		4.24±0.22		1.22±0.19			
8.1						2.64±0.11		5.03±0.19		5.14±0.19
7.4	3.90±0.03	6.93±0.21	4.02±0.21	4.81±0.19	1.50±0.17	8.56±0.08	5.72±0.09		6.45±0.16	
6.7								2.48±0.08		2.43±0.09
6.3	2.23±0.09	1.28±0.10	2.29±0.12	6.34±0.09	4.07±0.00					
5.3	6.69±0.12	4.67±0.15	2.23±0.01							
5.1					2.72±0.03	4.59±0.23	3.43±0.04		3.12±0.13	

Fig. 2. SDS-PAGE patterns of myofibrillar proteins extracted with phosphate buffer from nine moray eel species with Coomassie blue staining. M, protein standards; GFA, *Gymnothorax favagineus*; GFI, *G. fimbriatus*; GFL, *G. flavimarginatus*; GME, *G. meleagris*; GPS, *G. pseudothyrsoideus*; GUN, *G. undulates*; GAL, *G. albimarginatus*; GJA, *G. javanicus*; GKI, *G. kidako*; CS: causative sample.

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