CHARACTERIZATION OF THE SIPHONAL MANTLE OF *TRIDACNA CROCEA*

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Abstract

Among the eight species of Family Tridacnidae, Tridacna crocea is the smallest and identified as the most abundant tridacnids in reefs around the Philippine archipelago. This investigation describes the characteristic mantle pattern and color of T. crocea and correlates them to genetic structure; verifies the characteristic mantle color and pattern of T. crocea underwater and differentiates it from its closely related species, T. maxima in the field. Tissue samples of Tridacna crocea and T. maxima were collected and preserved in 95% alcohol. Prior to any mantle collection, each clam was photographed.Thirteen mantle patterns were identified from the 174 Tridacna crocea individuals of six reef areas: Pamilacan, Tanon Strait, Carbin, Camiguin, Southeastern Samar and Spratlys. Results revealed that Tridacna crocea can be distinguished from T. maxima in the field by the appearance and arrangement of their hyaline organs. Moreover, analysis on genotype-phenotype correlation using the T. crocea mantle morphology/color, found no significant relationship between the mantle morphs and genetic structure of the individuals.

Keywords: *siphonal mantle, iridophores, hyaline organs, Tridacna crocea, T. maxima*

Introduction

Along with the eight species of Family Tridacnidae, *Tridacna crocea* is the smallest and the most abundant tridacnids in reefs around the Philippine archipelago even with the existence of its commercial harvesting (Ravago-Gotanco *et al*., 2007; Juinio-Menez, *et al*., 2003; Calumpong and Cadiz, 1993; Gomez & Alcala, 1988; Junio, et al., 1988; Alcala, 1986). This species, in its natural environment, can be recognized by its pattern of being entirely locked up in coral pockets with free margins of its valves nearly flush with the substrate. (Rosewater, 1965). This habit distinguishes *Tridacna crocea* from its closely related species, *T. maxima* which subsists in relatively shallower burrows in coral; therefore its shell obtrudes halfway from the coral rubble, but which is also firmly fastened to the substrate by a byssus.

The widely established taxonomic classification of the family Tridacnidae was based on shell morphology and by Rosewater (1965). He described *Tridacna crocea* and

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T. maxima as having opposed valves with a well-defined byssal orifice without tightly fitting teeth. However, the valves of *T. crocea* are usually quite smooth and stouter with depressed sculpture and are more triangularly ovate in shape (Fig. 4.1A); although sometimes nearly scaleless, *T. maxima* usually does not have the shell sculpture so reduced and the shape of its valves tend to be more triangularly elongated. (Fig.4.1B). Rosewater (1965) noted other dissimilarities between the two which include the interdigitating projections of the dorsal margins of the valves, the number of riblets on the radial folds, the relative lengths of byssal orifices and the shapes of the adductor-retractor muscle scar complexes.

However, there is a considerable overlap in mantle coloration and pattern between these two species. The mantle is an extension of the inhalant and exhalent siphons and is also referred to as siphonal tissue. It consists of the bulk of the zooxanthellae as well as the fixed cells known as iridophores that hold pigments which primarily shelter the clam against unwarranted light and UV radiation. These pigments have color range of blue to brown or green to yellow. These pigments and their combinations are the reason for the extensive chains of colors and patterns that are found in these clams.

As cited by Rosewater (1965), both species are remarkably variable in these characters, but this may be owed to convergence where these characters are subject to no strong selective pressures. Like *T. maxima*, mantle color in *T. crocea* runs an array of brilliant green, blue, purple and brown with great pattern variation. According to Rosewater (1965), color brilliancy in *T. maxima* may vary in widely separated geographic regions. Observation showed that the mantle colors tend to be extremely bright in Eniwetok, marshal Island, as well as in the Great Barrier Reef. However, the colors were observed to be more subdued like in *T. crocea* at Andaman Sea in Malaysia and Thailand and off southern Sumatra. This phenomenon may be due to differences in the conditions of the animals at the time the observations were made, to a real geographic variation based on genetic difference, or to environmental factors. The distinction between the two species as to their hyaline organs which tend to be concentrated along the edge of the mantle on papillae in *T. maxima* while in *T. crocea* they are more diffuse on the mantle surface is accentuated by Rosewater (1965). Even so, this character may be confusing underwater particularly when the two species crop up together in reef flats and coral rubble.

Based on the amendment done by Lucas *et al*. (1991) on Rosewater's taxonomy using morphologic characters, Family Tridacnidae has eight extant recognized species with two genera and three subgenera (*Tridacna*: subgenus *T. sensu stricto* (*T. gigas* Linnaeus, 1758); subgenus *Chametrachea*: *T. maxima* Roding, 1798, *T. squamosa* Lamarck, 1819, and, *T. crocea* Lamarck, 1819; subgenus *Persikima*: *T. derasa* Roding, 1798 and *T. tevoroa* Lucas, Ledua and Braley, 1991); and *Hippopus*: *H. hippopus* Linnaeus, 1758 and *H. porcellanus* Rosewater, 1982) (Rosewater, 1965, 1982; Lucas et al., 1991). The ensuing main groups was confirmed by Benzie and Williams (1998) using protein electrophoresis, on the other hand, they recommended that several characters, such as, the lack of boring into coral, the small byssal orifice, the lack of attachment to the substrate as adults and the dorsally extended ctenidia, are not dependably diagnostic of the genus, subgenus or species levels. Many of the characters shared by pairs of subgenera inside *Tridacna* are also located in *Hippopus*, and are likely to be primitive and as a result give miniature clue to the evolutionary associations of the taxa concerned. The first three mentioned above are also part of a set of ecologically plastic and functionally inter-related characters.

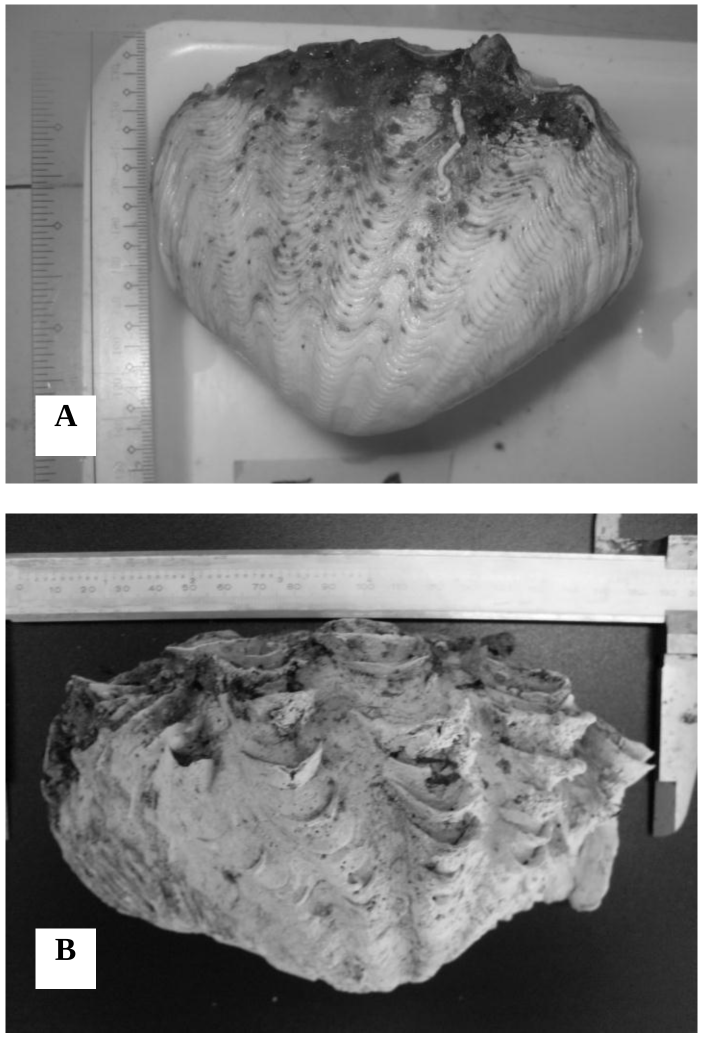


Fig. 4.1. Lateral view of the shell valves of A. *Tridacna crocea*. B. *T. maxima*.

This investigation describes the characteristic mantle pattern and color of T. crocea and differentiates it from its closely related species, T. maxima. This study verifies the characteristic mantle color and pattern of both species using genetic analysis. Furthermore, an attempt to correlate the clams' mantle morphology to their genetic structure was also done.

Research Method and Design

Phenotypic Differentiation (Mantle Color and Morphology). Sampled were fifty clams from each of the following reefs: Bolisong(Tanon Strait), Camiguin Island, Pamilacan(Bohol), Carbin (Sagay, Negros Occidental) Spratlys (South China Sea) and Camanga (Southeastern Samar). Each clam was photographed using an underwater digital camera, before a piece of mantle was cut from each clam. Pictures were downloaded and sorted according to mantle color and pattern. Samples were then grouped according to categories listed in Table 4.1.

Relating Mantle Morph to Genetic Structure. To scrutinize the genetic configuration that may be allied with the mantle pattern and color of the Tridacna crocea, only nine mantle characters (morphs 1 to 9) were considered. This was based on the individuals with DNA that were successfully sequenced. The DNA sequences were converted into a FASTA format and analyzed for genetic variation using the analysis of molecular variance (AMOVA) implemented in the ARLEQUIN version 3.

Differentiating Tridacna crocea from T. maxima. Tissue samples of Tridacna crocea and T. maxima were collected and preserved in 95% alcohol. Ten clams were picked up and identified as Tridacna crocea (5 individuals) and T. maxima (5 individuals) via a jury of three giant clam specialists from the underwater pictures acquired in the above-mentioned sampling. Selection and identification were based on mantle pattern and color. These were labeled TcP1-5 and TmP1-5, respectively. Another five T. crocea and six T. maxima that were positively identified by their shell and mantle morphology (collected and maintained in the hatchery for spawning purposes) were also sampled for comparison and were labeled TcL1-5 and TmL1-6. Six more samples which were randomly collected from the field as T. crocea were also included.

Genomic DNA was extracted using standard phenol-chloroform extraction method utilizing TNES-urea digestion buffer (6 M urea, 1M Tris-HCl pH 7.5, 5 M NaCl, 0.5 M EDTA, and 1% SDS or sodium dodecyl sulfate) as described in Wasko et al. (2003) and Proteinase K treatment. Partial sequences (500 bp) of the mitochondrial cytochrome c oxidase 1 (COI) gene were amplified with a specific primer for Tridacna crocea (Tridacna 1F 5'- ACC CTT TAY TTT TTA TTA GCA Y- 3'; Tridacna 3R 5'-CAA TGC TGT AAT CGC CAA TGA C–3') designed by Barber (2006). PCR products were visualized using 1% (w/v) agarose gel electrophoresis. Clones (forward and reverse stands) were sequenced on an ABI 377 or an ABI 3730 automated sequencer using Big Dye (Applied Biosystems, Foster City, CA) terminator chemistry. Nucleic acid sequences were subjected to BLAST/N (Altschul et al., 1990) searches at the National Center for Biotechnology Information (NCBI).ChromasPro version 1.33 available at http://www.technelysium.com.au/ ChromasPro.html, sequences were downloaded and subjected to BLAST search at http://0-www.ncbi.nlm.nih.gov.csulib. ctstateu.edu/blast/ blast FAQs.html.

Table 4.1. Mantle color and patterns generated from the field and picture observations used as criteria in grouping the samples. Photographs are found in Fig. 4.1. * not included in the AMOVA.

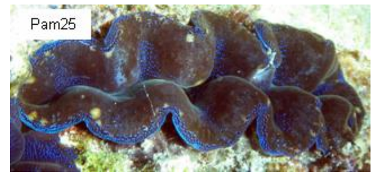
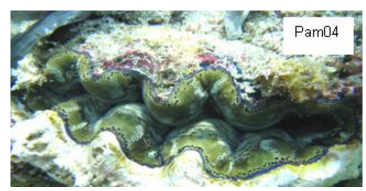
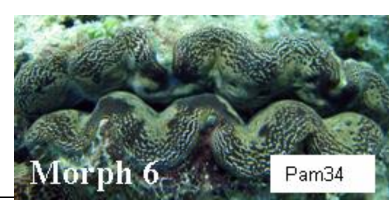
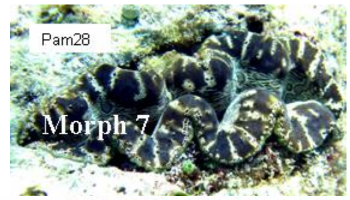
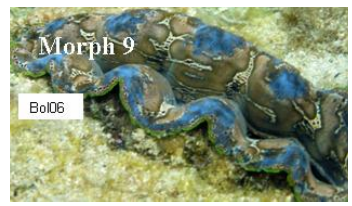
Mantle Colormorph	Description	Photo
1	Dark blue to chocolate brown overall mantle color. Eyespots or hyaline organs bounded by Iridescent blue circles. Mantle margins are also iridescent blue.	
2	Light brown with thin cream/white specks on central part of siphonal mantle. Hyaline organs/eyes are black in color bounded by thinner cream/tan margins. Mantle margins are usually light green.	
3	Light brown/tan with specks of dark brown, tan and cream/white. Hyaline organs black bounded by cream to white margins. Occasional warty protuberances are found on the lateral side of siphonal mantle folds. Mantle margins are generally yellow green and prominent.	
4	Tan with specks of white with blue mantle edge/margin.	
5	Dark brown with iridescent yellow or tan eye margins. Central siphonal mantle appeared to be plain.	
6	Dark brown with cream or white fine lines (horizontal) along siphonal mantle outer fold.	

Table 4.1. continued…

Mantle Colormorph	Description	Photo
7	Dark brown either with white specks horizontally spread on the siphonal mantle or white or cream rays regularly spaced on the outer mantle fold. Yellow green mantle margins.	
8	Tan to olive green with green or cream irregular spots scattered on the siphonal mantle. White stripes maybe present along the lateral side. Mantle margins are usually yellow green.	
9	Light to dark brown with scattered blue specks. Mantle margin yellow green.	
10*	Light brown on lateral side of siphonal mantle with warty protuberance. Dark brown on central portion with mint green specks.	
11*	Olive green mantle. Hyaline organs are black bounded by white margins. They occur in two or three layers or are scattered.	
12*	Dark brown with specks of blue on inner mantle fold. Mantle margins yellow green.	
13*	Siphonal mantle plain without obvious pattern. Sky blue to deep blue mantle margins. Hyaline organ margin same color with mantle margin.	

Results

Phenotypic Differentiation (Mantle Color and Morphology). Thirteen mantle patterns (Table 4.1) were identified from the 174 *Tridacna crocea* individuals of six reef areas: Pamilacan, Tanon Strait, Carbin, Camiguin, Southeastern Samar and Spratly. The occurrence and distribution of mantle patterns in each population are summarized in Fig.4.3. Pamilacan displayed nine mantle patterns (1, 2, 3,4, 5,6,7, 8 and 11), Spratly, only three (1, 2 and 4), Tañon Strait displayed 10 (1,3,5,6,7,8,9,10, 12 and13), Carbin has six (1,2,3, 6,7 and 13), Camiguin, five (1,2,3,7 and 8) and Southeastern Samar, nine (1, 2, 3, 4, 5, 6, 7, 8 and 13). Mantle morph 1 was the most common in all populations. Fig. 4.4 presents the percentage of each population exhibiting each mantle pattern. As shown, all patterns except for morphs 9, 10, 11 and 12 were found in all six sites.

Relating Mantle Morph to Genetic Structure. A total of 64 clam DNA sequences from five populations: Pamilacan, Carbin, Bolisong, Spratly and Southeastern Samar (Table 4.1) were analyzed. Pamilacan samples displayed six mantle patterns (morphs 1, 2, 4, 6, 7 and 8), Guiuan and Bolisong have five (morphs 1, 2, 3, 4 and 5 and morphs 1, 3, 5, 6 and 9, respectively), Carbin has three (morphs 1, 2 and 6) and Spratly, three (morphs 1, 2 and 4).

The 64 DNA sequences were grouped according to the nine mantle patterns and subjected to analysis of molecular variance (AMOVA). Results revealed no significant genetic variation among the morphs (F_{ST} = -0.03737, p = 0.93842) at 1023 permutations. As indicated in Table 4.2, different mantle morphs may even share the same haplotypes like in the case of haplotype 7. This result is in conformity with the results obtained by Laurent *et al.* (2002) on *Tridacna maxima* using allozymes. They found no relationship between color of the mantle and genetic structure of *T. maxima*, with individuals of different patterns showing similar genetic structures. Rosewater (1965) had reported variation in mantle color of giant clams in which he described several morphs *Tridacna maxima*. According to McMichael (1974), such variation in color can be due to genetic variation in the clam. However, Rosewater (1965) emphasized the role of zooxanthellae variation.

Differentiating Tridacna crocea from T. maxima. Sequences were subjected to BLAST search (BLASTN 2.2.18, Zheng Zhang et al., 2000). Among the photograph-based samples, all probable *Tridacna crocea* were verified exactly as *T. crocea*,(maximum score=800-900, but, the probable *T .maxima* turned out to be *T. crocea*. For the *T. maxima* (TmL1-6) and *T. crocea* (TcL1-6) samples identified by their mantle and shell morphology were exactly *T. maxima* and *T. crocea,* excluding TmL5 which failed to complement among any of the tridacnid CO1 sequences in GeneBank via the NCBI basic Tool Alignment Search Tool (BLAST). On the other hand, sequences of the seven "*T. crocea*" samples randomly collected from the field all matched with *T. maxima cytochrome c oxidase I* sequence during the BLAST search.

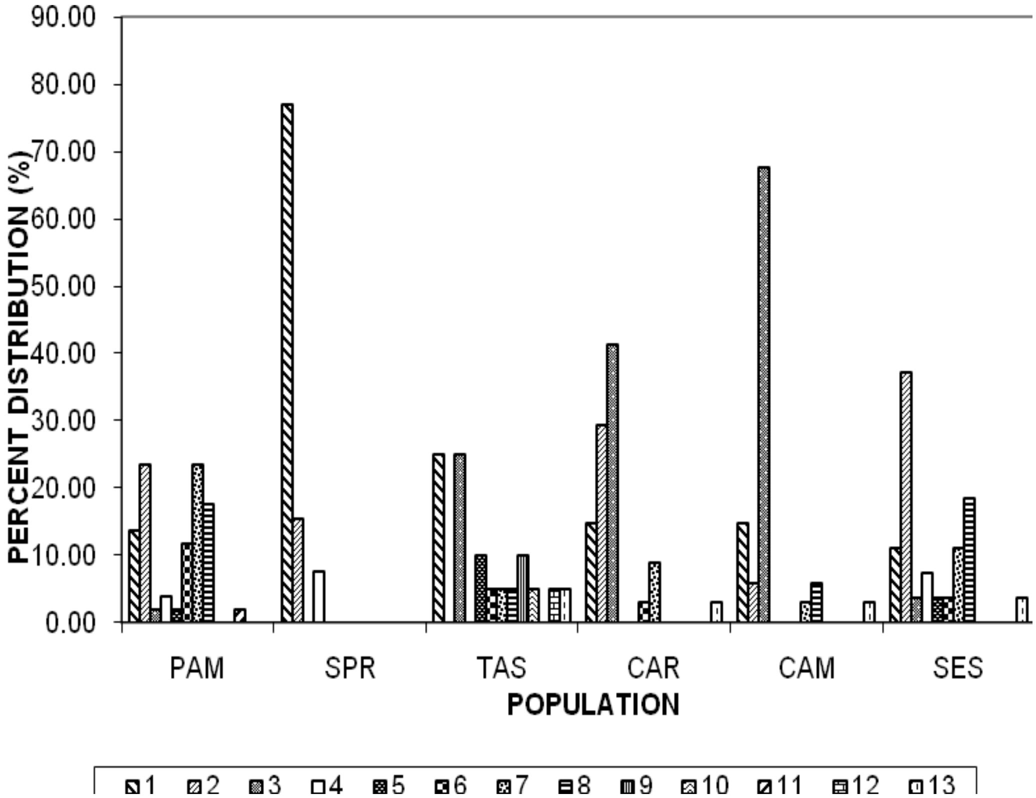


Fig. 4.3 Percent distribution of mantle patterns in each of the six populations. PAM- Pamilacan, SPR- Spratly, TAS –Tanon Strait, CAR- Carbin, CAM-Camiguin, SES- southeastern Samar.

Discussion

The only description Rosewater (1965) has given to differentiate *T. maxima* from *T. crocea* was wherein the former, they tend to be concentrated along the edge of the mantle on papillae in terms of their hyaline organs arrangement (Fig. 4.4. A) whereas in the latter, they are more disperse on the mantle surface (Fig.4.4.B & C). This difference has been observed in the present study. Specifically, these “dark spots” or “eyes” appeared as a distinct continuous line along the siphonal mantle margin in *T. maxima*. Moreover, these eye-like structures as called by Stasek (1966), typically were bounded by lighter margins usually white, cream and blue in *Tridacna crocea*, whereas in *T. maxima*, were not (Fig 4.4.B & C). This feature was consistently observed in the 64 samples of *T. crocea* with BLAST results matching with the *T. crocea* sequences in GeneBank. Likewise, 94.4% of the sequenced *T. maxima* samples exhibited the unbounded hyaline organs.

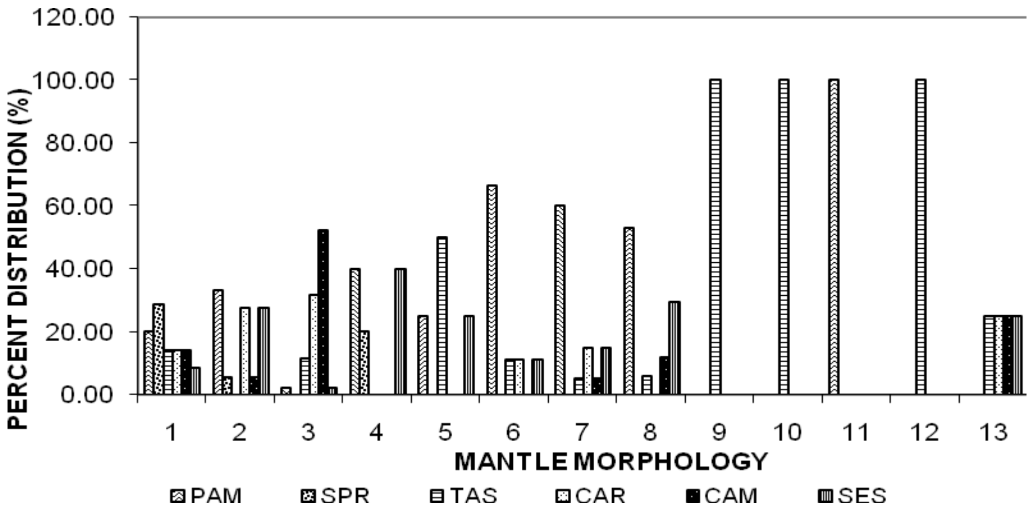


Fig. 4.4. Percentage of population exhibiting each mantle pattern. Codes are given in Fig. 4.2.

Relating genetic structure to mantle morphology in the present study does not provide new insights since no significant genetic difference according to mantle morphology was observed. To sum it up, according to Laurent *et al*., 2002, the lack of difference is not definite, given that it can be inferred as genetic homogeneity of *Tridacna crocea* and mantle pattern like color maybe a result of zooxanthellae and iridophores variation or local adjustment. Moreover, the non-significant variation may be simply due to the reason that the genetic marker (mitochondrial *cytochrome c oxidase 1*) used in the study is not linked to color pattern regulation. Moreover, *Tridacna crocea* can be distinguished from *T. maxima* in the field by the appearance and arrangement of their hyaline organs. These organs are bounded by white, iridescent blue or yellow circles. *T. crocea* has distinct mantle patterns but colors are overlapping with *T. maxima*.

Fig. 4.4. A comparison of the morphology and arrangement of the hyaline organs between *Tridacna maxima* and *T. crocea*. A. *T. maxima*. B. *T. crocea*. Please note the white circles around the *T. crocea* hyaline organs. C.

T.crocea showing the hyaline organs scattered on the siphonal mantle. *Photography by Dr. J.Estacion*.

Table 4.2. Haplotypes of *Tridacna crocea* and their mantle morphology.

Haplotype	Mantle Morphology								
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
2			1						
3									1
4			2				1		
5					1				
6			1						
7	2	4	2	1		3	4	2	1
9							1		
10	1								
11	3			1	1				
12	1								
13		1	1					1	
19							1		
20			1						
28	1								
29	2		1			1			
30		1							
31			1						
32	1								
33							1		
34		1							
36								1	
37	2							1	
41	1								
42	1								
43	1								
44	1								
50	2	1							
51					1				
52				1					
53	1								
54			1						

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Gotangco of UPMSI, Dr. Edgar Balbuena and Dr. Francisco Tabiliran of Jose Rizal Memorial State College.

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