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# Male Genetic Diversity of Siwa Brahmin Clan in Bali Based on Y-Chromosomal Microsatellites DNA

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## Abstract

The Brahmin clan in Bali consisted of two groups, that are Siwa Brahmin the descendant of Dang Hyang Nirartha and Boda Brahmin, the descendant of Dang Hyang Astapaka. Dang Hyang Nirartha came to Bali around 1480 and descended Siwa Brahmin clan, with consist of 5 sub-clans (Kemenuh, Manuaba, Keniten, Mas and Patapan). This Research was conducted to determine the molecular characters of Siwa Brahmin clan.

The research was conducted by employing the molecular marker microsatellite Y-chromosomal DNA from June to October 2013. There was 8 sizes of allele found, one in the DYS19 locus (200bp), three in DYS390 locus ( 203, 207 and 211bp) and two alleles were found in DYS393 locus ( 129 and 133bp) and in DYS395 locus (127 and 131bp). The highest frequency of haplotype, from 6 haplotypes recorded, was found in haplotype 2 (0.54) with the allele combination of 200, 207, 129, 127. This haplotipe was found in all of sub-clans: Kemenuh, Manuaba, Keniten, Mas, including sample from unidentified sub-clan. Haplotype 2 could be as the original haplotype of Siwa Brahmin.

**Keywords**: Siwa Brahmin clan, DNA microsatellite, allele, haplotype

## 1. Introduction

Balinese Hindu society today is divided into groups based on paternal genealogical lineage called clan or *soroh* (Balinese). The society developed from the acculturation between people who came to Bali island earlier, which is known as *Bali Mula* and people who came later that brought new thought what is called as Religion based on holly book of Hindu, Veda. Based on the history, Bali Island was inhabited by people who came from various place such as China, India, Central and East Java (Covarubias, 1956; Bellwood, 1985; Ardika, 1996; Wikarman, 1994). The profile of mtDNA collected from the teeth excavated from the tomb at Sembiran village, Buleleng Regency was found similar to that of Indian (Lansing *et al.,*, 2004). Karafet *et al.,*, (2005) found that 83.7% of Balinese men have Austronesian genes, 12% have Indian genes and only 2,2% representing gene from pre-Neolithic society. The new comers from various places enlarging Balinese community certainly brought various new genetic profiles and culture which will enrich the genetic structure, as well as the culture of Balinese.

Many people in Balinese society groups are function as Brahmin (Balinese: *Sulinggih*) to assist the cultural ceremony. They are many Sulinggih that are called differently depend on the clan. For example, Brahmin from Brahmin (Brahmana) clan is called *Pedanda*, Brahmin from Ksatria clan called Begawan, and Brahmin from Pasek clan called Sri Mpu. Brahmins (Pedandas) descendant in Balinese society was based genealogical lineage, which is grouped into two from two ancestors. Those are the group or clan of Siwa (Siva) Brahmin who descendant of Dang Hyang Nirartha and the clan of Boda Brahmin, the descendant of Dang Hyang Astapaka. The descendants of Brahmin clan was characterized by the name begin with *Ida Bagus* for the men and *Ida Ayu* for the woman (Dharma Gosana of Regency Jembrana, 2008; Wiana and Santeri, 1993). The existence of Brahmin in Bali was known far earlier than those the division of Brahmin clans (Siwa and Boda). They existed since the arrival of Rsi Markandea at the Caka Year of 80 (152 AD), the founder of the *Besakih* Temple (the largest Temple in Bali), then followed by Mpu Gana , Mpu Semeru, Mpu Gnijaya, and Mpu Kuturan (Gingsir, 2000; Dharma Gosana Kab. Jembrana, 2008; Sastrodiwiryo, 2010).

The population of Siwa Brahmin in Bali was more then of Boda Brahmin, which represented by the number of people and families. The Siwa Brahmin and its *Pedanda* were widespread through out Bali, while the Boda Brahmin was limited and can only be found in Bodakeling and Wanasari villages, Karangasem Regency; Tusan village, Klungkung Regency, and Sukawati village, Gianyar Regency. The dynasty or clan of Siwa Brahmin was started in fifty century (1480 AD) when the founding father, Dang Hyang Nirartha came from Java to Bali with his family. At that time, Gelgel was the empire of Bali governed by the King of Watu Renggong (Sidemen Dkk, 1983; Gingsir, 2000; Dharma Gosana Kab. Jembrana, 2008; Sastrodiwiryo, 2010). However, Riana (2011) reported that Dang Hyang Nirartha arrived in Bali about year 1489. The Siwa Brahmin was devided into five groups (sub-clans) which based on their wives of Dang Hyang Nirartha. He had five wives, the first was from Daha (Kediri-East Java) who descended Brahmin sub-clan Kemenuh. The second wife from Pasuruan (East Java) descended Brahmin sub-clan Manuaba. The third wife from Blambangan (East Java) desdended Brahmin sub-clan Keniten. The fourth wife from Mas (Gianyar-Bali) descended Brahmin sub-clan Mas, and the fifth wife

from Gading Wani (Jembrana-Bali) descended Brahmin sub-clan Patapan (Bek, 1959; Gingsir, 2000).
The molecular DNA microsatellite markers represent part of DNA that does not encode protein, so it does not related the quality human being. Microsatellite genetic markers have been used widely to asses the genetic variation among population (Weber and Wong, 1993; Bowcock *et al.,* 1994; Slatkin, 1995). Microsatellite DNA have the high mutation rate, thereby it is good to be employed to detect genetic differences among individuals and population (Weber and Wong, 1993, Bowcock *et al.,* 1994; Slatkin, 1995 and Hillis *et al.,* 1996; ). Microsatellite chromosom-Y DNA markers that are located at chromosome Y and descended from father to the sons only. Therefore, it is good to be used to asses the genetic lineage of Balinese who the families are based on patrilineal system that is the woman (bride) move and live to the man (bridegroom) house when they are married (Junitha, 2007). Microsatellite chromosom-Y markers has been used to determine genetic flows between caste in India (Mitchell *et al.,* 2006), Balinese society (Karafet *et al*., 2005) and for genealogical groups or clans in Bali (Junitha and Sudirga, 2007; Junitha *et al*., 2009; Junitha *et al*., 2012).
This research aim to investigate the type of DNA haplotype of Siwa Brahmin sub-clan Kemenuh, Manuaba, Keniten, Mas and Patapan in Bali, using four microsatellite chromosome-Y markers, DYS19, DTYS390, DYS393 and DYS395. The results were recorded and used to enrich the DNA database of clan exist in Balinese society.

## 2. Research Method

The research was conducted by employing the molecular marker microsatellite Y-chromosomal DNA from June to October 2013. Samples were collected with purposive sampling method, by identifying people of Siwa Brahmin clan from their name, from 1 city (Denpasar) and 8 regencies (Badung, Gianyar, Bangli, Klungkung, Karangasem, Buleleng, Negara and Tabanan) in Bali. The epithelium mucosa cell was swab using sterilized cotton buds from fifty five volunteers. Before sample collection, all volunteers were given the explanation about the research and they has to sign the inform concern as an agreement. DNA was extracted using phenol-chloroform method with alcohol precipitation. DNA samples were amplified in PCR using Mastermix Solution (*i*-Taq$^{TM}$) kit with four Y-specific microsatellite DNA primers (DYS19, DYS390, DYS395 and DYS395). The PCR was run 30 cycles in 52-55$^{o}$C annealing temperatures, then visualized by staining in silver nitrate (Tegelstorm, 1986). The amplicons were electrophoresis on 6% polyacrilamide gel (PAGE) running in 110 volt for 90 minutes. DNA typing was determined by plotting the distance migration of DNA amplicons on semi-log paper plot (Hutscinson, 2001). The genetic diversity was calculated following Parra *et al*., (1999).

## 3. Research Result

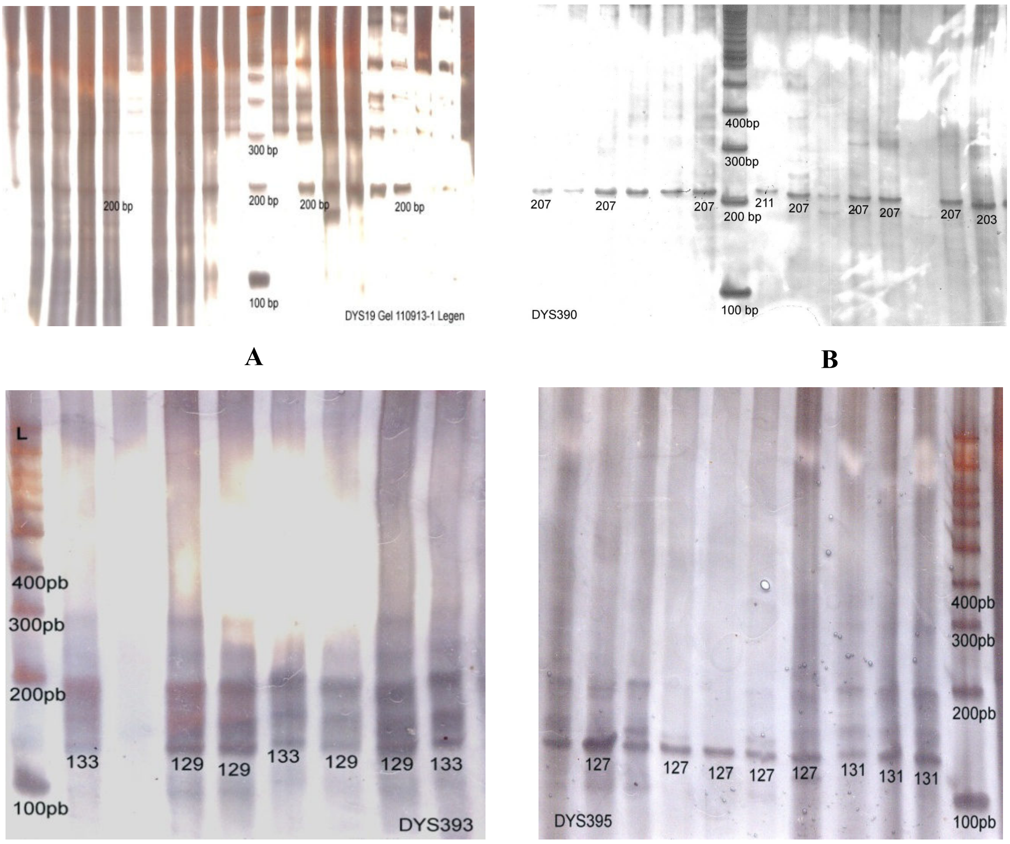
Fifty five buccal cells samples were collected from male Siwa Brahmin clan. Those were collected from Denpasar City and other eight regencies in Bali, namely: Karangasem, Klungkung, Bangli, Gianyar, Badung, Tabanan, Jembrana and Buleleng. Unfortunately, Patapan sub-clan could not be because, therefore the samples were derived only from four sub-clans, which were nine from Kemenuh, 29 from manuaba, 11 from Keniten and five from Mas. One of 55 volunteer probandus was collected from Jembrana Regency, but his sub-clan was not known (Table 1).

Tabel 1. The distribution of Probandus of sub-clan of Siwa Brahmin in Bali.

| No | Regency/city | Jumlah probandus | Kemenuh | Manuaba | Keniten | Mas | NK |
|---|---|---|---|---|---|---|---|
| 1 | Karangasem | 6 | | 6 | | | |
| 2 | Klungkung | 3 | 2 | | 1 | | |
| 3 | Bangli | 7 | | 6 | 1 | | |
| 4 | Gianyar | 8 | | 6 | 1 | 1 | |
| 5 | Denpasar | 7 | | 3 | 3 | 1 | |
| 6 | Badung | 4 | | 3 | | 1 | |
| 7 | Tabanan | 9 | 1 | 4 | 2 | 2 | |
| 8 | Jembrana | 3 | 1 | 1 | | | 1 |
| 9 | Buleleng | 8 | 5 | | 3 | | |
| | Total | 55 | 9 | 29 | 11 | 5 | 1 |

NK= unknown sub-clan

Out of all 55 buccal cell samples collected, two of them leaked on the tilting process, therefore only 53 samples were amplified. However, one of those 53 samples, one sample was not amplified in two primers out of four primers employed, which were not showing any band in electrophoreses results (Fig. 1A – 1D).



A B

C D

Figure 1. Amplification results and electrogram of A: locus DYS19; B: locus DYS390, C: locus DYS393, and D: locus DYS395. Note: L= DNA ladder (from 100bp). The number on each tract is the sample sizes. The blank tract is the sample that was fails to be amplified.

The allele sizes of an amplicon were expressed as a set of numbers of nucleotide. Allele size of each locus and its frequency are presented in Table 2. There were eight alleles found from all of the primer used. One of them was monomorphic in DYS19 locus, and polymorphic with three allele sizes were found in DYS390 (203, 207 and 211 bp), and two allele sizes were found in DYS393 (129 and 133 bp) and DYS395 (127 and 131 bp).

Table 2. Locus, Alelle size and allele frequency

| Locus | Alelle size (base pair/bp) | Frequancy |
|---|---|---|
| DYS19 | 200 | 1,00 |
| DYS390 | 203 | 0,38 |
| | 207 | 0,58 |
| | 211 | 0,04 |
| DYS393 | 129 | 0,96 |
| | 133 | 0,04 |
| DYS395 | 127 | 0,96 |
| | 131 | 0,04 |

Genetic diversity was varied on each locus (Table 3). The highest diversity 0.782 ± 0.026 was found in DYS390 locus and the lowest (0.00) was found in DYS19 locus, with mean diversity of 0.267 ± 0.023.

Table 3. Genetic diversity of each locus

| Locus | Diversity ± SE |
|---|---|
| DYS19 | 0,00 ± 0,00 |
| DYS390 | 0,782 ± 0,026 |
| DYS303 | 0,143 ± 0,034 |
| DYS395 | 0,143 ± 0,034 |
| Mean | 0,267 ± 0,023 |

This research was founded six haplotype based on alleles combination for four loci (DYS19,DYS390,DYS393 and DYS395), there are haplotype no 1 to 6 is presented at table 4. The haplotype 2 owning highest frequency with alleles combination(200,207,129,127 bp) 0.538 folowed by haploype no 1 (200,203,129,127) 0.384 , haplotype no 3, 4, 5 and no 6 with each frequency are 0.19.

Tabel 4. Haplotype, alelles combinations, frequency and the distribution of sub-clan of Balinese Siwa Brahmin

| Hapl | Alelles DYS: 19, 390,393,395 | Indiv | Freq | Kemn | Manb | Kenit | Mas | NK |
|---|---|---|---|---|---|---|---|---|
| 1 | 200,203,129,127 | 20 | 0,384 | 2 | 11 | 4 | 3 | 0 |
| 2 | 200,207,129,127 | 28 | 0,538 | 5 | 15 | 5 | 2 | 1 |
| 3 | 200,207,133,131 | 1 | 0,019 | 0 | 0 | 1 | 0 | 0 |
| 4 | 200,207,133,127 | 1 | 0,019 | 0 | 1 | 0 | 0 | 0 |
| 5 | 200,211,133,131 | 1 | 0,019 | 1 | 0 | 0 | 0 | 0 |
| 6 | 200,211,133,127 | 1 | 0,019 | 1 | 0 | 0 | 0 | 0 |
|  |  | 52 | 1 | 9 | 27 | 10 | 5 | 1 |

Note: Hapl=haplotype, Indiv= number of individu, Freq= Frequency
Kemn= Kemenuh, Manb= Manuaba, Kenit= Keniten, and
NK= unknown sub-clan

## 4. Discussion

The result shows that there was no Patapan sub-clan of Siwa Brahmin found in this study. Based on the information from history of Dang Hyang Nirartha written by Bek (1959), the clan that legalized to be a Siwa Brahmin was merely 4 sub-clans, those are sub-clan Kemenuh, Manuaba, Keniten and Mas which was familiarly called Catur Dwija. Patapan sub-clan, however, commonly did not proclaim as Patapan, but they might be announced themselves as Mas sub-clan. This is true that the Patapan sub-clan was also called Mas-Alitan (Bek , 1959).

Out of 53 samples amplified using 4 pair of primers (DYS19, DYS390, DYS393 dan DYS395), one sample was not amplified in DYS19, DYS390 loci, but was amplified in the other two. This may due to the failure in sample processing or due to mutation occurred on the primer site which is referred as null allele (Dankin and Avise, 2004).

The length of alleles varied among loci. Eight variety of alleles were found, which the highest frequency (1.0) was found in DYS19 locus that was the allele of 200bp. This means that there was no mutation occurred since the founding father, Dang Hyang Nirartha. This allele was common found in Balinese (Junitha dan Sudirga, 2007; Junitha dkk, 2009; Junitha *et al.* 2012) and was also commonly found in the world (Hammer and Horai, 1995; Ruiz-Linares *et al.* 1996; Hammer *et al.*, 1997). The allele of 207bp, 127bp, and 129bp were found highest in the locus of DYS390 (0.58), DYS395 (0.96) and DYS393 (0.96) respectively. The allele of 207bp was detected in almost every caste or social group in India (Ramana *et al.*, 2001; Battacharyya *et al.*, 1999). However, the earlier study in other Balinese clan (Kayu Selem) found that the highest frequency of allele in DYS390 211bp (Junitha dkk., 2009).

The highest Genetic diversity was found in DYS390 with the value of 0.78±0.23. This due to the number of allele found in this locus was the highest (3). The total average of genetic diversity was low (0,27 ± 0,02). This because of the low diversity of allele on each locus and Siwa Brahmin clan was relatively established recently (15 century) (Sastrodiwiryo, 2010). Genetic diversity of Siwa Brahmin clan was lower than Terunyan society (0.28), but higher than the men of Tenganan Pegringsingan traditional society (0.14) (Junitha et al, 2012; Junitha dkk 2009, Junitha dan Sudirga 2007; Junitha, 2004).

From six haplotype were found, haplotype 2 has the highest frequency and widely spread in all sub-clans including the sample with unidentified clan. The haplotype 2 has allele combinations of 200, 207, 129, 127, which could be the original haplotype of Dang Hyang Nirartha, the founding father of Siwa Brahmin clan. The frequency of haplotype 1 was 0.38, the second highest of all haplotypes. The data shown that haplotype 1 with the haplotype combinations of 200, 203, 129, 127 and was distributed in all sub-clan, occurred later than haplotype 2. The mutated allele was the allele of 207 to 203, 1 step mutation, in the locus of DYS390 (Gusmao et al. 2005). The mutation of haplotype 1 occurred far earlier than the other haplotypes (3,4,5 and 6) to the original haplotype (haplotype 2).

## 5. Conclusion

It was found that the Siwa Brahmin clan has low genetic diversity with 6 different haplotypes. The haplotype 2 with the allele combinations of 200,207,129,127 could be as the original haplotype of Siwa Brahmin.

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