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Phylogenetic Analysis of *Malayopython reticulatus* (Schneider, 1801) from Southern Sulawesi based on Morphological and Molecular Character

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Abstract. *Malayopython reticulatus* is widely distributed from South Asia to Southeast Asia, including most of the Indonesian islands except Papua and surrounding apart. *Malayopython reticulatus* divided into three subspecies based on morphological characters and molecular data. The geographical barrier does exist among populations which result in the formation of a new subspecies. The latest two subspecies described from a population of Selayar Island and Tanahjampea (South Sulawesi). However, there are some populations have distinctive morphological characters and not recorded yet such Karumpa, Honey/Madu and Kalaotoa Islands (South Sulawesi). This study purpose was to identify morphological and molecular characters of *Malayopython reticulatus* population of related islands and examine the phylogenetic relationship among them. The study was divided into two steps, first was to identify morphological character including morphometric, head scale character and body scales. Later step was DNA isolation and sequencing of *cytochrome b.* BLAST software, DNA Baser, ClustalX and Mega5 were used to analyze the data and construct phylogenetic trees by using the neighbor-joining method. The result showed that these samples have a very close relationship each other as close as to *M. r. reticulates* and *M. r. jampeanus* rather than to *M. r. saputrai.* It was highly suspected that the samples were missing links which can be used as "stepping stone" of *M. reticulates* dispersal from Lesser Sunda to Jampea Island.

Keywords: *Cytochrome b, Malayopython reticulatus* (Schneider, 1801), morphological character, molecular character, phylogenetic analysis.

INTRODUCTION

Indonesia is a megabiodiversity country, which is dispersed to more than 17 000 islands. Indonesia located between two continents and show a different pattern of animal dispersal from each continent, thereby making Indonesia becomes very potential resource for many kinds of systematical researchers due to the difference of dispersal flow from each continent. Indonesia has 726 reptiles species out of 10 272 of total reptile species all over the world and 353 of them consist of snakes distributed in Indonesia [1].

Sulawesi/Celebes is the largest fifth island in Indonesia. This island is dominated with highland especially in the north-side. Sulawesi is formed by the collision of former continents resulting some unique well-developed species due to the different plate had taken their part in the formation of this islands [2]. Sulawesi consists of K-like shape mainland and some smaller islands. Many small islands were arisen near the mainland due to the lowering of sea level million years ago resulting many isolated population organisms in each small island.

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Malayopython reticulatus is widely distributed in many Indonesia Islands. This snake can reach size of 9 m to 10 m in captivity and for its beautiful appearance had become as one of the most widely traded snakes for the last decades, making this snake more vulnerable to the extinction [1]. This snake is heavily exploited to fulfill market demands whether as fashion commodities or traded in live condition as a pet. Indonesia has exported about 112 000 wild snakes skin annually since 2000 [3].

Malayopython reticulatus is divided into three subspecies according to the area they have founded based on morphological approach and supported by molecular data; they are *M. r. reticulatus, M. r. saputrai,* and *M. r. jampeanus*. Two latest subspecies discovered in Southern Sulawesi and some smaller island around it (*M. r. saputrai* specimen was collected in South Sulawesi and Selayar Island. *M. r. jampeanus* specimen was collected from Jampea Island, far south from Selayar Island) [4].

The study of this heavily exploited snake in Indonesia is very limited due to many islands can not be reached easily caused by limited water/sea transportation route. But ironically, many hunters have the opportunity to trade their wild caught snakes collected from that isolated area in large number. With this lack of study and overwhelming trade attempts, the keeper of this snakes could be easily crossbreed one of the specific isolated population to another without knowing the impact of what they have done. This crossbreeding can be fatal to future mislead identification.

The observation of some others locality of this snake showed that there are still many other populations potentially as an isolated population that has been traded for last decade. But the most isolated and potential to shows specific morphological and genetical character were from Kalaotoa, Madu and Karumpa Island, which are located in the Flores Sea, the southernmost of Sulawesi mainland (Fig.1).

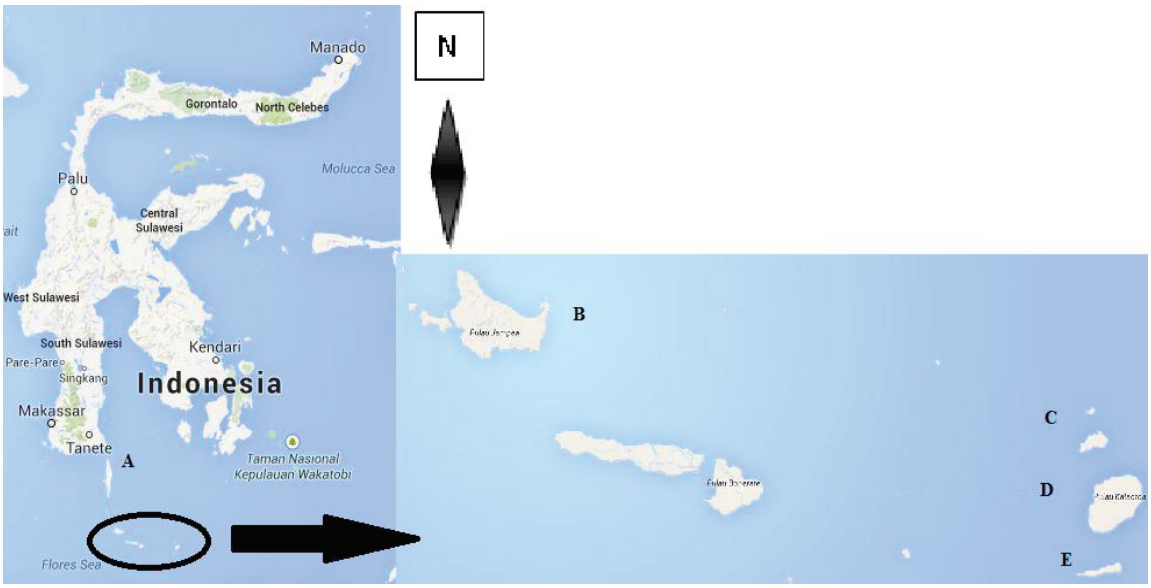

FIGURE 1. South Sulawesi smaller islands where smaller retics population collected. A: Selayar Island; B: Jampea Island; C: Karumpa Island; D: Kalaotoa Island; E: Madu Island.

This research aimed to discover and compare the morphological character of snake from mentioned population supported by molecular data.

MATERIALS AND METHODS

Specimens were obtained from Kalaotoa, Madu and Karumpa Islands by using Visual Encounter Survey from July–Agustus 2013 and April–May 2014. Twenty of the total population was examined [4]. Head scale was characterized for each population. The number of ventral, dorsal anterior, dorsal mid, dorsal posterior and subcaudal scales was counted.

Some tissues of the related specimen were collected from euthanized specimens and prepared in 96 % alcohol. We used liver, heart and musculus subcutaneous tissue). DNA isolation, amplification of *cyt b gene* and electrophoresis conducted in Laboratory of Genetics, Universitas Gadjah Mada. DNA isolated using Genomic DNA

Mini kit Geneaid and amplified by using KAPA fast ready mix for 35 cycles; each cycle consists of 3 minutes at 94 °C, one minute at 50 °C, and one minute at 72 °C. We used primer cytbL14841 (F) (5'-AAA AAG CTT CCA TCC AAC ATC TCA GCA TGA TGA AA-3') and *cytb* H15149 (R) (5'-AAA CTG CAG CCC CTC AGA ATG ATA TTT GTC CTC A- 3') [4]. Samples result were sent to Genetics Science for following DNA sequencing.

Morphological characters and Sequenced DNA of *Cytochrome b* of three surveyed specimen was compared according to Auliya [4]. Sequenced DNA was analyzed using BLAST (Basic Local Allignment Search Tool), ClustalX, DNA Baser and Mega5. The phylogenetic tree was constructed using the Neighbour-Joining method.

RESULT

Morphological Approach

The morphological comparison showed that Kalaotoa population has the widest range of ventral scale and mid dorsal scales count number (Table 1).

TABLE 1. Scales count of Malayopython reticulates

Population	Ventral Scales	Anterior Dorsal Scales	Mid-dorsal Scales	Posterior Dorsal Scales	Subcaudal Scales
P. r. reticulates [5]	304–325		68–78		
P. r. jampeanus [5]	290–301		64–68		
P. r. Saputrai [5]	330–334		77–81		
Pulau Kalaotoa	291–318	55–62	62–75	29–36	80–88
Pulau Madu	295–305	54–67	62–67	32–36	85–89
Pulau Karumpa	302–306	52–59	60–64	31–38	83–91

Three population of *M. reticulatus* collected showed specific coloration and dorsal pattern. Population from Kalaotoa island showed darkish grey or silver with longer pattern. Population from Madu has yellowish or brown clear and brighter dorsal pattern. While Population from Karumpa island often showed smaller pattern with many variotion of color (Fig. 2 to Fig. 4).

FIGURE 2. Specimens of *M. reticulatus* collected from Kalaotoa Island. They have dark specific coloration with the longer dorsal pattern.

FIGURE 3. Specimens of *M. reticulatus* collected from Karumpa Island. They are the newest population being popular in the pet trade. They have yellowish-orange up to gray coloration with the smaller pattern on their dorsal.

FIGURE 4. Specimen of *M. reticulatus* collected from Madu Island. This specimen mostly have brighter coloration and wider dorsal pattern.

We discovered that each population shares the same dorsal head scales combination similar to *M. reticulatus jampeanus* [4] except for Kalatoa population. Some of Specimens from Kalaotoa population showed some addition in prefrontal scale while some others did not. This addition scale did not change even after some shedding occurred. (Fig. 5).

FIGURE 5. Specimen of *M. reticulatus* collected from Kalaotoa Island shows differs

Molecular Data

We found that some sample of these three population of *Malayopython reticulatus* forms a large group along with *M. reticulatus jampeanus* and *M. reticulatus reticulatus*. Some specimen has a closer relationship to *M. r. reticulatus,* another specimens closer to *M. r. jampeanus*, only *M. reticulatus saputrai* has significant distance and forming an exclusive group of their own (Fig. 6).

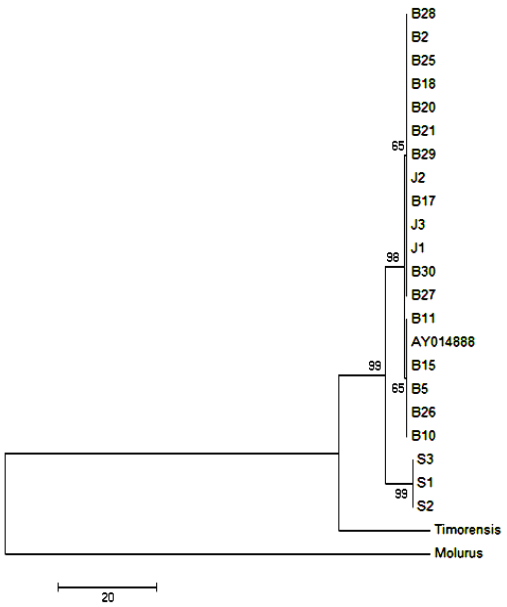

FIGURE 6. A phylogenetic tree formed by using the Neighbour-Joining method. 1 = Timorensis: *Malayopython tumorgenesis* (AF241398); 2 = Molurus: *Python molurus* (FJ936560); 3 = S1–S3: *M. r. saputrai* [5] (AY014892, AY014893, AY014894); 4 = J1–J3: *M. r. jampeanus* [5] (AY014885, AY014886, AY014887); 5 = V: *M. reticulatus* isolate Vietnam (AY014895); 6 = M: *M. reticulatus* isolate Malaysia (AY014889); 7 = B2–B15: *M. reticulatus* Kalaotoa population; 8 = B17–B25: *M. reticulatus* Madu population; 9 = B26–B30: *M. reticulatus* Karumpa Population; 10 = *Another ascension*: isolate of *M. reticulatus*

DISCUSSION

Malayopython reticulatus also known as *Pyhton reticulatus* of which has been recognized lately as distinct genus by Rawlings [5] as *Broghammerus reticulatus* has close relationship to *Python timorensis.* However, according to Kaiser [6], the name Broghammerus authorized by Hoser included in one of taxonomical vandalism which caused destabilized of nomenclature. Even so Rawlings [5] found some interesting fact that split *Python reticulatus* from others Python group along with *Python timorensis.*

Malayopython reticulatus collected from Kalaotoa, Madu and Karumpa Island differs from any other population. They have a smaller size than any other *M. reticulatus* found in Indonesia. The largest sample we found reached 3 m in total length collected from Kalaotoa Island. We predict there are some larger specimen up to 4 m based on locals testimony. Livestock being eaten is rarely occur but they did prey on small-medium lamb once after some years which tell us there are some snakes can reach a larger size than 3 m but still that size is unusual to this population.

Larger population predicted to be from Kalaotoa Island, because out of these three islands, Kalaotoa is the only island that larger prey can be found (wild boar only found in Kalaotoa Island) but the more natives inhabit other islands, that statement should be questioned. Many local people keep livestock such goat, horse and lamb. This mean other population can prey larger food that can boost their growth faster. However, retics which longer than 3 meters is very rare to find in these islands.

According to Table 1. Kalaotoa population has a closer number of ventral scales and mid dorsal scales number count to *M. r. reticulatus,* which has 68 to 67 mid dorsal scales and 304 to 325 of ventral scales count [4], whereas Madu and Karumpa population are relatively closer to *M. r. jampeanus* for both ventral scales count and mid dorsal scales count. Some specimens closer to one subspecies, some other has a closer relationship to other subspecies. We can see a pattern here and predict that these three populations were connecting one population to another long time ago.

Table 1 also shows that *M. r. saputrai* is far separated to others population here, indicating that retics from South Sulawesi and Selayar Island distributed from Sulawesi mainland[4] *Malayoputhon reticulatus* has 330 of ventral scale count. This sample has a high possibility collected from Sulawesi mainland. As for other two subspecies and three population we observed, has a very possibility come from Lesser Sunda.

Specimen collected from Madu and Karumpa Island showed consistency in dorsal head character. They are very similar to *M. reticulatus jampeanus* which has one row of posterior prefrontal scales as described by Auliya [4]. However, we found some specimen from Kalatoa Island, which has one additional scale in the middle of prefrontal as if they form additional uncompleted another row of prefrontal scale (shows in blue arrow) (Fig. 5).

Fig. 2, Fig. 3 and Fig. 4 shows each population has differs general coloration. Kalaotoa retics well known for their lack of yellow pigment and dominated by dark gray or plain yellow in color. They usually have longer net pattern at dorsal body. Some specimens show brighter color than others, but their color relatively lacks contrast and have many dark spots.

Madu population have brighter yellow and lack of a spot in their dorsal body. Their anterior-dorsal body has less granite pattern compared to their neighbor population. Although they most likely have bright yellow colour, there are some specimen have grayish color with spotless. They may have the widest dorsal net pattern out of these three population (Fig. 4)

Karumpa population have just introduced in late of 2012–2013 more or less (personal communication with local snakes hunter). We encountered vary coloration from orange-yellowish to grayish. Our specimens showed for having the smaller net dorsal pattern and lack of lateral white spots in some of the area. Some specimen show granite pattern in the dorsal-anterior body, but graniteless for another (Fig. 3).

We manage to isolate *Cytochrome b* gene to support our study on this snake. Our specimens were compared to another discovered and published sequences obtained from *Genebank* using Neighbour-Joining method using total difference with bootstrap 100 replication (Fig. 6). We use pattern homogenous uniform rates by complete deletion for missing data.

The most significant distance showed from *M. r. saputrai* (South Sulawesi and Selayar Island population). Three of Selayar population separately forming their group. Other than a group of Selayar population, there are two other groups formed which consist of mixed of our specimen and *M. r. reticulatus* and *M. r. jampeanus*. This result supports our previous statement and makes the dispersal route clearer. This snake should be distributed from two ways when entering South Sulawesi Islands. One of them come from Sulawesi mainland which reaches Selayar Island; another group predicted from Lesser Sunda through smaller islands in the Flores Sea when the sea water level lowered in late Cretaceous and Tertiary up to Pleistocene [4] and succeeded in reaching Jampea Island. There should be very deep ocean trenches between Selayar and Jampea Island.

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