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## DNA HOMOLOGICAL ANALYSIS OF THE *GELOINA* SHELL IN REULEUNG LEUPUNG MANGROVE ECOSYSTEM, ACEH BESAR DISTRICT, ACEH PROVINCE, INDONESIA

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### Abstract

Three species of *Geloina* shells were found in the Reuleung Leupung mangrove ecosystem area, Aceh Besar District, Indonesia which consists of *Geloina erosa*, *G. expansa*, and *G. coaxana*. The phylogenetic relationship of the three closely related species is currently not established. In this study, we analyze the DNA sequence homology of the three *Geloina* species and compare them with other mangrove shells species stored at NCBI GenBank. The research activities were conducted from July to September 2017, in the area of the Reuleng Leupung mangrove ecosystem where *Geloina* shells were obtained using destructive sampling method. The results showed that the three *Geloina* shells have a high similarity with *Polymesoda carolina* Voucher, *Cyrenoida floridana* Partial, *Cyrenoida floridana* Voucher, *Glaucanome rugosa* Voucher, *Corbicula fluminea*, *Glaucanome viren* Partial, where a very close level of phylogenetic relationship was shown at *Polymesoda carolina* Voucher sequence homology of 99%. We conclude that due to 99% DNA homology between *G. erosa*, *G. expansa*, and *G. coaxan* and *Polymesoda carolina*, they are of the same species.

## INTRODUCTION

Mangrove ecosystems are brackish water forest areas with biodiverse flora and fauna. Specific flora that makes up the mangrove ecosystem included *Rhizophora apiculata*, *R. mucronata*, *Sonneratia alba*, *S. caseolaris*, *Nypa fruticans*, *Acrosticum auretum*, *Bruguera* sp., and *Acanthus* sp. [1]. The fauna inhabited in the mangrove ecosystem consists of terrestrial fauna and aquatic fauna [2]. Terrestrial fauna found in the mangrove ecosystem, include *Egretta media*, *E. alba* and *Varanus* sp. The benthic fauna in the mangrove ecosystem includes *Scylla serrata*, *Geloina erosa*, *G. expansa*, *G. coaxan*, *Faunus ater*, *Pyramidella sulcata*, *Crassostrea* sp. [3]. The coastal area of Reuleung Leupung in Aceh Besar District, Aceh Province, Indonesia includes a major mangrove ecosystem. The aquatic fauna found in the Reuleung Leupung mangrove ecosystem (RLME) include *Alcedo attis*, *Amourornis phenicurus*, *Varanus* sp., *Macaca fascicularis*, and *Sus crofa*. The terrestrial fauna inhabited in this area were *Faunus ater*, *Geloina erosa*, *G.*

*expansa*, *G. coaxan*, *Scylla serrata* and *Macrobrachium rosenbergi* [1].

*Geloina erosa*, *G. expansa* and *G. coaxan* are common shells found in the water of the RLME [1]. These three shells belong to the Genus *Geloina*, Family Corbiculidae, Order Corbicula and Class Bivalvia which have a morphological appearance of shells with various shapes. *G. erosa* has the same shell length on both sides, *G. expansa* has asymmetrical shell length, and *G. coaxan* has a thick shell so that the body thickness is more dominant when compared to its length and width.

Although different in morphology, the three species that live in the RLME are thought to have a high DNA sequence similarity. Here, we analyze the DNA sequence homology of the three *Geloina* species and compare them with other mangrove shells species stored at NCBI GenBank. Specifically, we study of the DNA homology of the three *Geloina* species with six other species of mangrove shells found in various mangrove ecosystems in the world.

## MATERIALS AND METHODS

### Site and time

The sampling of *G. erosa*, *G. expansa*, and *G. coaxan* was conducted in the RLME, Aceh Besar District, Aceh Province, Indonesia in July 2017. The DNA analysis was conducted at the Research Laboratory of the Faculty of Veterinary Medicine, Universitas Syiah Kuala, Banda Aceh, Indonesia.

### Tissue sample collection

The *Geloina* shells were obtained by scraping the bottom of the waters with small knife in the RLME. After the *Geloina* shells were identified, they were cleaned from the mud attached to the shell and then collected in sample bag. *Geloina* shells that have been collected are then separated by species before proceeding to the DNA analysis.

### DNA extraction

The principle of DNA isolation is to lyse, precipitate, and extract DNA from solid materials such as cellulose and protein [4, 5]. In this study, we isolate 16S rDNA. rDNA isolation from each species was conducted by taking  $\pm 1$  mm of tissue which is then ground using mortar by adding 10 ml of aquadest. The ground materials were then transferred to 1.5 ml microtube to separate the rDNA from debris such as carbohydrates, fats, and proteins. The amplification process was conducted using PCR and then run through electrophoresis using agarose gel. The nucleotide sequences of each shell were then used for phylogenetic construction to see the phylogenetic relationship with other mangrove shells recorded in the GenBank.

### Homologous analysis of DNA of *Geloina* with shells in GenBank

DNA homology analysis was conducted using the BLASTN program. The similarity or homology of the DNA sequences of three species of mangrove shells found in the RLME with six species of shells deposited in GenBank, can be seen through the alignment of sample DNA sequences with nucleotide data at the National Center for Biotechnology Information (NCBI) via the <http://www.ncbi.nlm.nih.gov>. [6, 7].

### Phylogenetic analysis

The phylogenetic relationship between the three shells species found in the RLME and the mangrove shells species recorded in the GenBank was conducted descriptively using the MEGA 7.0 program [8].

## RESULTS AND DISCUSSION

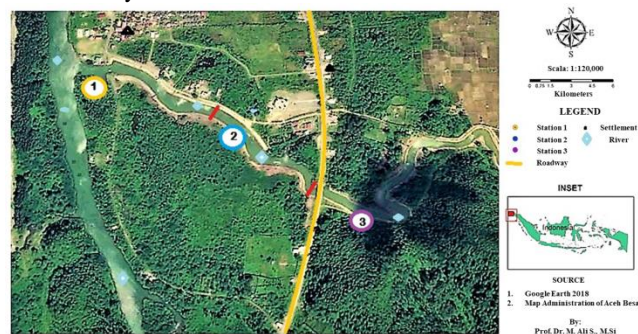
The results of DNA homology analysis showed that *G. erosa*, *G. expansa*, and *G. Coaxan* (GenBank accession no: MG946720, MG946718 and MG763131) found in the RLME in Aceh Besar District had a similarity value 99% with *Polymesoda caroliniana* (GenBank accession no: KX713338.1) (**Table 1**). According to Puillandre *et al.* [9] and Armani *et al.* [10], DNA sequences that have similarities value  $> 98\%$ , are considered members of the species mentioned in GenBank. However, if the percentage of homology value is less than 97%, it is considered to be of a different species [11]. According to Hebert *et al.* [12], a species is said to be different if the DNA variation reaches 3% (DNA identity  $< 97\%$ ). Thus, 99% DNA homology value between the

three *Geloina* shells and *Polymesoda caroliniana* means that all isolates can be grouped as one species.

**Table 1.** Homology level of *Geloina* shells from the mangrove ecosystem of Reuleung Leupung, Aceh Besar District, Indonesia

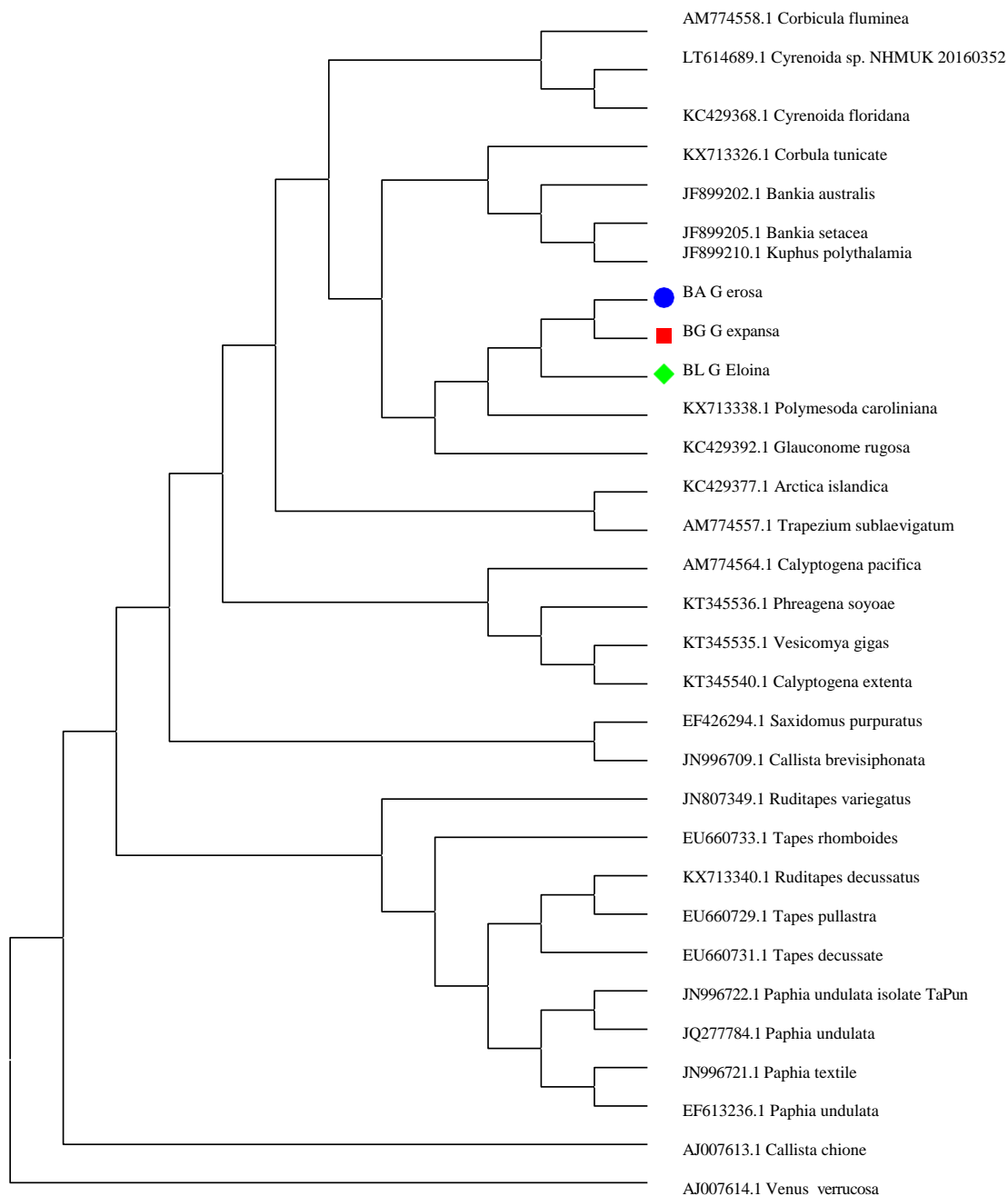
Species	The homologous species name in GenBank	Identity	Homology
<i>G. erosa</i> <i>G. expansa</i> <i>G. Coaxan</i>	<i>Polymesoda caroliniana</i> voucher BivAToL- 281 18S ribosomal RNA gene, partial sequence	99%	1. The nucleotide base arrangement has a homology level of 99%  2. The shells has two plates, so the homology level is 100%  3. Equally included in the Class Bivalvia, Order Corbicula and Family Corbiculidae, with a level of similarity of 100%

The results of the phylogenetic tree analysis showed a high phylogenetic relationship among the three shells species (*G. erosa*, *G. expansa*, and *G. coaxan*) that lived in the RLME in Aceh Besar District (**Figure 1**). These results showed from the branch of the phylogeny in one monophyletic cluster. This is consistent with the statement of Combosch *et al.* [13], monophyletic species from the Bivalve family have very close phylogenetic relationship levels and relatively similar DNA structures.



**Figure 1.** Research map in the Reuleung Leupung mangrove ecosystem in Aceh Besar District, Indonesia

The *G. erosa*, *G. expansa*, and *G. coaxan* that live in the RLME in Aceh Besar District, have close phylogenetic relationship with *Polymesoda caroliniana*, which recorded in GenBank. This is indicated by the presence of three species of *G. erosa*, *G. expansa*, and *G. coaxan* shells in one phylogenetic branch and directly related to *Polymesoda caroliniana* in one phylogenetic branch. The level of similarity that occurs is based on a sequence of nucleotide bases that have a very close degree of similarity between each of these species. Notredame [14] state that phylogenetics is a phylogeny that compares genes that are equivalent to various species to reconstruct the tree of life and find out which species are most dean related to others, so that life history can be reconstructed and can explain the level of diversity of living things. According to Livi *et al.* [15], species positioned in one quadrant within a phylogenetic tree have close phylogenetic relationship [16]. This phylogenetic relationship is indicated by the DNA base arrangement that composes the DNA bands in each of these shells species, which have the same pair structure of DNA bases.



**Figure 2.** The dendrogram of phylogenetic tree of three species (blue, red and green colour) of Reuleung Leupung Mangrove Ecosystem, Aceh Besar District and the level of homology with other bivalve families

Previous research conducted by Carter [17] mentions that *G. erosa*, *G. expansa*, *G. coaxan* is the synonym name of *Polymesoda erosa*, this conclusion is based on several marine Biodiversity Information Facilities (SeaLifeBase and Catalog of Life, WoRMS and OBIS). Furthermore, other studies also mention that *Geloina* is a synonym for *Polymesoda* taxa [18- 21].

Therefore, *G. erosa*, *G. expansa*, and *G. coaxan* were identified as *Polymesoda* taxa, thus forming a monophyletic cluster with *Polymesoda caroliniana* in the phylogenetic tree (**Figure 2**).

## CONCLUSIONS

In the Reuleung Leupung mangrove ecosystem in Aceh Besar District based on morphological identification, there are three species of shells, namely *G. erosa*, *G. expansa*, and *G. coaxan*. The results of 16S rDNA genomic DNA analysis showed *G. erosa*, *G. expansa*, and *G. coaxan* formed a monophyletic in the phylogenetic tree with *Polymesoda carolina* species. Furthermore, blast results show *G. erosa*, *G. expansa*, and *G. coaxan* have high similarity with *Polymesoda carolina* (99% DNA identity). Therefore, it is concluded that *G. erosa*, *G. expansa*, and *G. coaxan* found in the RLME in Aceh Besar District are *Polymesoda carolina* species.

## CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this manuscript.

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