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Molecular Identification and Phylogenetic Analysis of the Coral *Pocillopora* in the Gulf of Thailand

Makamas Sutthacheep[a,*], Thamasak Yeemin [a], Chinarong Ruangthong[b], Charernmee Chamchoy[a], Sittiporn Pengsakun[a], Wanlaya Klintong[a]

[a]Marine Biodiversity Research Group, Department of Biology, Faculty of Science, Ramkhamhaeng University, Huamark, Bangkok 10240, Thailand
[b] Chumphon Marine National Park Operation Center 1, Department of National Parks, Wildlife and Plant Conservation, Chumphon Province, Thailand

*Corresponding author: *smakamas@hotmail.com*

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Abstract. Molecular genetic studies have showed cryptic species associated with the *Pocillopora* '*damicornis*-like' coral. All specimens of this species in Thailand have been identified as *P. damicornis*, based on morphological characteristics. The objective of this study is to examine molecular genetic information for taxonomic identification of morphologically *Pocillopora* '*damicornis*-like' coral in the Western Gulf of Thailand. The *Pocillopora* samples were investigated using mitochondrial DNA data and morphology. Molecular phylogenetic analyses showed that most *Pocillopora* '*damicornis*-like' coral in the Western Gulf of Thailand are *P. acuta* instead of *P. damicornis*. Regional collaboration research programs should analyze genetic samples from reef sites in the Western Pacific region for a better understanding on historical distributions of *P. acuta, P. damicornis* and other cryptic coral species.

Keywords: taxonomy, cryptic species, phylogenetic analysis, *Pocillopora*, Gulf of Thailand

1. Introduction

In the last decade, the use of DNA genetics has become a key tool that has led to discoveries of an increasing number of cryptic and/or parentage of endemic lineages (Pfenninger and Schwenk, 2007). Most of which are waiting to be new discoveries and proving species validity. Historically, morphological criteria traits were used to classify and identify species, but it is now widely accepted that several approaches to species delimitation should be combined to improve our understanding of ecological and evolutionary patterns and processes, and integrative taxonomic studies should be conducted (Padial et al. 2010).

Identification of hard coral taxonomy is generally reliant on skeletal morphology (Veron 2000). Many coral species present morphological variation (Foster 1977, Foster 1983, Veron 1995), which can arise from genetic differences or phenotypic plasticity responses to the environment (Todd et al. 2004, Pinzón et al. 2013). As morphological identification of coral taxonomy is dependent on skeletal, such variability can potentially blur species boundaries and make identification difficult due to morphological variation and lack of taxon knowledge. In addition, hybridization between closely related species can confuse taxonomic boundaries.

The scleractinian coral within the genus *Pocillopora* (Lamarck, 1816), which has more than 40 described species, of which only about 17 are generally accepted species (Veron 2013). It is widely is distributed throughout the tropical Indo-Pacific region and the Red Sea (Veron 2000; Pinzón et al. 2013). Previously, the genus *Pocillopora* was classified based on morphological criteria such as the shape and structure of branches and verrucae, and this classification was debatable due to substantial intraspecific colony diversity. (Wells, 1972; Veron 2000, Flot and Tillier 2006, Schmidt-Roach et al. 2014). Hence, using DNA-based techniques develop our understanding of the genetic structure of coral populations (Avise 1993).

Recently, the genus *Pocillopora* has been investigated to characterize species boundaries

using DNA-based techniques and the development of a variety of genetic markers for understanding the genetic structure of coral populations (Flot et al. 2006, 2007, 2008, 2010; Pinzón et al. 2011, 2013; Forsman, et al. 2013; Schmidt-Roach, et al. 2013, 2014; Martí-Puig et al. 2014; Gélin et al., 2017). Schmidt-Roach et al. (2013) focused on *P. damicornis* ecomorphs described in Veron and Pichon (1976) and sequenced mitochondrial ORF, revealing five different lineages that were further termed *P. damicornis* types a, b, c, d, and e, furthermore, these five lineages were formally ascribed to the taxonomic species *P. damicornis*, *P. acuta*, *P. verrucosa*, *P. aliciae*, and *P. cf. brevicornis* respectively (Schmidt-Roach et al., 2014). Recently, new evidence showed that most Pocillopora 'damicornis -like' corals in Singapore are *P. acuta* instead of *P. damicornis* (Poquita-Du et al. 2017). This study aims to revisit molecular genetic information for taxonomic identification of morphologically closely related *Pocillopora* species in the Western Gulf of Thailand.

2. Materials and Methods

2.1 Sample collection

Pocillopora samples were collected from Mu Ko Chumphon (Ko Ngam Yai, Ko Mattra, Ko Rangka Chiu, Ko Kula) and Mu Ko Angthong (Ko Thaiphlao, Ko Hindap, North of Ko Sam Sao, West of Ko Sam) in the Western Gulf of Thailand (Figure 1). In each locality, we photographed each colony of *Pocillopora* in the field before part of it was collected (Figure 2). Coral fragments were randomly taken at each sampling site from selected colonies that were at least 3 m apart. Small coral fragments (1-2 cm) were preserved in 100% ethanol in 1.5- ml Eppendorf tubes for molecular analysis and were then transported to the laboratory.

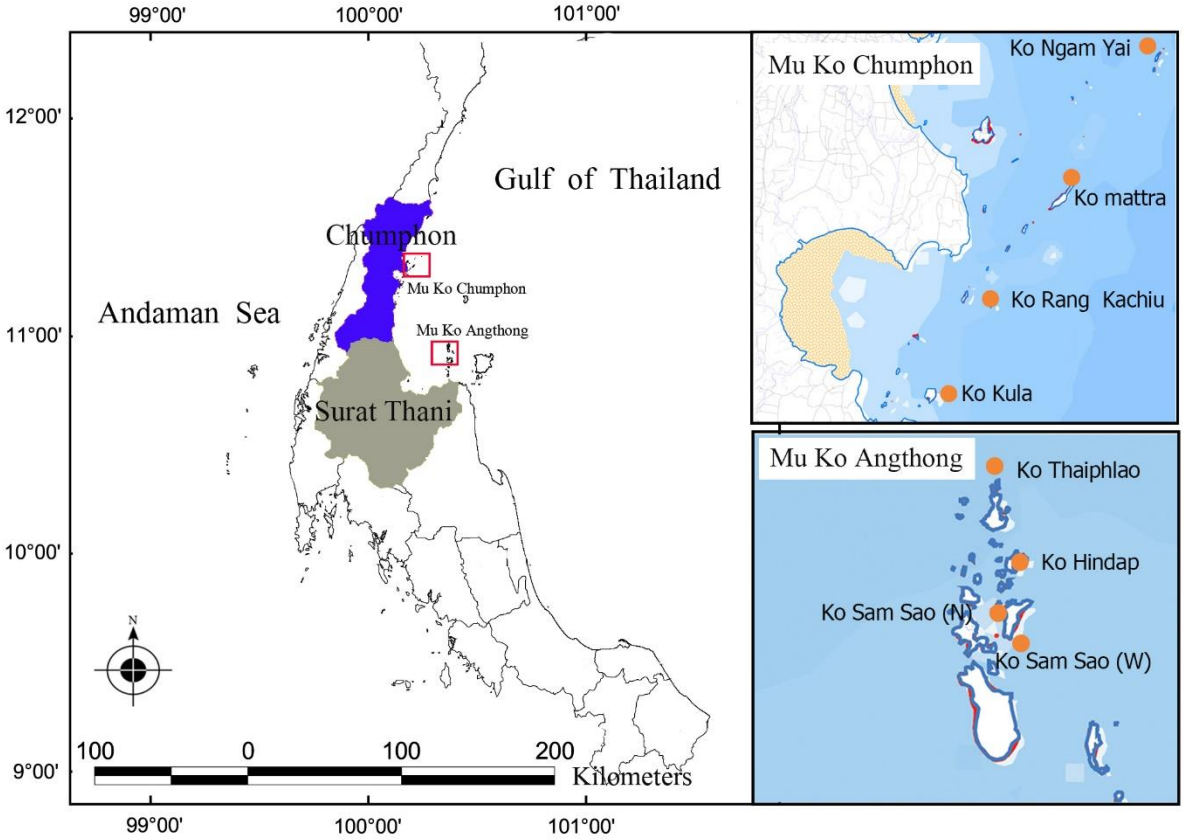

Figure 1. Map showing study sites in Mu Ko Chumphon and Mu Ko Angthong, the Western Gulf of Thailand

Mu Ko Chumphon

a) Ko Ngam Yai b) Ko Rang Kachiu c) Ko Kula

Mu Ko Angthong

d) Ko Hindap e) Ko Thaiphlao f) Ko Sam Sao (west)

Figure 2. Morphological variation of *Pocillopora* samples observed at Mu Ko Chumphon (a, b, c) and Mu Ko Angthong (d, e, f) in the Western Gulf of Thailand.

2.2 DNA extraction, amplification and sequencing

Total DNA was extracted and purified from each coral sample using the DNAeasy® Tissue kit (QIAGEN, Qiagen Inc., Valencia, California, USA), following the standard protocol. The mitochondrial open reading frame region (mtORF) was useful for the identification of genetic types of pocilloporids (Flot et al., 2008; Flot et al., 2011). Polymerase chain reaction (PCR) amplifications were then performed in 50 µL and contained template DNA more than 50 ng/mL: 0.5 µL AmpliTaq Gold 360 Master Mix (Thermo Fisher Scientific), 0.5 µL DNA template, and 0.4 µL of each primer (10 mM) for mtORF: FATP6.1 (5′-TTTGGGSATTCG TTTAGCAG-3′) and RORF (5′- SCCAATATGTTAAACASCATG TCA-3′), following the manufacturer's instructions. The thermo-cycling conditions were as follows: 94 °C for 1 min, followed by 40 cycles at 94 °C for30 s, 53 °C for 30 s, and 72 °C for 75 s, with a final extension at 72 °C for 5 min. PCR products were purified with QIAquick PCR purification kit (QIAGEN) according to the manufacturer's instructions and sent to Macrogen Inc. (Korea) for sequencing on an ABI 310 XL sequencer using ABI dye-terminator chemistry. DNA sequences were edited using MEGA7.

2.3. Data analysis

Sequences obtained were aligned with mtORF sequences available in GenBank (Flot et al. 2011; Schmidt-Roach et al. 2012; Marti-Puig et al. 2014; Nakajima et al. 2017; Poquita-Du et al. 2017; Torres et al. 2018) to identify the genetic species of *Pocillopora*. Reconstruction of phylogenetic trees was based on 1000 bootstraps. Phylogenetic hypotheses were generated in MEGA7.

3. Results and Discussion

Molecular phylogenetic analyses were carried out based on the mitochondrial open reading frame region gene sequences of the isolates of *Pocillopora* species and some isolates published previously. BLAST results revealed that samples identified as *P. acuta* (n = 20) had 99–100% sequence identity with *P. acuta* ORF GenBank records. Phylogenetic trees are generated with two different methods: neighbor-joining (NJ) (Figure 3) and maximum likelihood (ML) (Figure 4) analyses, with *Pocillopora acuta*, *Pocillopora damicornis*, *Pocillopora verrucosa* and *Pocillopora meandrina*. The topologies of NJ and ML trees are very similar and only slightly differences in bootstrap support values are obtained among those.

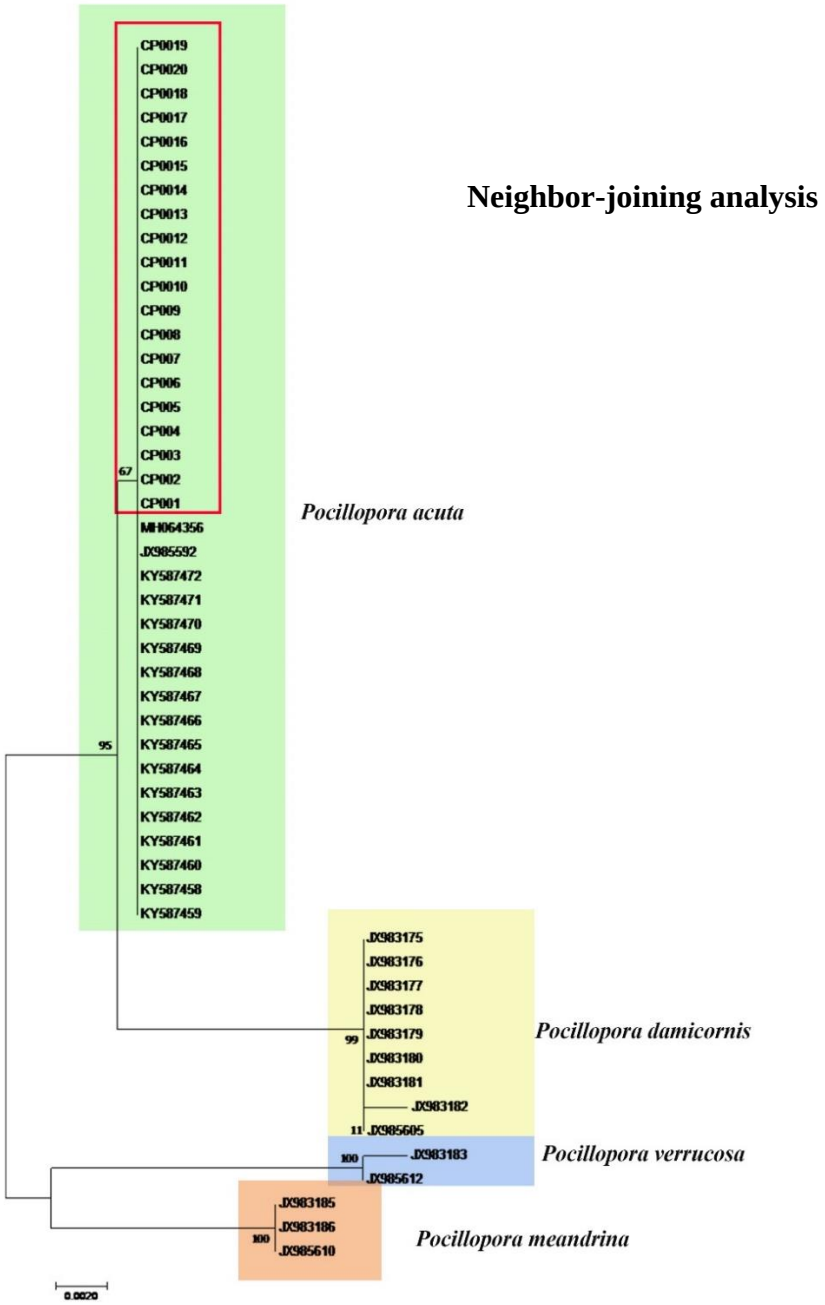

Figure 3. Phylogenetic relationships of genus *Pocillopora* isolates based on the mtORF sequences. Nodal support of >50% is shown neighbor-joining. Accession number and host origin are used to identify each sequence. Genotypes in the red quadrilateral are genotypes identified in this study.

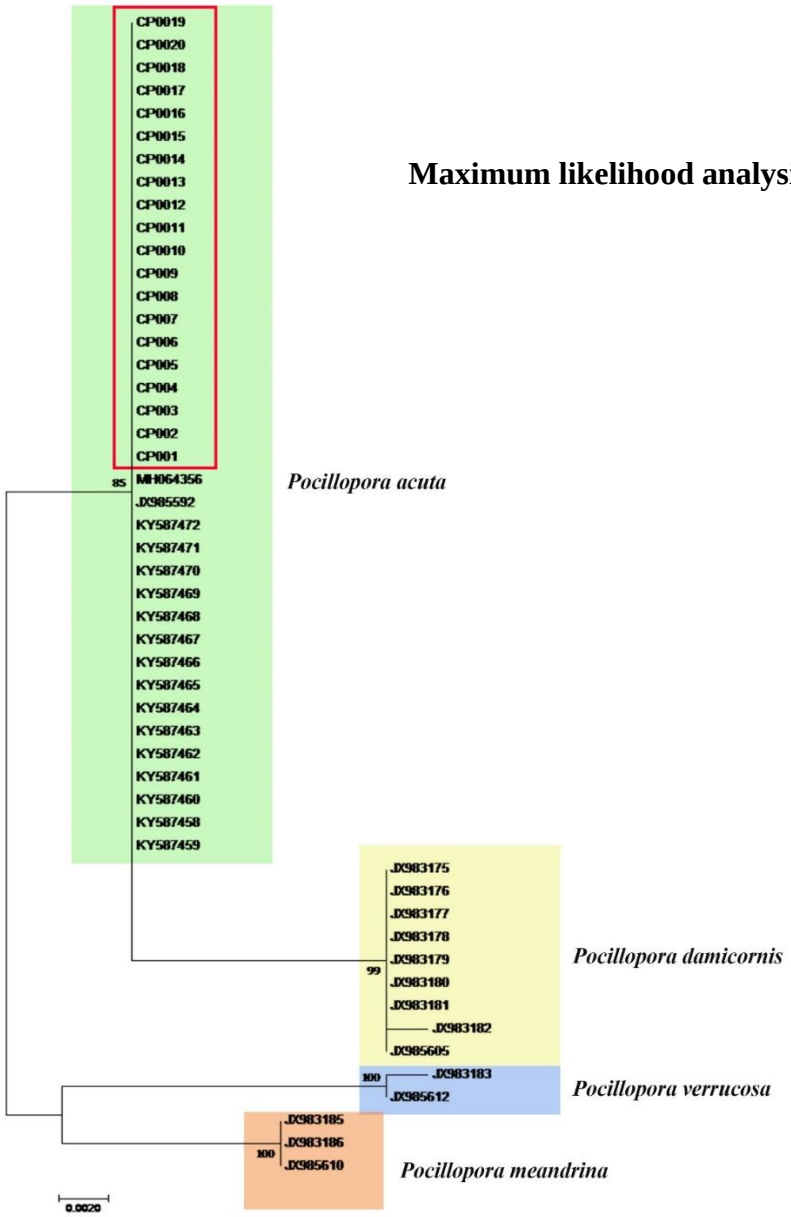

Figure 4. Phylogenetic relationships of genus *Pocillopora* isolates based on the mtORF sequences. Nodal support of >50% is shown maximum likelihood. Accession number and host origin are used to identify each sequence. Genotypes in the red quadrilateral are genotypes identified in this study.

This study shows no evidence of *P. damicornis* in the Western Gulf of Thailand. However, the high variation of colony branch thickness of *Pocillopora acuta* is clearly observed in the field surveys. Further studies should focus on a comprehensive analysis of *P. acuta* samples from different localities. The morphological variation may be associated with wave and current conditions, with thicker branches found in exposed reef sites (Poquita-Du et al. 2017). In general, *P. acuta* is recorded in Taiwan and Gulf of Thailand, but *P. damicornis* seems to be limited to the northern part of the South China Sea. In Japan, *P. damicornis* is found at Yaeyama Islands; however it is likely rare compared to *P. acuta* (Kitano et al. 2015). Regional investigation projects for analyzing genetic samples from various reef sites are required to explain the historical distributions of *P. acuta, P. damicornis* and other cryptic coral species. This study provides scientific data showing that most *Pocillopora* '*damicornis*-like' corals in the Western Gulf of Thailand are *P. acuta* rather than *P. damicornis*.

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