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MORPHOLOGICAL AND MOLECULAR EXAMINATIONS OF A NORTHWESTERN INDIAN OCEAN POPULATION OF THE AFRICAN ANGELSHARK, *Squatina* cf. *africana* Regan, 1908 (CHONDRICHTHYES: SQUATINIFORMES: SQUATINIDAE), WITH REMARKS ON INTRASPECIFIC VARIATIONS

Tassapon Krajangdara[1*], Jenjit Khudamrongsawat[2], Chanikarn Chaorattana[2], Pattarapon Promnun[2] and Simon Weigmann[3,4]

[1]*Phuket Marine Fisheries Research and Development Center, Wichit Subdistrict, Muang District, Phuket, 83000 Thailand*
[2]*Animal Systematic and Molecular Ecology Laboratory, Department of Biology, Faculty of Science, Mahidol University, Bangkok, 10400 Thailand*
[3]*Elasmo-Lab, Elasmobranch Research Laboratory, Hamburg, 22609 Germany*
[4]*Center of Natural History, University of Hamburg, Hamburg, 20146 Germany*
**corresponding author: tassapon@gmail.com*

ABSTRACT: Morphological and molecular examinations of angelshark samples from the northwestern Indian Ocean, collected by a Thai fishing vessel in February 2017, offered an opportunity to explore the diversity of angelsharks in this region. All specimens were similar to *Squatina africana* Regan, 1908 on several characteristics, such as the presence of simple nasal barbels with narrow, tapering tip, smooth to weakly fringed bases, enlarged denticles on snout, and the absence of paired ocelli on back, distinguishing them from *S. tergocellata* McCulloch, 1914, *S. pseudocellata* Last and White, 2008, and *S. legnota* Last and White, 2008, in the adjacent Indo-West Pacific region. However, morphological characteristics of these samples also showed intraspecific variation, particularly concerning size and coloration as well as many measurements, that were different from *S. africana*. The application of DNA barcoding using fragments of COI gene could not confidently support species identification. Although COI sequences of our samples indicated a monophyletic grouping with the known *S. africana* that was separated from other congeneric species with high statistical support, the genetic distance within this clade was greater than intraspecific genetic variation commonly reported in most elasmobranchs. Therefore, it was appropriate to identify as *S.* cf. *africana* until further confirmation with additional samples. Nevertheless, the new data on intraspecific variation found in our samples and comparison with *S. africana* fill in the knowledge gap of shark diversity and contribute to a much-needed conservation plan for angelsharks in western Indian Ocean.

Keywords: African angelshark, *Squatina* cf. *africana*, Indian Ocean, species identification, morphological variation, DNA barcoding

INTRODUCTION

Angelsharks of the genus *Squatina* Duméril, 1805 (Squatiniformes, Squatinidae) are distributed in tropical to warm temperate seas in coastal areas and on continental shelves and upper slopes, currently comprising 22 valid species that attain maximum sizes of 787 to 2,440 mm total length (TL) and occur in 0 to 1,289 m depths (updated from Weigmann 2016, 2017). The diversity of angelsharks in the Indian Ocean is poorly known (Berg *et al*. 2002; Van der Elst *et al*. 2012). The extreme southeastern Indian Ocean extending to the western extremity of the Indo-Australian region harbors *S. tergocellata* McCulloch, 1914, *S. pseudocellata* Last and White, 2008, and *S. legnota* Last and White, 2008, while only a single species, *S. africana* Regan, 1908 has been reported from the western Indian Ocean (Compagno 1984; Last and White 2008; Ebert 2013; Weigmann 2016). The range of *S. africana* includes the Cape Coast of South Africa, and the coasts of Mozambique, southern Madagascar and Tanzania (Ebert 2013). Type locality of *S. africana* is at Durban Bay, South Africa (Regan 1908). A more recent record from the southwestern coast of India (Ambily *et al*. 2018) suggested this species

has a wider distribution range in the Indian Ocean than previously recognized. The collection of angelsharks caught in the northwestern Indian Ocean in February 2017 offered an opportunity to test this hypothesis.

Samples from the aforementioned collection of angelsharks from northwestern Indian Ocean were tentatively identified as *Squatina* cf. *africana* (Krajangdara *et al*. 2019) based on the absence of paired ocelli on dorsal surface and nasal barbels weakly or not bifurcate (Ebert 2013). As the morphology of different species of angelsharks is rather similar, with few well-defined morphological characteristics and very limited information available from their original descriptions, misidentification remains a common issue (Theiss and Ebert 2013). Detailed examination of morphological characteristics and the use of molecular approaches was considered desirable for our specimens both to confirm species identification and to collect information regarding intraspecific variations (Ward *et al*. 2005; Holmes *et al*. 2009). As angelsharks in coastal areas suffer from anthropogenic threats as a result of both intentional and accidental capture (Ebert *et al*. 2015; Lawson *et al*. 2020; Ellis *et al*. 2021), exacerbated by their localized habits and low fecundity (Bridge *et al*. 1998; Baremore 2010), species-specific biological and fisheries data of angelsharks are necessary for evaluating current levels of exploitation, assessing conservation status and developing sustainable fishery management leading to maintenance of biodiversity of angelsharks (Ellis *et al*. 2021). This study aimed to describe morphological characteristics and explore intraspecific variations in morphology and genetics of the collected samples tentatively identified as *Squatina* cf. *africana.*

MATERIALS AND METHODS

Sample collection

Fourteen individuals of angelsharks were collected from northwestern Indian Ocean (Latitude: 10°27′ N to 10°42′ S and Longitude: 61°35′ E to 60°45′ E) by a Thai fishing vessel using single boat bottom otter trawls (Fig. 1) and landed at Ranong fishing port on 23 February 2017. The specimens were stored in a -20°C freezer at Phuket Marine Fisheries Research and Development Center in Phuket Province, Thailand. Pelvic fin tissues measuring 10 mm x 10 mm of the samples were cut and preserved in absolute ethanol at -20°C for genetic examination. After this study, all specimens were deposited at the Reference Collection of Phuket Marine Biological Center (PMBC), Thailand (catalogue numbers: PMBC 21219.01-PMBC 21219.14)

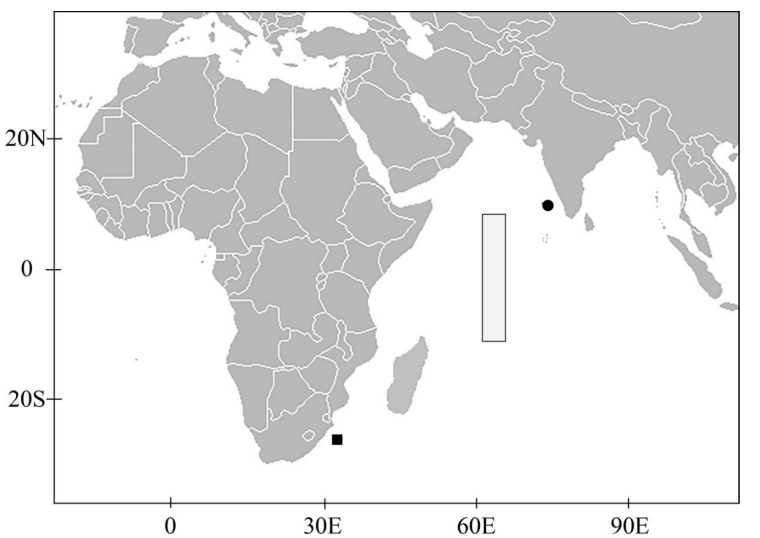

Figure 1. Map displaying locations of samples in the present study (rectangle: fishing area of trawler), a reference of *Squatina africana* from India (black circle; Ambily *et al*. 2018) and southwestern population (black square; Stelbrink *et al.* 2010).

Morphological examination

Morphological measurements were taken following Theiss and Ebert (2013) and Vaz and Carvalho (2013, 2018). Measurements were taken to the nearest 0.1 mm using a digital Vernier caliper and measuring tape. Differences between males and females were compared using nonmetric multidimensional scaling (NMDS) based on 78 morphometric measurements excluding total length and clasper characteristics. Grouping of data was pre-defined into two groups based on sex. The significant difference between groups was tested using PERMANOVA (permutations = 9999 and distance = Bray-Curtis distance). Descriptive diagnostic morphological characteristics were compared with the original description and other described individuals of *S. africana* (Bass *et al*. 1975; Compagno 1984; Ebert 2013; Ambily *et al*. 2018) and further comparison with other species. Intraspecific variation of ratios between specified morphological characteristics that could be used as numerical diagnostic characteristics was also explored for comparison with published information on *S. africana* (Compagno 1984; Ebert 2013; Ambily *et al.* 2018). These characteristics were quantified by sample mean, standard deviation (sd), and 95% confidence interval of the mean as well as using a Bayesian approach. The posterior mean (mu), posterior standard deviation (sigma), and their 95% credible intervals were estimated in R v4.0.4 (R Core Team 2021) and RStudio v1.3 (RStudio Team 2020), "wiqid" package v0.3.0 (Meredith 2020), with non-informative priors due to lack of available data of this species.

Another specimen labeled as "African angelshark" measuring 1,150 mm TL is currently deposited at Chulalongkorn University Museum of Natural History in Bangkok, Thailand. This specimen was collected in the Andaman Sea in 1981 at 267 to 303 meters depths and reported as *S. japonica* Bleeker, 1858 (Wongratana 1982). Due to its weakly bifurcate nasal barbels, the museum staff labeled this specimen as "*S. africana*". In order to clarify its identity, we tried to examine this specimen. As the specimen was old and in fragile condition, we could not carry out detailed morphometric examinations but only observe its external morphology based on a few diagnostic characteristics for species identification and comparison with *S. tergocellata*, *S. pseudocellata*, and *S. legnota*, which inhabit the nearby region.

Molecular examination

Genomic DNA was extracted from pelvic fin tissues using the NucleoSpin® tissue kit (Macherey-Nagel) following the manufacturer's protocols. Since the DNA was highly degraded, primers specific for short regions of COI gene were designed (forward primers: SqtCOI_L1 5'-ACCTCTGTCCACGGGACTAC-3', SqtCOI_L2 5'- ATGGCTTTCCCACGAATAAA-3'; reverse primers: SqtCOI_R1 5'- GATTGCTA-AATCTACGGATGCTC-3', SqtCOI_R2 5'- TGGGCT-CAAACGATAAAACC-3') using software Primer3 (ver. 0.4.0) (Koressaar and Remm 2007; Untergasser *et al.* 2012) based on sequences of other congeneric species described in Stelbrink *et al*. (2010). Fragments of cytochrome c oxidase I (COI) were amplified using AccuStart II GelTrack PCR SuperMix (Quanta BioSciences) 0.5 pmole of each primer and 20 ug of DNA sample. PCR condition was set as the following: initial denaturation at 95°C for 2 min followed by 30 cycles of 95°C for 15 sec, 50°C for 15 sec and 72°C for 30 sec following standard PCR protocol. PCR products were purified using AccuPrep® PCR Purification Kit (Bioneer Corporation) and sent for sequencing analyses with the Applied Biosystems BigDye Terminator v3.1 Cycle Sequencing Kit following the protocol from the manufacturer. The sequences were aligned using MEGA X (Kumar *et al.* 2018). Final alignment of COI was 615 base pairs (bp). All sequences were translated into coding proteins and deposited in GenBank database (GenBank accession No. MW680886-MW680899).

Basic Local Alignment Search Tool (BLAST) was used to find matching species in Genbank database (Altschul *et al*. 1990) based on available COI sequences. The relationship of our samples with congeners was inferred based on Bayesian Inference (BI) phylogenetic approach. The best evolutionary model was selected under Bayesian information criterion (BIC; Schwarz 1978) using Kakusan4 (Tanabe 2007). The BI trees were constructed using MrBayes v.3.2.7 (Ronquist *et al.* 2012) on CIPRES Science Gateway, with a Metropolis-coupled, Markov chain Monte Carlo (MC-MCMC), started from random tree and run twice in parallel with a four-chain analysis for 1 million generations. The trees were sampled every 500 generations. 25% of the generations were discarded as "burn-in". Effective Sample Size (ESS; >200) of outputs were checked in Tracer v1.7.1 (Rambaut *et al*. 2018). The posterior probability (95% or higher) estimated

from the remaining trees was considered as significant support (Larget and Simon 1999). The constructed phylogenetic trees were illustrated and edited in FigTree v.1.4.3 (Rambaut 2009). Genetic distance based on Kimura 2-parameters (K2P) was also calculated using MEGA X (Kimura 1980).

RESULTS AND DISCUSSION

Our samples constituted eight females and six mature males ranging from 537 to 800 mm with an average of 687 mm, which was comparable with the holotype of *Squatina africana* (BMNH: 1906.11.19.21, TL = 800 mm). Proportional measurements of our samples (PMBC 21219.01–PMBC 21219.14) are shown in Table 1. The samples revealed characteristics that mostly matched with the description of *S. africana* and the following characters: body relatively stocky and depressed, width at pectoral fin insertions (TRW) about 1.2 to 1.6 times head length, and 3.4 to 4.5 in precaudal length (PCL-D), dorsal coloration greyish brown with numerous light spots and flecks, but the dorsal coloration is somewhat variable (Fig. 2A), and whitish at ventral side (Fig. 2B); head width about 4.3 to 5.3 in precaudal length, head length 5.3 to 5.9 in precaudal length and 1.1 to 1.3 in head width; interorbital space concave; eye length 3.1 to 4.0 in interorbital space, mostly preorbital length greater than eye length, eye-spiracle space less than eye length; spiracle width greater than, but sometimes equal (1.0 to 1.3 times) to eye length, interspiracular space greater than or subequal to interorbital space; non-bifurcate nasal barbels with simple narrow tapering and some with spatulate tips and smooth to weakly fringed bases (Fig. 2C, 2D); teeth on both jaws erect and narrow cusped (Fig. 2 E), tooth row counts 18 to 20 on upper jaw and 18 to 19 on lower jaw; moderately large thorns or denticles present on snout, on interorbital space above and medial to eyes (Fig. 2F), adult males with enlarged thorns on anterior dorsal margins of pectoral fins and a few on pelvic fins; underside of body without denticles except for anterior margins of pectoral fins, apices of pelvic fins; lateral head folds low with single lobes in some specimens (Fig.

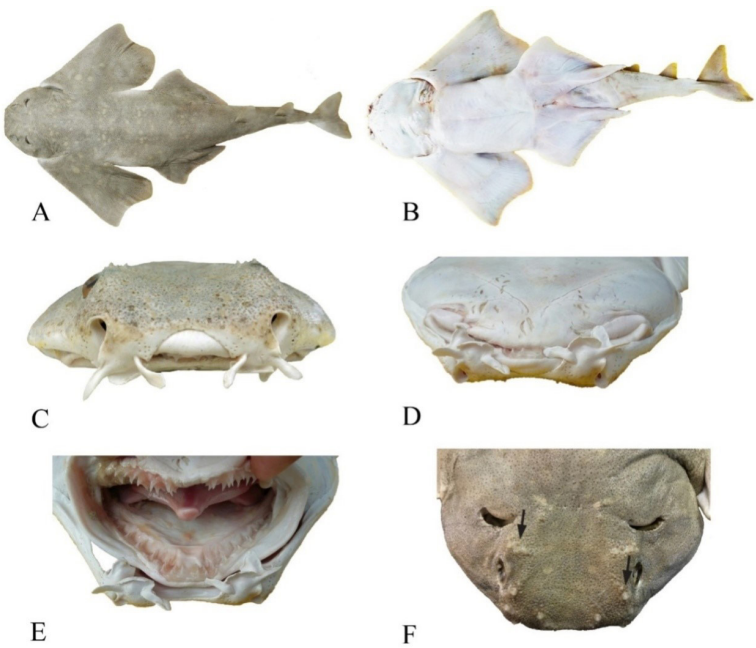

Figure 2. Morphological characteristics of collected samples including: (A) dorsal surface, showing broadly angular pectoral fins, (B) ventral side, (C) weakly fringed nasal barbels, smooth or weakly fringed nasal flaps, (D) ventral side of head, (E) teeth, and (F) denticles on head.

3); angle of pectoral fin apex narrowly obtuse, usually slightly greater than a right angle, pectoral fin anterior margins straight, not forming a distinct anterior shoulder, apices angular to narrowly rounded, posterior margin shallowly concave, rear tips usually broadly rounded or sometimes narrowly rounded, inner margins broadly convex and rounded, distance from anterior tip of pectoral fin to insertion (P1W) about 0.6 to 0.7 of pectoral fin length (P1L), pectoral fin length about 31.1 to 35.2% of total length, free rear tip of pectoral fin usually closer to pelvic fin origin than pelvic fin apex; first dorsal fin origin opposite pelvic fin free rear tips but sometimes posterior to them, first dorsal fin base about 1.6 to 2.5 in interdorsal space and 1.6 to 2.2 times in postdorsal space; postventral caudal margin oblique.

The size of head region in our samples was smaller than of *S. africana* and some specimens had single lobe at anterior part of lateral head folds that differed from the description of *S. africana* (lateral head folds without triangular lobes; see Ebert 2013), while the absence of paired ocelli on the back distinguished our samples from *S. tergocellata* and their weakly or not bifurcated nasal barbels with smooth or weakly fringed nasal flaps also separated them from *S. pseudocellata.* Furthermore, our samples were very similar to *S. legnota* in showing the presence of weakly or not bifurcated nasal barbels, they differed in the absence of two dark saddles below the dorsal fins and in not showing a blackish anterior ventral surface of pectoral fins. Nevertheless, our species is apparently much smaller than *S. legnota* that attains a maximum size of 1,341 mm TL, with three mature males ranging from 1,252 to 1,341 mm TL (Last and White 2008).

Our measurements of morphometric characteristics yielded information on intraspecific variations in the species (Table 2). The characteristics present in our samples were similar to those described for *S. africana* by Compagno (1984) and Ebert (2013). However, neither author mentioned the number of specimens of *S. africana* examined. Their mean values of measurements may not be universally representative throughout all populations of the species since they emanated from geographical areas different from our samples. An analysis of variation based on Bayesian Inference was performed and indicated with high probability that some of the measured characteristics and description of our samples fell within the range that included the described specimens of *S. africana* but differences were also observed. Ratios between pectoral fin width and pectoral fin length (P1W: P1L), first dorsal fin base length and first dorsal fin height (D1B: D1H), interdorsal space and dorsal-caudal space (IDS: DCS), precaudal length ventrally and head width (PCL-V: HDW) and pectoral fin length (P1L) appeared to be larger than the description of *S. africana.* On the other hand, ratios between tail width and vent-caudal length (TAW: VCL), spiracle length and eye length (SPL: EYL) and internarial space and nostril width (INW: NOW) were smaller than those of *S. africana*. As the number of samples may influence the measurements, our samples could not positively be identified as *S. africana* based on morphology just yet but should rather be called as *S.* cf. *africana*. Nevertheless, our data added information on morphological variation of this species, which can be used for more accurate species identification and examination of the variability of this species in future research.

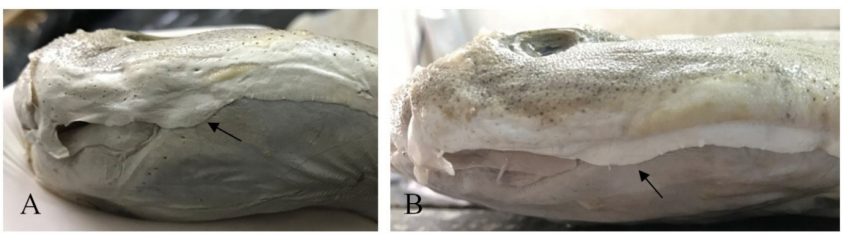

Figure 3. Lateral head folds of collected samples: (A) male and (B) female.

Table 1. Morphometric measurements of our samples (*Squatina* cf. *africana*: PMBC 21219.01-PMBC 21219.14), expressed as percentage of total length.

Catalogue numbers: PMBC 21219.01–14	**01–08 (Female = 8)**		**09–14 (Mature male = 6)**		**01–14 (Total = 14)**	
Morphometric characters	**Min.**	**Max.**	**Min.**	**Max.**	**Min.**	**Max.**
Total length (TL)	537 mm	800 mm	645 mm	691 mm	537 mm	800 mm
Precaudal length at dorsal side (PCL-D)	83.15	85.77	83.41	86.86	83.15	86.86
Precaudal length at ventral side (PCL-V)	80.92	86.12	82.02	85.99	80.92	86.12
Pre-second dorsal fin length (PD2)	73.26	75.05	73.02	75.62	73.02	75.62
Pre-first dorsal fin length (PD1)	62.67	64.38	61.87	63.36	61.87	64.38
Pre-pelvic fin length (PP2)	38.02	43.02	37.08	39.02	37.08	43.02
Pre-pectoral fin length (PP1)	15.08	19.97	17.81	19.12	15.08	19.97
Prebranchial length (PG1)	13.50	15.33	13.75	14.45	13.50	15.33
Prespiracular length (PSP)	7.54	8.63	8.06	8.47	7.54	8.63
Pre-ocular length (PEY)	4.50	5.64	4.23	5.79	4.23	5.79
Preorbital length (POB)	2.14	2.90	2.48	3.12	2.14	3.12
Head length (HDL)	14.50	16.01	14.88	15.96	14.50	16.01
Head width (HDW)	16.73	19.56	16.02	18.96	16.02	19.56
Head width in front of eyes (HWEY)	11.21	14.18	12.03	13.35	11.21	14.18
Head width in front of spiracles (HWSP)	17.29	20.55	18.31	19.07	17.29	20.55
Head height (HDH)	5.16	6.96	4.65	5.99	4.65	6.96
Mouth-inner width (MOW-I)	10.84	12.80	11.51	12.11	10.84	12.80
Mouth-outer width (MOW-O)	12.57	14.20	12.69	13.51	12.57	14.20
Upper lip arch width (UAW)	3.26	4.44	3.30	3.90	3.26	4.44
Upper lip arch height (UAH)	1.12	1.36	1.20	1.43	1.12	1.43
Interorbital space (INO)	7.40	8.81	7.74	8.32	7.40	8.81
Eye length (EYL)	1.91	2.62	2.08	2.56	1.91	2.62
Eye width (EYW)	1.13	1.69	1.46	1.75	1.13	1.75
Eye-spiracle space (ESL)	1.63	2.11	1.48	2.14	1.48	2.14
Nostril width (NOW)	1.61	1.88	2.12	2.59	1.61	2.59
Anterior nasal flap length (ANF)	1.10	1.84	1.85	2.24	1.10	2.24
Internarial space (INW)	6.11	7.41	6.23	6.72	6.11	7.41
Interspiracular space (INS)	7.28	8.20	7.27	7.74	7.27	8.20
Spiracle length (SPL)	2.35	3.41	2.35	2.89	2.35	3.41
Intergill width (ING1)	8.14	9.34	7.97	9.55	7.97	9.55
Intergill length (ING)	2.14	2.91	2.23	2.92	2.14	2.92
Interdorsal space (IDS)	6.83	7.49	6.51	7.88	6.51	7.88
Dorsal-caudal space (DCS)	6.32	7.30	6.97	7.36	6.32	7.36
Pectoral-pelvic space (P1P2S)	10.95	14.23	9.99	14.89	9.99	14.89
Pelvic (origin)-caudal space (P2OCS)	42.09	47.38	44.62	48.31	42.09	48.31

Catalogue numbers: PMBC 21219.01–14	**01–08 (Female = 8)**		**09–14 (Mature male = 6)**		**01–14 (Total = 14)**	
Morphometric characters	**Min.**	**Max.**	**Min.**	**Max.**	**Min.**	**Max.**
Pelvic-caudal space (P2CS)	31.66	34.52	34.92	37.96	31.66	37.96
Width at pectoral origins (WP1)	15.73	18.08	13.50	15.99	13.50	18.08
Trunk width (TRW)	19.40	25.13	18.89	20.39	18.89	25.13
Tail width (TAW)	12.35	14.79	10.32	14.09	10.32	14.79
Tail height (TAH)	3.91	6.81	4.45	6.51	3.91	6.81
Pectoral-fin length (P1L)	33.75	35.20	31.08	32.40	31.08	35.20
Pectoral-fin anterior margin (P1A)	28.75	31.14	27.85	28.84	27.85	31.14
Pectoral-fin base length (P1B)	11.08	12.57	10.52	11.24	10.52	12.57
Pectoral-fin width (P1W)	19.71	21.71	19.83	21.35	19.71	21.71
Pectoral-fin inner margin (P1I)	17.19	18.68	17.06	18.14	17.06	18.68
Pectoral-fin posterior margin (P1P)	12.66	15.34	12.31	15.52	12.31	15.52
Pelvic-fin length (P2L)	23.97	25.66	23.08	25.26	23.08	25.66
Pelvic-fin width (P2W)	10.80	15.45	11.08	14.58	10.80	15.45
Pelvic-fin inner margin (P2I)	10.13	13.04	11.38	13.49	10.13	13.49
Pelvic fin posterior margin (P2P)	17.08	22.49	17.36	19.69	17.08	22.49
First dorsal-fin length (D1L)	6.37	7.02	6.35	7.18	6.35	7.18
First dorsal-fin base length (D1B)	2.98	4.23	3.57	4.31	2.98	4.31
First dorsal-fin anterior margin (D1A)	8.13	9.39	8.61	9.65	8.13	9.65
First dorsal-fin height (D1H)	4.80	6.79	5.19	7.60	4.80	7.60
First dorsal-fin inner margin (D1I)	2.45	3.13	2.33	3.21	2.33	3.21
First dorsal-fin posterior margin (D1P)	4.63	5.64	5.25	6.19	4.63	6.19
Second dorsal-fin length (D2L)	5.85	6.48	6.15	6.73	5.85	6.73
Second dorsal-fin base length (D2B)	2.75	3.70	3.10	3.80	2.75	3.80
Second dorsal-fin anterior margin (D2A)	7.50	8.13	8.03	8.96	7.50	8.96
Second dorsal-fin height (D2H)	4.45	5.77	4.15	6.92	4.15	6.92
Second dorsal-fin inner margin (D2I)	2.50	3.20	2.63	3.18	2.50	3.20
Second dorsal-fin posterior margin (D2P)	4.09	5.19	4.34	5.60	4.09	5.60
Dorsal caudal-fin margin (DCM)	12.00	13.83	12.76	14.04	12.00	14.04
Preventral caudal-fin margin (CVM)	15.13	18.23	15.88	18.46	15.13	18.46
Caudal-fin height (CAH)	11.92	16.95	11.24	17.36	11.24	17.36
Lower post ventral caudal margin (CPL)	4.16	5.62	4.11	5.22	4.11	5.62
Upper post ventral caudal margin (CPU)	5.99	8.15	6.22	8.27	5.99	8.27
Sub-terminal caudal fin margin (CST)	3.00	4.53	3.88	4.87	3.00	4.87
Clasper inner length (CLI)	-	-	18.41	20.41	18.41	20.41
Clasper outer length (CLO)	-	-	15.30	17.32	15.30	17.32
Clasper base width (CLB)	-	-	2.65	2.99	2.65	2.99
Snout-vent length (SVL)	44.28	48.75	43.54	47.18	43.54	48.75

Catalogue numbers: PMBC 21219.01–14	01–08 (Female = 8)		09–14 (Mature male = 6)		01–14 (Total = 14)	
Morphometric characters	**Min.**	**Max.**	**Min.**	**Max.**	**Min.**	**Max.**
Vent-caudal length at anterior cloaca (VCL)	53.58	57.30	55.19	58.54	53.58	58.54
Pre-caudal length ventrally to origin of long precaudal ridge	75.98	82.56	76.92	81.02	75.98	82.56
Pelvic (origin)-caudal distance excluding the precaudal ridge	35.57	41.64	37.69	42.04	35.57	42.04
Pelvic-caudal distance excluding the precaudal ridge	22.91	29.36	27.75	30.66	22.91	30.66
Preventral caudal-fin margin including precaudal ridge	84.43	88.43	84.81	89.93	84.43	89.93
Distance from base of tail to origin of first dorsal	20.11	21.53	20.46	23.07	20.11	23.07

Intraspecific variations in morphological characteristics of our samples revealed information regarding sexual differences. Although the 95% confident interval indicated potential size overlapping between males and females probably due to small sample size, the differences in measurements of these groups were observed ($p<0.05$) (Fig. 4). Sexual size dimorphism in angelsharks was observed in *S. tergocellata* (Bridge *et al*. 1998) and *S. oculata* Bonaparte, 1840 (Capapé *et al*. 2002) but not in *S. guggenheim* Marini, 1936 (Awruch *et al*. 2008).

The single specimen at Chulalongkorn University Museum of Natural History labeled as "*Squatina africana*" was slightly damaged but its nasal barbels appeared weakly bifurcate in contrast to *S. pseudocellata* (Fig. 5A, 5B). Paired ocelli were absent on its back (Fig. 5A) distinguishing this specimen from *S. tergocellata*, which further differs from that specimen in having greatly enlarged orbital thorns. Based on the weakly bifurcate nasal barbels and the total length of the specimen, it is re-identified here as *S*. cf. *legnota*. Although whitish ventral side and no black saddles are detectable below the dorsal fins (Fig. 5C, 5D), these are not always present in *S. legnota* (see Last and White 2008). Furthermore, the specimen is generally faded and, thus, any saddles could have become indistinct after decades of preservation. For further clarification, it would be desirable to carry out molecular analyses, but it is unclear if the specimen was fixed in formaldehyde and if it is still possible to obtain useable tissue samples. *Squatina legnota* is currently known only from off eastern Indonesia (Last and White 2008; Krajangdara 2017). Therefore, this record would represent a range extension if confirmed.

Molecular data revealed unclear species verification. Although all COI sequences of our samples matched with the reference sequences of *S. africana* (Stelbrink *et al*. 2010) with 99.84% maximum percent identity and the BI phylogenetic tree showed monophyly with *S. africana* with strong statistical support (Fig. 6). Our samples formed a separate clade from *S. africana* (except for the sample from India) with the K2P genetic distance of 2.4%, which was greater than 2% variation suggested for intraspecific variation of individuals of the same species (Ward *et al*. 2008; Ward 2009). Although species affinity based on DNA barcode is not well-defined, 2% or less genetic variation of a single species was reported for 96% of known elasmobranchs (Ward *et al*. 2008; Naylor *et al*. 2012). In addition, Ward *et al*. (2008) further suggested a factor of 15 or greater obtained from the ratio of genetic distance between and within locality of a species that would require extensive examination of other characteristics as it may be cryptic species. The southwestern Indian Ocean population showed no intraspecific variation while our samples and the Indian sample revealed 0.2% variation. Our data yielded a factor of 12 but would be 24 if excluding the sample from India. It was apparent that this criterion highly depended to the number of samples included in the calculation and variation within localities since the addition of samples might challenge the result. To avoid

Table 2. Average morphological variations presented in minimum and maximum of ratios of morphometric characters in our samples (N = 14) compared with other references of *Squatina africana.*

Ratios of characteristics	Our samples (PMBC 21219.01-PMBC 21219.14)							References of *Squatina africana*		
	range	mean	sd	95% cf	posterior mean (mu)	posterior sd (sigma)	mu lower-upper ci	Compagno (1984)	Ebert (2013)	Ambily *et al.* (2018)
P1W: P1L	0.57-0.68	0.62	0.03	0.60-0.64	0.62	0.03	0.60-0.64	0.60	NA	0.83
TAW: VCL	0.18-0.26	0.23	0.02	0.22-0.25	0.23	0.03	0.22-0.25	0.25	NA	NA
D1B: D1H	0.47-0.81	0.65	0.10	0.60-0.70	0.65	0.10	0.59-0.70	0.50	NA	0.57
D1B: D2B	0.94-1.32	1.13	0.09	1.08-1.18	1.13	0.10	1.08-1.18	≥ 1	NA	1.05
IDS: DCS	0.89-1.17	1.04	0.09	0.99-1.08	1.04	0.09	0.99-1.09	≤ 1	NA	0.43
SPL: EYL	0.94-1.31	1.17	0.11	1.12-1.23	1.17	0.12	1.11-1.24	NA	1.20-1.40	1.41
INO: INS	0.94-1.31	1.05	0.03	1.04-1.07	1.05	0.04	1.03-1.07	NA	1.10-1.30	1.05
INW: NOW	2.50-4.43	3.35	0.60	3.03-3.66	3.35	0.64	3.01-3.71	NA	3.70-4.80	4.34
IDS: D1B	1.63-2.51	1.85	0.22	1.74-1.97	1.85	0.23	1.73-1.98	NA	1.70-2.40	2.07
PCL-V: HDW	4.23-5.23	4.69	0.33	4.51-4.86	4.69	0.35	4.50-4.88	NA	3.30-4.00	4.10
INO: EYL	3.12-3.95	3.48	0.30	3.33-3.64	3.48	0.31	3.32-3.66	NA	3.10-4.00	3.66
POB: EYL	0.86-1.50	1.18	0.18	1.09-1.27	1.18	0.19	1.08-1.28	NA	>1	1.35
ESL: EYL	0.59-0.98	0.80	0.10	0.75-0.86	0.81	0.11	0.75-0.87	NA	≤ 1	0.89

* sd = standard deviation; cf = confidence interval; ci = credible interval, all abbreviations of ratios are shown in Table 1.

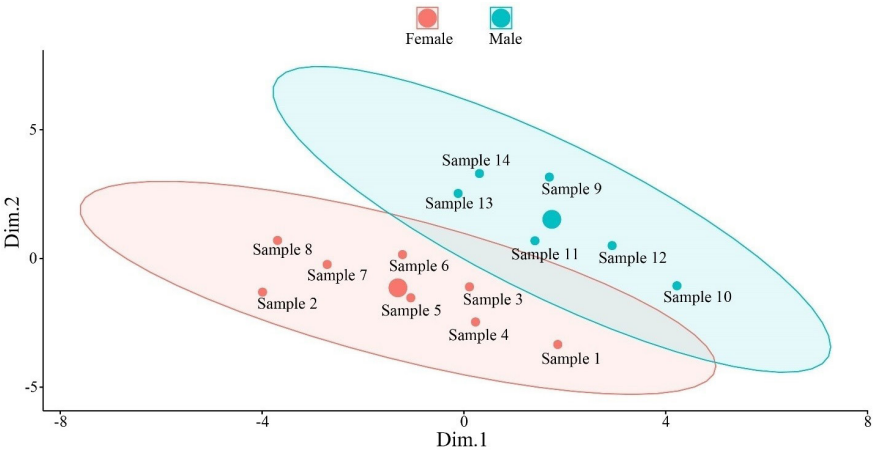

Figure 4. Multidimensional scaling plot showing variation among samples; grouping based on sexes; pink ellipse = 95% confident interval of female; blue ellipse = 95% confident interval of male; big pink circle = centroid of female data; big blue circle = centroid of male data.

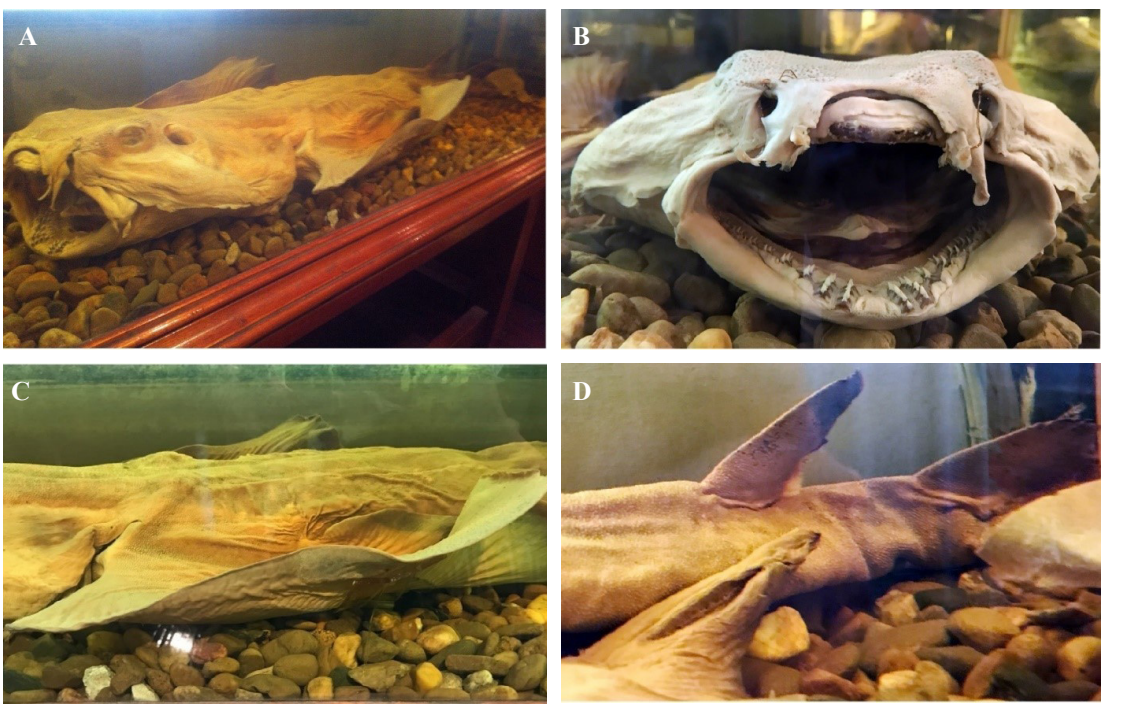

Figure 5. A specimen at Chulalongkorn University Museum of Natural History, Bangkok, Thailand: (A) the specimen stored in a glass tank, (B) its nasal barbels, (C) whitish ventral side, and (D) lack of black saddles below dorsal fins.

a misidentification, our samples were tentatively identified as *S*. cf. *africana* in concordance with the morphological data. The sample from India sharing the same clade with our samples may require further examination of morphology and genetics as it showed slight differences in morphological characteristics compared to our samples.

Despite that fact that species verification could not be confirmed, our data provided evidence of the existence of angelsharks in the northwestern Indian Ocean and contributed additional information of geographic distribution of this group of sharks in the Indian Ocean. The lack of conservation plans at all scales in this ocean as well as the need for more research will likely jeopardize the populations of angelsharks in the future. The information from our present study, especially a potential distinctive angelshark species as well as their intraspecific variation in both morphology and genetics, could be incorporated into future conservation and management plans of sharks in the Indian Ocean.

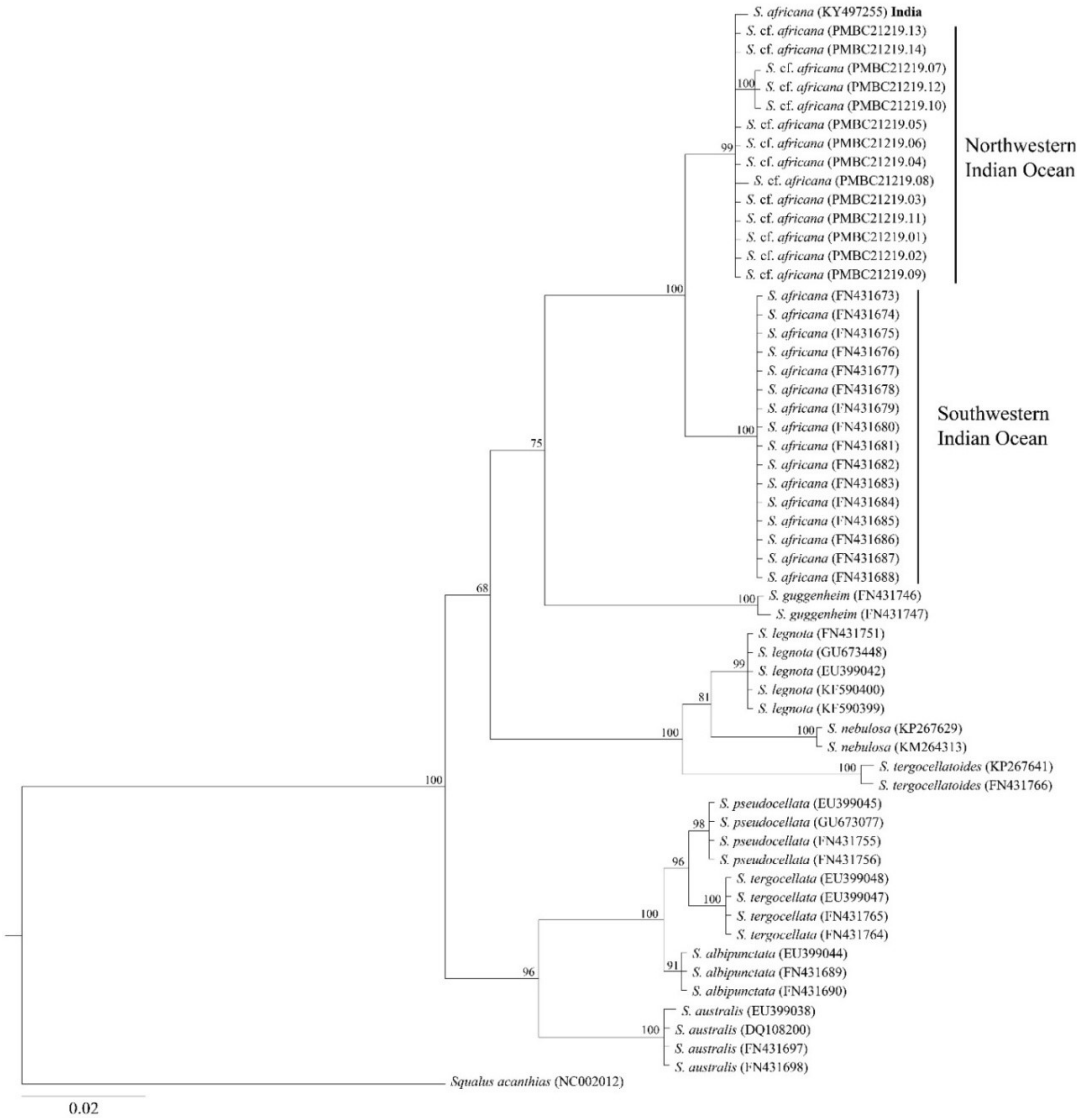

Figure 6. Bayesian Inference phylogenetic tree based on COI gene of *Squatina* cf. *africana* (this study: PMBC 21219.01-PMBC 21219.14), *S. africana* from coast of India (Ambily *et al*. 2018), *S. africana* (Stelbrink *et al*. 2010). Numbers on nodes represent BI posterior probability.

CONCLUSIONS

Fourteen angelshark samples from the northwestern Indian Ocean collected by a Thai trawler in February 2017 were morphologically similar to *Squatina africana* Regan, 1908 based on several characteristics. However, thorough morphological examination of these samples revealed intraspecific variation, particularly concerning many morphometric measurements and some characters that were different from those of *S. africana*. The application of DNA barcoding using fragments of COI gene could not confidently support species identification. Although COI sequences of our samples indicated a monophyletic grouping with the known *S. africana* and clear separation from other congeneric species with high statistical support, our samples formed distinct clade that was separated from *S. africana.* With this uncertainty, the samples were identified as *S*. cf. *africana* until further confirmation with additional samples. Furthermore, a specimen deposited at Chulalongkorn Museum of Natural History that was labeled as "*S. africana*" was unlikely *S. africana* due to great size difference and questionable morphological characteristics and therefore should be identified as "*S*. cf. *legnota*" based on close morphological similarities and geographic distance. Our findings on intraspecific variation in morphology and genetics of African angelsharks revealed hidden diversity of sharks in the Indian Ocean, which may contribute to a much-needed conservation plan for chondrichthyans in this region.

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