

Morphological and Genetic Evidence Confirmed Three New Records of Ghost Shark Species (Chimaeriformes) From the Andaman Sea of Thailand

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ABSTRACT.— Three species of ghost sharks (Chimaeriformes) were recorded for the first time from the Andaman Sea of Thailand during a deep-sea trawl survey conducted from October 1-15, 2018. Morphological characteristics primarily revealed species described as the sicklefin chimaera, *Neoharriotta pinnata* (Rhinochimaeridae), longspine chimaera, *Chimaera* aff. *macrospina* (Chimaeridae) and Philippine chimaera, *Hydrolagus* cf. *deani* (Chimaeridae). The presence of *N. pinnata* in the Andaman Sea of Thailand provided a plausible extension of its distributional range, but the record of the other two ghost sharks were far outside their known ranges and remained tentative. Using DNA barcoding, the *Chimaera* aff. *macrospina* sample was different from Australian *C. macrospina* and any other *Chimaera* species whose DNA sequences were available in databases. The sample of *Hydrolagus* cf. *deani* showed slight differences in morphology with the known *H. deani*, *H. mitsukurii* and *H. africanus*. It was close to *H. africanus* based on the genetic information, but state morphologically, especially shape of second dorsal fin, this specimen was most similar to *H. deani*.

KEY WORDS: *Chimaera*, *Hydrolagus*, *Neoharriotta*, morphological, DNA barcoding, Andaman Sea, Thailand

INTRODUCTION

Ghost sharks or chimaeras (Chimaeriformes) are a little-known group of cartilaginous fishes consisting of three families, (Chimaeridae, and Rhinochimaeridae) of which primarily occurs in the deep-sea and (Callorhynchidae) a shallow water family (Nelson et al., 2016; Ebert, 2014). The deep-sea chimaerids are distributed circumglobally in most oceans except in Antarctic waters (Ebert and Winton, 2010). The Indo-Pacific region has the highest diversity with at least 30 species

(Didier et al., 2012; Ebert, 2014; Weigmann, 2016). In the Indian Ocean region, at least 21 species and five genera of chimaeroids are known to occur in this region (Ebert, 2014; Clerkin et al., 2017; Walovich et al., 2017). As a part of the eastern Indian Ocean region (FAO Major Fishing Area 57), information on chimaeroids in the Andaman Sea of Thailand are still very few. Since 1975, studies on deep-sea fishes in the Andaman Sea of Thailand have been conducted. So far only one family and two species (*Chimaera* cf. *phantasma* and an unidentified *Hydrolagus* sp.)

have been recorded from the Andaman Sea of Thailand (Ali et al., 2014; Krajangdara, 2017). The 2018 Deep Sea Expedition in the Andaman Sea of Thailand by the Dr. Fridtjof Nansen Research Vessel has obtained three specimens of ghost sharks that provided an opportunity to explore diversity of this group of fish in this region.

The objective of this study was to identify species of these three specimens based on their morphological characteristics. In case of uncertainty, a mitochondrial DNA barcoding study was conducted for confirmation. These records not only enrich the diversity of deep-sea chondrichthyans of Thailand waters but could potentially provide information on the extended distribution of those species in the eastern Indian Ocean region.

MATERIALS AND METHODS

Morphological examination

A deep-sea survey under the Department of Fisheries (DoF), Thailand and FAO project were conducted in the Andaman Sea of Thailand using Dr. Fridtjof Nansen Research Vessel from October 1-15, 2018. The bottom trawl sampling was performed at various depths from 212 to 781 meters (Fig. 1). A mature male of *Neoharriotta pinnata* (Schnakenbeck, 1931) was caught on October 7, 2018 at depth 506-510 m (Long. 97.01°E and Lat. 08.17° N), while an immature male of *Chimaera* aff. *macrospina* Didier, Last and White, 2008 and a female of *Hydrolagus* cf. *deani* (Smith and Radcliffe, 1912) were collected on October 11, 2018 at depth 772-775 m (Long. 96.99° E and Lat. 07.54° N). Identification of those three species was based on identification keys for chimaeroids in Didier and Stehmann (1996), Compagno (1999), Last and Stevens (2009), Ebert (2014), and

comparisons with description papers of related species and records from other areas (Smith, 1912; Didier et al., 2008; Jawad et al., 2012; Suresh and Raffi, 2012; Walovich et al., 2015).

Morphological measurements were taken following terms and morphometric standards from FAO (Compagno, 1999; Ebert, 2014), Didier et al. (1996), Walovich et al. (2015) and Clerkin et al. (2017). All measurements were taken using measuring tape and digital vernier caliper to the nearest mm (Table 1). All specimens were labelled and deposited at the Reference Collection of Phuket Marine Biological Center (PMBC), Thailand.

Genetic examination

Selection of gene fragments for analyses was based on reference sequences available in GenBank and the Barcode of Life Data System (BOLD) databases. This part was performed in the putative *Chimaera* aff. *macrospina* and *Hydrolagus* cf. *deani*. Identification of the samples was based on the use of COI sequences for *C.* aff. *macrospina* and ND2 sequences for *H.* cf. *deani*, using Basic Local Alignment Search Tool (BLAST), which were the only reference sequences available in GenBank and the Barcode of Life Data System databases.

Genomic materials of the two specimens of these ghost sharks were extracted (taken from pelvic fin). Initial amplification of mitochondrial COI and ND2 sequences was conducted using universal primers (Naylor et al., 2012; Ward et al., 2005) but failed to produce clear sequence information possibly due to tissue degradation. As DNA templates were degraded into short fragments, specific primers needed to be designed for PCR amplification. External and internal primers for the amplification of the COI gene were designed using the complete mitochondrial DNA sequence of *Chimaera fulva* (GenBank accession No. HM147138.1) and the COI

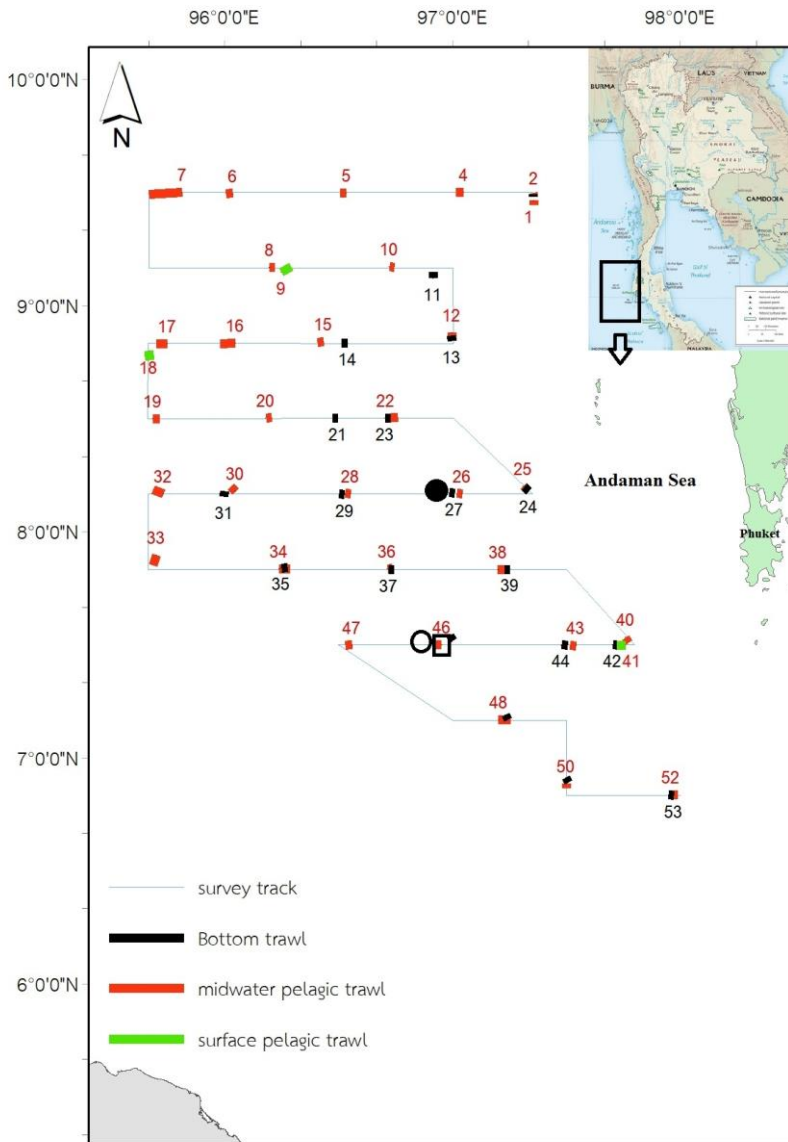


FIGURE 1. Map of the record localities of *Neoharriotta pinnata* (●), *Chimaera* aff. *macrospina* (○) and *Hydrolagus* cf. *deani* (□) in the Andaman Sea of Thailand.

sequence of *C. macrospina* (BOLD ID: FOAF581-07), respectively. For amplification of the ND2 region, the whole mitochondrial DNA sequence of *Hydrolagus lemures* (GenBank accession No. HM147139.1) was used to design external primers. The ND2 sequence of *H. mitsukurii* (GenBank accession

No. KF927898.1) was used to design internal primers. Short regions of these genes were then specified, and primers were designed to cover these fragments using Primer3 (ver. 0.4.0) (Koressaar and Remm, 2007; Untergasser et al., 2012) (Table 2).

TABLE 1. Morphometric measurements of three chimaeroids from the Andaman Sea of Thailand, expressed as percentage of body length.

| Species | <i>Neoharriotta pinnata</i> | <i>Chimaera aff. macrospina</i> | <i>Hydrolagus cf. deani</i> |
|------------------------------------|-----------------------------|---------------------------------|-----------------------------|
| Collection number | PMBC 30401 | PMBC 30399 | PMBC 30400 |
| Sex | Mature male | Immature male | Female |
| Total length (TL) | 1092 mm | 508 mm | 739 mm |
| Precaudal length (PCL) | 797 mm | 360 mm | 362 mm |
| Body length (BDL) | 478 mm | 276 mm | 292 mm |
| Preorbital length (POB) | 45.6 | 10.1 | 13.0 |
| Prenarial length (PRN) | 39.8 | 13.0 | 12.7 |
| Preoral length (POR) | 38.7 | 11.2 | 9.3 |
| Snout-vent length (SVL) | 105.9 | 71.4 | 56.9 |
| Pre-first dorsal (PD1) | 66.3 | 29.7 | 26.7 |
| Pre-second dorsal (PD2) | 100.8 | 48.2 | 44.9 |
| Pre-pectoral (PP1) | 68.2 | 33.0 | 25.7 |
| Pre-pelvic (PP2) | 108.2 | 65.9 | 59.9 |
| Snout width (SWF) | 5.6 | 8.0 | 3.8 |
| Snout width at base (SWB) | 9.8 | 9.1 | 7.9 |
| Snout height at base (SHB) | 9.2 | 9.4 | 7.2 |
| Head length (HDL) | 65.7 | 30.4 | 24.0 |
| Head height (HDH) | 25.7 | 24.3 | 19.9 |
| Head width (HDW) | 14.9 | 12.0 | 13.7 |
| Eye length (EYL) | 7.7 | 9.8 | 10.6 |
| Eye height (EYH) | 5.5 | 6.2 | 6.5 |
| Interorbital space (INO) | 3.8 | 6.9 | 0.3 |
| Mouth length (MOL) | 5.9 | 4.4 | 3.8 |
| Mouth width (MOW) | 10.1 | 8.0 | 6.9 |
| Trunk width (TRW) | 22.4 | 15.9 | 11.6 |
| Trunk length (TRL) | 43.9 | 40.2 | 38.4 |
| Pectoral-pelvic space (PPS) | 32.4 | 26.1 | 32.9 |
| Dorsal-caudal space (DCS) | 10.3 | 1.8 | 0.7 |
| Anal-caudal space (ACS) | 1.1 | 1.5 | - |
| Interdorsal space (IDS) | 8.0 | 5.8 | 6.9 |
| Pelvic-caudal space (PCA) | 53.6 | 62.3 | 61.6 |
| Pectoral fin max. length (PIL) | 29.5 | 36.2 | 37.0 |
| Pectoral fin anterior margin (PIA) | 29.5 | 35.1 | 37.0 |

TABLE 1. (Continue)

| | | | |
|---|------|------|-------|
| Pectoral fin base (PIB) | 8.6 | 8.7 | 9.9 |
| Pelvic fin max. length (P2L) | 19.3 | 17.0 | 21.2 |
| Pelvic fin anterior margin (P2A) | 19.3 | 15.9 | 21.2 |
| Pelvic fin base (P2B) | 5.2 | 5.8 | 4.8 |
| First dorsal fin anterior margin (D1A) | 25.3 | 23.9 | 22.9 |
| First dorsal fin base (D1B) | 24.3 | 15.6 | 18.5 |
| First dorsal fin height (D1H) | 19.7 | 21.7 | 19.9 |
| Dorsal spine height (DSA) | 24.3 | 27.2 | 25.3 |
| Second dorsal fin base (D2B) | 50.2 | 79.7 | 80.5 |
| Maximum height of anterior of second dorsal fin | 5.4 | 6.2 | 6.2 |
| Maximum height of the middle of second dorsal fin | - | - | 1.7 |
| Maximum height of posterior of second dorsal fin | 5.4 | 6.2 | 3.8 |
| Second dorsal fin length (D2L) | 54.2 | 81.2 | 80.8 |
| Second dorsal fin inner margin (D2I) | 2.5 | 2.9 | 0.5 |
| Anal fin length (ANL) | 14.6 | 6.5 | - |
| Anal fin base (ANB) | 9.8 | 3.3 | - |
| Anal fin height (ANH) | 10.9 | 4.7 | - |
| Dorsal caudal margin length (CDM) | 36.6 | 19.9 | 26.0 |
| Ventral caudal margin length (CVM) | 49.0 | 22.8 | 37.7 |
| Caudal filament length (CFI) | 24.3 | 34.1 | 103.8 |
| Total caudal length (CTL) | 63.0 | 54.0 | 128.1 |
| Maximum height of upper lobe of caudal fin (CDH) | 2.3 | 2.9 | 2.4 |
| Maximum height of lower lobe of caudal fin (CVH) | 3.8 | 2.9 | 2.1 |
| Origin of D1 to origin of P1 (D1P1) | 19.0 | 17.0 | 12.7 |
| Origin of D1 to origin of P2 (D1P2) | 45.6 | 39.5 | 33.9 |
| Origin of D2 to origin of P1 (D2P1) | 34.7 | 23.2 | 24.0 |
| Origin of D2 to origin of P2 (D2P2) | 19.2 | 22.5 | 18.8 |

Regions of COI and ND2 were amplified following standard PCR protocol. Successfully amplified products were visualized, purified, and sequenced. Sequences were corrected and aligned using MEGA7 (Kumar et al., 2016; Tamura et al., 2004). Final alignments of COI and ND2 were 641 base pairs (bp) and 1,044 bp, respectively. All sequences were deposited in NCBI database (GenBank

accession Nos. MN626332 and MN626333). Phylogenetic trees were constructed using maximum likelihood (MEGA7). The selected model for ML was HKY+G (bootstrap support values = 1,000 iterations). Calculation of genetic pair wise distance based on Kimura 2-parameter (K2P) using bootstrap support values of 1,000 iterations was performed in MEGA7.

TABLE 2. Lists of primers used for gene amplification.

| DNA barcode region | Name of primer | Primer sequence (5'→3') |
|--------------------|----------------|-------------------------|
| COI | ChiCOI_L1 | CGCCTAAACTCAGCCATCTT |
| | ChiCOI_R1 | AGTACCCGCACCTGCTTCTA |
| | ChiCOI_L2 | CGCCCTAATGGGAGATGAT |
| | ChiCOI_R2 | ACCGGCTGCTAGAACAGGTA |
| | ChiCOI_L3 | CCCTCTAGCAGGGAATCTAGC |
| | ChiCOI_R3 | TCCAAATCCGGGTAGAATTA |
| ND2 | HydND2_L1 | GGCCCATACCCCAAACAC |
| | HydND2_R1 | GTGGAGAGAAGTGCCAAGGT |
| | HydND2_L2 | AGCCTAAAACCTGGGCCTTG |
| | HydND2_R2 | TGTTGTCATTGAGAGGGAGTTG |
| | HydND2_L3 | ACCTTGGCACTTCTCTCCAC |
| | HydND2_R3 | TGTCTGGGTTGCATTGAGAG |

RESULTS AND DISCUSSION

Neoharriotta pinnata

A mature male of longnose chimaera specimen (PMBC 30401; 1,092 mm TL) was initially identified as the sicklefin chimaera, *Neoharriotta pinnata* (Schnakenbeck, 1931); Family Rhinochimaeridae Garman, 1901 (Fig. 2). It was obtained on October 7, 2018, Andaman Sea, Thailand (Long. 97.01° E and Lat. 08.17° N) at the depth of 506-510 m.

A longnose chimaerid with characteristics as follows: body flabby, elongate, tapering to a caudal fin with a filamentous tail. Head large, its length about 0.4 times precaudal length and 65.7% of body length (BDL). Snout long and pointed, widely based; preoral length 58.9% of head length and 2.6 times in body length; snout width at base 1.8 times snout width. Eyes relatively large, eye length 11.7% head length and its height 0.2 times head height. Oral and preopercular lateral line canals well separated; lateral line on trunk relatively straight, not undulating. Pectoral fins relatively broad and short, not

reaching the origin of pelvic fins. Pelvic fins relatively small, broadly rounded on the posterior margin; the maximum length 1.5 times in pectoral maximum length. First dorsal fin small, its base 24.3% BDL and the height 0.2 times BDL. Dorsal spine straight and relatively long, about 1.2 times first dorsal fin height. The origin of the dorsal spine just opposite the pectoral fin origin. Second dorsal fin low, prolonged and slightly convex; its height 3.7 times in first dorsal fin height; the base 50% BDL and 2.1 times first dorsal fin base. First dorsal and second dorsal fins are well separated, connected with a low membrane; the interdorsal space 8% BDL. Anal fin present, the position of anal fin origin is in front of the second dorsal fin insertion. Anal fin separated from lower caudal fin lobe by a deep notch; its base 0.2 times the lower caudal fin lobe. The lower caudal fin longer and greater than upper lobe, its length 1.3 times and height 1.7 times the upper lobe. Tail filament 24.3% BDL and 0.7 times the length of upper caudal lobe.

The adult male specimen has a pair of long and slender claspers (122 mm; 25.5% BDL) equipped with a pair of prepelvic tenaculæ and a frontal tenaculum (Fig. 3). Prepelvic tenaculæ prominent, blade-like, with five denticles along the medial edge of left tenaculum (no scar of missing denticles) and seven denticles on the right one (Fig. 3); frontal tenaculum well developed, knob-

like, its base anterior of supraorbital. Body coloration uniformly dark brown, without any spots or stripes. All fins have similar color with the body.

The sicklefin chimaera, *Neoharriotta pinnata* can be distinguished to its congeners by having well separated between oral and preopercular lateral line canals, rounded pelvic fins, and uniformly second dorsal fin



FIGURE 2. *Neoharriotta pinnata* (PMBC 30401), from Thailand-Andaman Sea



FIGURE 3. Prepelvic tenaculæ of *Neoharriotta pinnata* (PMBC 30401), from Thailand-Andaman Sea

height (Didier and Stehmann, 1996). This species was previously known to occur from the East Atlantic (from the southern Bay of Biscay to West Africa) to the Indian Ocean (from the Gulf of Aden, the Arabian Sea, the southwest of India to the Bay of Bengal) (Manilo and Movchan, 1989; Ali et al., 2009; Suresh and Raffi, 2012; Diez and Mugerza, 2017). The record of *N. pinnata* in the Andaman Sea is an extended distribution of this species to the eastern Indian Ocean.

Chimaera aff. macrospina

An immature male chimaerid specimen (PMBC 30399; 508 mm TL) was initially identified as the longspine chimaera, *Chimaera macrospina* Didier, Last and White, 2008; Family Chimaeridae Bonaparte, 1831 (Fig. 4). It was obtained on October 11, 2018, Andaman Sea, Thailand (Long. 96.99° E and Lat. 07.54° N) at the depth of 772-775 m.

This short nose chimaerid showed characteristics as follows: body elongate, tapering to a caudal fin with a filamentous tail. Head large, its length 0.2 times precaudal length and 30.4% BDL. Snout short, bluntly pointed; preorbital snout 0.1 times body length, preoral length 2.7 times in head length. Eyes relatively large, eye length 32.2% head length and eye height 0.6 times its length. Body slightly compress,

lateral line canal originating at level of upper eye, forming a notch anteriorly below the dorsal spine origin; lateral line on trunk relatively straight, not undulating and running along to caudal filament. Pectoral fins relatively broad and long, semi-falcate, with slightly convex on anterior margin; its length 36.2% body length and reaching slightly posterior to the origin of pelvic fin. Pelvic fins moderately broad and large, paddle-shape with angular apex; its maximum length about 2.1 times in pectoral maximum length. First dorsal fin relatively long with narrow base, its base 15.6% body length and its height 4.6 times in body length. Dorsal spine straight and long, its length more than 1.3 times first dorsal fin height and 1.1 times in head length. The origin of the dorsal spine just over the pectoral fin origin. Second dorsal fin moderately low and prolonged, the upper margin relatively straight with similar height; its height 3.5 times in first dorsal fin height; its base 79.7% body length and 5.1 times first dorsal fin base. First dorsal and second dorsal fins are well separated, connected with a low membrane; the interdorsal space 5.8% body length. Anal fin present, the position of anal fin insertion slightly behind the second dorsal fin



FIGURE 4. *Chimaera aff. macrospina* (PMBC 30399), from Thailand-Andaman Sea

insertion. Anal fin separated from lower caudal fin lobe by a deep notch; its base 14.5% lower caudal fin lobe. The lower caudal fin slightly longer than upper lobe, its length about 1.1 times but the height is similar to the upper lobe. Tail filament longer than caudal fin lobes, 1.7 times the length of upper caudal lobe and 34.1% body length.

The immature male specimen has a pair of undeveloped and short claspers, equipped with a pair of not well developed prepelvic tenaculae. Denticles on the medial edge not prominent and a frontal tenaculum that is not fully developed. Body coloration uniformly dark brown, without any spots or stripes. All fins have slightly darker color than the body.

Morphological characteristics of this sample revealed many similarities to *Chimaera macrospina*, whose distribution was recorded in Australia waters (Didier et al., 2008; Kemper et al., 2014; Last and Stevens, 2009) and eastern Indian Ocean and south-western Pacific Ocean (Ebert, 2014; Weigmann, 2016). It was distinguished to its congeners by having long dorsal spine and uniformly chocolate brown coloration

(Didier et al., 2008; Kemper et al., 2014). It differs from *C. notafriicana*, a species of *Chimaera* distributing in the Indian Ocean, in head length, eye size, pectoral and first dorsal fin shape, and the coloration (Kemper et al., 2010; Ebert, 2014). However, as the sample was an immature male, several characteristics were not fully grown making morphological identification tentative.

Although this sample revealed similarities with *Chimaera macrospina* and morphologically distinct from other congeners, the presence of this species in Thailand was initially considered as a new record for Thailand since this species was usually recorded only in Australia waters, eastern Indian Ocean and south-western Pacific Ocean. The result from DNA barcoding study challenged morphological identification. The maximum likelihood tree showed that the *C. macrospina* sample formed a separate clade and did not cluster with Australian *C. macrospina* nor with any other *Chimaera* species whose sequences were available for comparison (Fig. 5). The pair wise distance value between this sample and the reference *C. macrospina*

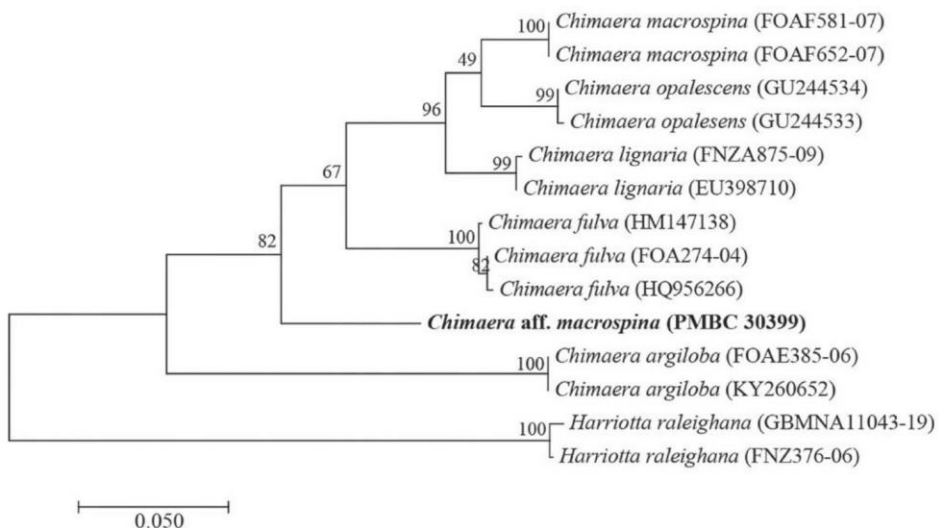


FIGURE 5. Maximum likelihood tree based on mitochondrial COI sequences representing the relationship of the unidentified *Chimaera aff. macrospina* (PMBC 30399), from Thailand-Andaman Sea. Numbers at nodes indicate posterior probability.

was 10.3%, while between our sample and other *Chimaera* species ranged from 10.7 to 16.0%. Based on this result, the specimen from the Andaman Sea of Thailand is identified as “*Chimaera* aff. *macrospina*” as it may represent an undescribed taxon.

The use of DNA barcoding showed contradictory result with the morphological identification. The sample formed its own cluster separating from other congeners, especially *C. macrospina*. Unfortunately, because of limited number of reference sequences available in public databases, the analysis that depended on these references may not be thorough. It was suspected that the *Chimaera* specimen from Thai Andaman waters was an undescribed taxon, although was not temporarily labelled as “*Chimaera* aff. *macrospina*” for future references.

Hydrolagus* cf. *deani

A female specimen of a long tailed chimaerid (PMBC 30400; 739 mm TL) was obtained on October 11, 2018, Andaman Sea, Thailand (Long. 96.99° E and Lat. 07.54° N) at the depth of 772-775 m (Fig. 6). This specimen has some characteristics as

follows: body elongate, tapering to a caudal fin with a long filamentous tail. Head moderately large, its length about 0.2 times precaudal length and 24.0% body length. Snout obtuse; preorbital snout 13.0% body length, preoral length 2.6 times in head length. Eyes very large, eye length 44.2% head length and eye height 0.6 times its length. Body rather slender, lateral line canal originating at level of upper eye, forming a shallow notch in front of the dorsal spine origin; lateral line on trunk relatively straight, not undulating and running along to caudal filament. Pectoral fins broad and triangular, its apex pointed, with posterior margin slightly convex, broadly rounded on base; its length 37.0% body length and reaching beyond to the origin of pelvic fin; anterior margin 1.8 times pelvic anterior margin. Pelvic fins rather long, paddle-shape with pointed apex, posterior margin slightly concave; its maximum length about 1.8 times in pectoral maximum length. First dorsal fin moderately long, triangular and short-based; its base 18.5% body length and its height 5.0 times

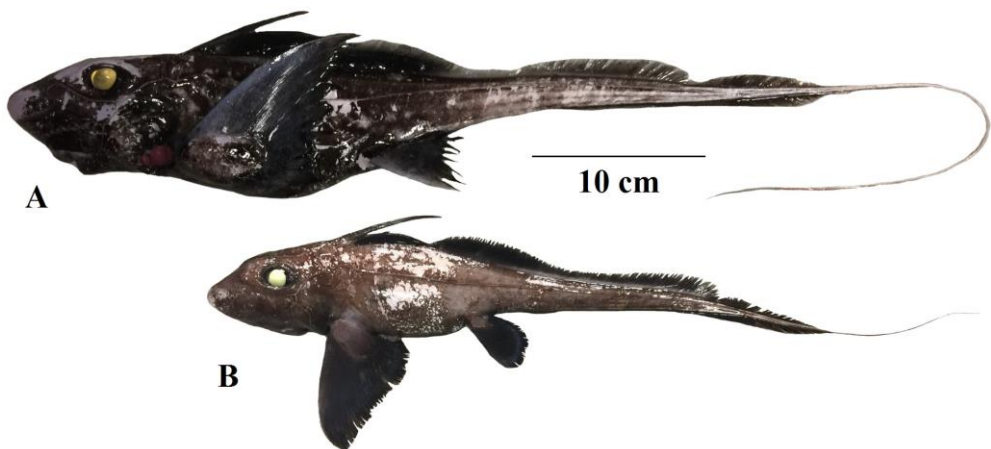


FIGURE 6. Specimen of *Hydrolagus* cf. *deani* (PMBC 30400) from Thailand-Andaman Sea (A), and photo of the fresh specimen taken on the Research Vessel (B).

in body length. Dorsal spine slender and slightly curved, its length 1.3 times first dorsal fin height and almost similar to head length. The origin of the dorsal spine slightly behind pectoral fin origin; the tip of dorsal spine reaches beyond the origin of second dorsal fin when depressed. Second dorsal fin moderately low and prolonged, the upper margin curved with the lowest in middle fin; anterior part of second dorsal fin is the highest, its height 1.6 times the posterior portion and 3.7 times the lowest fin height in the middle part. Second dorsal fin base 80.5% body length and 4.4 times first dorsal fin base. First dorsal and second dorsal fins are well separated, connected with a low membrane; the interdorsal space 6.9% body length. Posterior margin of the second dorsal rounded; there is no distance between second dorsal fin insertion and upper caudal fin lobe, only separated by a deep notch. Upper caudal fin lobe shorter than the lower caudal lobe, its base 26.0% body length and 0.7 times lower caudal lobe. Anal fin absent. The lower caudal fin origin well anterior the second dorsal fin insertion, its height is slightly lower than the upper lobe (87.5%). Tail filament very long, almost similar to body length and almost 4 times the length of upper caudal lobe. The body coloration of this female specimen is uniformly light brown, without any spots or stripes; head slightly darker than trunk. All fins have darker color than the body, the base of second dorsal fin has lighter color.

This specimen has distinctive characters that can be distinguished from its congeners by having a very low indentation in the middle of second dorsal fin and an elevated anterior of second dorsal fin. Those characters are similar to the description of *Hydrolagus deani* from the Philippines as stated in Smith (1912) and Compagno

(1999). However, *H. deani* was suggested as a junior synonym of *H. mitsukurii* (Compagno, 2005; Compagno et al., 2005; Weigmann, 2016). Didier (1995), previously separated between *H. deani* and *H. mitsukurii*, but in later publications (Didier, 2004; Didier et al., 2012), he confirmed that only *H. mitsukurii* was considered as a valid species. However, there are some regional checklists listed *H. deani* as a valid species (e.g. Randall and Lim, 2000; Iwamoto and McCosker, 2014), but more specimens are needed to be examined for confirmation. In contrast, the specimen from the Thailand-Andaman Sea differs from *H. mitsukurii* as described in Didier (2004) by having an indentation in the middle part of the second dorsal fin rather than not indented fin (Table 3).

Hydrolagus mitsukurii known to occur in the Western North Pacific from Japan to the Philippines (Didier et al., 2012). In 2017, a specimen recorded from Papua New Guinea waters was designated as *H. cf. mitsukurii* (White et al., 2017; White and Ko'ou, 2018) based on the similarity in morphology, such as having low second dorsal fin with a slight indentation at the middle part, and also had close related to *H. mitsukurii* in mitochondrial DNA.

Another congener species distributing in the Western Indian Ocean with morphological character by having indentation in the middle of dorsal fin is *Hydrolagus africanus* (Walovich et al., 2015). However, this species also differs from our specimen in the shape of second dorsal fin and eye size. Our specimen has a lower indentation in the middle of second dorsal fin (1.7% vs 2.8-5.0%) and larger eyes (see Table 3), which are similar to another congener from the Western Atlantic, *H. mirabilis* (Didier, 2002; Didier et al., 2012).

TABLE 3. Morphometric measurements of *Hydrolagus africanus* from southern Africa (Walovich *et al.*, 2015), *H. mitsukurii* (KAUM-I.55544), from Kagoshima of Japan (Fukui *et al.*, 2015) and *Hydrolagus cf. deani* (PMBC 30400), from the Andaman Sea of Thailand, expressed as percentage of body length.

| Measurement | Percentage of BDL | | | | | |
|-------------|-------------------|--------------------|---------------------|-----------------------|---------------|------------|
| | Neotype male | Range all (n = 65) | Range male (n = 34) | Range female (n = 31) | KAUM-I. 55544 | PMBC 30400 |
| BDL | 388 mm | 221-465 mm | 292-415 mm | 221-465 mm | 297 mm | 292 mm |
| TL | 154.6 | 117.2-293.8 | 117.2-251.4 | 119.9-293.8 | 232.0 | 253.1 |
| PCL | 121.4 | 116.1-130.0 | 116.5-125.3 | 116.1-130.0 | 124.2 | 124.0 |
| SVL | 53.9 | 51.9-77.9 | 51.9-77.9 | 54.0-74.7 | 59.3 | 56.9 |
| TRL | 35.6 | 30.9-46.8 | 30.9-45.1 | 34.0-46.8 | 34.0 | 38.4 |
| PD2 | 46.4 | 41.1-57.5 | 41.3-51.7 | 41.1-57.7 | 50.1 | 44.9 |
| PD1 | 25.5 | 20.3-33.9 | 20.6-29.8 | 20.3-33.9 | - | 26.7 |
| POB | 11.3 | 8.5-15.0 | 8.5-13.7 | 8.8-15.0 | 8.1 | 13.0 |
| D2B | 79.6 | 69.6-86.7 | 69.6-86.7 | 70.5-78.3 | 74.7 | 80.5 |
| D2AH | 4.9 | 4.3-7.5 | 4.3-6.9 | 4.5-7.5 | 2.6 * | 6.2 |
| D2PH | 4.1 | 3.4-6.5 | 3.5-6.5 | 3.4-5.9 | 2.7 | 3.8 |
| D2MH | - | 2.8-5.0 | 2.8-5.0 | 2.8-4.4 | - | 1.7 |
| D1B | 9.0 | 9.0-18.9 | 9.0-17.5 | 13.0-18.9 | 20.7 | 18.5 |
| DSA | 25.8 | 18.6-28.3 | 21.2-28.3 | 18.6-25.8 | 19.9 | 25.3 |
| D1H | 19.3 | 11.8-20.5 | 13.2-20.5 | 11.8-19.7 | 17.0 | 19.9 |
| CDM | 21.1 | 16.0-25.6 | 16.0-25.6 | 16.1-23.7 | 2.2* | 26.0 |
| CDH | 2.6 | 2.3-5.0 | 2.3-4.4 | 2.4-5.0 | 2.1 | 2.4 |
| CTL | 34.3 | 33.8-163.0 | 33.8-127.7 | 34.4-163.0 | - | 128.1 |
| CVM | 33.8 | 22.8-44.1 | 22.8-44.1 | 25.8-43.0 | - | 37.7 |
| CVH | 2.8 | 2.0-5.0 | 2.0-4.1 | 2.4-5.0 | - | 2.1 |
| HDL | 21.6 | 17.9-31.3 | 18.2-26.5 | 17.9-31.3 | 31.6 | 24.0 |
| P1A | 37.1 | 29.3-41.4 | 31.5-39.7 | 29.3-41.4 | 33.5 | 37.0 |
| P2A | 19.3 | 16.3-23.5 | 16.3-22.1 | 16.3-23.5 | 16.7 | 21.2 |
| IDS | 12.6 | 2.1-12.6 | 2.1-12.6 | 3.9-11.9 | 8.8 | 6.9 |
| DCS | 0.0 | 0.0-1.3 | 0.0-1.3 | 0.0-1.0 | - | 0.7 |
| PPS | 28.4 | 25.5-37.6 | 25.5-35.7 | 27.8-37.6 | 25.8 | 32.9 |
| D1P1 | 14.7 | 14.4-21.9 | 14.4-20.4 | 15.7-21.9 | 16.9 | 12.7 |
| D1P2 | 38.9 | 25.6-44.0 | 25.6-42.7 | 37.6-44.0 | 32.4 | 33.9 |
| D2P1 | 27.6 | 23.5-34.1 | 23.5-34.1 | 25.3-32.8 | 27.8 | 24.0 |
| D2P2 | 23.5 | 18.8-27.7 | 18.8-25.7 | 20.0-27.7 | 15.8 | 18.8 |
| EYL | 8.2 | 5.1-9.7 | 5.1-8.3 | 5.8-9.7 | 14.8 | 10.6 |
| EYH | 4.9 | 2.9-5.9 | 3.8-5.9 | 2.9-5.7 | 5.7 | 6.5 |

* maybe mistaken in measurement

There are some morphological differences between our specimen (PMBC 30400) and other congeners based on comparisons with specimens of *Hydrolagus africanus* from

southern Africa (Walovich *et al.*, 2015) and *H. mitsukurii* (KAUM-I.55544) from Kagoshima of Japan (Fukui *et al.*, 2015). Some characters of *H. mitsukurii* differ from our specimen,

such as the height of posterior of second dorsal fin (D2PH), first dorsal fin base (D1B), dorsal-caudal height (CDH), head length (HDL), distance from the origin of second dorsal to pelvic fin (D2P2), and eye length (EYL). While *H. africanus* differ from our specimen in the second dorsal mid height (D2MH), dorsal caudal margin (CDM), distance from first dorsal to pectoral origin (D1P1), and eye size (EYL and EYH) (Table 3).

Based on the comparison of second dorsal fins of *H. deani* from the Philippines, *H. mitsukurii* from Taiwan and *H. africanus* from southern Africa, the shape of second dorsal fin of specimen from the Thailand-Andaman Sea was most similar to *H. deani* (Fig. 7).

Genetic analysis using the comparison of ND2 sequences revealed that this specimen had 97% similarity with *Hydrolagus mitsukurii*

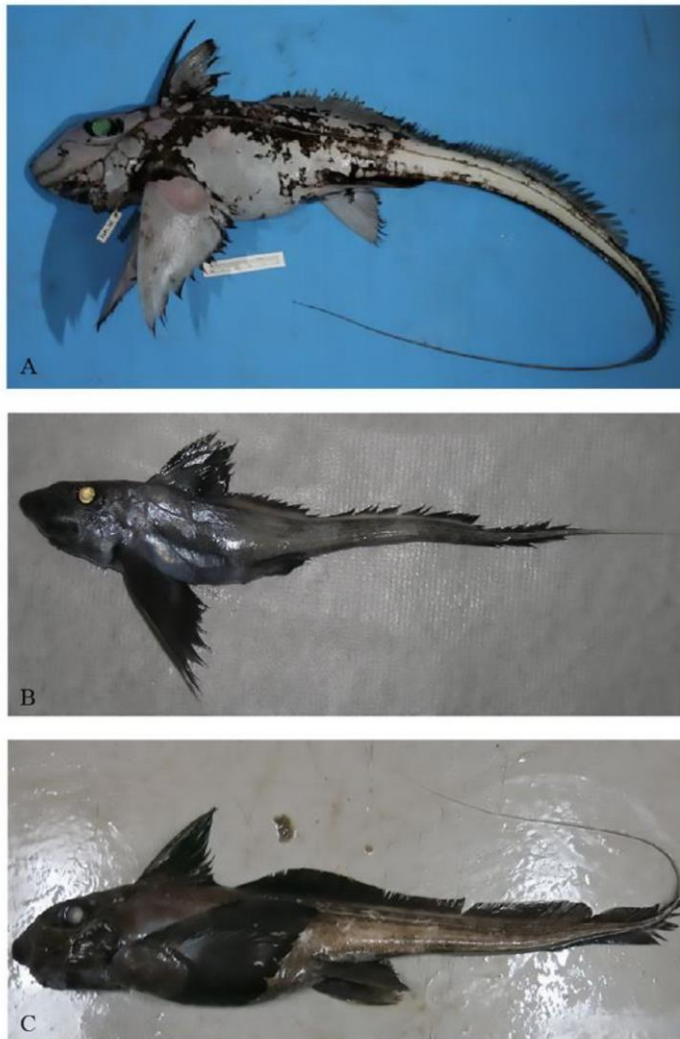


FIGURE 7. *Hydrolagus deani* (DC 1413) from the Philippines (A), and *H. mitsukurii* from Taiwan (B) and *H. africanus* from southern Africa (C)

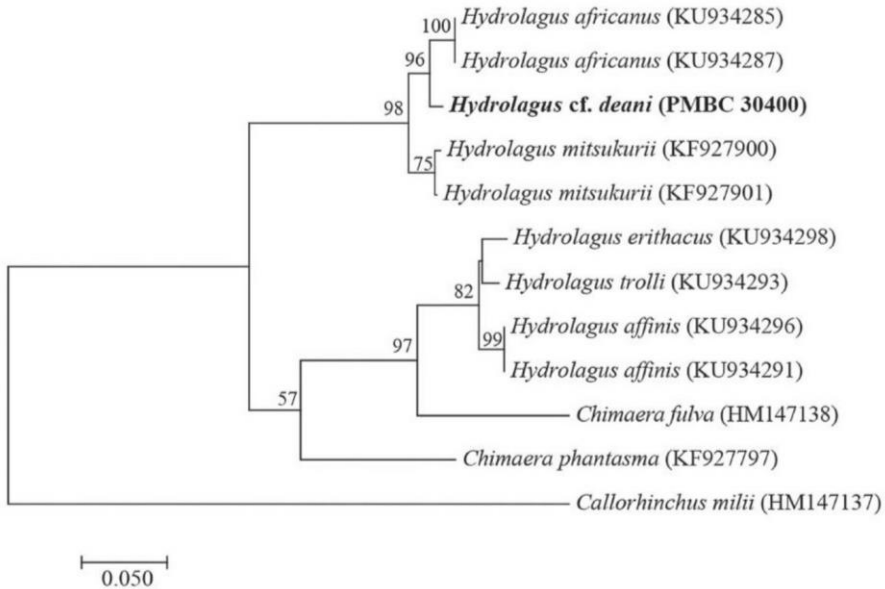


FIGURE 8. Maximum likelihood tree based on mitochondrial ND2 sequences representing the relationship of *Hydrolagus cf. deani* (PMBC 30400), from Thailand-Andaman Sea. Numbers at nodes indicate posterior probability.

from off of Taiwan (GenBank No. KF927901) and 98% similarity with *H. africanus* from South Africa (GenBank Nos. KU934286 and KU934287). The maximum likelihood analysis showed that this specimen clustered with *H. africanus* rather than *H. mitsukurii* (Fig. 8). Average pair wise distance value between the sample and *H. mitsukurii* was 3.2-3.5% but was only ca. 2% when compared to *H. africanus*. This specimen differed by more than 18% when compared with other *Hydrolagus* species.

The ND2 sequence of the specimen from the Andaman Sea indicated a high similarity to *H. africanus*, while *H. mitsukurii* represented as a sister group. Although species delimitation based on DNA sequences was not fully resolved, a 2% difference among conspecifics was suggested to hold for over 90% of fishes (Ward, 2009). It was close to *H. africanus* based on the genetic information, but state morphologically, especially shape of second dorsal fin, this specimen was most similar to *H. deani*. Therefore, we suggested this specimen to be referred as “*Hydrolagus cf. deani*”.

CONCLUSIONS

The exploration of deep-water Andaman Sea exposed substantial species diversity of cartilaginous fishes, and many of them present new record or undescribed species. The 2018 Deep Sea Expedition to the Andaman Sea of Thailand by the Dr. Fridtjof Nansen Research Vessel revealed three species of Chimaeriformes which were described as *Neoharriotta pinnata* (PMBC 30401), *Chimaera aff. macrospina* (PMBC 30399), and *Hydrolagus cf. deani* (PMBC 30400) based on morphological and genetic examinations. The application of DNA barcoding provided useful information that clarified species identification. Accurate and reliable identification requires comparison with reference samples, which appears to be insufficient in this study. Nevertheless, the discovery of these ghost sharks uncovers the hidden diversity in Thailand waters and will present important information for Thailand’s National Plan of Action for Conservation and Management of Sharks.

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