Egyptian Journal of Aquatic Biology & Fisheries
Zoology Department, Faculty of Science,
Ain Shams University, Cairo, Egypt.
ISSN 1110 – 6131
Vol. 26(4): 1337 – 1353 (2022)
www.ejabf.journals.ekb.eg

Validity of *Heteronarce prabhui* Talwar, 1981 and *Narke impennis* (Annandale, 1909) (Pisces: Narkidae) off Visakhapatnam, central - eastern coast of India

Ravali Vallangi , V. A. Iswarya Deepti *, K. Sujatha
Department of Marine Living Resources, Andhra University, Visakhapatnam- 530003, Andhra Pradesh, India.

*Corresponding Author: ishwarya.deep@gmail.com

ARTICLE INFO
Article History:
Received: July 12, 2021
Accepted: June 30, 2022
Online: Aug. 25, 2022

Keywords:
Sleeper rays,
Diagnostic characters,
Multivariate analysis,
DNA barcodes

ABSTRACT

Sleeper rays of family Narkidae are represented in demersal shrimp trawl by-catches of Visakhapatnam (Lat. 17°01' N to 19°22' N; Long. 83°23' E to 85°14' E), in the central-eastern coast of India. A total of 158 specimens belonging to two genera *Heteronarce* Regan, 1921 (three species) and *Narke* Kaup, 1826 (two species) were collected from July 2015 to March 2018. These species are often misidentified in the catches due to several overlapping characters. Multivariate analysis including Principal Component Analysis (PCA) has been carried out to identify distinguishing characters to help in differentiating these species. In addition, DNA barcodes were generated for two species in genus *Narke*, and a phylogenetic tree was constructed with reference sequences. Molecular phylogenetic results were assessed and integrated with sound morphological evidence. Thus, the present paper helps in clarifying taxonomic ambiguities by providing diagnostic characteristics, comparing morphometric data of closely related species, and discussing the validity of *Heteronarce prabhui* Talwar, 1981 and *Narke impennis* (Annandale, 1909). In the present study, *H. prabhui* represents the first report from the east coast of India; barcode sequences were generated for the first time for *N. impennis*. Additionally, Cytochrome C Oxidase I (COI) gene sequences were generated for the first time from the Indian waters for *N. dipterygia* (Bloch and Schneider, 1801).

INTRODUCTION

Sleeper rays of family Narkidae are caught as minor by-catches of offshore trawl fisheries and probably inshore artisanal net fisheries. They possess paired kidney-shaped electric organs on the base of pectoral fins and can deliver a strong shock to the unwary when disturbed or caught,; however, they are usually inoffensive to people. Their prey ranges are limited, feeding on small invertebrates on the bottom, polychaete worms and perhaps small organisms inside mud pellets **(Compagno & Last, 1999)**.

Last *et al.* (2016) have provided complete pictorial atlas of world's ray fauna, where the occurrence of *Heteronarce mollis* (Lloyd, 1907) and *Narke dipterygia* (Bloch and Schneider, 1801) was reported from the Indian waters. Detailed descriptions of *H. mollis*, *H. regani* von Bonde and Swart, 1923, *H. natalensis* Fowler, 1925, *H. garmani* Regan, 1921, *N. capensis* (Gmelin, 1789), *H. bentuviai* (Baranes and Randall, 1989), *N. dipterygia* and *H. rierai* Lloris and Rucabado, 1991 were provided in the studies of **Lloyd (1907)**, **von Bonde and Swart (1923)**, **Fowler (1925a)**, **Smith (1961)**, **Smith and Heemstra (1986)**, **Baranes and Randall (1989)**, **Monkolprasit (1990)**, **Lloris and Rucabado (1991)**, respectively.

Ramaiyan and Sivakumar (1991), **Krishnan and Mishra (1993)**, **Sujatha (2002)**, and **Raje *et al.* (2007)** reported the occurrence of *N. dipterygia* from the Indian waters. But due to various overlapping characters between *N. dipterygia* and *N. impennis* (Annandale, 1909), the latter is frequently misidentified as *N. dipterygia.* Hence, the afore- mentioned reports conducted on the occurrence of *N. dipterygia* may include specimens of *N. impennis.* In addition, **Ravali *et al.* (2018)** reported *N. impennis* from the Visakhapatnam coast.

In Visakhapatnam, three species of the genus *Heteronarce* Regan, 1921 - *H. garmani, H. mollis, H. prabhui* Talwar, 1981 and two species of genus *Narke* Kaup, 1826 - *N. diptertygia* and *N. impennis* of family Narkidae are represented in the by-catch of trawl fisheries **(Sujatha *et al.*, 2021)**. The conservation status of these species according to IUCN Red List criteria of threatened species (2021-1) **(www.iucnredlist.org)** is Data Deficient (DD), except for *H. garmani* that was placed in Near Threatened (NT, nearly meeting Vulnerable A2d) Category as this species has undergone a population reduction of 20-29% over the past three generations (15 years) due to the levels of exploitation (by-catch). There is a lack of population trend data, and this species is exposed to trawl fishing pressure across its range.

The number of dorsal fins varies across the two genera: single (*Narke*), and two dorsal fins (*Heteronarce*), which originate completely posterior to the pelvic fin bases. Dorsal surface of the body and tail: brown or reddish brown or grey, either plain or with paired creamish, with white spots and streaks on either side of the tail. Ventral surface uniform creamish - white/grayish with brown margins towards the edges of disc and pelvic fins. There exists confusion in correct identification of species of these genera due to various overlapping characters, Principal Component Analysis (PCA) that aids in identifying major distinguishing characters was carried out. DNA barcoding proposed by **Hebert *et al.* (2003)** was used as an additional aid to traditional taxonomy for accurate identification of species. Thus, the present paper provided diagnostic characters,

biometric data and colored illustrations for five species of family Narkidae that help in resolving taxonomic ambiguities existing among these species.

MATERIALS AND METHODS

A total of 158 specimens with length range from 43-227mm total length (TL) of sleeper rays were collected during July 2015 to March 2018 from shrimp trawl low value by-catch of Visakhapatnam fisheries harbor. Catches from shrimp trawlers (13-15 m OAL) operating in coastal waters off Visakhapatnam (Lat. 17°01' N to 19°22' N; Long. 83°23' E to 85°14' E), central east coast of India, at a depth ranging from 20 to 200 m were landed (Fig. 1). Moreover, the collections included samples from Chennai fisheries harbor. Colored photographs were taken and coloration of the specimens was noted in fresh condition. The total length of specimens was taken from the tip of snout to caudal fin end (TL) and was measured to the nearest millimeter. Specimens were identified to species level following the standard taxonomic works of **Compagno and Last (1999)**, **Carvalho (2016)** and **Nelson *et al*. (2016)**. After identification, morphometrics and terminology for morphological characters including abbreviations followed the descriptions in the studies of **Compagno and Heemstra (2007)** and **Last *et al*. (2016)**.

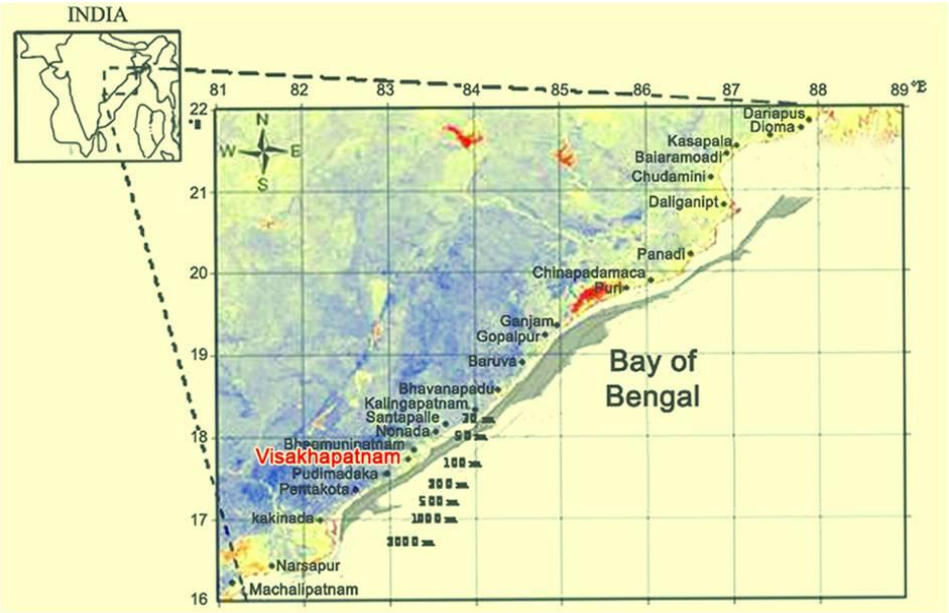

Fig. 1. A map of the central east coast of India showing the sample collection centerss

PCA was carried out for all morphometric characters of the five species under study. Before computation, all these characters were adjusted to pool information from different characters into a comparable scale following **Thorpe (1983)**. This analysis was

carried out using SPSS version 14.0 software **(Coakes & Steed, 2007)**. The Tukey test was performed only for those characters that became significantly different. Characters for which loadings are above 0.90 were considered significant.

For molecular analysis, the total DNA was extracted from muscle tissue preserved in 95% ethanol following the standard phenol chloroform method **(Sambrook *et al*., 1989)**. After extraction, quality and quantity of DNA was measured with UV Spectrophotometer and DNA diluted to 100ng/µl for further use. Approximately, 655bp of Cytochrome C Oxidase I gene (COI) of mitochondrial DNA was amplified using universal primers Fish F1 (5'TCAACCAACCACAAAGACATTGGCAC3') and Fish R1 (5'TAGACTTCTGGGTGGCCAAAGAATCA3') designed in **Ward *et al*. (2005)**. Amplification was performed in a 25µl reaction mixture. Thermocycler conditions included initial preheating at 95˚C for 5min, denaturation at 94˚C for 30 sec, annealing at 58˚C for 1min and extension at 68˚C for 1 min repeated for 35 cycles followed by a final extension for 5min at 72˚C. PCR products were examined on 1 % agarose gels purified with Promega PCR purification kit and sequenced with the automated sequencer using the dye-termination method. Amplicons were sequenced in both forward and reverse directions. For analysis, sequences were trimmed to approximately 650bp and submitted to BOLD, and BIN numbers were obtained for two species of the genus *Narke*. Sequences were aligned using Clustal W and pairwise evolutionary distance was determined by the Kimura 2-Parameter method **(Kimura, 1980)** using the software programme MEGA 7.0 **(Kumar *et al*., 2016)**. Reference sequences that are available for sleeper rays were retrieved from GenBank, and a phylogeny tree was constructed using the Neighbour Joining (NJ) method. To verify the robustness of the internal nodes of the NJ tree, bootstrap analysis was carried out using 1,000 pseudo replicates **(Felsenstein, 1985)**. *Neotrygon kuhlii* (Müller and Henle, 1841) was used as an out-group in this tree.

RESULTS

Diagnostic characters of five species of family Narkidae collected during present study are given below.

1. Diagnostic characters of three species of genus *Heteronarce*:

1.1. *H. garmani* Regan, 1921: oval disc, circular spiracles, first dorsal fin originating opposite to pelvic fin base, dorsally uniform pale brown in color with few irregular whitish markings on the disc, ventrally creamish - white (Figs. 2, 3). Teeth - 10 to 12 rows in both jaws, out of which five rows were with prominent pointed cusps (Fig. 4).

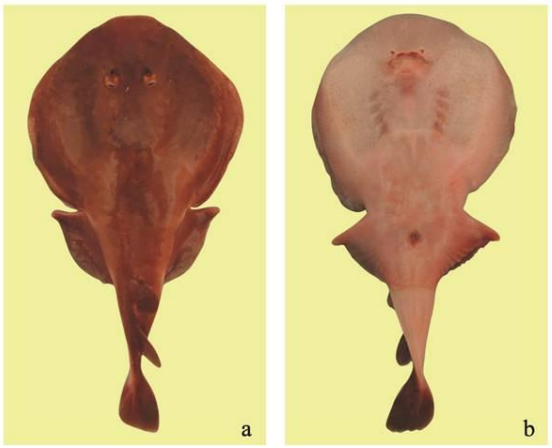

Fig. 2. *Heteronarce garmani* (Female – 181 mm TL)

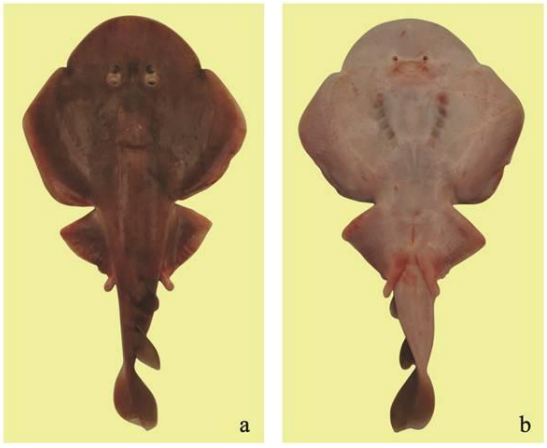

Fig. 3. *Heteronarce garmani* (Male – 204 mm TL)

(a) Dorsal view (b) Ventral view

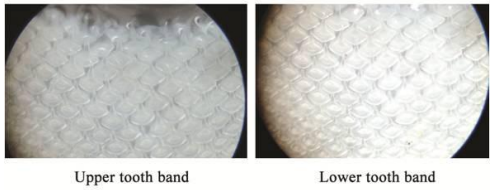

Fig. 4. *Heteronarce garmani* – Teeth

1.2. *H. mollis* (Lloyd, 1907): oblong disc, circular spiracles, first dorsal fin originating little behind the pelvic fin base, dorsally uniform umber in color devoid of markings, ventrally uniform grayish (Figs. 5, 6). Teeth - 13 to 15 rows in both jaws, out of which six rows were with prominent pointed cusps (Fig. 7).

Fig. 5. *Heteronarce mollis* (Female – 166 mm TL)

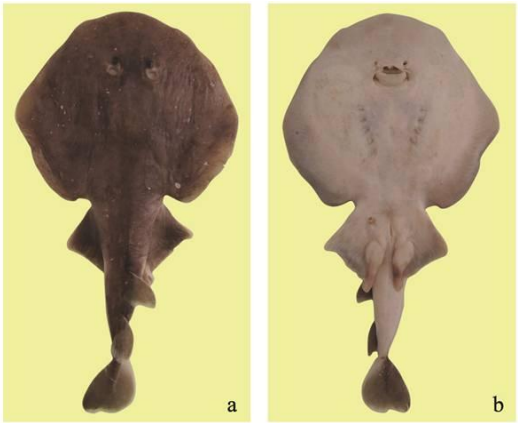

Fig. 6. *Heteronarce mollis* (Male – 172 mm TL)

(a) Dorsal view (b) Ventral view

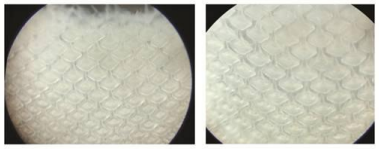

Fig. 7. *Heteronarce mollis* – Teeth

1.3. *H. prabhui* Talwar, 1981: circular disc, crescent shaped spiracles, first dorsal fin originating just opposite to pelvic fin base, dorsally uniform grayish-brown in colour with pale markings on either side of the tail, ventrally creamish - white (Figs. 8, 9). Teeth - 11 to 12 rows in both jaws, out of which five rows were with prominent pointed cusps (Fig. 10).

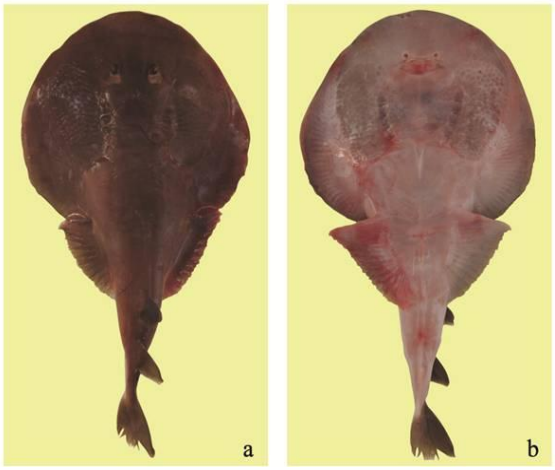

Fig. 8. *Heteronarce prabhui* (Female – 178 mm TL)

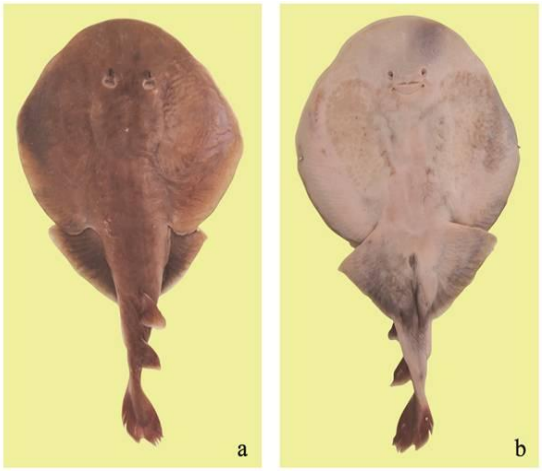

Fig. 9. *Heteronarce prabhui* (Male – 179 mm TL)

(a) Dorsal view (b) Ventral view

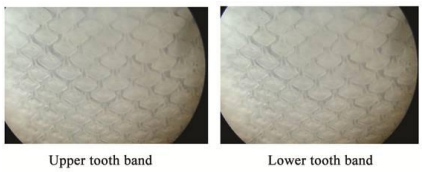

Fig. 10. *Heteronarce prabhui* – Teeth

Morphometric data for the above three species are given in Table (1). Based on PCA, the variance explained by the first two components was 42.99%. Considering the factor loadings of the first factor, major distinguishing characters were identified and Tukey test was carried out for those characters that revealed significant difference (P<0.01) (Table 2).

Table 1. Morphometric measurements expressed as percentage of total length (TL) of three species of genus *Heteronarce* represented in the catches of Visakhapatnam

	H. garmani		***H. mollis***		***H. prabhui***	
Total length, (TL) mm	149-227, n=34 (♀=24;♂=10)		93-216, n=40 (♀=12;♂=28)		112-216, n=19 (♀=16;♂=3)	
Measurements	Range	$\bar{X}$±SD	Range	$\bar{X}$±SD	Range	$\bar{X}$±SD
Disc width	40.98-57.29	50.24±3.94	46.06-62.36	52.51±3.49	46.76-59.82	53.47±3.43
Disc length*	44.88-51.08	47.38±1.29	43.23-51.55	47.80±1.86	44.44-54.46	48.93±2.20
Snout length	11.71-14.29	12.95±0.59	11.46-24.73	13.87±1.99	11.85-16.07	13.32±1.00
Snout, preorbital	13.17-16.11	14.52±0.69	13.02-17.02	15.50±0.88	13.07-18.75	14.94±1.26
Snout, preoral*	12.68-12.56	14.07±0.71	12.50-15.89	14.37±0.79	13.27-17.86	14.57±1.05
Snout, prenasal	11.21-13.33	12.14±0.61	10.85-14.61	12.86±0.90	10.65-16.07	12.65±1.16
Eye length	2.36-4.03	3.00±0.30	1.82-3.87	2.58±0.53	2.31-3.60	2.92±0.33
Interorbital distance	5.50-7.30	6.34±0.43	5.21-7.45	6.52±0.51	5.97-9.82	7.05±0.88
Spiracle cavity length	1.79-3.41	2.58±0.41	1.57-4.00	2.58±0.46	1.90-4.32	2.82±0.55
Spiracle width	1.89-3.90	2.81±0.45	1.99-4.00	2.99±0.38	2.56-4.49	3.23±0.45
Interspiracular distance	4.25-6.19	5.41±0.42	4.17-7.28	6.04±0.72	5.09-8.04	6.06±0.71
Orbit+spiracle length	4.76-6.60	5.77±0.45	4.46-6.74	5.33±0.51	5.03-6.72	5.66±0.41
Pectoral base	38.60-48.89	42.72±2.15	34.90-45.34	40.84±2.61	40.68-49.11	43.34±1.83
Height of first dorsal fin	6.40-10.94	9.37±0.85	7.00-11.24	9.27±0.87	8.09-10.67	9.37±0.78
Length of first dorsal fin	6.11-8.33	7.17±0.63	6.19-8.06	7.11±0.51	6.36-8.99	7.52±0.74
Height of second dorsal fin	7.88-11.46	9.93±0.81	7.00-11.56	9.58±0.89	8.52-10.95	9.86±0.60
Length of second dorsal fin	6.04-8.85	7.54±0.63	6.44-8.99	7.54±0.58	6.92-8.43	7.66±0.47
Distance between second dorsal and caudal fins	1.69-3.72	2.50±0.53	1.76-4.90	3.12±0.85	1.69-4.50	2.78±0.81
Caudal height upper lobe	16.18-20.60	18.28±1.12	9.87-19.86	17.32±1.62	16.07-19.42	18.00±0.87
Caudal height lower lobe	11.82-15.50	13.92±0.84	11.93-15.63	13.75±0.90	12.17-16.50	13.80±0.87
Caudal margin length	5.91-12.64	8.79±1.79	6.45-12.59	9.71±1.42	7.46-12.32	9.60±1.34
Tail, height at caudal origin	2.14-3.52	2.78±0.35	2.01-4.00	2.94±0.39	2.26-3.57	2.84±0.34
Tail, width at caudal origin	2.76-3.93	3.36±0.30	2.31-4.00	3.10±0.37	2.68-3.79	3.33±0.31
Lateral tail fold length	16.67-25.98	19.88±1.87	13.40-26.88	20.44±2.86	18.71-25.84	20.86±1.81
Head length; ventral*	29.72-36.56	33.85±1.42	30.21-38.41	34.32±1.67	32.23-36.82	34.23±1.35
Head length; dorsal*	19.02-21.67	20.42±0.72	17.71-23.40	20.75±1.05	18.98-23.21	20.70±1.04
Mouth width	4.93-7.27	5.96±0.50	4.63-7.97	5.94±0.79	5.47-8.04	6.14±0.65
Upper tooth band width	2.51-4.00	3.37±0.33	3.02-4.30	3.52±0.37	2.99-4.00	3.45±0.28
Lower tooth band width	2.51-4.00	3.37±0.33	3.02-4.30	3.52±0.37	2.99-4.00	3.45±0.28
Internarial width	4.90-6.99	6.16±0.50	5.21-7.53	6.36±0.48	5.62-7.14	6.29±0.44
Nasal curtain length	1.86-3.52	2.69±0.39	2.08-4.00	2.75±0.41	2.25-3.39	2.71±0.34
Nasal curtain width	5.39-7.54	6.59±0.53	5.21-7.89	6.81±0.62	6.29-8.38	7.10±0.57
Width of first gill slit	1.86-2.98	2.47±0.29	1.64-3.00	2.30±0.30	1.99-2.99	2.51±0.30
Width of third gill slit	2.33-3.32	2.78±0.22	1.68-3.29	2.43±0.33	1.99-3.57	2.58±0.39
Width of fifth gill slit	1.55-2.98	2.20±0.32	1.42-3.00	2.08±0.39	1.66-2.88	2.25±0.35
Distance between first gill openings	12.24-15.63	13.90±0.86	11.46-16.00	13.98±1.01	12.92-16.07	14.62±0.73
Distance between third gill openings	10.05-13.26	11.65±0.70	9.79-13.82	11.58±1.06	10.95-14.29	12.41±0.89
Distance between fifth gill openings	6.64-9.94	8.22±0.90	6.60-9.94	8.15±0.93	7.46-10.43	8.68±0.65
Pelvic fin length	8.29-19.09	11.24±1.80	10.32-20.62	13.93±2.30	8.96-18.96	11.98±2.07
Pelvic fin width	15.58-38.64	21.45±3.79	11.83-39.33	19.71±5.32	17.50-37.91	23.77±4.35
Pelvic fin to dorsal origin	6.16-20.00	8.25±2.20	4.63-9.87	7.23±1.26	6.97-9.55	8.16±0.83
Anterior margin of pelvic fin	9.76-15.71	13.26±1.29	11.86-15.60	13.60±1.05	11.37-15.64	13.29±1.10
Posterior margin of pelvic fin	13.76-26.40	21.09±2.43	14.53-25.26	19.86±3.23	18.41-27.34	22.97±2.64
Snout to pelvic origin*	43.90-50.56	47.41±1.42	41.67-54.61	47.49±2.23	44.38-55.36	47.82±2.41
Snout to cloaca length*	52.20-56.67	54.83±1.22	48.96-58.62	54.45±2.27	51.12-60.71	55.35±2.11
Snout to first dorsal fin length	61.32-81.06	64.91±3.34	60.94-69.29	64.51±1.88	62.36-74.11	65.21±2.58
Snout to second dorsal fin length	70.72-78.02	74.62±1.75	72.28-79.50	75.40±1.98	71.76-84.82	75.45±2.74
Distance between dorsal fins	2.25-5.00	3.48±0.64	2.87-5.21	4.13±0.61	2.56-4.62	3.70±0.57

Snout to maximum greatest disc width*	33.17-43.98	38.64±2.54	32.29-42.55	38.30±2.34	36.57-46.07	40.98±2.29
Snout to first gill slit*	22.27-26.11	23.94±0.91	21.84-26.95	24.73±1.29	21.76-27.68	24.44±1.57
Mid of cloaca to caudal fin length	46.23-52.26	49.15±1.68	47.02-53.13	49.69±1.36	47.75-54.46	49.57±1.64
Electric organ length	22.64-27.96	24.86±1.33	22.92-29.71	25.77±1.33	22.47-28.50	24.99±1.63
Electric organ greatest width	8.29-13.26	11.77±1.00	8.39-14.89	12.39±1.37	11.11-15.18	13.08±1.19
Electric organ width	6.83-10.71	9.16±0.87	7.81-12.06	9.95±0.99	8.33-11.73	10.13±0.85
Tail width	16.08-23.08	19.91±1.78	12.43-25.00	18.48±2.70	17.00-23.81	20.96±2.09
Cloaca length	3.50-5.52	4.63±0.50	3.55-7.00	4.76±0.66	3.85-5.68	4.90±0.45
For males						
Clasper length	4.00-6.04	4.76±0.58	3.23-6.90	5.19±0.84	3.98-6.15	4.71±1.24
Clasper outer length	11.50-14.06	12.70±0.74	8.60-15.94	13.41±1.31	11.94-14.53	12.99±1.36
Clasper-cloaca length	13.24-15.00	14.08±0.67	10.75-17.39	14.57±1.28	13.93-15.08	14.34±0.65

*significant characters in PCA

Table 2. Results of Tukey test for morphometric characters between three species of genus *Heteronarce*: *H. garmani, H. mollis* and *H. prabhui* represented in the catches of Visakhapatnam

Characters	**F value**	***P*- value**
Disc length	1.432	P<0.001
Preoral length	3.456	P<0.001
Head length ventral	2.768	P<0.001
Head length dorsal	8.437	P<0.001
Snout to cloaca length	4.512	P<0.001
Snout to pelvic origin	2.114	P<0.001
Snout to greatest disc width	5.476	P<0.001
Snout to first gill slit	4.657	P<0.001

2. Diagnostic characters that help in identification of two species of genus *Narke*:

2.1. *N. dipterygia* (Bloch and Schneider, 1801): circular disc, eyes large, round and protruded, circular spiracles, posterior margins of pelvic fins convex, dorsal surface has paired, large circular white spots on either sides of the base of disc on a uniform light brown background (Figs. 11, 12). Teeth - 10 rows linearly and 11 rows laterally in upper jaw; nine rows linearly and 11 rows laterally in lower jaw out of which five rows were with prominent pointed cusps (Fig. 13).

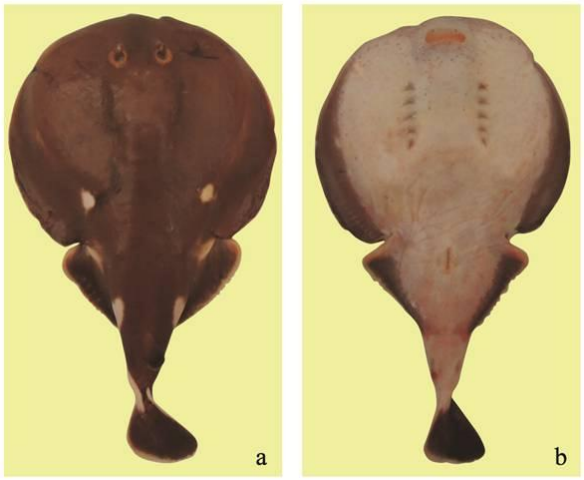

Fig. 11. *Narke dipterygia* (Female – 173 mm TL)

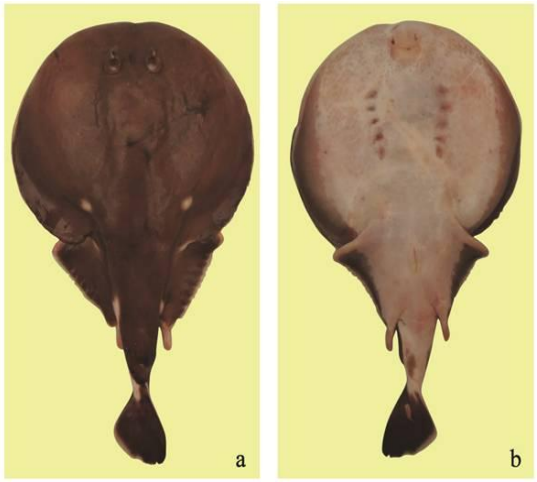

Fig. 12. *Narke dipterygia* (Male – 152 mm TL)

(a) Dorsal view (b) Ventral view

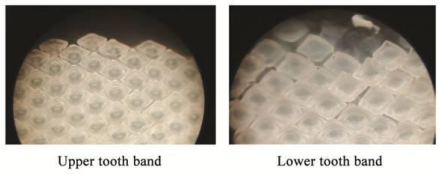

Fig. 13. *Narke dipterygia* – Teeth

2.2. *N. impennis* (Annandale, 1909): oval disc, very small, round and deeply sunk eyes, crescent-shaped spiracles, posterior margins of pelvic fins concave, dorsal surface has paired, large oval creamish-white spots on either sides of the base of disc on a dark chocolate brown coloured background (Figs. 14, 15). Teeth - 14 rows linearly and eight rows laterally in upper jaw; 17 rows linearly and eight rows laterally in lower jaw out of which six rows were with prominent pointed cusps (Fig. 16).

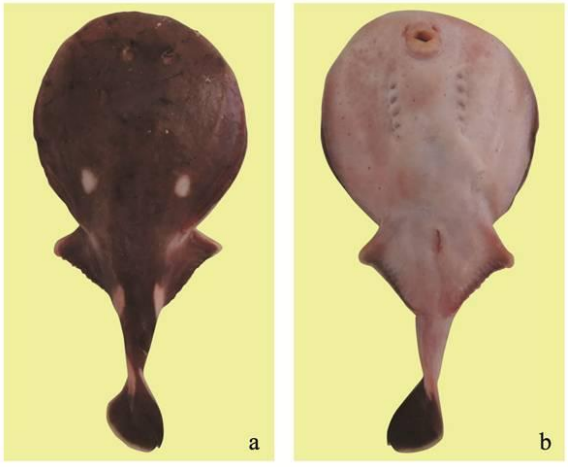

Fig. 14. *Narke impennis*

Fig. 15. *Narke impennis*

(Female – 112 mm TL) (Male – 118 mm TL)

(a) Dorsal view (b) Ventral view

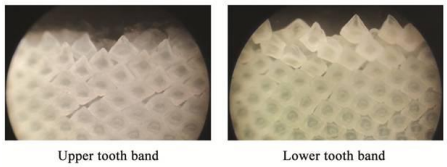

Fig. 16. *Narke impennis* – Teeth

Morphometric data for the above two species are given in Table (3). Based on PCA, the variance explained by the first two components was 35.848%. Considering the factor loadings of the first factor, major distinguishing characters were identified and Tukey test was carried out for those characters that showed a significant difference (P<0.01) (Table 4).

Table 3. Morphometric measurements expressed as percentage of total length (TL) of two species of genus *Narke* represented in the catches of Visakhapatnam

	N. dipterygia		***N. impennis***	
Total length, TL (mm)	91-208, n=28 (♀=12;♂=16)		43-206, n=37 (♀=15;♂=22)	
Measurements	Range	$\bar{X}$±SD	Range	$\bar{X}$±SD
Disc width*	52.41-65.81	58.67±3.27	43.48-62.14	52.51±4.86
Disc length*	48.45-58.24	53.03±2.43	44.57-57.14	50.75±3.29
Snout length	6.49-13.19	8.26±1.27	6.45-12.22	8.82±1.56
Snout, preorbital	9.09-17.58	11.51±1.68	8.09-14.10	10.82±1.57
Snout, preoral	8.26-14.29	9.78±1.23	7.69-13.95	9.56±1.22
Snout, prenasal	5.84-12.09	8.12±1.18	6.45-11.63	8.42±1.14
Eye length	1.92-4.40	2.73±0.53	1.65-4.65	2.57±0.68
Interorbital distance	5.42-8.79	6.85±0.82	4.68-9.30	6.61±1.18
Spiracle cavity length	1.53-4.40	2.58±0.55	1.73-4.65	2.61±0.57
Spiracle width	2.41-4.40	3.24±0.51	2.17-4.65	3.01±0.59
Interspiracular distance	4.35-6.77	5.52±0.56	4.00-9.30	5.45±1.08
Orbit+spiracle length	4.33-6.59	5.51±0.51	3.88-9.30	5.08±0.94
Pectoral base	43.48-54.70	48.13±3.08	30.19-55.24	44.67±4.88
Height of dorsal fin	6.21-9.87	8.16±0.86	5.74-11.32	7.78±1.13
Length of dorsal fin	6.21-8.63	7.65±0.64	4.92-10.29	7.33±1.11
Distance between dorsal and caudal fins	3.45-7.83	5.11±0.97	3.52-14.44	6.11±1.91
Caudal height upper lobe	19.58-25.27	22.27±1.38	17.65-27.91	22.32±2.18
Caudal height lower lobe	14.29-17.99	16.48±1.03	11.95-20.93	16.36±1.95
Caudal margin length	7.69-15.97	12.94±1.86	10.33-17.61	13.87±1.75
Tail, height at caudal origin	3.19-4.61	3.76±0.38	2.82-10.00	4.38±1.18
Tail, width at caudal origin	3.30-5.59	4.39±0.55	2.11-11.11	4.60±1.44
Lateral tail fold length	13.53-26.37	18.95±2.78	11.97-52.22	18.52±6.49
Head length; ventral	27.66-38.46	31.82±2.29	21.83-38.46	31.07±3.20
Head length; dorsal	14.18-20.88	16.59±1.47	10.56-63.33	16.76±8.10
Mouth width	4.51-8.16	6.07±0.82	4.23-26.67	6.77±3.60

Upper tooth band width	3.25-5.49	4.31±0.47	3.09-26.67	5.15±4.36
Lower tooth band width	3.25-5.49	4.31±0.47	3.09-26.67	5.15±4.36
Internarial width	4.35-6.63	5.24±0.55	4.12-17.78	5.71±2.17
Nasal curtain length	2.78-4.40	3.53±0.48	2.27-12.22	3.68±1.57
Nasal curtain width	2.80-6.92	6.04±0.78	4.23-17.78	6.60±2.07
Width of first gill slit	1.53-3.47	2.73±0.44	1.28-15.56	2.91±2.33
Width of third gill slit	2.04-3.37	2.74±0.28	1.63-6.98	2.85±0.88
Width of fifth gill slit	1.48-3.19	2.04±0.35	0.97-4.65	1.90±0.69
Distance between first gill openings	12.36-16.67	14.21±1.00	11.63-17.95	14.62±1.52
Distance between third gill openings	11.73-16.48	13.40±1.25	9.30-16.38	13.26±1.50
Distance between fifth gill openings	10.84-15.60	13.37±1.38	9.30-15.79	13.00±1.69
Pelvic fin length	7.80-24.48	12.93±4.98	3.64-23.03	11.98±3.18
Pelvic fin width	20.11-40.89	27.97±6.31	11.69-35.76	22.58±4.51
Pelvic fin to dorsal origin	6.43-11.04	8.57±1.14	5.13-81.40	10.63±12.05
Anterior margin of pelvic fin	9.03-15.13	12.47±1.58	9.15-15.63	12.08±1.65
Posterior margin of pelvic fin	4.29-26.11	18.75±7.62	1.79-29.92	16.09±9.00
Snout to pelvic origin*	43.98-54.95	47.96±2.44	42.93-55.13	49.00±2.62
Snout to cloaca length*	52.60-60.44	55.90±2.12	52.17-60.00	56.22±2.31
Snout to dorsal fin length	67.42-78.02	70.97±2.45	66.30-81.40	71.61±3.33
Snout to greatest disc width*	38.55-51.06	44.48±2.75	34.04-49.61	41.09±4.44
Snout to first gill slit*	17.02-25.27	20.54±1.60	17.42-44.24	25.24±7.80
Mid of cloaca to caudal fin length	46.15-55.56	50.74±2.10	48.12-57.14	52.65±2.24
Electric organ length*	27.95-32.97	30.53±1.33	25.00-37.18	31.13±3.06
Electric organ greatest width	9.43-16.43	13.35±1.72	8.81-20.93	13.49±2.47
Electric organ width	9.04-15.00	11.81±1.36	8.81-18.60	11.61±1.85
Tail width	20.48-30.71	24.64±2.59	18.87-28.17	23.92±2.46
Cloaca length	3.94-7.14	5.47±0.68	4.49-11.63	6.13±1.31
For males				
Clasper length	4.40-7.64	6.35±0.94	4.49-11.69	7.03±1.49
Clasper outer length	13.19-19.48	16.37±1.61	9.30-19.39	14.72±2.25
Clasper-cloaca length	16.48-22.22	18.91±1.87	16.28-23.30	19.17±1.74

*significant characters in PCA

Table 4. Results of Tukey test on the morphometric characters between two species of genus *Narke*: *N. dipterygia* and *N. impennis* represented in the catches of Visakhapatnam

Characters	**F value**	***P*- value**
Disc width	3.442	P<0.001
Disc length	1.276	P<0.001
Snout to cloaca length	6.982	P<0.001
Snout to pelvic origin	5.476	P<0.001
Snout to greatest disc width	8.657	P<0.001
Snout to first gill slit	3.254	P<0.001
Electric organ length	4.768	P<0.001

During the present study, DNA barcodes were generated for two species of genus *Narke – N. dipterygia* (n = 2) and *N. impennis* (n=1). Three sequences were submitted to

BOLD (Table 5). For both the species, we got the same BIN number as COI gene sequences were not available for *N. impennis* in BOLD. The overall percentage mean genetic distance within these two species showed a value of 0.32±0.08. Sequence analysis revealed base compositions with GC% 38.08. The average percentage of base composition of COI gene in these two species were 32.84 (T), 20.72 (C), 17.36 (G) and 29.08 (A). These base compositions are well within the range of those estimated for other Indian chondrichthyan species reported in the study of **Bineesh *et al*. (2017)**. The estimated transition/ transversion ratio was 2.78.

Table 5. Details of sleeper ray species barcoded from Visakhapatnam coast

Species	**Code**	**TL in mm**	**BIN number**
Narke dipterygia	NRKD1IFV1516-17	173	BOLD ADL5801
N. dipterygia	NRKD2 IFV1523-17	152	
N. impennis	NRKI3IFV1522-17	142	

Phylogeny tree was constructed with 22 sequences, among which three sequences are from the present study (*N. dipterygia* – 2; *N. impennis* – 1) as mentioned above, while the remaining 19 are reference sequences retrieved from Genbank including *Neotrygon kuhlii* which was used as an out-group to strengthen the position of sequences obtained from this region. Estimates of evolutionary divergences between COI sequences of sleeper rays are shown in Fig. (17). Phylogeny tree revealed two major clades of which one is formed by reference sequences of *Narke japonica* (Temminck and Schlegel, 1850) and the other is from the species under study. *N. dipterygia* formed one clade and *N. impennis* branched from *N. dipterygia* clearly distinguishing these two species. Barcode sequences were generated for the first time for *Narke impennis* in the present study. So far there are no reference sequences for this species in BOLD/Genbank. For *Narke dipterygia,* COI gene sequences generated for the first time from Indian waters.

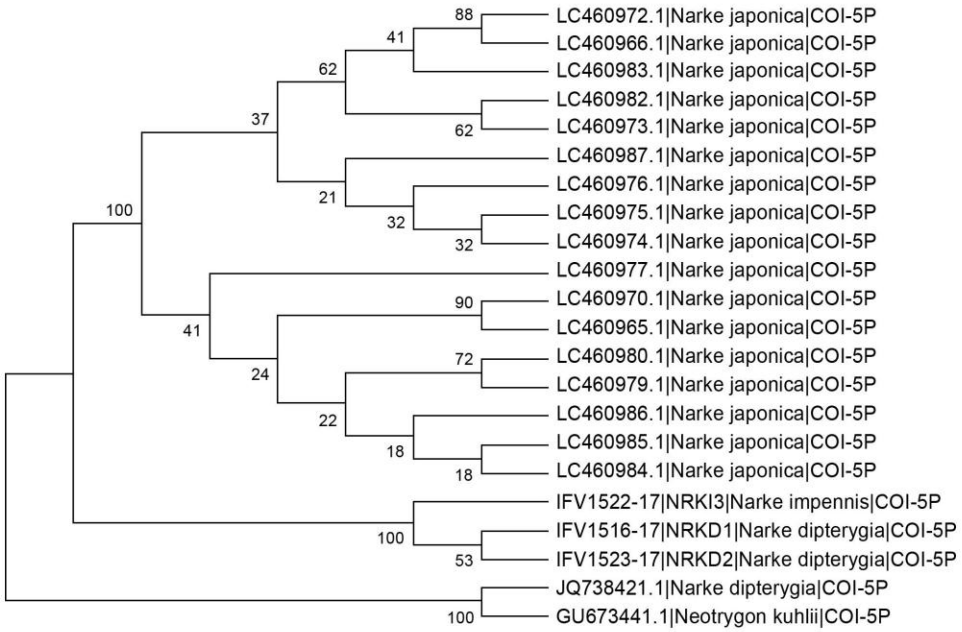

Fig. 17. Estimates of evolutionary divergences between COI sequences of sleeper rays

DISCUSSION

In genus *Heteronarce* - *H. garmani* differs from its closely related species *H. mollis* in certain characters. **Regan (1921); Fowler (1941); Wallace (1967c); Ebert (2014); Carvalho (2016)** differentiated these two species in various characters such as eyes, spiracles, snout, mouth, nasal valves, disc, proportions of the head and dorsal colouration. In the present study, considerable difference in the size of eyes and spiracles as well as dorsal coloration was observed. *H. mollis* closely resembles *H. prabhui* but differs slightly in its dorsal color and disc shape.

H. prabhui is a little known numbfish, known only from Quilon, Arabian Sea, India at around 300m depth region **(Talwar, 1981a)**. **Carvalho (2016)** synonymised *H. prabhui* with *H. mollis* without clearly mentioning the reasons. **Ebert (2014)** made *H. prabhui* a junior synonym of *H. mollis* but **Weigmann (2016)** stated that it is a questionably valid species. On the other hand, **Compagno (1999)**, **Manilo and Bogorodsky (2003)**, **Compagno and Heemstra (2007)** and **Akhilesh *et al.* (2014)** considered it as a valid species. In the present study, based on the results from multivariate analysis, PCA the major distinguishing characters that aid in differentiating *H. prabhui* from *H. mollis* were clearly identified (Table 1). Thus, it can be stated that *H. prabhui* is a valid species, and this is the first report from the east coast of India.

In genus *Narke*, there is some confusion regarding the validity of *Narke impennis* as only *N. dipterygia* is well supported. The description of *Bengalichthys impennis* as a distinct species was given by **Annandale (1909)** where he stated that, the strong muscles of the disc are liable to undergo great changes in shape; the degeneracy of the pectoral fins causes the disc to terminate, and the mouth is similar to *N. dipterygia* but is protrusible only to some extent. **Garman (1913)** in his memoir combined Annandale's new genus *Bengalichthys* with *Narke* without giving any reasons. Nevertheless, according to **Prashad (1920)**, the two genera are quite distinct,. In addition, the two specimens described in the study of **Day (1878)** as *Astrape dipterygia* actually belong to the genus *Bengalichthys*, and the figures given in his study for *N. dipterygia* refer to *B. impennis* (Pl. CXCII Fig. 4). **Munro (1955)** stated that *N. impennis* is brown above with diffuse blackish cloudings medially, and this character was observed in the present study. The description of *B. impennis* was reported in the study of **Misra (1969)** from India, and even though **Misra (1969)** considered *N. impennis* as a valid species, latter authors **(Compagno & Heemstra, (2007); Akhilesh *et al*., 2014)** synonimized *N. impennis* to *N. dipterygia.*

During the present study, the two species, *N. dipterygia* and *N. impennis*, were encountered in this region, the latter being the dominant sleeper ray species of trawl by-catches and both were represented by male and female specimens. However similar they might morphologically appear, based on the present study, the *N. impennis* is considered as a valid species.

CONCLUSION

Species identification based on biometric characters, color patterns and morphology can be considered as one of the greatest challenges due to various overlapping characters existing among congeners of sleeper rays of the Order Torpediniformes. The approach involving confirmation of the validity of taxonomically problematic sleeper ray species integrating traditional taxonomy and DNA barcoding has proven to be promising. Thus, the present study would be helpful in resolving ambiguities in the identification, status and uncertainties of species of the family Narkidae of the Indian waters, and furtherly aid in defining the species composition of fishery and mapping out distribution ranges.

ACKNOWLEDGEMENTS

The authors are thankful to the Head ot the Department of Marine Living Resources, Andhra University for providing facilities to carry out this research work. The authors are grateful to the Ministry of Earth Sciences (MoES) – Centre for Marine Living Resources and Ecology (CMLRE), Kochi for providing financial assistance to conduct this research work. The authors are also grateful to the University Grants Commission (UGC) in New Delhi for providing fellowships to the authors.

REFERENCES

Akhilesh, K. V.; Bineesh, K. K.; Gopalakrishnan, A.; Jena, J. K.; Basheer, V. S. and Pillai, N. G. K. (2014). Checklist of chondrichthyans in Indian waters. J. Mar. Biol. Ass. India., 56(1): 109-120. http://dx.doi.org/10.6024/jmbai.2014.56.1.01750s-17

Annandale, N. (1909). Report on the fishes taken by the Bengal fisheries steamer "Golden Crown". Part I - Batoidei. Mem. Indian Mus. 2(1): 1-60. http://dx.doi.org/10.5962/bhl.title.13766

Baranes, A. and Randall, J. E. (1989). *Narcine bentuviai*, a new torpedinoid ray from the northern Red Sea. Israel. J. Zool. 36(2): 85–101. http://dx.doi.org/10.1080/00212210.1989.10688627

Bineesh, K. K.; Gopalakrishnan, A.; Akhilesh, K. V.; Sajeela, K. A.; Abdussamad, E. M.; Pillai, N. G. K.; Basheer, V. S.; Jena, J. K. and Ward, R. D. (2017). DNA barcoding reveals species composition of sharks and rays in the Indian commercial fishery. Mitochondrial DNA A DNA Mapp. Seq. Anal. 28(4): 458-472. http://dx.doi.org/10.3109/19401736.2015.1137900

Carvalho de, M. R. (2016). Sleeper rays, Family Narkidae. In: "Rays of the World." Last, P. R.; White, W. T.; Carvalho, M. R. de.; Séret, B.; Stehmann, M. F. W. & Naylor, G. J. P. (Eds.). CSIRO publishing, Cornstock publishing Associates, Melbourne, pp. 170-181.

Coakes, S. J. and Steed, L. G. (2007). SPSS version 14.0 for Windows: Analysis without anguish. Milton, QId: John Wiley and Sons, Australia.

Compagno, L. J. V. (1999). Checklist of living elasmobranchs. In: "Sharks, Skates and Rays – The Biology of Elasmobranch Fishes." Hamlett, W. C. (Ed.). The Johns Hopkins University Press, Baltimore, MD, pp. 471-498.

Compagno, L. J. V. and Heemstra, P. C. (2007). *Electrolux addisoni*, a new genus and species of electric ray from the east coast of South Africa (Rajiformes: Torpedinoidei: Narkidae), with a review of torpedinoid taxonomy. Smithiana Bull., 7(7): 15-49.

Compagno, L. J. V. and Last, P. R. (1999). Narkidae. In: "FAO identification guide for fishery purposes. The living marine resources of the Western Central Pacific." Carpenter, K. E. & Niem, V. H. (Eds.). FAO, Rome, pp. 1443-1446.

Day, F. (1878). The fishes of India; being a natural history of the fishes known to inhabit the seas and freshwaters of India, Burma and Ceylon. Dawson and Sons, London, Vol. 1 and 2+ 778 pp.

Ebert, D. A. (2014). Beyond Jaws: Rediscovering the "Lost Sharks" of Southern Africa. Abstract. In: Program and Abstracts of Shark International, Durban, 57 pp.

Felsenstein, J. (1985). Confidence limits on phylogenies: an approach using the bootstrap. Evolution, 39(4): 783-791. https://dx.doi.org/10.2307/2408678

Fowler, H. W. (1925a). Descriptions of three new marine fishes from the Natal coast. Annals of the Natal Museum, 5(2): 195-200. Figs. 1-3.

Fowler, H. W. (1941). Fishes of the Philippine islands and adjacent Seas. United States National Museum, Bulletin. No. 100, 13: 332-354.

Garman, S. (1913). The Plagiostomia: sharks, skates and rays. Mem. Mus. Comp. Zoology Harv. Coll., 36(1), 515 pp.

Hebert, P. D. N.; Cywinska, A.; Ball, S. L. and Ward, J. R. (2003). Biological identifications through DNA barcodes. Proc. Biol. Sci., 270(1512): 313-22. https://dx.doi.org/10.1098/rspb.2002.2218

IUCN. (2021). The IUCN Red List of Threatened Species. Version 2021-1. http://www.iucnredlist.org. (Last accessed on 20th June 2021)

Kimura, M. (1980). A simple method for estimating evolutionary rate of base substitutions through comparative studies of nucleotide sequences. J. Mol. Evol., 16(2): 111-120. https://dx.doi.org/10.1007/BF01731581

Krishnan, S. and Mishra, S. S. (1993). On a collection of fish from Kakinada- Gopalpur sector of the east coast of India. Rec. Zool. Surv. India., 93(1-2): 201-240.

Kumar, S.; Stecher, G. and Tamura, K. (2016). MEGA7: Molecular Evolutionary Genetics Analysis version 7.0 for bigger datasets. Mol. Biol. Evol., 33: 1870-1874.

Last, P. R.; White, W. T.; Carvalho, M. R. de.; Séret, B.; Stehmann, M. F. W. and Naylor, G. J. P. (2016). Rays of the World. CSIRO publishing, Cornstock publishing Associates, Melbourne, i-ix+pp. 1-790.

Lloris, D. and Rucabado, J. A. (1991). *Heteronarce rierai*, a new narkid ray from Mozambique, western Indian Ocean. Japan. J. Ichthyol., 37(4): 327–332. https://dx.doi.org/10.11369/jji1950.37.327

Lloyd, R. E. (1907). Contributions to the fauna of the Arabian Sea, with descriptions of new fishes and crustacean. Rec. Indian Mus, Calcutta, 1, pp. 1-12.

Manilo, L. G. and Bogorodsky, S. V. (2003). Taxonomic composition, diversity and distribution of coastal fishes of the Arabian Sea. J. Ichthyol., 43(suppl. 1): S75-S149.

Misra, K. S. (1969). Elasmobranchii and Halocephali. In: "The fauna of India and adjacent countries." Pisces, (Second Edition). Roonwal, M. L. (Ed.). Zool. Sur. India., Government of India Press, Faridabad, pp. 1-276.

Monkolprasit, S. (1990). The electric rays found in Thailand. Kasetsart Journal: Natural Science., 24: 388-397.

Munro, I. S. R. (1955). The marine and freshwater fishes of Ceylon. Department of External Affairs, Canberra, 351 pp.

Nelson, J. S.; Grande, T. C. and Wilson, M. V. H. (2016). Fishes of the world. John Wiley and Sons. Fifth ed. Hoboken, New Jersey, pp. 83-84.

Prashad, B. (1920). Notes from the Bengal Fisheries laboratory, No. 7. On some Indian Torpedinidae from the Orissa coast. Rec. Indian Mus., 19: 97-105.

Raje, S. G.; Sivakami, S.; Mohan Raj, G.; Manoj Kumar, P. P.; Raju, A. A. and Joshi, K. K. (2007). An Atlas on the Elasmobranch fishery resources of India. CMFRI., Spl. Publ., 95, pp. 200-201.

Ramaiyan, V. and Sivakumar, R. (1991). Sharks, Skates and rays: An aid to the identification of sharks, skates and rays of the parangipettai coast, CAS in Marine Biology, Annamalai University, India, pp. 1-55.

Ravali, V.; Sujatha, K. and Iswarya Deepti, V. A. (2018). Length-weight relationship, growth and reproductive biology of *Narke impennis* (Annandale, 1909) (Pisces: Narkidae) of Visakhapatnam coast, Western Bay of Bengal, India. J. Mar. Biol. Ass. India., 60(2): 75-85. https://dx.doi.org/10.6024/jmbai.2018.60.2.2022-11

Regan, C. T. (1921). New fishes from deep water off the coast of Natal. Ann. Mag. Nat. Hist., 9(7): 412-420. https://doi.org/10.1080/00222932108632540

Sambrook, J.; Fritsch, E. F. and Maniatus, T. (1989). Molecular Cloning: A Laboratory Manual, second edition, Cold Spring Harbor Laboratory Press, New York, xxxviii+ 1546 pp.

Smith, J. L. B. (1961). The Sea Fishes of Southern Africa. Central News Agency, Ltd., South Africa, pp. 73-75.

Smith, M. M. and Heemstra, P. C. (1986). Smith's seafishes. Springer-Verlag, Publication, pp. 112-114.

Sujatha, K. (2002). Batoid fishes off Visakhapatnam, north-east coast of India. J. Mar. Biol. Ass. India., 44(1 and 2): 155-162.

Sujatha, K.; Iswarya Deepti, V. A.; Ravali, V. and Jha, S. (2021). Finfish constituents of shrimp trawl low value by-catch off Visakhapatnam, India. Indian J. Mar. Sci., 50(02): 165-167. http://nopr.niscair.res.in/handle/123456789/56313

Talwar, P. K. (1981a). The electric rays of the genus *Heteronarce* Regan (Rajiformes; Torpedinidae), with the description of a new species. Bull. Zool. Surv. India., 3(3): 147-151.

Thorpe, R. S. (1983). Review of the numerical methods for recognizing and analyzing ratial variables. In: "Numerical taxonomy." Felsenstein, J. (Ed.). Springer-Verlag, Berlin, pp. 404-423.

von Bonde, C. and Swart, D. B. (1923). The Platosomia (skates and rays) collected by the S.S. "Pickle". Reports of the Fisheries and Marine Biological Survey of the Union of South Africa, 3(5): 1-22.

Wallace, J. H. (1967c). The batoid fishes of the east coast of southern Africa. Part III. Skates and electric rays. Investigational Report, Oceanographic Research Institute, Dubran, 17: 1-62.

Ward, R. D.; Zemlak, T. S.; Innes, B. H.; Last, P. R. and Hebert, P. D. (2005). DNA barcoding Australia's fish species. Philos. Trans. R. Soc. Lond. B. Biol. Sci., 360 (1462): 1847-1857. https://dx.doi.org/10.1098/rstb.2005.1716

Weigmann, S. (2016). Annotated checklist of the living sharks, batoids and chimaeras (Chondrichthyes) of the world, with a focus on biogeographical diversity. J. Fish. Biol., 88(3): 837-1037. https://dx.doi.org/10.1111/jfb.12874