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The perplexing mysteries in sexual dimorphism: Morpho-histological variations in *Thalassoma lunare* (Linnaeus1758), a hermaphroditic reef fish from the Indian coast

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ABSTRACT

Thalassoma lunare is one of the most popular species of the family Laridae, and it has several attractive colour patterns in addition to a longer life span in the aquarium. In some species of Labridae, there is a colour pattern that enables discrimination between sexes and that colour variation, is missing in *T. lunare*. Therefore, it is challenging to distinguish sexes through colour patterns or external markings. The study aims to clarify the morphometric differences between the sexes of moon wrasses and detect the most valuable characters which assist in discriminating between males and females. All transformed relative percentages of morphometric values concerning standard length, head length, and body depth were subjected to non-metric multidimensional scaling (NMDS) for examining the parameters that help discrimination between sexes. The sex determination of the moon wrasse *Thalassoma lunare* was confirmed after a histological examination of gonads, and the samples were sorted into primary males, secondary males, and females. In multivariate morphometric space, primary males and females formed different clusters. Comparison of size-corrected morphometric characters between sexes suggested that two characters, longer caudal fin, and pelvic fin in males, were significant even after adjusting for family-wise error rate and indicated some degree of sexual dimorphism in primary males and females. Such differences represent an important criterion for selecting large male individuals for sexual selection in mating. Both primary and secondary males are found, indicating that this is a diandric species, and there is a remarkable variation in the histological structure between the two types of male testes; the study confirmed the sex reverse of female to secondary male.

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1. Introduction

Family Labridae is the second largest family of marine fishes and one of the most diversified fish families in shape, colour, and size (Nelson *et al.,* 2016). Labrids are most common in shallow waters, associated with various habitats, such as rocky reefs, sand, and seagrass, and contain about 548 species (Parenti and Randall, 2018). Most species bury themselves in the sand at night, and some small species clean larger fishes off their ectoparasites. Feeding habits in the group are diverse, and the food includes gastropods, bivalves, crustaceans, fishes, coral mucous, zooplankton, ectoparasites, and algae. The commercial importance of labrid fishes lies primarily in their popularity as aquarium fishes, although many species are prized food fishes (Carpenter and Niem, 2001; Nelson *et al.,* 2016).

Wrasses in the genus *Thalassoma* comprise 28 recognized species that occur predominantly on coral reefs and subtropical rocky reefs worldwide (Bernardi *et al.,* 2004). The Lunare Wrasse *Thalassoma lunare* is also named the Moon Wrasse because its yellow caudal fin shape resembles a crescent moon with long upper and lower lobes. This species usually leads a solitary life and is often found on coastal reefs, lagoons, and reefs protected from open seas at depths from 1 to 20 m (Myers,1991; Allen and Erdmann, 2012). *T. lunare* is diandric (Choat, 1969), and that means both primary males (a male borne as such) and secondary males (a male resulting from the sex change of female; protogynous hermaphrodite) are present in the same population. Describing the morphological differences between the sexes of *T. lunare* can help us to understand its reproductive biology, besides helping in sex segregation in aquaria. For ichthyo-taxonomical studies, morphometric analyses were mainly used to find out interspecies variations and species identifications, to regulate fisheries, and to determine the effects of environmental improvement (Burchett, 1983; Deepti *et al.,* 2013; Sajina *et al.,* 2013; Jawad, 2015; Lawson and Whenu, 2010; Mazlan *et al,* 2012; Mahilum *et al.,* 2013; Masood *et al.,* 2015; Zubia *et al.,* 2015; Mahmoud *et al.,* 2016; Mohan and Williams, 2016; Soliman *et al.,* 2018; Gul *et al.,* 2019).

Few studies are available on moon wrasses, such as identification (Alzahaby and Biju Kumar, 2023), distribution, and general information (Fischer and Bianchi, 1984; Alzahaby, 2022), feeding ecology (Amalina *et al.,* 2016), and length-weight relationship (Alzahaby and Biju Kumar, 2020). There are no previous studies to distinguish between the sexes (primary males and females) due to the phenomenon of sexual transformation in the family Laridae. Only Robertson and Choat (1974) discriminate secondary males from primary males of *T. lunare* through colour phases and found that all "Gaudy" (more brightly coloured) individuals were secondary males, and a duller "Drab" may be either females or primary males. Due to the presence of two types of males, one of them is secondary "Gaudy", easy to recognize, and the other is the primary, which is very similar to females (both of them are "Drab"); this paper documents the histological variations of gonads and, upon it finds out the external morphometric differences

between primary males and females of *T. lunare* and detect the most common characters which discriminate between them precisely and finding the last missing ring in sexual dimorphism of the species.

2. Materials and Methods

2.1. Sample and data collection

The *T. lunare* specimens were collected from Vizhinjam Bay (Fig. 1) in Kerala, southwest of India (8º22'35.30 N; 76º59'30.80 E) using a non-return valve trap net and gill net between December 2019 and November 2020. Local artisanal fishers were employed for the setting of fishing gear and the collection of fish. Wherever possible, fishes were brought to the laboratory and photographed, examined fresh, and preserved in 10% neutral buffered formalin for further morphometric analysis. Sex was determined by dissection and verification by histological gonad inspection. Specimens with undeveloped gonads were considered juveniles. All morphometric measures of both primary males and females were measured to the nearest 0.1 cm using a digital calliper. Thirty-four adult specimens of moon wrasse, *T. lunare*, comprising eight primary males and 26 females, formed the material for the present study. Twenty-six morphometric and ten meristic characters (Table 1; Fig. 2) were used in the present study.

2.2. Histology and sex determination

For sex confirmation of each fish, the gonads of all collected samples were dissected, cut into small pieces, fixed in 10% neutral buffered formaldehyde for 72 hours, dehydrated through graded ethanol, cleared in xylene and embedded in paraffin wax (56-58ºC). Routine microtomy (Leica RM2125 RTS) was followed to obtain 5µ thin sections of the tissues. Sections were stretched on clean glass slides, and the transverse sections were stained with Harri's haematoxylin and Eosin stain. The stained slides were examined under an Olympus BX43 light microscope. Microphotographs of the sections were taken using a Magcam DC 10 (Magnus) digital camera attached to the microscope.

2.3. Statistical analysis

Since the morphometric data showed linear correlations with standard length (SL), head length (HL), and body depth (BD), we corrected the morphometric data for size by taking percentage values concerning SL, HL, and BD. We performed non-metric multidimensional scaling (NMDS) using Euclidian distances to understand whether primary males and females of *T. lunare* form different clusters in multivariate morphometric space. To check whether the NMDS model fit the original data well, we plotted Shephard's plot between the target, obtained ranks, and calculated stress value. To check the null hypothesis that the primary males and females do not form significantly different clusters, we analysed similarity (ANOSIM) using Euclidian distances. We also performed t-tests for each size-adjusted character to understand which character significantly differed between primary males and females. Since multiple t-tests were completed, we corrected for family-wise error rates using sequential Bonferroni correction. Statistical analysis was performed in PAST 4.03 (Hammer *et al.*, 2001).

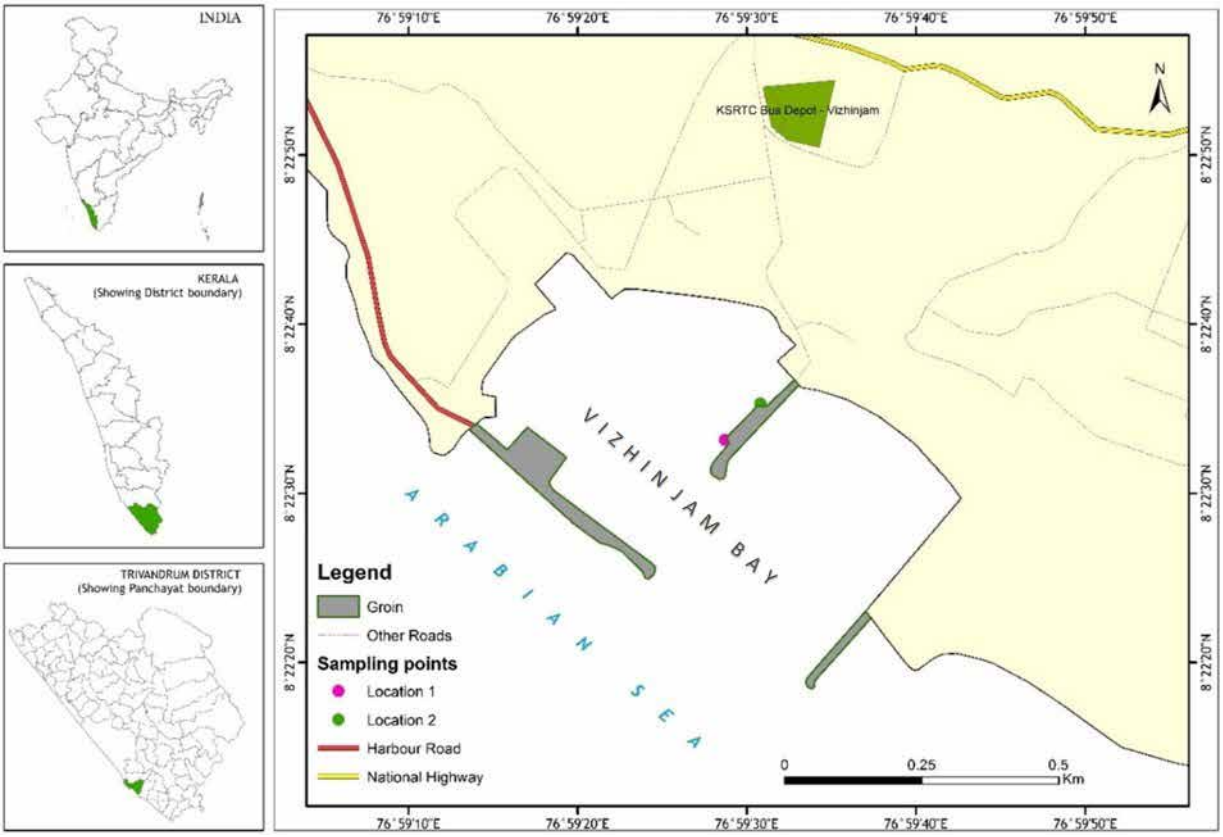

Fig. 1. Map of India showing the sampling sites of *Thalassoma lunare* from Vizhinjam Bay, Kerala, India

Table 1. Description of morphometric measurements of moon wrasse (*Thalassoma lunare*); The number refers to Fig. 2.

No.	Characters	Acronym	Description
1	Total length	TL	Distance from the tip of snout to the tip of the longest caudal-fin ray
2	Standard length	SL	Distance from the tip of snout to origin of the caudal fin
3	Body depth	BD	Maximum depth from the base of the dorsal fin to the ventral surface
4	Body width	BW	Width at its broadest region
5	Head length	HL	Distance from the tip of the snout to posterior margin of operculum
6	Head width	HW	Width of the head at its broadest region
7	Eye diameter	ED	Distance between the anterior and posterior bony edge of the orbit in the horizontal axis
8	Snout length	SnL	Distance from the tip of snout to the anterior edge of orbit
9	Post orbital length	POL	Distance from the posterior margin of orbit to posterior margin of operculum
10	Pre dorsal fin length	PDFL	Distance from the tip of snout to anterior margin of a dorsal fin
11	Pre pectoral fin length	PPFL	Distance from the tip of snout to the upper end of the pectoral fin base
12	Pre pelvic fin length	PPvFL	Distance from the tip of snout to the base of pelvic fin
13	Pre anal fin length	PAFL	Distance from the tip of snout up to the anterior margin of anal fin
14	Caudal peduncle length	CPL	Distance from the base of last anal-fin ray to the middle of caudal fin fold or end of the vertebral column
15	Caudal peduncle depth	CPD	Distance between the dorsal and ventral insertion of caudal peduncle at the narrowest part
16	Caudal peduncle width	CPW	Width of caudal peduncle
17	Dorsal fin length	DFL	Distance from the anterior tip of dorsal fin base to the posterior tip of the longest ray of the dorsal fin
18	Dorsal fin base	DFB	Distance from the base of the first dorsal spine to the base of the last dorsal ray
19	Pelvic fin length	PvFL	Distance from the base to the tip of pelvic fin
20	Pectoral fin length	PF L	Distance from the base to the tip of pectoral fin
21	Pectoral fin base	PFB	Distance between the dorsal and ventral insertion of pectoral fin
22	Anal fin length	AFL	Distance from the anterior tip of anal fin base to the posterior tip of the longest ray of anal fin
23	Anal fin base	AFB	Distance from the base of the first anal spine to the base of last anal ray
24	Dorsal fin hight	DFH	Length of the longest ray or spine of the dorsal fin
25	Anal fin hight	AFH	Length of the longest ray or spine of anal fin
26	Caudal fin length	CFL	Length from the end of the vertebral column to the tip of longest caudal fin ray

3. Results

3.1. Body morphology

The body of *T. lunare* (Fig. 2) is elongated and bilaterally compressed with vertical red bars. The head is green with irregular pink strips. Morphometric and meristic data of primary males and females are given in Tables 2 and 3, respectively. The body is covered with ctenoid scales except for the head region, with 26–28 pored scales along the lateral line and 7–11 scales between the dorsal and the anal fin origin. The lateral line canal opens on the surface of some scales by three-branched pores. The mouth is relatively small and terminal, usually with prominent lips. Jaws are highly protrusible to make the fish able to get food between the rocks and branches of the coral reef. The jaw teeth are conical in shape and composed of outer uniserial pointed teeth. Two curved canine teeth are in front of the upper and lower jaws. The dorsal fin is supported by eight spines and 13 soft rays (the last ray unbranched). The pectoral fin is supported by 14 soft rays (the first two are unbranched, and the rest branched). The anal fin is supported by two solid spines and 11–12 soft rays. The pelvic fin is supported by 1 spine and 5 soft rays (Table 3). The yellow crescent caudal fin identifies adults. Juveniles have two large black spots: one on the upper back at the middle of a dorsal fin and the other at the caudal-fin base.

3.2. Morpho-histological variations of gonads

3.2.1. General morpho-histology structure of the testes

The male reproductive organs of moon wrasse comprise two testes (two testicular lobes) situated ventrally to the

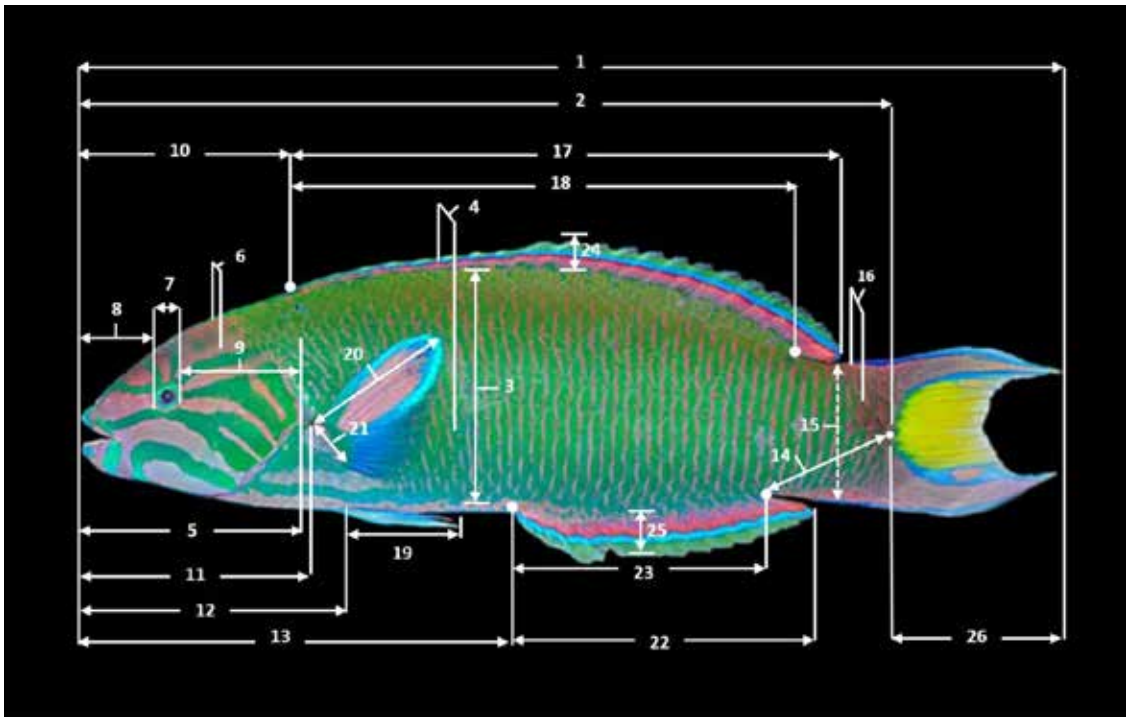

Fig. 2. Morphometric measurements of moon wrasse (*Thalassoma lunare*) from Vizhinjam Bay, Kerala, India. Character numbers 1 to 26 are explained in Table 1.

kidneys at the posterior region of the abdominal cavity and dorsal to the alimentary canal. The two testes are elongated structures, have the genital blood vessel running along their inner side, and are attached to the dorsal peritoneum by mesorchia. The testicular lobes ducts fused posteriorly and form a short common sperm duct leading to the urinogenital aperture. The testicular lobes are generally unequal in size; one is short than the other and exhibits a remarkable variation in shape, size, colour, and texture during the successive developmental stages (Fig. 3A).

Generally, in the cross-sections, the parenchyma of the testicular tissue consisted of seminiferous lobules (lobular type testes), which are held together through inter-lobular septa, mainly of connective tissue and surrounded by tunica albuginea. The protrusions of tunica albuginea extend into the testicular parenchyma, forming seminiferous lobules. The lobules are composed of two compartments: (i) The interstitial compartment, which contains the blood vessels and steroidogenic Leydig cells (Fig. 3B). (ii) The germinal compartment contained numerous seminiferous lobules (Fig. 3B). Each seminiferous lobule comprises germ cells or spermatogenic cells (functional tissue) and somatic Sertoli cells.

Histologically, there are two types of male testes; primary male and secondary male testes, which are similar in morphological shape but differ in histological structure. The primary male testis (males developing directly from the juvenile) was characterized by the solid construction of lobules and one central vas deferens packed with mature spermatozoa (Fig. 3B). The secondary male testes were characterized by a central lumen (a remnant of the ovarian lumen which is considered as evidence for prior function as females) and more than longitudinal sperm duct running through the gonad wall such that secondary ducts or sinus (secondary vas deferens) are a characteristic feature of secondary male testes (Fig. 3C).

3.2.2. General morpho-histology structure of the ovaries

The ovaries of moon wrasse are bilobed, situated ventral to the kidneys in the abdominal cavity, and suspended in the body cavity by mesovarium. The two oviducts join posteriorly, forming a short common oviduct leading to the urinogenital aperture. The two ovary lobules are unequal in size and exhibit a remarkable variation in shape, size, colour, and texture during the successive developmental stages (Fig. 4A).

Generally, the ovary of *Thalassoma lunare* is circular in cross-section and exhibits remarkable variation in diameter during the successive developmental stages. The ovarian wall comprises three layers; the outermost is a thin peritoneum layer. The middle thick layer is tunica albuginea, composed of blood vessels, connective tissue, and muscle fiber. The innermost layer is the germinal epithelium, which extends into the ovary's central lumen in the form of ovigerous lamellae, composed of epithelial cells and oogonia. The ovigerous lamellae are seats for all stromal compartments containing the different oocyte stages. The ovarian wall is thick during the early phases of maturation and thin and highly vascular during the ripe stage. The germ cells or oogonia originate from the germinal epithelium and appear in clusters or groups on the lamellae. The oogonia undergoes several successive stages to produce the mature ova or oocyte.

Histologically, some sections confirmed the transition

Table 2. Morphometric data of primary male and female Moon wrasse (*Thalassoma lunare*), collected from Vizhinjam Bay, Kerala, India. Statistical comparison based on t-test is provided only for size-adjusted characters

Character	Primary male			Female			Comparison	
	Mean	sd	Range	Mean	sd	Range	t	P
TL (cm)	21.3	4.8	10.6–25.6	14.7	3	8.9–19.8		
SL (cm)	16.4	3.2	9.1–19.1	12.3	2.3	7.5–16.4		
BD (cm)	4.7	1.2	2.8–6.2	3.2	0.7	1.5–4.4		
HL (cm)	4.5	0.9	2.5–5.3	3.4	0.7	2.2–4.6		
%SL								
TL	129.2	11	116.5–152.4	119.3	3.7	110.2–124.8	3.982	0.000**
BD	28.6	3.5	23.8–33.5	25.7	3.1	17–31	2.3	0.028*
BW	11.3	1.1	10.1–13.7	10.2	1.3	8–12.3	2.2	0.035*
HL	27.6	0.9	26.5–29.1	28	1.3	25–30.2	-0.879	0.386
PDFL	27.5	1.5	24.3–29.1	28.4	1.5	25.4–31.9	-1.392	0.173
PPFL	26.7	2.1	23.1–28.6	26.9	1.4	23.9–29.6	-0.283	0.779
PPvFL	30.3	1.9	27.5–33.7	30.1	2.1	26.6–35.9	0.218	0.829
PAFL	54.1	2	50–56	56.3	2.5	52.4–64.6	-2.295	0.028*
CPL	15.3	1.6	13.4–17.6	16.4	1.1	13.5–18.6	-2.16	0.038*
DFL	66.3	2.6	61.5–69.8	64.1	2.9	55.6–68.7	1.868	0.071
DFB	57.1	2.5	53.5–60.4	56.7	3.2	47.1–62.7	0.301	0.765
PvFL	15.7	2.7	11.2–18.8	13.3	1.9	10.2–19.7	2.841	0.008**
PFL	19.8	2.3	15.4–23.6	19	1.5	15.5–21.6	1.235	0.226
PFB	5.7	0.4	5–6.3	5.3	0.8	3.4–6.5	1.242	0.223
AFL	37.8	2.6	33.1–41.3	36	3.4	26.6–40.9	1.344	0.188
AFB	29.5	0.7	28.6–30.8	28.2	3	18.6–32	1.239	0.224
CFL	26.3	7.3	15.4–35.1	19.3	2.7	14.3–24.7	4.166	0.000**
%BD								
CPD	61	5	55.7–70	64.3	9	47.8–84.2	-0.964	0.342
CPW	14.1	2.8	10.7–18.6	16.3	4.6	11.1–26.7	-1.248	0.221
DFH	24.5	4.8	19.4–34.2	29.8	6.3	13–47.4	-2.195	0.036*
AFH	23.4	3.8	18–30	26.3	4.9	13–36.7	-1.501	0.143
%HL								
HW	37.9	2.5	34.1–41.7	35.7	4.8	29.2–50	1.247	0.222
ED	15	3.7	12.5–24	15.7	3.3	12.1–29.2	-0.502	0.619
SnL	30.6	4.8	20–34.8	30.4	2.6	25–36.6	0.151	0.881
POL	54.9	4	50–61	50.8	5	42.9–64.7	2.093	0.044*

* Not significant after sequential Bonferroni correction, ** significant even after sequential Bonferroni correction

process from the ovary to the testis (sex reverse to secondary males). Most ovaries were found in the initial phase (IP), with oocytes in several stages of maturation. In some hermaphroditic fish, mature spermatozoa inside secondary sperm ducts and oocytes are found simultaneously (synchronous hermaphrodites). The type of hermaphrodite, in this case, is delimited because the testicular and ovarian tissues are separated by a membrane of connective tissue (Fig. 4B, 4C). In another case, the transitional ovary contains atretic oocytes and clusters of spermatids or spermatozoa mixed, and the type of hermaphrodite, in this case, is mixed undelimited because the testicular and ovarian tissues are mixed (Fig. 4D, 4E).

3.3. Morphometric analysis

NMDS revealed that primary male and female in the multivariate morphometric space formed distinct clusters (Fig. 5a). The Shephard's stress was close to one (Fig. 5b), indicating that the NMDS model was a good fit for the actual data. There was a significant difference in the centroids of clusters of primary males and females (ANOSIM, R = 0.2747, P = 0.0175, Fig. 5c), suggesting that primary males and females showed sexual dimorphism in morphometric values. Although nine characters significantly differed between primary males and females at $\alpha = 0.05$ (indicated by one and two asterisks in Table 2), only three were significant when sequential Bonferroni correction was

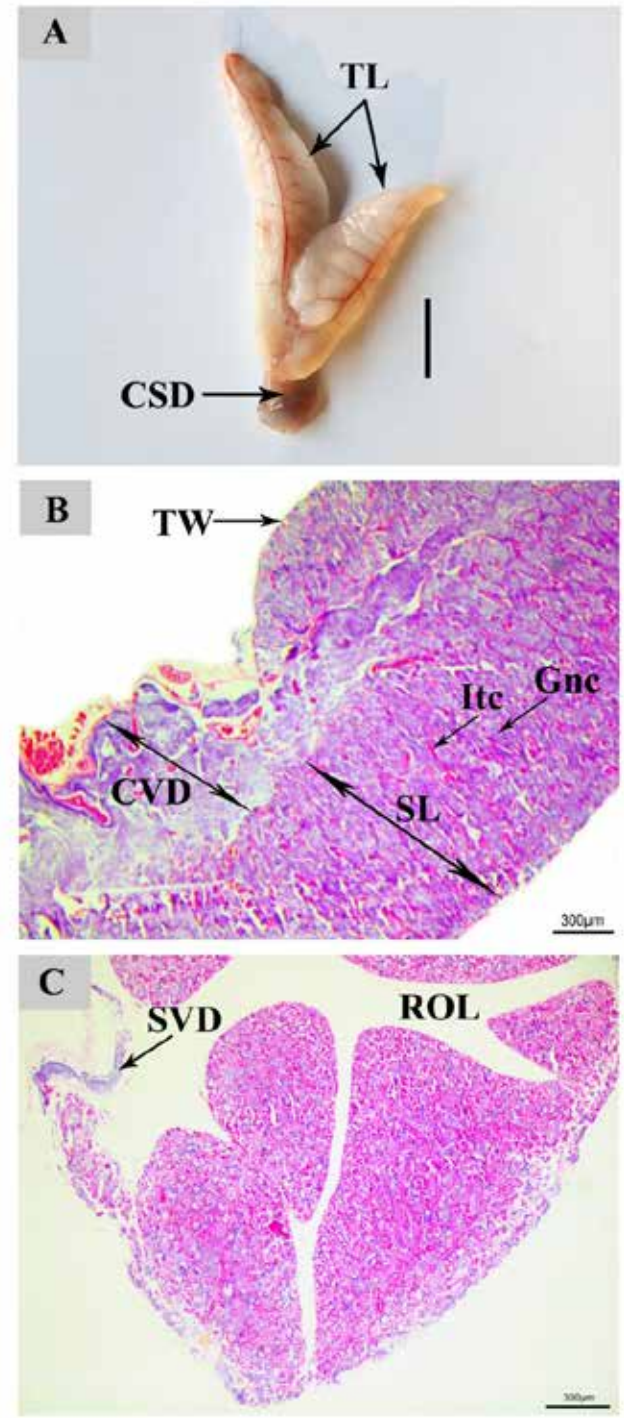

Fig. 3. Light photograph and light photomicrographs of *Thalassoma lunare* testicular structure showing histological variations between primary males and secondary males testes; stained by hematoxylin and eosin: **A** General morphology of male testis, showing two testicular lobes (TL) united at the hind to form a common spermatic duct (CSD); **B** Cross-sections of primary male testes, showing solid construction of lobules with central vas-deferens (CVD), a characteristic feature of primary male testes, testicular wall (TW), seminiferous lobules (SL), germinal compartment (Gnc) interstitial compartment (Itc). **C** T.S of secondary male testes, showing remnant ovarian lumen (ROL), a characteristic feature of secondary male (the sex inversion from female to male; protogynous sex inversion); note the right side of the secondary vas deferens (SVD) or sinus containing spermatozoa. Scale bar: **A**=10mm; **B**, **C**=300µm.

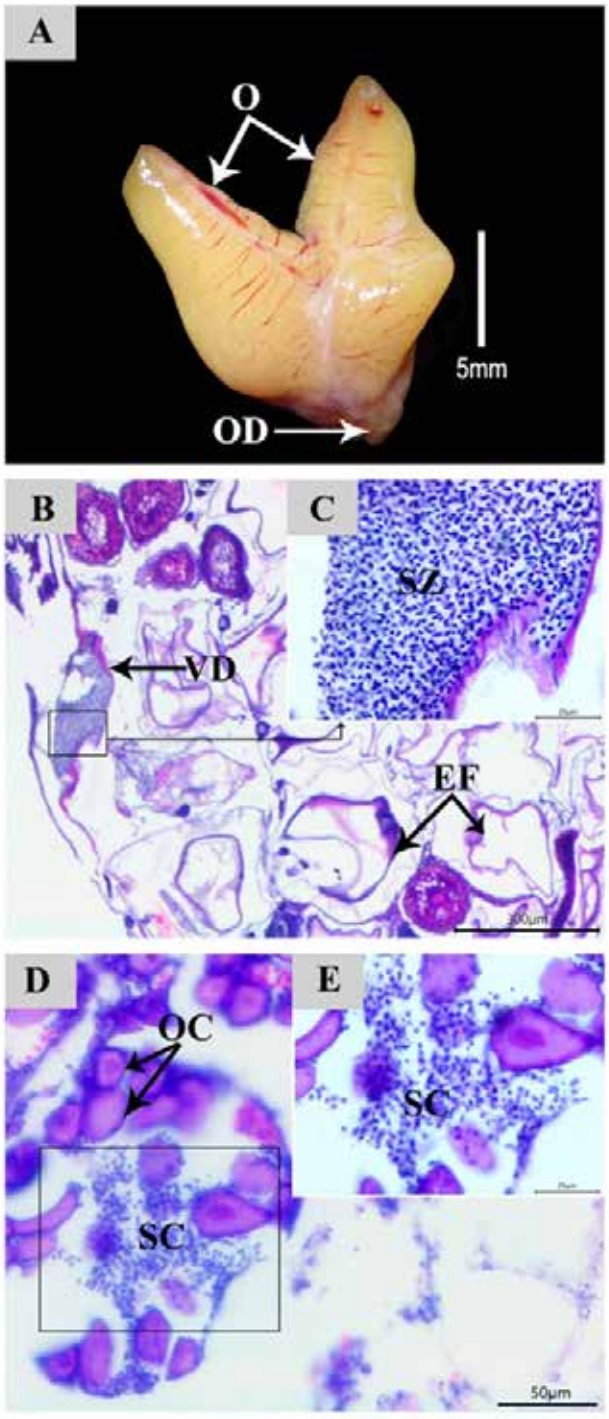

Fig. 4. Light photograph and Light photomicrographs of *Thalassoma lunare* ovarian structure: **A** General ovary morphology shows two ovaries (O) united at the hind to form oviduct (OD). **B** T.S. in the spent ovary, showing on the right side the secondary vas deferens (VD) containing spermatozoa, a characteristic feature of undergoing sex inversion and empty follicle (EF). **C** A higher magnified portion from the previous section shows spermatozoa (SZ). **D**, **E** T.S. of the transitional gonad showing the dense clusters of spermatocytes (SC) distributed amongst the oocytes (OC). Scale bar **A**=5mm; **B**=300µm, **C**, **E**=25µm; **D** = 50µm.

Table 3. Meristic counts range for primary males and females moon wrasse (*Thalassoma lunare*), collected from Vizhinjam Bay, Kerala, India

Characters	Primary males	Females
Dorsal fin spines	8–8	8–8
Dorsal fin soft fin rays	13–13	13–13
Pectoral fin soft fin rays	14–14	14
Pelvic fin spines	1	1
Pelvic fin soft fin rays	5	5
Anal fin spines	2	2
Anal fin soft fin rays	11–12	11–12
Lateral line pored scales	26–28	21–28
Transverse scale rows from the origin of the dorsal fin to the ventral surface	7–11	7–9
Rakers on first-gill arch	14–16	14–17

applied to α (Table 2). Even after Bonferroni correction, the important characteristics include total length, pelvic fin length, and caudal fin length, all expressed as a percentage of standard length. Total length as a percentage of standard length was significant because of longer caudal fins in primary males compared to females after size correction. As a result, only the two most prominent characters are sexually dimorphic in *T. lunare*, namely, the longer caudal fin and pelvic fin.

4. Discussion

Many investigators used the morphometric and meristic indices as they played significant roles in the identification, species taxonomy, and even differentiation between sexes, in addition to assessing the evolutionary adaptation of a species to its environment (Mekkawy, 1991; Mahmoud *et al.,* 2016; Alzahaby, 2015; Aguirre and Akinpelu, 2010; Mohan and Williams, 2016; Ulicevic *et al.*, 2018). In the present study, the morphometric and meristic characters of *T. lunare* were like those recorded by Fischer and Bianchi (1984). However, variations in the number of anal fin spines and pectoral fin rays were observed in addition to black spots associated with dorsal and caudal fins, which disappear in large individuals. This variation may be due to the geography, impact of environmental factors, or genetic structure of the population in Vizhinjam Bay.

Generally, hermaphroditism is a common and highly successful reproductive strategy between many Labridae species like *Notolabrus tetricus* (Warner and Robertson, 1978), *Achoerodus viridis* (Gillanders, 1995), and *Halichoeres trimaculatus* (Kuwamura *et al.,* 2007). Many researchers have earlier reported similar morphology in different teleost gonads like *Hirundichthys affinis* (Oliveira et al., 2015), *Cheilinus lunulatus* (Alzahaby, 2015), *Peprilus medius* (Maldonado-Amparo *et al.*, 2017), and *Siganus rivulatus* (Abdelhak *et al.*, 2020). The results indicated that both initial phase males (primary) and secondary male testes are similar in morphological shape but differ in histological structure. This was seen among other labrid fishes (Barrett, 1995; Ross, 1984; Smith *et al.*, 2003).

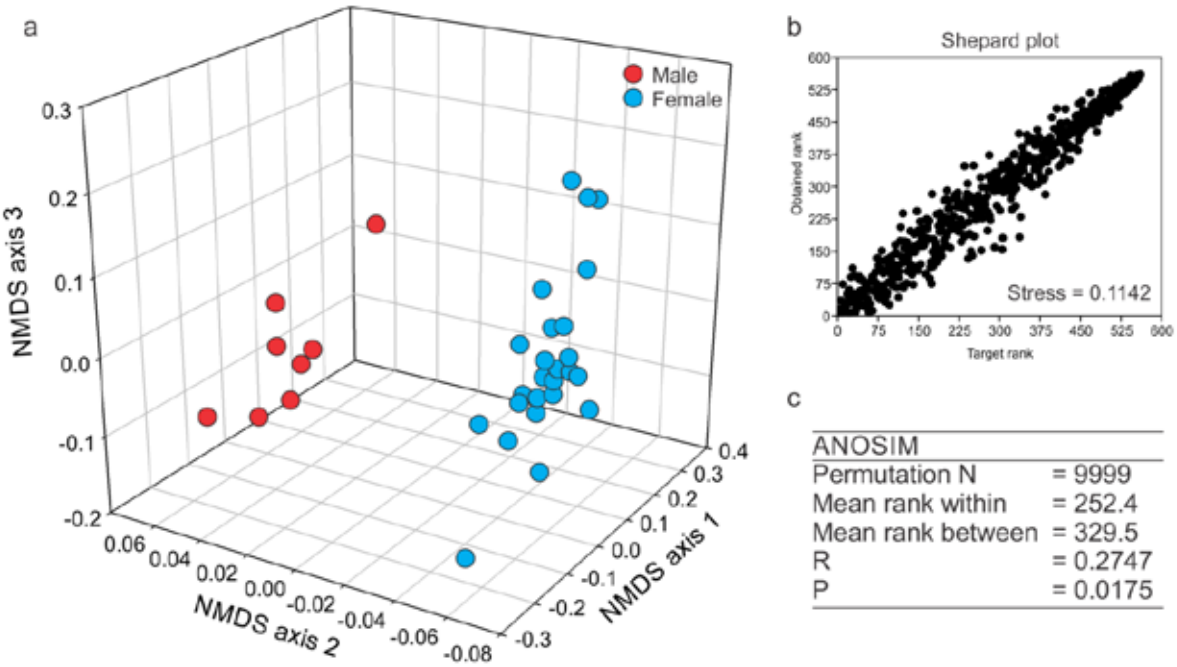

Fig. 5. Multivariate analysis of moon wrasse (*Thalassoma lunare*) based on size-corrected morphometric data: **a** Non-metric multidimensional scaling (NMDS) using Euclidian distances. **b** Shepard plot depicting the goodness of fit based on stress value for target and obtained ranks. **c** Analysis of Similarity (ANOSIM) results for checking the null hypothesis that there is no difference in morphometric data of primary male and female moon wrasse *T. lunare*. Both NMDS and ANOSIM analysis suggests that there is a significant difference in morphometry of primary male and female moon wrasse *T. lunare*

The histological analysis of terminal phase testes (secondary male) revealed that some females reverse sex when they attain large sizes to males, and the crypts of resting germ cells watched dormant in the lateral walls of the gonad or small clusters at the borders of the lamellae walls and sometimes distributed mange oocytes (Barrett 1995). The light microscope examination cannot differentiate these cells as oogonia or spermatogonia (Reinboth, 1965). On the other hand, these cells were examined by electron microscope in other protogynous wrasses, *Thalassoma dupery,* and they can be differentiated, and these cells are bipotential (i.e., able to differentiate into oogonia or spermatogonia), according to the gonadal tissue present. Also, these cells are associated with hormone levels in plasma (i.e. testosterone remained low throughout the sex change of females to males, but a second androgen, 11-ketotestosterone, increased gradually in parallel to the increased numbers of Leydig cells and spermatogonia (Nakamura *et al.,* 1989). Sex change in *Thalassoma lunare* females begins with the presence of these cells in most areas of the ovary and then a breakdown of the lamellae cell wall and atresia of oocytes along with the degeneration of female tissue. The same was documented in many protogynous serranid fishes (Reinbot, 1965; Hasting, 1989) and other wrasses like Mediterranean rainbow wrasse *Coris julis* (Guraya, 2000; Alonso-Fernández *et al.,* 2011). On the other hand, in the initial phase, males devoted a significantly higher proportion of their body weight to gonads than females (Warne, 1982). In the present study, the testes of *Thalassoma lunare* are made up of two parts, the germinal and interstitial compartments, quite similar to the testes of *Symphodus mediterraneus* (Raposeiro and Azeved, 2009), *Notolabrus fucicola* (Denny and Schie, 2002), *Thalassoma duperrey* (Hourigan *et al.,* 1991), *Labrus beryl* and *Labrus ossifagus* (Dipper and Pullin, 1979) and *Notolabrus tetricus* (Smith *et al.,* 2003).

Histological sections of gonads showed large differences in the gonadal lumen, lamellar form, and sperm ducts which run longitudinally through the gonad wall. This variation helped recognise both primary and secondary males and females. Thus, the morphometric measurements were precisely raised for morphometric analysis. These differences agree with many diandric species (Warner and Robertson, 1978; Sadovy and Domeier, 2005). The presence of primary and secondary males indicates that this species is a diandric hermaphrodite (Warner and Robertson, 1978). In the present study, the number of primary males is low and represents a small proportion of the total catch, and this may be a significant cause of female sex inversion. Dipper and Pullin (1979) reported that the primary males of *L. ossifagus* were low in number but did not seem to take part in reproduction.

The present study showed significant differences in the centroids of clusters of sexes, indicating sexual dimorphism between primary males and females. That means most body-related measurements display strong sexual dimorphism, as observed in both sexes of two labrid species *Cheilinus lunulatus* and *Halichoeres hortulanus* (Sarhan *et al.,* 2019). This study's highly significant morphometric characters are total length, caudal fin length, and pelvic fin length, which showed notable growth compared to the other body parts. This could be due to the differential rates of growth that maintain more or less constant ratios to one. These differences can easily escape the notice of the casual observer. The morphometric variation may be due to the diet, which causes variation in morphology not only in fish but also in most organisms (Meyer, 1988) or the genetic makeup of an individual, as well as environmental influences, such as development, growth rate, and nutrition (Wimberger, 2008). In the sexual dimorphism of *T. lunare* of Vizhinjam Bay in India, the striking characters that discriminate between the primary male and female are longer caudal fins and pelvic fins of primary males.

Environmental signals have a marked effect on fish's physiological and biochemical processes, and a raised temperature regime has a complex impact on fish's reproductive, nervous, and endocrine systems (Azab *et al.,* 2015). This means that the environmental cues may affect morphological variation between males and females of the same species differently. Each sex does not receive or is affected by external signals (photoperiod and temperature) at the same level, resulting in a differential growth rate between males and females. In *T. lunare,* the shape of the tail changes from truncate to lunate in adults, and our study shows that the only discriminating character between primary male and female fish is the longer caudal and pelvic fins of primary males. However, this study could not substantiate the possible reason for longer caudal and pelvic fins in moon wrasse primary males and their functional role.

5. Conclusion

The study demonstrated morphometric and histological significance differences between primary and secondary males and females of moon wrasses. These differences can be relied on in the separation between the sexes (primary males and females), and among the most prominent of these morphometric parameter's total length, pelvic fin length, and caudal fin length. The study documented the reverse of the sex of female to male and confirmed hermaphroditism of *T. lunare.*

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