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RESEARCH ARTICLE

Color Polymorphism in the Sea Slug *Berthellina citrina* (Rüppell and Leuckart, 1828) (Heterobranchia: Notaspidea: Pleurobranchidae) from the Red Sea

Alaa Y. Moustafa, Fathy E. Soliman, Tarek G. Ismail, Safa M. El-Masry
Zoology Department Faculty of Science, Sohag University, Sohag 82524, Egypt

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***Corresponding Author**

Alaa Y. Moustafa

Abstract

Berthellina citrina (Rüppell and Leuckart, 1828) is a common Pleurobranchid gastropod in the intertidal area of the Red Sea. It has distinct color pattern variation from pale yellow, orange to red. The present study aimed to evaluate whether the variation of these color patterns are due to polymorphism, or may be speciation. Anatomically, the three color patterns showed variations in their digestive systems especially in the shape of the crop, stomach and the intestine. However, the reproductive system showed variations in the prostate gland, penis, the **c**onnection of receptaculum seminis with busra copulatrix and the convolution of both distal part of vas deference and penial gland. Statistically, morphometric analysis, linear regression and discrimination function using length and width measurements of the main taxonomic characters; shells, buccal masses, radulae and jaws separated the red color pattern from the other two color patterns. Also, cluster analysis of the three color patterns based on length and width ratios of the previous taxonomic characters revealed that, the red color pattern comprised a separated group and the pale yellow and orange color patterns are very close. So, the present data revealed that there is a significance variations between the orange and pale yellow color pattern from the red color one and these may be led to isolation of the red color pattern as a separate species.

Introduction

Opisthobranch sea slugs are a large and miscellaneous group of worldwide marine gastropods occupying great diverges of ecological niches (Tullrot, 1998; Rudman and Willan, 1998). Reduction or loss of the protective shell is the main evolutionary trend of all the lineages within opisthobranchs. Therefore, they led to several active methods of defense, amazing array of body forms and bright color patterns (Martin and Walther, 2002; Wägele, 2004; Yonow, 2008). The color pattern variations (polymorphism) have been considered to play defensive role involve aposematic warning coloration and camouflage (Edmunds, 1987; Gosliner and Behrens, 1990; Rudman, 1991, Ornelas et al. 2011; Valdes et al. 2013). Noteworthy, color pattern within this group constitutes an important character for species identification (Dayarat, 2010, Gosliner et al. 2008; Gosliner, 2011).

Of opisthobranchs, *Berthellina citrina* (Rüppell and Leuckart, 1828) is a remarkably species belonging to the family Pleurobranchidae with color pattern variation from pale yellow, orange to red, (Willan, 1984; Rudman, 1999). *B. citrina* is extensively distributed in the shallow waters of Indo-West Pacific region (Rudman, 1999). In the Red Sea it was recorded from the Egyptian coast (O'Donoghue, 1929; Gohar and Abul-Ela, 1957; Yonow, 2000), Gulf of Aqaba (Engel and Van Eeken, 1962; Fishelsen, 1971; Marbach and Tsurnamal, 1973; Hughes 1977; Mastaller 1979; Yonow, 2008). In addition to Sudanese Red Sea (Heller and Thompson, 1983) and Saudi Arabia (Yonow, 2000).The identification of *B. citrina* is still confusing and needs a precise revision to solve the interference among them (Hermosillo, 2004; Rudman, 2007; Bertsch and Marlett, 2011; Cervera, 2012 (per. com). Recently, we gave new insight to the main taxonomic characters of the three color patterns of *B. citrina* comprised the shells, buccal masses, radulae, jaws and gills, in addition to their abundance, local and temporal distribution (Soliman et al. 2013).

The goal of the present study is to investigate whether the variation of three color patterns of *B. citrina* are due to polymorphism, or may be speciation by using gross anatomy, environmental and statistical studies.

Materials and Methods

Animal Collection and anatomical studies

Specimens of *B. citrina* were collected from seven sites along the North Western Coast of the Red Sea of Egypt, extending from the south of Al-Qusair city to the south of Hurghada city (Fig. 1A). The specimens were collected during the low tide at the day-time from 2009 to 2011. They were handly picked from lower surfaces of the stones and dead coral blocks and kept in plastic containers containing seawater. In the laboratory, they were sorted according to color pattern; pale-yellow, orange and red and counted for each site (Fig. 1B-E). Then, the specimens were relaxed before preservation in 5% formalin-sea water solution by adding few crystals of menthol to the arenas water for about 2 hours. Specimens were dissected; buccal masses, radulae and jaws, digestive and reproductive systems were removed. Drawings of the different parts of the dissected specimens were done using a camera lucida. Measurements were made by a calibrated ocular micrometer to the nearest 0.1 mm

Statistical analysis

Since the environmental conditions in the seven collecting sites were almost similar during collecting dates, the data were pooled for statistical analysis. Comparison of the mean values describing the length and width of shells, buccal masses, radulae and jaws of the three color patterns of *B. citrina* were carried out using ANOVA. The relative size of shells, buccal masses, radulae and jaws were assessed by calculating ratios between length and width (L/W). Also, the relationships between the length and width of the shells, buccal masses, radulae and jaws of the three color patterns were assessed using the linear regression analysis. Then, comparison of the slopes and coefficients of the regression were carried out using ANCOVA described by Zar (1984). Cluster and discrimination functional analysis were applied to detect similarities between the three color patterns of *B. citrina*. In order to compare the abundance of the species at the seven studied sites, the ANOVA test was applied.

Results

Environmental data

The three color patterns were recorded in five of the seven sites, where only orange color pattern collected from sites 5 and 7. However, color frequencies differed among seven sites (Fig. 2A). Also, on the same substrates where adults of *B. citrina* are present, eggs are laid in clusters in the form of ribbons. The eggs are connected to each other and the substrate by a transparent gelatinous material that has a color similar to that of their parents, i.e., pale yellow, orange and red (Fig. 2B-D)

Digestive system of *B. citrina*

In the present study, the digestive systems of three color patterns consist mainly of the following parts: mouth opening, oral tube, buccal mass, salivary gland, oesophagus, crop, stomach, digestive gland and intestine (Fig. 3, Fig 4). The following description concerns specimens of the pale yellow color pattern. And differences in the structure of the digestive system among the three color patterns are summarized in Table 1.

The mouth opening is semi-circular in shape and locates at the mid-ventral side of the head between the foot and the oral veil. The mouth leads to an extensible oral tube, which protrudes out of the mouth during feeding (Fig. 3D).

The oral tube leads to the buccal mass which consists of two lateral jaws and an elongated central radula. The structure of the jaws and the radula were described in details previously (Soliman et al. 2013*)*

A pair of salivary glands opens at the junction of the oral tube and the buccal mass with two separate ducts and extends posteriorly on both sides of the oesophagus. The buccal mass leads to a short and tubular oesophagus passes posteriorly to join the crop.

The crop is semi-circular, creamy whitish in color, thin and its posterior part is embedded in the upper part of the digestive gland. It leads to a short narrow duct that extends posteriorly and bulges to give the stomach which is completely embedded in the digestive gland. Precise dissection showed that, two ducts originate from the digestive gland and unit together to form a single duct before entering the stomach.

The digestive gland is a brownish compact voluminous gland constitutes the largest part of the digestive system and occupies the posterior half of the body cavity. It covers the stomach and the intestine. The digestive gland consists of a large number of follicles.

The stomach leads to a narrow tubular creamy white intestine. The latter extends backwardly in a groove inside the digestive and the hermaphroditic glands. The rectum is the posterior part of the intestine but there is no clear separation was noticed between them. The intestine including the rectum curves forwardly to open by the anus dorso-lateral to the end of the attachment of the gill with the body.

The reproductive system

B. citrina is hermaphrodite, its reproductive system opens externally via the genital aperture that locates on the right side, merely in front of the gill and surround by a collar-like ridge (Figs. 1D).The reproductive system of each of the three investigated color patterns (10 individuals of each morph having similar body lengths, (*ca.* 20 mm, in the preserved state) consists mainly of the following parts: ovotestis, hermaphroditic duct, male and female reproductive organs (Fig. 5 & Fig. 6). The following description concern specimens of the pale yellow morph and the differences in the reproductive systems among the three color patterns are presented in Table 2.

The ovotestis is located in the postero-dorsal part of the body cavity of the animal and intimately associated with the digestive gland. In live specimens, it has a purple color that distinguishes it from the brownish digestive gland. It is a large granulated compact mass occupies a large portion of the visceral coelom.

The hermaphroditic duct is a single duct arises from the ovotestis and runs between the ovotestis and the nidamental gland (female gland mass). Gross anatomy of the ovotestis showed that there are several gonoducts that combine together to form the hermaphroditic duct. Directly after leaving the ovotestis, the hermaphroditic duct widens to form an ampullar long duct. The male and female organs are intimately embedded inside the nidamental gland. The ampulla finally becomes narrow before branching into the vas deferens and the oviduct.

The male reproductive ducts begin with the vas deferens. The proximal part of the vas deferens is short and passes through the lobes of a wide yellow prostate gland while its distal part is thin, undulating and enters the base of the penis. A long convoluted tubular penial gland joins the distal part of the vas deferens before entering the penis base. It has a rounded bulging distal end. The penis is a smooth curved conical structure, enveloped in a muscular sheath.

The female reproductive ducts begin with the oviduct that branched from the hermaphroditic duct. It runs as a long, narrow and slightly convoluted tube through the lobes of the nidamental gland. Gross anatomy shows that the oviduct convolutes several times inside the nidamental gland and leaves it to open into the proximal part of the vagina. The nidamental gland is a large globular structure locates dorso-lateral to the ovotestis. It has a bright yellow color in live specimens and it is responsible for building the gelatinous egg masses. In addition, two seminal vesicles open into the vagina. The first is bursa copulatrix which is a large, rounded has a delicate membranous wall that leads to the bursa copulatrix duct (stalk). It is pale-yellow in color changes to brownish-red when filled with sperms. The second is the receptaculum seminis which is smaller than the former, oval in shape has a thin muscular wall and a narrow long muscular duct (stalk). The bursa copulatrix and receptaculum seminis ducts open side by side into the proximal part of the vagina. Finally, the vagina opens to the exterior via genital aperture that is surrounded by the genital papilla.

Statistical analysis of the morphometric characters

Morphometric characters as length and width of the shells, buccal masses, radulae and jaws (Fig. 7 & Fig 8) were recorded for 42 individuals of the pale yellow color pattern, 35 individuals of the orange color pattern and 48 individuals of the red color pattern.

The data showed that, the red color pattern has the longest and the widest shell ($F=64.9$, $P<0.000$; $F= 235.02$, $P<0.000$), buccal masses ($F=13.5$, $P<0.000$; $F= 9.39$, $P<0.000$) and radulae ($F=13.48$, $P<0.000$; $F= 8.82$, $P<0.000$) followed by the orange and the pale yellow morphs; respectively (Table 2). The red morph has the longest jaw ($F=7.97$, $P<0.000$) whereas, the jaw widths of the three color patterns were more or less equal ($F=0.83$, $P=0.437$)

The means of length/width (L/W) ratios for the shells, buccal masses, radulae and jaws of the three color patterns of *B. citrina* are shown in (Table 3, 4 and Fig. 9). The red morph recorded the lowest L/W ratio for shell ($F=161.2$, $P<0.000$) and highest L/W ratio for jaw ($F=27.62$, $P<0.000$) compared with the other two morphs.

The length-width relationships (linear regression) of the shells, buccal masses, radulae and jaws of the three morphs are shown in Table 5 and Fig 10. The linear relationships of the shells and jaws showed complete separation of the red color pattern from the pale yellow and orange color patterns However, the linear regression of the buccal masses and radulae revealed that the red color pattern can be separate from the other two color patterns that are nearly close to each other.

Comparison among the determined coefficients (R^2) of three color patterns regression equations was applied using ANCOVA analysis. The analysis revealed that length of both shell and jaw has a significant effect on corresponding width (ANCOVA, shell: $F= 85.83$, $P= 0.001$; and jaw: $F= 61.926$, $P= 0.00$). However, this effect is similar (allometery) among the three color patterns in case of the buccal mass (ANCOVA, $F = 0.88$, $P = 0.417$) and the radula (ANCOVA, $F= 1.555$, $P= 0.215$). (Table 5)

Cluster analysis for the three color patterns based on the shells, buccal masses, radulae and jaws length-width ratios is shown as a dendrogram (Fig. 11). The cluster revealed two groups: the red morph group and the pale yellow and the orange patterns group.

The discrimination function analysis was carried out using length and width measurements of the buccal masses, jaws, radulae and shells, for the three color patterns. All data were transformed logarithmetically to increase their fit to normal distributions, and then it was subjected to canonical analysis. Canonical analysis dealt with the data as two groups, one mono group, containing the red color pattern, while the other group is a bi-group containing orange and pale yellow color patterns. The majority of variation, 99.5 % was explained by the canonical root1 that separate the red morph from the other two color patterns (orange and pale yellow). While, 0.5 % of the variation was explained by the canonical root2. Root1 indicated that the orange and the pale yellow morphs can be differentiate from the red one as shown by the positive scores (1.34 and 2.09), versus negative scores for the red color pattern (-2.81) (Fig. 12). On the other hand, the root2 seems to provide a weak discrimination between the pale yellow and the orange color patterns. Shell width contributes much in discrimination among the three color patterns (Wilk's lambda= 0.27, F= 42.1, P< 0.0000), followed by the jaw width (Wilk's lambda= 0.19, F= 13.5, P< 0.0000) and jaw length (Wilk's lambda= 0.17, F= 5.4, P< 0.0000); respectively (Table 6).

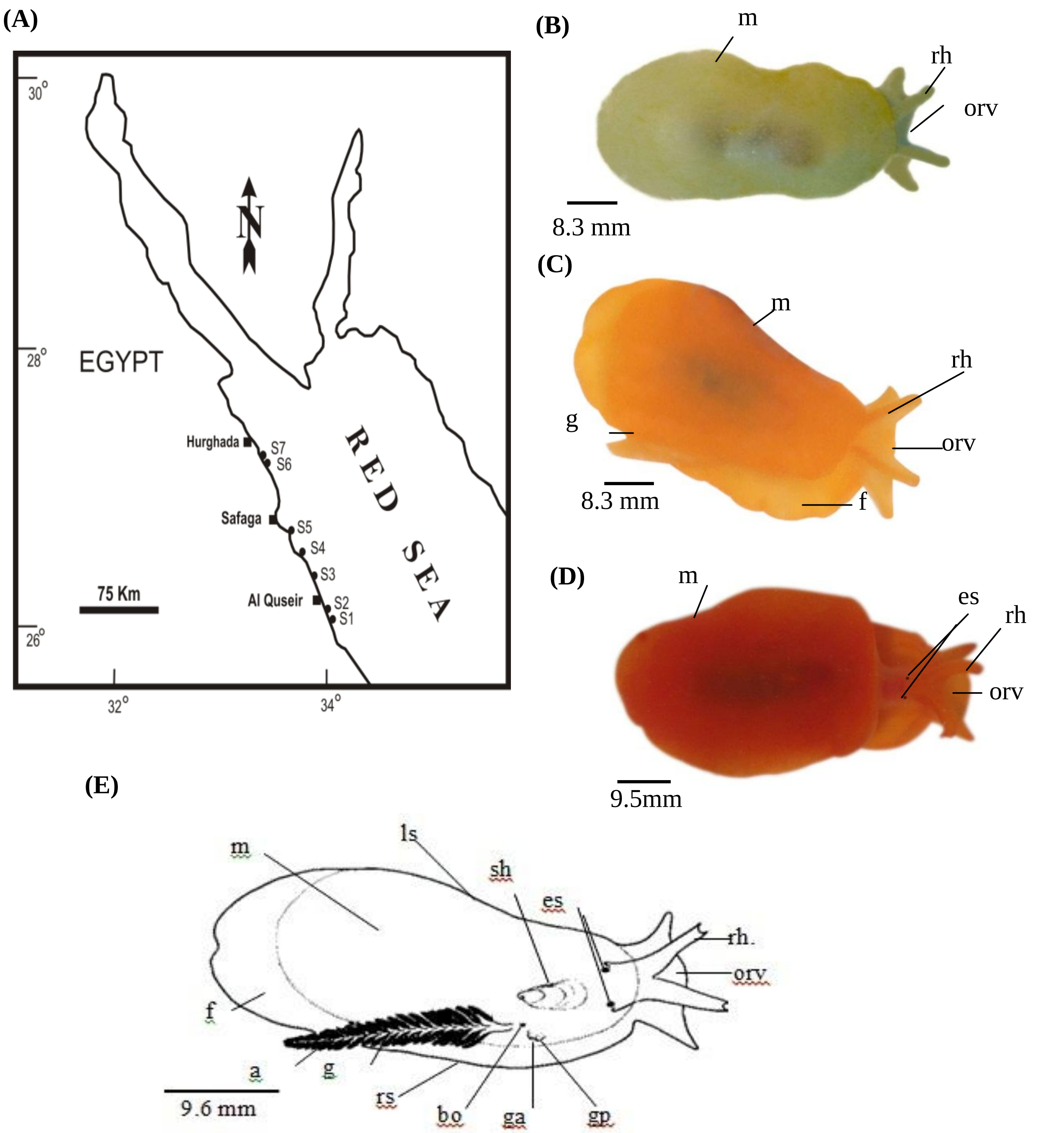

Fig. 1. A: A map of the northern part of the Red Sea showing the studied sites (S1-S7), S1=13 km South of Al-Qusier city, S2, S3, S4=10, 25, 44 km North of Al-Qusier city, respectively, S5=17 km South of Safaga city, S6= 40 km South of Hurgada city (Sharm El-Nagha), S7= 30 km South of Hurgada city (Makady Bay). **B-D:** Photographs of the dorsal view of the three color patterns of *B. citrina* showing (B): The pale yellow color pattern **(C)**: The orange color pattern (**D**): The red color pattern. **E**- A diagrammatic drawing showing the external color pattern morphology of the pale yellow color pattern of *B. citrina* after removing the mantle (modified after Soliman, et al. 2013), (a= anus, es= eye spot, f= foot, g= gill, ga= genital aperature, gp= gential papillae, ls= left side, m= mantle, orv= oral veil and rh= rhinophore(s), bo=branchial opening, rs= right side).

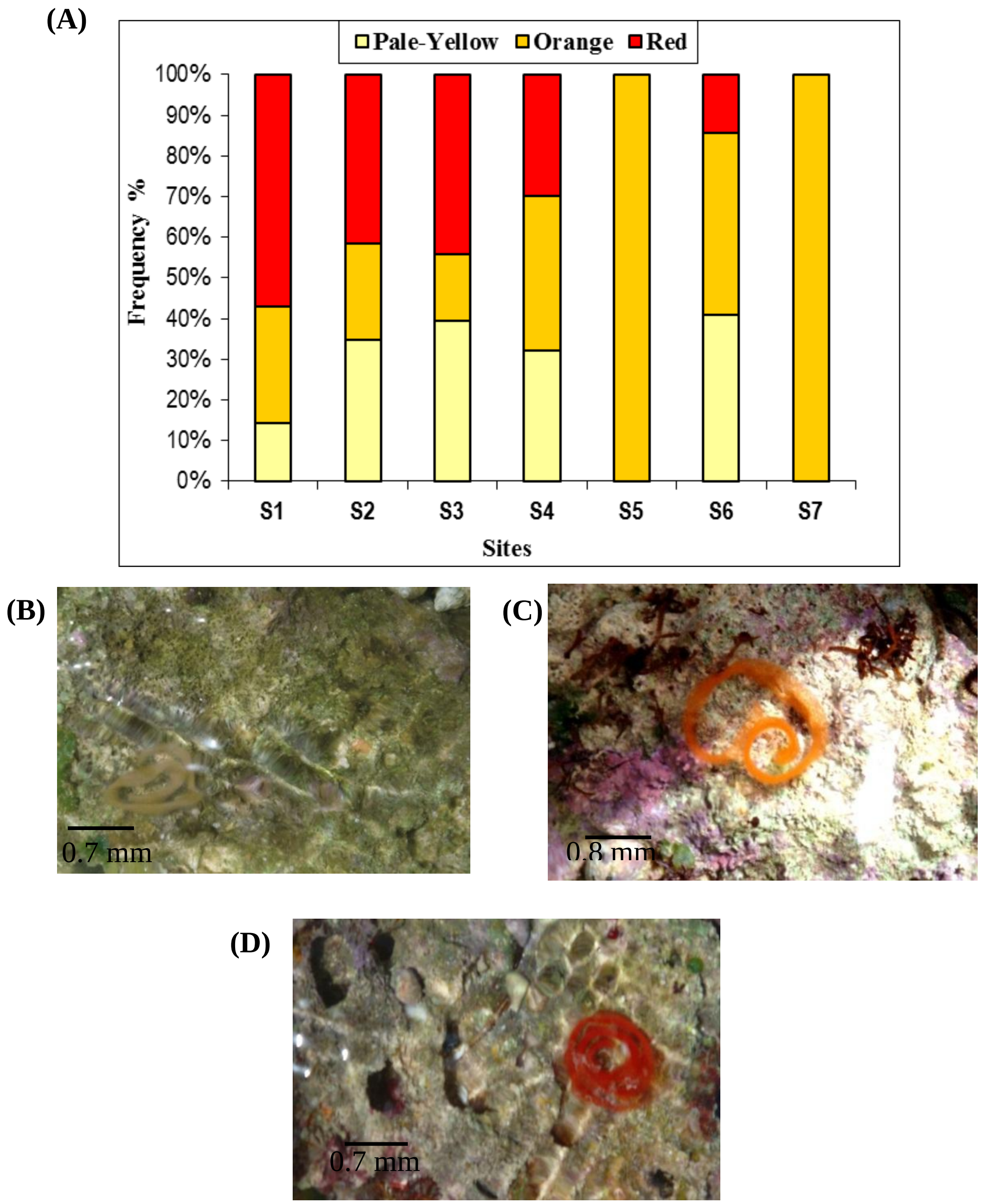

Fig 2: **A**- Distribution of the three color patterns of *B. citrina* within different sites of collection. **B-D**: Photographs of the egg masses three color patterns of *B. citrina* showing **(B)**: The pale yellow color pattern **(C)**: The orange color pattern **(D)**: The red color pattern.

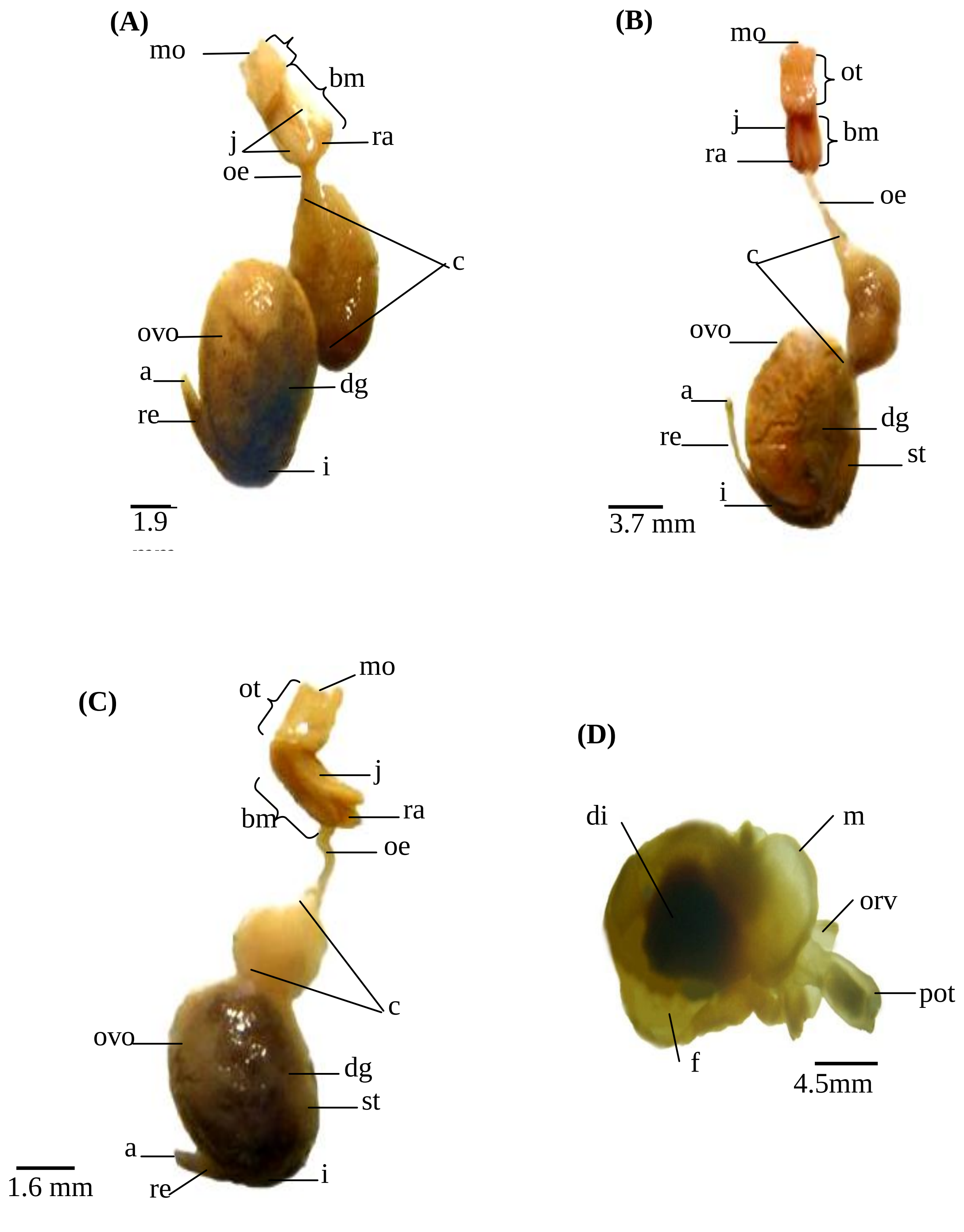

Fig.3. A-C photographs showing the digestive system of *B. citrina* (without salivary glands) (**A)**: The red color pattern **(B):** The orange color pattern**: (C)** The pale yellow color pattern. (**D)**: Photograph showing the oral tube protrudes from the mouth in the pale yellow color pattern (a= anus, bm= buccal mass, c= crop, dg= digestive gland, dgd= digestive gland duct,f= foot, i=intestine, j = jaw,m= mantle, mo= mouth, oe= oesophagus, orv= oral veil, ot= oral tube, pot= protruded oral tube, ra= radula, re= rectum, sag= salivary gland, st= stomach).

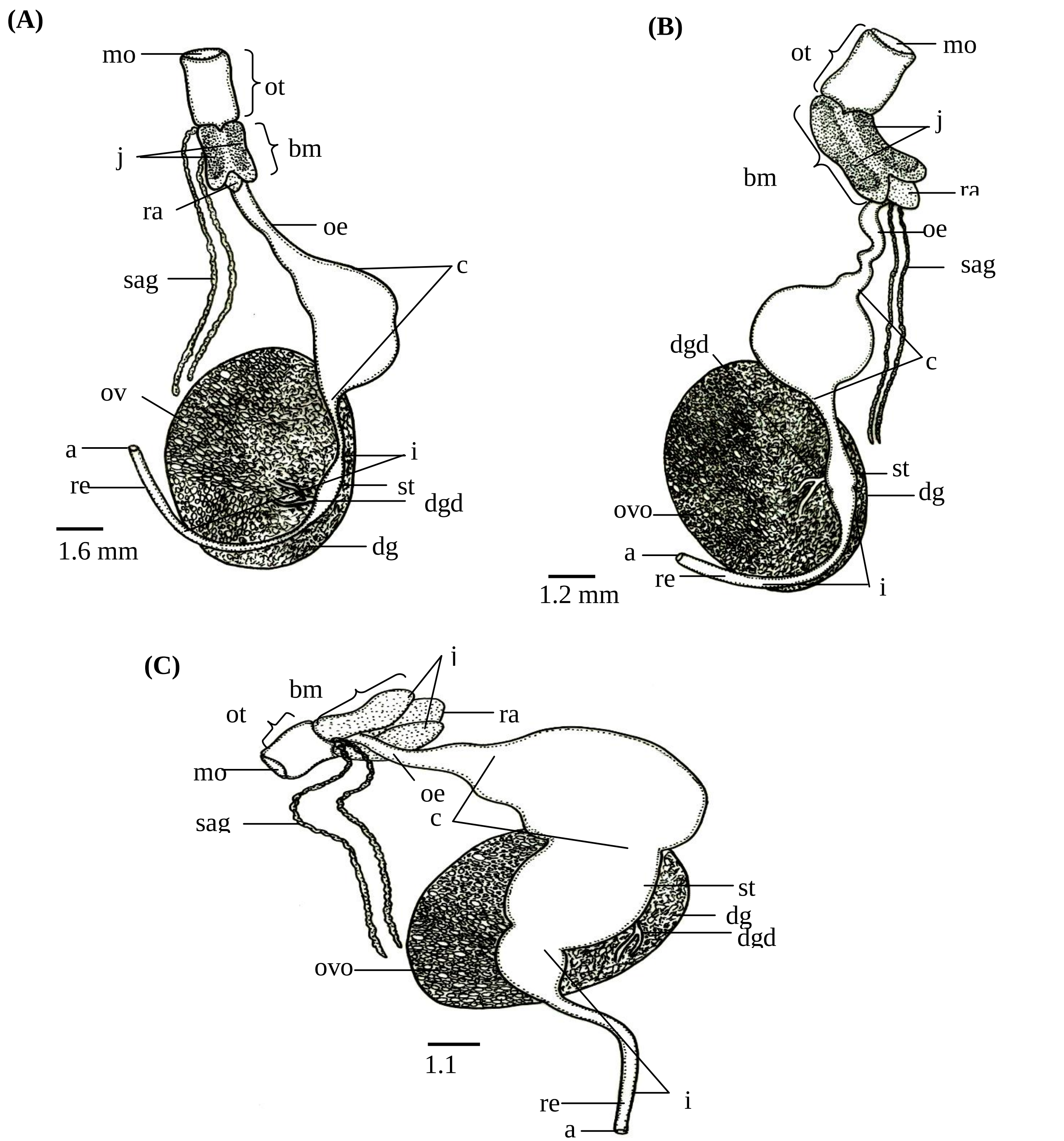

Fig.4. A-C: Diagrammatic drawings showing the digestive system of three color patterns of *B. citrina* **(A):** The red color pattern (**B):** The pale yellow color pattern **(C):** The red color pattern (a= anus, bm= buccal mass, c= crop, dg= digestive gland, dgd= digestive gland duct, i=intestine, j = jaw, mo= mouth, oe= oesophagus, ot= oral tube, pot= protruded oral tube, ra= radula, re= rectum, sag= salivary gland, st= stomach).

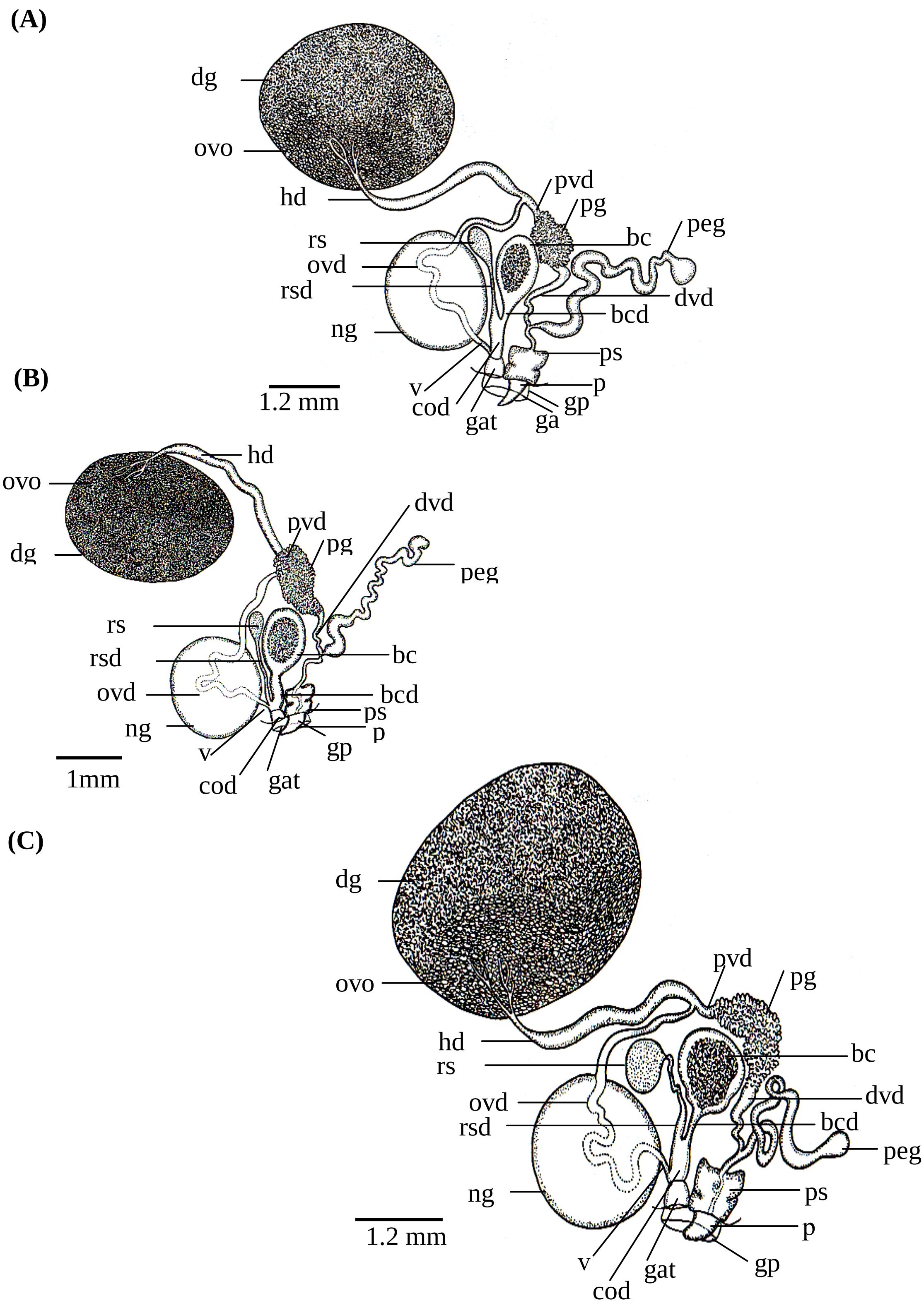

Fig. 5. A-C: Diagrammatic drawing showing the ventral view of the reproductive system of the three color patterns of *B. citrina* **(A):** The pale yellow color pattern **(B):** The orange color pattern (**C):** The red color pattern (bc= bursa copulatrix, bcd=bursa copulatrix duct, cod= common duct, dvd= distal portion of vas deferens, ga= genital aperture, gat= genital atrium, gp= genital papillae, hd= hermaphrodite duct, ng= nidamental gland, ovd= oviduct, ovo= ovotestis, p= penis, ps= penis sheath, peg= penial gland, pg= prostate gland, pvd= proximal portion of vas deferens, rs= receptaculum seminis, rsd= receptaculum seminis duct, v= vagina).

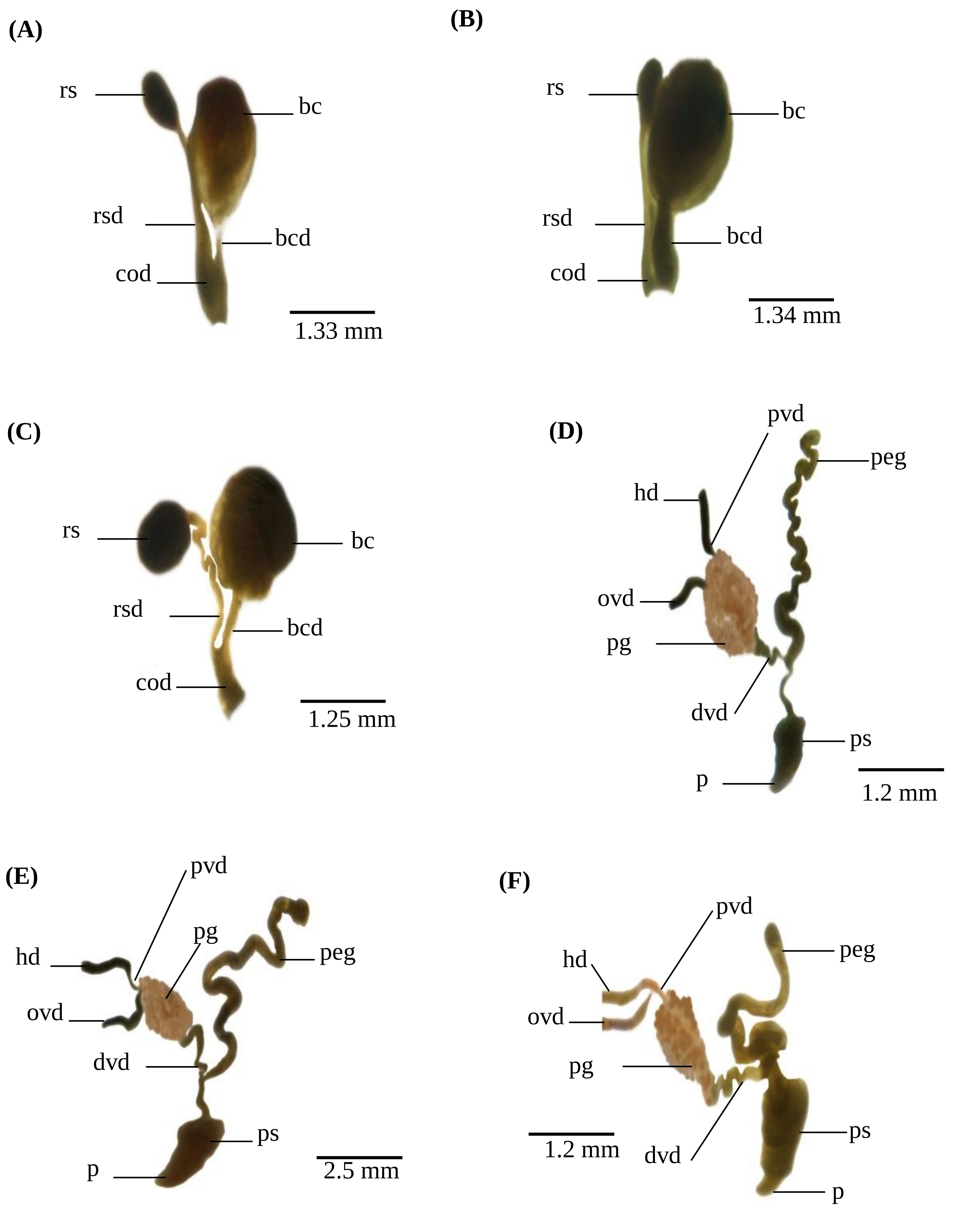

Fig. 6.**A- C**: Photographs showing isolated bursa copulatrix and recepticulum seminalis with their ducts of *B. citrina* **(A):** The pale yellow color pattern **(B)**: The orange color pattern and **(C):** The red color pattern. **D-F**: Photographs showing isolated male reproductive organs and their ducts of *B. citrina* **(D):** The pale yellow color pattern **(E)**: The orange color pattern (**F**)**:** The red color pattern (bc= bursa copulatrix, bcd=bursa copulatrix duct, cod= common duct, dvd= distal portion of vas).

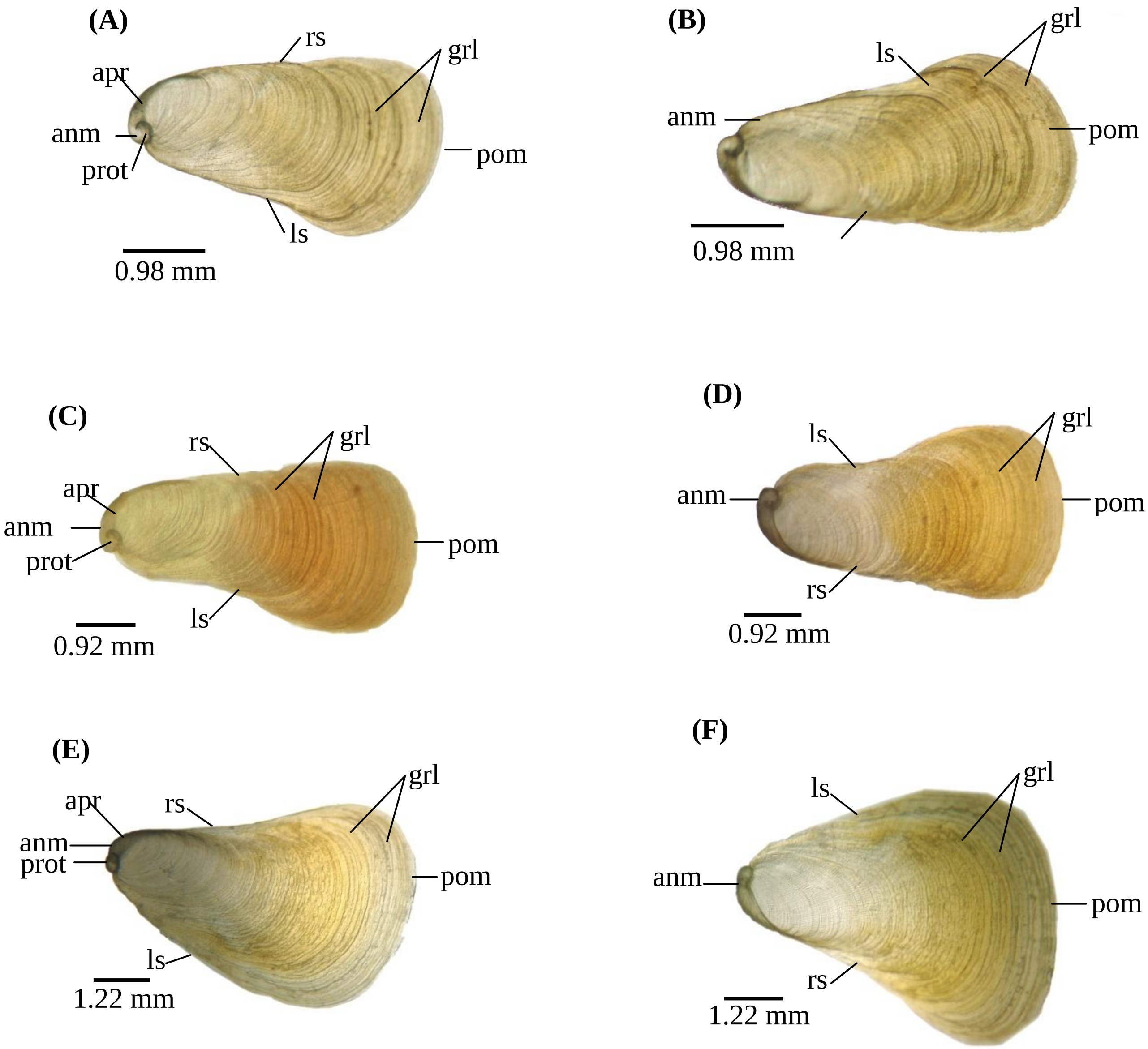

Fig.7. A-F: Photographs showing the dorsal and the ventral view for the shells of the three color patterns of *B. citrina* (A): The dorsal view of the pale yellow color pattern (after Soliman, et al. 2013) **(B)**: The ventral view of the pale yellow color pattern (**C, D)**: The dorsal and the ventral view of the color pattern, respectively (**E, F)**: The dorsal and the ventral view of the red color pattern, respectively (anm= anterior margin, apr= apical region, grl= growth line(s), ls= left side, prot= protoconch, rs= right side, pom= posterior margin).

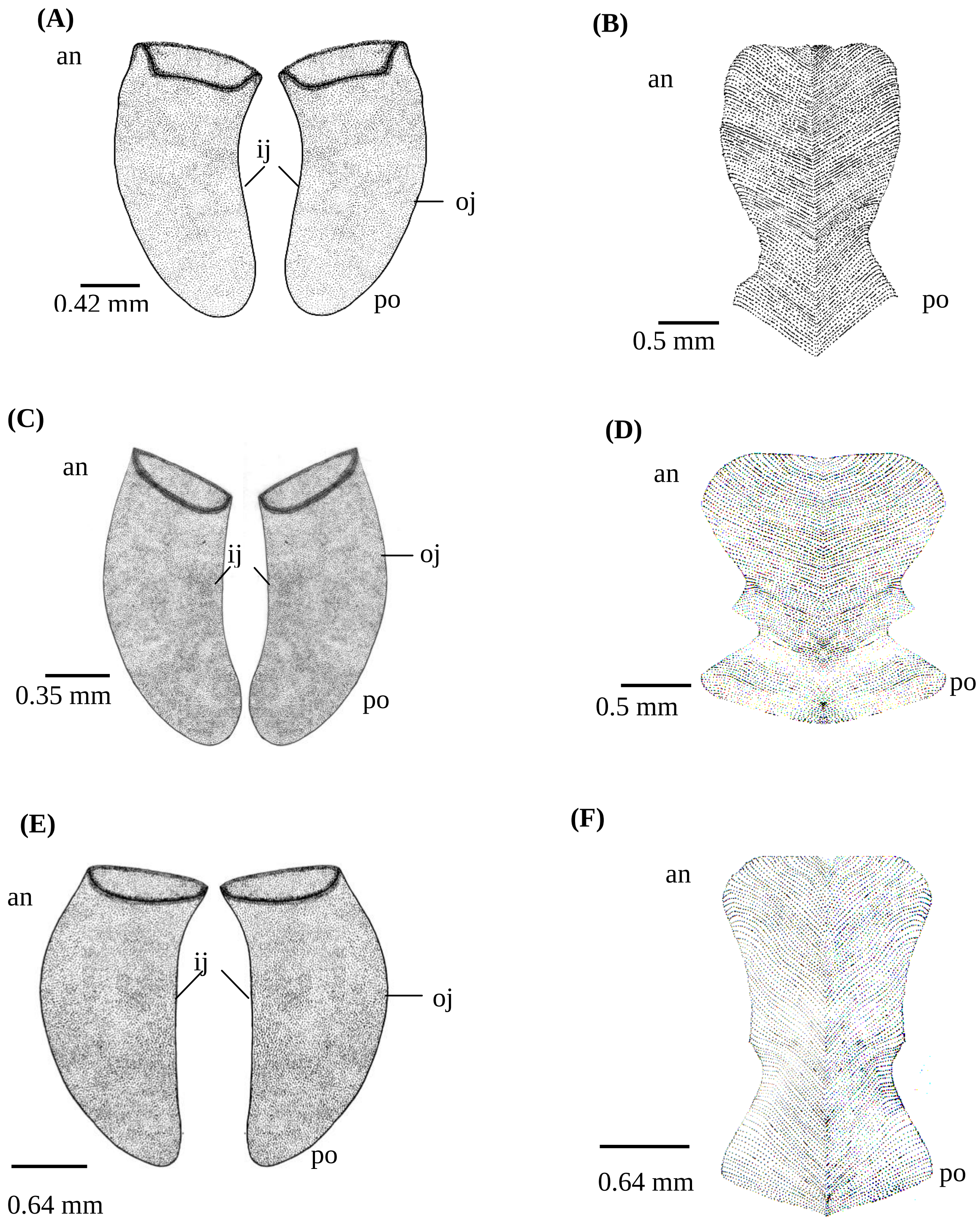

Fig. 8. A-F: Diagrammatic drawings showing the jaws and radulae of the three color patterns of *B. citrina* **(A)**: The jaws of the pale yellow color pattern (**B)**: The upper view of the radula of the pale yellow color pattern **(C)**, The jaws of the orange color pattern (**D)**: The upper view of the radula of the orange color pattern (**E)**: The jaws of the red color pattern (**F)**: The upper view of the radula of the red color pattern (an= anterior, ij= inner edge of jaw, oj= outer edge of jaw and po= posterior).

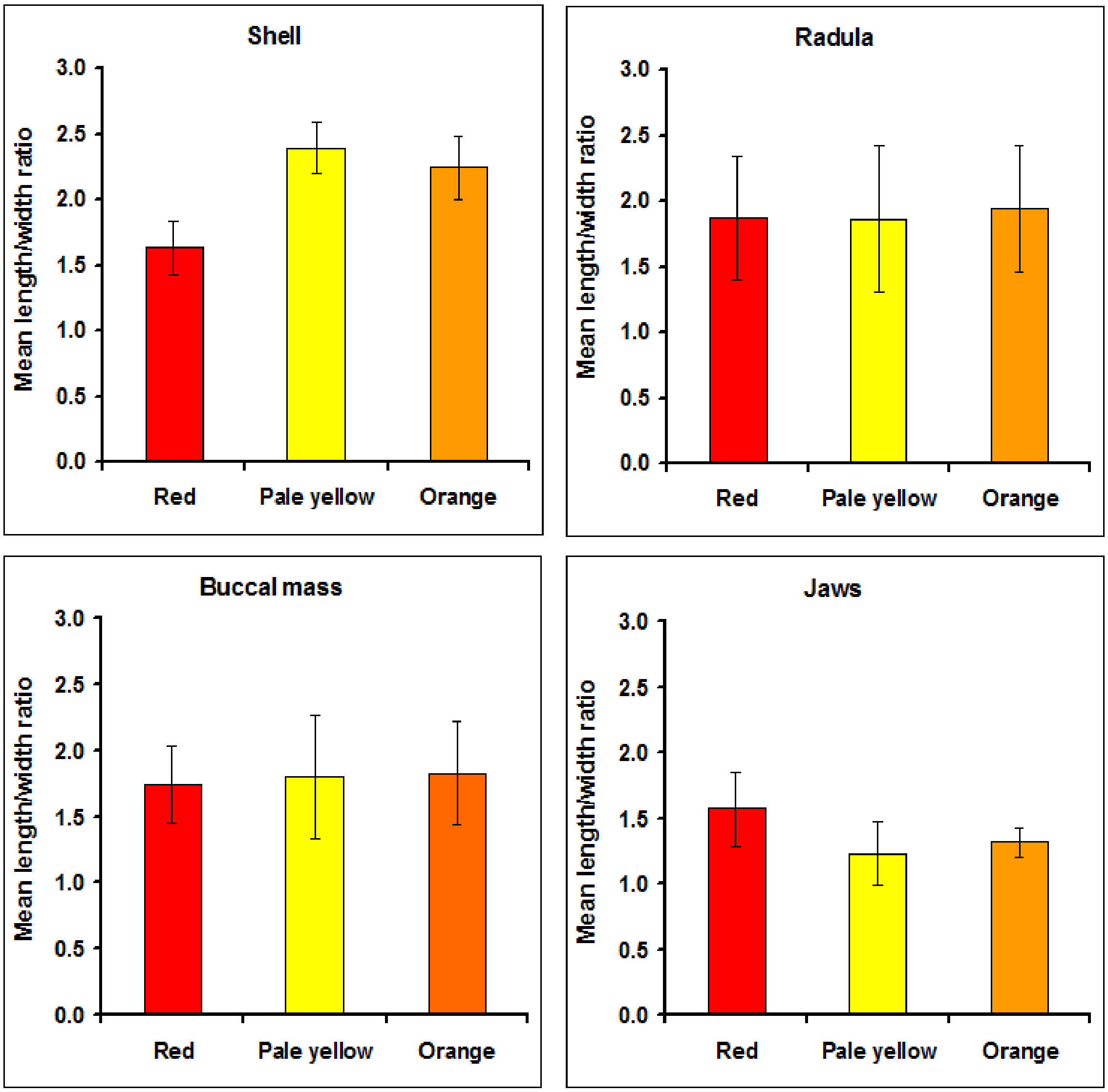

Fig. 9. Means of the shells length and width ratios of the three color patterns of *B. citrina*.

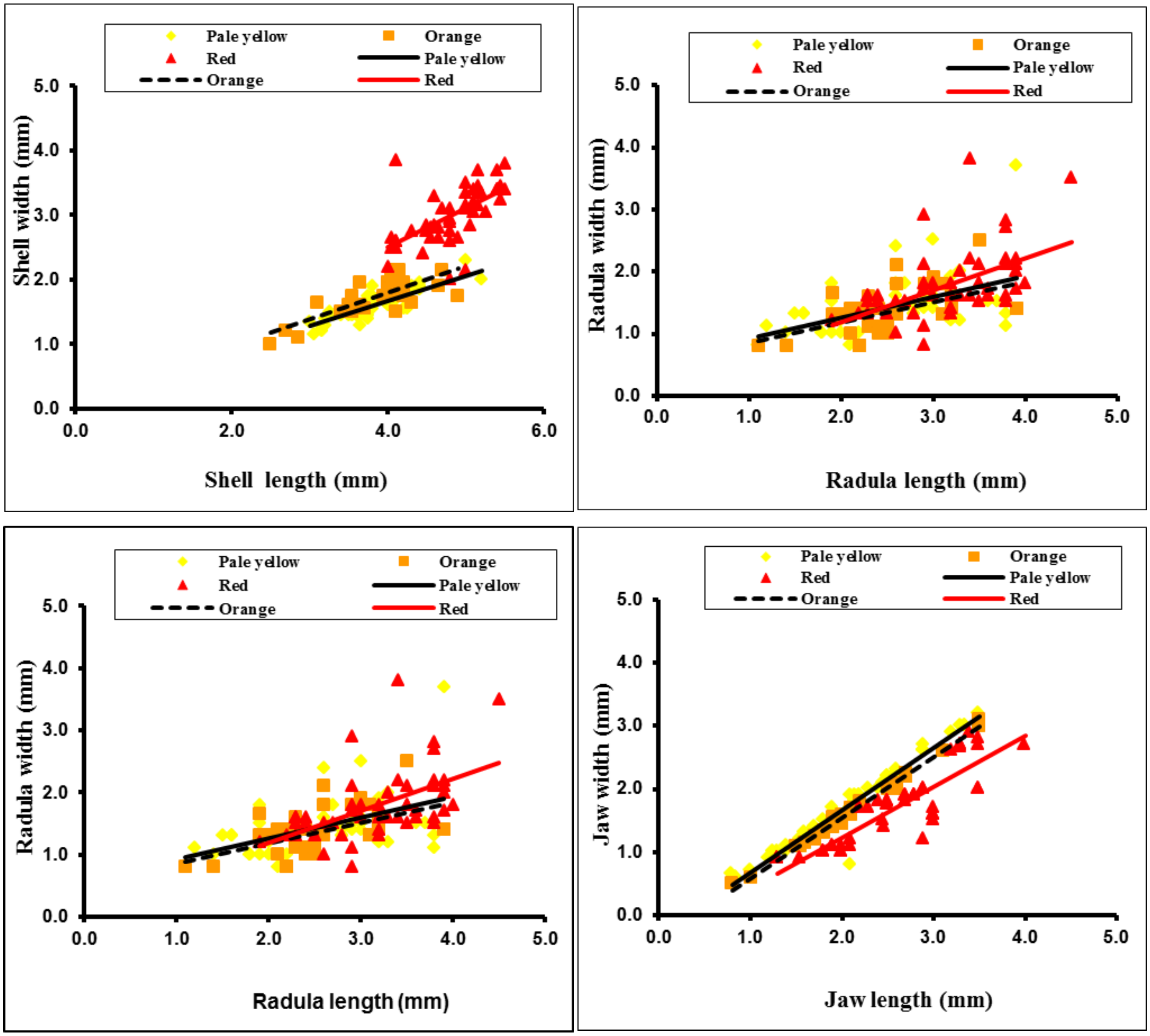

Fig. 10. Length-width relationships of the shells, buccal mass, radula and jaws of the three color patterns of *B. citrina*.

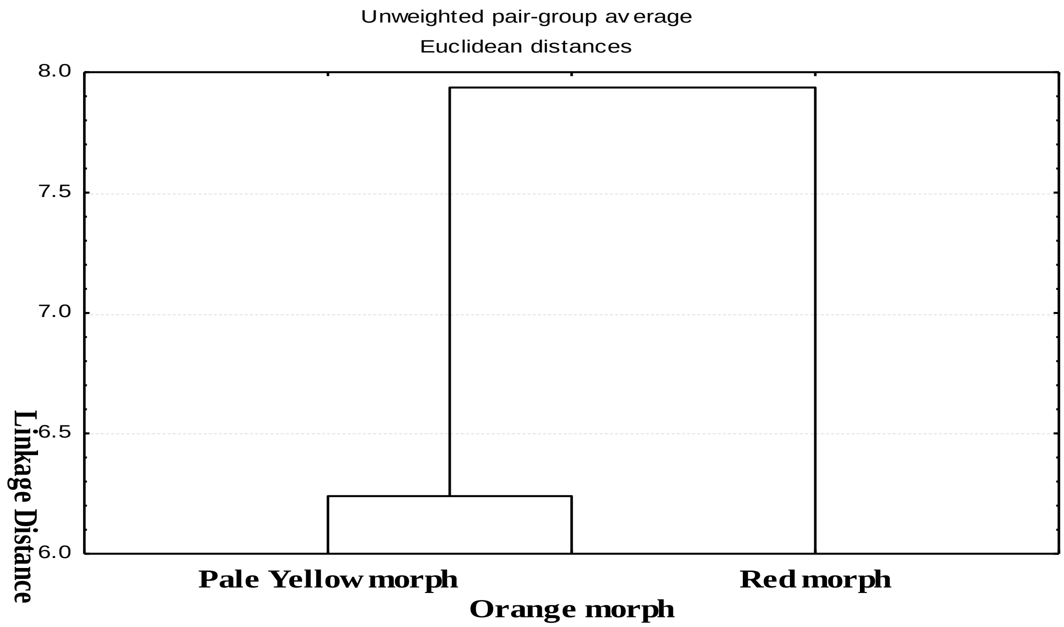

Fig. 11. Clustering analysis of the three color patterns of *B. citrina* depending on the shells, buccal masses, radulae and jaws length/width ratios.

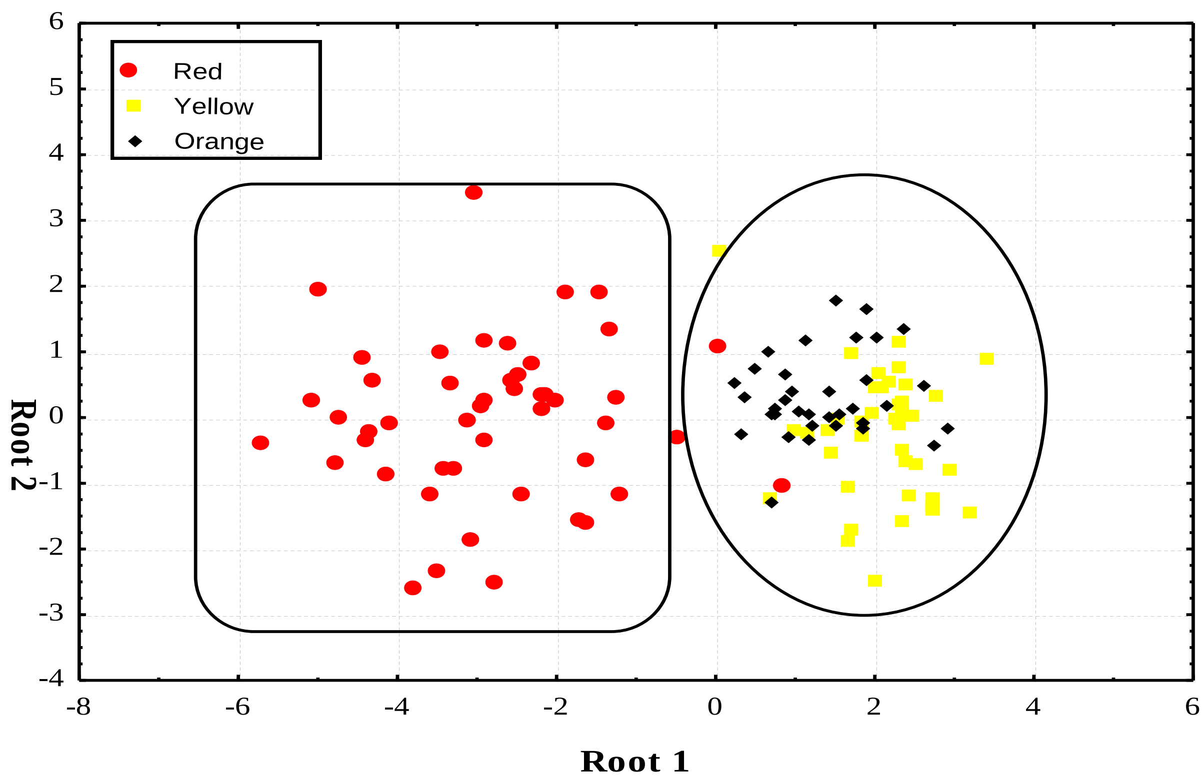

Fig. 12. Canonical analysis. A plot of the first discriminant function (root 1) against the second discriminant function (root 2), depicting any discrimination by selected variables of the three color patterns

Table 1. Differences in the structure of the digestive and reproductive systems among the three color patterns of *Berthellina citrina*.

Characters	Pale yellow color pattern	Orange color pattern	Red color pattern
Crop shape	Semi-circular	Circular	Semi-circular
Stomach	Narrow long tube leads to the ovate stomach.	Short tube leads to the ovate stomach.	There is no tube between the crop and the stomach.
Intestine	One tubular part	One tubular part	Two parts; large swallow anterior one and tubular posterior one
Prostate gland size	Small	Moderate	Moderate
Distal part of vas defrence	Simple	Simple	Convoluted
receptaculum seminis shape	Ovate	Ovate	Globular
receptaculum seminis duct	Connected with busra copulatrix duct in nearly in the middle	Connected with busra copulatrix duct at the end	Connected with busra copulatrix duct in the middle
Penial gland shape	Long convoluted tube, with rounded bulging end	Long, mild convoluted tube without bulging end	Short, less convoluted tube without bulging end
Penis	pale yellow color	orange color	red color

Table 2 Maximum and minimum values of lengths and widths of the Shell, buccal masses, Jaw and radula of the three color patterns of *B. citrina*.

Characters		Color pattern		
		Pale yellow	Orange	Red
Shell length	Max.	5.2	4.9	5.50
	Min.	3.0	2.5	4.0
	Mean± SD	3.75±0.51	3.87±0.52	**4.82±0.43***
Shell width (mm)	Max.	2.3	2.15	3.85
	Min.	1.15	1	2.0
	Mean± SD	1.58±0.24	1.75±0.29	**2.99±0.43***
Buccal mass length (mm)	Max.	4	3.6	4.65
	Min.	1.25	1.25	2
	Mean± SD	2.68±0.65	2.59±0.52	**3.2±0.60***
Buccal mass width (mm)	Max.	3.80	2.6	3.95
	Min.	0.75	0.6	0.95
	Mean± SD	1.55±0.52	1.47±0.4	**1.89±0.49***
Jaw length (mm)	Max.	3.9	3.9	4.50
	Min.	1.10	1.10	1.9
	Mean± SD	2.56±0.74	2.54±0.62	**3.18±0.60***
Jaw width (mm)	Max.	3.70	2.5	3.80
	Min.	0.8	0.80	0.8
	Mean± SD	1.44±0.52	1.36±0.39	**1.79±0.58***
Radula Length (mm)	Max.	3.50	3.50	4.0
	Min.	0.8	0.80	1.3
	Mean± SD	2.16±0.7	2.10±0.61	**2.62±0.63***
Radula Width (mm)	Max.	3.2	3.10	2.9
	Min.	0.6	0.50	0.9
	Mean± SD	1.8±0.73	1.64±0.59	1.7±0.59

ANOVA test * = significant at P< 0.0001

Table 3. Means of the four morphometric characters in the three color patterns of *B.citrina*.

Color patterns	L/W of shell		L/W of buccal mass		L/W of radula		L/W of jaw	
	Mean	**±SD**	**Mean**	**±SD**	**Mean**	**±SD**	**Mean**	**±SD**
Pale yellow	2.39	±0.20	1.8	±0.47	1.8	±0.52	1.23	**±0.24**
Orange	2.24	±0.24	1.8 3	±0.39	1.94	±0.48	1.32	**±0.11**
Red	**1.63**	**±0.21**	**1.74**	**±0.29**	**1.87**	**±0.47**	**1.57**	**±0.28**

Table 4. Comparison among the three color patterns of *B. citrina* depending on the four morphometrical ratios (Data analysis using ANOVA test).

Color pattern	Shell	Radula	Buccal mass	Jaw
Red X Pale yellow	**F= 315.45 P=0.000***	F= 0.00969 P= 09218. ns	F= 0.56747 p= 0.45327 ns	**F= 38.620 P= 0.000***
Red X Orange	**F= 156.318 P=0.000***	F= 0.43598 p= 0.51094 ns	F= 1.53404 p= 0.21908 ns	**F= 26.89 P= 0.000***
Orange X Pale yellow	**F= 9.03003 P= 0.0036***	**F = 0.44531 p = 0.50662 ns**	**F= 0.09424 p= 0.75971 ns**	**F= 3.61423 p= 0.06113 ns**

* = significant at P< 0.01, ns = not significant at P> 0.05, F = result of ANOVA test

Table 5. Linear regressions of the length and width of the shells of the three color patterns of *B. citrina*. Shell width= SW, Shell length= SL, Buccal mass width= BW and Buccal mass length= BL, Radula width= RW and Radula length= RL, Jaw width= JW and Jaw length= JL (R2 analyzed using ANCOVA test).

		R^2	F	P	Slop t	Slop p	Color pattern
Shell equation	SW=0.07+0.61 SL	**0.36**	26.8	0.00005	8.46	<0.0001	**Red**
	SW=0.14+0.415 SL	0.57	46.6	0.00001	**6.82**	<0.00001	**Orange**
	SW=0.12+0.389 SL	0.69	93.1	<0.00001	9.64	<0.00001	**Pale yellow**
Buccal mass equation	BW=0.13+0.545 BL	**0.47**	43.2	<0.00001	**6.57**	<0.00001	**Red**
	BW=0.52+0.33 BL	0.30	15.5	<0.004	3.94	<0.0004	**Orange**
	BW=0.37+0.442 BL	0.29	17.7	<0.0001	4.21	<0.0001	**Pale yellow**
Radula equation	RW=0.15+0.52 RL	**0.27**	18.27	<0.0001	**4.27**	<0.0001	**Red**
	RW=0.35+0.43 RL	**0.25**	12.55	<0.001	**3.54**	<0.001	**Orange**
	RW=0.58+0.336 RL	**0.21**	11.88	<0.001	**3.44**	<0.001	**Pale yellow**
jaws equation	JW= -0.38+0.81 JL	**0.75**	140.7	<0.0001	**11.9**	<0.0001	**Red**
	JW=0.38+0.96 JL	0.98	325.2	<0.00001	**57**	<0.0001	**Orange**
	JW= -0.31+0.99 JL	**0.95**	**760**	**<0.00001**	**27.6**	**<0.0001**	**Pale yellow**

Table 6. Discrimination function analysis.

N=125	Wilks' Lambda	Partial Lambda	F	P
Radula length	0.158084	0.978319	1.27427	**0.283553**
Radula width	0.157398	0.982583	1.01924	**0.364105**
Shell length	0.157429	0.982387	1.03087	**0.359967**
Shell width	0.267890	0.577313	42.09945	**0.000000**
Buccal mass length	0.156867	0.985909	0.82181	**0.442200**
Buccal mass width	0.156041	0.991124	0.51491	**0.598924**
Jaw length	0.169142	0.914360	5.38554	**0.005811**
Jaw width	**0.190951**	**0.809926**	**13.49417**	**0.000005**

Discussion

With regard to the taxonomic status of genus *Berthellina* Gardiner, 1936, it is still controversy and it represented by eight or nine worldwide species (Cervera et al., 1999; Cervera, 2002). There is still unidentified species *Berthellina sp.* from Canary (Cervera, 2000), From Reunion (Bidgrain, 2007) and from South Africa (Bertsch and Marlett, 2011). There are some unpublished data suggesting that may be there is more than one orange *Berthellina* species in the Indo-West Pacific region (Rudman, 2007). Narayanan, (1969) described the spotted form of *Berthellina* from the Gulf of Kutch *as Berthellina minor* Bergh, 1905 and changed it to *B. citrina* (Narayanan, 1970). Gosliner (1987) changed the name of specimens form South African belonging to *B. citrina* to *B. granulata* (Krauss, 1848). The white speculated form of *Berthellina* was described as *Berthellina cf. citrina* (Apte and Bhave, 2013). Moreover, until recently there is confusion in the identification between genera of *Berthella* and *Berthellina*; Hermosillo and Valdés (2008) was described a new species named as *Berthella grovesi* from the pacific coast of Mexico which previously known *Berthellina* sp. By Behrens and Hermosillo (2005) and Hermosillo et al. (2006).

The investigated three color patterns of *Berthellina citrina* showed some differences among each other regarding to the digestive and reproductive systems (Table 1). These differences in the digestive systems were represented in the shape of the crop, stomach and the intestine. There is a narrow tube leads to an ovate stomach in the pale yellow and the orange color patterns but it was shorter in the latter. Whereas, the tube is absent in the red color pattern. The crop is semi-circular in the pale yellow and red the color patterns, whereas, it is circular in the orange color pattern. In addition to the intestine in the Red color pattern could be distinguished into two parts; large swallow anterior one and tubular posterior one. Although, Willan (1983) reported that Pleurobranchidae showed little variation in the basic structure of the alimentary canal and only radulae and jaw elements are the best diagnostic characteristics for determining genera.

Like other pleurobranchids, *B. citrina* had a triaulic reproductive system (Willan, 1987; Wagele and Hain, 1991; Cervera et al. 2000). The present findings showed clear differences in the reproductive system of the three color patterns represented in the prostate gland size, penis, the connection of receptaculum seminis with busra copulatrix, the degree of convolution of both distal part of vas deference, and penial gland as shown in Table 1.

In the present species, the receptaculum seminis and busra copulatrix are united with their stalks and the oviduct enters the female gland mass and then it meet the two seminal vesicles leading to the exterior by vagina. This typically one type of the triaulic genital system which represented in other three genera involved *Berthella*, *Pleurehdera* and *Tomthompsonia* (Wagele and Hain, 1991; Cervera et al. 2000). Noteworthy, the triaulic reproductive system in this group has three types (Cevera, et al. 2000). The three color patterns of *B. citrina* had rudiments of the oviducts inside the nidamental glands. Although, Willan (1983) reported that, no connection exists between the vagina and the oviduct in *B. citrina*. It is likely to assume that, the oviduct may be damaged during egg ribbon formation.

According to Willan and Bertsch (1987) the penial glands is existing in all the family Pleurobranchidae with the exception of *Bathyberthella antarctica*. However, Gohar and Abul-Ela (1957) did not mention the presence of this gland in the genital system of *B. citrina*. Also, they stated that the vas deferens is short, provided with compact prostate gland that slightly coiled and opens into the penis. These variations of the genital system support the idea that species belonging to the genus *Berthellina* are separated by differences in their genitalia (Gosliner, 2006).

Herein, Morphometric measurements (length and width relationships) and linear regression between length and width of the shells, buccal masses, radulae and jaws of the three color patterns led to isolation of the red color pattern. Also, cluster analysis according to the ratios of shells, buccal masses, radulae and jaws revealed that, the red color pattern comprises a separate group, while pale yellow and orange color patterns are found to be very close. Furthermore, the discrimination function analysis using the length and width measurements of the shells, buccal masses, radulae and jaws separated the red color pattern from the other ones. All these tests support the idea of the isolation of the red color patterns as a separate species. In addition, we described previously in details the external morphology, gills, shells, radulae and jaws of the three color patterns of *B. citrina* and they showed some remarkable variations in these taxonomic characters (Soliman et al., 2013).

The significance and maintenance of color polymorphisms for traits related to fitness is a dominant theme in ecology and evolutionary biology (Toonen and Pawlik, 2001; Ross et al. 2003; Tarjuelo et al. 2004). The combination of phenotypic and environmental data used in our study of *Berthellina citrina* allows us to evaluate several plausible explanations for the color polymorphism in this species. Our phenotypic data showed that although the various *B. citrina* color patterns were sympatric, their relative frequencies among sites with a dominate red color pattern in sites 1, 2 and 3 and orange and pale-yellow predominant in sites 4 and 6. This distribution may reflect genetic differentiation, either through drift or via local selection, or environmentally driven plasticity (Harley et al. 2006).

In conclusion, the present environmental, anatomical and statistical data of the three color patterns of *Berthellina citrina* disclosed there is a significance variation between the orange and pale yellow color pattern from the red one and these may be led to isolation of the red color pattern as separate species. Further molecular studies are needed to support the present data.

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