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First record of a-sexual reproduction by fission in *Holothuria (lessonothuria) verrucosa* (Selenka, 1867) from coastal waters of Karachi, Pakistan

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Abstract

An experiment of a-sexual reproduction by fission was conducted on sea cucumber, *Holothuria (Lessonothuria) verrucosa* (Selenka, 1867) for 98 days. Anterior fission was observed on Day 1 with the formation of fission on the body of the sea cucumber. Initial length of 14 cm and weight of 21 g was measured at Day 1 in an aquarium under laboratory condition. Complete fission was observed on Day 7 of experiment by formation of 2 specimens; A and P. Initial length and weight for A was 4.5 cm and 3.18 g, while P was 9.5 cm and 17.30 g respectively. Increased weight for specimen A was observed on Day 20 with the first observation of the formation of anal pore. Tentacles started to form on specimen P on Day 34. Complete formation of anus on specimen A was seen on the 98th day of experiment with final weight of 3.84 g and length of 4.6 cm. Large growth of tentacle was also seen on Day 98 of the experiment with a final weight of the specimen P as 17.56 g and length as 9.7 cm.

Keywords: Asexual reproduction, fission, *Holothuria verrucosa*, Pakistan

Introduction

A total of 19 species of sea cucumbers belonging to the class Holothuroidea are reported from Pakistan. Eight of them belong to the family Holothuriidae, 3 to the family Cucumariidae, 2 to the family Synaptidae, 2 to the family Sclerodactylidae and 4 to the family Phyllophoridae. Taxonomic detail of *Holothuria (Lessonothuria) verrucosa* (Selenka 1867) from Karachi coast was published by Ahmed et al. (2016). However, no data are available on the a-sexual and sexual reproduction of holothurians from Pakistan coasts.

The objective of the present study was to establish the information regarding a-sexual reproduction of sea cucumber under laboratory conditions. This study will provide information related to the holothurians a-sexual reproduction under laboratory conditions in Pakistan.

Studies on the asexual reproduction of holothurians by fission are relatively scarce (Emson and Wilkie 1980; Lawrence 1987; Smiley et al. 1991; Mladenov and Burke 1994). Asexual reproduction through fission is a feature of many of the most abundant Aspidochirote holothuroids in the Indo-Pacific region (Uthicke 1997 and 2001). Fissiparous species split in half and the two halves regenerate to make complete individuals. Thus far, six *Holothuria* and two *Stichopus* species are known to be fissiparous and asexual reproduction appears to be a major form of reproduction used in maintaining populations (Ebert 1978; Harriott 1982; Conand and de Ridder 1990; Chao et al. 1994; Conand et al. 1997; Uthicke 2001).

The aim of this study was to observe a-sexual reproduction in *H. verrucosa* by fission during November 2015 to April 2016 under laboratory conditions.

Materials and Methods

A total of 71 sea cucumbers were collected from the Buleji (24°50'20.41" N, 66°49'24.15" E) and Sunehri (24°52'33.49" N, 66°40'40.20" E) coast during November 2015 to April 2016. Animals were brought to the laboratory by keeping them in polythene bag filled with sea water and supplied with aeration. The area of study was shown on the map in Figure 1. A-sexual reproduction was observed in one of the *H. verrucosa* collected from Buleji coast on 25th of November 2015, with a tide of -0.30 m from intertidal area on rocky shores under hard rock. The initial length and weight of the animal was

recorded. The physicochemical parameters such as water temperature (°C), salinity and pH were measured by multi-parameters. For morphological and a-sexual reproduction characteristics, length (cm) and weight (g) were examined in laboratory on daily basis. Animal was externally examined for signs of fission, changes in physiology and anatomy were noted, and its movement was observed as well. Water salinity was maintained every 15 days by adding a 25% of fresh sea water into the aquarium. Experiment was conducted for 98 days. Microphotography was also performed using a digital camera (Fujifilm 16 MP).

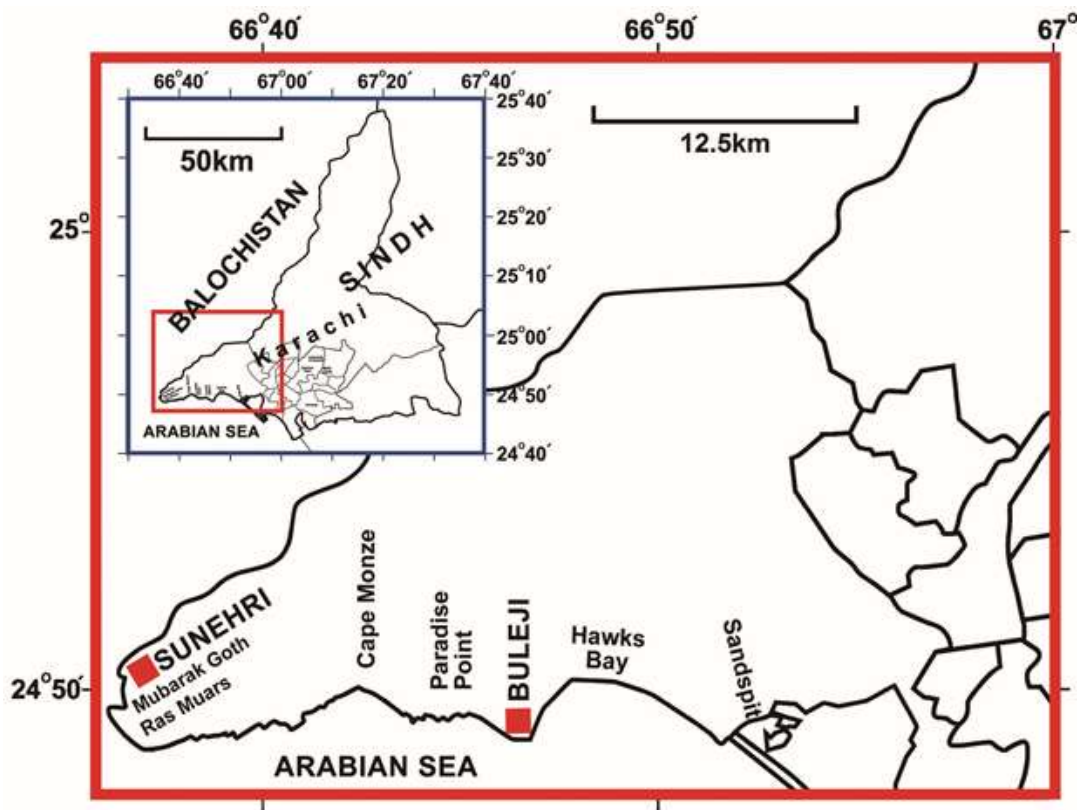


Fig 1. Study area map of Sunehri and Buleji coast

Results

During routine surveys of holothurians on Buleji coast on 25th November 2015 a specimen of *H. verrucosa* was observed to constrict slightly from anterior (Fig.2A) and it was collected carefully and transported to a laboratory aquarium with running seawater for observation.

Table 1 shows physico-chemical parameters of the study area on Buleji coast recorded on 25th November 2015 when the animal was collected. Table 2 shows maintained hydrological parameters in the aquarium throughout the experiment.

Water temperature was $30.60 \pm 0.82^\circ\text{C}$, salinity was 36.80 ± 0.83 (‰) and pH was 8.10 ± 0.12 in the aquarium during the experiments in April and February (Table 2). A-sexual characteristics by fission were observed in *H. verrucosa* collected from Buleji coast from the intertidal area on rocky shores under the hard rock. Anterior fission was observed on the body wall of *H. verrucosa*. Coloration of fresh specimens was burnt sienna and fission point was located on the anterior end of the specimen (Figure 2 A-B). An initial length of 14 cm and weight of 21 g were measured at Day 1 of the experiment. On Day 2, animal was observed to be moving in anti-clock wise rotation. Animal was also seen twisting, turning, whirling and rotating which eventually led to formation of fission on the body wall (Figure 2C).

On Day 6, animal was continuously twisting on the opposite side direction and fission point was very clear, consequently a thin body wall between A and P developed and looked like a loop. A loop-like body wall was more stretched between the opposite sides (Figure 2 D). On Day 7, complete fission was achieved, and two animals were formed into specimen A and specimen P: A= anterior specimen with an existing mouth at one end and a closed wound; P = a posterior specimen with an anus at one end and a closed wound at the other. The length and weight of specimen A were 4.5 cm and 3.18 g, respectively. Specimen P had a length of 9.5 cm and a weight of 13.30 g (Figure 2 E-G).

On Day 20, anal pore formation was observed in specimen A, the measured length and weight were 4.5 cm and 2.04 g, respectively. No obvious gross anatomical development was observed on specimen P but there was a slight

weight increase to 13.46 g. Both specimen A and P were in relaxed and active condition (Figure 2 I-J). On Day 29, the weight of specimens continued to increase to 2.95 g in specimen A and 13.84 g in specimen P. Small outgrowth of anus was observed in specimen A. Specimen A and P were seen in active and relaxed condition (Figure 2 K-L).

Formation of tentacle outgrowth was observed on specimen P on Day 34, and large anal pore formation with thin membrane was observed on specimen A on the same day (Figure 2 M-N). On Day 41, tentacle growth formation was observed on specimen P and an anus pore covered with thick yellowish membrane was clearly observed on specimen A (Figure 3 A-C). Weight of specimen P showed a great increase from 13.84 g to 16.53 g on Day 29, however, the length remained the same. Specimen A was more active than specimen P. On Day 48, the anus pore was seen covered with thick membrane on the inner side of the wall. Small tentacle formation was seen on specimen P (Figure 3 E-F).

A tiny, peltate shaped tentacle was formed on the Day 82 of the experiment on specimen P (Figure 4 D-E). Complete formation of anal pore on specimen A was seen on Day 98 of experiment and the length and weight recorded were 4.6 cm and 3.84 g, respectively. The tentacle formation was also clearly seen on specimen P on Day 98 with recorded length and weight as 9.7cm and 17.56 g, respectively. The specimens were also seen active on the last day of the experiment. The specimens were seen attached on the aquarium wall and base during the 98-days of the conducted experiment.

Table 1. Physico-chemical parameters on Buleji coast on 25th November 2015

Tide (m)	Water temperature ($^\circ\text{C}$)	pH	Salinity (‰)
-0.30	26	8	38

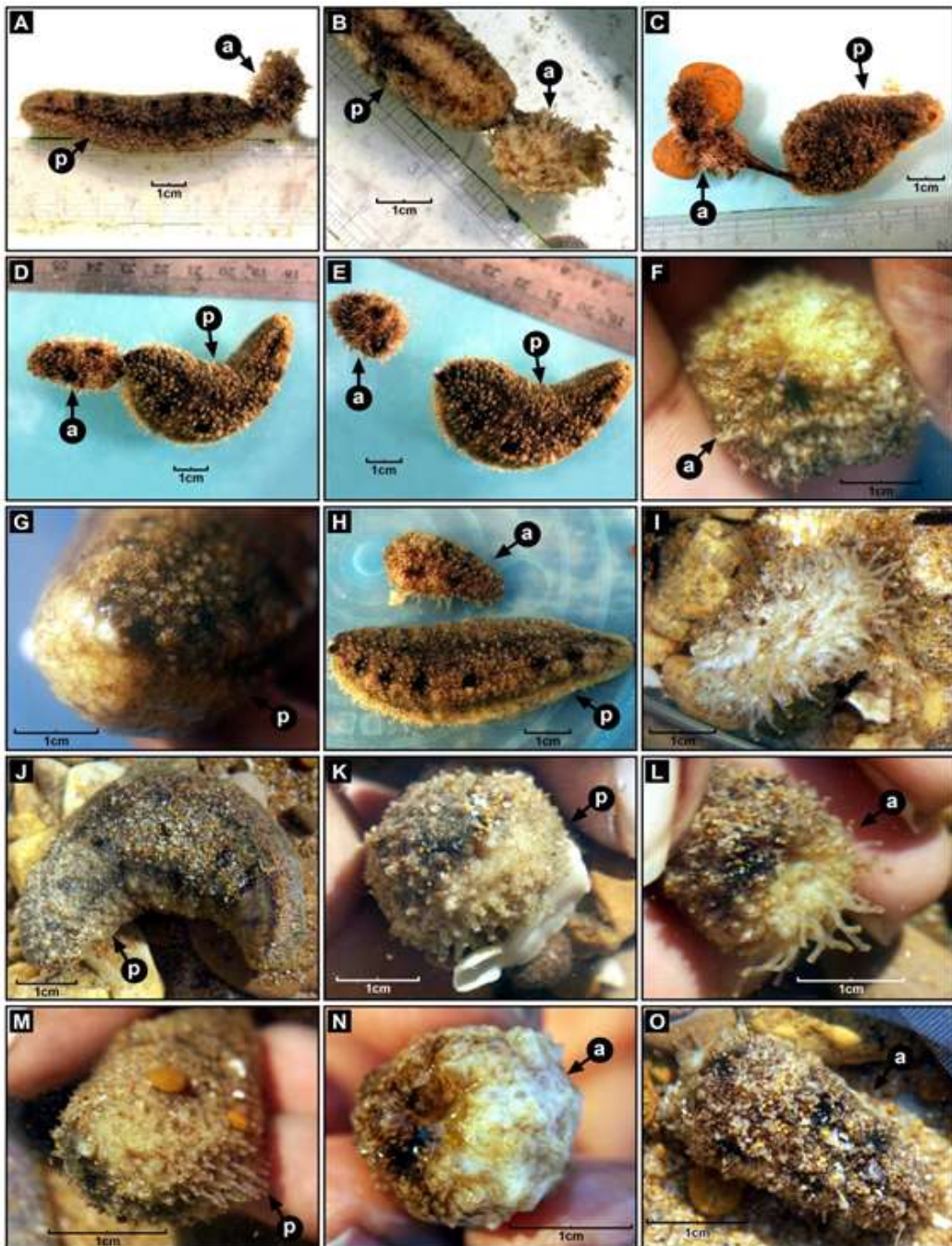


Fig 2. *Holothuria verrucosa* fission occurs from anterior side of the animal [A; Day 1 of experiment, animal (dorsal), B; Day 1 of experiment, animal (Ventral), C; Day 2 (twisting), D; Day 6 twisting continue, E-G; Day 7, (fission completed with two separate specimen form) ‘A’ (anterior specimen with mouth and tentacles) and ‘P’ (posterior specimen with anus), H; Day 7, A and P specimen in relax condition, I-J Day 20, specimen A (ventral) P (dorsal), K-L Day 29, small outgrowth for tentacles formation in specimen P and anal pore formation in specimen A, M-N; Day 34, tentacle outgrowth formation in specimen P and large anal pore formation with thin membrane was observed in specimen A. O; Day 38, dorsal view of specimen A in relaxes condition.

Table 2. Physico-chemical parameters were maintained in aquarium during the experiment

Months	Water temperature (°C)	Salinity (‰)	pH
	Mean ±SD (Min-Max)	Mean ±SD (Min-Max)	Mean ±SD (Min-Max)
December	29.10±0.89 (28.00-30.00)	36.60±0.54 (36.00-37.00)	8.04±0.89 (8.00-8.20)
January	29.60±0.82 (28.50-30.50)	36.60±0.89 (36.00-38.00)	8.06±0.13 (8.00-8.30)
February	29.70±0.44 (29.00-30.00)	36.80±0.83 (36.00-38.00)	8.10±0.12 (8.00-8.30)
March	30.20±0.57 (20.50-31.00)	36.40±0.89 (36.00-38.00)	8.04±0.89 (8.00-8.20)
April	30.60±0.82 (30.00-32.00)	36.66±0.54 (36.00-37.00)	8.06±0.13 (8.00-8.30)

Discussion and Conclusion

Dolmatov (2014) discussed asexual reproduction, features of fission, and regeneration of anterior and posterior fragments in holothurians in his review article. He described a-sexual reproduction in the larval stages and in adult holothurians by fission mechanism. Monticelli (1896) differentiated three fission mechanisms in *O. planci* by constriction, twisting, or stretching. In the present study, the specimen was divided into two specimens by the same process. Conand and Ridder (1990) observed a-sexual reproduction in *Holothuria atra*, the body divided into two fragments with an anterior: posterior length ratio of 4: 5. In the present study the *H. verrucosa* divided into two specimens with a ratio of 1:2. Conand et al. (1997) observed asexual reproduction in *H. leucospilota*, the length of the anterior fragments constitutes approximately 19% of the total body length. Jessica et al. (2008) observed asexual reproduction by fission in *Holothuria hilla*, the fission products of the specimen was 1:2. Asexual reproduction by

fission in *Holothuria atra* was reported by Asha and Diwakar (2015). During the field surveys, the specimen was observed in twisting pattern moving in opposite direction and it constricted its mid body part throwing out internal organs, particularly the gonad, and eventually the body was separated into two halves. The process of asexual reproduction by transverse fission in *Stichopus chloronotus* observed in laboratory aquarium the specimen showed the posterior half of the individual remained stationary, while the anterior end continued to move forward this resulted in a more distinct constriction (Uthicke 2001). Present studies were indicated similar observation during the experiment. Fission is considered to play a significant role in maintaining populations of several holothuroids by compensating for mortality and migration (Ebert 1978; Harriott 1982; Chao et al. 1994; Uthicke 2001). This is the first description of the process of asexual reproduction in *H. verrucosa*. However, the observations are based on only one individual and experiment was carried out in aquarium conditions.

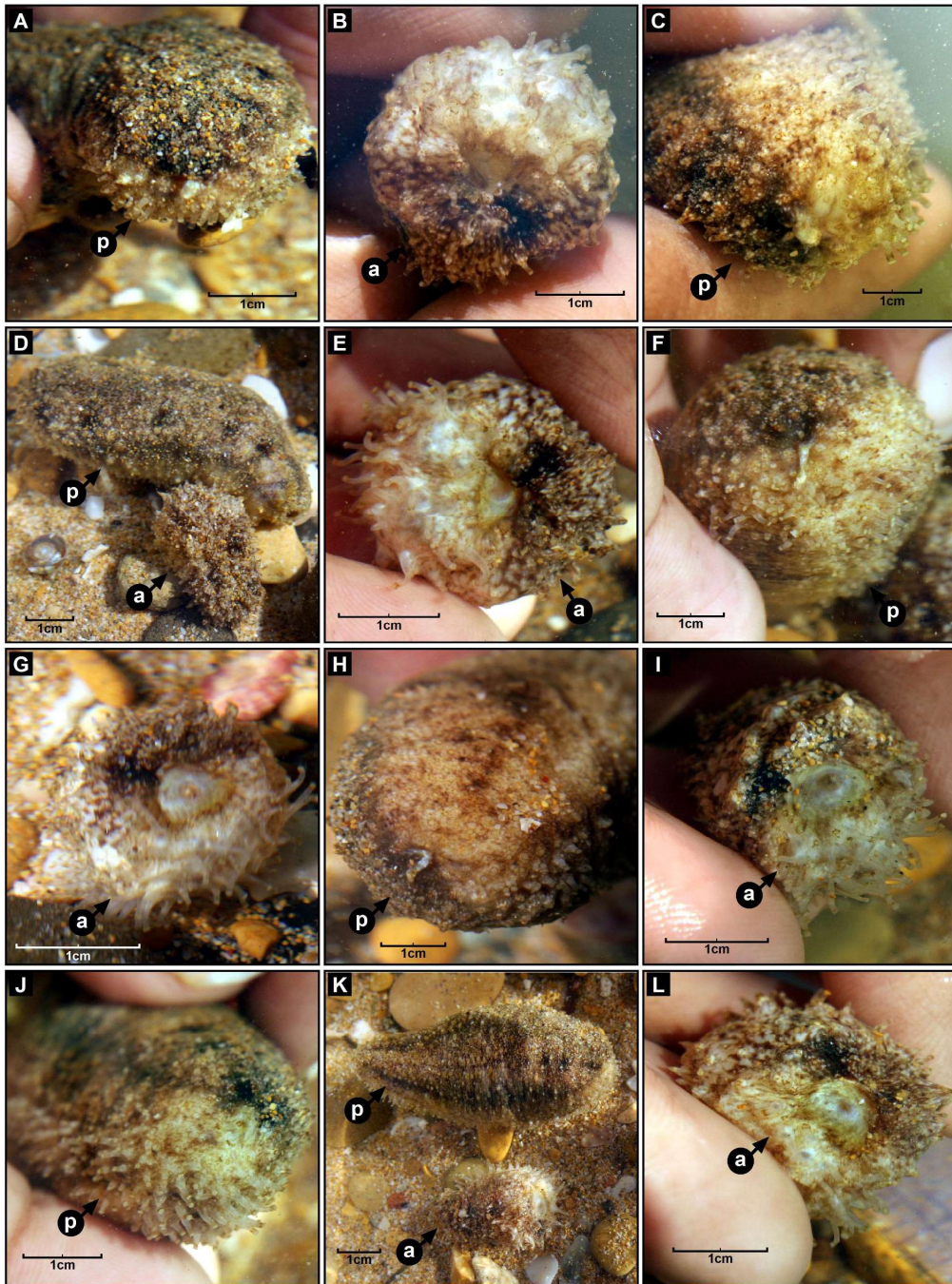


Fig 3. A-C; Day 4, tentacle formation in specimen P and anus formation in specimen A, D; specimen A and P in relax condition, E-F; Day 48, anus pore formed, inner side covered with thick membrane in specimen A and tentacle formation in progress, no tentacle observed on specimen P. G-H; Day 55 anus pore was observed clearly on specimen A but no tentacle observed on specimen P, I-J; Day 64, small outgrowth was observed outside on specimen A and tiny tentacles formation around the mouth of the specimen P, K; Specimen A and P in relax condition. L; outgrowth of anus in specimen A.

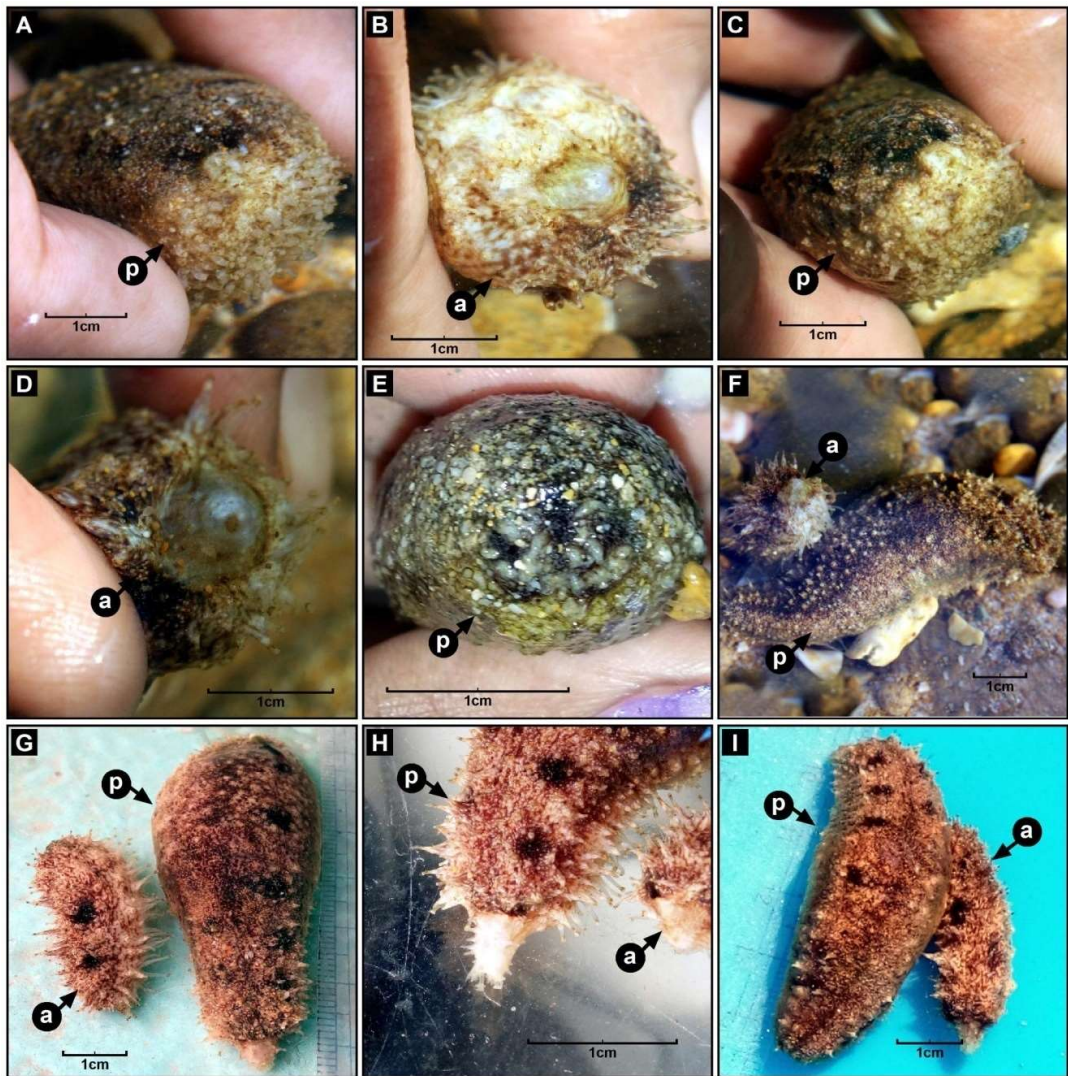


Fig 4. Day 71, A-C; Tentacle and anus formation were seen on specimen P and A, D-E; Day 82, anus outgrowth with clear anus pore and tiny peltate shape tentacle formation. F-G; Day 90, both specimen relaxed in aquarium, H-I; Day 98, tentacle formation with large outgrowth.

The present study is first description of the process of asexual reproduction in *Holothuria (Lessonothuria) verrucosa* (Selenka, 1867) from Karachi coastal waters of Arabian Sea. This study may provide useful data since no previous studies have been conducted on population density of holothurians from Pakistan coasts.

Conflict of interest statement

We declare that we have no conflict of interest.

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