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Farhana S Ghory

Marine Reference Collection &
Resource Centre University of
Karachi, Karachi, Pakistan

Qadeer M Ali

Marine Reference Collection &
Resource Centre University of
Karachi, Karachi, Pakistan

Shumaila Mubarak

Marine Reference Collection &
Resource Centre University of
Karachi, Karachi, Pakistan

First record of *Aplysia (Varria) cornigera* Sowerby, 1869 (Mollusca: Gastropoda: Aplysiidae) from the Karachi coast, with observations on developmental stages under laboratory conditions

Farhana S Ghory, Qadeer M Ali and Shumaila Mubarak

Abstract

The present study is an investigation of the taxonomical features, spawning behavior, structure of egg masses, and egg development in *Aplysia (Varria) cornigera* Sowerby, 1869. Adult specimens of *A. cornigera* were collected from Buleji on 23rd of January, 2017 and kept in aquaria under controlled laboratory conditions until they laid eggs and hatching occurred. This is the first report of *Aplysia (V.) cornigera* from Pakistan.

Keywords: *Aplysia (Varria) cornigera*; taxonomy; developmental stages

Introduction

Most gastropod molluscs exhibit gonochorism, and some clades such as the Opithobranchia and Pulmonata are typically simultaneous hermaphrodites (Lee *et al.*, 2014) [9]. *Aplysia*, commonly known as sea hares, is a benthic herbivorous opisthobranch mostly living in tropical and subtropical marine waters. The genus *Aplysia* has 50 species that are distributed worldwide (Beeman, 1968, Klussmann-Kolb, 2004, Lee *et al.*, 2014) [1, 8, 9]. *Aplysia* species use inks to escape from predators, using a variety of mechanisms (Derby and Aggio, 2011) [4]. They also have a long series of planktonic larval stages before reaching the juvenile stage. *Aplysia* is among the least studied fauna of Pakistan. Previously, *Aplysia oculifera*, *Aplysia benedicti*, and *Aplysia* sp. were reported by Kazmi *et al.*, 1996 [7], from Pakistan, but she did not describe the taxonomical features of those species. Ours is the first report of *Aplysia (Varria) cornigera* from Pakistan.

Materials and Methods

Two adult specimens of *Aplysia (Varria) cornigera* were obtained during a routine collection trip from the intertidal zone of Buleji (long 66°49' 12" E, lat 24°50' 12" N) on 23rd of January, 2017. *Aplysia* species were usually found when turning over stones. Specimens were kept in the laboratory in filtered seawater with a salinity of 37 ppt, pH 7.8, room temperature 22°C, and water temperature 20°C. A mating pair was observed in the aquaria, and newly spawned egg clusters were collected from the aquaria on 25th of January, 2017. The filtered seawater was changed daily and vigorously aerated until hatching occurred. The specimens were viewed with the help of Olympus BX51 microscope (magnifications WHN10X/22 x10 and 20) and Nikon UFX-DX (10XD/21, 0.66) binocular microscopes. The spent female and the remaining larvae were deposited in the Marine Reference Collection and Resource Centre, University of Karachi (Cat. No.GAS- 225, Acc. No. 2038).

***Aplysia (Varria) cornigera* (Figs. 1-15).**

Aplysiacornigera G. B. Sowerby I, 1869 – Eales, 1960:302-304 (biology).

Tethys cornigera Macri, 1816: synonym of *Tethys fimbria* Linnaeus, 1767, pp. 1-532.

Tethys (Aplysia): synonym of *Aplysia* Linnaeus, 1767- Bouchet and Gofas, 2017:12-20.

Results

Size: Two specimens. 46 - 68 mm long; 30 - 35 mm wide.

Corresponding Author:

Farhana S Ghory

Marine Reference Collection &
Resource Centre University of
Karachi, Karachi, Pakistan

Description:

The skin is soft, smooth, and flabby. Olive green in color with reticulations. Head and neck broad and short. Cephalic tentacles well developed, flat, leaf-like, wholly lateral and slightly rolled at the edges. Eyes small, distinct. Rhinophores slender, rounded close together. Foot narrow, thick, soft, but becomes hard in contraction, its edges well defined, front edges rounded, tail short and blunt (Figs.1& 2).

Penis short, flat, sickle-shaped, moderately broad at the base, the spermatic groove laying along its edge (Fig. 3). Parapodia short, rounded, edges irregular but not fimbriated, jointed low down on the foot, making a platform. Mantle foramen closed, with radial markings around it. Anal siphon short, thin, tubular, frilled on the edges (Fig.2). A deep purple secretion from the ink gland. Mantle cavity small and neat. Opaline gland compound and uniporous. Shell delicate, thin and fragile, large and broad, deeply concave with strong concentric lines. Apex small plate-like, rather shallow (Fig.4). Jaws pale and delicate curved at the tips (Fig.5).

Radula about 40 rows, formula 30.1.30. The rhachidian teeth have a broad triangular basal plate, short cusp with small blunt denticles, the two basal ones larger than the others (Fig.6). The lateral teeth have denticulate main cusps, with one denticle being the largest, the outermost three to five teeth are vestigial (Fig.7).

Caecum lies flat on the surface of the digestive gland (Fig. 8). The visceral ganglia are partially fused, the cerebral ganglia fused, flat, band like (Fig. 9).

Distribution: India, Sri Lanka, Southeast Asia, the Philippines, and West Pacific.



Fig 1: *Aplysia (Varria) cornigera* Sowerby, 1869. Adult specimens.

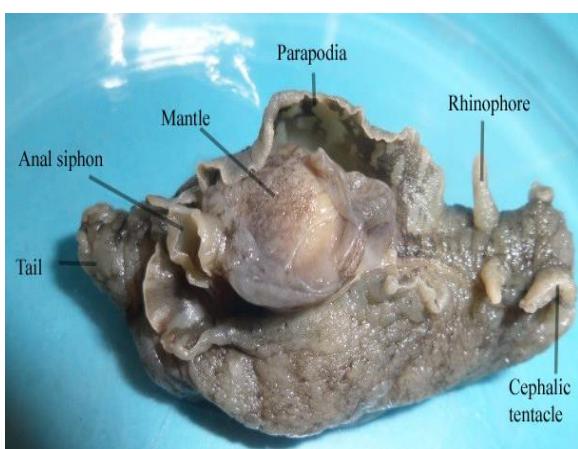


Fig 2: Lateral view.



Fig 3: Penis.



Fig 4: Jaws

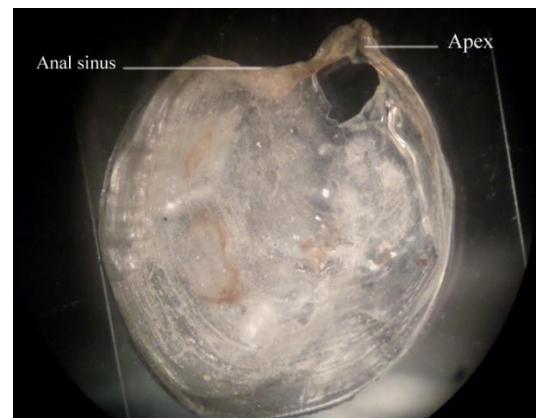
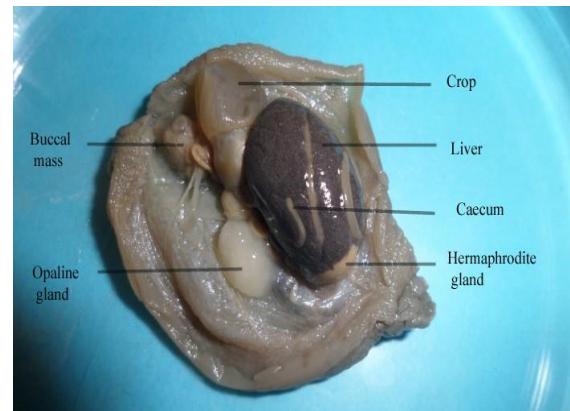
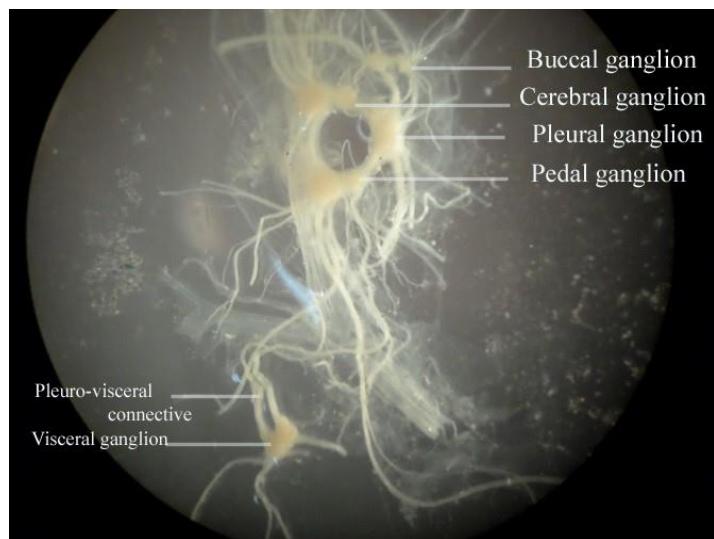


Fig 5: Shell, dorsal view



Fig 6: Radula with rhachidian and lateral teeth

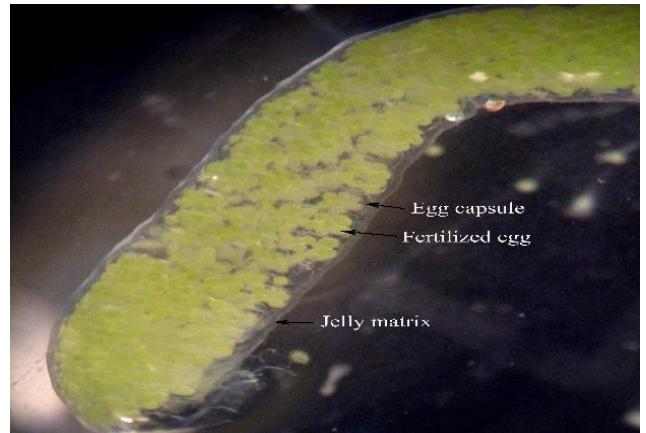
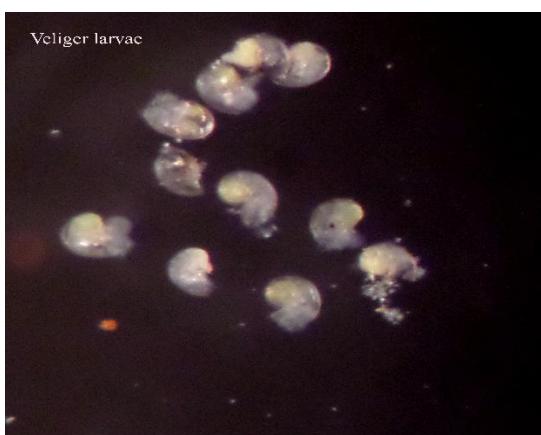
**Fig 7:** Radula with lateral and marginal teeth**Fig 8:** Visceral mass, position of the caecum, and hermaphrodite gland.**Fig 9:** Nervous system, dorsal view.**Table 1:** Morphological differences between *Aplysia (Varria) cornigera* Sowerby, 1869 from Pakistan (present study) and from India.

Characters	<i>Aplysia (V.) cornigera</i> Present study	<i>Aplysia (V.) cornigera</i> Eales, 1960	<i>Aplysia (V.) cornigera</i> Farran, 1905
Foot: front edges	rounded	long pointed tail	not mentioned
Shell: Apex:	Broad small plate-like	Ovate inturned but weak	Broad in turned but weak

Description of Larvae

Aplysia (Varria) cornigera produced fertilized eggs by internal fertilization via copulation. The fertilized egg mass was laid in a form of cylindrical cord that was implanted in layers of mucopolysaccharidemucus (Figs. 10-12). The egg masses were orange in color after spawning, (Fig. 10) but changed color during their development (Fig. 13). As the embryos developed from trophophore stage to veliger stage, the color of the egg masses became brownish (Fig. 14). At 5 to 6 days after spawning, the embryos developed into the trophophore stage and began to rotate within the egg capsule (Fig. 14). After 3 days, they transitioned to the veliger stage. The veligers broke out of the egg capsules and hatched as free swimming larvae (Fig. 15). Unfortunately, all larvae died as veligers, probably because of unfavorable rearing conditions, such as bacterial contamination or unsuitable food availability, etc.), and their long developmental time, suggesting that these stages might be particularly sensitive to environmental changes (Reverol *et al.*, 2004) [11].

**Fig 10:** Copulation and egg masses

**Fig 11:** Egg strings.**Fig 12:** Egg masses just after spawning**Fig 13:** Change in color of egg masses over developmental stage.**Fig 14:** Trochophore stage.**Fig 15:** Veliger stage

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