Morpho-taxonomic analysis of *Onchidium stuxbergii* (Westerlund, 1883) and *Onchidium melakense* (Dayrat & Goulding, 2019) (Mollusca: Gastropoda: Onchidiidae) from the Karachi coast, Northern Arabian Sea

Naz Khatib A.[1]; Ghory F.S.[1*]

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Abstract

The genus *Onchidium* (Onchidiidae) is typically associated with mangrove swamps and intertidal mudflats. Species of this genus are readily recognized in the field by two diagnostic features: large conical papillae on the dorsal notum and long, slender ocular tentacles (Dayrat *et al*., 2019). Unlike related gastropods, Onchidium lacks dorsal branchial gills but bears eye spots on the dorsal papillae.

In this study, a morpho-taxonomic examination was conducted on two species, *Onchidium stuxbergii* (Westerlund, 1883) and *Onchidium melakense* (Dayrat & Goulding, 2019), collected from the Karachi coast, Northern Arabian Sea. This investigation represents the first taxonomic record of Onchidium species from this region, thereby contributing new insights into the distribution and morphological variation of the genus.

Keywords: Taxonomy, *Onchidium stuxbergii, Onchidium melakense,* Gastropoda, Onchidiidae, Karachi coast, Northern Arabian Sea

1-Marine Reference Collection and Resource Centre University of Karachi, 75270, Karachi, Pakistan

*Corresponding author's 'Email: farhanaghory@yahoo.com

Introduction

The genus *Onchidium* belongs to the family Onchidiidae it's one of the most well-known genera of air-breathing slugs (Dayrat *et al*, 2016). These slugs are found primarily in intertidal zones of brackish or marine environments, often in the mudflats, mangrove swamps, and other coastal ecosystems, particularly in regions with significant tidal changes (Dayrat, 2009; Zhang *et al*., 2016; Goulding *et al*., 2021). Some species are specifically adapted to life in mangrove swamps, where they can tolerate fluctuating salinity and oxygen levels. They can often be seen clinging to rocks or vegetation in these environments, using their muscular foot to move.

Onchidium species are typically flattened, elongated, and soft-bodied. Their bodies often have a noticeable dorsal ridge that runs along the back, which gives them a distinctive appearance (Dayrat *et al*., 2019). They have tentacles on their heads, though these are not as prominent as in other gastropods. Their body is usually a combination of brownish, greenish, or grayish hues, which helps them blend into the mud and other substrate they live on.

Like other Onchidiidae, *Onchidium* slugs breathe air through a lung-like structure (a modified mantle cavity) (Abdellatif *et al*., 2017), though they can also absorb oxygen through their skin when submerged. This makes them well-adapted to living in areas where the air exposure is regular and where they may occasionally be submerged during high tides.

They feed primarily on algae, detritus, and other organic matter they encounter on the mudflats or coastal surfaces. Their diet is a mix of vegetation and small organisms that settle in the substrate.

Onchidium species are generally slow-moving and spend much of their time foraging or resting in sheltered spots during high tide. They are nocturnal to some extent, becoming more active at night to avoid the heat of the day and possible predators. They also tend to be territorial and can exhibit aggressive behaviors toward other slugs when there's a competition for food or space.

Onchidium species, like many other onchidiid slugs, are hermaphroditic. However, they still need to mate with another individual to exchange genetic material. They lay their eggs in small clutches, and the larvae go through a direct development process, meaning they skip the free-living larval stage that many other snails undergo and hatch directly into miniature versions of the adult.

They play an important role in their ecosystems by helping to break down organic matter and graze on algae. This keeps the environment balanced and prevents algae overgrowth. These slugs also serve as food for a variety of predators, including birds, fish, and some invertebrates. Their ability to hide in the substrate helps them avoid predation.

Presently we described the morpho-taxonomy of two *Onchidium* species *Onchidium stuxbergii* (Westerlund, 1883) and *Onchidium melakense* (Dayrat and Goulding, 2019) and

compared with earlier studied of same species.

Materials and methods

Study area

Onchidium species were obtained from the mangrove sampling site (Baba Island, Manora Channel) (Lat. 24º 48' 31"N Long. 66º 58' 27" E) Karachi coast during low tide. Photographs of specimens were taken in the field, in their natural habitat. Physico-chemical parameters i.e pH, water and air temperature (ºC), and salinity (ppt) were measured.

Preservation of collected specimens

Specimens anaesthetized with gradual adding of small quantities of magnesium sulphate, magnesium chloride or menthol. Specimens were initially preserved in a 5% solution of formalin for a day, and then transferred to 70% alcohol.

Microscopic observations

Internal anatomy was examined using a Leica WILD M3C microscope. The buccal mass was removed and boiled in 5% KOH to separate the radula. The radula, male reproductive organs and digestive organs were separately cleaned and prepared for scanning electron microscope for further anatomical examinations. Photographs were made by using an Olympus BH2 microscope (1.25X4, 10, 20 and 40 magnifications) with Nomarski Differential Interference Contrast (D/C) *camera lucida* attachment and Leica WILD M3C microscope. The total length (TL) of specimen was determined from the anterior end to posterior end. Measurements are in millimeters (mm). Comparison was made between species as recorded by previous authors and the present study was carried out and reported in Tables 1 and 2.

***Onchidium stuxbergii* (Westerlund, 1883) (Figs. 1 and 2)**

Material examined: 8 specimens

Locality: Baba Island (Manora Channel)

Size range: 19mm-24mm

Habitat: Found on mangrove mud.

Morphological characters: Body of the specimen examined is 24mm**,** elongated to oval in shape, with light gray dorsal notum. Notum is covered with pointed, spicule-like papillae with eyes scattered all around which are more visible when the animal was alive. A central retractile papillae is present having 2-3 eye spots. The ocular tentacles are elongated and reddish brown in appearance. Male opening is present below the right eye tentacle, on its left side. Hypnotism is bright yellowish to brown in some individuals. The color of the foot is dark yellow. The male aperture is below the right eye tentacle, on its left. Pneumostome is median in position, just above the anal opening on the ventral side.

Anatomical characters examined

Radula: It has rows of teeth. In every row there is a median or rachidian tooth with 2 half rows of lateral teeth, of equal size and shape. The rachidian teeth are tricuspid with a pointed median cusp.

The lateral teeth are unicuspid with a prominent hook. The radular formula is 50(90/1/90) (Fig.2A and B).

Intestinal loop: The intestinal loop is of type III with a transitional loop at 3 o'clock (Fig. 1D).

Copulatory Apparatus: .The accessory pineal gland has a hollow spine which is narrow, elongated and slightly curved. The pineal sheath has a penis which is a tube like structure without spines (due to small size of animals, immature specimens). The retractor muscle is short (Fig. 2C).

Distinctive Diagnostic Features: This species of *Onchidium* is characterized by a combination of unique characters: bright yellow foot color and intestinal loop of type III.

Distribution : It is distributed along Myanmar, western Peninsular Malaysia**,** Brunei**,** Bohol, Philippines, Vietnam and eastern Sumatra, Thailand (Gulf of Thailand) southernmost tropical China and Pakistan.

Figure 1: ***Onchidium stuxbergii*** **(Westerlund, 1883). A, in lab; B and C, in its habitat; D, intestinal loop.**

***Onchidium melakense* (Dayrat and Goulding, 2019) (Fig. 3)**

Material examined: 6

Size range: 21mm-26mm

Locality: Baba Island (Manora Channel)

Habitat: In the mud of the mangrove area, inside the crevices.

Morphological characters: Body of the examined specimen is 26mm. It is oval to elongated, with a light brown dorsal notum. It is covered with numerous pointed papillae bearing eyes. A central large papillae present having 2-3 eyes. The hyponotum is pale white with a foot, grayish white in color. The eyes are present on the tips of exceptionally elongated tentacles, brown in color. Male opening is present below the right tentacle, on its left.

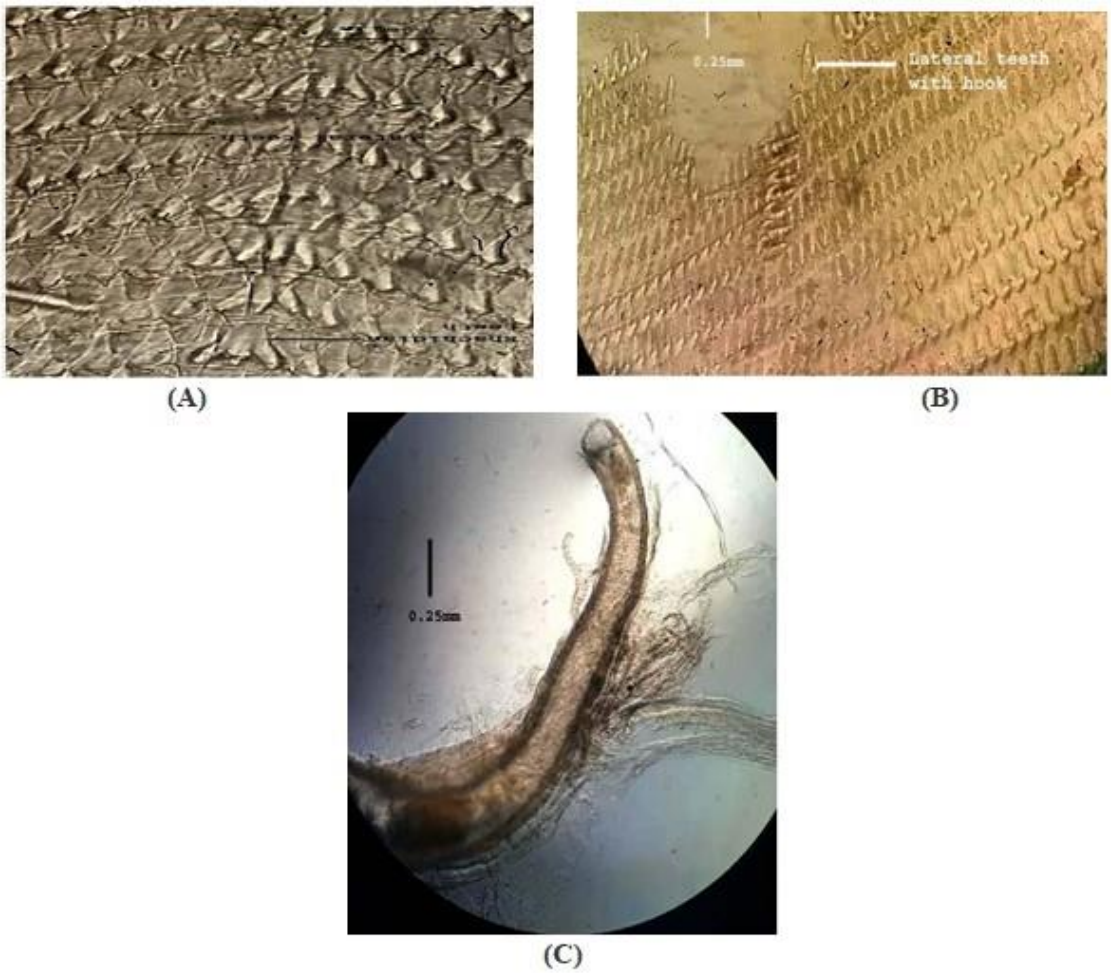

Figure 2: ***Onchidium stuxbergii*** **(Westerlund, 1883). A, radula; B, showing lateral tooth with hook; C, hollow spine of penis gland.**

Anatomical Characters examined:

Radula: It has rows of teeth, each row has a median tooth and half rows of lateral teeth, on its both sides. The lateral teeth are pointed and bear pointed hook. The radular formula is 45(80/1/80) (Fig.3C).

Intestinal loop: It is of type III with transitional loop at 5 o'clock (Fig. 3D).

Male copulatory organs: The pineal gland and the pineal sheath forms the male reproductive system, sharing the same vestibule. The hollow spine is present inside the tube like a pineal gland. The pineal sheath is an elongated tube but due to the small size of the specimen it has an undeveloped penis. The retractor muscle is long.

Distinctive diagnostic features: It has a unique combination of anatomical characters, with a light brown notum and pale yellow foot, and intestinal loop of type III.

Distribution: Known distribution is Andaman Islands, Western Peninsular Malaysia, Eastern Sumatra and Pakistan (Fig. 3).

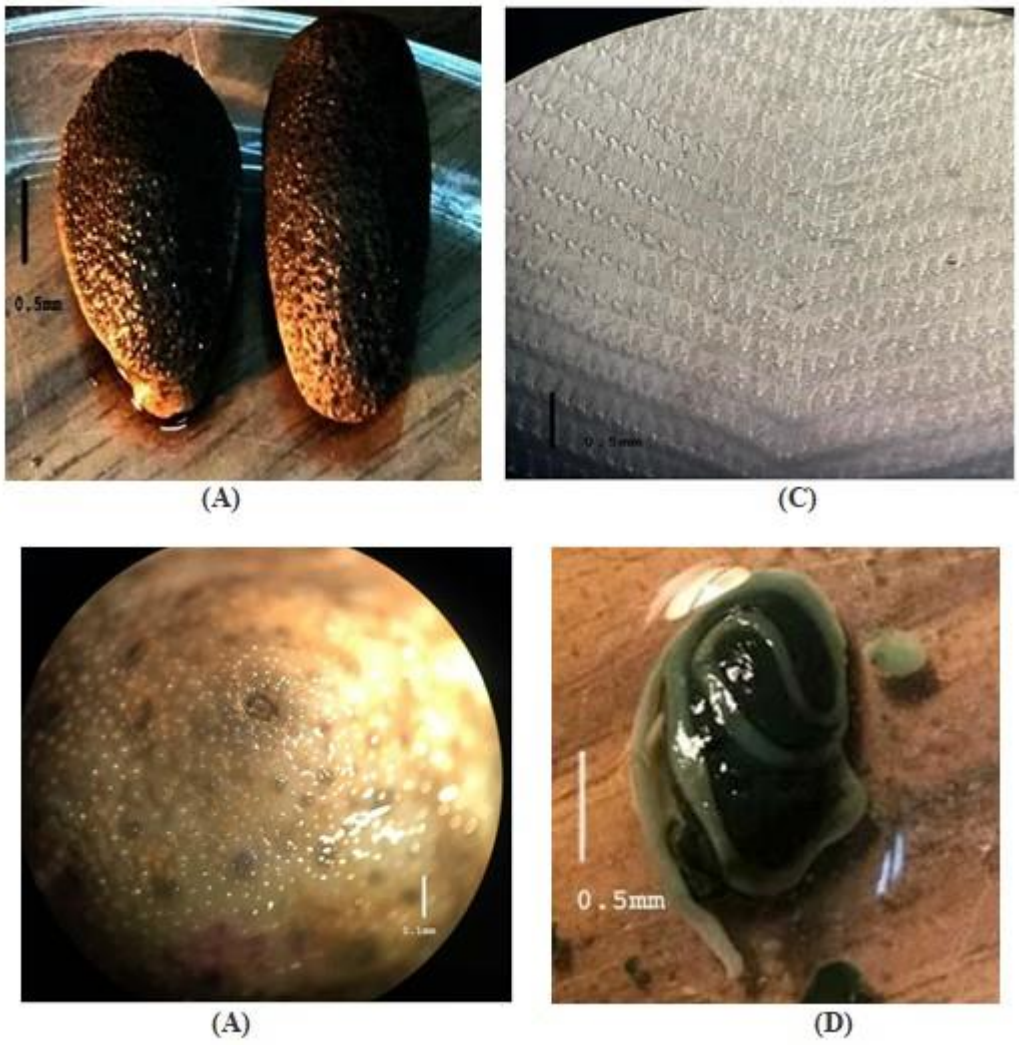

Figure 3: O*nchidium melakense* (Dayrat and Goulding, 2019). A, dorsal view; B, retractable central papillae with eyes; C, radula; D, intestinal loop.

Discussion

The genus *Onchidium* is the type genus of the family Onchidiidaehas (Dayrat *et al.,* 2016). There are four known species in the genus *Onchidium* Buchannan, 1800 (Dayrat *et al.,* 2019). Some *Onchidium* species are considered quite hardy and can survive being exposed to

air for long periods, even during low tides when the water level drops significantly. They have developed behavioral and physiological adaptations to avoid dehydration during these periods.

Identifying *Onchidiidae* species typically involves examining key morphological features such as body shape, coloration, size, presence of tentacles, foot characteristics, and internal anatomy. Additionally, genetic analysis through DNA sequencing can provide valuable information for species identification within the Onchidiidae family. Reference guides, taxonomic keys, and expert consultation may also be necessary for accurate species identification due to the subtle differences between species (Maniei *et al*., 2020).

On the basis of two external features: large, conical, pointed papillae on the dorsal notum and very long and thin ocular tentacles the *Onchidium slugs* can be easily identified in the field (Dayrat *et al.,* 2019). *Onchidium* have been recorded in two species so far as per our observation; *O. stuxbergi* and *O. melakense.* Morphologically we got the smaller sized individual from the mangrove sampling site (Baba Island, Manora Channel). In comparison to Dayrat's *et al.* (2016, 2019) observations, morphological identifications are recorded that differ quite significantly (Tables 1 and 2).

Table 1: The anatomical characters of *Onchidium stuxbergi* (Westerlund,1883), present study, is compared with the previously described same species studied by Dayrat *et al*.,2016.

O. stuxbergi	**Present study**	**Dayrat *et al*., 2016**
Size range	19mm-24mm	12 mm-35mm
Color of d. notum	Light gray	brown
Color of foot	Bright yellow	Bright orange
Color of hyponotum	Yellow to brown	Grayish yellow

Table 2: The anatomical characters of *Onchidium melakense* (Dayrat and Goulding, 2019), present study, is compared with the previously described same species studied by Dayrat *et al.,* 2019.

O. melakense	**Present study**	**Dayrat *et al*., 2019**
Size range	21mm-26mm	25mm-45mm
Color of d. notum	Light brown	Light brown
Color of foot	Grayish white	Pale yellow
Color of hyponotum	Pale white	Pure white

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