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Morpho-taxonomic study of *Peronia verruculata* (Cuvier, 1830) (Mollusca: Gastropoda: Onchidiidae) from the Karachi coast (Northern Arabian Sea)

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ABSTRACT

Peronia verruculata, a species of air-breathing, warty sea slug, plays a significant role in its ecosystem through various ecological interactions and processes. The *P. verruculata* resides in rocky intertidal zones, in crevices of large rocks along streams, and in manmade structures such as seawalls. This species can thrive on large, isolated boulders on sandy beaches. Furthermore, it can adapt to different coastal environments effectively. Dayrat et al., (2020) conducted a review of the systematics of *P. verruculata*. We have conducted a detailed taxonomic study of *P. verruculata*, comparing it with previously described instances of the same species.

Keywords: Taxonomy, *Peronia verruculata*, Gastropoda, Onchidiidae, Karachi coast, Northern Arabian Sea.

1. INTRODUCTION

The diverse climates and subtropical regions support the species *Peronia* (Fleming, 1822). Onchidiid slugs with ramified appendages on their dorsal notum, or dorsal gills, comprise the genus *Peronia*. Currently, 31 species-group names are available for onchidiids with dorsal gills, but their application is unknown (Dayrat et al., 2020). People have avoided studying Peronia's taxonomy for decades due to its complexity (Labbé, 1934a; Labbé, 1934b). The genus *Peronia* has undergone systematic revisions, which have led to a better understanding of its species and their relationships. These revisions have also helped to clarify the taxonomy of *P. verruculata* and its position within the family Onchidiidae. Molecular phylogenetic analyses have confirmed the monophyly of *P. verruculata* and its relationships within the family Onchidiidae.

These studies have also revealed that the species is part of a larger clade of onchidiid slugs with dorsal gills (Mörch, 1872a:28; Mörch, 1872b:325). A large number of species of onchidiids occur during the summer, usually when their populations are at their highest (Dey, 2006). In contrast, recorded abundance with

most excellent density during winter season. Tidal activity significantly affects *P. verruculata*, with the species being more active during the daytime when the tide is low. This correlation between activity and tidal cycles is crucial for understanding the species' behavior and habitat preferences (Hamaguchi and Yoshioka, 2002). As a species sensitive to environmental changes, *P. verruculata* can serve as an indicator of climate change and habitat degradation, providing insights into the health of the ecosystem.

Its grazing activities can create feeding trails and modify the intertidal environment, potentially influencing the distribution and abundance of other species. These ecological roles highlight the importance of *P. verruculata* in maintaining the balance and diversity of its intertidal habitat. Dayrat et al., (2020), Griffiths and Florens, (2006), and Hamaguchi and Yoshioka, (2002) reviewed the taxonomy of *P. verruculata*. provided preliminary information on the distribution patterns of *Onchidium verruculatum* in Pakistani waters. The present study has provided a complete morphological and anatomical description of *P. verruculata.*

2. MATERIAL AND METHODS

Study area

Random samples were collected during low tide from the intertidal areas of Mubarak Village (Lat. 24o 50′ 44″N Lon. 66o 39′ 35″ E), Sandspit (Kaka pir village) (Lat. 24o50′24″N Lon. 66o54′24″E) and Buleji (Lat. 24o 50′ 12″N Lon. 66o 49′ 12″ E) (Table 1 and Figure 1). Mature specimens were collected from each site. The physico-chemical parameters measured included pH, water temperature (°C), air temperature (°C), and salinity (ppt).

Maintenance in aquaria under laboratory conditions

The collected specimens were placed in aquaria filled with seawater. Aquaria were maintained according to the collection site in terms of physico-chemical parameters, including temperature (°C), salinity (ppt), and pH.

Preservation of collected specimens

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

Microscopic observations

Leica WILD M3C microscopes were used to examine the internal anatomy. 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 taken 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 specimen's total length (TL) was measured from its anterior end to its 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 (Table 2). Specimens have been deposited at the Marine Reference Collection and Resource Centre, University of Karachi. The dissected specimen was cataloged (Cat. No. MRC&RC-UOK-GAST- 228).

Habitat

Mostly inhabit rocky intertidal areas.

Distribution

P. verruculata is the most widespread of all onchidiid species. The species is found from Singapore to eastern Australia, New Caledonia, Japan, Sumatra, Andaman, Malaysia, Singapore, Western India, Pakistan, Persian Gulf, Mozambique, Madagascar and Red Sea.

Table 1 Summary of Material Examined.

Name of species	Date of collection	Locality	Size of specimens	Number of species
Peronia verruculata	Jan. 2023	Mubarak village	47mm-60mm	2
	Jan. 2023	Sandspit (Kaka pir village)	32mm-75mm	5
	Jan. 2023	Buleji	55mm-70mm	12
	Oct. 2023	Buleji	60mm-65mm	2

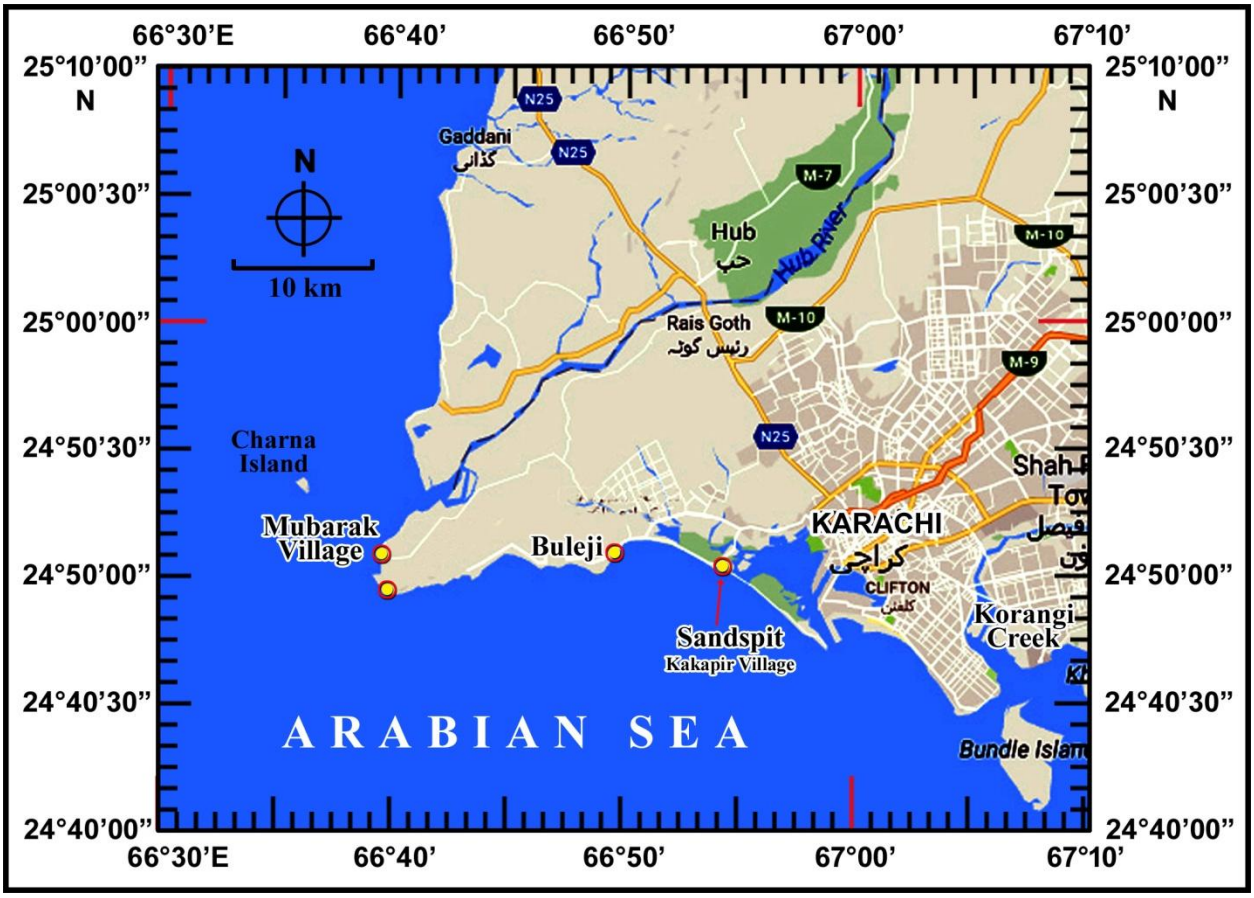


Figure 1 Map showing the collection sites.

***Peronia verruculata* (Cuvier, 1830) (Figures 2 to 6)**

Synonyms: *Onchidium verruculatum* Cuvier, 1830: 281; Semper, 1880: 255–257, pl. 22, figs 3, 4; 1882: pl. 21, fig. 1 [only in part]; Bergh, 1884a: 148–151, pl. VII, figs 7–12, pl. VIII, fig. 14; Farran, 1905: 358–359, pl. VI, figs 13–22; Odhner, 1919: 23; Hoffmann, 1928: 44, 72–75 [only in part].

Peronia verruculata (Cuvier, 1830): Keferstein, 1865a: pl. CIV, figs 9–12; Britton, 1984: 183–184, fig. 2 [only in part]; Sun et al., 2014: 63; Liu et al., 2015: 753–754; Chang et al., 2018: 149–165, figs 1–8; Xu et al., 2018: 3.

Onchidium ferrugineum Lesson, 1831a: 128–130; Lesson 1831b: 300–302; Lesson, 1832: 36–37, fig. 32.

Peronia ferruginea (Lesson, 1831a): Lesson, 1833: 3 pp. with no pagination, pl. 19, figs 1, 2; Oken, 1834b: 269–270; Gray, 1850: 117; Adams and Adams, 1855: 235; Tapparone Canefri 1883: 214.

Peronia savignii Récluz, 1869: 61. Syn. nov.

Peronia mauritiana: Mörch, 1872a: 28; 1872b: 325 [non *Peronia mauritiana* Blainville, 1824].

Onchidium branchiferum Plate, 1893: 141, 183–185, pl. 11, figs 63, 64; Hoffmann, 1928: 68, 75. Syn. nov.

Peronia branchifera (Plate, 1893): Labbé, 1934a: 194.

Onchidium elberti Simroth, 1920: 297–298, pl. XX, figs 51–54.
Onchidium astridae Labbé, 1934b: 77–78, figs 18, 38, pl. I, fig. 5. Syn. nov.
Scaphis astridae (Labbé, 1934b): Labbé, 1934a: 213, fig. 46.
Peronia gaimardi Labbé, 1934a: 194–195, fig. 8. Syn. nov.
Peronia anomala Labbé, 1934a: 195–196. Syn. nov.
Paraperonia gondwanae Labbé, 1934a: 199–200, figs 19–22 [only in part]. Syn. nov. *Scaphis viridis* Labbé, 1934a: 207–208, figs 31–34. Syn. nov.
Scaphis carbonaria Labbé, 1934a: 208–209, figs 35, 36. Syn. nov.
Scaphis gravieri Labbé, 1934a: 209–211, figs 37–40. Syn. nov.
Scaphis tonkinensis Labbé, 1934a: 211–212, figs 41–43. Syn. nov.
Scaphis lata Labbé, 1934a: 212, figs 44–45. Syn. nov.
Onchidium durum Labbé, 1934a: 220–221, figs 55–57. Syn. nov.
Peronia sp. ("group V"): Tagaki et al., 2019: 34.
Peronia persiae Maniei et al., 2020a: 507–514, figs 2–10. Syn. nov.

3. RESULTS AND DESCRIPTION

Morphological Characters

The specimen measures 60 mm in length and has an elongated oval-shaped body. The body is not dorsoventrally flattened. The dorsal notum is colored dark brown to olive green, featuring prominent vertical lines in some specimens. The color of the dorsal notum varies among individuals; in some, it appears blackish-green with reddish-brown patches. The number of papillae on the dorsal notum mostly bears eye spots. Usually, 10-16 papillae have eye spots depending on the size of the specimen. The eye spots are 1-2-3 or sometimes four present on each dorsal papillae.

The dorsal papillae are more numerous in the center of the notum and less abundant around the edges. The gills are present on the posterior end of the dorsal notum, which are more visible while the animal is undisturbed or in water. The hyponotum is pale white with a large foot grayish green in color. The pneumostome is median in position, located just above the anal opening at the posterior end of the hyponotum. A pair of eyes present on short ocular tentacles present anteriorly just above the buccal cavity. The male opening is located just below the right ocular tentacle, on its left side.

Distinctive Diagnostic Features

This species of *Peronia* is characterized by a unique set of anatomical features: intestinal loop is the type I with a transitional loop oriented between 3-6 o'clock, the muscular sac of the accessory pineal gland is not more than 10 mm in length with needle spine not less than 2.2 mm long and a very long retractor muscle inserted at the end of the visceral cavity.

Anatomical Characters Examined

Radula: The radula has rows of teeth; each radular row has one median or rachidian teeth with two half rows of lateral tooth on both sides. The median teeth are unicuspid. The lateral teeth have the same size and shape with as a basal spine. The innermost lateral teeth are pointed. The radial formula is 76(89 / 1 / 89) (Figures 6A & C).
The Intestinal loop is a type I with a transitional loop oriented between 4-5 o 'clock (Figure 6B).
The Male Reproductive System comprises a pineal sheath and an accessory pineal gland both sharing the same vestibule and the male opening anteriorly. The penis is a long, narrow, soft, and hollow tube closely intact inside the pineal sheath joined by a retractor muscle and a different duct at its proximal end. The pineal sheath protects the penis through its entire length. The pineal hooks are smaller in size and less in number at the distal end while more in number and more significant in size and densely packed till the mid of the penis, while the rest of the penis is without hooks. The length of the pineal hook is 50 micrometers. The retractile muscle is very long and thread-like in appearance, inserted at the end of the visceral cavity. The accessory pineal gland at its distal end has a hollow needle spine. The pineal gland is transformed into a thick muscular sac (less than 10 mm in length) and ends into a tube with a dead end. The needle spine of the accessory pineal gland is 2.4 mm long and red in color. The bottom of the hollow spine has a cone shape, with a width of 200 micrometers, while the tip measures 90 micrometers across (Figures 5A & B).

(A)

(B)

Figure 2 *Peronia verruculata* (Cuvier,1830). A) in its habitat; B) in lab.

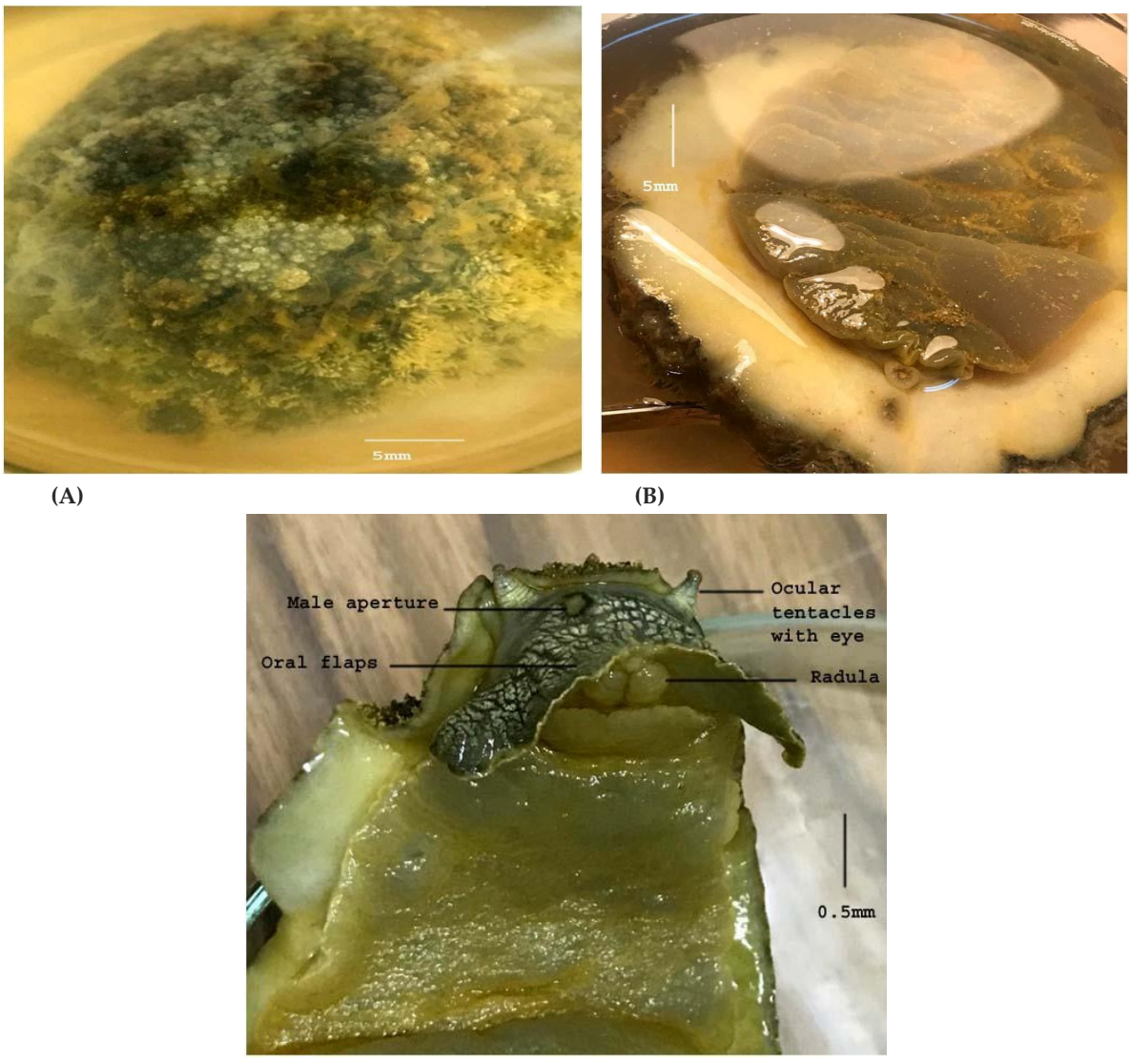


Figure 3 *Peronia verruculata* (Cuvier, 1830). A) dorsal view of the specimen; B) ventral view with pneumostome; C) anterior region, dorsoventral view.

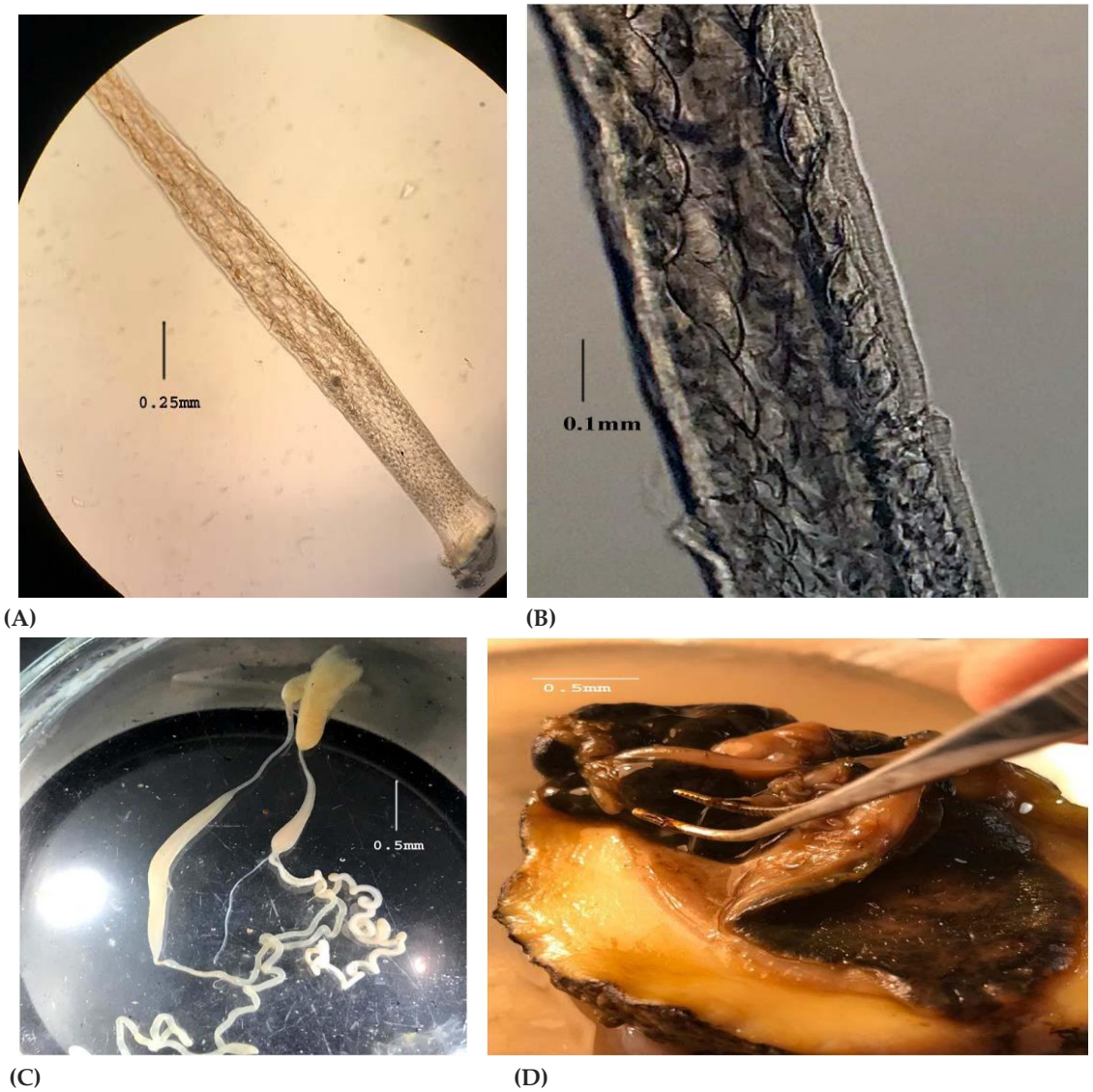


Figure 4 *Peronia verruculata* (Cuvier,1830). A) penis; B) penial hooks; C) male reproductive system; D) retractile muscle inserted into the visceral cavity.

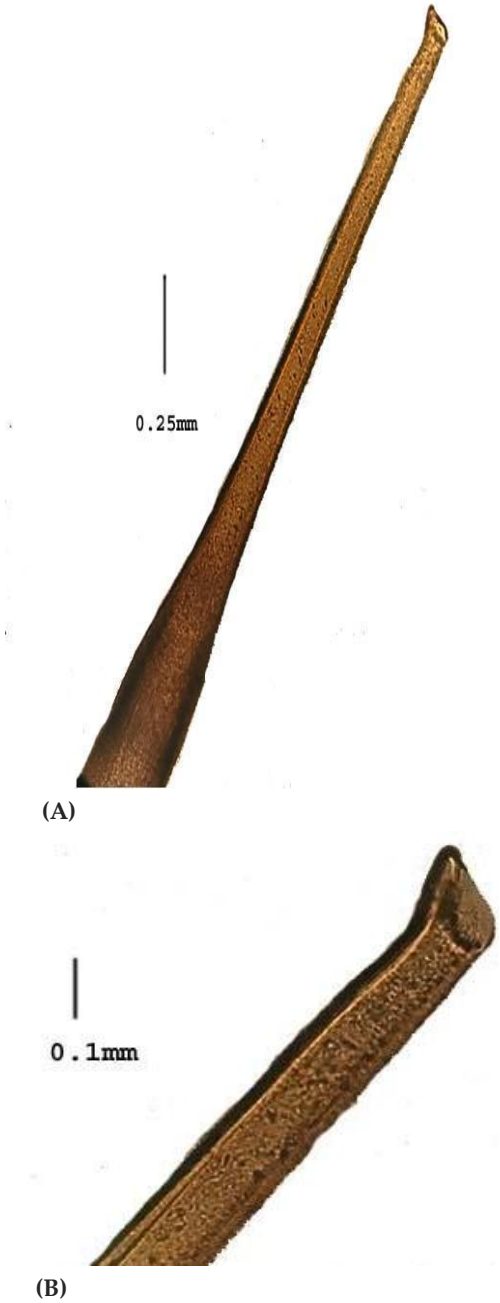
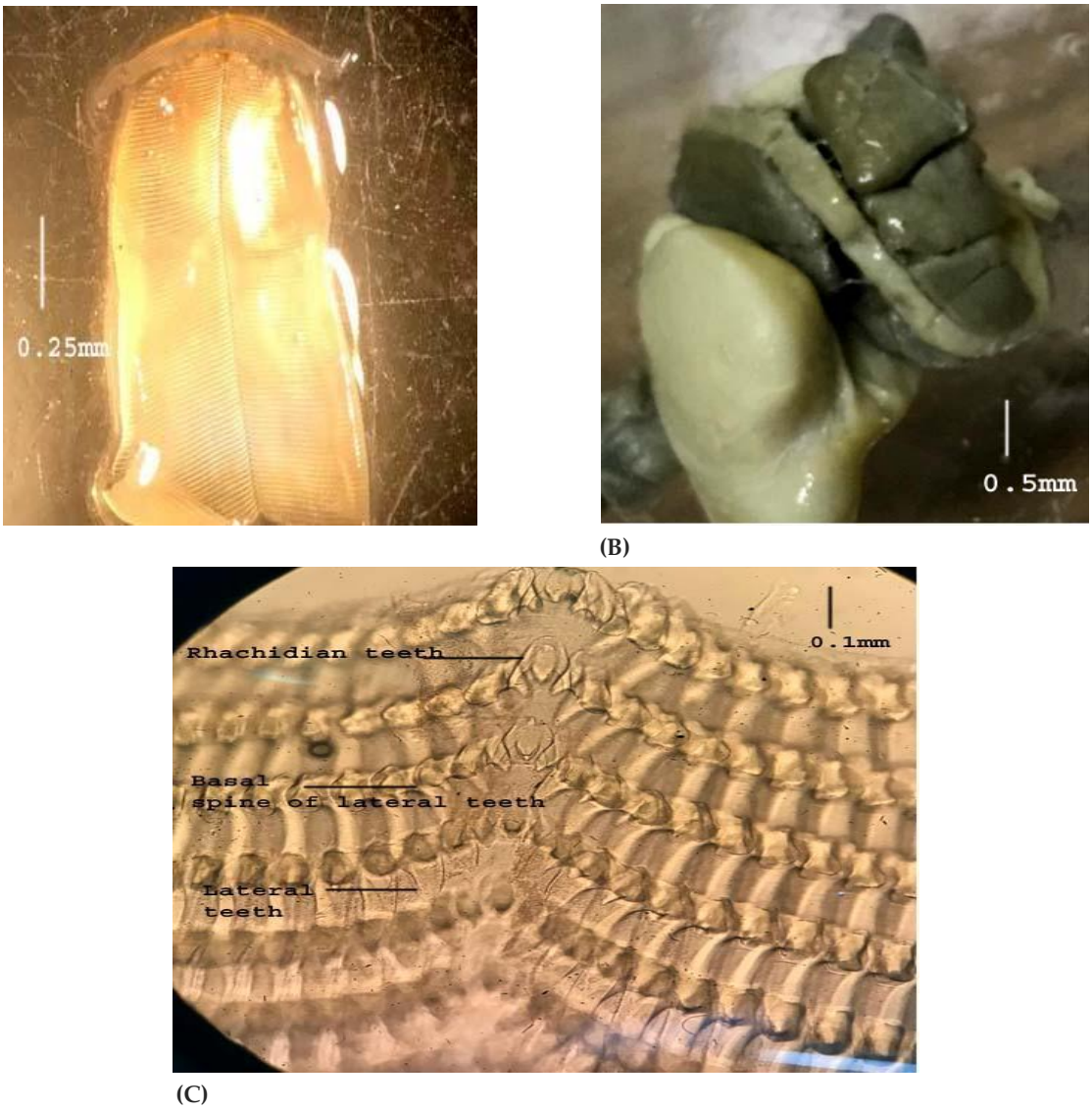


Figure 5 *Peronia verruculata* (Cuvier, 1830). A) needle spine; B) tip of needle spine (enlarged).



(A) (B) (C)

Figure 6 *Peronia verruculata* (Cuvier,1830). A) radula; B) intestinal loop; C) showing lateral and rachidian teeth and basal spine of the lateral teeth.

4. DISCUSSION

The *Peronia* species are externally cryptic species that can hardly be distinguished based on the internal anatomy because they are separated by minute anatomical features (Maniei et al., 2020a). During the present study, we found that the gills are located on the posterior end of the dorsal notum in *P. verruculata* while they are observed to cover nearly the back half of the upper parts in other Peronia species, besides those intestinal loops are differing in *Peronia* species. *P. verruculata* has intestinal loop type I and a transitional loop positioned at 4-5 o'clock. Radial formulas and the shape, color, and size of needle spines vary among different species of *Peronia.* Examining the anatomy of the genitalia can help detect morphological differences between closely related onchidiid species (Chang et al., 2018).

Table 2 Differences between *P. verruculata* (Cuvier, 1830), the present study, and previously studied the same species by (Dayrat et al., 2020).

Characters	*P. verruculata* (present study)	*P. verruculata* Dayrat et al., 2020.
Size	60mm	60mm
Papillae with dorsal eyes	10–22	10–22
Retractor muscle insertion (near the heart or end of the visceral cavity)	End of the visceral cavity	End of the visceral cavity
Muscular sac length	15mm	10mm
Needle spine length	2.3mm	2.2mm
Type of intestinal loop	Type I (Tloop b/w 4-5 o'clock)	Type I (Tloop b/w 3-6 oclock)
Accessory penial gland spine length (mm)	1.4–2.0	1.8–2.2
Accessory penial gland spine diameter base (μm)	100–270	200–270
Accessory penial gland spine diameter tip (μm)	35–50	35–50
Penis hooks length (μm)	<60	<60

Author Contributions

ANK performed all the experiments. FSG supervised and drafted the main manuscript text. Both authors reviewed and approved the final version of the manuscript.

Ethical approval

In this article, as per the animal regulations followed in Marine Reference Collection and Resource Centre, University of Karachi, Pakistan, the authors observed the morpho-taxonomic of *Peronia verruculata* (Cuvier, 1830) (Mollusca: Gastropoda: Onchidiidae) from the Karachi coast (Northern Arabian Sea). The Animal ethical guidelines are followed in the study for species observation, identification & experimentation. Specimens of the present study have been deposited at the Marine Reference Collection and Resource Centre, University of Karachi, Pakistan. The dissected specimen was cataloged (Cat. No. MRC&RC-UOK-GAST- 228).

Informed consent

Not applicable.

Funding:

The study has not received any external funding.

Conflicts of interests:

The authors declare that there are no conflicts of interests.

Data and materials availability

All data associated with this study are present in the paper.

REFERENCES

1. Adams H, Adams A. The Genera of Recent Mollusca: arranged according to their organization. J Van Voorst, London 1853–1858; [two volumes of text and one volume of plates; for a collation see p. 661 of the text vol. 2; pp. 232–235 (vol. 2) on onchidiids published in September 1855; pl. 81 with onchidiids published in April 1855]. doi: 10.5962/bhl.title.4772

2. Bergh R. Report on Nudibranchiata. Report of the Scientific Results of the Voyage of the H.M.S. Challenger during the years 1873-76. Zoology, 1884a; Band 10, Theil 26. C.W. Kreidel, Wiesbaden, 154 pp. [pls 1–14].
3. Blainville HMDde. Mollusques. In: Cuvier F (Ed) Dictionnaire des Sciences Naturelles (Vol. 32). F. G. Levrault, Strasbourg 1824; 392.
4. Britton KM. The Onchidiacea (Gastropoda, Pulmonata) of Hong Kong with a worldwide review of the genera. J Molluscan Stud 1984; 50:179–191. doi: 10.1093/oxfordjournals.mollus.a065863
5. Chang JJM, Tay YC, Ang HP, Tun KPP, Chou LM, Meier R, Huang D. Molecular and anatomical analyses reveal that *Peronia verruculata* (Gastropoda: Onchidiidae) is a cryptic species complex. Contrib Zool 2018; 87(3):149–165. doi: 10.1163/18759866-08703002
6. Cuvier G. Le règne animal distribué d'apres son organisation, pour servir de base a l'histoire naturelle des animaux et d'introduction a l'anatomie comparée. Vol. 3. 2nd Edition. Déterville et Crochard [includes Polychaeta], Paris, 1830; 504.
7. Dayrat B, Goulding TC, Apte D, Aslam S, Bourke A, Comendador J, Khalil M, Ngô XQ, Tan SK, Tan SH. Systematic revision of the genus *Peronia* Fleming, 1822 (Gastropoda, Euthyneura, Pulmonata, Onchidiidae). ZooKeys 2020; 972:1–224. doi: 10.3897/zookeys.972.52853
8. Dey A. Handbook on mangrove associate molluscs of Sundarbans. Zoological Survey of India, Kolkata, 2006; 96.
9. Farran GP. Report on the opisthobranchiate Mollusca collected by Professor Herdman, at Ceylon, in 1902. Report to the government of Ceylon on the pearl oyster fisheries of the Gulf of Mannar 1905; Part 3, Suppl. Report XXI: 329-364.
10. Fleming J. The Philosophy of Zoology: or a General view of the Structure, Functions and Classification of Animals Vol. 2. Archibald Constable, Edinburgh 1822; 618.
11. Gray ME. Figures of molluscous animals, selected from various authors. Etched for the use of students (Vol. 2). Longman, Brown, Green & Longmans, London 1850; [137 plates numbered 79–199 and a few additional plates marked with an asterisk; onchidiids are on pls 181–183].
12. Griffiths OL, Florens VFB. A field guide to the non-marine molluscs of the Mascarene Islands (Mauritius, Rodrigues and Réunion) and the northern dependencies of Mauritius. Bioculture Press: Mauritius 2006; Pp. i–xv, 1–185, pls 1-32.
13. Hamaguchi H, Yoshioka E. Activity Rhythms, Homing and Reproductive Behavior of the Intertidal Pulmonate Mollusc Peronia verruculata (Cuvier, 1830) on Sesoko Island, Okinawa. Venus (Journal of the Malacological Society of Japan) 2002; 61(1-2):49-60.
14. Hoffmann H. Zur Kenntnis der Oncidiiden. Zoologische Jahrbuecher 1928; 55:29–118.
15. Keferstein WM. Lungenschnecken: Pulmonata. In: Bronn, HG, Ed., Klassen und Ordnungen der Weichthiere (Malacozoa), wissenschaftlich dargestellt in Wort & Bild. Bd. 1865a; 3(2). Winter, Leipzig & Heidelberg: 1160 – 1306.
16. Labbé A. Les Silicodermés Labbé du Muséum d'Histoire Naturelle de Paris. Première partie: classification, formes nouvelles ou peu connues. Annales de l'Institut Océanographique 1934a; 14:173–246.
17. Labbé A. Opisthobranches et Silicodermés (Onchidiadés). Résultats scientifiques du voyage aux Indes Orientales Néerlandaises de LL. AA. RR. le Prince et la Princesse Léopold de Belgique. Mémoires du Musée Royal d'Histoire Naturelle de Belgique, Hors Série 1934b; 2(14):1–83. [1 pl.]
18. Lesson RP. Voyage autour du monde, Exécuté par Ordre du Roi, Sur la Corvette de Sa Majesté, la Coquille, pendant les années 1822, 1823, 1824 et 1825, sous le ministère et conformément aux instructions de S. E. M. le Marquis de Clermont-Tonnerre, ministre de la Marine; Et publié sous les auspices de Son Excellence Mgr le Cte de Chabrol, ministre de la Marine et des Colonies, par M. L. I. Duperrey, capitaine de Frégate, chevalier de Saint-Louis et membre de la Légion d'Honneur, commandant de l'Expédition. Zoologie, par M. Lesson. Tome Second. 1re Partie. Arthus Bertrand, Libraire-Editeur, Paris 1830–1831b; 471 pp. [pls 1–16; pp. 241–471 published on November 15, 1831; plates on mollusks published between January 9, 1830 and December 22, 1831; plate 14 published on November 15, 1831; for a collation see Cretella (2010)]. doi: 10.5962/bhl.title.57936
19. Lesson RP. Illustration de Zoologie ou Recueil de figures d'animaux peintes d'après nature. Arthus Bertrand, Paris 1832–1835; [60 pls; pl. 19 was published in 1833; for a collation see Sherborn (1922: lxxx)]
20. Lesson RP. Mollusques: Nouvelle espece d'Onchidie, Onchidium ferrugineum. Memorial encyclopedique et progressif des connaissances humaines 1832; 2:36 – 37.
21. Lesson RP. Note sur deux Onchidies nouvelles. Bulletin des Sciences Naturelles et de Géologie 1831a; 25:128–130.
22. Liu C, Wei L, Shen HD, Zhu HC, Zhou N. Complete mitochondrial genome of *Peronia verruculata* (Gastropoda: Pulmonata: Systellommatophora: Onchidiidae). Mitochondrial DNA 2015; 26:753–754. doi: 10.3109/19401736.2013.850679
23. Maniei F. Espeland M. Movahedi M. Wägele H. Description of a new *Peronia* species (Gastropoda: Eupulmonata: Onchidiidae) from Iran, Persi Gulf. Zootaxa 2020a; 4758(3): 501–531. doi: 10.11646/zootaxa.4758.3.5

24. Mörch OAL. Synopsis Molluscorum a cl. J. Reinhardt lectorum in circumnavigatione orbis terrarum Danicae navis Galatheae dictae. Oversigt over de af Prof. J. Reinhardt paa Corvetten Galatheas Reise omkring Jorden indsamlede Bloeddyr. Videnskabelige Meddelelser fra den naturhistoriske Forening i Kjoebenhavn 1872a; 9-35.
25. Mörch OAL. Catalogue de Mollusques terrestres et fluviatiles des anciennes colonies danoises du golfe du Bengale. *Journal de Conchyliologie* 1872b; 20:303–327.
26. Odhner NJ. Contribution a la faune malacologique de Madagascar. Arkiv foer Zoologi 1919; 12:1–52. doi: 10.5962/bhl.part.789
27. Oken L. Illustrations de Zoologie ou choix de Figures peintes d'après nature des espèces inedites et rares etc. par *Lesson* Isis von Oken 1834b; 268–273. [title in French, article in German]
28. Plate L. Studien über opisthopneumone Lungenschnecken, II, Die Oncidiiden. Zoologische Jahrbücher, Anatomie und Ontogenie der Thiere 1893; 7:93–234. [pls 7–12]
29. Récluz C. Observations sur l'*Onchidium celticum* de Cuvier. Actes de la Société linnéenne de Bordeaux 1869; 27:59–62.
30. Semper C. Dritte Familie, Onchididae [for Onchidiidae]. In: Semper C (Ed.) Reisen im Archipel der Philippinen, Zweiter Theil. Wissenschaftliche Resultate. Dritter Band. Landmollusken. CW Kreidel's Verlag, Wiesbaden 1880–1885; 251–264 [1880], 265–290 [1882] [pls 19–20, 22–23 (1880), pl. 21 (1882), pls 24–27 (1885); for a collation see Johnson (1969)].
31. Simroth H. Über einige Nacktschnecken vom Malayischen Archipel von Lombok an ostwärts bis zu den Gesellschafts-Inseln. Abhandlungen der Senckenbergischen Naturforschenden Gesellschaft 1920; 35:261- 302.
32. Sun B, Chen C, Shen H, Zhang K, Zhou N, Qian J. Species diversity of Onchidiidae (Eupulmonata: Heterobranchia) on the mainland of China based on molecular data. Molluscan Res 2014; 34:62–70. doi: 10.1080/13235818.2013.868860
33. Tapparone-Canefri C. Fauna malacologica della Nuova Guinea e delle isole adiacenti. Parte I. Molluschi estramarini. Annali del Museo Civico di Storia Naturale di Genova 1883; 19:5–313. [pls 1–9]
34. Xu G, Yang T, Wang D, Li J, Liu X, Wu X, Shen H. A comprehensive comparison of four species of Onchidiidae provides insights on the morphological and molecular adaptations of invertebrates from shallow seas to wetlands. PLoS ONE 2018; 13(4):e0196252. doi: 10.1371/journal.pone.0196252