

Further SEM assessment of radular characters of the limpets *Patella caerulea* Linneaus 1758 and *P. rustica* Linneaus 1758 (Mollusca: Gastropoda) from Antalya Bay, Turkey

Beria FALAKALI MUTAF*, Deniz AKŞİT

Akdeniz University, Faculty of Aquatic Sciences and Fisheries, Dumlupınar Bulvarı, 07059 Antalya - TURKEY

Received: 05.07.2007

Abstract: This paper aims to elucidate the structure and design of radulae of 2 species of limpet, namely *Patella caerulea* and *P. rustica*. Samples were examined by light microscope and SEM. Although the general formula is the same, the pattern of each dentition differed between the 2 species. General morphology and histology of odontophore showed structural significance of the organ, suggesting a further study to confirm ecological choice of the genus at different intertidal levels.

Key words: Mollusca, Gastropoda, *Patella caerulea*, *Patella rustica*, radula

Antalya Körfezi *Patella* türlerinde SEM’de ayrıntılı radula karakterlerin belirlenmesi, *Patella caerulea* Linneaus 1758 ve *P. rustica* Linneaus 1758 (Mollusca: Gastropoda)

Özet: Çalışmada iki *Patella* türü *Patella caerulea* ve *P. rustica*, olarak adlandırılan radula sıralanış modeli ve yapısının ayrıntılı olarak ortaya konulması amaçlanmıştır. Örnekler ışık ve taramalı elektron mikroskoplarında incelenmiştir. Genel formül aynı olmasına rağmen, iki tür arasında diş yapılarında farklılıklar bulunmaktadır. Odontoforun genel morfolojisi ve histolojisi organın yapısal önemini göstermiş olup, daha ileriki çalışmalarda cinsin farklı gel-git seviyelerinde ekolojik tercihlerinin araştırılması önerilmektedir.

Anahtar sözcükler: Mollusca, Gastropoda, *Patella caerulea*, *Patella rustica*, radula

Introduction

Genus *Patella*, known as a limpet, is one of the most widespread gastropods on rocky shores and is characterized by a cap-shaped shell.

Phenotypic variations among species have been reported for many particulars of *patellids*, including

shell form and size, foot color and radular characteristics (Corte-Real et al., 1996). Being a herbivorous, grazing gastropod, limpet’s radula has been of interest of much research (Blinn et al., 1989; Guralnick et al., 1999; Cabral et al., 2003; Sasaki et al., 2006). The differences in its dentition pattern have

* E-mail: fmberia@akdeniz.edu.tr

been reported and used as diagnostic character for taxonomic purposes (Corte-Real et al., 1996; Öztürk and Ergen, 1996). Given that radula reflects taxonomic differences are better than the intraspecific variations in shells and foot morphology, they are under the influence of environmental factors.

Patella caerulea, characterized by a pearl in the inner surface of its shell, is a common species in the Mediterranean Sea. *P. rustica*, however, is known to spread mainly in the Atlantic Ocean. These 2 species are very common in Turkish seawaters, with *P. caerulea* having a wider distribution (Öztürk and Ergen, 1996).

The present study investigates the external morphology of radulae of the 2 *Patella* species on the basis of further systematic diagnosis and intends to contribute additional knowledge regarding the morphology of radula.

Materials and methods

Collection

Specimens of *Patella caerulea* (Linnaeus, 1758) of 98 individuals and *P. rustica* (Linnaeus, 1758) of 45 individuals were collected from Antalya Bay (between 36°53'04.26 N - 36°36'25.22 N and- 31° 46'31.30 E- 30°42'03.62E) on the southwestern coast of Turkey (Figure 1).

Morphological analyses

Shell morphometrics, i.e. shell length (SL), shell width (SW), and shell height (SH), were measured by precision calipers.

Radulae of all samples were studied by light and scanning electron microscopy. The extracted radulae was cleaned in 10% KOH for 24 h, washed with deionized water and then, dehydrated through alcohol series. After air-drying, the radulae was mounted flat on aluminum stubs and coated with gold-palladium (Andrade and Solferini, 2005). We examined the external morphology of radular teeth by using a Zeiss Leo 1430 Scanning Electron Microscope (SEM) at Akdeniz University Medical University EM Unit (TEMGA).

Visceral mass was dissected and fixed in Bouin's solution. Samples were taken from 30% to absolute alcohol, cleaned in xylene and embedded in wax. 5 µm-7 µm sections were stained by Gomori Trichrome (Drury and Wallington, 1973), examined by Olympus CX31 and photographed at 3× digital zoom.

Results

Morphometry

The shell and radula lengths, as well as relative radula lengths of *Patella caerulea* (Linnaeus, 1758) and *P. rustica* (Linnaeus, 1758) were calculated in average and tabulated accordingly (Table 1). The 2 species varied markedly in size. Although the radular

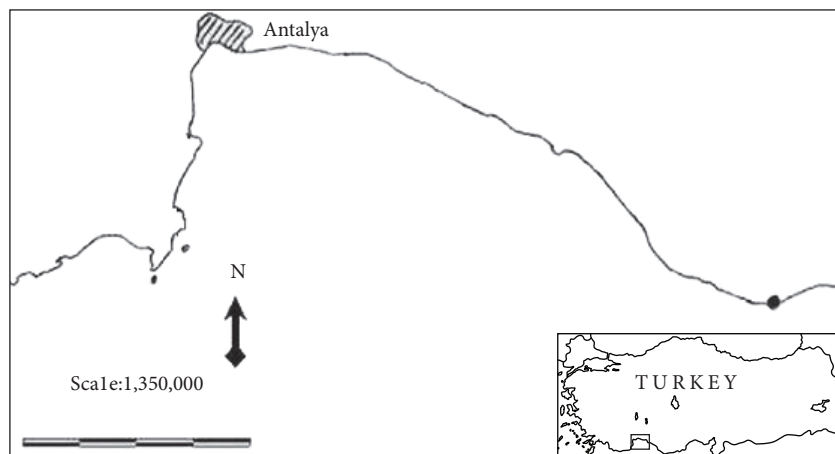
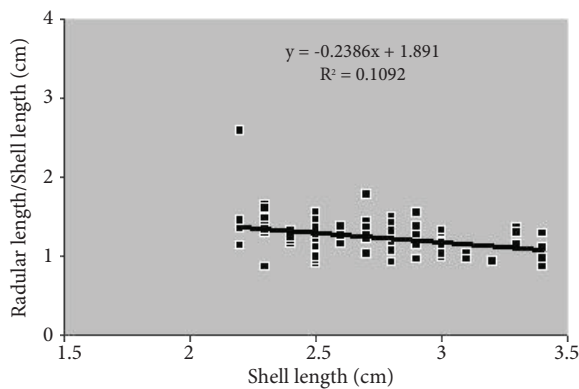


Figure 1. Map of sampling area.

Table 1. Average values of metric measurements and relative radula length of 2 *Patella* species.

Character	Species (n) Mean $\pm$ SD	
	<i>P. caerulea</i> (n = 98)	<i>P. rustica</i> (n = 45)
Shell length	2.8 $\pm$ 0.3	2.4 $\pm$ 0.3
Radular length	3.3 $\pm$ 0.5	4.3 $\pm$ 1.0
Relative radula length	1.255 $\pm$ 0.219	1.775 $\pm$ 0.378

ribbon was longer in *P. rustica* in contrast to its smaller shell size, there was no close relationship between the shell length and relative radula length in both species as shown in Figures 2 and 3.

Figure 2. Relationship between relative radular length and shell length in *P. caerulea*. ($P > 0.5$).

Radula morphology

Radula was found to extend through 2 bi-lobed odontophoral cartilages enclosed by a voluminous muscle tissue (Figure 4). A canal exists on the inner face of the cartilages for the radular ribbon to run through (Figure 5). A bulbous structure was bulging out at the anterior end as a licker (Figure 6).

The radular ribbon consists of several tens of transverse rows of teeth based on an elastic basal plate. The teeth formula was 3 + [1+2] + 1 + [2 + 1] + 3 in both species but there were marked morphological differences in shape.

The angles at the curved end of the inner laterals slightly differed, much apart in *P. caerulea* (Figure 7). The central rachidian is very indiscreet in both species, being much smaller in *P. rustica* (Figure 8).

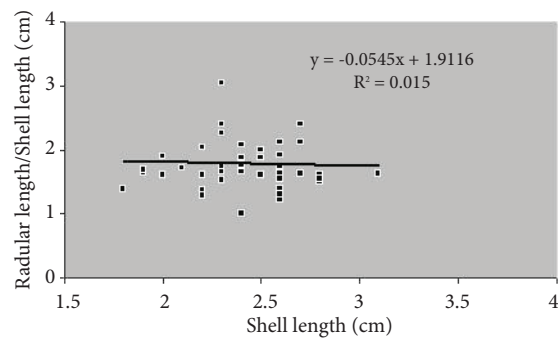
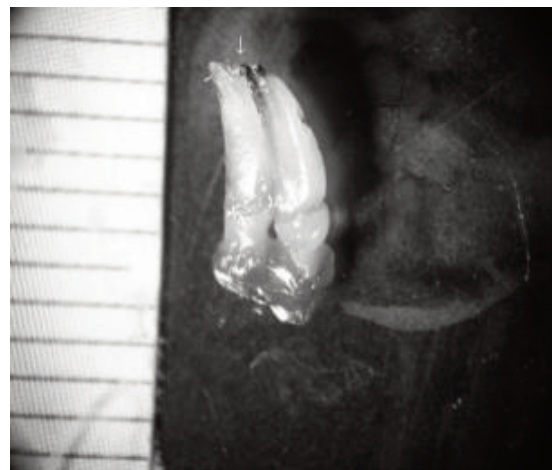
Figure 3. Relationship between relative radular length and shell length in *P. rustica*. ($P > 0.5$).Figure 4. General morphology of odontophoral organ of *Patella*.

Figure 5. Supporting odontophoral cartilage pair stripped off its musculature. ↓, odontophoral groove.

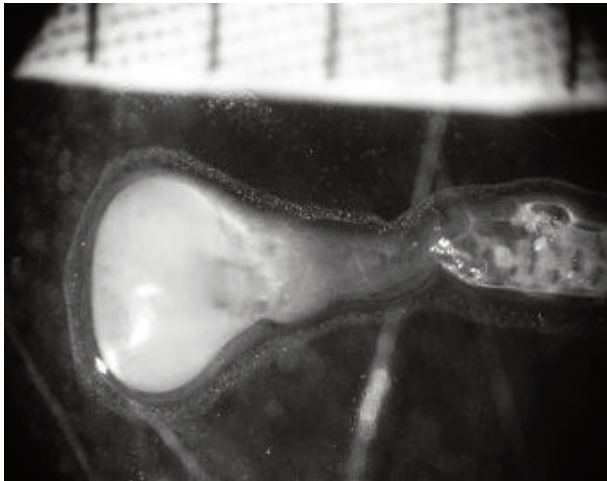


Figure 6. A bulbous bulge (licker) at the tip of radula.

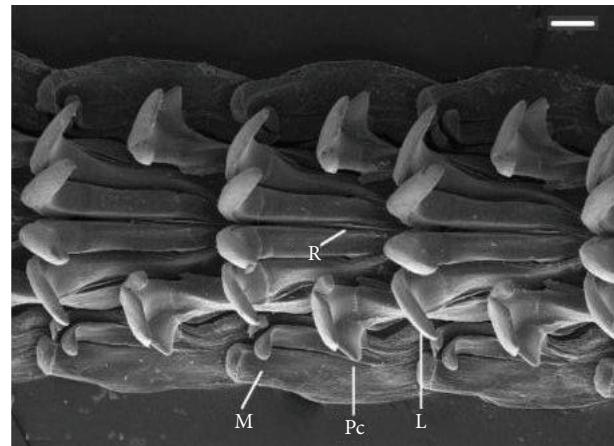


Figure 7. SEM view of *P. caerulea*'s radular ribbon; scale bar = 60 μ m. L, lateral teeth; M, marginal teeth; Pc, pluricuspid teeth; R, rachidian teeth.

Outer laterals were the most dominant teeth, lined behind the inner ones, and showed pluricuspid shape in both species, being bicuspid in *P. rustica* and tricuspid in *P. caerulea*. Cusps were easily breakable from the basal part and they showed inequality as seen on a mitten-like piece of *P. caerulea*'s radula (Figure 9a). A protuberance was apparent on the innerside of the major cusp in *P. rustica* (Figure 9b).

Radula/odontophore is located within the buccal cavity and embedded in the anterior part of

the visceral mass. The elastic subradular membrane contains layers of muscle tissue related to radular movement and the odontophore is fixed to the body by muscles at anterior and posterior ends (Figure 10).

Teeth were buried in the odontophoral body as seen in histological sections of the radula. Intra- and extra-cellular secretion activity at proximate of a tooth was presumed a continuous development and ultimate mineralisation of the teeth (Figure 11).

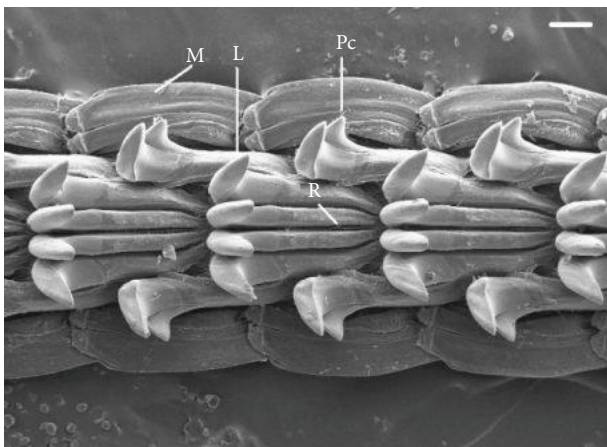


Figure 8. SEM view of *P. rustica*'s radular ribbon; scale bar = 60 μ m. L, lateral teeth; M, marginal teeth; Pc, pluricuspid teeth; R, rachidian teeth.

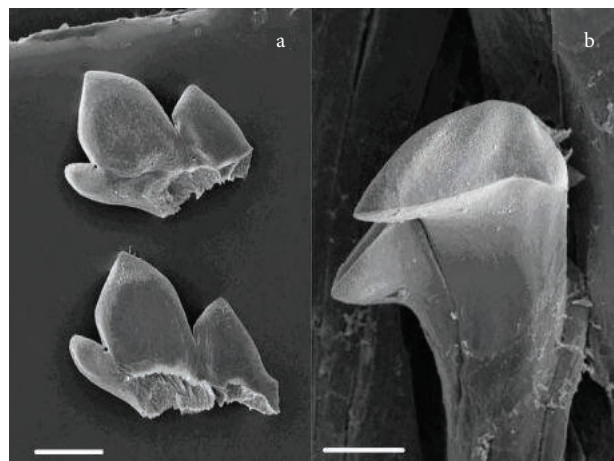


Figure 9. SEM view of pluricuspid teeth. **a.** *P. caerulea*'s radula; scale bar= 60 μ m. **b.** *P. rustica*'s radula; scale bar = 30 μ m.

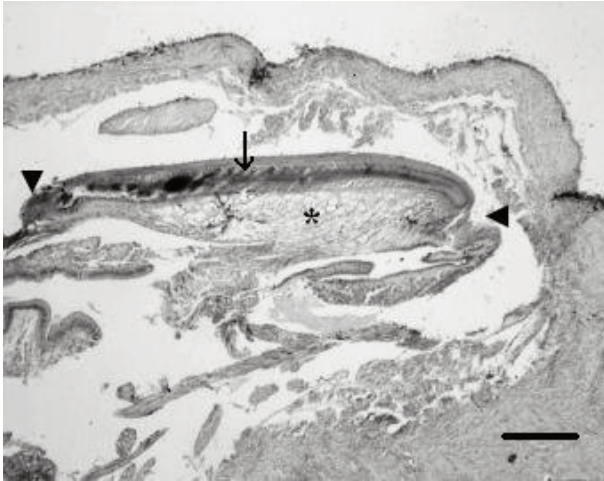


Figure 10. Vertical section of anterior part of visceral mass. Odontophore location is seen at the buccal region; scale bar = 40 μ . $\blacktriangle$, odontophoral muscle; $\downarrow$, subradular muscle; *, cartilage.

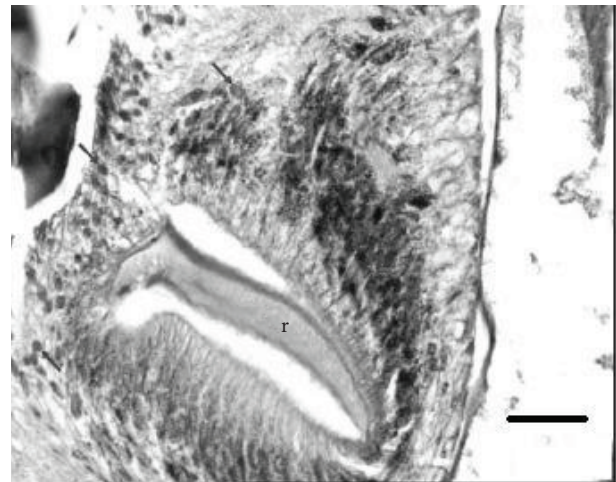


Figure 11. Longitudinal section of radula with a lateral teeth. Secretory activity clearly observable; scale bar = 5 μ . r, radula; $\rightarrow$, gland cells.

Discussion

Patellids are common organisms in the Mediterranean (Cachia et al., 1991) and are well distributed in Turkish waters (Öztürk and Ergen, 1996). They are found on rocky coasts browsing on algae. They graze and scrape off microscopic algae from the substratum by means of radula, a teeth-bearing chitinous ribbon.

Radula is characteristic to all molluscs, except bivalves (Purchon, 1977). Radular particularities have been used in the definition of generic groups, as its morphology is often found relatively uniform within a clade (Haszprunar and McLean, 1996; Reid and Mak, 1999).

The radulae of *Patella* species have been illustrated for taxonomic purposes (Côte-Real et al., 1996), because intraspecific variations in shell and foot morphology have often been questioned for use in taxonomic descriptions as they are influenced by environmental factors (Christiaens, 1973; Côte-Real et al., 1992). A considerable distinct relation on the length of radula relative to shell length within a species was found in this study, which corresponds to some previous research (Padilla et al., 1996). Shell length and width were considered here as objective characters in distinguishing 2 very similar species.

The type of radula of limpets is well known as docoglossan (Purchon, 1977). A licker, apparent at the anterior tip of radula, is functionally assumed to be a sensory organ tasting the substratum to find the algal material before the teeth are erected for working angle. The licker was found as a smooth structure, although this sub-radular organ has been described as lamellated in *Patella* in the literature (Fretter and Graham, 1994; Sasaki et al., 2006). The odontophoral cartilages were mentioned as three to five pair type in the Patelloidea (Sasaki et al., 2006), but there were only 2 bilobed cartilages found in the specimens of this study. This pair was characterized by a groove for the radular ribbon; thus, the disassociation of radula from the cartilages was structurally obvious.

Teeth number on the transverse rows was well in accordance with the references (Powell, 1973; Côte-Real et al., 1996). Although Powell (1973) gives the radular formula for this genus as $3 + 1 + (2 + 1 + 2) + 1 + 3$, the teeth are formulated as $3 + (1 + 2) + 1 + (2 + 1) + 3$ in this study, the outer and inner laterals were taken into consideration together. The rachis was found vestigial in all sexually mature specimens of both species and observable only by scanning electron microscopy. It was indicated to disappear during development in *Patella vulgata* (Côte-Real et al., 1996); there, however, was no such case in these 2

species studied. The major difference between *P. rustica* (Linnaeus, 1758) and *P. caerulea* (Linnaeus, 1758) was in the pluricuspid tooth, differing in cusp number and size, similarly to that described for *P. rustica* and *P. piperata* (Côrte-Real et al., 1996). The most inner laterals have different angles, much apart in *P. caerulea*. Marginal teeth have a more prominent scoop in *P. caerulea* than those of *P. rustica*. The cuspid tooth of the limpet has been reported as the main effective element of feeding and has been shown to be composed of organic materials as well as mineral-rich crystals (Mann et al., 1986; Van der Wal, 1989; Linddiard, et al., 2005). The shape and angles of the inner laterals observed in this study imply a most likely functional significance, at least for maintaining a good grip of grazed materials until they are forwarded into the digestive system.

Odontophoral body, well furnished with muscles, was embedded in the buccal region of the visceral cavity as seen in histological sections. The muscle tissue around sub-radular membrane ensures flexibility of the radula. The cartilage lies adjacent to and appressed to the radula accounting for its undoubted support during the feeding processes as

shown for *Bathycyma* (Sasaki et al., 2006), and as described to serve as a pulley wheel (Guralnick and Smith, 1999). The secretion activity observed around the tooth is supposed for the addition of organic material for tooth replacement as they wear off in usage (Van der Wal, 1989).

Some previous research has indicated a close relationship between radular form and diet in littorinids (Reid and Mak, 1999; Andrade and Solferini, 2005). A further study is required to confirm an association between radular morphology and substrate choice for *P. caerulea* and *P. rustica*, because of their ecological success on rocky shores are at different intertidal levels.

Acknowledgement

This study was made possible by the support of Akdeniz University Scientific Research Projects Unit (2004.02.0121.029) and The Scientific and Technological Research Council of Turkey (105 O 052). We also thank Assist. Prof. Dr. P. G. A. Glover (Akdeniz Univ., Fac. Education) for his useful comments on the manuscript.

References

- Andrade, S.C.S. and Solferini, V.N. 2005. Transfer experiment suggests environmental effects on the radula of *Littoraria flava* (Gastropoda: Littorinidae). J. Molluscan Stud. 72: 111-116.
- Blinn, D.W., Truitt, R.E. and Pickart, A. 1989. Feeding ecology and radular morphology of the freshwater limpet *Ferrissia fragilis*. J. N. Am. Benthol. Soc. 8: 237-242.
- Cabral, J. P. and Silva, A.C. F. 2003. Morphometric analysis of limpets from an Iron-Age shell midden found in northwest Portugal. J. Archaeological Sci. 30: 817-829.
- Cachia, C., Mifsud, C. and Sammut, P. M. 1991. The marine shelled mollusca of the Maltese Islands (Part One: Archaeogastropoda), Grima Printing, Malta.
- Christiaens, J., 1973. Révision du genre *Patella* (Mollusca: Gastropoda). Bulletin da Museum National d'Histoire Naturelle. Paris. 182: 1305-1392.
- Côrte-Real, H.B.S.M., Hawkins, S.J. and Thorpe, J.P. 1992. Genetic confirmation that intertidal and subtidal morphs of *Patella ulyssiponensis aspera* Röding (Mollusca: Gastropoda: Patellidae) are conspecific. Arquipélago. 10: 55-66.
- Côrte-Real, H.B.S.M., Hawkins, S.J. and Thorpe, J.P. 1996. An interpretation of the taxonomic relationship between the limpets *Patella rustica* and *P. piperata*. J. Mar. Biol. Assoc. U.K. 76: 717-732.
- Drury, R.A., Wallington, E.A. 1973. Carleton's histological technique, Fourth Edition. Oxford University Press London. London.
- Fretter, V., Graham, A. 1994. British Prosobranch Molluscs. Scientific Medical and Technical Publications Press, Pp: 900.
- Guralnick, R. and Smith, K. 1999. Historical and biomedical analysis of integration and dissociation in molluscan feeding, with special emphasis on the true limpets (Patellogastropoda: Gastropoda). J. Morphology. 241: 175-195.
- Haszprunar, G. and McLean, J.H. 1996. Anatomy and systematics of bathyphytophilid limpets from the northeastern Pacific (Mollusca, Archaeogastropoda). J. Zool. Scripta. 25: 35-49.
- Linddirad, K.J., Hockridge, J.G., Maecey, D.J., Webb, J. and Bronswijk, W. 2005. Mineralisation in the teeth of the limpets *Patelloida alticostata* and *Scutellastra laticostata* (Mollusca: Patellogastropoda). Molluscan Res. 24 (1): 21-31.
- Mann, S., Perry, C.C., Webb, J., Luke, B. and Williams, R. 1986. Structure, morphology, composition and organization of biogenic minerals in limpet teeth. Proc. Royal Soc. Biol. Sci. 227: 179-190.
- Öztürk, B. and Ergen, Z. 1996. Saros Körfez'inde (Kuzey Ege Denizi) dağılım gösteren *Patella* (Archaeogastropoda) türleri. Turk. J. Zool. 2: 513- 519.

- Padilla, D.K., Dittman, D.E., Franz, J. and Sladek, R. 1996. Radular production rates in two species of *Lacuna turton* (Gastropoda: Littorinidae). J. Molluscan Stud., 62: 275-280.
- Ponder, W.F. and Lindberg, D.R. 1997. Towards a phylogeny of gastropods molluscs: an analysis using morphological characters. Zool. J. Linn. Soc. 119: 83-265.
- Powell, A. 1973. The patellid limpets of the world. Indo-Pacific Mollusca, 3,75-206.
- Purchon, R.D. 1977. Bio. Mollusca, 2nd. Edit. Pergamon Press, Oxford.
- Reid, D. G. and Mak, Y. M. 1999. Indirect evidence for ecophenotypic plasticity in radular dentition of Littoria Species (Gastropoda: Littorinidae). J. Molluscan Stud., 65: 355-370.
- Sasaki, T., Okutani, T. and Fujikura, K. 2006. Anatomy of *Bathymacrea secunda* Okutani, Fujikura & Sasaki. 1993. (Patellogastropoda:Acmaeidae). J. Molluscan Stud. 72: 295-309.
- Van der Wal P. 1989. Structural and material design of mature mineralized radula teeth of *Patella vulgata* (Gastropoda). J. Ultrastruct. Mol. Struct. Res. 102: 147-161.