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The giant keyhole limpet radular teeth: A naturally-grown harvest machine

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ABSTRACT

The limpet radula is a feeding organ, which contains more than 100 rows of teeth. During their growth the teeth mature and advance in position along the radula. The simpler doccoglossan radulae operate by grinding rocky substrates, extracting the algae by rasping and scraping with the teeth functioning as shovels. Less is known about the rhipidoglossan radulae, used as rakes or brooms that brush and collect loose marine debris. This type of radula is found in the giant keyhole limpet (*Megathura crenulata*). The large size of this organism suggests that the rhipidoglossan radula entails a technological superiority for *M. crenulata* in its habitat. The structure and function of the radulae teeth have however not been reported in detail. Using a combination of 2D and 3D microscopy techniques coupled with amino acid analysis and X-ray scattering, we reveal the working components of *M. crenulata*'s radula. It is characterized by numerous marginal teeth surrounding a pair of major hook-like lateral teeth, two pairs of minor lateral teeth and a large central tooth. The mature major lateral teeth show pronounced signs of wear, which gradually increase towards the very front end of the radula and are evidence for scraping. An abrupt change in the amino acid composition in the major lateral teeth and the concurrent formation of a chitinous fiber-network mark the onset of tooth maturation. In comparison to the simpler rock-scraping doccoglossate limpets, the radula of *M. crenulata* forms an elaborate feeding apparatus, which can be seen as a natural harvest machine.

1. Introduction

The radula is a unique feeding apparatus specific to mollusks. It is an elongated flexible membrane to which numerous teeth are anchored (Cruz and Farina, 2005; Rinkevich, 1993; Runham et al., 1969; Shaw et al., 2008). The radular teeth are used to graze or cut food (algae, detritus, plants or even animals) as the membrane is manipulated by various muscles and cartilages (Guralnick and Smith, 1999). There exist seven basic types of radula morphology, considered by some to reflect the various stages in the evolution from carnivorous to herbivorous feeding habits of the different mollusks (Steneck and Watling, 1982). Herbivorous mollusks that graze for algae need more teeth with different functions to scrape, grind and brush the food from the substrate to which they attach (Steneck and Watling, 1982). Limpets (Mollusca, Gastropoda) are a large group of primitive marine mollusks characterized by their inverted cone-shaped shells. They mostly inhabit intertidal zones but are also found in deep-sea hydrothermal vent sites (Cruz and Farina, 2005). The limpet radula is a toothed structure (Fig. 1), which contains more than 100 rows of teeth of various kinds, but only the outermost few rows are actively used at any given time (Lowenstam and Weiner, 1989). During their growth and maturation, the teeth advance in position along the radula in a conveyor belt manner and eventually enter the scraping zone, i.e. the front end of the radula, which is in contact with the substrate while feeding (Lowenstam and Weiner, 1989). Once the teeth start to function, they begin to wear down until they are discarded, at an average rate of one tooth row per day (Kirschvink and Lowenstam, 1979). At the same time, a new row of teeth emerges (Isarankura and Runham, 1968; Shaw et al., 2008). Limpets are predominantly herbivorous and their radular morphology is either doccoglossan or rhipidoglossan (see schematic in Fig. 2) (Goldberg, 2013). The doccoglossan ('docco' is the Latin word for beam and 'glossa' for tongue) radula (Fig. 2, left panel) is structurally rather primitive, with only two types of teeth (marginal and lateral), whereas the rhipidoglossan ('rhipido' is the Latin word for fan)

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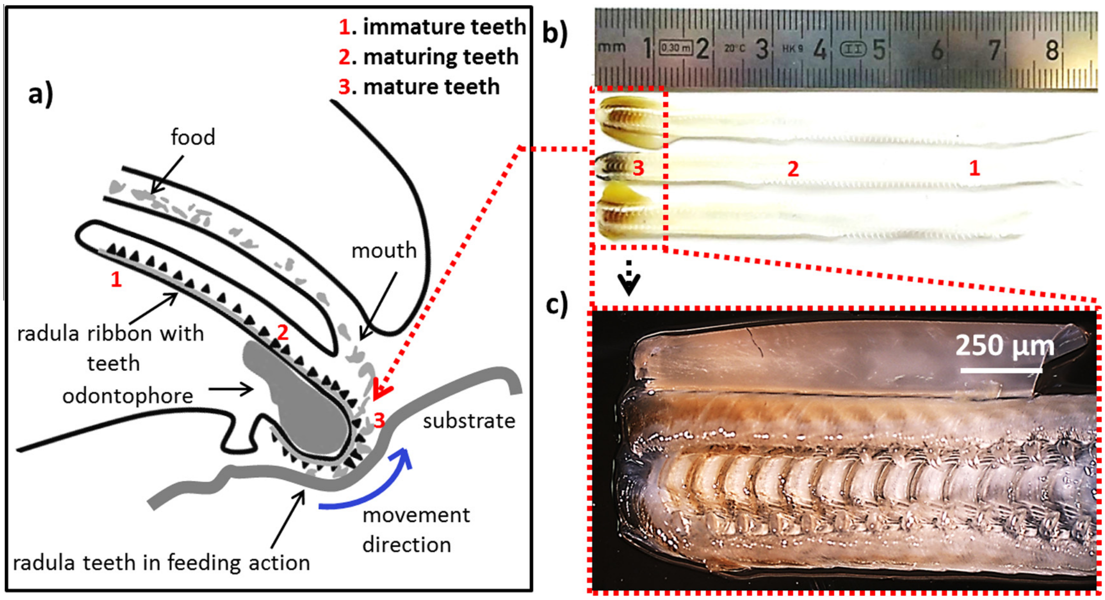

Fig. 1. (a) Schematic of the limpet radula in feeding action. The blue arrow denotes the direction of radula movement during the stroke. (b) A photograph of extracted radula of three adult giant keyhole limpets depicting the inter-specimen variability. (c) Optical micrograph of the mature end of the radula.

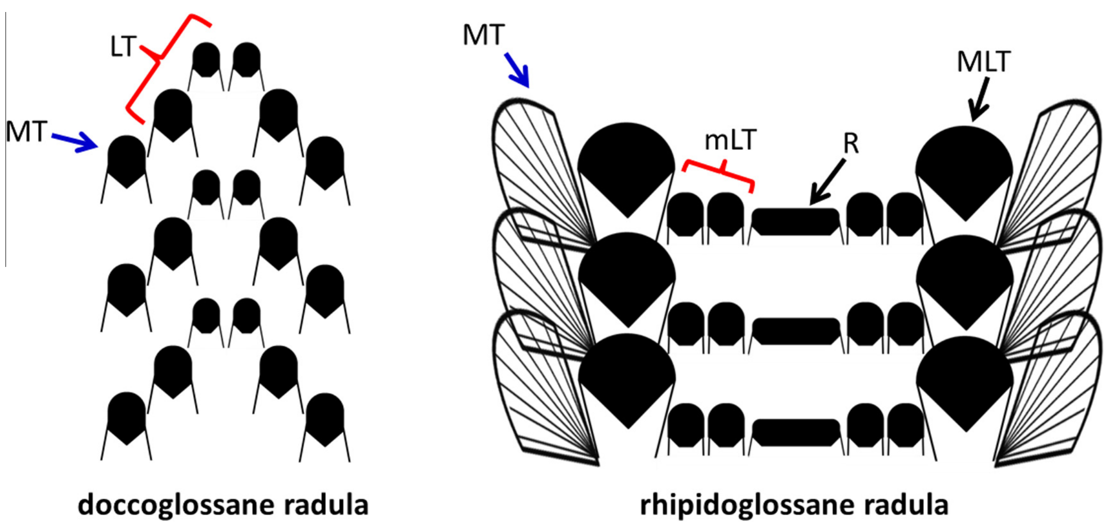

Fig. 2. Schematic representations of the two major types of herbivorous limpet radulae: (left) doccoglossane radula and (right) rhipidoglossane radula. The abbreviations denoting various teeth are: MT = marginal teeth, LT = lateral teeth, mLT = minor lateral teeth, MLT = major lateral teeth and R = central or rachidian tooth.

radula (Fig. 2, right panel) has a variety of different teeth (marginal, two types of lateral and central).

In the doccoglossan radula (Fig. 2, left panel), there is typically a small central tooth, up to three lateral teeth and up to 3 marginal teeth in each row (Steneck and Watling, 1982). In some doccoglossate species, the central tooth is missing. The teeth are stiff and fixed, and of limited mobility due to the low number of radula muscles (Steneck and Watling, 1982). Doccoglossate radulae are found in the earliest limpets (families Patellidae, Lottiidae, Lepetidae) and function like chains of shovels scraping hardened macroalgae (Cruz and Farina, 2005; Isarankura and Runham, 1968; Kirschvink and Lowenstam, 1979; Lowenstam and Weiner, 1989; Shaw et al., 2008; Steneck and Watling, 1982). The large spaces between the teeth are not suitable for collecting microalgae from substrates (Steneck and Watling, 1982), such that the doccoglossate limpets are less adaptable to different food resources, i.e. they are limited to feeding on larger algae. These early limpet families have mineralized teeth, containing either crystalline goethite and silica (in shallow-water limpets (Faivre and Ukmar Godec, 2015; Grime et al., 1985; Lowenstam, 1962; Lowenstam, 1971; Sone et al., 2005; Sone et al., 2007; Towe and Lowenstam, 1967; van der Wal, 1989)) or amorphous silica and amorphous iron oxide (in the deep-sea hydrothermal vent limpet (Cruz and Farina, 2005)). The combined effects of mineralized teeth on a rigid radula give rise to an enhanced stroke with impressive excavating ability of doccoglossate limpets whilst feeding (Steneck and Watling, 1982). The mineral fiber length in the doccoglossate limpet teeth was suggested to be optimized to ensure maximal efficiency during mechanical loading (Lu and Barber, 2012). Doccoglossate limpet teeth were found to have an absolute material tensile strength that is the highest recorded for a biological material (Barber et al., 2015).