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Morphological study on radula of nine cephalopods in the coastal waters of China

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Abstract: The radula of nine cephalopods are compared on the basis of scanning electron microscopic observation and morphological measurements. Results indicate that all of them consist of 7 longitudinal rows of teeth: a median tooth and the 1st, 2nd, and 3rd lateral or lateral, inner marginal, and outer marginal teeth. The formula of radula is 3·1·3. Sepioidea (*Sepia robsoni*, *S. esculenta*, *S. pharaonis*, *S. latimanus*, *S. aculeata*, *Sepiella maindroni*, *Euprymna berryi*) has only one cusp in the median tooth, and a marginal plate is absent. The median tooth has 3 and/or 5 cusps and marginal plates are found in the two species of *Octopus ocellatus* and *O. variabilis*. Other differences in the radula structure among the nine species are illustrated in detail. The relationships of radula of the cuttlefishes, octopuses and squids are discussed. It is suggested that radula should be thought in the cuttlefish taxonomy.

Key words: cephalopods; cuttlefish; radula; morphology; scanning electron microscope (SEM)
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1 Introduction

Anatomical characters have traditionally been used in fisheries biology to describe geographic variation in some exploited species of cephalopods[1－4]. Unlike molecular genetic markers, it is obvious that phenotypic variation is influenced by environmental factors, and the genetic component of such variance is uncertain[5]. Nigmatullin[6] proved that morphologically based methods of stock identification of *Illex argentinus*, at least of soft body parts, had limited value. A study on hard structures in the ommastrephid squid *Todarodes sagittatus*[4] indicates that characters such as the gladius, beaks, may be more effective discriminatory tools than conventional soft body parts. If it becomes possible, it will be a new way to explore phenotypic variation in relation to the genotypic component.

China is one of the major countries of cephalopod fishing. It boasts rich species resources. 95 species are discovered and belong to 6 orders, 21 families and 45 genus[7]. Aspects of their population biology are generally poorly documented, especially the population genetic structure and diversity. The radula is peculiar digestive organ in Mollusca. As a kind of important hard structure, it has been widely used for species identification and taxonomy of Gastropoda[8,9]. Until recently, there hasn't been systemic research in cephalopods by scanning electron microscope (SEM).

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In this study we report the results of the radular morphological analysis of nine cephalopods based on SEM. We are interested in determining relationships of different species and populations, and seeking after phenotypic variation related to genetic composition and phylogenesis.

2 Materials and Methods

Natural specimens of the nine cephalopods were collected (Fig. 1, Tab. 1). The radula were observed based on the scanning electron microscopy as the following protocol: radula were firstly removed from buccal cavity with the forceps and washed with diluted water three times to scar away adhesive materials such as food residue, then, the tissues were immerged into 1% KOH solution for a day. Prefixation stayed overnight in 2.5% glutaraldehyde at 4℃, and postfixation in 0.5% osmium tetroxide for 2 hours.

Tab. 1 Samples used for scanning electron microscope

species	No. of individuals	date	location
Sepia esculenta	8	2000－06	Rizhao
S. robsoni	9	2000－10	Zhanjiang
S. aculeata	6	2000－10	Zhanjiang
S. pharaonis	6	2000－11	Shanghai
S. latimanus	7	2000－05	Nan'ao
Sepiella maindroni	12	2000－05	Nan'ao
Euprymna berryi	7	2000－05	Nan'ao
Octopus variabilis	6	1999－10	Luoyuan
O. ocellatus	7	2000－05	Qingdao

After being washed with diluted water three times for 10 mins, respectively, the tissues were dehydrated rapidly through a graded ethanol series for each 10 mins, and replaced through a grade isoamyl acetate series. The tissues were CO_2 critical point dried, coated with gold. Finally, they were examined with JSM-840 scanning electron microscope.

3 Results and Discussion

In all the nine species, their radula consist of 7 longitudinal rows of teeth. The formula of radula is 3·1·3. There is a median tooth and the 1st, 2nd, and 3rd lateral (or lateral, inner marginal, and outer marginal) teeth on the both sides (Plate－1～9). A marginal plate is present on each edge in *Octopus variabilis* and *O. ocellatus* (Plate－8, 9), but it is absent in the seven species of Sepioidea studied.

3.1 *Sepia* (**Plate－1～5**)

The median, 1st and 2nd lateral teeth are alike in the morphological characteristic and the arrangement pattern. Each of them has a sharp cusp and a base with a concave. Their surface is smooth, not protuberant and medial zygomorphic, except the 2nd lateral tooth in *S. esculenta*. The 3rd lateral tooth is a long sable-like cusp. It is the longest one.

3.1.1 *Sepia robsoni* (**Plate－1**)

The median tooth (275.04±16.44μm) is much wider than the 1st (229.05±1.07μm) and 2nd lateral teeth (192.36±12.09μm). It is also the widest median tooth in all the 5 species of *Sepia* studied.

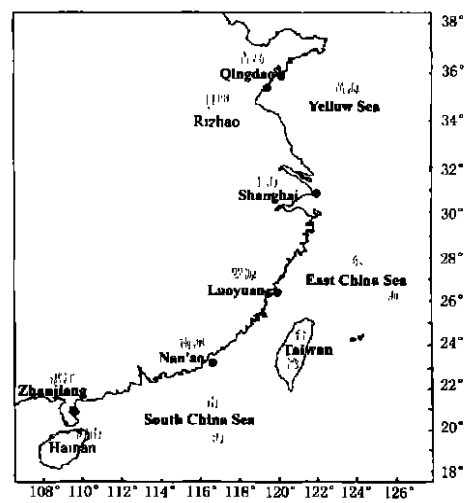

Fig. 1 Distribution of samples of nine cephalopods in the coastal waters of China

3.1.2 *Sepia esculenta* (**Plate－2**)

Both the median and 1st lateral teeth have a sharp cusp and an oblong-oval base.. The latter (258.04±11.28μm) are wider than the former (219.19±10.94μm). The 2nd lateral tooth, which cusp is asymmetrical, is nearer to the 1st lateral tooth. The cusp of the 3rd lateral tooth is a bit blunt. Its base is quadrate.

3.1.3 ***Sepia aculeata*** **(Plate—3)**

The width of the 1st and 2nd lateral teeth is almost the same, which is 203.49±17.42μm, 206.35±13.02μm, respectively. The median tooth (182.04±20.46μm) is a bit narrower than them. The cusp of the 3rd lateral tooth is a bit blunt. Its base is quadrate.

3.1.4 ***Sepia latimanus*** **(Plate—4)**

The median, lateral teeth (including 1st, 2nd and 3rd lateral teeth) are all the narrowest in the 5 species of *Sepia* studied. On the contrary, the rate of height/width is highest in each of the median, the 1st and 2nd lateral teeth. The cusp of the 3rd lateral tooth seems very blunt.

3.1.5 ***Sepia pharaonis*** **(Plate—5)**

The median and the 1st lateral teeth are alike in the shape and size, which look stocky: a cusp and an oblong-oval base. The 2nd lateral tooth is much bigger than them. The cusp of the 3rd lateral tooth seems very blunt like *S. latimanus*.

In brief, the width relationships of teeth of the 5 species are the following: the median tooth > the 1st lateral tooth > the 2nd lateral tooth in *S. robsoni*; the median tooth < the 1st lateral tooth ≈ the 2nd lateral tooth in *S. aculeata*; the median tooth ≈ the 1st lateral tooth < the 2nd lateral tooth in *S. pharaonis*; the median tooth < the 1st lateral tooth in *S. esculenta* which has the asymmetrical 2nd lateral tooth; the median and lateral teeth of *S. latimanus* are the narrowest in the five species, respectively.

3.2 *Sepiella* (Plate—6)

Sepiella maindroni.. There is only one cusp and protuberant in both sides of base in the median and the 1st, 2nd lateral teeth. The width relationship is the median tooth > the 1st lateral tooth > the 2nd lateral tooth.. But the height is contrary, which is the median tooth < the 1st lateral tooth < the 2nd lateral tooth. The 3rd lateral tooth is a long sable-like cusp.

3.3 *Euprymna* (Plate—7)

Euprymna berryi.. The median tooth has one cusp, which lie in the middle. The 1st lateral tooth has a dagger-like cusp and its base is like lune. It is dissociative between the teeth. The 2nd lateral tooth has also a dagger—like cusp, which tends to the direction of the median tooth as the same of the 1st lateral tooth. Its base is wider than the 1st lateral tooth and like rectangle. The 3rd lateral tooth is a long sable-like cusp. The teeth of *E. berryi* are the smallest in the species of Sepioidea. Whatever length and width, they are one third to half of the species of *Sepia* and *Sepiella*.

3.4 *Octopus*

3.4.1 ***Octopus variabilis*** **(Plate—8)**

A median tooth has 5 symmetrical and sharp dagger-like cusps. The shape of the median tooth varies on neighboring transverse bows, but it is exactly repeated in each third (seriation of the radula). The 1st lateral tooth with one cusp is the smallest one, the cusp of which is medial. The 2nd lateral tooth has a wide heel, and one dagger-like cusp that tends to 1st lateral tooth. It has one bulge near the 1st tooth and base is a bit concave. The 3rd lateral tooth has a short base and a long sable-like cusp..

3.4.2 ***Octopus ocellatus*** **(Plate—9)**

A median tooth has two kinds of cusps: 3 and 5 symmetrical and sharp dagger-like cusps. The 1st lateral tooth with one cusp is the smallest one like *O.. variabilis*' s, but its cusp is not medial but tends to the side closer to the 2nd lateral tooth. The 2nd lateral tooth has a wide heel, and one dagger-like cusp that tends to the

1st lateral tooth. It has one bulge near the cusp of the 1st tooth and base is a bit concave. The 3rd lateral tooth is a long sable-like cusp. But differed with *O. variabilis*, its base has a pommel.

On the whole, Octopodidas were very easy to be identified from Sepiidae (including *Sepia* and *Sepiella*) and Sepiolidae (e.g. *Euprymna*) according to the arrangement pattern and morphological characteristic of teeth, especially the number of cusps, which have 3 or/and 5 cusps in the median tooth in the former and have only one cusp in the latter. *Euprymna* can be distinguished from *Sepia* and *Sepiella* with the size and shape of the radula, which is one third to half of the latter whatever height and width of every rows of teeth. According to whether there is protuberant in both sides of base in the median or not, we can identify *S. maindroni* from other species of *Sepia*. The 5 species can be distinguished by compared with the width of the median, 1st and 2nd lateral teeth as well as the cusp symmetry, respectively.

Furthermore, Nesis[10] thought that in squids and cuttlefishes, the 2nd and 3rd lateral teeth are alike: a short base and a long saber-like or dagger-like cusp. In our studies, the 2nd lateral tooth is much more like the 1st lateral tooth (*E. berryi*) and/or median tooth (such as *Sepia*) than the 3rd lateral tooth. Nesis[10] mentioned that the radula in the squids, cuttlefishes, and argonautoidea mainly served for gripping the pieces of food bitten off by the beak and for transferring them into the pharynx. Therefore, the 2nd and the 3rd lateral teeth, which held the pieces of food, were highly developed, whereas the rhachidian (median) tooth, which served to prevent the pieces from slipping along the radular band, was relatively weaker in development. There are somewhat difference in our research. The median tooth isn't weaker than the 2nd lateral tooth in each of the seven cuttlefish species. For instance, the median tooth is obviously wider than the 2nd one in *S.. robsoni*. The tooth maybe plays a more important role in holding the pieces of food. *Sepioteuthis lessoniana* Lesson, a kind of squid, has a developed median tooth with three sharp cusps[11]. The radula, especially the median tooth of it, might have more functions in food digestion in the squids and cuttlefishes.

According to different life habit and edible speciality of biology, the radula has different morphological characteristics[12]. In the benthic octopuses, the function of the radula is different from that of cuttlefishes and squids: they use the median tooth for piercing the shells of the bivalves and gastropods and for scraping pieces of food out of crab legs. The median tooth is very large and strong. This also explains the great development of the marginal plates serving as a secure support for the teeth raised into a working position[10]. However, the lateral teeth of octopuses such as *O. ocellatus* and *O. variabilis*, particularly the 1st one, are smaller. It is not known what their functions are. Dong[13] thought that predatism played an important role in radula of differentiation. Radula, as a kind of hard structure, shows difference in these species collected.. We make suggestion that radula structure should be thought in the cuttlefish taxonomy. Meanwhile, a study on other hard structures (e.g. statolith, beak, especially the upper beak) in the cuttlefishes is being carried out, and their role will be discussed.

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中国沿海九种头足类齿舌的形态学

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摘要: 采用扫描电镜技术对9种头足类的齿舌进行了显微观察和比较研究。结果显示所研究的物种的齿舌均有7列纵向的齿组成, 齿式为3·1·3。乌贼目的7个种(罗氏乌贼 *Sepia robsoni*、金乌贼 *S. esculenta*、虎斑乌贼 *S.. pharaonis*、白斑乌贼 *S. latimanus*、目乌贼 *S. aculeata*、曼氏无针乌贼 *Sepiella maindroni*、柏氏四盘耳乌贼 *Euprymna berryi*)中央齿一列, 具有一个齿尖; 侧齿位于中央齿两侧各3列, 分别称为第一、第二和第三侧齿, 也称为侧齿, 内缘齿和外缘齿; 无缘板结构。长蛸 *Octopus variabilis* 和短蛸 *O. ocellatus* 中央齿一列, 分别有5个、3和5个齿尖, 侧齿各3列, 具有缘板。文中对九个种的齿舌结构以及彼此的异同进行了详细的描述和比较, 并对乌贼类、蛸类和枪乌贼类彼此之间的齿舌关系以及功能差异进行了讨论。

关键词: 头足类; 乌贼; 齿舌; 形态学; 扫描电镜
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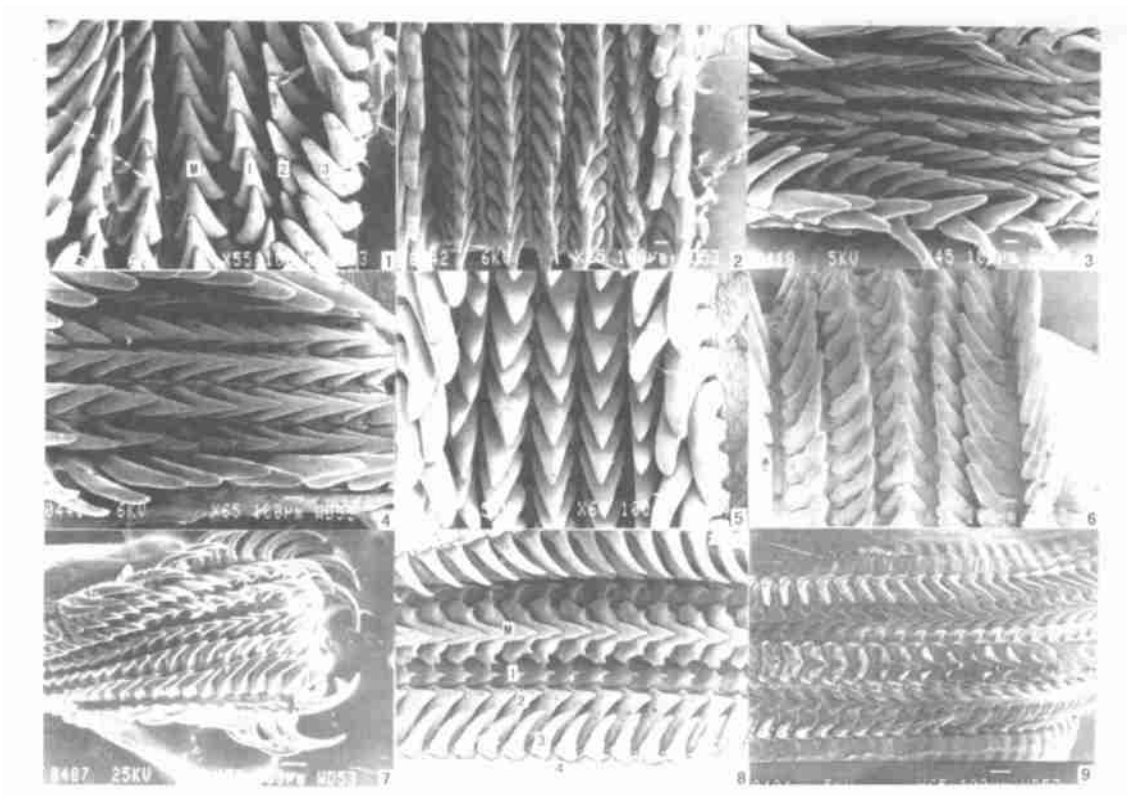

Plate Scanning electron micrographs of the radula of nine cephalopods

1. *Sepia robson*; 2. *S. esculenta*; 3. *S. aculeate*; 4.. *S. latimanus*; 5. *S. pharaonis*; 6. *Sepiella maindroni*; 7. *Euprymna berryi*; 8. *Octopus variabilis*; 9. *O. ocellatus*.

1: lst lateral tooth; 2: 2nd lateral tooth; 3: 3rd lateral tooth; 4. marginal plate; M: median tooth.